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Standard Paper

Phylogenetic study of the *Cladonia cervicornis* group (*Cladoniaceae*, *Lecanorales*) discloses a new species, *Cladonia teuvoana*

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Abstract

The *Cladonia cervicornis* group comprises lichen-forming fungi characterized by having scyphi with central proliferations. It includes *c.* 20 species globally. The taxonomy of this group is poorly resolved, with many species not thoroughly disentangled. The focus of this study is the European species in the *C. cervicornis* group. In order to estimate the phylogenetic relationships of these species, six loci were used: ITS rDNA, IGS rDNA, *RPB*1, *RPB*2, *ef1α* and *cox1*. Species delimitation methods (ASAP, PTP and GMYC) were used to infer the species boundaries based on four loci, ITS rDNA, IGS rDNA, *cox1* and *RPB*2. A morphological analysis based on multivariate methods was performed to assess the importance of phenotypic differences among the lineages. The phylogenetic reconstructions placed the species of this group in the subclade Cladonia. Five lineages were recovered, corresponding to *C. cervicornis*, *C. macrophyllodes*, C. *pulvinata*, *C. verticillata* and a new lineage that we describe here, *C. teuvoana*. Our analyses revealed that *Cladonia cineracea, C. stricta* and *C. trassii* are polyphyletic.

Keywords: *Lecanoromycetes*; lichens; morphology; taxonomy

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Introduction

The genus *Cladonia* is one of the most speciose genera of macrolichens (Lücking *et al.* 2017), with more than 475 accepted species described (Stenroos *et al.* 2019). Most of the species are terricolous, growing in open areas that have a certain amount of moisture (Ahti 2000). The genus has a subcosmopolitan distribution. It is notably diverse in the Neotropics (*c.* 195 species), particularly Eastern Brazil, and Australasia-Melanesia with around 40% of species endemic for this region (Ahti & Aptroot 1992; Ahti 2000; Aptroot *et al.* 2021), while in Europe *c.* 105 species have been reported (Litterski & Ahti 2004). The genus *Cladonia* is easily recognizable by usually having a dimorphic thallus, consisting of a squamulose or crustaceous primary thallus and a fruticose secondary thallus. However, species identification within this genus can be challenging (Burgaz *et al.* 2020; Černajová *et al.* 2022). Most of the taxonomic characters used to distinguish *Cladonia* species are associated with the secondary thallus, called podetium (Ahti 2000). Due to the limited number of characters available, distinguishing species based on squamule morphology is often difficult (Burgaz *et al.* 2020). However, many species have a dominant primary thallus and rarely develop podetia (Ahti 2000; Pino-Bodas *et al.* 2010*a*, 2012). In these cases, morphological characters associated with the primary thallus are indispensable for distinguishing species. The study of secondary metabolites can also be of great use for species circumscription (Huovinen & Ahti 1982; Culberson 1986), since morphologically similar species sometimes contain different secondary metabolites, for example *C. subulata* (L.) F. H. Wigg. and *C. rei* Schaer. or *C. humilis* (With.) J. R. Laundon and *C. conista* (Nyl.) Robbins (Dolnik *et al.* 2010; Pino-Bodas *et al.* 2010*b*, 2013*a*).

This study focuses on the *C. cervicornis* (Ach.) Flot. group in Europe. Members of this group are characterized by scyphose podetia with a corticate surface and by having proliferations arising from the centre of the scyphi. There are *c.* 20 species of *Cladonia* globally with these features (Ahti 2007). The most recent phylogeny of the genus placed them within the clade Cladonia, subclade Cladonia. This clade also includes species of the former sections *Cladonia, Helopodium* and *Unciales*, including the type species of the genus, *C. subulata* (Stenroos *et al.* 2019). Numerous authors have paid attention to the taxonomy of species with central proliferations in different geographical regions (Abbayes 1949; Ahti & Marcelli 1995; Ahti 1978, 1980, 1983, 1998, 2007; van Herk & Aptroot 2003; Pino-Bodas *et al.* 2010*a*; Charnei & Eliasaro 2013). However, many taxa are still not well understood (Ahti 2000, 2007) and phylogenetic studies have shown that some are polyphyletic (Pino-Bodas *et al.* 2013*b*; Stenroos *et al.* 2019).

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Nine species in the *C. cervicornis* group have been previously reported from Europe: *C. cervicornis*, *C. firma* (Nyl.) Nyl., *C. macrophyllodes* Nyl., *C. pulvinata* (Sandst.) Van Herk & Aptroot, *C. stricta* (Nyl.) Nyl., *C. subcervicornis* (Vain.) Kernst., *C. trassii* Ahti, *C. uliginosa* (Ahti) Ahti, and *C. verticillata* (Hoffm.) Schaer. (Ahti & Stenroos 2013). However, phylogenetic studies have shown that *C. firma* and *C. subcervicornis* do not belong to this group as they are phylogenetically distant from the rest of the species (Stenroos *et al.* 2019). In addition, there is another species restricted to Macaronesia, *C. microphylla* Ahti & Aptroot (Ahti & Aptroot 2009). *Cladonia cervicornis*, *C. pulvinata* and *C. verticillata* have a wide distribution in Europe (van Herk & Aptroot 2003; Ahti & Stenroos 2013; Burgaz *et al.* 2020; Pino-Bodas *et al.* 2021), while *C. stricta*, *C. trassii* and *C. uliginosa* are primarily distributed in the arctic or arctic-boreal regions of Eurasia and North America, with some outposts in the Alps, although their distribution is not yet well known (Ahti 1998; Brodo *et al.* 2001; Ahti & Stenroos 2013; Pino-Bodas *et al.* 2021). *Cladonia macrophyllodes* is more widely distributed, but outside arctic regions it is restricted to mountainous areas (Goward & Ahti 1997; Ahti & Stenroos 2013; Burgaz *et al.* 2018, 2020). The distribution patterns of *C. cervicornis*, *C. pulvinata* and *C. verticillata* differ, although in several regions of Europe they often coexist, and morphological discrimination can be difficult (Ahti 1980; Ahti & Stenroos 2013; Søchting 2017; Gheza *et al.* 2018; Burgaz *et al.* 2020). Morphological studies by van Herk & Aptroot (2003) showed that the coloration and morphology of the primary thallus can be used to distinguish these three taxa. In addition, the presence of psoromic acid allows *C. pulvinata* to be distinguished from the other two species, which do not synthesize this compound. *Cladonia cervicornis* is a highly variable species and some authors have indicated the need for further studies to clarify its taxonomy (Ahti & Stenroos 2013; Burgaz *et al.* 2020). Specimens of these taxa have been the subject of some molecular research (Pino-Bodas *et al.* 2010*a*, 2013*b*; Osyzcka *et al.* 2018; Stenroos *et al.* 2019), the results of which indicate that *C. cervicornis*, *C. pulvinata* and *C. verticillata* form independent lineages. However, it should be noted that sampling has been mostly focused on the Mediterranean region, where *C. cervicornis* is particularly common (Burgaz *et al.* 2020), while less sampling has been carried out in Central and Northern Europe where *C. verticillata* is more frequent and more difficult to distinguish from *C. cervicornis* (Ahti & Stenroos 2013). Phylogenetic studies have shown *C. verticillata* to be polyphyletic (Pino-Bodas *et al.* 2013*b*; Stenroos *et al.* 2019), and more extensive sampling will be necessary to determine whether European specimens belong to a single species.

The aims of this study were: 1) to evaluate the monophyly of the species within the *C. cervicornis* group reported for Europe; 2) to estimate the phylogenetic relationships of the species of the *C. cervicornis* group within the subclade Cladonia; 3) to carry out a morphological study that characterizes each of the lineages found.

Material and Methods

Taxon sampling

This study is based on 160 specimens from 18 countries. Specimens representing all known European species with central proliferations were included in the phylogenetic analyses. New study specimens included: *C. cervicornis* ($n = 57$), *C. cineracea* Ahti ($n = 1$), *C. macrophyllodes* ($n = 26$), *C. microphylla* ($n = 7$), *C. pulvinata* ($n = 15$), *C. stricta* ($n = 4$), *C. trassii* ($n = 6$), *C. uliginosa* ($n = 2$) and *C. verticillata* ($n = 32$). In addition, a selection of specimens from other regions of the world were included. A small number of specimens of *C. rappii* A. Evans, a species difficult to distinguish from *C. verticillata* in North America, were newly sequenced (Table 1). The specimens studied are deposited in C, H, E, K, MACB and TROM. Types of *C. cervicornis* (lectotype H-ACH 1672B and isolectotype BM-ACH 722A), *C. macrophyllodes* (H-ACH 1673), *C. pulvinata* (lectotype H), and representative specimens of *C. sobolifera* Nyl. (H-NYL-0608, 0638, 38780, 38784, 38785, 387986) were studied to compare their morphological features with our specimens.

Phenotypic study

The secondary metabolites of each specimen were studied by thin-layer chromatography (TLC). The standard protocol was used as described in White & James (1985) and Orange *et al.* (2010), with solvents A (toluene:1,4-dioxane:acetic acid) and C (toluene:acetic acid). The specimens were identified following van Herk & Aptroot (2003), Ahti (2007), Ahti & Stenroos (2013) and Burgaz *et al.* (2020). The morphological study was focused on *C. cervicornis*, *C. pulvinata* and *C. verticillata* which are the more common taxa in Europe and difficult to distinguish from each other. Based on previous studies (van Herk & Aptroot 2003; Ahti 2007; Ahti & Stenroos 2013; Burgaz *et al.* 2020), 13 quantitative traits were selected to conduct the morphological analyses: length of squamules, width at the middle of squamules, maximum width of squamules, squamule incision depth, length of the blackened base of squamules, thickness of squamules, length of first scyphi, total length of podetium, number of proliferations, number of tiers, widening in lower half of podetium, width of scyphi, and thickness of podetial wall. To better represent morphological patterns, the length ratio of the variables 'width of squamules', 'width of scyphi', 'squamules incisions depth' and 'blackened base of squamules' were used. To determine whether the widening of the scyphi is gradual from the base or abrupt above the middle of the podetium, the difference in width of the podetium was analyzed, corrected for length (width at the middle – width at the base / 1/2 (podetium length)). These traits were examined in a selection of 63 specimens, representing all phylogenetic lineages, by measuring three squamules and three podetia per specimen (whenever the amount of material permitted) under a dissecting microscope (Nikon SMZ21000) using a digital caliper. In addition to quantitative characters, the following qualitative features were also studied: colour of the underside of the squamules, colour of the upper side of the squamules, morphology of the squamules, morphology of the squamule lobes, presence of veins on the underside of the squamules, presence of rhizines and continuity of the podetial algal layer.

All statistical analyses were conducted in R v. 4.1.2 (R Core Team 2022). The normality and homoscedasticity of the quantitative variables were assessed using the Shapiro-Wilk and Bartlett tests, respectively. It was found that only 'depth of incisions' was normally distributed. Due to the lack of normality, the variables did not meet the assumptions of a principal component analysis (PCA), so the dataset was analyzed using non-metric multidimensional scaling (NMDS; Kruskal 1964), a more robust method for this type of data (Austin 1985; Minchin 1987). NMDS was conducted with the Bray-Curtis distances in the *vegan* package (Oksanen *et al.* 2013). Permutational

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Table 1. List of subclade *Cladonia* specimens included in the study with voucher information, secondary metabolites detected by thin-layer chromatography and GenBank accession numbers. New sequences are in bold.

Taxa	DNA code	Voucher specimens	Secondary metabolites	ITS rDNA	IGS rDNA	*cox1*	*RPB2*	*RPB1*	*ef1α*
C. albonigra	CL335	Russia, Primorye Territory, *T. Ahti* 72042 (H)		MK179466	MK141792	—	MK179764	MK180176	MK152559
C. aleuropoda	CL287	Bolivia, *P. Rodriguez* & *A. Flakus* 21998 (H)		MK179468	MK141794	—	—	MK180178	MK152561
C. aleuropoda	CL272	Bolivia, *P. Rodriguez* & A. *Flakus* 2202 (H)		MK179467	MK141793	—	—	MK180177	MK152560
C. andesita	CL418	Costa Rica, San José, *P. Clerc* & *C. Rojas* PC2013/121 (H)			—	—	—	—	—
C. andesita	AT679	Kenya, *Chuah-Petiot* 947 (TUR)		MK214455	MK141799	—	MK179770	—	MK152568
C. apodocarpa	CL86	USA, New York, *S. Stenroos* 5757 (H)		MK179743	MK141801	—	MK179772	MK180184	MK152570
C. boryi	LK6	Canada, Nova Scotia, *T. Ahti* 57176 (H)		AF455163	KR019448	—	MK179801	—	MK152602
C. brevis	CL114	USA, Pennsylvania, *J. Lendemer* 13914 (H)		MK179660	MK141824	—	MK179806	MK180213	MK152607
C. caespiticia	LK72	Canada, Nova Scotia, *T. Ahti* 57084 (H)		AF455205	MK141825	—	—	—	MK152608
C. calycantha	CL279	Bolivia, *A. Flakus* & *O. Plata* 20358 (H)		MK179501	MK141828	—	—	MK180215	MK152610
C. calycantha	CL280	Bolivia, *A. Flakus* & *O. Plata* 20399 (H)		MK179654	MK141829	—	—	—	MK152611
C. calycantha	CL316	Bolivia, *M. Kukwa* 8943 (H)		MK179531	MK141830	—	—	—	MK152612
C. centrophora	CL222	France, Réunion, *M. Brand* 58833 (H)		MK179502	MK141850	—	—	—	
C. ceratophyllina	CL172	France, Réunion, *F. Schumm* & *J.P. Frahm* 15187 (H)		MK179709	MK141855	—	MK179830	—	MK152638
C. ceratophyllina	CL159	Madagascar, *Baranow* & *Szlachetko* (H)		MK179504	MK141854	—	MK179829	—	MK152637
C. ceratophyllina	CL115	France, Réunion, *P.* & *B. van der Boom* 40320 (H)		MK179503	MK141853	—	MK179828	—	MK152636
C. cervicornis	CL1080	Albania, Shkodër, Malësi e Madhe, Dedaj, *A. R. Burgaz* (MACB 124079)	FUM	**PQ256170**	**PQ252398**	**PQ252547**	**PQ274471**	—	—
C. cervicornis	CL1087	Albania, Vlorë, Finiq, Muzinë, *A. R. Burgaz* (MACB 127516)	FUM	**PQ256177**	**PQ252404**	**PQ252552**	**PQ274474**	—	—
C. cervicornis	CL1507	Algeria, Kala National Park, Capa Rosa, *M. A. Ahmed* & *S. Boudiaf*	FUM	OQ267629	**PQ252498**	**PQ252614**	**PQ274537**	—	—
C. cervicornis	CL1506	Algeria, Kala National Park, Capa Rosa, *M. A. Ahmed* & *S. Boudiaf*	FUM	OQ267625	**PQ252497**	**PQ252613**	**PQ274536**	—	—
C. cervicornis	CL1508	Algeria, Kala National Park, Capa Rosa, *M. A. Ahmed* & *S. Boudiaf*	FUM	OQ267628	**PQ252499**	**PQ252615**	**PQ274538**	—	—
C. cervicornis	CL1247	Bulgaria, Blagoevgrad, Garmen, Krushevo, Dolno, *A. R. Burgaz* (MACB 124085)	FUM	**PQ256222**	**PQ252445**	**PQ252591**	**PQ274515**	—	—
C. cervicornis	CL1246	Bulgaria, Sliven, Sliven, Balkan Mts, Slivenska Planina, *A. R. Burgaz* (MACB 124081)	FUM	**PQ256221**	**PQ252444**	**PQ252590**	**PQ274514**	—	—
C. cervicornis	CL1084	Cyprus, Kantara, Kyrenia Mts (Pentadactylos Mts), Kantara Forest, *A. R. Burgaz* (MACB 110034)	FUM, ATR	**PQ256174**	**PQ252401**	**PQ252549**	**PQ274548**	—	—
C. cervicornis	CL1081	Cyprus, Pano Lefkara, road Lythrodontas, *A. R. Burgaz* (MACB 127535)	FUM, ATR	**PQ256171**	—	—	**PQ274472**	—	—
C. cervicornis	CL1082	Cyprus, Pano Panagia, Troodos Mts, Paphos Forest, *A. R. Burgaz* (MACB 127535)	FUM, ATR	**PQ256172**	**PQ252399**	**PQ252548**	**PQ274482**	—	—

(*Continued*)

Table 1. (*Continued*)

Taxa	DNA code	Voucher specimens	Secondary metabolites	ITS rDNA	IGS rDNA	*cox1*	*RPB2*	*RPB1*	*ef1α*
C. cervicornis	CL1083	Cyprus, Paphos, Neo Chorio, Akamas Peninsula, *A. R. Burgaz* (MACB 127517)	FUM	**PQ256173**	**PQ252400**	—	**PQ274473**	—	—
C. cervicornis	CL1123	Czech Republic, C Bohemia, Praha, Natural Park Košíře, *Z. Palice* 24916 (MACB 124101)	FUM	—	**PQ252421**	**PQ252571**	**PQ274492**	—	—
C. cervicornis	CL1067	Czech Republic, C Bohemia, Prague, Motol, Natural Park Kšiř-Motol, *T. Ahti* 74315, *J. Steinová*, *J. Liška* & *Z. Palice* (H)	FUM	**PQ256160**	**PQ252387**	**PQ252538**	**PQ274467**	—	—
C. cervicornis	CL1129	Czech Republic, N Bohemia, Dist. Česká Lípa, Sosnová, *Z. Palice* 24927 (MACB 124098)	FUM	—	**PQ252426**	**PQ252576**	**PQ274498**	—	—
C. cervicornis	CL1068	Denmark, Bornholm, Sandkås, *E. S. Hansen*, lichenes Danici Exsiccati 598 (H)	FUM	**PQ256161**	**PQ252388**	**PQ252539**	**PQ274549**	—	—
C. cervicornis	CL1415	Denmark, Jutland, Bredevandsbakker, *U. Søchting* OSE5151 (C-L 75833)	FUM	**PQ256241**	**PQ252481**	—	**PQ274534**	—	—
C. cervicornis	CL1409	Denmark, Jutland, Vandflod, N of Oksby, *U. Søchting* 12449 (C-L 75835)	FUM	**PQ256235**	**PQ252475**	**PQ252602**	**PQ274528**	—	—
C. cervicornis	CL1408	Denmark, Kølvrå, *U. Søchting* DMS-9207986 (C-L 75842)	FUM	—	**PQ252474**	—	**PQ274527**	—	—
C. cervicornis	CL1406	Denmark, Nordmarken, *U. Søchting* DMS-931277 (C-L 75838)	FUM, fatty acid	**PQ256233**	**PQ252462**	**PQ252600**	**PQ274526**	—	—
C. cervicornis	CL1412	Denmark, Spidsbjerg, *U. Søchting* DMS-9320520 (C-L 75829)	FUM	**PQ256238**	**PQ252478**	**PQ252603**	**PQ274531**	—	—
C. cervicornis	CL1411	Denmark, Spidsbjerg, *U. Søchting* DMS-9320521 (C-L 75830)	FUM	**PQ256237**	**PQ252477**	—	**PQ274530**	—	—
C. cervicornis	CL1414	Denmark, Spidsbjerg, *U. Søchting* DMS-9320522 (C-L 75827)	FUM	**PQ256240**	**PQ252480**	**PQ252605**	**PQ274533**	—	—
C. cervicornis	CL1413	Denmark, Spidsbjerg, *U. Søchting* DMS-9320523 (C-L 75828)	FUM	**PQ256239**	**PQ252479**	**PQ252604**	**PQ274532**	—	—
C. cervicornis	CL1194	France, Provence-Alpes-Côte D'Azur, Piste de Vidauban, *A. R. Burgaz* (MACB 112582)	FUM	**PQ256215**	**PQ252439**	**PQ252587**	**PQ274509**	—	—
C. cervicornis	CL1195	France, Provence-Alpes-Côte D'Azur, Tanneron, Les Marjoris-Tanneron, *A. R. Burgaz* (MACB 110701)	FUM	**PQ256216**	**PQ252440**	**PQ252588**	**PQ274510**	—	—
C. cervicornis	CL1189	France, Provence-Alpes-Côte D'Azur, Var, Chemin de Patusque, *A. R. Burgaz* (MACB 112576)	FUM	**PQ256210**	**PQ252435**	**PQ252582**	**PQ274504**	—	—
C. cervicornis	CL1191	France, Provence-Alpes-Côte D'Azur, Var, Langoustaou *A. R. Burgaz* (MACB)	FUM, CPH1	**PQ256212**	**PQ252437**	**PQ252584**	**PQ274506**	—	—
C. cervicornis	CL1192	France, Provence-Alpes-Côte D'Azur, Var, Colgolin, *A. R. Burgaz* (MACB 112579)	FUM	**PQ256213**	**PQ252438**	**PQ252585**	**PQ274507**	—	—
C. cervicornis	CL1089	France, Provence-Alpes-Côte D'Azur, Var, Esterel Massif, *A. R. Burgaz* (MACB 112576)	FUM	**PQ256179**	**PQ252406**	**PQ252553**	**PQ274476**	—	—
C. cervicornis	CL1193	France, Provence-Alpes-Côte D'Azur, Var, Gonfaron, *A. R. Burgaz* (MACB 112577)	FUM	**PQ256214**	—	**PQ252586**	**PQ274508**	—	—
C. cervicornis	CL1190	France, Provence-Alpes-D'Azur, Var, Les Pierrons, *A. R. Burgaz* (MACB 112578)	FUM	**PQ256211**	**PQ252436**	**PQ252583**	**PQ274505**	—	—
C. cervicornis	CL1186	Germany, *V. Otte* 7408 (GLM)	FUM	**PQ256209**	**PQ252432**	—	—	—	—
C. cervicornis	CL1092	Greece, Crete, Chania, Kissamos, *A. R. Burgaz* (MACB 110846)	FUM, ± PHY	**PQ256182**	**PQ252409**	**PQ252555**	**PQ274478**	—	—

(*Continued*)

Table 1. (*Continued*)

Taxa	DNA code	Voucher specimens	Secondary metabolites	ITS rDNA	IGS rDNA	*cox1*	*RPB2*	*RPB1*	*ef1α*
C. cervicornis	CL1093	Greece, Crete, Chania, Platanias, *A. R. Burgaz* (MACB 110823)	FUM, ± PHY	**PQ256183**	—	**PQ252556**	**PQ274479**	—	—
C. cervicornis	CL1091	Greece, Crete, Heraklion, Malevizi, *A. R. Burgaz* (MACB 124087)	FUM, CPH1	**PQ256181**	**PQ252408**	—	**PQ274477**	—	—
C. cervicornis	CL1090	Greece, Crete, Herajlion, Hersonissos, *A. R. Burgaz* (MACB 124089)	FUM, ± PHY, ± ZEO	**PQ256180**	**PQ252407**	**PQ252554**	**PQ274555**	—	—
C. cervicornis	CL1088	Greece, Macedonia-Tracia, Thásos island, Theologos, *A. R. Burgaz* (MACB)	FUM	**PQ256178**	**PQ252405**	—	**PQ274475**	—	—
C. cervicornis	CL1208	Italy, Calabria, Reggio Calabria, San Luca, *A. R. Burgaz* (MACB 124091)	FUM	**PQ256217**	**PQ252441**	—	**PQ274511**	—	—
C. cervicornis	CL1085	Italy, Sardinia, Nuoro, Sassari, Tempio Pausania, *A. R. Burgaz* (MACB 124078)	FUM	**PQ256175**	**PQ252402**	**PQ252550**	**PQ274553**	—	—
C. cervicornis	CL1086	Italy, Sardinia, South Sardinia, Villasimius, Notteri, *A. R. Burgaz* (MACB 127519)	FUM	**PQ256176**	**PQ252403**	**PQ252551**	**PQ274554**	—	—
C. cervicornis	CL1144	Italy, Sicily, Catania, Caltagirone, Santo Pietro, *A. R. Burgaz* (MACB 124096)	FUM	**PQ256207**	—	**PQ252581**	**PQ274503**	—	—
C. cervicornis	CL1142	Italy, Sicily, Catania, Linguaglossa, P. dell'Etna, *A. R. Burgaz* (MACB 124094)	FUM	**PQ256205**	—	**PQ252579**	**PQ274501**	—	—
C. cervicornis	CL1145	Italy, Sicily, Catania, Zafferana Etnea, P. dell'Etna, *A. R. Burgaz* (MACB 124095)	FUM	**PQ256208**	**PQ252430**	—	—	—	—
C. cervicornis	CL1141	Italy, Sicily, Messina, Castanea delle Furìe, *A. R. Burgaz* (MACB 124093)	FUM	**PQ256204**	—	**PQ252578**	**PQ274500**	—	—
C. cervicornis	CL1143	Italy, Sicily, Palermo, Monreale, Ficuzza, *A. R. Burgaz* (MACB 124097)	FUM	**PQ256206**	—	**PQ252580**	**PQ274502**	—	—
C. cervicornis	CL1040	Spain, Toledo, Belvis de la Jara, *R. Pino-Bodas* (MACB 127536)	FUM	**PQ256157**	**PQ252384**	**PQ252535**	**PQ274550**	—	—
C. cervicornis	CL1301	Spain, Toledo, Sevilleja de la Jara, *R. Pino-Bodas* (MACB)	FUM	—	**PQ252449**	—	**PQ274519**	—	—
C. cervicornis	CL1112	The Netherlands, Drenthe, Gasteren, *A. Aptroot* 76156 (MACB 127515)	FUM	**PQ256195**	**PQ252416**	**PQ252567**	**PQ274488**	—	—
C. cervicornis	CL1114	The Netherlands, Noord-Brabant, Loonse en Drunense, *A. Aptroot* 76168 (MACB 124104)	FUM	—	**PQ252418**	**PQ252569**	**PQ274490**	—	—
C. cervicornis	CL1113	The Netherlands, Utrecht, Zeist, *A. Aptroot* 76145 (MACB 127514)	FUM	**PQ256196**	**PQ252417**	**PQ252568**	**PQ274489**	—	—
C. cervicornis	CL1317	UK, England, East Kent, Canterbury, Stodmarch NNR, *P. F. Cannon* (K-M)	FUM, ZEO	**PQ256227**	**PQ252452**	**PQ252594**	**PQ274521**	—	—
C. cervicornis	CL1384	UK, England, Isles of Scilly, St. Mary´s Peninnis Head, *H. Thüs* S143b (BM 001084827)	FUM	—	—	—	**PQ274525**	—	—
C. cervicornis	CL1315	UK, England, Surrey, Esher Common, Round Hill, *B. M. S. Sponer* & *M.B. Aguirre-Hudson* (K-M)	FUM, ZEO	—	**PQ252451**	—	—	—	—
C. cervicornis	CL1512	UK, Hampshire, New Forest National Park, Lyndhurst, *R. Pino-Bodas* & *N. Sanderson* (MACB 127520)	FUM	**PQ256252**	**PQ252500**	**PQ252616**	**PQ274539**	—	—
C. cervicornis	CL1513	UK, Hampshire, New Forest National Park, Lyndhurst, *R. Pino-Bodas* & *N. Sanderson* (MACB 127521)	FUM	—	**PQ252501**	**PQ252617**	**PQ274540**	—	—
C. cervicornis	CL1461	UK, Surrey, Thursley Natural Reserve, *R. Pino Bodas* (K-M)	FUM	**PQ256248**	—	—	—	—	—
C. cervicornis	CL1439	UK, Surrey, Thursley Natural Reserve, *R. Pino Bodas* (K-M)	FUM	**PQ256246**	—	—	—	—	—
C. cervicornis	CL1459	UK, Surrey, Thursley Natural Reserve, *R. Pino Bodas* (K-M)	FUM	**PQ256247**	—	—	—	—	—

(*Continued*)

Table 1. (*Continued*)

Taxa	DNA code	Voucher specimens	Secondary metabolites	ITS rDNA	IGS rDNA	*cox1*	*RPB2*	*RPB1*	*ef1α*
C. cervicornis	CL1420	UK, Surrey, Thursley Natural Reserve, *R. Pino Bodas* (MACB 127537)	FUM	**PQ256245**	**PQ252486**	**PQ252607**	**—**	—	—
C. cervicornis	CL1064	Ukraine, Crimea, Alushta District, *O. Nadyeina* (H)	FUM	**PQ256158**	**PQ252385**	**PQ252536**	**PQ274465**	—	—
C. cervicornis	CL1066	Ukranie, Crimea, NE vicinities of Kerch city, *O. Nadyeina* (H)	FUM	**PQ256159**	**PQ252386**	**PQ252537**	**PQ274466**	—	—
C. cervicornis	9CER	Spain, Cuenca, *A. R. Burgaz* (MACB 90718)	FUM	FM205906	—	FM208171	—	—	—
C. cervicornis	1CER	Spain, Cádiz, *A. R. Burgaz* (MACB 91631)	FUM	FM211897	MK141856	—	—	MK180239	MK152639
C. cervicornis	5CER	Spain, Guadalajara, *A. R. Burgaz* (MACB 90738)	FUM	FM205904	—	—	FM2057578	—	MK152640
C. cervicornis	13CER	Spain, Huelva, *A. R. Burgaz* (MACB 91610)	FUM	FM205916	—	—	—	—	—
C. cervicornis	CL198	Greece, *F. Schumm* & *Düll* 13410 (H)		MK179619	MK141859	**PQ252621**	MK179833	MK180242	MK152643
C. cervicornis	CL193	Spain, Mallorca, *F.* Schumm, *J. P. Frahm* & *Lüth* 16330 (H)		MK179618	MK141857	**PQ252620**	MK179831	MK180240	MK152641
C. cervicornis	7CER	Spain, Madrid, *A. R. Burgaz* (MACB 90840)	FUM	FM205905	—	FM208170	—	—	—
C. cervicornis	CL230	Greece, *H. Sipman* & *T. Raus* (H)		MK179594	MK141858	**PQ252626**	MK179832	MK180241	MK152642
C. aff. *cervicornis*	CL1078	USA, Maine, Washington County, T10 SD, Donnell Pond Public Reserve Lands, *J. Lendemer* 22968 (H)	FUM	**PQ256168**	**PQ252455**	**PQ252545**	**PQ274470**	—	—
C. cineracea	CL1331	Russia, Kamchatka Peninsula, Koryak Okrug, Olutorsky District, Koryak State Reserve, N to Kuyakynvayam River, *D. E. Himelbrant* & *I. S. Stepanchikova* Kor-Goven-26-2018 (H)	FUM, ATR	—	**PQ252460**	**PQ252597**	**PQ274523**	—	—
C. cineracea	CL117	Russia, Sakha Republic, *T. Ahti* 64967 (H)		MK141867	MK179710	—	—	—	MK152651
C. cineracea	CL1332	Russia, Kamchatka Peninsula, Koryak Okrug, Olutorsky, *D. E. Himelbrant* & *I. S. Stepanchikova* Kor-Goven-26-2018 (H)	FUM	**PQ256231**	**PQ252461**	**PQ252598**	—	—	—
C. clathrata	LK83	Brazil, Minas Gerais, *S. Stenroos* 5085a (TUR)		AF455185	—	—	MK179840	—	MK152652
C. cornuta ssp. *cornuta*	10CORN	Finland, Uusima, *R. Pino-Bodas* (MACB)	FUM	JN811385	JN811350	—	JN811428	MK180269	MK152678
C. farinacea	4FARIN	Chile, Magallanes, *A. R. Burgaz* (MACB)	FUM	KR818327	KR818411	—	KR818502	—	—
C. fimbriata	CL259	Finland, *V. Haikonen* 28468 (H)		MK141945	MK179532	—	MK179898	MK180305	MK152729
C. firma	CL1517	UK, E. Cornwall, Hemar Tor, Lanlivery, *N. A. Sanderson* 2679	FUM, ATR	**PQ256255**	—	**PQ252619**	—	—	—
C. firma	1FIRM	Spain, Burgos, *A. R. Burgaz* (MACB 91615)	FUM, ATR	FM205909	MK141946	FM208168	FM207577	MK180306	KC526124
C. foliacea	16FOL	UK, Scotland, *B. Coppins* (MACB 95602)	FUM, USN	FM205898	MK141950	FM208145	FM207566	MK180309	MK152733
C. furcata	1FURC	Spain, León, *A. R. Burgaz* (MACB 91055)	FUM	KR818310	KR818394	—	KR818488	—	MK152739
C. furfuraceoides	LK60	Guyana, *S. Stenroos* 4794 (TUR)		AF455202	MK141956	—	MK179907	MK180316	MK152741
C. gracilis ssp. *gracilis*	1GRAC	Spain, *A. R. Burgaz* (MACB)		JN811386	JN811386	—	JN811412	MK180321	MK152747
C. graeca	CL211	Greece, *H. Sipman* & *T. Raus* 53957a (H)		MK179510	MK141965	—	—	MK180326	MK152754
C. grayi	CL87	USA, New York, *S. Stenroos* 5752 (H)		MK141970	MK179716	—	—	—	MK152759
C. isabellina	1ISABEL	Ecuador, *R. Pino-Bodas* (MACB)		MK179717	MK141982	—	—	—	MK152771

(*Continued*)

Table 1. (*Continued*)

Taxa	DNA code	Voucher specimens	Secondary metabolites	ITS rDNA	IGS rDNA	*cox1*	*RPB2*	*RPB1*	*ef1α*
C. isabellina	CL288	Bolivia, *A. Flakus* & *O. Plata* 22705 (H)		MK179482	MK142029	—	MK179972	MK180381	MK152824
C. isabellina	CL347	Costa Rica, *P. Clerc* & *C. Rojas* PC2013/170 (H)		MK179476	MK141986	—	MK179929	—	MK152773
C. isabellina	CL278	Bolivia, *P. Rodriguez* 1210 (H)		MK141984	MK179475	—	—	—	—
C. isabellina	CL269	Bolivia, *P. Rodriguez* & *A. Flakus* 1103 (H)		MK141983	MK179474	—	—	MK180339	MK152772
C. isabellina	CL282	Bolivia, *A. Flakus* & *O. Plata* 21705 (H)		MK179571	MK141985	—	—	—	—
C. islandica	CL255	USA, Alaska, *S. Talbot* AML008-X-13Ab (H)		MK179511	MK141987	—	—	MK180340	MK152774
C. islandica	CL262	USA, Alaska, *S. Talbot* AML008-X-13A (H)		MK141988	MK179512	—	MK179930	MK180341	MK152775
C. itatiaiae	AT563	Brazil, Minas Gerais, *S. Stenroos* 4996 (H)		—	MK141989	—	—	—	MK152776
C. krempelhuberi	CL71	New Zealand, *S. Stenroos* 6012 (H)		MK179477	MK141991	—	—	—	MK152781
C. latiloba	1LATILOB	Brazil, Santa Catarina, *Sanders* s.n. (H)			MK141995	—	JN621967	—	JN621998
C. macrophyllodes	CL2216	Andorra, Encamp, Soldeu, Port d'Envalira, *R. Pino-Bodas* 373-2022 & *A. R. Burgaz* (MACB 127526)	FUM, ATR	**PQ256257**	—	—	—	—	—
C. macrophyllodes	CL2217	Andorra, Encamp, Soldeu, Port d'Envalira, *R. Pino-Bodas* 375-2022 & *A. R. Burgaz* (MACB 127527)	FUM, ATR	**PQ256258**	—	—	—	—	—
C. macrophyllodes	CL2219	Andorra, Encamp, Soldeu, Port d'Envalira, *R. Pino-Bodas* 378-2022 & *A. R. Burgaz* (MACB 127528)	FUM, ATR	**PQ256259**	—	—	—	—	—
C. macrophyllodes	CL2223	Andorra, Ordino-Arcalís, *R. Pino-Bodas* 450-2022 & *A. R. Burgaz* (MACB 127530)	FUM, ATR	**PQ256261**	**PQ252503**	**PQ252623**	—	—	—
C. macrophyllodes	CL2226	Andorra, Ordino-Arcalís, *R. Pino-Bodas* 452-2022 & *A. R. Burgaz* (MACB 127531)	FUM, ATR	**PQ256263**	**PQ252515**	**PQ252624**	—	—	—
C. macrophyllodes	CL2228	Andorra, Ordino-Arcalís, *R. Pino-Bodas* 458-2022 & *A. R. Burgaz* (MACB 127532)	FUM, ATR	**PQ256264**	**PQ252505**	**PQ252625**	—	—	—
C. macrophyllodes	CL2224	Andorra, Ordino-Arcalís, *R. Pino-Bodas* 456-2022 & *A. R. Burgaz* (MACB 127529)	FUM, ATR	**PQ256262**	**PQ252504**	—	—	—	—
C. macrophyllodes	CL1302	Bulgaria, Blagoevgrad, Bansko, Pirin Mts, *A. R. Burgaz* (MACB)	FUM, ATR	**PQ256226**	**PQ252450**	—	**PQ274520**	—	—
C. macrophyllodes	CL1299	Bulgaria, Blagoevgrad, Bansko, Pirin Mts, around Vihren, *A. R. Burgaz* (MACB)	FUM, ATR	**PQ256225**	**PQ252448**	**PQ252593**	**PQ274518**	—	—
C. macrophyllodes	CL2461	Canada, British Columbia, Cathedral Lake, *G.W. Scotter* QLL02 (CANL)	FUM, ATR	**PQ256267**	**PQ252507**	—	—	—	—
C. macrophyllodes	CL2460	Canada, British Columbia, Osoyoos Division Yale Land, *G.W. Scotter* JPL20 (CANL)	FUM, ATR	**PQ256266**	**PQ252506**	—	—	—	—
C. macrophyllodes	CL2385	Montenegro, Crna Gora, Plav, Meteh Babino Polije, *A. R. Burgaz* (MACB 111751)	FUM, ATR	—	—	**PQ252627**	—	—	—
C. macrophyllodes	CL1577	Montenegro, Plav, Meteh, *A. R. Burgaz* (MACB 111751)	FUM, ATR	**PQ256256**	—	—	—	—	—
C. macrophyllodes	CL1484	Norway, Nord-Trøndelag, Snåsa, *H. Holien* 12195 (TRH L-13141)	FUM, ATR	—	**PQ252496**	—	—	—	—

(*Continued*)

Table 1. (*Continued*)

Taxa	DNA code	Voucher specimens	Secondary metabolites	ITS rDNA	IGS rDNA	*cox1*	*RPB2*	*RPB1*	*ef1α*
C. macrophyllodes	CL3018	Norway, Østerdalen, Innlandet, Alvdal, Tron Mountain, *R. Pino-Bodas* 645-2023 (MACB 127553)	FUM, ATR	—	**PQ252522**	**PQ252632**	**—**	—	—
C. macrophyllodes	CL2251	Portugal, Mihno, Peneda-Gerês National Park, *R. Pino-Bodas* 307-2022 (MACB 127552)	FUM, ATR	**PQ256265**	—	—	—	—	—
C. macrophyllodes	CL3036	Switzerland, Kallarmes Schneetälchen, *M. Vust* TL22JG4107	FUM, ATR	—	**PQ252528**	—	—	—	—
C. macrophyllodes	CL2746	Switzerland, *M. Vust* 3849	FUM, ATR	—	**PQ252520**	—	—	—	—
C. macrophyllodes	CL2740	Switzerland, UR, Realp, Col de la Furka, *M. Vust* 5966 & *C. Scheidegger*	FUM, ATR	—	**PQ252514**	—	—	—	—
C. macrophyllodes	CL2742	Switzerland, VS, Bagnes, La Ly, *M. Vust* 6815	FUM, ATR	—	**PQ252517**	—	—	—	—
C. macrophyllodes	CL2733	Switzerland, Canton Uri, Alps, road between Wassen, *R. Pino-Bodas* 457_2023 (MACB 12755)	FUM, ATR	—	**PQ252513**	—	—	—	—
C. macrophyllodes	CL2744	Switzerland, UR, Realp, Col de la Furca, *M. Vust* & *C. Scheidegger* 6044	FUM, ATR	—	**PQ252519**	—	—	—	—
C. macrophyllodes	CL2747	Switzerland, VS, Bagnes, La Ly, *M. Vust* 654	FUM, ATR	—	**PQ252521**	—	—	—	—
C. macrophyllodes	CL2741	Switzerland, VS, Bagnes, Pte du Parc, *M. Vust* 961	FUM, ATR	—	**PQ252516**	—	—	—	—
C. macrophyllodes	CL2743	Switzerland, VS, Obergoms, Col de la Furka, *M. Vust* & *C. Scheidegger* 6091	FUM, ATR	—	**PQ252518**	—	—	—	—
C. macrophyllodes	LK79	Greenland, *E. S. Hansen*, Lich. Groenl. Exs. 683 (H)		AF455173	MK142013	—	MK179959	—	MK152806
C. magyarica	2MAGY	Hungary, *L. Lökös* (MACB 98243)		KC526136	KC526045	—	KC526075	MK180368	KC526114
C. mauritiana	CL113	France, Réunion, *P. & B. van der Boom* 40842 (H)		MK179620	MK142022	—	—	—	MK152815
C. mauritiana	CL191	France, Réunion, *F. Shumm* & *J.P. Frahm* 15496 (H)		MK179600	MK142023	—	MK179967	MK180375	MK152816
C. mauritiana	AT584	Seychelles, *Saaristo* (TUR)		AF453846	MK142020	—	MK179965	—	MK152813
C. mauritiana	CL101	Cameroon, *Piatek* & *Piatek* 2 (KRAM)		MK179616	MK142021	—	MK179966	MK180374	MK152814
C. mawsonii s. lat.	LK12	Kerguelen, *Poulsen* RSP-1044 (TUR)		AF455178	MK142025	—	—	—	MK152818
C. mawsonii	CL30	New Zealand, *S. Stenroos* 5901 (H)		MK179480	MK142024	—	MK179968	MK180376	MK152817
C. melanopoda	CL277	Bolivia, *A. Flakus* 21788 (H)		MK179481	MK142028	—	—	—	—
C. mateocyatha	CL123	USA, Pensylvania, *Harris* 54245 (H)		MK179479	MK142019	—	—	—	MK152812
C. microphylla	CL1218	Cape Verde, Porto Santo, loc. 894, *S. Pérez-Ortega* 6035 (MA)	FUM	**PQ256218**	**PQ252442**	**—**	**PQ274512**	—	—
C. microphylla	CL1221	Cape Verde, Porto Santo, loc. 899, *S. Pérez-Ortega* 6120 (MA)	FUM	**PQ256220**	**PQ252443**	**PQ252589**	**PQ274513**	—	—
C. microphylla	CL1070	Portugal, The Azores, Pico island, Candelária, *R. Pino-Bodas* (H)	FUM	**PQ256163**	**PQ252390**	**PQ252541**	**PQ274551**	—	—
C. microphylla	CL1071	Portugal, The Azores, Faial island, Cedros, *R. Pino-Bodas* (H)	FUM	**PQ256164**	**PQ252391**	**PQ252542**	**PQ274552**	—	—
C. microphylla	CL817	Portugal, The Azores, Terceira Island, *R. Pino-Bodas* (H)	FUM, CPH2	**PQ256276**	**PQ252529**	**PQ252640**	**PQ274547**	—	—
C. microphylla	CL1100	Portugal, The Azores, Terceira, Algar do Carvão, *J. Etayo* 31054	FUM	**PQ256186**	**PQ252410**	**PQ252557**	**—**	—	—
C. microphylla	-			MN586949	—	—	—	—	—
C. neozelandica	CL32	New Zealand, *S. Stenroos* 5903 (H)		MK179539	MK142053	—	MK179993	MK180398	MK152849

(*Continued*)

Table 1. (*Continued*)

Taxa	DNA code	Voucher specimens	Secondary metabolites	ITS rDNA	IGS rDNA	*cox1*	*RPB2*	*RPB1*	*ef1α*
C. nipponica	CL213	USA, Alaska, *K. Dillman* 19 (H)		KR019397	KR019473	—	MK179999	MK180404	MK152855
C. nitens	FH346	Russia, Sakha Republic, *T. Ahti* 64841 (H)		MK179513	MK142059	—	MK180001	MK180406	MK152857
C. petrophila	CL227	USA, North Carolina, *Perlmutter* 2538 (H)		MK179679	MK142069	—	MK180011	MK180415	MK152868
C. peziziformis	AT631	USA, DC, *S. Stenroos* 5198 (TUR)		AF455221	KR019478	—	—	—	MK152870
C. phyllophora	CL2222	Andorra, Ordino-Arcalís, *R. Pino-Bodas* 455-2022 & *A. R. Burgaz* (MACB)	FUM, ATR	**PQ256260**	—	**PQ252622**	—	—	—
C. phyllophora	CL3035	Norway, Østerdalen, Innlandet, Dovre Municipality, *R. Pino-Bodas* 754b-2023 (MACB 127554)	FUM	—	**PQ252527**	**PQ252637**	**PQ274545**	—	—
C. phyllophora	CL3033	Norway, Østerdalen, Innlandet, Venabygb, Venabu, *R. Pino-Bodas* 461-2023 (MACB 127534)	FUM	**PQ256272**	**PQ252525**	**PQ252635**	**PQ274557**	—	—
C. phyllophora	CL3031	Norway, Østerdalen, Innlandet, Venabygb, Venabu, *R. Pino-Bodas* 463-2023 (MACB)	FUM	**PQ256271**	**PQ252524**	**PQ252634**	**PQ274543**	—	—
C. phyllophora	CL3034	Norway, Østerdalen, Innlandet, Alvdal, Tron Mountain, *R. Pino-Bodas* 648-2023 (MACB 127533)	FUM	—	**PQ252526**	**PQ252636**	**PQ274544**	—	—
C. phyllophora	LK15	Finland, *S. Stenroos* 5161 (TUR)		AF455170	MK142071	—	MK180012	MK180417	MK152872
C. polycarpoides	CL1252	Bulgaria, Blagoevgrad, Sandanski, Gorno Spanchevo, *A. R. Burgaz* (MACB 127538)	NSTIC, CNSTIC	**PQ256224**	**PQ252446**	**PQ274517**	—	—	—
C. polycarpoides	FH354	Russia, Leningrad Region, *T. Ahti* 68339 (H)		MK179607	MK142084	—	—	MK180427	MK152886
C. polyscypha	LK73	Guyana, *S. Stenroos* 4789 (TUR)		AF453847	MK142088	—	MK180025	MK180430	MK152890
C. pulvinata	CL1405	Denmark, Harrild Hede, *U. Søchting* DMS-9196414 (C-L 75839)	PSO, CPSO	**PQ256232**	**PQ252471**	**PQ252599**	—	—	—
C. pulvinata	CL1407	Denmark, Lodbjerg Fyr, *U. Søchting* DMS-9316490 (C-L 75837)	PSO, CPSO	**PQ256234**	**PQ252472**	**PQ252601**	—	—	—
C. pulvinata	CL1475	Norway, Sør-Trøndelag, Åfjord, Storfjellet, *H. Holien* 14946 (TRH L-16327)	PSO, CPSO	**PQ256249**	**PQ252473**	**PQ252609**	—	—	—
C. pulvinata	CL1096	Portugal, Beira Alta, Penhas Douradas, Serra da Estrela, *A. R. Burgaz* (MACB)	PSO, CPSO	**PQ256185**	**PQ252464**	—	—	—	—
C. pulvinata	CL1094	Spain, Ávila, Candelario, P. Nat. S° de Candelario, *A. R. Burgaz* (MACB 95387)	PSO, CPSO	**PQ256184**	**PQ252463**	—	—	—	—
C. pulvinata	CL1098	Spain, Burgos, Pineda de la Sierra, S° de Mencilla, *A. R. Burgaz* (MACB 98143)	PSO, CPSO	—	**PQ252466**	—	—	—	—
C. pulvinata	CL1097	Spain, Cáceres, Guadalupe, Sª de Guadalupe, subida al pico Villuercas, *A. R. Burgaz* (MACB 95141)	PSO, CPSO	—	**PQ252465**	—	—	—	—
C. pulvinata	CL2661	Spain, Guipúzcoa, Urbia, *R. Pino-Bodas* 227-2023 (MACB 125072)	PSO, CPSO	**PQ256268**	**PQ252511**	**PQ252630**	**PQ274542**	—	—
C. pulvinata	CL1095	Spain, Soria, Muriel Viejo, *A. R. Burgaz* (MACB 98303)	PSO, CPSO	—	—	—	**PQ274480**	—	—
C. pulvinata	CL1106	The Netherlands, Gelderland, Kootwijk, *A. Aptroot* 76138 (MACB 124102)	PSO, CPSO	**PQ256191**	**PQ252467**	**PQ252562**	**PQ274483**	—	—
C. pulvinata	CL1110	The Netherlands, Gelderland, Wekerom, *A. Aptroot* 76141 (MACB 124105)	PSO, CPSO	—	—	**PQ252565**	—	—	—
C. pulvinata	CL1109	The Netherlands, Noord-Brabant, Loonse en Drunense, *A. Aptroot* 76167 (MACB 124107)	PSO, CPSO	**PQ256194**	**PQ252469**	**PQ252564**	**PQ274486**	—	—

(*Continued*)

Table 1. (*Continued*)

Taxa	DNA code	Voucher specimens	Secondary metabolites	ITS rDNA	IGS rDNA	*cox1*	*RPB2*	*RPB1*	*ef1α*
C. pulvinata	CL1107	The Netherlands, Utrecht, Zeist, *A. Aptroot* 76143 (MACB 124103)	PSO, CPSO	**PQ256192**	**PQ252468**	**—**	**PQ274484**	—	—
C. pulvinata	CL1103	The Netherlands, Utrecht, Zeist, *A. Aptroot* 76144 (MACB 124106)	PSO, CPSO	**PQ256189**	—	**PQ252560**	**PQ274481**	—	—
C. pulvinata	CL1321	UK, Scotland, VC92, Aberdeenshire, Glen Quioch, *A. Delaney* & *R. Yahr* 5363 (E 007188)	PSO, CPSO	—	**PQ252470**	—	—	—	—
C. pulvinata	9PUL	Spain, Segovia, *A. R. Burgaz* (MACB 95598)	PSO, CPSO	—	—	—	—	—	—
C. pulvinata	7PUL	Portugal, Tras-Os-Montes, *A. R. Burgaz* (MACB 94339)	PSO, CPSO	—	—	—	—	—	—
C. pulvinata	4PUL	Spain, Orense, *A. R. Burgaz* (MACB 91646)	PSO, CPSO	FM205911	KC526048	FM208172	FM207579	—	MK152905
C. pulvinata	CL132	The Netherlands, *A. Aptroot* 59984 (H)		MK142101	MK179725	—	MK180039	—	MK152906
C. ramulosa	CL344	Denmark, *P. Corfixen*, *S. Christensen* & *E. S. Hansen* (H)		MK142113	MH183220	—	MH183038	—	MK152919
C. rappii	CL648	Canada, New Brunswick, *T. Ahti* 74421 & *S. Clayden* (H)	FUM	**PQ256273**	**PQ252456**	—	—	—	—
C. rappii	CL649	Canada, New Brunswick, *T. Ahti* 74439 & *S. Clayden* (H)	FUM	**PQ256274**	**PQ252457**	—	—	—	—
C. rappii	CL1329	Canada, Newfoundland & Labrador, Island of Newfoundland, SW coast, Isle aux Mort, *T. Ahti* 75979 & *J. M. McCarthy* (H)	FUM	—	**PQ252458**	—	—	—	—
C. rappii	CL350	Costa Rica, *P. Clerc* & *C. Rojas* PC2013/155 (H)		MK179492	MK142128	—	—	—	MK152933
C. rappii	CL273	Bolivia, *A. Flakus* & *O. Plata* 21564 (H)		MK179491	MK142127	—	—	MK180465	MK152932
C. rappii	CL183	Australia, Western Australia, *F. Schumm* & *Stocker* 15647 (H)		MK179687	MK142215	—	MK180149	MK180549	MK153032
C. rappii	CL174	Australia, Western Australia, *F. Schumm* & *Stocker* 15800 (H)		MK179499	MK142219	—	—	—	MK153035
C. rappii	AT707	Australia, New South Wales, *Wall* s.n. (TUR)		AF455177	MK142129	—	MK180062	MK180466	MK152934
C. rappii	LK29	Bhutan, *U. Søchting* 8205 (H)		AF453843	MK142130	—	MK180063	MK180467	MK152935
C. signata	AT562	Brazil, Minas Gerais, *S. Stenroos* 4955 (TUR)		AF455233	MK142152	—	MK180082	MK180483	MK152955
C. staufferi	AT738	Australia, Victoria, *Hammer* 7051 (H)		AF455179	MK142164	—	MK180088	MK180491	MK152964
C. stricta	CL1424	Greenland, Narsaq, Mt Nuluk S of Igaliku, *S. Svane* (C-L75516)	FUM, ATR	—	**PQ252488**	—	—	—	—
C. stricta	CL1422	Greenland, Narsaq, Narsaq Kommune, *V. Alstrup* 7609 (C)	FUM	—	**PQ252487**	—	—	—	—
C. stricta	CL1339	Iceland, Mýrasýla, Norðurárdalur, Túnþýði farm, *P. F. Cannon* (K-M 201921)	FUM	—	—	—	**PQ274524**	—	—
C. stricta	CL1482	Norway, Nord-Trøndelag, Namssos, *H. Holien* 11342 (TRH L-1279)	FUM	—	**PQ252489**	—	—	—	—
C. sobolescens	1SUBCARI	USA, New Jersey, *J. Lendemer* et al. 1037 (H)		JN621936	MK142169	—	JN621969	—	JN622000
C. subcervicornis	1SUBCER	Portugal, *A. R. Burgaz* (MACB)		MK179610	MK142171	—	MK180096	MK180498	MK152974
C. subcervicornis	CL99	UK, Scotland, *S. Stenroos* 6090 (H)		MK179681	MK142173	—	MK180098	MK180499	MK152976
C. subcervicornis	CL135	Faeroe Islands, *H. Väre* L1841 (H)		MK179726	MK142172	—	MK180097	—	MK152975
C. subchordalis	LK90	Chile, Osorno, *Feuerer* 60406 (TUR)		AF455174	—	—	MK180100	MK180501	MK152978
C. subchordalis	AT512	Chile, Magallanes, *Feuerer* 60166 (TUR)		AF455175	—	—	MK180099	MK180500	MK152977

(*Continued*)

Table 1. (*Continued*)

Taxa	DNA code	Voucher specimens	Secondary metabolites	ITS rDNA	IGS rDNA	*cox1*	*RPB2*	*RPB1*	*ef1α*
C. subsquamosa	CL296	Bolivia, *A. Flakus* & *O. Plata* 20894 (H)		MK179630	MK142182	—	MK180110	—	MK152989
C. subulata	CL336	Finland, *T. Ahti* 71379 (H)		MK179686	MK142198	—	MK180129	—	MK153007
C. subulata	LK64	Germany, *A. Thell* 9932 (TUR)		AF455181	—	—	MK180130	MK180527	MK153008
C. teuvoana sp. nov.	CL1130	Czech Republic, C Bohemia, Praha, Natural Park Košíře, *Z. Palice* 24917 (MACB 127523)	FUM	**PQ256203**	**PQ252426**	**PQ252577**	**PQ274499**	—	—
C. teuvoana sp. nov.	CL1410	Denmark, Jutland, Harrild, Hede, *U. Søchting* 12520 (C-L 75831)	FUM	**PQ256236**	**PQ252476**	**—**	**PQ274529**	—	—
C. teuvoana sp. nov.	CL2663	Spain, Guipúzcoa, Urbia, *R. Pino-Bodas* 229-2023 (MACB 125083)	FUM	**PQ256269**	**PQ252512**	**PQ252631**	**—**	—	—
C. teuvoana sp. nov.	CL1111	The Netherlands, Drenthe, Balloo, *A. Aptroot* 76154 (MACB 127525)	FUM	**PQ252415**	—	**PQ252566**	**PQ274487**	—	—
C. teuvoana sp. nov.	CL1108	The Netherlands, Drenthe, Gasteren zand, *A. Aptroot* 76155 (MACB 127524)	FUM	**PQ256193**	**PQ252414**	**PQ252563**	**PQ274485**	—	—
C. teuvoana sp. nov.	CL1515	UK, Hampshire, New Forest National Park, Lyndhurst, *R. Pino-Bodas* & *N. Sanderson* (MACB 127522)	FUM	—	**PQ252502**	**PQ252618**	**PQ274541**	—	—
C. teuvoana sp. nov.	CL129	Sweden, *Løfall* 10970 (H)		MK179606	MK142083	—	—	—	MK152885
C. trassii	CL1480	Norway, Nordland, Grane, Holmvassdalen, *H. Holien* 11879 (TRH L-12715)	FUM, ATR	—	**PQ252492**	—	—	—	—
C. trassii	CL1481	Norway, Nord-Trøndelag, Namssokogan, W of Steinfjellettunnelen, *H. Holien* 11753 (TRH L-12481)	FUM, ATR	**PQ256251**	**PQ252493**	**PQ252612**	**—**	—	—
C. trassii	CL1478	Norway, Trøndelag, Namdalseid, *H. Holien* 12184 (TRH L-13132)	FUM, ATR	—	**PQ252490**	**PQ252610**	**—**	—	—
C. trassii	CL1479	Norway, Trøndelag, Snåsa, S of Grønhaugen, *H. Holien* 12184 (TRH L-13132)	FUM, ATR	**PQ256250**	**PQ252491**	**PQ252611**	**—**	—	—
C. trassii	CL1330	Russia, Kamchatka Peninsula, Koryak Okrug, Olutorsky, *Himelbrant & I.S. Stepanchikova* Kor-Goven-10add-2018 (K)	FUM, ATR	**PQ256230**	**PQ252459**	**PQ252596**	**PQ274522**	—	—
C. trassii	CL1323	UK, Scotland, VC94 Banffshire, Cairngorms, Beinn, Macduibh, Garbh Uisge Mor, *P. Harrold* 11328 (E0075565)	FUM, ATR	**PQ256228**	**PQ252453**	**PQ252595**	**—**	—	—
C. turgida	AT645	Finland, *Jääskeläinen* (TUR)		AF455203	MK142207	—	MK180138	MK180536	MK153017
C. uliginosa	CL1328	Russia, Kamchatka Peninsula, Koryak Okrug, Olutorsky, *D. E. Himelbrant* & *I.S. Stepanchikova* Kor-Galin-26-2018 (K)	FUM, ATR	**PQ256229**	**PQ252454**	—	—	—	—
C. uliginosa	CL327	Finland, *V. Haikonen* 29139 (H)		MK179498	MK142208	—	—	MK180537	MK153018
C. verticillata	CL1251	Bulgaria, Vidin, Ruzhintsi, Pleshivets, Vidin-Kulata road, *A. R. Burgaz* (MACB 127541)	FUM	**PQ256223**	**PQ252447**	**PQ252592**	**PQ274516**	—	—
C. verticillata	CL869	Canada, New Brunswick, Saint John, *T. Ahti* 74443 & *S. R. Clayden* (MACB 124110)	FUM	**PQ256277**	**PQ252530**	**—**	**—**	—	—
C. verticillata	CL1073	Canada, Newfoundland & Labrador, Island of Newfoundland, Humber East District, *T. Ahti* 74744 (H)	FUM	**PQ256165**	**PQ252392**	**PQ252543**	**PQ274468**	—	—
C. verticillata	CL1419	Canada, Newfoundland & Labrador, Island of Newfoundland, *T. Ahti* 75602 & *J. McCarthy* (H)	FUM	**PQ256244**	**PQ252485**	**—**	**—**	—	—
C. verticillata	CL1074	Canada, Newfoundland, White Bay South region, *T. Ahti* 70697 (H)	FUM	**PQ256166**	**PQ252393**	**—**	**—**	—	—

(*Continued*)

Table 1. (*Continued*)

Taxa	DNA code	Voucher specimens	Secondary metabolites	ITS rDNA	IGS rDNA	*cox1*	*RPB2*	*RPB1*	*ef1α*
C. verticillata	CL2487	Canada, Quebec, Bas-St-Laurent, *C. Boudreault* 20191024A9 (H)	FUM	—	—	**PQ252629**	—	—	—
C. verticillata	CL2486	Canada, Quebec, *C. Boudreault* 20210724A4 (H)	FUM	—	**PQ252510**	—	—	—	—
C. verticillata	CL1125	Czech Republic, N Bohemia, National Park České, *Z. Palice* 24886 (MACB 127545)	FUM	**PQ256199**	—	**PQ252572**	**PQ274494**	—	—
C. verticillata	CL1124	Czech Republic, N Bohemia, National Park České, *Z. Palice* 24897 (MACB 127546)	FUM	**PQ256198**	**PQ252422**	—	**PQ274493**	—	—
C. verticillata	CL1131	Czech Republic, N Bohemia, Dist. Česká Lípa, Sosnová, *Z. Palice* 24928 (MACB 124111)	FUM	—	**PQ252428**	—	—	—	—
C. verticillata	CL1122	Czech Republic, N Bohemia, Dist. Česká Lípa, Sosnová, *Z. Palice* 24935 (MACB 127549)	FUM	**PQ256197**	**PQ252420**	**PQ252570**	—	—	—
C. verticillata	CL1121	Czech Republic, N Bohemia, Jetřichovice, National Park, *Z. Palice* 24921 "České Švýcarko" (MACB 124110)	FUM	—	**PQ252419**	—	**PQ274491**	—	—
C. verticillata	CL1132	Czech Republic, N Bohemia, National Park České, *Z. Palice* 18869 (MACB 124108)	FUM	—	**PQ252429**	—	—	—	—
C. verticillata	CL1127	Czech Republic, N Bohemia, National Park České Švýcarsko, *Z. Palice* 24898 (MACB 127547)	FUM	**PQ256201**	**PQ252424**	**PQ252574**	**PQ274496**	—	—
C. verticillata	CL1128	Czech Republic, N Bohemia, National Park České Švýcarsko, *Z. Palice* 24937 (MACB 127548)	FUM	**PQ256202**	**PQ252425**	**PQ252575**	**PQ274497**	—	—
C. verticillata	CL1417	Denmark, Ålbæk Klitplantage, *U. Søchting* DMS-9198468 (C-L 75840)	FUM	**PQ256242**	**PQ252483**	—	**PQ274535**	—	—
C. verticillata	CL1418	Denmark, Hanstholm Vildtresevat, *U. Søchting* DMS-9245802 (C-L 75841)	FUM	**PQ256243**	**PQ252484**	**PQ252606**	—	—	—
C. verticillata	CL1416	Denmark, Hulsig Hede, *U. Søchting* DMS-9198104 (C-L 75834)	FUM	—	**PQ252482**	—	—	—	—
C. verticillata	CL870	Finland, Uusimaa, Espoo, *R. Pino-Bodas* s.n. & *T. Ahti* (MACB 124109)	FUM, ± PHY	**PQ256278**	**PQ252531**	**PQ252641**	—	—	—
C. verticillata	CL1069	Finland, Uusimaa, Espoo, Takkula, *T. Ahti* 74359 & *R. Pino-Bodas* (MACB 127551)	FUM	**PQ256162**	**PQ252389**	**PQ252540**	—	—	—
C. verticillata	CL1185	Germany, Halbeldorf, *V. Otte* (GLM-L 30348)	FUM	—	**PQ252431**	—	—	—	—
C. verticillata	CL1126	Germany, Sachsen-Anhalt, Ergebirge, *Z. Palice* 24972	FUM	**PQ256200**	**PQ252423**	**PQ252573**	**PQ274495**	—	—
C. verticillata	CL1188	Germany, Sarnser, Oberlasia, *V. Otte* (GLM-L 39356)	FUM	—	**PQ252434**	—	—	—	—
C. verticillata	CL1187	Germany, Sarnser, Oberlasia, *V. Otte* (GLM-L 39419)	FUM	—	**PQ252433**	—	—	—	—
C. verticillata	CL1079	Hungary, Baranya, Cserkút, Mts. Mecsek, *A. R. Burgaz* (MACB 112583)	FUM	PQ256169	**PQ252397**	**PQ252546**	—	—	—
C. verticillata	CL1474	Norway, Sør-Trøndelag, Åfjord, Storfjellet, *H. Holien* 14948 (TRH L-16326)	FUM	—	—	**PQ252608**	—	—	—
C. verticillata	CL3030	Norway, Østerdalen, Innlandet, Venabygb, Venabu, *R. Pino-Bodas* 479-2023 (MACB 127539)	FUM	**PQ256270**	**PQ252523**	**PQ252633**	—	—	—
C. verticillata	CL806	Russia, Leningrad Region, Bolshoy Tuters island, *I. S. Stepanchikova* 12-2015 (H)	FUM	**PQ256275**	**PQ252495**	**PQ252639**	**PQ274546**	—	—

(*Continued*)

Table 1. (*Continued*)

Taxa	DNA code	Voucher specimens	Secondary metabolites	ITS rDNA	IGS rDNA	*cox1*	*RPB2*	*RPB1*	*ef1α*
C. verticillata	CL1101	The Netherlands, Drenthe, Gasteren, *A. Aptroot* 76157 (MACB 12544)	FUM	**PQ256187**	**PQ252411**	**PQ252558**	**—**	—	—
C. verticillata	CL1105	The Netherlands, Gelderland, Kootwijik, *A. Aptroot* 76139 (MACB 127542)	FUM	**PQ256190**	**PQ252413**	**PQ252561**	**—**	—	—
C. verticillata	CL1102	The Netherlands, Noord-Brabant, Loonse en Drunense, *A. Aptroot* 76169 (MACB 127543)	FUM	**PQ256188**	**PQ252412**	—	**PQ274556**	—	—
C. verticillata	CL311	The Netherlands, Gelderland, Garderen, *T. Ahti* 72002 & *A. Aptroot* (H)	FUM	—	—	**PQ252638**	—	—	—
C. verticillata	1947	Canada, Ontario, *A. R. Burgaz* (MACB 103716)	FUM	KC776933	**PQ252382**	**PQ252532**	**PQ274463**	—	—
C. verticillata	1vertti	Spain, Burgos,Villasur de Herreros, *A. R. Burgaz* (MACB 103717)	FUM	KC776932	—	**PQ252534**	—	—	—
C. verticillata	1948	USA, New Hampshire, *A. R. Burgaz* (MACB 103718)	FUM	KC776934	**PQ252383**	**PQ252533**	**PQ274464**	—	—
C. verticillata	CL1483	Norway, Leirfjord, E of Fagervika, *H. Holien* 10714 (TRH L-11882)	FUM	—	**PQ252494**	—	—	—	—
C. verticillata	CL1076	Canada, British Columbia, Nass Ranges, *T. Goward* 196 (H)	FUM	**PQ256167**	**PQ252395**	**PQ252544**	**PQ274469**	—	—
C. verticillata	CL2485	Canada, Quebec, *C. Boudreault* 20180817B3	FUM	—	**PQ252509**	**PQ252628**	—	—	—
C. verticillata	LK49	Canada, Newfoundland, *T. Ahti* 56951 (H)		AF453845	MK142217	—	MK180151	MK180551	MK153034
C. verticillata	CL134	USA, Pennsylvania, *J. Lendemer* 16481 (H)		—	MK142218	—	—	—	—
C. vescula	CL275	Bolivia, *A. Flakus* & *O. Plata* 20543 (H)		MK179667	MK141900	—	MK179863	MK180277	MK152686

ATR = atranorin; FUM = fumarprotocetraric acid; CNSTIC = connorstictic acid; CPH-2 = confumarprotocetraric acid; CPSO = conpsoromic acid; NSTIC = norstictic acid; PHY = physodalic acid; PSO = psoromic acid; USN = usnic acid; ZEO = Zeorin

multivariate analysis of variance (PERMANOVA) was used to test differences between clades and species, with the Bray-Curtis dissimilarity, 999 permutations and Bonferroni correction for multiple comparisons.

Contingency tables were used to assess the association between qualitative characters and different clades. The morphological characters studied were: upcurled dry squamules, upper surface colour, lanceolate lobes, colour of base squamules, continuous algal layer and basal colour of podetia (alternative states are listed in Supplementary Material Table S1, available online). The analyses were performed using the *abind* package (Plate & Heiberger 2016). As the number of specimens in clade C was low, Fisher's test was used instead of the chi-square test (Agresti 2018).

DNA extraction, PCRs and sequencing

A single podetium per specimen was used for DNA extraction. Exceptionally, for the molecular biology study, specimens with poorly developed podetia or without them were included. Before DNA extraction, the fragment was soaked in acetone for 1 h to remove secondary metabolites. The acetone extracts were used to conduct TLC. Total genomic DNA was extracted using the E.Z.N.A. Forensic Kit (Omega) following the manufacturer's instructions; DNA was dissolved in 100 µl of elution buffer (10 mM Tris-Cl). Based on preliminary studies (Pino-Bodas *et al.* 2010*a*, 2013*a*, *c*; Stenroos *et al.* 2019), the regions ITS rDNA, IGS rDNA, *RPB*2 and *cox1* were selected to conduct the revision of the *C. cervicornis* group. The primers and PCR conditions are described in Pino-Bodas *et al.* (2010*b*) and Stenroos *et al.* (2019). PCR reactions were carried out using BIOTAQ polymerase (Bioline), including 0.3 µl of Taq polymerase, 2.5 µl of 10× PCR buffer, 1.4 µl of $MgCl_2$ (50 µm $µl^{-1}$), 1.6 µl of dNTPs (2.5 µm $µl^{-1}$), 1 µl of BSA (1 µm $µl^{-1}$), 1 µl of each primer (10 µm $µl^{-1}$), and 1 µl of extracted DNA. PCR products were sequenced at Macrogen Spain (http://www.macrogen.com).

Phylogenetic analyses

The dataset used for the phylogenetic analyses included the specimens newly sequenced from the *C. cervicornis* group, sequences of *Cladonia* species with central proliferation from other regions of the world included in clade Cladonia, subclade Cladonia, and other species without central proliferations but belonging to this clade (Stenroos *et al.* 2019). Additionally, specimens of *Cladonia* species representing other major lineages within the clade Cladonia (Stenroos *et al.* 2019) were included in the phylogenetic analyses (Table 1). *Cladonia boryi* Tuck. and *C. nipponica* Vain., belonging to clade Boryia, were used as an outgroup. The sequences were assembled using Sequencher v. 4.1.4 (Gene Codes Corporation, Inc., Ann Arbor, Michigan, USA). The alignments of each genetic region were performed using the online version of MAFFT (Katoh & Standley 2013), with default parameters. Ambiguous positions of ITS rDNA and IGS rDNA alignments were removed with Gblocks v. 0.91b (Castresana 2000) using less restricted options (allowing final blocks, gap positions and less strict flanking positions).

Maximum likelihood (ML) analyses were performed in RAxML v. 7.04 (Stamatakis *et al.* 2008) for each locus, using 500 pseudoreplicates of rapid bootstrap and the GTRGAMMA model. The congruence among loci was inspected manually, based on the method described by Kauff & Lutzoni (2002, 2003). No incongruences were detected, and the datasets were combined. In addition to the genetic regions amplified in the specimens of the *C. cervicornis* group, concatenated analyses also included *RPB*1 and *ef1α* loci, which were used to estimate the phylogeny of *Cladonia* (Stenroos *et al.* 2019) and numerous sequences for specimens of the Cladonia subclade were available. This approach increased the number of loci sampled within the clades in the phylogeny. We verified that *RPB*1 and *ef1α* were not represented exclusively in specimens of some branches of the phylogeny. The concatenated dataset was restricted to specimens represented by at least two loci. The concatenated dataset was analyzed by ML and Bayesian inference. JModelTest (Posada 2008) under the Akaike information criterion (AIC) was used to select the best substitution model for each region. The selected models were: TrNef + G for ITS rDNA, IGS rDNA and *RPB*2; K80 + G for *cox1*; SYM + G for *ef1α* and TrNef + I + G for *RPB*1. The partitioning scheme used to analyze the concatenated datasets considered 15 partitions: ITS rDNA, IGS rDNA and each codon position for the loci *cox1*, *ef1α*, *RPB*1 and *RPB*2. ML analysis was conducted in RAxML using the GTRGAMMA model for each partition and 1000 pseudoreplicates of rapid bootstrap. The Bayesian analyses were performed using MrBayes v. 3.2.6 (Ronquist *et al.* 2012). Posterior probabilities were approximated by sampling trees using Markov chain Monte Carlo (MCMC). Two simultaneous runs, each with 20 000 000 generations and employing four chains, were executed. Every 1000th tree was saved into a file. The first 25% of trees was discarded as burn-in. Convergence between chains was assessed using Tracer v. 1.7 (Rambaut *et al.* 2018). The likelihood versus generation number and the average standard deviation of split frequencies (≤ 0.01) were plotted. Branches with posterior probabilities ≥ 0.95 and bootstrap values ≥ 70% were considered strongly supported. The phylogenetic analyses based on the concatenated dataset were performed on the CIPRES Science Gateway web portal (Miller *et al.* 2010).

Species delimitation analyses

In addition to the clade delimitation obtained in the phylogenetic reconstructions, three species delimitation methods were employed to assess the number of species within the *C. cervicornis* group: Automatic Partitioning (ASAP) (Puillandre *et al.* 2021), Poisson Tree Processes (PTP) (Zhang *et al.* 2013) and General Mixed Yule Coalescent (GMYC) (Pons *et al.* 2006). These algorithms belong to the category of 'discovery' species delimitation methods, since they do not require any prior information on species boundaries. These analyses included only putative species represented by more than two sequences, removing singletons that may create artefacts (Lim *et al.* 2012; Pentinsaari *et al.* 2017). ASAP was implemented on the web server (https://bioinfo.mnhn.fr/abi/public/asap/) using the Jukes-Cantor (JC69) model to compute the genetic distances. PTP analyses were performed using the best ML tree estimated in RAxML for each locus. The analyses were conducted on the webserver (http://species.h-its.org/) with default parameters that included 500 000 generations, with a thinning rate of 100 and 10% burn-in. GMYC was conducted using iTaxoTools (Vences *et al.* 2021), using a single threshold. ML trees of each locus were transformed into ultrametric trees with the 'chromos' function of the *ape* package (Paradis & Schliep 2019) for R v. 4.3.3 (http://www.rproject.org/) and used for GMYC analyses.

Results

In this study, 469 new DNA sequences were generated (119 of ITS rDNA, 145 of IGS rDNA, 95 of *RPB*2 and 110 of *cox1*). The

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concatenated dataset was composed of 221 specimens and 3798 characters. The ML analysis resulted in a tree with −Lnl = 27581.920. The Bayesian analyses produced a consensus tree with an arithmetic mean of −Lnl = 102147.35. ML and Bayesian consensus trees showed similar topologies. Figure 1 shows the Bayesian 50% majority-rule consensus tree, which is consistent with the topology of the subclade Cladonia generated in the phylogeny of Stenroos *et al.* (2019). The subclade Cladonia was revealed as monophyletic with high support, while the centrally proliferating species are not monophyletic. All specimens of *C. verticillata* from Europe form a monophyletic clade, although this is supported only in the Bayesian analysis (PP = 1/ Bootstrap = 64). A small number of specimens from North America are included in this clade, which is closely related to *C. subulata*. The results appear to suggest that *C. trassii* is polyphyletic; one specimen of *C. trassii* is basal to the clade formed by *C. verticillata* and *C. subulata*, while three other specimens occur distantly, close to a specimen of *C. cineracea* and *C. rappii*. *Cladonia pulvinata* was monophyletic and highly supported in both analyses. Specimens identified as *C. cervicornis* form two well-supported clades. One clade (clade C), with seven specimens, is closely related to a clade comprising *C. ceratophyllina* (Nyl.) Vain., *C. centrophora* Müll. Arg., *C. andesita* Vain. and *C. nitens* Ahti. Clade D includes most of the specimens of *C. cervicornis* in addition to all the specimens studied of *C. microphylla*. *Cladonia macrophyllodes* is found to be monophyletic and closely related to *C. uliginosa*, which is represented by two specimens in the phylogeny. One specimen (*Lendemer* 22968) morphologically related to *C. cervicornis* from North America appears to be related to *C. mateocyatha* Robbins.

The results of species delimitation methods are presented in Fig. 2. The number of inferred species ranges from 2 to 115. In general, these methods showed little congruence with each other and with the current species delimitation. However, several of the methods for different loci identified clade C of *C. cervicornis* as a different species. Specifically, this putative species was inferred by ASAP for ITS rDNA, *RPB2* and *cox1*, by PTP for ITS rDNA and *RPB2*, and by GMYC for *RPB2*.

Table 1 shows the secondary metabolites detected by TLC in each specimen studied. The results show a high chemical homogeneity within the taxa. As a major substance, all the specimens of *C. pulvinata* contained psoromic acid, and all the specimens of *C. verticillata* contained fumarprotocetraric acid. Most *C. cervicornis* samples contained fumarprotocetraric acid, except for a small number of samples from Cyprus which also contained atranorin. Traces of other substances such as physodalic acid, confumarprotocetraric acid and a fatty acid with an R_f compatible with rangiformic acid were detected in specimens from different geographical origins (Table 1).

Table 2 presents the PERMANOVA pairwise comparison. PERMANOVA analyses showed a clear morphological differentiation between *C. cervicornis* and *C. verticillata*, and between *C. cervicornis* and *C. pulvinata*. However, there were no significant differences between *C. verticillata* and *C. pulvinata*. Our analyses showed that the specimens of clade C were morphologically very similar to *C. pulvinata*. These results were confirmed by NMDS analysis (Fig. 3), where specimens of *C. cervicornis* and *C. verticillata* clearly form separate clusters, while those of *C. pulvinata* and clade C overlap.

The contingency table analyses showed significant associations between the clades and the characters: lower surface colour (*P*-value = 0.0001034), upper surface colour (*P*-value = 0.01213), lanceolate/rounded lobes (*P*-value = 0.001965), basal colour of squamules (*P*-value = 1.917e-11), and basal colour of podetia (*P*-value = 0.01323), although there were no significant associations among clades for continuous/discontinuous algal layer (*P*-value = 0.11).

Discussion

Species delimitation of the Cladonia cervicornis *group in Europe*

It is well known that many species of *Cladonia* present a high level of morphological intraspecific variation. This variation may be due to different stages of development, to changes produced by different environmental conditions, such as different light intensity or humidity, or a response to anthropogenic disturbances (Osyzcka & Rola 2013; Osyczka *et al.* 2014, 2018; Pino-Bodas *et al.* 2015). Therefore, determining which phenotypic traits can be used as diagnostic characters is often challenging. Species delimitation in *Cladonia* has been hampered by the low genetic variability of the genus, which means that the markers used in phylogenetic and species delimitation studies in fungi often do not have sufficient resolution (Pino-Bodas *et al.* 2011, 2013*c*; Stenroos *et al.* 2019). Several studies have shown that phenotypically and ecologically disparate species cannot be genetically distinguished even using multiple loci (Stenroos *et al.* 2019; Steinová *et al.* 2022). The low genetic variation among *Cladonia* species may be attributed to recent divergence among species or to the dominance of asexual reproduction (Ahti 2000; Stenroos *et al.* 2019; Steinová *et al.* 2022).

This research investigates the morphological and genetic variation of the *C. cervicornis* group in Europe, focusing on the three most common species, *C. cervicornis*, *C. pulvinata* and *C. verticillata*. Characters traditionally used to distinguish species of the *C. cervicornis* group have been: the number of central proliferations, the number of tiers, the presence of a melanotic region at the base of the podetia, gradual or abrupt podetial tapering, morphology of squamules, and the secondary metabolites (Ahti 2000, 2007; van Herk & Aptroot 2003). Although preliminary phylogenetic studies indicated that these three taxa were monophyletic and constituted independent lineages (Pino-Bodas *et al.* 2010*a*, 2013*b*; Stenroos *et al.* 2019), the large morphological variation documented in *C. cervicornis* and the difficulty in distinguishing some specimens from *C. verticillata* (Ahti & Stenroos 2013; Søchting 2017; Burgaz *et al.* 2020) prompted us to perform a new phylogenetic study. In this study, we used an integrative approach, combining the results of phylogenetic analyses, different methods for molecular species delimitation and the phenotypic study of specimens, to address species delimitation in the *C. cervicornis* group in Europe. Phylogenetic analyses showed five clades (Fig. 1), one corresponding to *C. verticillata* (clade A), another to *C. pulvinata* (clade B), two clades including specimens previously identified as *C. cervicornis* (clades C and D) and one corresponding to *C. macrophyllodes* (clade E). Nevertheless, these five clades were not identified as distinct species by the different species delimitation methods employed (Fig. 2). In fact, the results of the different species delimitation methods displayed great inconsistencies between them (Fig. 2), most showing an unrealistic amount of oversplitting. Although species delimitation methods have been widely used to establish species boundaries more objectively within different groups of lichen-forming fungi (Parnmen *et al.* 2012; Del-Prado *et al.* 2016; Pérez-Ortega *et al.* 2016; Wei *et al.*

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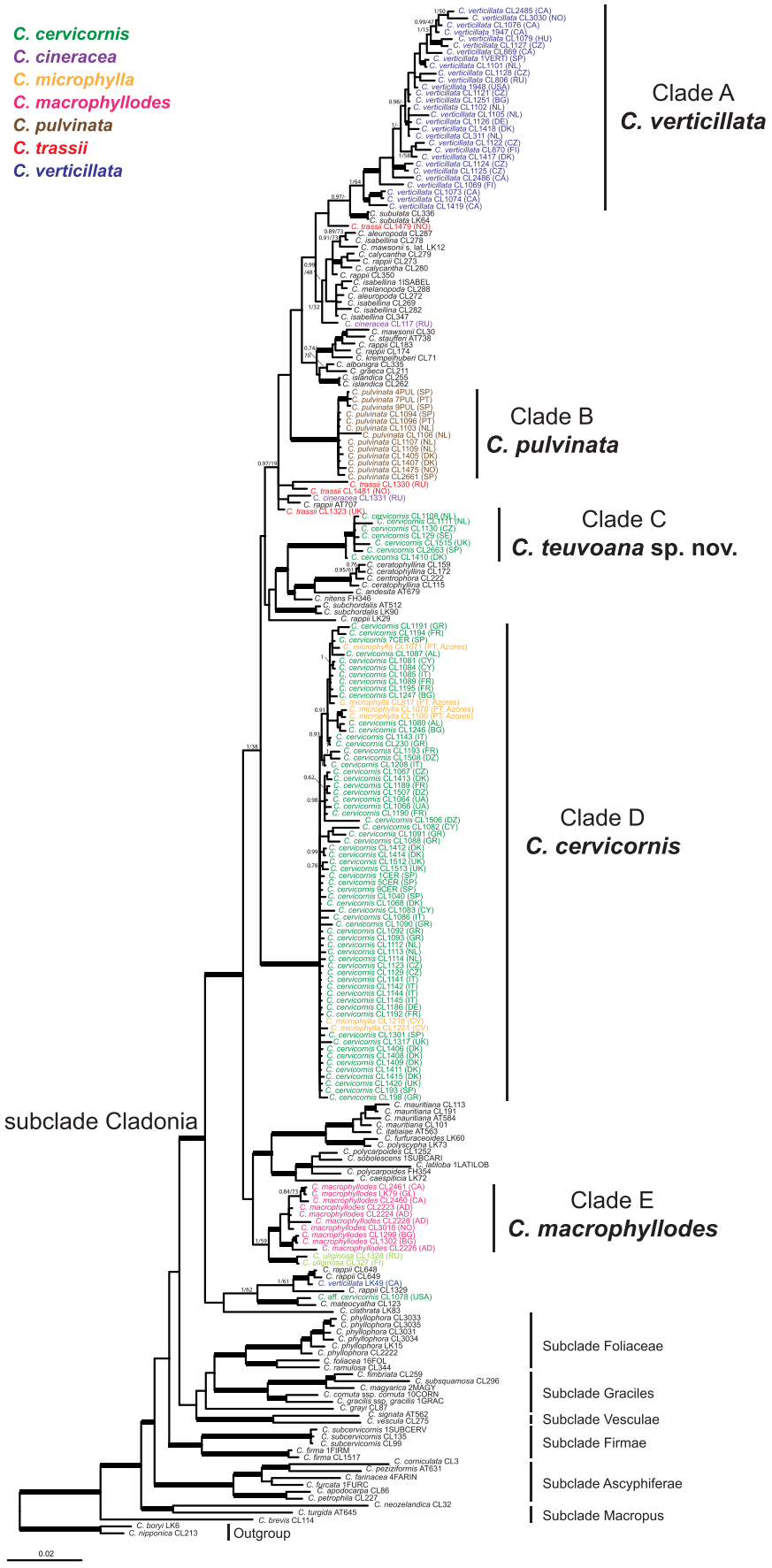

Figure 1. Phylogeny of subclade Cladonia, based on concatenated ITS rDNA, IGS rDNA, *RPB*1, *RPB*2, *ef1α* and *cox1,* showing the 50% majority-rule consensus tree of the Bayesian analysis. Branches with a bootstrap value of > 70% and a posterior probability of > 0.95 are in bold. Species of the *C. cervicornis* group are indicated in different colours. The country of origin of the specimen is indicated in brackets using the ISO abbreviations. In colour online.

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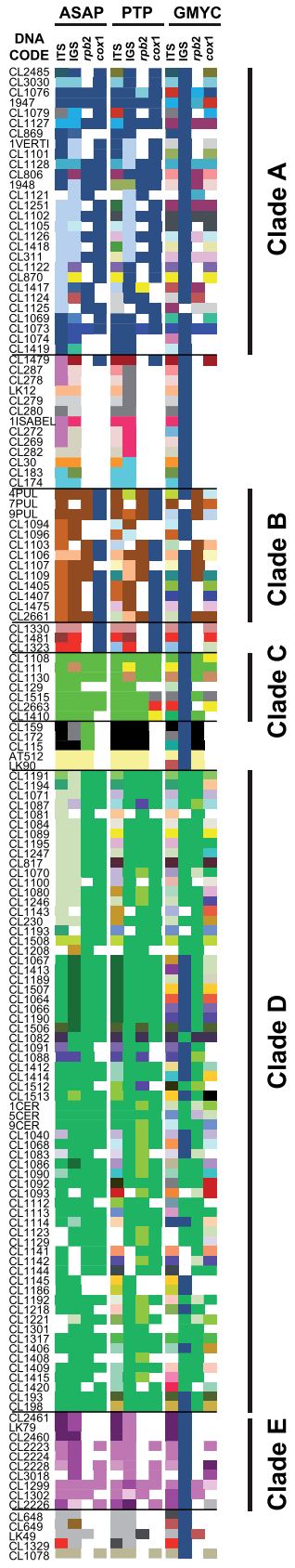

Figure 2. Results of species delimitation analyses (Automatic Partitioning (ASAP) (Puillandre *et al.* 2021); Poisson Tree Processes (PTP) (Zhang *et al.* 2013); General Mixed Yule Coalescent (GMYC) (Pons *et al.* 2006)) in the *Clandonia cervicornis* group, based on ITS rDNA, IGS rDNA, *cox1* and *RPB2*. The same colour indicates that the specimens were inferred to belong to the same species. The absence of colour indicates that the specimens were not analyzed. The DNA code corresponds to that shown in Fig. 1. Further details of the specimens can be found in Table 1. In colour online.

Table 2. PERMANOVA results of morphological pairwise comparison among subclade *Cladonia cervicornis* group. Significant *P*-values are in bold.

Taxon comparisons	*P*-value
C. cervicornis-C. pulvinata	**0.012**
C. cervicornis-C. teuvoana	**0.006**
C. cervicornis-C. verticillata	**0.006**
C. pulvinata-C. teuvoana	0.528
C. pulvinata-C. verticillata	0.078
C. verticillata-C. teuvoana	**0.006**

2016; Košuthová *et al.* 2020), it has been common to obtain inconsistent or unrealistic results in terms of the number of species delimited (Pino-Bodas *et al.* 2018; Mercado-Díaz *et al.* 2020; Myllys *et al.* 2023). Inconsistencies may be attributed to breaking assumptions of different methods, employing low numbers of specimens, lack of sampling across the geographical range of species, or recent divergence (Carstens *et al.* 2013; Magoga *et al.* 2021). Although our sampling was extensive, with a large number of specimens analyzed, it was focused on Europe and might be geographically biased for some lineages. Reciprocal monophyly has been used as a criterion for species delimitation, since it is indicative of sufficient divergence between lineages (De Queiroz 1998; Lücking *et al.* 2021). Based on this criterion and our knowledge of genetic variation in *Cladonia*, we consider that the lineages found in the phylogenetic analyses represent different species. In addition, most of these lineages are supported by phenotypic differences. Our morphological analyses not only document variation in each lineage, but also show morphological differences among most of them (Fig. 3, Table 2), confirming the results of van Herk & Aptroot (2003).

Our analyses show that all European *C. verticillata* specimens belong to a single lineage, clade A. Therefore, this clade represents *C. verticillata* s. str. Furthermore, morphological analyses indicate morphological differences with the specimens of *C. cervicornis*. Clade A not only included European specimens, but also some from North America. Within this clade, two well-supported subclades could be distinguished, one containing three specimens from North America, the other with all European specimens and some from North America. However, these subclades were not delimited as independent species by any of the species delimitation methods. Other specimens of *C. verticillata* from other regions were not included in this clade, which confirms that a worldwide study is required to clarify the taxonomy of *C. verticillata* (Ahti 2007; Pino-Bodas *et al.* 2021).

Clade B consists exclusively of *C. pulvinata* specimens. According to our morphological analyses, the distinction between *C. pulvinata* and *C. verticillata* is difficult, since they display a large amount of overlap (Fig. 3). However, in regions where the species grow side-by-side, they can usually be reliably distinguished (van Herk & Aptroot 2003), and the presence of psoromic acid in *C. pulvinata* can always be used as a diagnostic character to identify the specimens of this lineage.

Clade C encompasses specimens with a more or less Atlantic distribution. Morphological analyses indicate differences from the rest of the lineages of the *C. cervicornis* group. This lineage was also supported by some species delimitation analyses (Fig. 2). From a morphological point of view, this lineage is distinguished by the narrower scyphi compared to other taxa within

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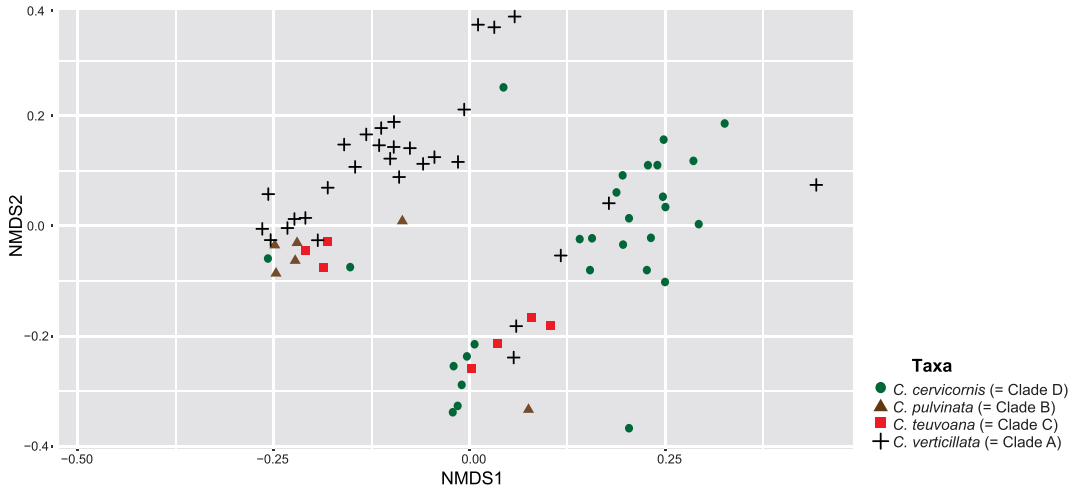

Figure 3. Non-metric multidimensional scaling (NMDS) plot, illustrating the morphological variation among the species belonging to the *Cladonia cervicornis* group. Stress = 0.14. In colour online.

the group. These scyphi display abrupt widening and no tier proliferations have been observed. The squamules are smaller than those of the other lineages and may have a blackened zone on the underside, reaching up to 1/3 of the length of the squamules, which are white at the ends. Study of the type material has allowed us to determine that *C. cervicornis* s. str. corresponds to clade D. The primary thallus of the *C. cervicornis* type (lectotype and isolectotype) is morphologically similar to that of the specimens of this clade (Fig. 4), characterized by large, deeply incised squamules and an underside with purplish-greyish tinged ends. Possible designations applicable to clade C would be *C. cristata* Hoffm. or *C. sobolifera*. However, neither the iconography presented by Dillenius (1742) nor the description by Hoffmann (1796) matches the morphology of the specimens in this clade and we therefore rule out applying *C. cristata* to this clade. We did not have access to the type of *C. sobolifera* but we studied the Nylander specimens in the Helsinki herbarium and conclude that they belong to *C. cervicornis* (Fig. 4). Therefore, we consider clade C to be a new species that we describe here under the name *Cladonia teuvoana*.

Clade D consists of the specimens of *C. cervicornis* s. str. together with all the analyzed specimens of *C. microphylla* (Fig. 1), a Macaronesian endemic species characterized by deeply divided squamules (Ahti & Aptroot 2009). Although the analyses performed here did not distinguish this species from *C. cervicornis*, we do not propose any taxonomic changes since we did not undertake an in-depth morphological study of this taxon. In addition, phylogenomic analyses have resolved relationships between closely related species of lichen-forming fungi that conventional markers (e.g. ITS rDNA, LSU rDNA, *RPB2*) used in fungal phylogenies failed to separate (Grewe *et al.* 2018). We expect that analyses at the genomic scale will provide a better understanding of the boundaries between *C. cervicornis* and *C. microphylla*. *Cladonia cervicornis* specimens grouped in this clade show a high morphological variation (Fig. 3), but this variation is not related to genetic variation. Despite the low genetic variation found, several of the species delimitation methods divided the clade into several species. Given the lack of congruence among the delimitation methods, we opted for the most conservative option, which is to keep this lineage as a single species, a hypothesis that was supported only by ASAP for ITS rDNA and *RPB2*. *Cladonia cervicornis* has been reported from North America (Hammer & Ahti 1990; Hammer 1995; Ahti & Hammer 2002) but the identity of these specimens is uncertain. Our results, based on the study of a single specimen, indicate that *C. cervicornis* s. str. is probably not present in North America (Fig. 1).

Clade E includes all the analyzed specimens of *C. macrophyllodes*. No circumscription problems have been reported for this species; although the primary thallus could be confused with that of *C. subcervicornis*, the two species have somewhat different habitat requirements (Ahti & Stenroos 2013; Burgaz *et al.* 2020). However, as for the remaining lineages, most species delimitation analyses inferred more than one species within this lineage, with the exception of ASAP and PTP for *cox1*. In the absence of other evidence, we maintain *C. macrophyllodes* as a single species.

Phylogenetic relationships within the Cladonia cervicornis *group*

The phylogenetic placement of species with central proliferations has been examined in previous studies (Stenroos *et al.* 2002, 2019; Beiggi & Piercey-Normore 2007; Pino-Bodas *et al.* 2010*a*, 2013*b*). However, in the present study the sampling is expanded substantially, including specimens from numerous countries and species not sampled to date, or poorly studied, such as *C. macrophyllodes*, *C. trassii* or *C. stricta*. The phylogenetic placement of the species of the *C. cervicornis* group is consistent with the results obtained by Stenroos *et al.* (2019), with all of them belonging to the Cladonia subclade.

The phylogenetic relationships of species in the *C. cervicornis* group, within the Cladonia subclade, are uncertain. Our results indicate that *C. verticillata* s. str. is related to *C. subulata*, a sorediate species without central proliferations, although this relationship was only supported in the Bayesian analysis (Fig. 1). This is a similar result to that found by Beiggi & Piercey-Normore (2007). However, the phylogeny of Stenroos *et al.* (2019) showed a possible relationship between *C. verticillata* and *C. pulvinata*, although it was not supported.

In our phylogeny, some clades consisted exclusively of species with central proliferations, but these clades did not always include species with morphological or biogeographical similarities. An example is the clade formed by *C. andesita*, *C. centrophora*, *C.*

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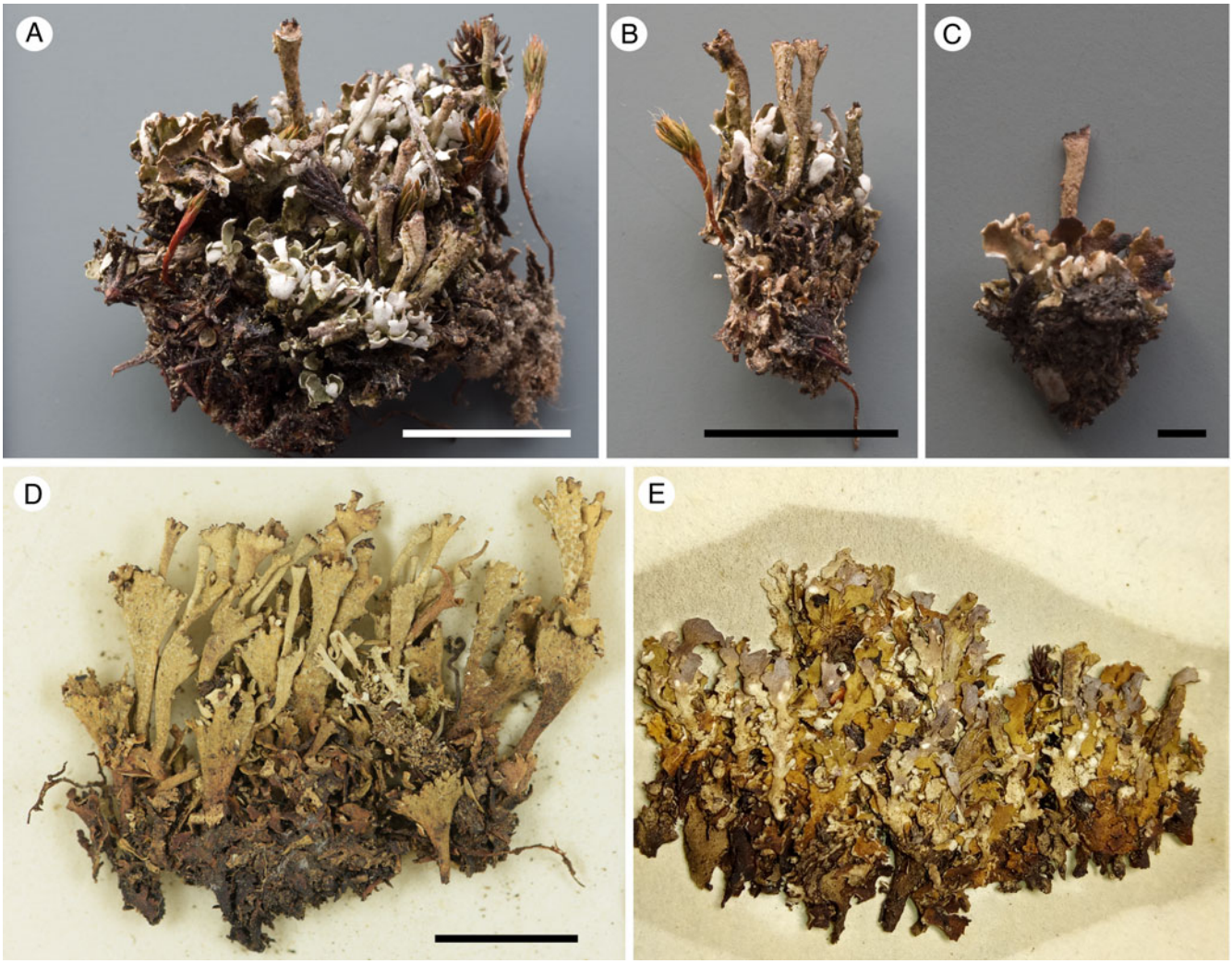

Figure 4. A–C, *Cladonia teuvoana* sp. nov. (MACB 127525—holotype). D, *Cladonia sobolifera* (H-NYL 0608). E, *Cladonia cervicornis* (BM-ACH 722A—isolectotype). Scales: A, B & D = 1 cm; C = 0.5 cm. In colour online.

ceratophyllina, *C. nitens* and *C. teuvoana*. While *C. teuvoana* is restricted to Europe, the rest of the species have very different distribution patterns. *Cladonia ceratophyllina* and *C. centrophora* are two morphologically similar African species (Swinscow & Krog 1988; Ahti & Aptroot 1992). *Cladonia andesita* was originally described from Colombia, but other populations later found in Africa, with some morphological differences, could represent a different species (Swinscow & Krog 1988; Ahti 2000). *Cladonia nitens* has an amphi-Beringian distribution and is characterized by having slender, shiny podetia and a large melanotic zone (Ahti 2007).

Another clade, consisting exclusively of species with central proliferations, is formed by *C. aleuropoda* Vain., *C. calycantha* Delise ex Nyl., *C. cineracea*, *C. isabellina* Vain., *C. mawsonii* C.W. Dodge, *C. melanopoda* Ahti and a specimen of *C. rappii*. This clade was previously shown in the phylogeny of Stenroos *et al.* (2019) but without support. With the exceptions of *C. mawsonii*, which has a disjunct distribution between Australasia and Tierra del Fuego, and *C. rappii*, with a broader distribution (Stenroos & Ahti 1990; Ahti 2000), the remaining taxa in the clade are neotropical, abundant in the páramos of the Andes and morphologically very similar. The delimitation of neotropical species is still not well understood and needs further study (Ahti 2000).

The phylogenetic relationships of *C. trassii* are studied here for the first time. Although numerous specimens were selected for phylogenetic analysis, sequencing failed for most of the gene regions (Table 1). Only for IGS rDNA were a high number of sequences obtained. Phylogenetic analyses of this region revealed that the specimens identified as *C. trassii* formed a polyphyletic group. A similar result was found in the multilocus analysis, which included only those specimens with sequences for at least two loci. This result is not surprising, since *C. trassii* is an extremely variable species, difficult to distinguish from *C. stricta* and *C. uliginosa* (Ahti 1998; Ahti & Stenroos 2013). However, phylogenetic analyses did not show that *C. trassii* specimens are related to *C. uliginosa*. Our results point out a possible relationship between *C. uliginosa* and *C. macrophyllodes*, although it was supported only in the Bayesian analysis. These two species are easily distinguishable. *Cladonia macrophyllodes* has a well-developed and persistent primary thallus, while *C. uliginosa* has an inconspicuous and evanescent primary thallus. The two species also show considerable discrepancies in the morphology of

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the podetia. *Cladonia uliginosa* has podetia with a discontinuous cortex, with several tiers of proliferations and a strongly melanotic base. None of these characters are shared by *C. macrophyllodes*, which has podetia with a continuous cortex and abundant squamules on the scyphi, and tiers are rarely present. However, both species synthesize the same secondary metabolites: fumarprotocetraric acid and atranorin (Ahti 1978; Hansen & Ahti 2011; Ahti & Stenroos 2013).

Our analyses did not resolve the phylogenetic relationships of *C. stricta*. IGS rDNA analysis showed that the specimens of this species formed a polyphyletic group (based on three sequences) and the only *RPB2* sequence obtained for it showed a relationship with a specimen of *C. trassii*. However, these data are not enough to draw conclusions about the taxonomy and phylogenetic relationships of this species. Based on its morphological features, *C. stricta* has been related to *C. phyllophora* Hoffm., a species characterized by podetia with a strongly melanotic base, with lateral proliferations that open and rarely produce central proliferations (Osyczka 2006; Hansen & Ahti 2011; Ahti & Stenroos 2013). Our phylogenetic analyses included several specimens of *C. phyllophora*, which was resolved as a monophyletic group, confirming the findings of Fontaine *et al.* (2010). However, *C. phyllophora* does not belong to the subclade Cladonia, as the phylogeny of Stenroos *et al.* (2019) showed, but belongs to the subclade Foliosae.

The phylogeny of the subclade Cladonia is in general still poorly resolved (Fig. 1). Although some well-supported clades have been found within this subclade, the relationships among them are not supported. This result highlights the need for future studies, including sampling in other geographical regions, that will help to better understand both the phylogenetic relationships of species with central proliferations as well as species delimitation. Preliminary data for some species such as *C. rappii* reveal that they may include more than one species and therefore a thorough morphological and molecular study is necessary to clarify their taxonomy. The use of phylogenomic techniques that increase the number of loci will allow better-founded phylogenetic hypotheses to be established.

Taxonomy

Cladonia teuvoana Pino-Bodas, Burgaz & Aptroot

MycoBank No.: MB 855855

Primary thallus persistent, with small squamules, white on the lower surface. Podetia with narrow scyphi, abruptly widening, with a corticate surface.

Type: The Netherlands, Drenthe, Gasterense zand, on sand, 24 March 2018, *A. Aptroot* 76154 (MACB 127525—holotype). GenBank Accession nos.: PQ252415, PQ252566, PQ254487.

(Fig. 4A–C)

Primary thallus persistent, prostrate or ascendent, squamules 3.7–8.11(9.2) mm long × 3.4–5.8 mm wide, apices sometimes incurved, green to greenish brown on the upper side, white on the lower side, darkening towards the base (up to 1/3 of squamule length). *Podetia* 4.7–8.7 mm tall, slender, simple, scyphose, rarely with squamules at the base, brownish, without a melanotic base; scyphi regular, with margins entire, very narrow, 1–3 mm wide, abruptly tapering; surface corticate, cortex smooth in the upper part, verrucose in old parts, faintly pruinose towards the scyphi; podetial wall 183–223 µm wide. *Hymenial discs* infrequent, dark brown, no well-developed ones observed; ascospores not observed.

Conidiomata at margin of scyphi, globose, pycnidial slime hyaline; conidia 7–10 × 1 mm, hyaline, falciform.

Chemistry. PD+ red, K−, UV−; contains the fumarprotocetraric acid complex.

Etymology. This species is named in honour of Prof. Teuvo Ahti, on the occasion of his 90th birthday, for his great contribution to lichenology.

Habitat and distribution. *Cladonia teuvoana* occurs on heathlands and sandy areas, and is currently known from Denmark, the Czech Republic, the Netherlands, Spain, Sweden and the United Kingdom.

Remarks. The species differs from *C. cervicornis* s. str. in having a less well-developed primary thallus with a white underside and narrower scyphi that abruptly widen. It can be distinguished from *C. pulvinata* by the absence of psoromic acid and differs from *C. verticillata* in having narrow scyphi and a smaller primary thallus. *Cladonia teuvoana* probably has a wider distribution in Europe. To achieve a more realistic understanding of its distribution, it would be necessary to conduct a comprehensive study of herbarium material.

Additional specimens. **Great Britain:** *England*: **V.C. 11,** South Hampshire, New Forest National Park, Lyndhurst, Bolton Bench, heather with *Calluna vulgaris* and *Erica cinerea*, 50° 52′17.2″N, 1°33′23″W, alt. 36 m, 2019, *R. Pino-Bodas* & *N. Sanderson* (MACB).—**Denmark:** Jutland, Harrild, Hede, 62.07550°N 5.10650°E, 2016, *U. Søchting* 12520 (C-L 75831).—**The Netherlands:** *Drenthe*: Balloo, Ballooerveld, on sand, 2024, *Aptroot* 76155 (MACB).—**Czech Republic:** *C. Bohemia*: Praha, Natural Park Košíře-Motol, path of a heath with grove of *Quercus petraea*, *Betula pendula* and *Pinus sylvestris* with *Calluna vulgaris* in understorey, 50°03′45.6″N, 14°19′48.7″E, alt. 338 m, 2018, *Z. Palice* & *P. Uhlík* 24917.—**Spain:** *Guipúzcoa*: Oñate, Aizkorri-Aratz Natural Park, Malkorra, heathland with sandstone, 42°56′36.5″N, 2°21′51.9″W, alt. 1500 m, *R. Pino-Bodas* 254-2023 (MACB 125083).—**Sweden:** 2005, *B. P. Løfall* 10970 (H).

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