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Multilocus approach to species recognition in the *Cladonia humilis* complex (Cladoniaceae, Ascomycota)[1]

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- *Premise of the study:* The members of the *Cladonia humilis* complex are characterized by a well-developed primary thallus and broadly scyphose podetia. In the present study, this complex was phylogenetically analyzed to test the boundaries between the species and to determine the usefulness of the phenotypic characters to distinguish them. The species *C. conista*, *C. cyathomorpha*, *C. hammeri*, *C. humilis*, *C. kurokawae*, *C. nashii*, *C. pulvinella*, and *C. subconistea* were examined.
- *Methods:* Four DNA loci were sequenced and analyzed to test the monophyly of the species. For the phylogenetic reconstructions, maximum parsimony, maximum likelihood, and Bayesian methods were employed. The genealogical sorting index was used to quantify the exclusive ancestry of the nonmonophyletic species on the tree.
- *Key results:* The performed phylogenetic analyses showed that the *C. humilis* complex is not monophyletic. *Cladonia nashii* is not closely related to the remaining taxa within the complex. Seven monophyletic lineages were identified, most of which comprise specimens belonging to more than one chemotype. *Cladonia hammeri* and *C. pulvinella* are conspecific, and this taxon is not present in Europe.
- *Conclusions:* This study suggests that morphological characters and secondary metabolites have less taxonomical value than thought in the *Cladonia humilis* complex. Use of multilocus phylogeny is recommended to delimit species.

Key words: Ascomycota; *Cladonia*; Cladoniaceae; *ef1α*; genealogical sorting index; lichen; *rpb2*; species delimitation.

In recent years, much attention has been paid to the problem of species delimitation in different groups of organisms (Göker et al., 2009; Groeneveld et al., 2009; Meudt et al., 2009; Wedin et al., 2009; Carstens and Dewey, 2010; Pagès et al., 2010; Gazis et al., 2011; Sakalidis et al., 2011), a task which, in many cases, involves a great challenge owing to phenotypical variation, phenotypical convergence, morphological stasis due to strong selection, hybridization, or horizontal gene transfer. Currently, it is widely accepted that molecular data are useful to inspect species boundaries and to establish which groups need a taxonomical revision (Larsson et al., 2004; Göker et al., 2010; Lumbsch and Leavitt, 2011). In lichenized fungi, molecular data have been employed to assess the usefulness of the different phenotypical characters in circumscription of taxa. For many groups, it has been assessed whether the presence of certain secondary metabolites (extrolites) correspond with the limits among species. The results of some of these studies proved that, in certain cases, the presence of one or another secondary metabolite correspond with the existence of different species (LaGreca, 1999; Lücking et al., 2008), while in other cases this is not true (Buschbom and Mueller, 2006; Nelsen and Gargas, 2009). Wherever molecular data did not represent the traditional taxonomical outline, specimens were often reexamined, sometimes with the finding that phenotypic characters originally overlooked were relevant, such as those in the cortex surface, the cortex structure, the thickness of the ascospore wall, or the thallus morphology (Argüello et al., 2007; Lumbsch et al., 2008a, b; Lendemer, 2012). Several authors also found semicryptic species (Vondrák et al., 2009), i.e., phylogenetic species without phenotypically distinctive characters, but having different ecological requirements or a different distribution pattern (Hodkinson and Lendemer, 2011; Šoun et al., 2011). Finally, in some cases, molecular data have shown the existence of cryptic species (Molina et al., 2011).

In groups of closely related species, whenever the divergence time is comparatively short with respect to the ancestral population effective size in a certain locus, the species frequently do not appear as monophyletic, even if they are reproductively isolated, since monophyly is the final stage in divergence process (Cummings et al., 2008). This phenomenon, known as incomplete lineage sorting, has raised difficulties in establishing limits and relationships among species in numerous groups of organisms (Takahashi et al., 2001; Morando et al., 2004; Heckman et al., 2007; Willyard et al., 2009; Gurushidze et al., 2010). The cause of the incomplete lineage sorting is that allelic coalescence is not simultaneous in the different loci, due to several factors such as a high heterozygosity degree within the species, large effective population size or long generational times (Rosenberg, 2003). This phenomenon can lead to incongruities among loci in the phylogenies (Carstens and Knowles, 2007). Consequently gene trees do not represent the phylogenetic history of species. In lichenized fungi, evidence for incomplete lineage sorting have been found in *Cavernularia* (Printzen et al., 2003), *Xanthoria* (Lindblom and Ekman, 2005), *Peltigera*

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(Goffinet et al., 2003), *Porpidia* (Buschbom and Mueller, 2006), *Thamnolia* (Nelsen and Gargas, 2009), and *Cladonia* (Myllys et al., 2003; Piercey-Normore et al., 2010), among others. Recently Cummings et al. (2008) developed a new method to assess the degree of genealogic divergence, known as the genealogical sorting index (GSI). This method pursues a quantification of the exclusive ancestry of individuals in user-labeled groups on a rooted tree.

Among macrolichens, *Cladonia* is one of the genera with greatest number of species, approaching 500 recognized species, distributed the world over. It is characterized by a dimorphic thallus composed of a squamulose or crustose primary thallus, which can be persistent or evanescent, and a fruticose secondary thallus, which is morphologically very variable. The morphological characters linked to the secondary thallus, along with the extrolites (secondary metabolites), are the ones primarily used for species differentiation. Based on these characters, the genus *Cladonia* was divided by Ahti (2000) into seven sections (*Ascyphiferae*, *Cladonia*, *Cocciferae*, *Helopodium*, *Perviae*, *Strepsiles*, and *Unciales*), while *Cladina* was considered to be a different genus. Nevertheless, the phylogenetic reconstruction presented by Stenroos et al. (2002), based on the study of ITS rDNA and *β-tubulin* loci, seemed to indicate that the sections of the genus were polyphyletic, while *Cladina* was a monophyletic group within *Cladonia*. These authors proposed a provisional classification of the genus in three subdivisions and four supergroups. A considerable number of studies on *Cladonia* using molecular data have been carried out in recent years. Many of them had the aim of elucidating the taxonomic value of phenotypical characters to distinguish species (Lendemer and Hodkinson, 2009; Dolnik et al., 2010; Kotelko and Piercey-Normore, 2010; Fontaine et al., 2010; Pino-Bodas et al., 2010a, 2010b). Species delimitation in *Cladonia* is often not easy, owing to the high morphological variability and to the existence of numerous complexes of closely related species (Vainio, 1887; Ahti, 2000).

This study focuses on the *Cladonia humilis* complex, which comprises species characterized by a well-developed primary thallus, small-sized podetia, in general with wide scyphi and soredia or granules on the outer or inner surface of the scyphi (Pino-Bodas et al., 2012a). According to Ahti (2000), the complex includes the following species: *C. cyathomorpha* Stirt. ex Walt. Watson, *C. humilis* (With.) J. R. Laundon, *C. kurokawae* Ahti & S. Stenroos, *C. pulvinella* S. Hammer, and *C. subconistea* Asahina (Ahti, 2000). Later on, two more species that morphologically belong to this complex were described, *C. hammeri* Ahti and *C. nashii* Ahti (Ahti and Hammer, 2002). *Cladonia humilis* was considered by many authors as a species containing two chemotypes (Ahti, 2000; James, 2009), while in other authors' view each chemotype was a different taxon (Holien and Tønsberg, 1985; Archer, 1989). Recent phylogenetic work (Pino-Bodas et al., 2012a) indicated that these two chemotypes differ genetically, whereby two species, *C. humilis* and *C. conista* (Nyl.) Robbins, were recognized. Some specimens of these species are morphologically very similar. The species distinction within the *C. humilis* complex is currently based on the size of the soredia (or granules), on the presence or absence of corticate podetia and their characteristics if present, as well as on the secondary metabolites. The phenotypical differences among the species of the *C. humilis* complex are shown in Table 1. The taxonomical value of some of the mentioned characters has been questioned. For example, Paus et al. (1993) pointed out that the soredium size is probably influenced by environmental conditions or depends on the individual development stage.

The species diversity in this group is concentrated in warm temperate regions, including Mediterranean climatic regions of North America and Eurasia, although some species, such as *C. conista*, *C. cyathomorpha*, and *C. humilis*, have a wider distribution. *Cladonia hammeri* and *C. pulvinella* have been reported from North America (Ahti and Hammer, 2002; Hammer, 1995), Europe, and the Canary Islands (Burgaz and Ahti, 1998, 2009; Pérez-Vargas, 2008; Sicilia et al., 2009); *C. nashii* has only been found as yet in North America (Ahti and Hammer, 2002).

The purpose of our study is to carry out a phylogenetic reconstruction of the *C. humilis* complex using four nuclear genes to assess whether the phenotypical characters can be used to identify monophyletic lineages within the complex. Whenever species turn out to be nonmonophyletic, GSI will be applied.

MATERIALS AND METHODS

***Sampling taxa*—**Ninety samples of the *C. humilis* complex coming from different regions (Appendix 1) were selected for phylogenetic analyses. The samples were obtained from the herbaria MACB, H, F, OSC, or UC. The species included are *C. conista*, *C. cyathomorpha*, *C. hammeri*, *C. humilis*, *C. kurokawae*, *C. nashii*, *C. pulvinella*, and *C. subconistea.* Further, some specimens of putatively undescribed species (these samples are codified as *C. "laevis"* and *Cladonia* sp.) belonging to the complex were included in this study. *Cladonia "laevis"* has a smooth cortex, farinaceous soredia on the podetia, and granules inside the scyphi, while the *Cladonia* sp. samples have an areolate cortex and granules on the podetia. In addition, some samples of *C. pocillum* and *C. pyxidata* were included because these taxa could be phylogenetically close to the *C. humilis* complex (Stenroos et al., 2002). As outgroups, samples of *C. subturgida* Samp., *C. rangiformis* Hoffm., and *C. thomsonii* Ahti were chosen based on our own data (Pino-Bodas et al., 2012b; Appendix 1).

The species identifications in the *C. humilis* complex were mainly based on the podetium configuration, soredium size, presence of cortex on the podetia, and secondary metabolites according to Hammer and Ahti (1990), Hammer (1991), Ahti et al. (1995), Ahti (2000), and Ahti and Hammer (2002). The identifications of the specimens were examined before and after the molecular analyses by RP-B, TA and ARB.

***DNA extraction and amplification*—**Only one podetium of each specimen was selected for the DNA isolation. Before the DNA isolation, the secondary metabolites were extracted by soaking the samples in acetone for 2 h, and the liquid was used for thin-layer chromatography (TLC). The DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) was used to extract DNA, according to the manufacturer's instructions. The DNA was eluted in the final step in 200 μL of elution buffer provided by the manufacturer. The four following loci were amplified: nuclear ITS rDNA using the primer pair ITS1F (Gardes and Bruns, 1993)/ITS4 (White et al., 1990) or SSU1780/LSU012 (Piercey-Normore and DePriest, 2001), *rpb2* using the primer pairs RPB2-5F/RPB2-7R (Liu et al., 1999), RPB2dRaq/RPB2rRaq (Pino-Bodas et al., 2010a) or CLRPB2-5F/CLRPB2-7R (Yahr et al., 2006), *ef1α* using CLEF-3F/CLEF-3R (Yahr et al., 2006), and IGS rDNA using IGSf/IGSr (Wirtz et al., 2008). The amplifications of ITS rDNA, *rpb2*, and *ef1α* were carried out with Ready-to-Go-PCR Beads (GE Healthcare Life Sciences, Little Chalfont, UK). The volume of each reaction was 25 μL, with 0.4 mmol/L final concentration of primers. The amplification of IGS rDNA was carried out using Biotaq polymerase (Ecogen, Barcelona, Spain). The volume of reaction was 25 μL, with 0.3 μL of *Taq* polymerase, 2.5 μL of 10× PCR buffer, 1.4 μL of $MgCl_2$ 50 mmol/L, 1.6 μL of dNTPs (2.5 mmol/L), 1 μL of BSA (1 mmol/L), 1 μL of each primer (10 mmol/L), and 1 μL of extracted DNA. The amplification programs were (1) 94°C for 5 min; 5 cycles of 94°C for 30 s, 54°C for 30 s and 72°C for 1 min; and 33 cycles of 94°C for 30 s, 48°C for 30 s and 72°C for 1 min; with a final extension of 72°C for 10 min for nuclear ITS rDNA; (2) initial denaturation at 94°C for 5 min; 40 cycles of 95°C for 1 min, 52°C for 30 s and 72°C for 2 min; with a final extension at 72°C for 10 min for *rpb2* region: (3) initial denaturation at 94°C for 5 min; 35 cycles of 95°C for 1 min, 55°C for 30 s and 72°C for 1 min; with a final extension at 72°C for 10 min for *ef1α* region: (4) 95°C for 2 min; 35 cycles of 95°C for 30 s, 54°C for 30 s and 72°C for 1 min; with a final extension of 72°C for 10 min. PCR products were purified using the QIAquick gel extraction Kit (Qiagen, Hilden, Germany) or with ExoSAP-IT (USB Corporation, Cleveland,

TABLE 1. Diagnostic characters to distinguish the species within *Cladonia humilis* complex.

Species	Propagules	Cortex position	Cortex surface	Chemistry
C. conista	Farinose soredia	Reaching the scyphal edge	Smooth	FUM, BOU
C. cyathomorpha	Granules	Reaching the scyphal edge	Rugose-areolate	FUM, ± CYAT
C. hammeri	Granules	Lack cortex or limited to podetial base	Rugose	FUM
C. humilis	Farinose soredia	Reaching the scyphal edge	Smooth	FUM, ATR
C. kurokawae	Granules	Reaching the scyphal edge	Rugose	FUM, ATR
C. nashii	Farinose soredia	Lack cortex	—	FUM, ATR
C. pulvinella	Granules	Lack cortex	—	FUM, ATR, BOU
C. subconistea	Farinose soredia	Reaching the scyphal edge	Smooth	PSO, ATR

Notes: Farinose soredia < 50 μm, granules > 50 μm, ± = inconstant. ATR = atranorin, FUM = fumarprotocetraric acid, BOU = bourgeanic acid, PSO = psoromic acid, CYAT = unknown substance (Jølle, 1977).

OH, USA). The sequencing was performed at Macrogen (South Korea, Gason-Dong) service (http://www.macrogen.com), with the same primers used for the PCR.

***Phylogenetic analyses*—**Sequences were aligned manually with the program SE-AL v2.0a11 (Rambaut, 2002) for each locus separately. According to the methods of Lutzoni et al. (2000), five ambiguous positions were found in ITS rDNA matrix; these positions were excluded. The matrices of IGS rDNA, *rpb2* and *ef1α* did not contain ambiguous positions. Each region was analyzed by maximum parsimony (MP) and maximum likehood (ML), Appendix S1 (see Supplemental Data with the online version of this article). The MP analyses (PAUP* version 4.0.b.10; Swofford, 2003) used heuristic searches with 1000 random taxon-addition replicates with tree-bisection-reconnection (TBR) branch swapping and the MulTrees option, equally weighted characters, and gaps treated as missing data. For the confidence analysis, bootstrap was applied with 1000 replicates using the heuristic option. The ML analyses were implemented using the program RAxML 7.04 (Stamatakis, 2006), assuming a GTRGAMMA model. Congruence among the different topologies inferred from the loci was tested following Lutzoni et al. (2004): each clade with more than 75% bootstrap support was scanned for conflict among loci. We considered the existence of a conflict whenever a clade was supported with a bootstrap (more than 75%) in a locus, while it was not supported in other loci, and the individual sequences of this clade were part of another clade with bootstrap support ≥75%. No conflict among the loci was found.

The combined data set was analyzed by MP, ML (on the same conditions of each locus separately), and Bayesian approaches. The combined data set was assigned to eight partitions for the analyses: ITS rDNA, IGS rDNA, and each of three codon positions of *rpb2*, and each of three codon positions of *ef1α*, respectively. Bayesian analysis was carried out using the program MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001). The models applied to each partition were selected by the program MrModeltest (Nylander, 2004) under the Akaike information criterion (AIC), and are listed in Table 2. The posterior probabilities were approximated by sampling trees using a Markov chain Monte Carlo (MCMC) analysis. The posterior probabilities of each branch were calculated by counting the frequency of trees visited during the MCMC analysis. Two simultaneous runs with 20000000 generations, each starting with a random tree and employing 4 simultaneous chains, were executed. Every 1000th tree was saved into a file. The first 2000000 generations (i.e., the first 2000 trees) were deleted as the "burn in" of the chain. The program 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. The combined data set and the trees from each locus have been included in this paper as online supplementary data (Appendix S2, see online Supplemental data).

The following hypotheses were tested: (1) monophyly of *C. hammeri* s.l., (2) monophyly of *C. humilis*, (3) monophyly of *C. kurokawae*, (4) monophyly of *C. "laevis"*, (5) monophyly of *C. pulvinella* s.l., (6) monophyly of *Cladonia* sp., (7) monophyly of *C. subconistea*, (8) monophyly of *C. hammeri* samples from Europe, (9) monophyly of *C. pulvinella* samples from Europe, (10) monophyly of *C. pulvinella* samples from North America, and (11) monophyly of *C. pulvinella* and *C. hammeri* samples from North America containing bourgeanic acid. Alternative hypothesis tests included the Shimodaira-Hasegawa test (SH; Shimodaira and Hasegawa, 1999) and expected likelihood weight (ELW; Strimmer and Rambaut, 2002), that were performed using the program TREE-PUZZLE 5.2 (Schmidt et al., 2002).

Haplotypes networks under statistical parsimony with a confidential interval of 95% were generated with the program TCS 1.21 (Clement et al., 2000) for clade A with the combined data set (Fig. 2).

TABLE 2. Phylogenetic information of each locus and evolutionary models chosen by MrModeltest.

Data sets	Sites	Informatives sites	Tree length	−Ln L	Model
ITS rDNA	652	103	250	2491.5673	SYM+G
IGS rDNA	335	87	182	1448.3564	GTR+G
rpb2	870	159	291	2948.0680	SYM+G
ef1a	610	77	135	1674.7939	HKY+I
Combined	2467	424	922	9313.3980	—

***Genealogical sorting index*—**Whenever the species were not monophyletic, the genealogical sorting index (GSI) was used to assess the level of genealogical exclusivity for each putative species (Cummings et al., 2008). The GSI was calculated for ML tree of each locus. Each branch tip was assigned a group name corresponding to a morphological species (*C. humilis*, *C. pulvinella*, *C. subconistea*, *C. kurokawae*, *C. hammeri*, *C.* "*laevis*"). This method was also applied to test the monophyly of *C. hammeri* samples from Europe, *C. pulvinella* samples from Europe, and *C. pulvinella* samples from North America. The GSI_T was calculated for the set of ML trees of the four loci. The significance of the GSI and GSI_T was calculated using 10.000 permutations on the online platform at http://www.genealogicalsorting.org/.

***Genetic distances and fixation index* (F_{ST})—**The pairwise distances using the models indicated in Table 2 were calculated in PAUP*. According to Del-Prado et al. (2010), the intraspecific distances were calculated as the mean value of the pairwise distances between the samples of each species. The interclade distances were calculated as the mean value of the pairwise distances between the samples of two different clades.

The pairwise fixation index F_{ST} (Weir and Cockerham, 1984) was calculated with the program DnaSPv. 5 (Librado and Rozas, 2009) for each locus. According to Shaffer and Thomson (2007), and on the basis of the hybrid zone barrier approach (Porter, 1990), we used the F_{ST} value to estimate whether two sets of specimens should be considered as two species.

***Morphology and chemistry study*—**The specimens were studied under an Olympus SZX9 dissecting microscope; the morphology of the podetia and their soredia and granules was observed. Measurements of podetium length and scyphus width were taken (three podetia per specimens, or one podetium in the samples with scarce material). Microscopic measurements of the soredia (10 per podetium), podetial wall thickness, and each anatomical layer of the podetia were taken (three per specimen) at 400× with an Olympus CX41 light microscope. The hand-cut sections were made at the podetial base and mounted in distilled water. The measurements were done by R. Pino-Bodas. The secondary metabolites were checked using thin-layer chromatography according to the standardized procedures of White and James (1985), with solvent systems A and B. The fatty acids were detected (opaque spots) before developing the plates by heating.

RESULTS

***Phylogenetic results*—**In this study, 293 new sequences were generated. Sequence features for the individual genes,

parsimony information, and models of sequence evolution for each partition are listed in Table 2. The locus with the highest percentage of most parsimony informative characters was IGS rDNA (25.97%), followed by *rpb2* (18.16%) and ITS rDNA (15.13%); *ef1α* was the least informative (12.60%). No conflict among the loci was found, and the four genes were combined. The combined data set contained 2467 characters, 1862 of which were constant, and 424 parsimony-informative positions. The MP analysis generated trees of 922 steps long, with CI = 0.7278 and RI = 0.9427. The ML analysis yielded a tree with a likelihood value of −Ln L = 9313.3980; the mean likelihood of the Bayesian tree sampling was −Ln L = 9427.36. The trees of the three analyses had the same topology; only the Bayesian tree is shown (Fig. 1). *Cladonia nashii* did not appear closely related to the other species of the *C. humilis* complex (Fig. 1). Eight well-supported clades were distinguishable: clade A, constituted by samples of *C. humilis* (from Europe, North America, and Asia), *C. pulvinella* (from Mediterranean region in Europe), some samples of *C. hammeri* (from Mediterranean region in Europe), *C. "laevis"* and some samples of *Cladonia* sp.; clade B, formed by samples of *C. hammeri* (from Mediterranean region in Europe) and samples of *Cladonia* sp. and closely related with clade A; clade C, formed by samples of *C. hammeri* and *C. pulvinella* (from Mediterranean region in North America); clade D, that corresponded to *C. conista*, closely related to clade C; clade E, which included the samples of *C. kurokawae* and *C. subconistea*; clade F, that corresponded to *C. cyathomorpha* (this clade appeared at the base of *C. humilis* complex); clade G, constituted by *C. nashii*; the last clade (clade H) was formed by *C. pyxidata* and *C. pocillum* samples. One sample of *C. hammeri* from Andorra appeared closely related to the clade of *C. kurokawae* and *C. subconistea.*

The haplotype analysis of the clade A yielded one network with 29 haplotypes. No haplotype was shared between different morphospecies (Fig. 2).

Hypothesis contrast and GSI—The SH and ELW tests rejected the monophyly of *C. hammeri* s.l. as well as the monophyly of the European specimens of this species; the monophyly of *Cladonia* sp. and the monophyly of *C. pulvinella* s.l. (Table 3) were also rejected by both tests. The monophyly of *C. kurokawae, C. "laevis"*, the European specimens of *C. pulvinella*, and the North American specimens of *C. pulvinella* were rejected by the ELW test, but not by the SH test. As regards *C. humilis* and *C. subconistea*, none of the tests rejected the monophyly of these morphospecies.

The support of the monophyly by the GSI test varied according to the different genes (Table 4). Only for two species, *C. humilis* and *C. hammeri*, the GSI *P* was significant in all the four genes, but with somewhat moderate values. In ITS rDNA and IGS rDNA, the GSI *P* indicated that *Cladonia* sp. was close to reaching monophyly. The GSI *P* was significant for *C. "laevis"* in ITS rDNA and *rpb2*. For *C. kurokawae*, *C. pulvinella* s.l., the samples of *C. hammeri* coming from Europe and the samples of *C. pulvinella* coming from Europe, the GSI *P* was significant only in ITS rDNA. For the samples of *C. pulvinella* from North America the GSI *P* was significant only in IGS rDNA. The GSI *P* rejected the monophyly of *C. subconistea* for all the loci. The GSI_T was only significant for *C. humilis* and *C. hammeri.*

Genetic divergence and fixation index—The genetic distances within the clades showed a range of average values of 0.0016–0.0079 in ITS rDNA, of 0.0025–0.013 in IGS rDNA, of 0.0007–0.0047 in *rpb2*, and of 0.0-0.012 in *ef1α* (Table 5). The greatest distances within the clades occurred between clade A sequences for ITS rDNA, between clade B sequences for IGS rDNA, between clade E sequences for *rpb2* and between clade G sequences in *ef1α*. The average genetic distances interclades varied 0.0173–0.1257 in ITS rDNA, 0.0144–0.2571 in IGS rDNA, 0.004–0.1058 in *rpb2* and 0.0012–0.0819 in *ef1α*. The greatest divergence among lineages was found between C and G clades in IGS rDNA (0.2571).

F_{ST} values are displayed in Table 6. In most of the comparisons between loci, the values exceeded 0.5, except between clades A and B, where the values were 0.068332 in *rpb2* and 0.0 in *ef1α*. F_{ST} values between A and B clades in ITS rDNA and IGS rDNA were, however, 0.80433 and 0.64639 respectively. F_{ST} values between the subclades in clade A were <0.5 except for the A1-A2, A1-A4 and A2-A4 in ITS rDNA, which were >0.5. The same comparisons yielded F_{ST} values = 0 in other loci (Table 7). The average genetic distances intersubclades in clade A varied 0.0048–0.0111 in ITS rDNA, 0.0031–0.0115 in IGS rDNA, 0.0005–0.0058 in *rpb2* and 0.0011–0.0038 in *ef1α*.

Morphological and chemical variation of the clades—Table 8 outlines the morphological variation found within each clade. Clade A contains samples with farinose soredia (samples identified as *C. humilis*), samples with granular soredia (identified as *C. hammeri* and *C. pulvinella*), samples with microsquamules (labeled as *Cladonia* sp.); samples with farinose soredia on the podetial surface and granules inside the scyphi (labeled as *C. "laevis"*) were also found. As for the cortex morphology, clade A contains samples with a smooth cortex, others with areolate cortex and others that lack cortex or, if present, is limited to podetial base (*C. pulvinella*). Regarding chemical variability, samples are found with fumarprotocetraric acid and atranorin; with fumarprotocetraric and bourgeanic acids; and with fumarprotocetraric acid only (Fig. 1). Clade B contains samples with granules; samples with smooth cortex reaching near the podetial tip and samples with areolate cortex similar to that of *C. pyxidata* and *C. pocillum*. The samples within this clade contain fumarprotocetraric acid or fumarprotocetraric acid and bourgeanic acid. Clade C contains samples with granules, with areolate cortex at the base of the podetia, or samples that lack cortex (Fig. 3). This clade is heterogeneous as to secondary metabolites, with samples that only contain fumarprotocetraric acid, others with fumarprotocetraric acid and atranorin, and others with fumarprotocetraric acid, atranorin, and bourgeanic acid. Clade D includes samples with farinose soredia, smooth cortex, and containing only fumarprotocetraric and bourgeanic acids. Samples in clade E have farinose to granular soredia and a smooth or areolate cortex. As for secondary metabolites, samples are found which contain fumarprotocetraric acid and atranorin, while others have atranorin and psoromic acid. Clade F contains samples with granules and areolate cortex. All the samples in this clade contain fumarprotocetraric acid. Clade G includes samples with farinose soredia that cover most of the podetial surface, sometimes with a corticate inferior half (Fig. 3). This clade comprises samples that only contain fumarprotocetraric acid and samples with both fumarprotocetraric acid and atranorin.

DISCUSSION

Species delimitation in the* Cladonia humilis *complex—In this paper, we study the species delimitation within *C. humilis*

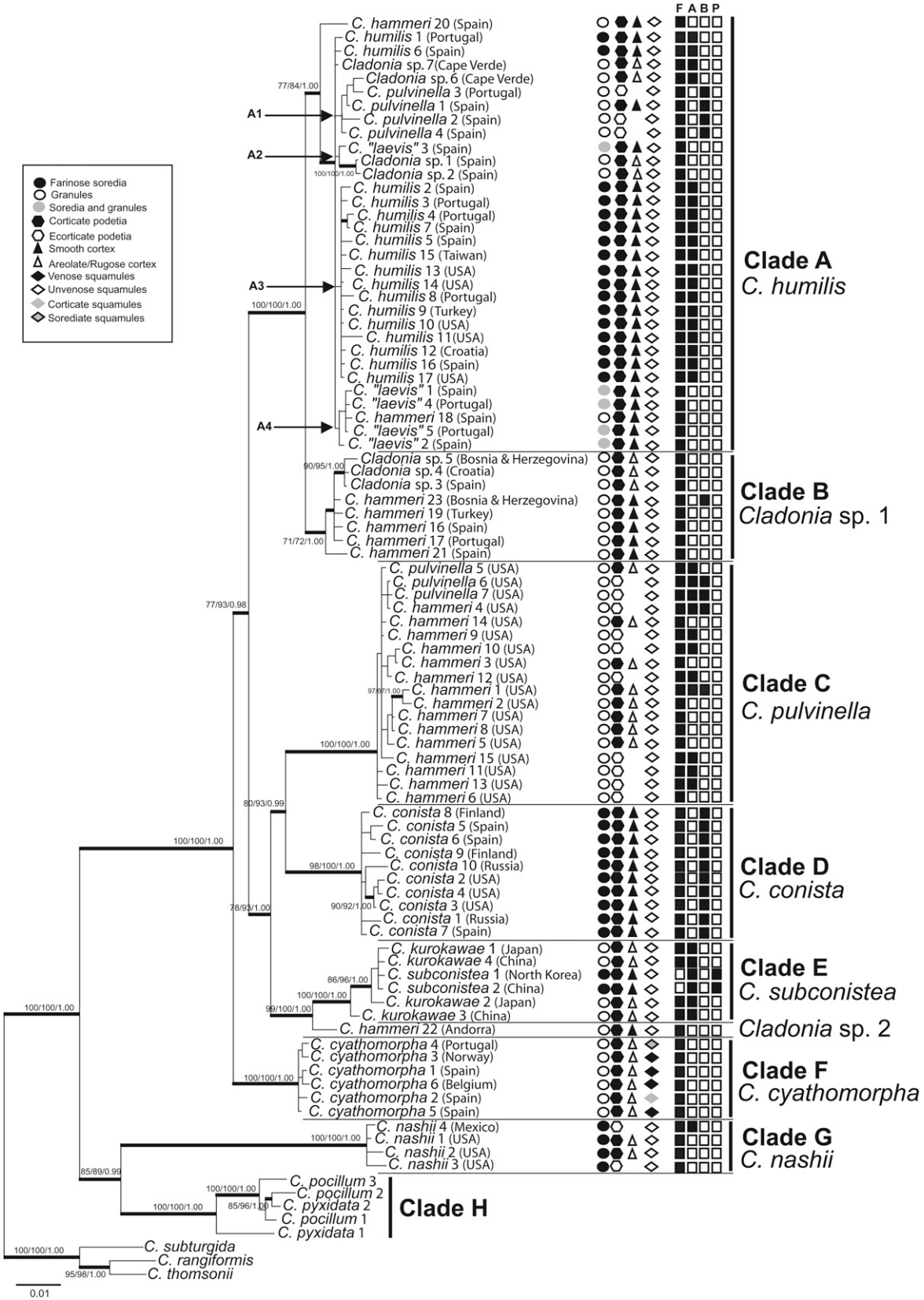

Fig. 1. Phylogeny of the *Cladonia humilis* complex based on the combined data set (ITS rDNA, IGS rDNA, *rpb2*, and *ef1α*). This is a 50% majority rule consensus tree of a Bayesian analysis. Branches supported with posterior probability ≥0.95 and bootstrap ≥70% are indicated in bold. A = atranorin, B = bourgeanic acid, F = fumarprotocetraric acid, P = psoromic acid. Black squares = presence, white squares = absence.

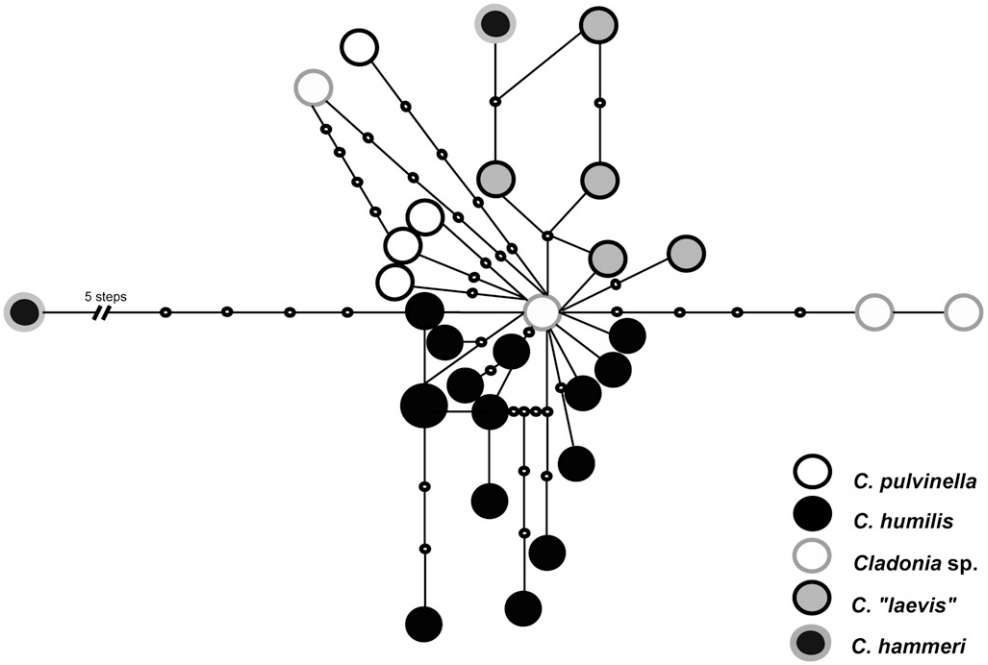

Fig. 2. Haplotype network at 95% probability for clade A (putative species identification) based on the combined data set.

complex, using multiple tools: the genealogical concordance phylogenetic species recognition (Taylor et al., 2000), the Shaffer and Thomson's method (2007), based on using F_{ST} and the method proposed by Cummings et al. (2008), that enables the acknowledgment of species before they reach monophyly. On the basis of our analyses, we diagnosed at least seven species within the traditional *C. humilis* complex. But not all these clades correspond to previously described species nor are all morphologically or chemically homogeneous.

Clade A contains the highest number of specimens and the largest morphological diversity. It comprises samples previously identified as *C. humilis*, *C. pulvinella*, *C. hammeri*, *C. "laevis"*, and *Cladonia* sp. Nevertheless, the samples that were identified as these species do not form independent subclades within clade A, nor do they separate in the haplotype network (Fig. 2). The four subclades found within clade A show certain morphological trends (Fig. 1). Thus, subclade A3 only includes specimens identified as *C. humilis* with a smooth podetial cortex, farinose soredia, and containing fumarprotocetraric acid and atranorin (Ahti, 1966). The subclade A4 contains four of the five samples identified as *C. "laevis"*, characterized by having farinose soredia on the external side of the scyphi and granules inside the scyphi, a smooth cortex, and by containing only fumarprotocetraric acid. However, this subclade also includes a sample identified as *C. hammeri*, which lacks farinose soredia. Subclade A1 contains the four samples identified as *C. pulvinella* from Europe, which lack a cortex and have a granulose podetial surface, plus a sample of *Cladonia* sp. with an areolate cortex. Finally, the subclade A2 comprises a sample identified as *C. "laevis"*, phenotypically similar to those in subclade A4 (Fig. 1), plus two samples of *Cladonia* sp. with areolate cortex and granules. The F_{ST} values indicate that gene flow exists among the different subclades within clade A (Table 7). F_{ST} values superior to 0.5 were obtained only between subclades A1-A2, A1-A4 and A2-A4 in ITS rDNA. The average genetic distances among the subclades of clade A are in general smaller than those obtained among the other clades (Table 7). In addition, the lengths of the branches between these subclades are comparatively short. All this evidence suggests the absence of divergence among the subclades of the clade A. In the different tests (SH, ELW, GSI) carried out to verify whether any evidence existed about the possible divergence among the species within clade A, only the samples identified as *C. humilis* were supported by SH, ELW and GSI for all the loci, and likewise by GSI_T. Though some putative species, such as *C. "laevis"* generated significant GSI values for certain loci, GSI_T, that integrates the genealogic pattern through the loci, giving a stronger evidence of divergence (Cummings et al., 2008), was not significant. Therefore, present-day data suggest that clade A is probably formed by a unique species subject to great phenotypical variability, *C. humilis*.

TABLE 3. Results of alternative topology tests for assessing the monophyly of different putative species, Shimodaira-Hasegawa test (SH) and likelihood weight test (ELW). * $P \leq 0.05$, statistically significant results. Significant results reject the monophyly.

Hypotheses	−Ln L	SH	ELW
Cladonia hammeri s.l.	10223.03	0.0000*	0.0000*
C. hammeri from Europe	10212.82	0.0000*	0.0000*
C. humilis	9671.77	0.9520	0.1853
C. kurokawae	9701.35	0.4890	0.0195*
C."laevis"	9689.65	0.6440	0.0256*
C. pulvinella s.l.	10081.14	0.0000*	0.0000*
C. pulvinella from Europe	9685.31	0.7380	0.0236*
C. pulvinella from North America	9686.32	0.7390	0.0108*
C. pulvinella and *C. hammeri* with bourgeanic acid	9708.58	0.0180*	0.0181*
C. subconistea	9669.56	1.0000	0.5073
Cladonia sp.	9811.41	0.0040*	0.0000*

TABLE 4. Genealogical sorting index and probability values under hypothesis null that the samples labeled as putative species are monophyletic.

Putative species	ITS rDNA		IGS rDNA		*rpb2*		*ef1α*		GSI_T	
	GSI	*P*	GSI	*P*	GSI	*P*	GSI	*P*	GSI_T	*P*
Cladonia hammeri s.l.	**0.5516**	**1e-04**	**0.3659**	**1e-04**	**0.2841**	**8e-04**	**0.3159**	**2e-04**	**0.3159**	**1e-04**
C. hammeri only samples from Europe	**0.4578**	**1e-04**	0.2326	0.0022	0.1582	0.1496	0.1844	0.0494	0.1844	0.0548
C. humilis	**0.6298**	**1e-04**	**0.272**	**2e-04**	**0.7971**	**1e-04**	**0.4041**	**1e-04**	**0.4041**	**1e-04**
C. kurokawae	**0.7414**	**1e-04**	0.4888	0.0016	0.3854	0.0036	0.4882	0.0013	0.4882	0.0011
C. "laevis"	**0.4186**	**2e-04**	0.3537	0.0011	**0.37**	**3e-04**	0.1802	0.0482	0.1515	0.1251
C. pulvinella s.l.	**0.2952**	**8e-04**	0.1333	0.1395	0.1385	0.3372	0.1155	0.4662	0.1155	0.4649
C. pulvinella from North America	0.2759	0.0031	**0.3537**	**9e-04**	0.2317	0.0229	0.1626	0.0674	0.1626	0.0717
C. pulvinella from Europe	**0.7414**	**1e-04**	0.1875	0.0265	0.1332	0.1585	0.1585	0.087	0.1585	0.0883
C. subconistea	0.2416	0.0541	0.2417	0.0575	0.494	0.0174	0.4942	0.0159	0.4942	0.0177
Cladonia sp.	**0.2941**	**9e-04**	**0.3073**	**5e-04**	0.2462	0.0061	0.2481	0.0046	0.2481	0.0057

Notes: Significant results ($P \leq 0.001$) are in boldface.

According to genealogical concordance phylogenetic species recognition, clade B would correspond to another phylogenetic species. Nevertheless, this clade includes samples that were previously identified as different species on the basis of phenotypical characters (Fig. 1). The samples identified as *Cladonia* sp. in clade B are indistinguishable from the samples of *Cladonia* sp. included in clade A, and the same is true for the samples identified as *C. hammeri*. The samples of *C. hammeri* in clade B come only from Europe, as do those samples of *C. hammeri* in clade A. The hypotheses contrasts rejected the monophyly of samples identified as *Cladonia* sp., and the monophyly of the samples from Europe identified as *C. hammeri*. GSI_T did not find either evidence of genealogic exclusivity among all the samples of *C. hammeri* from Europe, nor among all the samples of *Cladonia* sp. (Table 4). Therefore, in spite of being morphologically indistinguishable, the samples identified as *C. hammeri* and *Cladonia* sp. in clades A and B differ genetically and probably correspond to two different species. F_{ST} indicated the absence of gene flow between clades A and B in ITS rDNA and IGS rDNA but not in *rpb2* and *ef1α*, this test suggests that genetic isolation between clades A and B is incomplete.

A startling result is that the GSI for all the loci, and likewise the GSI_T, was significant, finding evidence of genealogical exclusivity for *C. hammeri* s.l. (including the samples from Europe and North America), despite the fact that this same test did not find evidence of genealogical exclusivity when only the *C. hammeri* samples from Europe were included (Table 4). The GSI result contradicts the SH and ELW test results, that reject the monophyly of the samples of *C. hammeri* from Europe and North America. Besides, F_{ST} values indicate that no gene flow exists between clade C (which includes the samples of *C. hammeri* from North America) and clades A and B (which include the remaining samples of *C. hammeri*), perhaps because the GSI presents a certain bias when groups with uneven number of samples are analyzed (Cummings et al., 2008). When it comes to interpreting results, it is important to take into account this GSI bias. On the basis of the remaining results, we conclude that *C. hammeri* is not present in Europe.

Clade C contains the samples from North America identified as *C. hammeri* and *C. pulvinella*. Both species, described from California (Hammer, 1991; Ahti and Hammer, 2002) are characterized by having propagules such as granules or schizidia on the podetia. However, the scyphus margin of *C. pulvinella* is irregularly dentate (Ahti and Hammer, 2002). The chemical diversity within clade C was notable (Fig. 1). The only secondary metabolite reported to date in *C. hammeri* is fumarprotocetraric acid (Ahti and Hammer, 2002). However, here we have found that in addition to this substance, atranorin, or atranorin along with bourgeanic acid, can also be present. In the same way, the only chemotype found in *C. pulvinella* from North America was the one containing fumarprotocetraric acid, atranorin, and bourgeanic acid. However, one sample of *C. pulvinella* lacked bourgeanic acid. The presence or absence of bourgeanic acid was an important character to distinguish *C. hammeri* from *C. pulvinella* (Ahti and Hammer, 2002). Yet the samples identified as *C. pulvinella* and *C. hammeri* containing bourgeanic acid do not group together. SH and EWL tests rejected the monophyly of these samples. Consequently, our results do not support *C. pulvinella* and *C. hammeri* as two different species.

Clade D, well supported in the three phylogenetic analyses of the combined matrix, and with high F_{ST} values for the three markers, corresponds to *C. conista*. This taxon is morphologically difficult to distinguish from *C. humilis* s.s., and both taxa

TABLE 5. Mean genetic distances (±SD) among and within the clade for every locus.

Group	ITS rDNA	IGS rDNA	*rpb2*	*ef1α*
Clade A	0.0079 ± 0.0076	0.0091 ± 0.0123	0.0026 ± 0.0019	0.0013 ± 0.0017
Clade B	0.0023 ± 0.0013	0.0130 ± 0.0168	0.0041 ± 0.0029	0.0012 ± 0.0015
Clade C	0.0026 ± 0.0019	0.0096 ± 0.0091	0.0017 ± 0.0017	0
Clade D	0.0034 ± 0.0022	0.0072 ± 0.0091	0.0031 ± 0.0013	0.0069 ± 0.0053
Clade E	0.0036 ± 0.0016	0.0025 ± 0.0019	0.0047 ± 0.0040	0
Clade F	0.0016 ± 0.0011	0.0063 ± 0.0029	0.0011 ± 0.0007	0.0056 ± 0.0045
Clade G	0.0078 ± 0.0044	0.0047 ± 0.0033	0.0007 ± 0.0007	0.0129
Clade A–Clade B	0.0281 ± 0.0061	0.0313 ± 0.0102	0.0040 ± 0.0028	0.0012 ± 0.0016
Clade A–Clade C	0.0285 ± 0.0041	0.0639 ± 0.0103	0.0682 ± 0.0031	0.0143 ± 0.0014
Clade A–Clade D	0.0316 ± 0.0049	0.0295 ± 0.0078	0.0711 ± 0.0026	0.0219 ± 0.0041
Clade A–Clade E	0.0315 ± 0.0315	0.0427 ± 0.0081	0.0582 ± 0.0021	0.0227 ± 0.0227
Clade A–Clade F	0.0223 ± 0.0042	0.0371 ± 0.0043	0.066 ± 0.0021	0.0091 ± 0.0014
Clade A–Clade G	0.1146 ± 0.0235	0.2279 ± 0.0068	0.1008 ± 0.0025	0.0692 ± 0.0020
Clade B–Clade C	0.0318 ± 0.0024	0.0547 ± 0.0118	0.0673 ± 0.0034	0.0141 ± 0.0012
Clade B–Clade D	0.0349 ± 0.0028	0.0206 ± 0.0094	0.0697 ± 0.0033	0.0218 ± 0.0041
Clade B–Clade E	0.0173 ± 0.0015	0.0321 ± 0.0080	0.0573 ± 0.0033	0.0225 ± 0.0044
Clade B–Clade F	0.0201 ± 0.0015	0.0273 ± 0.0063	0.0646 ± 0.0646	0.0090 ± 0.0012
Clade B–Clade G	0.1037 ± 0.0206	0.2155 ± 0.0091	0.0991 ± 0.0046	0.0691 ± 0.0022
Clade C–Clade D	0.0324 ± 0.0031	0.0401 ± 0.0119	0.0648 ± 0.0030	0.0104 ± 0.0041
Clade C–Clade E	0.0298 ± 0.0029	0.0516 ± 0.0099	0.0662 ± 0.0026	0.0117 ± 0.0042
Clade C–Clade F	0.0255 ± 0.0023	0.0462 ± 0.0091	0.0618 ± 0.0026	0.0189 ± 0.0004
Clade C–Clade G	0.1077 ± 0.0261	0.2571 ± 0.0113	0.1058 ± 0.0033	0.0722 ± 0.0033
Clade D–Clade E	0.0322 ± 0.0055	0.0239 ± 0.0054	0.0701 ± 0.0034	0.0185 ± 0.0063
Clade D–Clade F	0.0261 ± 0.0031	0.0144 ± 0.0070	0.0686 ± 0.0017	0.0269 ± 0.0042
Clade D–Clade G	0.1071 ± 0.0206	0.2033 ± 0.0084	0.0785 ± 0.0015	0.0805 ± 0.0111
Clade E–Clade F	0.0216 ± 0.0016	0.0319 ± 0.0031	0.0559 ± 0.0027	0.0275 ± 0.0044
Clade E–Clade G	0.0838 ± 0.0059	0.2332 ± 0.0048	0.0955 ± 0.0044	0.0819 ± 0.0072
Clade F–Clade G	0.1257 ± 0.0062	0.2133 ± 0.0058	0.0842 ± 0.0014	0.0748 ± 0.0010

have often been treated as conspecific (Ahti, 2000; James, 2009). But in molecular analyses, Dolnik et al. (2010) and Pino-Bodas et al. (2012a) found that these species differ genetically. Our results confirm this conclusion. A well-supported subclade contains the three specimens of *C. conista* from North America, but these samples only differ from others of *C. conista* in three polymorphisms (2 in IGS eDNA and 1 in *rpb2*).

Primarily owing to the presence or absence of psoromic acid, *C. subconistea* and *C. kurokawae* have been considered as separate species (Ahti et al., 1995; Awasthi and Ahti, 2007). However, clade E, strongly supported in all phylogenetic analyses, includes intermingled samples identified as one or the other. The ELW test rejected the monophyly of *C. kurokawae*, and GSI and GSI_T were not statistically significant for any species. Intraclade genetic distances were similar to those found in other clades constituted by a unique species (Table 5), which also suggests that this clade comprises one only species. In addition, *C. subconistea* and *C. kurokawae* are sympatric in China, Japan, and Korea (Awasthi and Ahti, 2007). Therefore, we conclude that *C. kurokawae* and *C. subconistea* are conspecific, being two chemotypes of the same species. The name *C. subconistea* has priority because it is the earliest legitimate name.

TABLE 6. Pairwise F_{ST} for each data set (ITS rDNA, IGS rDNA, *rpb2*, and *ef1α*).

Comparisons	ITS rDNA	IGS rDNA	*rpb2*	*ef1α*
Clade A-Clade B	0.80433	0.64639	*0.06832*	*0.00000*
Clade A-Clade C	0.88662	0.84343	0.97879	0.92070
Clade A-Clade D	0.89009	0.80161	0.94990	0.77683
Clade A-Clade E	0.84403	0.86183	0.92880	0.84157
Clade A-Clade F	0.89472	0.76562	0.97355	0.92070
Clade A-Clade G	0.96487	0.96902	0.98674	0.85202
Clade B-Clade C	0.81399	0.78192	0.96319	0.91429
Clade B-Clade D	0.80682	0.60044	0.93326	0.77319
Clade B-Clade E	0.75743	0.75813	0.90991	0.83682
Clade B-Clade F	0.80242	0.59578	0.95709	0.91429
Clade B-Clade G	0.92967	0.94513	0.97511	0.85065
Clade C-Clade D	0.88759	0.84486	0.96289	0.59683
Clade C-Clade E	0.84627	0.87417	0.95091	0.66667
Clade C-Clade F	0.89009	0.80215	0.98789	1.00000
Clade C-Clade G	0.95697	0.96051	0.99746	0.86486
Clade D-Clade E	0.67869	0.90604	0.92308	0.62055
Clade D-Clade F	0.89516	0.52174	0.96350	0.85146
Clade D-Clade G	0.96145	0.97953	0.97203	0.79840
Clade E-Clade F	0.78621	0.83969	0.93855	0.92308
Clade E-Clade G	0.94627	0.97826	0.96698	0.84729
Clade F-Clade G	0.96350	0.96367	0.99363	0.88095

Notes: Low values are in italics.

Some doubts have been expressed whether *C. cyathomorpha* is a well-delimited unit (Burgaz and Ahti, 2009; James, 2009), owing to the fact that it is morphologically variable, especially with respect to the primary thallus. In this study, we tried to include the whole morphological variation described for the species: samples with the lower side of squamules venose or sorediate or corticate (Figs. 1, 3). All of them grouped together into one monophyletic clade (clade F). Therefore, the differences in primary thallus only represent intraspecific variation. Similar presence or absence of soredia on primary thallus squamules does occur in other species of *Cladonia*, as *C. acervata* S. Hammer (Hammer, 2001), *C. borbonica* Nyl. (Ahti, 2000), *C. coniocraea* (Flörke) Spreng. (Ahti and Hammer, 2002), or *C. corniculata* Ahti & Kashiwadani (Ahti, 2000); in some of them, it is a developmental stage (Ahti, 2000). In most cases, *C. cyathomorpha* is easily distinguished from the other species within the group by the presence of pale pink veins on the lower face of the primary thallus. The material studied here only contains fumarprotocetraric acid; the chemically unknown substance identified by Jølle (1977) was not found in the present study material. Its constant presence is hardly a diagnostic character of the species.

TABLE 7. Genetic distances among and within the subclades of clade A (mean ± SD) and pairwise F_{ST} for each data set.

Comparisons	Genetic distances				F_{ST}			
	ITS rDNA	IGS rDNA	*rpb2*	*ef1α*	ITS rDNA	IGS rDNA	*rpb2*	*ef1α*
Clade A1-Clade A2	0.0111 ± 0.0023	0.0031 ± 0.0029	0.0043 ± 0.0021	0.0038 ± 0.0022	0.64205	0.00000	0.30769	0.40625
Clade A1-Clade A3	0.0087 ± 0.0050	0.0096 ± 0.0105	0.0028 ± 0.0013	0.0014 ± 0.0016	0.49341	0.29545	0.07347	0.22951
Clade A1-Clade A4	0.0086 ± 0.0023	0.0062 ± 0.0044	0.0005 ± 0.0010	0.0017 ± 0.0017	0.67391	0.00000	0.00000	0.00000
Clade A2-Clade A3	0.0071 ± 0.0046	0.0064 ± 0.0100	0.0058 ± 0.0058	0.0023 ± 0.0016	0.44862	0.44828	0.34127	0.47458
Clade A2-Clade A4	0.0074 ± 0.0013	0.0050 ± 0.0026	0.0032 ± 0.0018	0.0033 ± 0.0019	0.65574	0.22222	0.40000	0.38462
Clade A3-Clade A4	0.0048 ± 0.0044	0.0115 ± 0.0105	0.0023 ± 0.0009	0.0011 ± 0.0013	0.36998	0.34951	0.20779	0.00000
Clade A1	0.0050 ± 0.0027	0.0063 ± 0.0036	0.0011 ± 0.0012	0.0019 ± 0.0017	—	—	—	—
Clade A2	0.0032 ± 0.0028	0.00000	0.0043 ± 0.0028	0.0022 ± 0.0019	—	—	—	—
Clade A3	0.0044 ± 0.0053	0.0075 ± 0.0159	0.0022 ± 0.0018	0.0002 ± 0.0005	—	—	—	—
Clade A4	0.0017 ± 0.0011	0.0025 ± 0.0032	0.00000	0.0019 ± 0.0017	—	—	—	—

Clade G contains all the samples identified as *C. nashii*; all of them have farinose soredia (Table 8), but not all contain fumarprotocetraric acid and atranorin, which was the originally described chemotype of this species (Ahti and Hammer, 2002). The presence of atranorin seems to be inconstant. Morphologically, this species is rather similar to the others in the *C. humilis* complex, but it does not form a monophyletic group with them.

Clade H contains samples identified as *C. pyxidata* or *C. pocillum*. Our results agree with those by Kotelko and Piercey-Normore (2010), who on the basis of mainly Canadian material reported that neither species is monophyletic. The *C. pyxidata* group needs a through taxonomic revision based on larger material.

***Significance of phenotypic characters in the* C. humilis *complex*—**While phenotypic species concepts in *Cladonia* represent monophyletic lineages in some cases, as in *C. subulata*, *C. rei*, *C. maxima*, *C. phyllophora*, *C. subchordalis*, *C. multiformis*, *C. corsicana*, and *C. turgida* (Dolnik et al., 2010; Fontaine et al., 2010; Pino-Bodas et al., 2010a, 2012c), in other cases they do not, as, for example, in *C. arbuscula*, *C. pyxidata*, *C. pocillum*, or *C. gracilis* (Fontaine et al., 2010; Kotelko and Piercey-Normore , 2010; Piercey-Normore et al., 2010). Phenotypical features often do not characterize monophyletic lineages in the *C. humilis* complex. Cortical features (presence or absence of cortex, surface roughness) are often used as taxonomic characters in *Cladonia* (Ahti, 1980; Hammer, 1991, 1995). However, in the *C. humilis* complex, we find specimens with very different cortex characteristics, which appear lumped together within the same clade. For example, clade A contains samples without cortex, with areolate cortex and with smooth and continuous cortex. It is worth noting the case of *C. kurokawae*, with a cortex more or less smooth and continuous, and *C. subconistea*, with areolate verruculose cortex. The differences in the cortex type may represent different development stages, since in some species of *Cladonia*, cortex characteristics vary during development (Ahti, 2000). One of the many examples that we can quote is *C. cinerella* Ahti, which has a smooth cortex in early development stages, but soon becomes areolate (Ahti, 2000). It should also be noted that in general many of these characters are very difficult to interpret correctly in herbarium specimens, because the environmental conditions in different vegetation communities can strongly affect their phenotype (T. Ahti, University of Helsinki, personal observation). In the remaining clades of the *C. humilis*, complex cortical characters are more homogeneous.

Soredium size has been widely used to distinguish between closely related species in *Cladonia* (Hennings, 1983). Sometimes even a correlation between soredium size and secondary

TABLE 8. Morphological variation found in each clade of phylogenetic analyses.

Character	Clade A (*N* = 26)	Clade B (*N* = 8)	Clade C (*N* = 12)	Clade D (*N* = 10)	Clade E (*N* = 6)	Clade F (*N* = 5)	Clade G (*N* = 4)
Podetium morphology	Gradually flaring	Gradually flaring	Sharply flaring	Long stalk	Sharply flaring	Gradually flaring	Sharply flaring
Podetium size (mm)	3–10 (2.137)	2.8–10 (2.181)	3–10.1 (1.864)	4.75–12 (3.194)	2.9–8 (1.474)	4–12 (3.152)	3–5.5 (0.917)
Podetium thickness (μm)	147–330 (52.659)	245–300 (42.271)	147–387 (55.052)	160–338 (77.366)	145–370 (71.974)	145–300 (60.228)	230–425 (60.295)
Scyphus width (mm)	1–8 (1.972)	1.2–7 (1.910)	2–5.5 (1.061)	3.25–7.25 (1.587)	1–4 (0.944)	1.5–4 (1.050)	2–4 (0.665)
Cortex	Smooth, areolated, extends higher, at the base	Smooth or verrucose, extends higher	Verrucose, limited at base or half-podetia	Smooth, extends higher	Smooth, areolated-corticate, extends higher	Areolated, verrucose, half-podetia	Lack or only at the base
Soredium size (μm)	20–255 (42.544)	40–390 (74.753)	50–250 (76.200)	20-45 (6.759)	45–200 (37.118)	45–390 (89.600)	20–80 (25.378)
Chemistry	(I) FUM, PRO, ATR (II) FUM, PRO, BOU (III) FUM, PRO	(I) FUM, PRO	(I) FUM, PRO (II) FUM, PRO, ATR (III) FUM, PRO, ATR, BOU	(I) FUM, PRO, BOU	(I) FUM, PRO, ATR (II) PSO, CPSO, ATR	(I) FUM, PRO	(I) FUM, PRO (II) FUM, PRO, ATR
Distribution	North and Central America, Europe and Asia	Mediterranean Europe	North America	Europe and North America	East Asia	Europe	North America

Notes: Standard deviations are in parentheses; *N* = number of specimens measured. PRO = protocetraric acid, CPSO = conpsoromic acid (the other abbreviations are listed in Table 1).

Fig. 3. Morphology of some species of the *Cladonia humilis* complex. (A) *C. hammeri* (bar = 1 mm), (B) *C. humilis* (bar = 1 mm), (C) *C. nashii* (bar = 5 mm), (D–F) features of *C. cyathomorpha,* (D) *C. cyathomorpha* (bar = 5 mm), (E) Veins on lower face of the primary thallus (bar = 1 mm), (F) Cross section of the primary thallus stained with lactophenol cotton blue (bar = 25 µm).

metabolites has been found (Ahti, 1966; Kristinsson, 1971). However, in the *C. humilis* complex the clades A and E contain samples with farinose soredia and others with granules. In the other monophyletic entities, the soredia size is homogeneous.

Primary thallus characters such as squamule length, thickness, branching, and ascending vs. prostrate arrangement, have been used to distinguish some species of *Cladonia* (Ahti, 2000; Aptroot et al., 2001; Van Herk and Aptroot, 2003). In some cases, these characters proved useful to distinguish monophyletic lineages (Lendemer and Hodkinson, 2009). However, in *C. pocillum* and *C. pyxidata*, it became clear that the differences in the primary thallus are related to the soil pH and do not predict phylogenetic lineages. Therefore, the primary thallus in general is not often used for species delimitation in the *C. humilis* complex. However, in the case of *C. cyathomorpha,* the presence of veins on the largish squamules can be used as a taxonomic character to distinguish the species.

Secondary metabolites have been pivotal in distinguishing species in this and many other groups of lichens. However, in this study we found that most of the clades have more than one chemotype. Bourgeanic acid (a fatty acid), so far restricted to *C. conista* and *C. pulvinella*, appears also, though inconstantly, in samples of clade A. The presence or absence of fatty acids was considered likewise taxonomically significant, and in fact, in some lichenized fungi, phylogenetic lineages are correlated with the presence of fatty acids (Spribille et al., 2011). However, in other *Cladonia* species such as *C. subrangiformis*, the presence of bourgeanic acid was considered merely a chemotype (Burgaz and Ahti, 2009). In *C. subturgida*, the presence of protolichesterinic fatty acid is also inconstant (Pino-Bodas et al., 2012c). We have also found that atranorin and psoromic acid are inconstant in several lineages (A, C, E, G). As a matter of fact, it is frequent in *Cladonia* that these two substances inconstantly appear in numerous species (for example, atranorin in *C. pyxidata*, *C. furcata*, *C. scabriuscula*, *C. stricta*; Ahti and Hyvönen, 1985; Stenroos, 1995; Ahti, 1998). One example of a common secondary metabolite, in most species of *Cladonia*, seems to occur constantly is usnic acid (Huovinen et al., 1989, 1990). Phylogenetic studies have demonstrated that chemically polymorphic lineages are common in *Cladonia* (Lendemer and Hodkinson, 2009; Piercey-Normore et al., 2010; Pino-Bodas et al., 2012b). However, in many cases, the presence of certain

secondary metabolites is diagnostic in species identification. Such a case is exemplified by *C. subulata* and *C. rei*, where the presence or absence of homosekikaic acid is diagnostic (Dolnik et al., 2010; Pino-Bodas et al., 2010a).

Taxonomy—Some of the taxonomic conclusions discussed are formally presented below.

Cladonia pulvinella S. Hammer—Mycotaxon 40: 192 (1991). Type: USA, California, Marin County, Point Reyes National Seashore, Ridge Trail, 1987, *S. Hammer* 2023 [Holotype: FH, Isotype: H].

= *Cladoni hammeri* Ahti in Nash et al., Lichen Flora of the Greater Sonoran Desert Region 1: 144 (2002). Type: USA, California, Los Angeles Co., Santa Catalina Island, Parsons Landing, *T. H. Nash 32201* (Holotype ASU).

Cladonia subconistea Asahina—J. Jap. Bot. 17: 433 (1941). Type: Japan, Honshu, Prov. Shinano (Pref. Nagano), Mt. Yatsugadake ('Yatugadake'), 1926, *Y. Asahina* 704 (Lectotype, TNS, designated by Ahti [1993] as "holotype").

= *Cladonia kurokawae* Ahti & S. Stenroos in Ahti et al., Mycosystema 8–9: 54 (1996). Type: Japan, Prov. Hitachi (Pref. Ibaraki), Kuji-gun, Nakasatomura, Irishiken, 1950, *Y. Asahina* 25758 (Holotype TNS).

Conclusions—By weighing evidence from DNA sequences, morphology, and secondary metabolites, we accept seven species in the *C. humilis* complex. The traditional taxonomical concepts of *Cladonia conista*, *C. cyathomorpha*, and *C. nashii* represent monophyletic lineages. Despite its morphological affinities, *C. nashii* is not closely related to the remaining species in the complex. *Cladonia hammeri* and *C. pulvinella* are not present in Europe. The American samples of *C. hammeri* and *C. pulvinella* form a single lineage and they are regarded as a single species.

The ITS rDNA region has been the most used locus for species delimitation in the lichenized fungi. However, when the phenotypical variation does not coincide with the variation of this locus, the use of more independent loci becomes necessary, since the evolutionary history of one locus does not necessarily represent the evolutionary history of the species. In the *Cladonia humilis* group, combining four loci yielded more supported clades than any single locus (online Appendix S3). In addition, the inclusion of more loci in an analysis should reduce stochastic errors (Chen et al., 2008). Nevertheless, phylogenetic analyses only permit species recognition once they have attained monophyly. This is why, in species delimitation studies, tests to determine other statistics are useful, such as F_{ST}, which allows an analysis of whether some gene flow exists among the species before reaching reciprocal monophyly in all the genes of the genome.

Some studies have determined a threshold value for genetic distances in ITS rDNA. This threshold value can be used to mark out the limits among lineages. In Parmeliaceae, the intraspecific variation threshold was established to be between 0.015 and 0.017 (Del-Prado et al., 2010). Schoch et al. (2012) found that the average intraspecific distance in fungi is 0.025 for ITS rDNA. The intraclade genetic distance values found in the *C. humilis* complex vary from 0.0016 to 0.0079 in ITS rDNA, thus remaining within the range of the values known for other groups of fungi, although they are much lower than the average for fungi. More exhaustive studies, involving a representative number of species, will be necessary to establish a threshold value for intraspecific genetic distances in *Cladonia.*

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APPENDIX 1. Voucher information including GenBank accessions for samples included in this study.

Taxon: Collection locality, *Voucher*, Herbarium, GenBank accessions: ITS rDNA, *rpb2*, *ef1α*, IGS rDNA (identification name on the tree)

Cladonia conista: Russia, Kursk Region, Bubel, *N. I. Zolotuchin* 26a, H, JF926633, JF926568, JF926590, KC415837 (*C. conista* 1); USA, Connecticut, Litchfield County, *J. C. Lendemer et al.* 1358 Lich. E. N. Amer. Exs. 142, H, JF926634, JF926569, JF926591, KC415838 (*C. conista* 2); USA, New Jersey, Gloucester County, *J. C. Lendemer et al.* 15464, H, JF926635, JF926570, JF926592, KC415839 (*C. conista* 3); USA, Pennsylvania, Blair County, *J. C. Lendemer et al.* 11852, H, JF926636, JF926571, JF926593, KC415840 (*C. conista* 4); Spain, Castellón, Chóvar, *A. R. Burgaz* s.n., MACB 97591, JF926612, JF926566, JF926588, KC415817 (*C. conista* 5); Spain, Huesca, Oza, *A. R. Burgaz* s.n., MACB 92796, JF926613, JF926567, JF926589, KC415818 (*C. conista* 6); Spain, Orense, Cabeza de Manzaneda, *A. R. Burgaz* s.n., MACB 98080, JF926630, —, JF926596, KC415819 (*C. conista* 7); Finland, Laatokan Karjala, Parikkala, *T. Ahti* 62186, H, JF926629, JF926572, —, KC415827 (*C. conista* 8); Finland, Etelä-Häme, Nastola, *V. Haikonen* 21947, H, JF926632, —, JF926594, KC415828 (*C. conista* 9); Russia, Sakha Republic, Batagay, *T. Ahti* 65490, H, JF926619, JF926573, JF926595, KC415894 (*C. conista* 10).

Cladonia cyathomorpha: Spain, Ávila, Hoyocasero, *A. R. Burgaz* s.n., MACB 97180, KC415941, KC525275, KC525337, KC415871 (*C. cyathomorpha* 1); Spain, Madrid, Lozoya, *A. R. Burgaz* s.n., MACB 97543, KC415942, —, KC525339, KC415872 (*C. cyathomorpha* 2); Norway, Hordaland, Bergen, *T. Ahti* 68660 &. *T. Tønsberg,* H, KC415943, KC525276, KC525338, KC415870 (*C. cyathomorpha* 3); Portugal, Beira Alta, Penhas Douradas, *A. R. Burgaz* s.n., MACB 101278, KC415940, KC525274, KC525340, KC415874 (*C. cyathomorpha* 4); Spain, León, Igüeña, *A. R. Burgaz* s.n., MACB 101279, KC415944, KC525278, —, KC415875 (*C. cyathomorpha* 5); Belgium, Vielsalm, Cahay, *A. Aptroot* 67026, F, KC415945, —, KC525341, KC415873 (*C. cyathomorpha* 6).

Cladonia humilis: Portugal, Trás-os-Montes, Rebordainhos, *A. R. Burgaz* s.n., MACB 92885, JF926625, JF926584, JF926597, KC415820 (*C. humilis* 1); Spain, Toledo, Urda, *A. R. Burgaz* s.n., MACB 92807, JF926622, JF926585, JF926598, KC415821 (*C. humilis* 2); Portugal, Baixo Alentejo, Cavalheiro, *A. R. Burgaz* s.n., MACB 97326, JF926626, JF926587, JF926599, KC415822 (*C. humilis* 3); Portugal, Estremadura, Azoia, *A. R. Burgaz* s.n., MACB 92818, JF926627, JF926583, JF926600, KC415823 (*C. humilis* 4); Spain, Madrid, La Acebeda, *A. R. Burgaz* s.n., MACB 95913, JF926614, JF926575, JF926601, KC415824 (*C. humilis* 5); Spain, Gerona, Port de la Selva, *A. R. Burgaz* s.n., MACB 95931, JF926615, JF926576, JF926602, KC415825 (*C. humilis* 6); Spain, Mallorca, Sa Pobla, *A. R. Burgaz* s.n., MACB 92803, JF926628, JF926581, JF926603, KC415826 (*C. humilis* 7); Portugal, Madeira, Funchal, *P. Alanko* 120624, H, JF926616, JF926582, JF926604, KC415829 (*C. humilis* 8); Turkey, Trabzon, Akcaabat, *K. Yazici* s.n., H, JF926617, JF926577, JF926605, KC415830 (*C. humilis* 9); USA, California, Los Angeles County, *K. Knudsen* 4202, H, JF926618, JF926577, JF926605, KC415831 (*C. humilis* 10); USA, California, Sonoma County, *T. Ahti* 69196a, H, JF926620, JF926579, JF926607, KC415832 (*C. humilis* 11); Croatia, Dubrovnik-Neretva, Zamaslina, *A. R. Burgaz* s.n., MACB 101103, JF926621, JF926580, JF926608, KC415833 (*C. humilis* 12); USA, California, Marin County, *T. Ahti* 68970 *& L. St. Clair,* H, JF926624, JF926574, JF926610, KC415834 (*C. humilis* 13); USA, California, Marin County, *T. Ahti* 68963 *& L. St. Clair,* H, JF926623, JF926586, JF926609, KC415835 (*C. humilis* 14); Taiwan, Nantou County, *A. Aptroot* 52993, H, KC415922, KC525261, KC525342, — (*C. humilis* 15); Spain, Gerona, Olot, *A. R. Burgaz* s.n., MACB 97593, KC415933, —, KC525336, KC415856 (*C. humilis* 16); USA, Oregon, Sherman County, *H. T. Root* s.n., OSC 141889, KC415971, KC525305, —, KC415836 (*C. humilis* 17); Spain. Burgos, Urrez, *A. R. Burgaz* s.n., MACB 102979, KC415924, KC525257, KC525315, KC415853 (*C. "laevis"* 1); Spain, Albacete, Villapalacios, *A. R. Burgaz* s.n., MACB 102944, KC415925, KC525258, KC525316, KC415854 (*C. "laevis"* 2); Spain, Murcia, Alhama de Murcia, *A. R. Burgaz* s.n., MACB 102945, KC415926, KC525259, KC525317, KC415855 (*C. "laevis"* 3); Portugal, Alto Alentejo, Bencatel, *A. R. Burgaz* s.n., MACB 102946, KC415927, KC525260, KC525318, KC415857 (*C. "laevis"* 4); Portugal, Algarve, *A. R. Burgaz* s.n., MACB 102882, KC415972, KC525262, KC525348, KC415900 (*C. "laevis"* 5); Spain, Sevilla, Alanis, *A. R. Burgaz* s.n., MACB 93015, KC415928, KC525253, KC525319, KC415849 (*C. pulvinella* 1); Spain, Menorca, Es Mercada, *A. R. Burgaz* s.n., MACB 98059, KC415929, KC525254, KC525320, KC415850 (*C. pulvinella* 2); Portugal, Estremadura, Serra da Sintra, *A. R. Burgaz* s.n., MACB 92820, KC415930, —, KC525321, KC415851 (*C. pulvinella* 3); Spain, Almería, Rodalquilar, *A. R. Burgaz* s.n., MACB 97949, KC415931, KC525255, KC525322, KC415852 (*C. pulvinella* 4); Spain, Granada, Loja, *A. R. Burgaz* s.n., MACB 94396, KC415973, KC525265, KC525356, KC415862 (*Cladonia* sp. 1); Spain, Tarragona, Corberá d'Ebre, *A. R. Burgaz* s.n., MACB 93167, KC415974, KC525282, KC525357, KC415861 (*Cladonia* sp. 2); Cape Verde, St. Antão, Porto Novo, *M. P. Martín* 3262, MA-lichen, KC415923, KC525264, KC525306, KC415859 (*Cladonia* sp. 6); Cape Verde, St. Antão, Porto Novo, *M. P. Martín* 3263, MA-lichen, —, KC525263, KC525350, KC415858 (*Cladonia* sp. 7); Spain, Ávila, San Bartolomé de Bejar, *A. R. Burgaz* s.n., MACB 96093, KC415932, KC525249, KC525309, KC415860 (*C. hammeri* 18); Spain, Canary Island, Tenerife, *A. R. Burgaz* s.n., MACB 102877, KC415921, KC525251, KC525313, KC415867 (*C. hammeri* 20).

Cladonia nashii: USA, California, Santa Barbara County, Santa Rosa Island, (Topotype), *K. Knudsen et al.* 7721, H, KC415976, KC525284, —, KC415895 (*C. nashii* 1); USA, California, Marin County, *T. Ahti* 68967 & *L. St. Clair,* H, KC415977, KC525280, KC525325, KC415898 (*C. nashii* 2); USA, California, Sonoma County, *T. Ahti* 69200a, H, KC415978, KC525285, KC525363, KC415896 (*C. nashii* 3); Mexico, Baja California Norte, San Quintín peninsula, *P. Bowler& B. Bretz* s.n., H, KC415975, KC525281, —, KC415897 (*C. nashii* 4).

Cladonia pocillum: USA, Minnesota, Wabasha County, *A. Tehler* 7806, S, KC415982, KC525301, KC525358, KC415904 (*C. pocillum* 1); USA, Connecticut, Litchfield County, *J. C. Lendemer* 1357 & *A. L. A. Foray,* UPS L-160151, KC415981, KC525266, KC525359, KC415901 (*C. pocillum* 2); France, Corsica, Cinto, *E. Granda* s.n., MACB 102880, KC415979, KC525267, KC525351, KC415905 (*C. pocillum* 3).

Cladonia pulvinella: USA, Oregon, Gilliam County, *H. T. Root* s.n., OSC 141690, KC415958, KC525302, KC525367, KC415842 (*C. pulvinella* 5); USA, Oregon, Gilliam County, *H. T. Root* s.n., OSC 141689, KC415959, KC525303, KC525368, KC415843 (*C. pulvinella* 6); USA, Oregon, Gilliam County, *H. T. Root* s.n., OSC 141890, KC415960, KC525304, KC525367, KC415841 (*C. pulvinella* 7); USA, California, Sonoma County, *T. Ahti* 69200c, H, KC415957, KC525279, KC525324, KC415891 (*C. hammeri* 1); USA, California, Sonoma County, *T. Ahti* 69200b, H, KC415962, KC525286, KC525326, KC415893 (*C. hammeri* 2); USA, California, Marin County, *T. Ahti* 69191, H, KC415963, KC525287, KC525327, KC415890 (*C. hammeri* 3); USA, California, Sonoma County, *T. Ahti* 69198, H, KC415961, KC525288, KC525328, KC415885 (*C. hammeri* 4); USA, California, Marin County, *T. Ahti* 68885, H, KC415964, KC525289, KC525329, KC415886 (*C. hammeri* 5); USA, California, Marin County, *T. Ahti* 68970, H, KC415965, KC525290, KC525330, KC415887 (*C. hammeri* 6); USA, California, Sonoma County, *T. Ahti* 69201, H, KC415969, KC525271, KC525360, KC415888 (*C. hammeri* 7); USA, California, Sonoma County, *T. Ahti* 69200, H, KC415970, KC525272, KC525361, KC415889 (*C. hammeri* 8); USA, California, Orange County, Santa Ana Mountains, *K. Knudsen* 6041, H, KC415952, KC525291, KC525334, KC415844 (*C. hammeri* 9); USA, California, Santa Barbara County, *K. Knudsen* 7482, H, KC415953, KC525292, KC525335, KC415845 (*C. hammeri* 10); USA, California, Riverside County, *K. Knudsen* 6441.1 & S. Bukusgus, UCR 41270, KC415954, KC525292, KC525331, KC415846 (*C. hammeri* 11); USA, California, Riverside County, *K. Knudsen* 11005, UCR 204973, KC415955, KC525292, KC525332, KC415847 (*C. hammeri* 12); USA, California, Riverside County, *K. Knudsen* 6441.4, UCR 41270, KC415956, —, KC525333, KC415848 (*C. hammeri* 13); USA, California, Riverside County, *J. C. Lendemer* 2640 *& K. Knudsen*, H, KC415951, KC525270, KC525311, KC415884 (*C. hammeri* 14); USA, California, Riverside County, *J. C. Lendemer* 2640 *& K. Knudsen,* F, KC415966, KC525270, KC525349, KC415892 (*C. hammeri* 15).

Cladonia pyxidata: Ukraine, Luhans`k Oblast, Pereval`s`k district, *O. Nadyeina* s.n., H, KC415980, KC525268, KC525352, KC415903 (*C. pyxidata* 1); Greenland, Qeqertaq, *E. S. Hansen* Lich. Groenl. Exc. 941, H, KC415983, KC525269, KC525353, KC415902 (*C. pyxidata* 2).

Cladonia subconistea: Japan, Kyushu, Prov. Bungo, *H. Kashiwadani et al.* 45017, Lich. Minns Cong. Exc. 254, H, KC415946, —, —, KC415877 (*C. kurokawae* 1); Japan, Honshu, Ibaraki Prefi, *T. Ahti* 62068, H, KC415947, KC525297, KC525345, KC415878 (*C. kurokawae* 2); China, Yunnan, Kunming County, *A. Aprtoot* 55498, H, KC415948, KC525300, KC525343, KC415879 (*C. kurokawae* 3); China, Hunan, Sang-Zhi County, *T. Koponen et. al.* 55724, H, AF455207, KC525298, KC525346, KC415880 (*C. kurokawae* 4); South Korea, Gyonggy, Uhwang, *K. H. Moon* 7188, in Kashiwadani, Lich. Minus Cogn. Exs. 255, H, KC415949, KC525296, KC525344, KC415876 (*C. subconistea* 1); China, Hunan, Yan-Ling Co., *T. Koponen et al.* 55878, H, AF455210, KC525299, KC525347, KC415881 (*C. subconistea* 2)

***Cladonia* sp. 1**: Spain, Mallorca, Buyola, *A. R. Burgaz* s.n., MACB 102879, KC415968, KC525283, KC525355, KC415883 (*Cladonia* sp. 3); Croatia, Dubrovnik-Neretva, Palje Brdo, *A. R. Burgaz* s.n., MACB 101113, KC415967, KC525273, KC525354, KC415882 (*Cladonia* sp. 4); Bosnia and Herzegovina, Srpska Republic, Trebinje, *A. R. Burgaz* s.n., MACB 102885, KC415934, KC525277, KC525362, KC415869 (*Cladonia* sp. 5); Spain, Barcelona, Montseny, *A. R. Burgaz* s.n., MACB 95732, KC415937, KC525247, KC525307, KC415864 (*C. hammeri* 16); Portugal, Algarve, Monchique, *A. R. Burgaz* s.n., MACB 97323, KC415950, KC525248, KC525308, KC415865 (*C. hammeri* 17); Turkey, Giresum, Degirmenagzi, *K. Kinalioglu* 1444, H, KC415936, KC525250, KC525310, KC415866 (*C. hammeri* 19); Spain, Madrid, Somosierra, *A. R. Burgaz* s.n., MACB 102876, KC415939, KC525252, KC525312, KC415863 (*C. hammeri* 21); Bosnia and Herzegovina, Herzegovina-Neretva, Caplijina, *A. R. Burgaz* s.n., MACB 101115, KC415935, KC525256, KC525323, KC415899 (*C. hammeri* 23).

***Cladonia* sp. 2**: Andorra, Soldeu, Port d'Envalira, *A. R. Burgaz* s.n., MACB 102875, KC415938, KC525246, KC525314, KC415868 (*C. hammeri* 22).

Cladonia rangiformis: Netherlands, Zuid-Holland, *H. Van der Goes et al*. s.n., H, JN811400, JN811429, KC525364, JN811367.

Cladonia subturgida: Spain, Ciudad Real, Villamanrique, *A. R. Burgaz* s.n., MACB 99488, JF288793, JF288824, KC525366, KC415906.

Cladonia thomsonii: Russia, Krasnoyarsk, Severnaya Zemlya Archipelago, *M. Zhurbenko* 96457, H, JN811402, JN811431, KC525365, JN811369.