Taxonomy of *Cladonia angustiloba* and related species

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Abstract: The lichen species *Cladonia angustiloba* is characterized by a well-developed primary thallus, and narrow squamules, which show deep incisions and presence of usnic and fumarprotocetraric acids. Morphologically it is similar to *C. foliacea* and *C. convoluta*, from which it can be distiguished by the squamule size and morphology. In this study, the species delimitation within the *C. foliacea* complex has been studied by sequencing three loci, ITS rDNA, *cox1* and *rpb2*. The data were analyzed by means of phylogenetic and species delimitation methods (GMYC, PTP, ABGD and BPP). Our results show that none of the three species is monophyletic. Most of the species delimitation methods did not support the current species as evolutionary lineages. Only some of the BPP analyses supported that *C. angustiloba* is a different species from *C. foliacea* and *C. convoluta*. However, the hypothesis that considers the *C. foliacea* complex as constituted by a unique species obtained the best Bayes Factor value. Therefore, *C. angustiloba* and *C. convoluta* are synonymized with *C. foliacea*. A new, thoroughly checked synonymy with typifications of the whole *C. foliacea* complex is presented. An updated survey of the world distribution data was compiled.

Key words: *Cladonia*, lichens, Macaronesia, molecular systematics, species delimitation

Introduction

Cladonia is one of the most diverse macrolichen genera, with 470 species recognized at present (Ahti 2017, pers. comm). The podetium morphology and the surface features, along with the secondary metabolites, have commonly been the characters used for species identification (Ahti 2000). No characters of the hymenium are known that could be useful in order to distinguish species and the specimens often appear without apothecia. The problem of identification within the genus *Cladonia* is aggravated in those groups of species where the primary thallus is dominant and seldom develops podetia, since the number of taxonomical characters can be very low. In these cases, the morphological characters used for identification are the size and morphology of squamules, the presence of incisions and the chemistry (including coloration), but all these characters are strongly affected by the habitat conditions (Stenroos *et al.* 2002). One such group is composed of the species formerly included in the subsection *Foliosae* (Mattick 1940), namely *C. foliacea*, *C. convoluta* and *C. angustiloba*. They are characterized by a well-develped primary thallus, with green-yellowish upper side and yellow lower side, containing usnic and fumarprotocetraric acids, often additional zeorin, and rarely psoromic acid. The problem of the distinction between *C. foliacea* and *C. convoluta* has previously been examined using morphological (Burgaz *et al.* 1993) and phylogenetic (Pino-Bodas *et al.* 2010) approaches. Burgaz et al. (1993) did not find a clear separation between the squamules size of *C. foliacea* and *C. convoluta*, but they still recognized them as distinct species with some hesitation (Burgaz & Ahti 2009). The phylogenetic analyses by Pino-Bodas *et al.* (2010) did not find any phylogenetic lineage associated with the traditional circumscription of *C. foliacea* and *C. convoluta*, concluding that the observed phenotypical variation most likely is an adaptive response to the different environmental conditions, such as the substrate type or the degree of aridity. Ahti & Stenroos (2013) did include *C. convoluta* in *C. foliacea*, but some authors have still kept them as separate species (Çobanoğlu & Sevgi 2012; Bendaikha & Hadjadj-Aoul 2016; Catalano *et al.* 2016; Christensen 2016). *Cladonia angustiloba* Ahti & Aptroot is a species very similar to *C. foliacea*, from which it can be separated by having very narrow squamules with deep incisions (Ahti & Aptroot 2009). It was described as endemic to Macaronesia (Ahti & Aptroot 2009), but it has subsequently been found in Faroe Islands (Denmark) (Ahti & Stenroos 2012), Scotland and Ireland (Coppins 2016, pers. comm.). However, no molecular studies have been conducted to date in order to determine whether this species is an independent lineage. Owing to the large variation shown by the species within this genus (as seen in the *C.*

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foliacea/*C. convoluta* complex) and to the large number of polyphyletic species comprising the genus *Cladonia* (e.g. Stenroos *et al.* 2002; Steinová et al, 2013; Pino-Bodas *et al.* 2015a), it was necessary to check by means of DNA sequences whether *C. angustiloba* is an independent species. We also included additional specimens of *C. convoluta* and *C. foliacea* s. str. in our analyses.

The aim of this work was to study the circumscription of *C. angustiloba* using two nuclear loci, ITS rDNA and *rpb2,* and one mitochondrial locus, *cox1,* and species delimitation methods.

Material and Methods

Taxon sampling

In total, fourteen new specimens were sequenced (Table 1), five of *C. angustiloba*, six of *C. foliacea* and three of *C. convoluta*. The new specimens selected of *C. foliacea* and *C. convoluta* come from geographical areas poorly represented in the earlier molecular study (Pino-Bodas *et al.* 2010), such as Czech Republic, Cyprus, France, Greece, Hungary and Russia. The specimens of *C. angustiloba* come from two islands of the Azores (one of them nearby the type locality) and from Scotland (two specimens). The same loci used in the previous work by Pino-Bodas *et al.* (2010), have been studied here: ITS rDNA, *rpb2* and *cox1*. *Cladonia cariosa*, *C. cervicornis* and *C. firma* were selected as outgroups according to Pino-Bodas *et al.* (2013). The specimens were studied under Olympus SZX9 dissecting microscope and Olympus CX42 microscope. The secondary metabolites of each specimen were analyzed by thin layer chromatography (TLC) according to standardized procedures (White & James 1985; Orange 2001), using the solvents A, B and C.

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DNA extraction, PCR and sequencing

Secondary metabolites were extracted by soaking the samples in acetone for two hours previous to DNA isolation. The E.Z.N.A. Forensic DNA Isolation Kit (Omega Bio-Tek) was used to extract the genomic DNA. The DNA was eluted in 100 µl of elution buffer included in the kit. PCRs were carried out with Ready-to-Go-PCR Beads (GE Healthcare Life Sciences, UK). The volume of reaction was 25 µl for each tube, with 1

μl of each primer at 10 μM concentration, 3 μl of DNA, 2.5 units of PuReTaq DNA Polymerase, a concentration of 200 mM of dNTP and 1.5 mM of $MgCl_2$. The primers used to amplify each locus and PCR programs used are described in Pino-Bodas *et al.* (2010). PCR products were cleaned with ExoProStar TM 1-step (GE Healthcare). The Sanger sequencing reactions were done at Macrogen Europe service (www.macrogen.com), with the same primers used for the PCR.

The Sequencher 4.1.4 program (Gene Codes Corporation, Inc, Ann Arbor, Michigan, USA) was used to assemble the sequences. All sequence aligments were performed using MAFFT (Katoh & Standley 2013), then the alignments were checked and improved manually in BIOEDIT 7.0 (Hall 1999). The intron at the 3' end of 18S and 9-nucleotide tandem repeat motif of *cox1* were removed from the alignments. The ambiguous regions were removed using Gblock 0.91b (Castresana 2000) with the less stringent option, keeping 88% of the original positions.

Phylogenetic analyses

Each region was analyzed by maximum likelihood (ML). The ML analyses were implemented using RAxML (Stamatakis 2006) assuming the GTRGAMMA model. The bootstrap searches were conducted with 1000 pseudoreplicates using the rapid bootstrap algorithm. Congruence among the different topologies inferred from the loci was tested following Lutzoni *et al.* (2004). We consider nodes to be in conflict if different topologies are each supported with at least 75% bootstrap. The positions of three specimens were incongruent between ITS rDNA and *rpb2* datasets, and these samples were removed from the combined dataset. The combined dataset was analyzed by ML and Bayesian inference. The ML was run using GTRGAMMA model with five partitions (*cox1*, ITS and each codon position of *rpb2*). Two different analyses were run: 1) all specimens were included (specimens with one, two or three loci), complete matrix; 2) only the specimens with at least 2 loci were included. The results of both analyses were similar and the Bayesian analysis was run only for the complete matrix. The substitution model for each locus was selected with jModeltest (Posadas 2008) using the Akaike Information Criterion (AIC). The models selected were: TPMfuf for *cox1*, TrNef+I for ITS and TIM2ef+I for *rpb2*. The Bayesian analysis was carried out using MrBayes 3.2.6 (Ronquist *et al.* 2012) using five partitions and with jModeltest selected models except for *rpb2* partitions. TIM2ef+I is not implemented in MrBayes

and it was replaced by GTR+I according to Huelsenbeck & Rannala (2004). Two simultaneous runs with 20,000,000 generations, each one starting with a random tree and employing four simultaneous chains were executed. Every 1000th tree was saved into a file. After the analysis the convergence between runs was checked using the average standard deviation of split frequencies (it was below 0.005) and by plotting likelihood values across generations using Tracer 1.5 (Rambaut & Drummond 2009). The first ten million of generations were discarded as burn-in and the 50% majority-rule consensus tree was calculated using the 'sumt' command of MrBayes.

Polymorphisms and genetic differentiation

The number of fixed nucleotide differences, number of shared polymorphic positions and the pairwise fixation indices (Fst) between the species were estimated in DnaSp v5. (Librado & Rozas 2009).

Species delimitation analyses

According to Carstens *et al*. (2013) several analytical methods must be used in order to delimit species. Three "discovery" species delimitation methods were used to infer the species in the *Cladonia foliacea* complex, General Mixed Yule Coalescent approach (GMYC) method (Pons *et al*. 2006), Poisson Tree Processes (PTP) method (Zhang *et al*. 2013) and automatic barcode gap (ABGD) method (Puillandre *et al*. 2011). GMYC is a likelihood method that uses the different branching patterns of an ultrametric gene tree to establish the transition point between inter-and intra-species branching rates. GMYC analyses were run in R using the SPLITS packet (http://r-forge.r-project.org/projects/splits/). Single threshold and multiple threshold models were tested. The single threshold model assumes an unique bound of time; before it, all the nodes indicate diversification events. Once this bound is reached, the multiple threshold model searches alternative models that iteratively split and unite the species clusters. The log-likelihood ratio test (LTR) can be used to compare the alternative model (specimens are divided into n species) with the null model (all specimens belong to a single species). The analyses were run for each dataset (ITS rDNA, *cox1*, *rpb2* and combined dataset including only the specimens with three loci). The outgroup was included in the analyses.The ultrametric trees for GMYC were generated in BEAST 1.8 (Drummond *et*

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al. 2012) under uncorrelated lognormal clock and with a coalescent model of constant population size. The analysis ran 50,000,000 generations and sampled each 1,000. The convergence was calculated with TRACER 1.5. After discarding the first 5,000,000 generations the effective sample size for all the parameters of the evolutionary model reached values > 200. The tree was summarized with TREEANNOTATOR 1.7.5 (Rambaut & Drummond 2013) using maximum clade credibility tree option for the target tree type. PTP is a similar to GMYC method but uses the number of substitutions instead of the branching rates to delimit the species. Some studies have demostrated that this method gives more robust results than GMYC (Zhang et a. 2013; Ortiz & Francke 2016). PTP analyses were run using the best ML trees from RAxML anlyses for single locus. The analyses were implemented in the webserver (http://species.h-its.org/) for 500,000 generations, thinking = 100 and 10% of burn-in. ABGD approach is based on the distribution of genetic pairwise distances to detect the "barcode gap". ABGD was used to detect barcode gap in the ITS rDNA dataset, the barcode marker for fungi (Schoch *et al*. 2012). This analysis was conducted in the webserver (http://wwwabi.snv.jussieu.fr/public/abgd/) with default parameter: Jukes-Cantor (JC69) model was used to calculate the genetic distances, P_{min} = 0.001, P_{max} = 0.1, step = 10 and Nb bins = 20.

After these analyses, Bayesian phylogenetic and phylogeography (BPP) method (Rannala & Yang 2003) and Bayes factor (Leaché *et al.* 2014) were used to validate the species delimitation hypotheses obtained from the discovery methods. BPP was run using the program BPP v. 3.3 (Yang 2015; Yang & Rannala 2014) using "A11" algorithm (Yang 2015). The analyses were run with different sets of priors for population size (θ) and the age of the root in the species tree (τ) (Table 4) and automatic fine-tune adjustments of the parameters. Each analysis ran 200,000 generations, sampled every 2 generations and the first 10% of generations were discarded as burn-in. Owing to the fact that the analysis permits grouping different species into one, but does not permit to divide the predefined species, the analyses were carried out with two different criteria in order to assign individuals to species: in the first one (BPP1-BPP5 analyses in table 4) the specimens were assigned to the traditionally accepted species (*C. foliacea*, *C. convoluta*, *C. angustiloba*). In the second, the specimens were assigned to two possible species (BPP6-BPP10 analyses in table 4), such as suggested by some of the species delimitation analyses (GMYC and PTP for *rpb2*).

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Bayes factor uses a Bayesian coalescent-based reconstruction of species trees to compare the different species delimitation models, calculating the marginal likelihood and does not require a guide tree. This method was used to compare the different species delimitation models produced by other methods: a) model with the three current species, *C. foliacea*, *C. convoluta* and *C. angustiloba*; b) model with two species morphologically cryptic (supported in several analysis of GMYC); c) model with only one species in *C. foliacea* complex (supported by PTP results of ITS rDNA and *cox1*; results of GMYC single threshold for ITS rDNA and *cox1*; and ABGD). Marginal likelihood for each competing model was estimated using path sampling (PS) and stepping-stone sampling (SS) in *BEAST. The analyses were run with the same subtitution models used in MrBayes, under a strict clock for each locus, the Yule process model and constant population size for species tree priors. The MCMC chain was run for 200,000,000 generations, sampled every 2000th. The effective sample size (ESS) was evaluated in Tracer 1.5, all the values were > 200. Bayes factor is calculated as 2 x (marginal likelihood model1 - marginal likelihood model2). Negative values of Bayes factor support the model 1 and positive values support the model 2. To explore the effect of missing data on Bayes factor results, we ran the analyses with the combined dataset of at least two loci and with the complete combined dataset, in which all the specimens had sequences of three loci.

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Results

In this study 35 new sequences have been generated (13 of ITS rDNA, 14 of *rpb2* and 8 of *cox1*). The alignments for the respective loci contained 52 sequences and 589 positions for ITS rDNA, 40 sequences and 1045 positions for *rpb2* and 39 sequences and 717 positions for *cox1*. The concatenated dataset contained 49 sequences and 2080 characters, 128 of which were parsimony-informative. The ML analysis from concatenated dataset yielded a tree with -Lnl = 4354.334 and the Bayesian analysis yielded a tree with arithmetic mean of -Lnl = 4576.602. The tree topology from both analyses was similar, the 50% majority tree from the Bayesian analysis is shown in the Fig. 1. The three species were polyphyletic. A well supported clade comprised the specimens representing the three species, *C. angustiloba*, *C. convoluta* and *C. foliacea*. This clade contained one unsupported subclade. This subclade contained specimens

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from *C. convoluta* and *C. foliacea* from different geographical origins (Fig. 1). In the ML analysis all the specimens of *C. foliacea* complex formed a well-supported clade. This clade was split into two unsupported subclades (data not shown), each one containing specimens of more than one described species.

Table 2 shows the genetic divergence between the species. No fixed nucleotide differences were found between the species. The Fst values were close to zero, indicating no genetic differentiation between the species.

The results of GMYC analyses are summarized in Table 3. The multiple threshold analyses delimit a higher number of species than the single threshold analyses, with the exception of *rpb2* (same number of species delimited in both cases). The single threshold analyses of ITS rDNA and *cox1* (the latter is not significant) concluded the existence of a unique species in the *C. foliacea* complex, and three more corresponding to the outgroup. The analyses of *rpb2* (single and multiple threshold) and the single threshold analyses of the combined matrix inferred two species for the *C. foliacea* complex, one corresponding to the subclade found in the phylogenetic analyses, another corresponding to the remaining specimens.

The results of PTP analyses are listed in Table 4. The results of ITS rDNA and *cox1* analyses were congruent, both indicating that the *C. foliacea* complex (*C. angustiloba*, *C. convoluta* and *C. foliacea*) is one single species. However, this species has low support (Table 4). The *rpb2* analysis resulted in two species, corresponding to the subclade called entity A and the remaining specimens (called entity B), with low support (Table 4).

The results of ABGD analysis of ITS rDNA dataset estimated only one group in the *C. foliacea* complex.

The results of BPP analyses are summarized in Table 5. The BPP-1 to 5 analyses identified the whole *C. foliacea* plus *C. convoluta* as a single species with high probability. This analyses also determined that *C. angustiloba* is a different species from *C. foliacea* and *C. convoluta*. However, the BPP6 to 10 analyses (with different assignation criterion) considered the entity A and B as putative species, while A+B had low support.

The Bayes factor results are provided in Table 6. The species model that considers only one species in the *C. foliacea* complex was better than the other models, and the Bayes factor supported this model. The Bayes factor values based on the complete dataset were lower than those based on the dataset with at least two loci.

Discussion

In this study, molecular data were used in order to establish whether the species traditionally included in the *Cladonia foliacea* complex are independent lineages. This complex was already addressed by Pino-Bodas *et al.* (2010), who found no evidence of *C. foliacea* and *C. convoluta* being two independent species. The present work focuses on a recently described species of this group, *C. angustiloba* (hti & Aptroot 2009). Few populations are known of this species, most of them in the Macaronesian region (Azores and Madeira). Though it is relatively easy to distinguish this species morphologically from the other two that form the *C. foliacea* complex, the characters separating them are quantitative rather than qualitative (length of the squamules, width and depth of the incisions). Since similar characters were used as diagnostic characters to separate *C. foliacea* from *C. convoluta*, one might think that *C. angustiloba* is not an independent species. The results did not support the traditional species as independent lineages. The data did not show genetic isolation between the traditional species, Fst values were close to zero in most of the comparisons, they shared polymorphisms in several loci and fixed differences between species were not found (Table 2).

Though no discovering analysis separated the species traditionally accepted, the results of the different analyses are not totally congruent regarding the number of species and the limits among them. It is frequent for different methods of species delimitation to hold different delimitation hypotheses, often determining different number of species (Satler *et al.* 2013; Singh *et al.* 2015; Wei *et al.* 2016; Garrido-Benavent *et al.* 2017). It is generally assumed that GMYC overestimates the species diversity and that its results are very influenced by the sampling (Satler *et al.* 2013; Hamilton *et al.* 2014). Our GMYC analyses generate an unlikely number of entities in most cases (Table 3), though sometimes we obtain a low number of species (1 or 2). One single species was also delimited by several analyses (GMYC, PTP and ABGD, tables 3-4); while the GMYC single threshold analyses based on the combined matrix and *rpb2* were congruent with the PTP analyses based on *rpb2*, delimiting two species, one of them coincident with the subclade detected in the phylogenetic analyses, and another one constituted by the remaining specimens. The species validation tests based on BPP analyses do not fix the problem, since the results were different depending on the assignation criterion used (Table 5). *Cladonia angustiloba* was supported in some of the BPP analyses, however

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this species was not found in any of the discovery methods. Though many authors have considered BPP as a robust method for species delimitation (Zhang *et al.* 2011; Brown *et al.* 2012; Giarla *et al.* 2014), especially in those species complexes where speciation is recent (Leavitt *et al.* 2016), the usefulness of this method for delimiting species has been questioned (Sukumaran & Knowles 2017). These authors adduce that BPP overestimates the number of species, since it is not able to distinguish between population structure and barriers among species. Owing to the incongruities with the rest of the methods used, we consider that BPP could be detecting the existence of low levels of gene flow among populations which could be expected because of the great distance between the Azores populations and those in the European continent. This would be the reason why *C. angustiloba* appears to be a different species under this method, while it is not separated by the rest of the analyses. Another issue relative to BPP is the consistency of the results depending on the priors used. In the wayrecommended by Leaché & Fujita (2010), we carried out the analyses with different priors and conclude that they affect slightly the probability of delimiting multiple species (Table 5). The delimitation results were similar, except in that we obtained small differences in the probability of every species delimited. Nevertheless, according to our results it seems that the assignation of individuals to species is what most affects the results. The best values of Bayes factor in all the analyses were obtained with the hypothesis that considers only one species in the complex (model C). The Bayes factor indicated that both the models, B (two species, entity A, entity B) and C (one species) are better than the current taxonomy. However the Bayes factor slightly favoured the model with a single species in the complex (model C). The missing data did not affect the final result, but the value of Bayes factor was much lower in the analyses based on the complete dataset analyses than in the analyses based on at least two loci dataset. This result contrasts with those of Dembo et al. (2015). They found that missing data lead to low Bayes factor values and could not differentiate between the hypotheses.

The species delimitation hypothesis that circumscribes two species is not supported by the morphology. Specimens with different size of squamules are grouped together in both putative species. The morphological studies carried out by Pino-Bodas *et al.* (2010), based on measures of length, width, thickness of squamules and conidia length, indicated that the two subclades obtained by *rpb2* phylogeny were morphologically very variable. The putative two species were not associated with

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diffent types of substrate (Fig. 1). Though the cryptic species are very common in lichens (Crespo & Pérez-Ortega 2009; Crespo & Lumbsch 2010; Molina *et al.* 2011; Parnmen *et al.* 2012; Singh *et al.* 2015), we do not consider this to be the case in the *C. foliacea* complex, as the hypothesis of a single species was the one favoured by PTP analyses based on ITS rDNA and *cox1*, GMYC single threshold model of ITS rDNA and *cox1*, ABGD method and the Bayes factor. In absence of further evidence, we considered the hypothesis that circumscribes one single species in this complex as the most probable one. This conclusion is also based on the widely known fact that a number of species within this genus are morphologically highly variable (Ahti 2000). Therefore, *C. angustiloba* would not be a species different from *C. foliacea*. The populations of the islands often suffer a morphological divergence from the continental ones (Cody 2006; Lecocq *et al.* 2013) because they are subjected to different environmental conditions. The same occurs in other Macaronesian *Cladonia* species, whereby taxonomic differences were attributed to the continental and island populations. Nevertheless it has been recently proven that in some cases no genetic differences exist between continental and island populations. Consequently, some 'endemic' Macaronesian *Cladonia* taxa have recently been synonymized with widespread, continental species, for instance *Cladonia azorica*, *C. macaronesica* and *C. rangiformis* var. *gracillima* (Pino-Bodas *et al.* 2015b, 2016). Most likely the differences in the squamule morphology that characterizes *C. angustiloba* are a mere adaptation to local conditions.

A much overlooked feature causing variation in the morphology of the *Cladonia foliacea* complex is the accumulation of calcium oxalate in the thallus. The importance of this phenomenon to lichen morphology and taxonomy was noted by Schade in several papers (e.g., Schade 1964, 1965, 1966, 1967a, 1967b). He especially studied *Cladonia subrangiformis* Sandst., which he regarded as *C. furcata* which has an altered, swollen morphology due to high accumulation of calcium oxalate in calcareous habitats (Schade 1966). In fact, our earlier molecular study of this group was congruent with Schade; *C. subrangiformis* should be united with *C. furcata* (Pino-Bodas *et al.* 2015a). Schade actually listed *C. foliacea* 'var. *convoluta'*. *C. pyxidata* 'var. *pocillum*', *C. rangiformis*, and *C. 'symphicarpia'* (= C. *symphycarpa*) among some other lichens concentrating calcium oxalate in large amounts (e.g. Schade 1967b: 10), and even *Cladonia coniocraea* (Schade 1967b: 11). Also the status of *Cladonia pocillum* has been questioned by molecular studies (Kotelko & Piercey-Normore 2010), although

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many authors still accept that taxon. The status of *Cladonia macroceras* should likewise be re-examined in light of its apparent accumulation of calcium oxalate in certain regions (cf. Fontaine *et al.* 2010). Similarly the taxonomic status of the calcicolous (calcium oxalate containing) segregates of *Vulpicida (Cetraria) juniperinus*, viz. *V. tilesii* and *V. tubulosus*, recognized by the monographer Mattsson (1993), were doubted by Schade (1966) – and recently united with *V. juniperinus* on the basis of molecular analyses (Saag *et al.* 2014).

Thus the *Cladonia foliacea* complex may be the result of an influence on morphology from the calcareous nature of the habitat whereby thalli of *C. convoluta* are longer and wider than those of acidic or slightly calcareous habitats (*'C. angustiloba'*, *'C. foliacea* s. str.'). However, even in the Nordic countries and Siberia *C. foliacea* (with small squamules) is normally calciphilous, avoiding widespread acidic habitats.

The species delimitation studies should be united with the species names and to the formal descriptions of the newly delimited taxa. Therefore *C. angustiloba* and *C. convoluta* are here officially synonymized to *C. foliacea*.

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Taxonomy and nomenclature

Below all the important names at species level of the *Cladonia foliacea* complex are summarized, with indications of types as far as they are known. Many entries include additions or corrections to other sources, such as the Index Fungorum. Many authors have used varietal or subspecific epithets for the conspicuous morphs of this group. Schade (e.g. 1965) applied expressions like 'm. (= modification) subrangiformis'. Another informal expression is 'morph convoluta', if one desires to indicate a certain, commonly recognized and obvious environmental modification. As we mentioned in Pino-Bodas *et al*. (2010), a DNA extraction was made from an isoepitype of *C. foliacea* Leighton, Lich. Brit. Exs. No. 15, H), but the amplifications failed. Specimens similar to the types and collected close to the type locality were included in the analyses (*C. foliacea*, Scohland, MACB 95602; *C. convoluta*, France, Hérault, H; *C. angustiloba*, Azores. Pico Island, Candelaria, CL798, H).

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***Cladonia foliacea* (Huds.) Willd.**, Fl. Berol. Prodr. 363. 1787.

Basionym. *Lichen foliaceus* Huds., Fl. Angl. 457. Jan-Jun 1762.

Type: Icon in Dillenius, Hist. Musc., tab. 14, fig, 12A (1742), lectotype designated by Ahti & Stenroos 2013: 91; England, Salop (Shropshire), Haughmond Hill, W. A. Leighton in Leighton, Lich. Brit. Exs. No. 15 (BM–epitype, designated by Ahti & Stenroos 2013: 91; H, UPS—isoepitypes).

= *Lichen sterilis* Gouan, Illustr. 82. 1773. Type: France, A. Gouan (PC–syntype; H-ACH 1734, 1735– possible syntypes).

= *Lichen substerilis* Gouan, Illustr. 82. 1773. Type: France (not designated, but original material seen by Dufour 1821).

= *Cladonia alcicornis (*Lightf.*)* Fr., Lich. Exs. Suec. No. 210. 1826.

Basionym. *Lichen alcicornis* Lightf., Fl. Scot. 2: 872. 20-23 Sep 1777. Type: Scotland (not designated).

= *Lichen ambiguus* Latourr., Chlor. Lugd. 35. 1785. Type: France, M. Latourrette (PC–lectotype designated here).

= *Lichen nivalis* Vill., Hist. Pl. Dauphin. 3(2): 935. 12 Sep - 22 Oct 1789, nom. illeg. (later homonym, non *Lichen nivalis* L. 1753). Type. France (not seen; cf. Vainio 1894: 386).

= *Cladonia convoluta* (Lam.) Anders, Strauch- & Blattflechten Nordböhm. 29, 1906.

Basionym. *Lichen convolutus* Lam., Encycl. 3: 500. 13 Feb 1792.

Type: France, Paris, Bois de Boulogne, J. Deslongchamps s.n. (P-LA–lectotype).

Cladonia foliacea subsp. *convoluta* (Lam.) Suza, Lich. Bohemoslovakiae Exs. No. 225. 1933.

Cladonia foliacea var. *convoluta* (Lam.) Vain. Acta Soc. Fauna Fl. Fenn. 10: 394. Dec 1894.

= *Cladonia endiviifolia* (Dicks.) Fr., Lich. Eur. Ref. 212. Jun-Jul 1831.

Basionym. *Lichen endiviifolius* Dicks., Fasc. Pl. Crypt. Brit. 3: 17. Sep 1793, '*endiviaefolius'*. Type: England(not seen).

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= *Lichen crocatus* Dicks., Hortus Siccus Brit. 4: No. 24. 1795, nom. illeg. (later homonym, non *Lichen crocatus* L. 1771). Type: England, Dickson, Hortus Siccus Brit. No. 24 (BM, not seen).

= *Cenomyce damicornis* Schleich., Cat. Pl. Helv. (ed. 2): 32. 1807, '*damaecornis*', nom. nudum.

= *Cenomyce damicornis* Ach., Lichenogr. Universalis 530. Apr-Mai 1810, '*damaecornis*', nom. illeg, (superfl. for *Lichen convolutus* Lam. 1792).

= *Cladonia damicornis* Buch, Phys. Beschr. Canar. Ins. 199. 1828 (‘1825’), nom. illeg. superfl. (based on *Cenomyce damicornis* Ach., Lichenogr. Universalis 530. Apr-Mai 1810, nom. illeg. superfl. for *Lichen convolutus* Lam. 1792).
= *Lichen endiviolus* Brot., Fl. Lusit. 2: 459. 10 Jul 1805 (‘1804’). Type: Portugal (not designated).
= *Cladonia vaillantii* Dufour, Ann. Gén. Sci. Phys. 8(22): 52. Apr 1821, nom. illeg. superfl. for six species cited).
= *Cladonia cornucopiae* Spreng., Syst. Veg. 4: 272. 1827. Type: not designated.
= *Cladonia convoluta* var. *vagans* Follmann, Philippia 2: 208 1 Apr 1975 (‘1974’). Type: Spain, Teruel (‘Hispania, Aragonia’), Sierra de la Costera, near Fuentes Calientes, 1973, G. Follmann in Follmann, Lich. Exs. Casselenses, No. 145 (B–holotype; BCC, DUKE, H, LD, O, TNS, TUR, UPS–isotypes).
= *Cladonia angustiloba* Ahti & Aptroot, Biblioth. Lichenol. 99: 12, 2009. Type: Azores, Pico, São João, 30 m, on coastal lava, 7-VII-2007, A. Aptroot 67832 (H–holotype; ABL–isotype).

(Fig. 2)

Primary thallus persistent, dominant, squamulose, forming dense mats 3–10 cm in diameter with ascending or prostrate squamules; squamules well-developed, (0.5)6.6–21.6(38) X (0.3)1.3–3.7(6.3) mm (n = 133); upper side green-yellowish, smoothly corticate to maculate-verruculose; lower side pale yellow, arachnoid; margin irregularly lobate to deeply divided, with laciniate squamules; sometimes with black marginal hairs. *Squamule anatomy* (200)318–548(830) thick, cortex (37.5)54.3–99.7(166.7), algal layer (10)27.7–49.5(77.5), medulla (100) 201.5–415(680). *Podetia* infrequent, (0.3)3.3–11(20) mm tall (n = 51), (0.1)0.75–2(3) mm thick, green-yellowish, single with closed scyphi. *Surface of podetia* corticate, smooth to verrucose; *Conidiomata* on primary squamules, more rarely on the tip of the podetia, abundant, brown to black, (220)260–530(850) µm (n = 22) tall, subglobose to pyriform, constricted at base, with hyaline slime; *conidia* falciform to straight, hyaline, (5)5.7–(8.3(11) µm long (n = 182); *Hymenial discs* brown, (1)1.3–3.3(5) mm wide; *ascospores* simple, hyaline, (6)8–11.8(18) X (2)2.6–3.5(4) µm (n = 90).

Chemistry. Two chemotypes: 1) C–, K–, P+ orange to red, containing usnic acid (major), fumarprotocetraric acid (mayor) and protocetraric acid (minor). 2) C–, K–, P+

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orange to red containing usnic acid (mayor), fumarprotocetraric acid (mayor), protocetraric acid (minor), psoromic acid (mayor), conpsoromic acid (minor). The chemotype 1 is the most common. In addition, it contains other minor satellites of fumarprotocetraric acid, e.g. confumarprotocetraric acid (Cph-2) (Geyer 1985, Huovinen *et al.* 1989) and occasionally zeorin (Burgaz & Ahti 2009; Ahti & Stenroos 2013). The 'morph convoluta' seems to contain higher concentrations of usnic acid is therefore also more yellow (Geyer 1985).

Distribution. There are numerous local distribution maps of *C. foliacea* and *C. convoluta* (listed by Scholz 2007), and a world map of *C. convoluta* (Litterski & Ahti 2004). In the herbaria we have noticed that the limit of *C. foliacea* and *C. convoluta* has been variously interpreted, but there are large areas where only one morph is present. When the cited map of the 'morph convoluta' by Litterski & Ahti (2004) is supplemented with the other morphs and other new records, the range is widening to southern Scandinavia, Faroe Islands, and eastwards to Sakha Republic (Yakutia), Altay and Mongolia. In Africa the species extends to Cape Verde Islands, Mauritania, and Egypt. The species is then primarily known from southern and central Europe, northern Africa, Near East and western Siberia. Additions to the countries and provinces listed by Litterski & Ahti (2004: 211) are Albania, Altay, Cape Verde, Denmark, Faroes, Finland, Ireland, Netherlands, Latvia, Libya, Lithuania, Luxemburg, Mauritania, Mongolia, Norway, Sakha Republic, San Marino, and Syria.

Selection of specimens examined: **Albania**: Korçë, Pogradec Udënisht, SH3 Piskutat-Pogradec, shore Ohrid lake, 40º58'55"N 20º38'21"E, alt. 710 m, 22-IV-2017, *A. R. Burgaz* (MACB). — **Armenia**: Gegharkunik, Sevan, Sevanavank, 40º33'50"N 45º00'48"E, alt. 1950 m, 22-VI-2015, *A. R. Burgaz* (MACB).— **Bosnia and Herzegovina**: Federation of Bosnia and Herzegovina, Herzegovina-Neretva Canton, Bijakovići, Med-ugorje, way up to the Hill of Apparitions, 33TYH188845, 200 m, karstic limestone, 26-III-2010, *A. R. Burgaz* (MACB). — **Cape Verde**: São Antão, Lombo das Pedras, alt. 1350 m, 1987, *B. Mies* 490d (H). — **Croatia:** Dubrovnik–Neretva County, Zamaslina, Pelsejac peninsula, 33TYH231456, alt. 20 m, 31-III-2010, *A. R. Burgaz* (MACB).— **Georgia**: Shida Kartli-Kareli, Akhaltsikhe, Tsini-Giorgitsminda, 42°01′28″N 43°54′04″E, 12-VII-1968, *Tz. Inashvili* (TBI).— **Hungary**: Bács-Kiskum, Fülöpháza; 46º52'13"N, 19º25'22"E, alt. 125m; 25-V-2015; *A.R. Burgaz* (MACB). — **Kazakhstan**: Qaraghandy (Karaganda) Region, near Karkaraly (Karkaralinsk), alt. 800 m, 1963, *H. Aasamaa* (H). — **Mongolia**: East Khangay Mts.,

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Övörkhangay aimak, Mt. Ust-Uul, alt. c. 200 m, 1978 *L. G. Byazrov* 6451 (H). — **Portugal**: Madeira, Montado dos Pecegueiros, Levada by Caldeirão Verde, alt. 900 m, 25-III-1975, *A. Henssen* 22526L (H). — **Spain**: Álava, Leza, Sierra de Cantabria, pto. de Herrera, 30TWN2714, alt. 905 m, 26-VII-2006, *A. R. Burgaz* (MACB); Jaen, Otiñar, Sierra de Jaén, Quiebrajano River, 30SVG3273, alt. 570 m, 4-IV-2009, *A. R. Burgaz* (MACB). — Additional specimens studied can be found in Table 1; Table 1 of Pino-Bodas *et al.* (2010); Burgaz (2015).

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Fig. 1. Phylogeny of the *Cladonia foliacea* group based on a combined dataset (ITS rDNA, *rpb2* and *cox1*). This is a 50% majority rule consensus tree of a Bayesian analysis. Branches supported with bootstrap > 70% and posterior probability >0·95 are indicated by thick black lines. The specimens newly sequenced are indicated in bold, the rest of the sequences included in the tree come from Pino-Bodas *et al*. (2010). The

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specimens of *C. angustiloba* are marked in grey. *Cladonia cariosa*, *C. cervicornis* and *C. firma* were used as outgroups.

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Fig. 2. Morphological variation of *Cladonia foliacea*. A, C, F 'morph convoluta'; B, D, E, G 'morph foliacea'; H 'morph angustiloba'. A) Cyprus, Nicosia, Kakopetria *A. R. Burgaz* s.n. (CL852); B) France, Var, Esterel Massif, *A. R. Burgaz* s. n. (CL840); C) France, Bouches-du-Rhône, Auriol, *A. R Burgaz* s. n. (CL850); D) Hungary, Pest, Szobi, Kemence, *A. R. Burgaz* s.n. (CL853); E) Cyprus, Nicosia, Pedoulas, *A. R. Burgaz* (CL851); F) Hungary, Veszprém, Királyszentistván, *A. R. Burgaz* s. n. (CL838); G) Greece, Macedonia-Tracia, Chalkidiki, *A. R. Burgaz* s. n. (CL839); H) Portugal, The Azores Islands, Pico, Candelária, road to Pocinho, *R. Pino-Bodas* (CL798). Scale bars = 1cm.

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