



# *Cladonia subturgida* (Cladoniaceae, Lecanoromycetes), an overlooked, but common species in the Mediterranean region

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## Abstract

*Cladonia subturgida* is a Mediterranean species that has been overlooked. Apparently it was restricted to the Iberian Peninsula and Canary Islands. However, during the study of the genus *Cladonia* in the Mediterranean region, new populations from 44 localities were found in: south France, Sardinia, south Italian peninsula, Crete and continental Greece. Distribution models based on MaxEnt, GLM, GAM and MARS algorithms were used to estimate the potential distribution of *C. subturgida*. Sicily, Corsica and the north of Africa were regions with suitable climatic conditions for *C. subturgida* where it has not been reported yet. The climatic variables with greatest relative influence in the *C. subturgida* distribution were the Precipitation of Warmest Quarter and the Annual Precipitation. Additionally, the ITS rDNA region was used to study the genetic variation of this species across its distribution area. Eleven haplotypes were found, one of them widely distributed through its geographical range. AMOVA analyses indicated lack of geographical structure.

**Keywords** Cladoniaceae · Distribution modelling · Genetic diversity · Lichen forming fungi

## 1 Introduction

The Mediterranean basin is one of the world's biodiversity hotspots (Médail and Quézel 1999; Myers et al. 2000), concentrating 10% of all the known vascular plants, of which 80% are endemic (Fady-Welterlen 2005). Three factors are crucial to explain the high biodiversity of the Mediterranean basin: the complicated geology of the area, the climate, characterized by hot and dry summers, and the high impact of human activities, that have substantially altered the landscape (Thompson 2005).

There exist lichen catalogues for several Mediterranean countries (e.g. Litterski and Mayrhofer 1998; Llimona and Hladún 2001; Abbott 2009; Mayrhofer et al. 2013; Roux 2012; Nimis 2016). According to these inventories, more than 2500 lichen species grow in this region, though its diversity is

not absolutely well-known, since many areas are still poorly explored, especially in North Africa. For reasons of similarity to the Mediterranean biogeographical pattern of vascular plants (Thompson 2005), several authors have adopted this same pattern in lichens (Nimis and Poelt 1987; Nimis and Tretiach 1995; Galloway 2008). Yet the validity of this assumption has been questioned because very few endemic lichen species exist in the Mediterranean region (Barreno 1991; Nimis 1996; Nimis 2016), unlike what happens with plants.

The lichens of the genus *Cladonia* are characterized by a dimorphic thallus composed by a crustose or foliose primary thallus and a fruticose secondary thallus. In the Mediterranean region, the species of this genus mainly grow on roadside slopes, shrublands (as heaths), and pine groves (Burgaz and Ahti 2009), since most of the species are heliophilous. Though the genus *Cladonia* has been well studied in some countries of the Mediterranean basin, such as Spain, Croatia, Italy, France, Georgia (Burgaz and Ahti 2009; Nimis 2016; Burgaz and Pino-Bodas 2012; Burgaz et al. 2017; Roux 2017), the current knowledge for the whole region is still scarce, and the proof is that new records are regularly reported (Burgaz et al. 2017, 2019a, 2019b; Monia et al. 2018; Gheza et al. 2018; Kocakaya et al. 2018). To date, 90 species of this genus have been reported for the Mediterranean basin (Burgaz et al. 2020). The species of *Cladonia* present in the region show different distribution patterns; many of them have wide distributions that

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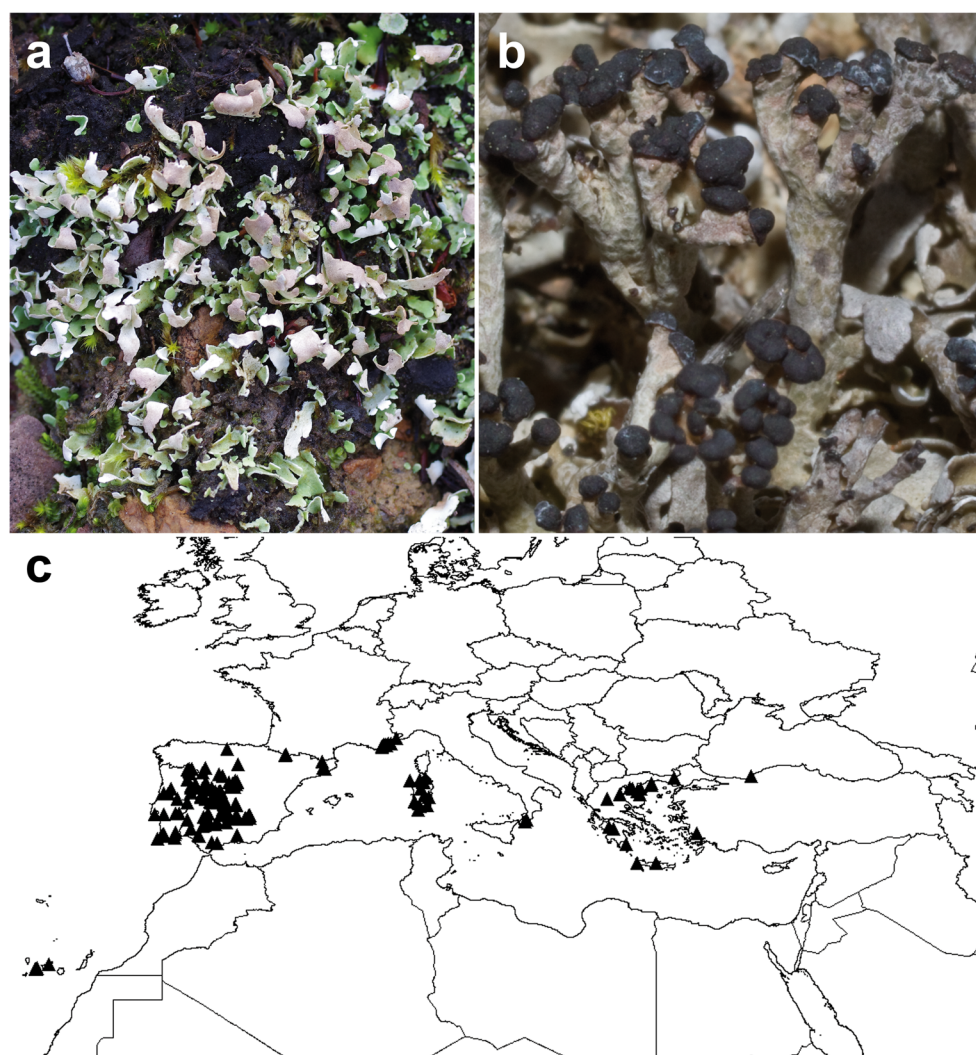
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embrace several continents (e.g., *C. furcata*, *C. humilis*, *C. pyxidata*, etc.), while others are restricted to Europe and Macaronesia (for instance *C. subcervicornis*, *C. cyathomorpha*). Some species of *Cladonia* characteristic of the Mediterranean region are, for example, *C. mediterranea*, *C. cervicornis*, *C. subturgida*, *C. foliacea*, *C. rangiformis* and *C. firma* (Litterski and Ahti 2004; Burgaz and Ahti 2009; Ahti and Stenroos 2013). Though these species do not restrict themselves to the Mediterranean region, it is there where they are most abundant (Litterski and Ahti 2004; Burgaz and Ahti 2009; Ahti and Stenroos 2013).

*Cladonia subturgida* is a species with a persistent and dominant primary thallus, often lacking a secondary thallus (Fig. 1a, b). It is distributed in the western area of the Iberian Peninsula and in the Canary Islands (Pino-Bodas et al. 2012). During our field work in different countries of the Mediterranean region (France, Italy, Greece) we found numerous specimens of *C. subturgida*. We hypothesize that

*C. subturgida* is a common species in the Mediterranean region, but poorly sampled, or mistaken for other species of *Cladonia* with dominant primary thallus, such as *C. cervicornis* and *C. firma*. In order to test this hypothesis, species distribution models have been used, based on all the known records. Species distribution models are helpful when it comes to understanding the environmental factors that determine the occurrence of species. These methods have been successfully used to predict the potential distribution of several epiphytic lichen species (Glavich et al. 2005; Bolliger et al. 2007; Pearson et al. 2018; Guttová et al. 2019), and likewise to assess the impact of the climatic change on the lichen distribution, to propose conservation plans (Allen and Lendemer 2016; Ellis et al. 2007; Wiersma and Skinner 2011; Pearson et al. 2018; Ellis 2019), and to determine zones that played the role of glacial shelters for certain species (Kukwa and Kolanowska 2016).

In this study we report new findings of *Cladonia subturgida* in several countries of the Mediterranean basin,



**Fig. 1** a Primary thallus of *Cladonia subturgida* b Podetia of *C. subturgida* c Distribution of *C. subturgida* based on specimens studied and literature references

the chemical variation of this species and its genetic diversity based on ITS rDNA region. Additionally, we model its potential distribution in order to identify the key environmental variable that shapes the ecological niche of *C. subturgida*.

## 2 Material and methods

### 2.1 Specimens studied

Specimens were collected from France, Italy, Sardinia, Greece and Crete between 2015 and 2018 ([Supplementary material](#)). The surveys were conducted on ca. 260 localities. All the new collections were deposited at MACB herbarium in Madrid and some duplicates were sent to Helsinki (H) and Trieste (TSB) herbaria. The secondary metabolites of each specimen were analysed by thin layer chromatography (TLC) according to standardized procedures (White and James 1985; Orange et al. 2001), using the solvents A, B and C.

Thirty eight newly collected specimens, in addition to the sequences from Pino-Bodas et al. (2012), were used to estimate the genetic diversity of *C. subturgida*. The specimens selected covered the whole distribution area (Fig. 1c). In total we included 18 specimens from Greece, three from Southern Italy, seven from Sardinia, nine from Spain, ten from France, one from Portugal and one from Canary Islands. The ITS rDNA region, the barcoding of fungi (Schoch et al. 2012), was selected to study the genetic diversity of *Cladonia subturgida*.

### 2.2 DNA extraction and amplification

The E.Z.N.A. Forensic DNA Isolation Kit (Omega Bio-Tek) was used to extract the genomic DNA, following the manufacturer's instructions. PCRs were carried out with Biotaq polymerase (Bioline). The volume of reaction was 25 µL, 0.3 µL of *Taq* polymerase, 2.5 µL of 10× PCR buffer, 1.4 µL of MgCl<sub>2</sub> 50 µM/µL, 1.6 µL of dNTPs (2.5 µM/µL), 1 µL of BSA (1 µM/µL), 1 µL of each primer (10 µM/µL), and 1 µL of extracted DNA. The primers used to amplify ITS rDNA region were ITS1F and ITS4 (White et al. 1990; Gardes and Bruns 1993) and PCR program was initial denaturation at 95 °C for 2 min; five cycles of 95 °C for 30 s, 58 °C for 30 s and 72 °C for 1 min and 34 cycles of 95 °C for 30 s, 56 °C for 30 s and 72 °C for 1 min; with a final extension at 72 °C for 10 min. PCR products were cleaned with ExoProStar™ 1-step (GE Healthcare). The sequencing reactions were done at Macrogen Spain service ([www.macrogen.com](http://www.macrogen.com)), with the same primers used for the PCR.

Sequencher 4.1.4 program (Gene Codes Corporation, Inc., Ann Arbor, Michigan, USA) was used to assemble the sequences. The alignment was made in MAFFT (Katoh and Standley 2013), then it was checked and improved manually in

BIOEDIT 7.0 (Hall 1999). A phylogenetic analysis based on ITS rDNA was carried out to test the monophyly of *Cladonia subturgida*. One hundred and sixty one species of *Cladonia* were included in this analysis based on the phylogenetic study of Stenroos et al. (2019). *Cladonia wainioi* was selected as outgroup. The ambiguous regions were removed using Gblock (Talavera and Castresana 2007) with the less stringent options. The alignment contained 211 sequences and 524 positions. Maximum likelihood analysis was implemented in RAxML 7.0.3 (Stamatakis et al. 2005) assuming the GTRGAMMA model. The bootstrap searches were conducted with 1000 pseudoreplicates using the rapid bootstrap algorithm.

### 2.3 Genetic diversity and haplotype analyses

The program DnaSP (Librado and Rozas 2009) was used to calculate the haplotype diversity, segregate sites and nucleotide diversity. Haplotype network under statistical parsimony were constructed in TCS 1.21 (Clement et al. 2000), considering the gaps as missing data. Mantel test was carried out to study the correlation between the ITS rDNA genetic distances and the geographical distances (using Euclidean distance) with 2000 random permutations to test the significance in VEGAN package (Oksanen et al. 2007) for R.

Analysis of molecular variance (AMOVA) was performed in Arlequin v 3.5 (Excoffier and Lischer 2000) in order to assess the proportion of the genetic variation attributed to different geographical regions (Iberian Peninsula, France, Italy and Greece). The single specimen from Canary Islands was excluded from these analyses.

### 2.4 Species distribution modeling

Species distribution modeling was used to estimate the potential distribution of *C. subturgida* under the current climatic conditions. The distribution modeling was based on 158 occurrence records whose identification has been verified by morphological studies according to Pino-Bodas et al. (2012) under dissecting microscope. The 19 bioclimatic variables at 2.5 min of spatial resolution were downloaded from the WorldClim website (<https://www.worldclim.org>; Hijmans et al. 2005). A pseudo-absences set was generated avoiding the overlap with the presences. Firstly the models were generated using all bioclimatic variables. Then, the models were estimated using only uncorrelated variables, according Kendall rank correlation coefficient, and selecting those variables which contributed more significantly in the first models. The variables selected were: Annual Mean Temperature (BIO1), Temperature Seasonality (BIO4), Mean Temperature of Driest Quarter (BIO9), Mean Temperature of Coldest Quarter (BIO11), Annual Precipitation (BIO12) and Precipitation of Warmest Quarter (BIO18).

Four modeling methods were used: Generalized additive models (GAM), generalized linear models (GLMs), Multivariate adaptive regression splines (MARS) and maximum entropy (Maxent). The models GAM, GLMs and MARS were implemented in R using the libraries GAM, DISMO and EARTH (Wood 2012; Milborrow et al. 2014; Hijmans et al. 2017). The method selected to assess the model fitting was the area under the receiver operating characteristic curve (AUC) (Fielding and Bell 1997). This value gives a measure of model discrimination accuracy, values close to 1 indicate a good discrimination. Jackknife test was run in Maxent, using 25 random points and 5 replicates to estimate the contribution of each variable to the model. This test compares the fitting of the models with and without a variable in order to assess the contribution of this variable to the distributional prediction (Phillips et al. 2006).

Then a consensus ensemble prediction from all individual models with selected variables was built.

### 3 Results

In this study 44 new records of *Cladonia subturgida* for three countries are presented. The specimens were collected in south France, Sardinia, south continental Italy, Crete and continental areas of Greece, representing the first records for all the countries. The complete distribution of *C. subturgida* is presented in Fig. 1c, based on the new data, our previous studies (Burgaz and Ahti 2009; Pino-Bodas et al. 2012) and a few literature reports (Kocakaya et al. 2018). It grows on bare soils or earth banks, preferably acidic or subneutrophilous soils of xerothermic evergreen vegetation dominated by *Cistus* shrubs, heathlands, *Pinus* or *Quercus* woodlands, in an altitude range from 25 to 1760 m.

Twelve populations were found in France at the Provence-Alpes-Côte d'Azur Region. Eleven from the Department of Var (Le Cannet des Maures, Massif des Maures and Massif de l'Esterel) and one from Alpes-Maritimes (Contes). The species was found on acidic substrate of *Quercus suber*, *Pinus pinea* and *P. pinaster* forests, 33–476 m altitude. In Italy 17 populations were found, 15 of them from Sardinia, growing on maquis shrubland with *Quercus suber* or deciduous *Quercus* and acidic substrate. Additionally, two populations were found in Calabria, on *Cistus monspeliensis* shrubs and deciduous *Quercus*, growing between 459 and 607 m altitude. Fifteen populations were found in Greece. Eight of them in Macedonia and Thrace, one in Thessaly, three in the Peloponnese, and one in West-Greece counties, growing on *Quercus coccifera* and *Pistacia lentiscus* formations, on acidic soils. In Crete island two populations were found, one of them in Heraklion and the another one in Chania, growing on *Arbutus unedo* and *Erica manipuliflora* shrubs.

The accompanying species were *Cladonia cervicornis*, *C. corsicana*, *C. firma*, *C. foliacea*, *C. humilis*, *C. pyxidata* or *C. ramulosa*.

Table 1 shows the chemical variation found in the new collections of *C. subturgida*. Eight different chemotypes were detected, the most common contains atranorin and protolichesterinic acid and the second one contains additionally zeorin. Greek populations were the most variable chemically, with seven different chemotypes. Five of them were present in Crete (Table 1). Intra-population chemical variation was detected on tree localities, all of them from Greece. In one locality in the Peloponnese the chemotypes I and III were detected, in one locality in Macedonia-Thrace the chemotypes I and IV were detected and in Chania, Crete Island, the chemotypes I, III and VII were found.

The new DNA sequences generated have been deposited in GenBank (MT510881–MT510918). The phylogenetic analysis shows that *C. subturgida* is monophyletic (Fig. 1S). A single haplotype network containing 11 haplotype without missing haplotypes was generated by TCS. One haplotype was widespread in the Mediterranean basin (Fig. 2), three haplotypes were unique to Greece, one was exclusive to south Italy, one was exclusive to France, one was restricted to Sardinia and two were exclusive to Iberian Peninsula. Three haplotypes were shared: one was shared between populations from Spain and France, other haplotype was shared between populations from Spain and Portugal and a third haplotype was shared between populations from Spain and Greece.

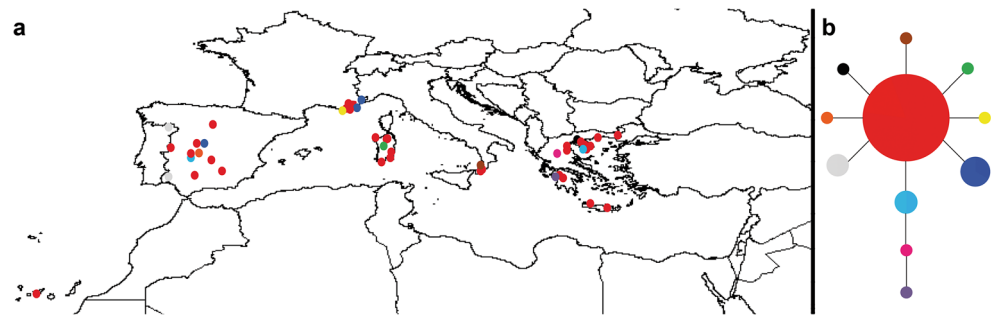
The genetic diversity of *Cladonia subturgida* is presented in Table 2. The populations from the Iberian Peninsula were the most diverse, following those from Greece. The AMOVA test did not show differentiation among the populations of different regions (Table 3). The Mantel test did not find any correlation between the genetic distance of *C. subturgida* and the geographical distance ( $r = 0.04239$ ,  $P$  value = 0.24338).

**Table 1** Chemical variation of *C. subturgida* found in the specimen newly collected

| Chemotypes          | France | Greece (Crete) | Italy | Total |
|---------------------|--------|----------------|-------|-------|
| ATR, PLIC           | 3      | 9 (1)          | 16    | 28    |
| ATR, PLIC, ZEO      | 6      | 2              |       | 8     |
| ATR, PLIC, FUM      |        | 4 (1)          |       | 4     |
| ATR, PLIC, FUM, ZEO | 2      | 1              |       | 3     |
| ATR, FUM            | 1      |                |       | 1     |
| ATR                 |        | 1 (1)          |       | 1     |
| FUM                 |        | 1 (1)          |       | 1     |
| FUM, PLIC           |        | 1 (1)          |       | 1     |

ATR Atranorin, PLIC Protolichesterinic acid, FUM Fumarprotocetraric acid complex, ZEO Zeorin

**Fig. 2** **a** Geographical distribution of the haplotypes of *Cladonia subturgida* **b** Haplotype network inferred by TCS based on ITS rDNA region. Each circle represents a haplotype, the circle size is proportional to haplotype frequency



### 3.1 Species distribution modelling

In total, 9 models were generated, 2 for each of the methods used, plus the consensus model of all models with selected variables. The AUC values obtained for each model with selected variables are shown in Table 4. All the models showed high AUC values (> 0.90), meaning a fine fitting. The overall agreement among models built using different methods was high (see supplementary material). Table 5 shows the relative contribution of every climatic variable. Annual Precipitation (37.3%) and Precipitation of Warmest Quarter (25.6%) were the variables with highest contribution to the models.

Figure 3 shows the consensus model, showing the areas with the highest suitability for *C. subturgida*. The predicted suitable areas for *C. subturgida* were the Iberian Peninsula, southern France, Corsica, Sardinia, Sicily, Italian Mediterranean area, Greece, western Turkey, north of Africa and a few spots on the southernmost parts of England.

## 4 Discussion

### 4.1 New records and distribution of *Cladonia subturgida*

This species was described for Portugal (Sampaio 1918) and for a long time known only from the type locality (Burgaz and Ahti 1998, 2009). Morphological similarities with *C. iberica* were noted (Burgaz and Ahti 1998, 2009)

**Table 2** Genetic diversity of *Cladonia subturgida* across its distribution range

|                     | N  | h  | H       | $\pi$   | S  |
|---------------------|----|----|---------|---------|----|
| Iberian populations | 12 | 5  | 0.66667 | 0.00143 | 4  |
| French populations  | 9  | 3  | 0.55556 | 0.00109 | 2  |
| Italian populations | 10 | 3  | 0.37778 | 0.00071 | 2  |
| Greek populations   | 17 | 5  | 0.42647 | 0.00137 | 4  |
| Total               | 49 | 11 | 0.54965 | 0.00146 | 10 |

N number of specimens, h number of haplotypes, H haplotype diversity, S number of polymorphic sites

and the phylogenetic studies confirmed that both taxa, *C. iberica* and *C. subturgida*, represented a single species phenotypically very variable, distributed in the Iberian Peninsula and Canary Island (Burgaz and Ahti 2009; Pino-Bodas et al. 2012). Recently, it has been reported for Turkey (Kocakaya et al. 2018) and the authors consider that this species should be common in the Mediterranean region, though reported only as scattered. Our results confirm that *C. subturgida* is widely distributed in the Mediterranean basin. The reasons why this species has been scarcely cited could be the following: 1) In general, only the primary thallus is developed; 2) It has been mistaken for other species; 3) Insufficient sampling in the territory. With the exception of some few species, the identifications of *Cladonia* based on the characters associated with the primary thallus are difficult (Ahti 2000). Although the colour and the morphology of the squamules of *C. subturgida* are very characteristic, the species can be difficult to identify for the non-specialists in the genus since it is morphologically very variable (Pino-Bodas et al. 2012). It is characterized by a dominant primary thallus with large and fragile, (6–25 mm long  $\times$  1.5–4 mm wide) undivided and lacinate or deeply lobate (Fig. 1). The upper surface is green glaucous to green olivaceous; lower surface white, purplish toward the margin. Podetia are rare, branched near the tips with open axils and corticate (Pino-Bodas et al. 2012; Burgaz et al. 2020).

In addition, *C. subturgida* is also chemically very variable. Pino-Bodas et al. (2012) reported six different chemotypes, five of which are also present in the newly collected material. In accordance with previous studies the commonest chemotype is the one containing atranorin and protolichesterinic acid. The latter substance is absent from most of the species morphologically closely related. But it is an aliphatic acid that can only be detected by TLC or HPLC methods and in many cases these techniques are not routinely used to identify *Cladonia* specimens (Haughland et al. 2018). The species morphologically close for which *C. subturgida* could have been mistaken are *C. firma* and *C. cervicornis*, both common in the Mediterranean region and with a dominant primary thallus (Burgaz and Ahti 2009; Pino-Bodas et al. 2012). Although both species have podetia with scyphi and

**Table 3** Analyses of molecular variance (AMOVA) among populations from different geographical areas (Iberian Peninsula, France, Italy and Greece)

|                    | d.f. | S.S    | Variance | % variation | Fst     | P value |
|--------------------|------|--------|----------|-------------|---------|---------|
| Among populations  | 3    | 1.043  | 0.00611  | 2.17915     | 0.02179 | 0.13196 |
| Within populations | 45   | 12.345 | 0.27433  | 97.82085    |         |         |
| Total              |      | 13.388 | 0.28044  |             |         |         |

*C. suburgida* never has scyphi, thalli without podetia are highly frequent (Burgaz and Ahti 2009).

Our result indicates that more lichen sampling in the Mediterranean region is needed, even in the countries where the lichens have been best studied, like Italy and France. Though terricolous lichens in the Mediterranean region have been studied (for instance Klement 1969; Alonso and Egea 1994, 1995; Martínez et al. 2006; Gheza et al. 2016; Cogoni et al. 2011), in general they are less well known than epiphytic ones (Nimis and Martellos 2004; Nimis 2016).

According to the potential distribution models, the regions with climatic conditions suitable for the growth of *C. suburgida*, but in which it has not yet been reported, are Sicily, Corsica, north of Africa (including the northern regions of Morocco, Algeria and Tunisia), certain enclaves in Cyprus, the south of England, the east of Ireland, the north of Scotland. Numerous localities were sampled in Sicily and Cyprus during the study of the family Cladoniaceae in the Mediterranean region (Burgaz et al. 2020), but *C. suburgida* was not found. Nevertheless we consider it plausible that some populations of *C. suburgida* exist in the northeast of Sicily (Monti Peloritani, Messina province) where the potential vegetation corresponds to oakwoods of *Quercus suber*. In Cyprus it is also likely for the species to be present in some spots of acid substrate. Corsica presents a large extension of acid substrates (Reille et al. 1997) and is another region where the species probably grows and should be looked for. In Italy *C. suburgida* has only been found in Calabria region to date, but the models point out for this country a wider distribution, broadly coincident with the humid, submediterranean, Tyrrhenian zone (Incerti and Nimis 2006). In Calabria, *C. suburgida* probably restricts itself to a narrow coastal strip, the true location of the Mediterranean vegetation (Nimis 2016). But more populations of this species are to

be expected in the Tuscany and in parts of the Puglia that share the same vegetation type. We are informed of a population of *C. suburgida* extant in the northwest of Algeria (Boudial et al. unpublished). There probably are still more populations in Algeria and Tunisia, very scarcely sampled regions whose lichen flora is poorly known (Seaward 1996; Amrani et al. 2018; Monia et al. 2018). Even in the south of England some populations of *C. suburgida* can be expected in habitats where *C. firma* and *C. cervicornis* have been reported.

It is helpful to keep in mind that the distribution models generated here only included climatic variables, but the soil conditions, key in the distribution of this species, were not included. *Cladonia suburgida* is restricted to acid pH substrates (Burgaz and Ahti 2009), and many of the areas potentially suitable for the species from a climatic standpoint present a basic pH (gypsisols or calcisols), therefore it is probable that this model overpredicts *C. suburgida* distribution. For example, a large part of the north of Morocco, a great part of Sicily, some of the selected areas of Cyprus, and some locations of southern England present calcareous substrates (Jones et al. 2010, 2013; Cohen et al. 2012; Soilscape, <https://www.landis.org.uk/soilscape>).

According to our results, the distribution of *C. suburgida* would be similar to that of *C. firma*, that grows in the southwest of Europe, the north of Africa, the Macaronesia, the west of Asia, occasionally the English Channel islands and southern Britain (Burgaz and Ahti 2009; James 2009; Nimis 2016). In general, both species live together in bare soils, roadside slopes and shrubland clearings in the Iberian Peninsula (Burgaz and Ahti 2009).

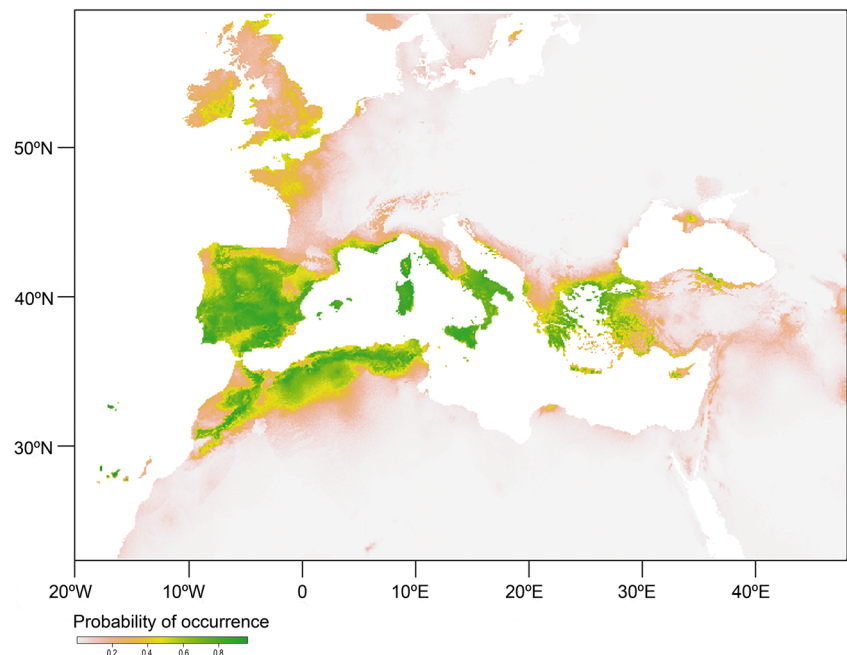
**Table 4** AUC values for the distribution models with selection of variables estimated

| Method | AUC value |
|--------|-----------|
| MAXENT | 0.989     |
| GAM    | 0.970     |
| GLMs   | 0.948     |
| MARS   | 0.960     |

**Table 5** Relative contribution of each bioclimatic variable to the Maxent model, calculated with jackknife test

| Bioclimatic variables               | Relative importance (%) |
|-------------------------------------|-------------------------|
| Precipitation of Warmest Quarter    | 25.6                    |
| Annual Precipitation                | 37.3                    |
| Temperature Seasonality             | 19.3                    |
| Mean Temperature of Driest Quarter  | 1.8                     |
| Annual Mean Temperature             | 9.9                     |
| Mean Temperature of Coldest Quarter | 6                       |

**Fig. 3** Consensus distribution model for *Cladonia subturgida* in Europe based on Maxent, GAM, GLM and MARS methods with selection of variables



The most relevant climatic variables in the distribution model for *C. subturgida* are those related to precipitation. Litterski and Ahti (2004) had already pointed out that humidity is the most important limitant climatic factor in the distribution of *Cladonia* species, in fact more than temperature. Species distribution models have proved that the variables related to precipitation are the key ones to predict the distribution of other Mediterranean species such as *Solenospora candidans*, *S. grisea* and *S. olivacea* subsp. *olbiensis* (Guttová et al. 2019).

#### 4.2 Chemical and genetic variation of *Cladonia subturgida* across its distribution

As previous studies have proved (Burgaz and Ahti 2009; Pino-Bodas et al. 2012), *Cladonia subturgida* is a chemically highly variable species. The study of the new specimens gathers together all the six chemotypes found by Pino-Bodas et al. (2012). In accordance with previous findings, the commonest chemotype is the one that contains atranorin and protolichesterinic acid, occasionally accompanied by zeorin. The chemical variation is not homogeneous across the geographical distribution of the species, the Greek populations being the most variable. Specifically five chemotypes have been found in Crete (Table 1).

Genetically *C. subturgida* is not a very variable species and has a weak geographical structure; there is gene flow among the different regions of the Mediterranean basin. This species reproduces both sexually and asexually but, due to the low frequency of apothecia, it is assumed that asexual reproduction, by means of the dispersion of thallus fragments, is dominant. Therefore, the low genetic variation found was

expected, since selection usually affects more directly the genetic variation in asexual species, making most of the loci to be effectively linked (Domaschke et al. 2012). However, a pronounced population structure would be expected due to a lower dispersal capacity of vegetative propagules against spores (Werth 2010; Seymour et al. 2005). But similar results were found in other lichens with dominant asexual reproduction (Werth and Sork 2008), which means that long-distance dispersal of the vegetative propagules is effective. In addition to wind (Muñoz et al. 2004), dispersion through seas (Bailey 1968; Jahns et al. 1976; Søchting and Castello 2012) and birds (Bailey and James 1979; Armstrong 1987; Wedin 1995) have been proposed as dispersal mechanisms in lichens.

Though it is not easy to establish comparisons with other studies (based on different markers and different geographical scales) it is necessary to note that the lack of a geographical structure is a recurrent pattern found in several species of the genus *Cladonia* (Myllys et al. 2003; Yahr et al. 2006; Park et al. 2012; Pino-Bodas et al. 2017). Which means that, in general, *Cladonia* species have a great dispersal ability and the success of the settlements will be determined by ecological factors.

In some studies about population genetics in the Mediterranean region, some patterns similar to those of *Cladonia subturgida* have been found, i.e. with scarce genetic variation and populations geographically little structured, for example in *Buellia zoharyi* (Chiva et al. 2019) and *Parmelina carporhizans* (Alors et al. 2017). The lack of geographical structure has been attributed to the high dispersal capacity of the species, to the absence of geographical barriers (Alors et al. 2017) and to the fact that the habitats were not affected by glaciations.

## 5 Conclusions

A knowledge of the species distribution as well as the genetic variation pattern needs to be substantial in order to predict the impact that anthropic disturbances and climatic change will have on them and consequently take appropriate measure for conservation purposes. In many cases, however, this implies a challenge difficult to confront, especially for those species difficult to identify (Allen and McMullin 2019). Therefore, species distribution models can be of great help to identify suitable areas for the species and to efficiently plan the samplings (Hao et al. 2020). Our data, along with the potential distribution models generated in this study, indicate that *C. subturgida* is a species widely distributed in the Mediterranean region in the Thermomediterranean, Mesomediterranean and Supramediterranean belts.

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