

Study of the genus *Cladonia* (Lecanorales, Ascomycota) in the southwest of Galicia (Pontevedra, SW Spain)

Estudio del género *Cladonia* (Lecanorales, Ascomycota) al suroeste de Galicia (Pontevedra, SW España)

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Abstract

This paper presents the results of a combined analysis of 300 specimens of the genus *Cladonia* collected in the southwest of province of Pontevedra. The species were classified based on to morphological, chemical (TLC) and genetic (ITS rDNA) characters. A total of 28 species were identified, including is the first record for the Iberian Peninsula of *Cladonia portentosa*, which contains fumarprotocetraric, perlatolic and usnic acids, as well as *Cladonia homosekikaica*, new for the Galician flora and *Cladonia ciliata*, a new record for the province of Pontevedra. It is important to note that morphological characteristics, chemistry and DNA sequences are essential in the taxonomy of the genus.

Keywords: lichenized fungi; Eurosiberian region; secondary metabolites; ITS rDNA.

Resumen

En este trabajo se presentan los resultados de un análisis combinado de 300 ejemplares del género *Cladonia* recolectados en el suroeste de la provincia de Pontevedra. Las especies se clasificaron en base a caracteres morfológicos, químicos (TLC) y genéticos (ITS rDNA). Se identificaron un total de 28 especies, incluyendo el primer registro para la Península Ibérica de *Cladonia portentosa*, que contiene ácidos fumarprotocetrárico, perlatólico y úsnico, así como *Cladonia homosekikaica*, nueva para la flora gallega y *Cladonia ciliata*, un nuevo registro para la provincia de Pontevedra. Es importante señalar que las características morfológicas, químicas y las secuencias de ADN son esenciales en la taxonomía del género.

Palabras clave: hongos liquenizados; región Eurosiberiana; metabolitos secundarios; ITS rDNA.



INTRODUCTION

Cladonia P. Browne is the genus with the highest number of species in the Cladoniaceae family (STENROOS *et al.* 2002). Identifying species within this genus is significantly difficult, as morphology varies notably during different stages of development and depending on environmental conditions. A wide variety of lichen substances have been identified in representatives of this genus, many can only be recognized through thin-layer chromatography (TLC) techniques. Although the identification of metabolites can be helpful, doubts and controversies regarding taxonomic status may still arise. Genetic tools, particularly in complex genera such as *Cladonia*, have significantly advanced the field. Molecular phylogenies were first attempted in the late 20th century, with STENROOS *et al.* (2002, 2019) conducting the most extensive works on the genus to date. It is important to note that relying solely on the ITS region for taxonomic identification within this group may not always be effective. This is because species within the same clade may have very similar ITS regions, which can lead to misidentifications (STENROOS *et al.*, 2019). Therefore, it should be complemented by morphological and chemical studies or additional genetic markers should be used.

BURGAZ & AHTI (2009) conducted a comprehensive monographic study on the genus *Cladonia* in the Iberian Peninsula. This was followed by a study that included all species from the Mediterranean Region (BURGAZ *et al.*, 2020). The only previous monographic work on this genus in Galicia was carried out by VALCÁRCEL *et al.* (1991) in the municipality of A Fonsagrada, Lugo province. BURGAZ (2015) updated the collected material for the volume of the Iberian Lichen Flora, contributing 62 species of the genus *Cladonia* for Galicia and 46 for Pontevedra. According to BURGAZ (2015), the most frequent and abundant species for Galicia are *Cladonia furcata*, *Cladonia coniocraea*, *Cladonia rangiformis*, *Cladonia portentosa*, and *Cladonia pyxidata*.

Materials and Methods

The study collected 300 specimens from various sampling points between September 2020 and March 2022, covering habitat variability. The voucher material has been deposited in the MACB herbarium of the Faculty of Biological Sciences at Complutense University of Madrid. The symbol ** is used to highlight new citations for the province of Pontevedra, * for new citations in the Galician community, and # for new chemotypes for the Iberian Peninsula.

Specimens of *Cladonia* were collected from various substrates in the municipality of O Rosal (Pontevedra) for a combined study of morphological, chemical, and molecular characters.

The study area is situated in the municipality of O Rosal, south of the province of Pontevedra, near the mouth of the Miño River. It covers an area of 41.13 km² and consists of a small urban core, with mountainous areas located in two parallel strips along the coast up to 500 m above sea level. These strips delimit the valley through which the Tamuxe River flows. Geologically, the mountainous areas are composed of alkaline granites, which are also present in the valley alongside Holocene deposits. In addition, there are metavolcanic rocks and schists that create acidic substrates (RODRÍGUEZ-GARCÍA 2004). Most of the mountainous areas are covered by plantations of *Pinus pinaster*, together with others that occupy a smaller area: *Pinus radiata* and *Eucalyptus globulus*. However, remnants of natural vegetation remain notably *Quercus robur* and *Q. suber*. The area is classified as a semi-hydro-oceanic bioclimate with a temperate thermoclimate according to the Rivas-Martínez climate classification. It is situated in a transition zone between the sub-Mediterranean temperate macroclimate and the typical

temperate bioclimate, making it an area of special interest as it allows species from the two climates to coexist (GUITIÁN & RAMIL, 2007; REES *et al.*, 2023). The average annual rainfall is 1,573 mm/m², and the average annual temperature is 15.0 °C. The average temperature of the coldest month is 9.5 °C, whereas the average temperature of the warmest month is 21.1 °C.

All the sampled localities belong to the municipality of O Rosal, in the province of Pontevedra.

List of sampling localities:

1. Alto de Campo do Couto, 41°57'32" N 8°51'59" W, 504 m, *Pinus radiata* and between alkaline granite soil and mosses.
2. Alto de Os Muíños do Folón, 41°57'48" N 8°50'18" W, 270 m, on alkaline granite soil and mosses.
3. Alto de Tabagón, 41°56'00" N 8°48'27" W, 117 m, *Quercus robur* and between schist, metavolcanic rocks, and mosses.
4. Campo do Couto, 41°57'14" N 8°51'33" W, 332 m, *Pinus pinaster*, *Quercus suber*, *Salix atrocinerea* and between alkaline granite soil and mosses.
5. Road to Campo do Torroso, 41°56'32" N 8°52'08" W, 165 m, *Quercus robur* and on alkaline granite soil.
6. Mirador de Tamuxe, 41°54'58" N 8°49'39" W, 5 m, *Quercus robur* on alluvial soil.
7. Muíños do Folón, 41°57'43" N 8°50'26" W, 173 m, *Pinus pinaster*, *Quercus robur*, dead wood and on alkaline granite soil and mosses.
8. Muíños do Picón, 41°57'36" N 8°50'07" W, 211 m, *Castanea sativa*, *Salix atrocinerea* and on alkaline granite soil and mosses.
9. Niño do Corvo, 41°56'55" N 8°48'40" W, 304 m, on schist and metavolcanic soil.
10. Petroglifos do Ghorghalado, 41°55'52" N 8°47'30" W, 80 m, on alkaline granite soil among mosses.
11. Playa das Eiras, 41°55'28" N 8°46'46" W, 3 m, *Alnus glutinosa*, *Pinus pinaster* and on alluvial soil.
12. Pozas da Pesqueira, 41°56'17" N 8°49'45" W, 15 m, *Pinus pinaster* and on alluvial soil.
13. Río Tamuxe-As Aceñas, 41°55'41" N 8°49'24" W, 17 m, *Alnus glutinosa*, *Quercus suber* and on alkaline granite soil and mosses.
14. Ascent to Alto do Torroso, 41°55'27" N 8°52'15" W, 338 m, *Pinus pinaster*, *P. radiata*, *Quercus robur*, *Q. Suber* and on alkaline granite soil and mosses.
15. Valdemiñotos, 41°56'04" N 8°49'27" W, 30 m, *Pinus pinaster*, *Quercus robur* and on soil and mosses.

Standard lichenology procedures were used to conduct morphological examination of specimens, using a Nikon SMZ445 stereomicroscope. Species identification primarily followed BURGAZ *et al.* (2020), with additional reference to other monographic works (BURGAZ & AHTI, 2009; PINO-BODAS *et al.*, 2021) and general keys (CLAUZADE & ROUX, 1985; SMITH *et al.*, 2009). Lichen substances were identified by thin-layer chromatography (TLC) techniques following the standardized method of WHITE & JAMES (1985), using solvents A, B, C and D; for substances not mentioned in this work and some doubtful cases, ORANGE *et al.* (2001) and SCHUMM & ELIX (2016) were used.

For genetic analysis, several specimens of each identified species were selected based on their morphological and chemical traits, ensuring representation of those with divergences.

The NZY PLANT/FUNGI gDNA ISOLATION kit (NZYTech, Portugal) was used for fungal gDNA extraction following the manufacturer's instructions. The resulting DNA was stored at -20 °C until PCR amplification. The ITS region of nrDNA was analyzed for species genetic characterization using the primers ITS1F and ITS4 (PINO-BODAS *et al.*, 2010). The amplification cycle was as follows: initial denaturation at 94 °C for 5 min; 35 cycles of denaturation at 94 °C for 30 s, annealing at 54 °C for 30 s, and extension at 72 °C for 40 s; and a final extension at 72 °C for 7 min. Amplifications were visualized on 2% agarose gel with TBE 1X buffer and GreenSafe Premium (NZYTech, Portugal) for DNA visualization, run for 25 min at 100 V. Amplified DNA was sent to the Genomics Service of the Scientific-Technological Research Support Center (University of Vigo) for purification with ExoSap (ThermoFisher) and bidirectional sequencing using the BigDye Terminator v3.1 sequencing kit (Life Technologies) and a SeqStudio Genetic Analyzer capillary sequencer (Applied Biosystems). Sequencer files containing chromatograms were processed using the Geneious Global Alignment algorithm (Geneious, Biomatters) to generate consensus sequences. Initial identification was assigned to each sample using the Basic Local Alignment Search Tool (BLAST) nucleotide search to verify identity. BLASTn search was performed using the nucleotide collection (nr/nt). Sequences analyzed have been deposited in the GenBank genetic sequence database; the corresponding reference for each species is indicated in the database. Table 1 shows the sequences from this study alongside the species with the highest identity percentage after BLAST search, with any discrepancies noted for each species.

Table 1. Summary of the species and their corresponding codes together with the result of their BLAST search (species with the highest percentage of identity with the one in this study). Comments on some of the percentages obtained are given for each species. For this study, results below 99% in most cases are considered inconclusive.

Species	Code GenBank	Species with highest % identity	Code GenBank
<i>Cladonia caespiticia</i>	OP479892	<i>C. caespiticia</i> (99,54%)	OK332962
<i>C. cervicornis</i>	OP479903	<i>C. cervicornis</i> (100%)	MK179618
<i>C. coccifera</i>	OP479895	<i>C. coccifera</i> (100%)	OM914269
<i>C. coniocraea</i>	OP479897	<i>C. coniocraea</i> (100%)	MN387221
<i>C. cryptochlorophaea</i>	OP479898	<i>C. cryptochlorophaea</i> (99,67%)	MK179507
<i>C. digitata</i>	OP479899	<i>C. digitata</i> (100%)	AF453701
<i>C. diversa</i>	OP479900	<i>C. diversa</i> (99,66%)	FR799158
<i>C. floerkeana</i>	OP479901	<i>C. floerkeana</i> (98,46%)	OQ717815
<i>C. furcata</i>	OP479904	<i>C. furcata</i> (99,75%)	OL625383
<i>C. glauca</i>	OP479905	<i>C. glauca</i> (99,76%)	OQ717816
	OP479906	<i>C. glauca</i> (98,76%)	OQ717816
<i>C. humilis</i>	OP479907	<i>C. humilis</i> (99,76%)	OL625557
	OP479908	<i>C. humilis</i> (100%)	OL625444
<i>C. mediterranea</i>	OP479910	<i>C. mediterranea</i> (100%)	KP941521
<i>C. merochlorophaea</i>	OP479911	<i>C. merochlorophaea</i> (99,96%)	OQ717818

Species	Code GenBank	Species with highest % identity	Code GenBank
<i>C. portentosa</i>	OP479913	<i>C. portentosa</i> (99,83%)	FR799168
	OP479914	<i>C. portentosa</i> (100%)	KP941519
	OP479922	<i>C. portentosa</i> (100%)	FR799167
	OP479923	<i>C. portentosa</i> (99,83%)	KP941499
	OP479924	<i>C. portentosa</i> (100%)	FR941519
<i>C. pyxidata</i>	OP479915	<i>C. pyxidata</i> (99,75%)	OL625502
	OP479925	<i>C. pyxidata</i> (100%)	OL625502
<i>C. ramulosa</i>	OP479916	<i>C. ramulosa</i> (100%)	OK332964
	OP479927	<i>C. ramulosa</i> (99,67%)	OK332964
<i>C. rangiformis</i>	OP479917	<i>C. rangiformis</i> (99,87%)	OL625552
<i>C. subcervicornis</i>	OP479918	<i>C. subcervicornis</i> (99,68%)	MK812661
<i>C. subulata</i>	OP479919	<i>C. subulata</i> 4 (99,65%)	FN86857
<i>C. uncialis</i> subsp. <i>biuncialis</i>	OP479921	<i>C. uncialis</i> subsp. <i>biuncialis</i> (99,47%)	KR019377

Results and Discussion

Of the 300 collected specimens, a total of 28 species of *Cladonia* were identified. The numbers of the locations correspond to the sampling localities list followed by the herbarium number where the specimens are deposited.

Despite significant human intervention, the study area displays a rich diversity of lichens (FERNÁNDEZ-COSTAS & GARCÍA-MOLARES, 2023). The surveyed area includes 28 species of the 62 *Cladonia* species cited for Galicia (BURGAZ *et al.*, 2020). Notably, the chemotype of *Cladonia portentosa*, containing fumarprotocetraric, perlatolic and usnic acids, is recorded for the first time in the Iberian Peninsula. Additionally, *C. homosekikaica* is a new addition to the Galician flora, and *Cladonia ciliata* is a new record for the Pontevedra province. The locality with the highest diversity is 'Petroglifos do Ghorghalado' (sample point 10), which is linked to areas with minimal human interference and a high level of moisture.

List of species.

Cladonia caespiticia (Pers.) Flörke

Our specimens correspond to the chemotype: Pd+ (red), K-, C-, KC-, UV-, containing fumarprotocetraric acid. The specimens were found growing on soil among mosses. It is found in temperate and warm regions of the Northern Hemisphere (GBIF, 2022). The species is widely distributed in the Eurosiberian Region, and has also been observed in other areas of the northern Mediterranean countries, such as southern France, northern Italy, Slovenia, and some isolated locations in northern Africa. In the Iberian Peninsula, it is mainly found in the northernmost areas (LLIMONA & HLADUN, 2001; BURGAZ *et al.*, 2020).

Sampling collection: 10 (MACB 117654). GenBank accession number: OP479892.

***Cladonia cervicornis* (Ach.) Flot.**

(Fig. 1c) The specimens are: Pd+ (red), K-, C-, KC-, UV-, containing fumarprotocetraric acid. Only two specimens were found on mossy rocks and soil. The species is not very abundant in the study area. It has a cosmopolitan distribution, although it is mainly concentrated in the Northern Hemisphere (GBIF, 2022). In Europe, it is widely distributed, with a higher number of records in western and southern Mediterranean territories. In the Iberian Peninsula, it is also widely spread (LLIMONA & HLADUN, 2001; BURGAZ *et al.*, 2020).

Sampling collections: 7 (MACB 117681, 117682). GenBank accession number: OP479903 for MACB 117681.

***Cladonia chlorophaea* (Flörke ex Sommerf.) Spreng.**

The species is easily confused with other closely related members, such as *C. cryptochlorophaea* Asah., *C. merochlorophaea* Asah., *C. grayi* G. Merr. ex Sandst., *C. novochlorophaea* (Sipman) Brodo & Ahti, and *C. homosekikaica* Nuno, due to many morphological similarities. Our specimens match the chemotype: Pd+ (red), K-, C-, KC-, UV-, containing fumarprotocetraric acid. Two specimens were found, on *Alnus glutinosa* and on *Pinus radiata*. This species has a subcosmopolitan distribution (GBIF, 2022). It is widely distributed throughout the Mediterranean Region (BURGAZ *et al.*, 2020), with numerous records known from the Iberian Peninsula. However, its presence may be overestimated due to its similarity to other species mentioned above.

Sampling collections: 1 (MACB 117787), 13 (MACB 117788).

**** *Cladonia ciliata* Stirt.**

Our specimens are compatible with the f. *flavicans*. The specimens belong to the chemotype: Pd+ (red), K-, C-, KC-, UV-, containing usnic and fumarprotocetraric acids. Several specimens were found directly on mossy soil. The species is distributed throughout the Northern Hemisphere. In Europe, it can be found at practically all latitudes (GBIF, 2022), f. *flavicans* is more abundant in the Mediterranean Region, where it occupies moister and warmer habitats than f. *ciliata* (BURGAZ *et al.*, 2020). In the Iberian Peninsula, most records are concentrated in the northern and western regions (LLIMONA & HLADUN, 2001; BURGAZ *et al.*, 2020). This is the first record in Pontevedra province, although it has been cited in Lugo and A Coruña (BURGAZ, 2015).

Sampling collections: 3 (MACB 117656), 10 (MACB 117657).

***Cladonia coccifera* (L.) Willd.**

The specimens are: Pd-, K-, C-, KC+ (yellow), UV-, containing zeorin, usnic and rhodocladonic acids. *Cladonia diversa*, with very similar chemistry, is distinguished by its densely squamulose-covered podetia. Several specimens were found growing on mossy soil. It is a subcosmopolitan species (GBIF, 2022), common up to the subalpine level of the Eurosiberian Region, while in the Mediterranean Region generally occurs at higher altitudes (BURGAZ *et al.*, 2020). In the Iberian Peninsula, most records are concentrated in the northern third (BURGAZ & AHITI, 2009).

Sampling collection: 4 (MACB 117655). GenBank accession number: OP479895.

***Cladonia coniocraea* (Flörke) Spreng.**

(Fig. 1g) All the specimens are Pd+ (red), K-, C-, KC-, UV-, containing fumarprotocetraric acid. In our case, *ochrochlora* morphology was observed on the ground and infrequently as an epiphyte, whereas specimens matching the *coniocraea* morphology were the most abundant, occurring on bark of different trees (*Quercus robur*, *Q. suber*, *Pinus pinaster* and *P. radiata*) and occasionally directly on soil. The species has a wide distribution in the Northern Hemisphere, as reported by GBIF (2022). In Europe, it is mainly found in the Eurosiberian Region, but it is also widely distributed in various locations of the Mediterranean Region (BURGAZ *et al.*, 2020). In the Iberian Peninsula, it has been reported several times, but most of the records are concentrated in the north (LLIMONA & HLADUN, 2001; BURGAZ & AHTI, 2009).

Sampling collections: *C. coniocraea* s. str.: 1 (MACB 117661), 6 (MACB 117660), 7 (MACB 117662, 117663), 10 (MACB 117658, 117659), 15 (MACB 117664). *C. coniocraea* morphology *C. ochrochlora*: 10 (MACB 117747), 13 (MACB 117748), 15 (MACB 117749). GenBank accession number: OP479897 for MACB 117662.

***Cladonia cornuta* (L.) Hoffm.**

(Fig. 1a). Our specimen is: Pd+ (red), K-, C-, KC-, UV-, containing fumarprotocetraric acid. A single specimen was found growing on soil. The occurrence of this species in the area is remarkable as it is common at higher altitudes (BURGAZ *et al.*, 2020). The molecular taxonomy techniques used to analyse the sequences resulted in a finding that is consistent with *C. gracilis* (L.) Willd., a species that has been found to be closely related in previous studies (FONTAINE *et al.*, 2010; PINO-BODAS *et al.*, 2012). However, the presence of soredia limited to the upper half of the podetia allows for its clear differentiation from the latter species. The taxon is subcosmopolitan, and more commonly found in cold regions of the Northern Hemisphere (GBIF, 2022). It is also abundant in the Eurosiberian Region and present in some mountainous locations of the Mediterranean Region. In the Iberian Peninsula, the records are concentrated in the northwestern quadrant (LLIMONA & HLADUN, 2001; BURGAZ *et al.*, 2020). It is reported from Ourense (CESPO-PARDO, 2016) and TEIXIDOR (1869) reported this species in Pontevedra.

Sampling collection: 2 (MACB 117665).

***Cladonia cryptochlorophaea* Asahina**

Our specimens are: Pd+ (orange), K+ (ochre), C± (weak yellow, almost imperceptible), KC+ (wine red), UV+ (pale yellow), containing cryptochlorophaeic and fumarprotocetraric acids. Several specimens grow on soil and trunks of *Quercus robur*, *Pinus pinaster* and *P. radiata*. It is the most common species in the study area. The species is mainly distributed in the Eurosiberian Region, but it can also be found in the territories of the Mediterranean Region. It has been reported in various locations in the northern and western areas of the Iberian Peninsula (LLIMONA & HLADUN, 2001; BURGAZ *et al.*, 2020).

Sampling collections: 7 (MACB 117667), 10 (MACB 117666), 14 (MACB 117669, 117670, 117671, 117672), 15 (MACB 117668). GenBank accession number: OP479898 for MACB 117669.

***Cladonia cyathomorpha* Stirt. ex Walt. Watson**

(Fig. 1b). Our specimen is: Pd+ (red), K-, C-, KC-, UV-, containing fumarprotocetraric and protocetraric acids. It is a rare species in this area. It grows on soil among mosses and on *Pinus radiata*, in the highest altitude of the municipality (527 m). *C. cyathomorpha* has a restricted distribution in Western Europe, reaching as far as the Canary Islands. It is probable that it has been disregarded or mistaken for other species within its group. Conversely, it has a broad distribution across the Iberian Peninsula (BURGAZ *et al.*, 2020).

Sampling collections: 1 (MACB 117789, 117790).

***Cladonia digitata* (L.) Hoffm.**

The specimen belongs to the chemotype: Pd+ (yellow), K+ (yellow), C-, KC-, UV-, containing thamnolic and rhodocladonic acids. Several specimens were found growing directly on soil, among mosses. The species is distributed across vast territories of the Northern Hemisphere (GBIF, 2022). Its presence is considered a good indicator of forest maturity (PINO-BODAS *et al.*, 2021). In the Iberian Peninsula, records are limited to the northernmost zone (BURGAZ *et al.*, 2020).

Sampling collection: 10 (MACB 117673). GenBank accession number: OP479899.

***Cladonia diversa* Asperges ex S. Stenroos**

Our specimens are: Pd-, K-, C-, KC+ (light yellow), UV-, containing zeorin and usnic acid. It is frequent and was located at different points of the area, developed on soil and rocks. The species has a wide distribution across Europe, Asia, North America and Macaronesia, although its exact distribution is not well-known (AHTI & STENROOS, 2013). In the Iberian Peninsula, the majority of records are concentrated in the northwestern quadrant (LLIMONA & HLADUN, 2001; BURGAZ *et al.*, 2020). STEINOVÁ *et al.* (2013) have identified it as a polyphyletic taxon.

Sampling collections: 2 (MACB 117676), 8 (MACB 117672), 9 (MACB 117678), 10 (MACB 117674), 14 (MACB 117679). GenBank accession number: OP479900 for MACB 117678.

***Cladonia fimbriata* (L.) Fr.**

Our specimen is: Pd+ (reddish-orange), K-, C-, KC-, UV-, containing fumarprotocetraric acid. A single specimen was located growing on soil. The species is cosmopolitan (AHTI & STENROOS, 2013), and is abundant in the Mediterranean Region. It has been widely cited in the Iberian Peninsula (LLIMONA & HLADUN, 2001; BURGAZ *et al.*, 2020).

Sampling collection: 7 (MACB 117680).

***Cladonia floerkeana* (Fr.) Flörke**

The specimens are: Pd+ (yellow), K+ (yellow), C-, KC-, UV-, containing barbatic and thamnolic acids, which does not fit with what it is indicated by BURGAZ *et al.* (2020), although PINO-BODAS *et al.* (2021) also consider this chemotype. Several specimens were found growing on moss debris on soil and bark of *Quercus robur*. This species has a subcosmopolitan distribution (GBIF, 2022) and is commonly found in oceanic areas (AHTI & STENROOS, 2013).

In the Iberian Peninsula, it is mostly recorded in the northwestern quadrant (LLIMONA & HLADUN, 2001; BURGAZ *et al.*, 2020).

Sampling collections: 4 (MACB 117684), 7 (MACB 117683). Sequence code: OP479901 for MACB 117683.

***Cladonia furcata* (Huds.) Schrad.**

Two chemotypes were found (fumarprotocetraric ± bourgeanic acids) both of them : Pd+ (red), K-, C-, KC-, UV-, . It is abundant in the study area. It was found on soil and occasionally among mosses, as well as on the bark of *Salix atrocinerea*. This is a subcosmopolitan species (AHTI & STENROOS, 2013; GBIF, 2022) spread across all latitudes and also very common in much of the Mediterranean Region and the Iberian Peninsula (LLIMONA & HLADUN, 2001; BURGAZ *et al.*, 2020).

Sampling collections: 1 (MACB 117687), 7 (MACB 117685), 8 (MACB 117686), 9 (MACB 117687, 117689), 14 (MACB 117690). GenBank accession number: OP479904 for MACB 117689.

***Cladonia glauca* Flörke**

Our specimens belong to chemotype: Pd-, K-, C-, KC-, UV+ (white), containing squamatic acid, and chemotype: Pd+ (yellow), K+ (yellow), KC±, (yellow), UV-, containing thamnolic and traces of barbatic acids. At once squamatic and thamnolic acids was found in some specimens. It is a very abundant species in some locations of the area of study. It grows on rocks, on soil, usually surrounded by mosses, although it was also found on *Pinus radiata* bark. According to GBIF (2022), it is mainly distributed in the Northern Hemisphere, particularly in areas with an oceanic tendency (AHTI & STENROOS, 2013). Previous studies have reported its presence in various locations in the western half of the Iberian Peninsula (LLIMONA & HLADUN, 2001; BURGAZ *et al.*, 2020).

Sampling collections: 1 (MACB 117696, 117697), 2 (MACB 117693), 9 (MACB 117694, 117695), 10 (MACB 117691, 117692). GenBank accession numbers: OP479905 for MACB 117691, OP479906 for MACB 117693.

*** *Cladonia homosekikaica* Nuno**

(Fig. 1d) Small squamules at the base of the podetia are frequently present. Our specimens belong to the chemotype: Pd-, K-, C-, KC-, UV+ (bluish-white), containing homosekikaic and sekikaic acids. It was found on soil, among mosses and on *Pinus radiata* bark in the highest altitude locality of the municipality (Alto de Campo do Couto). It is an uncommon species in the Northern Hemisphere and has also been observed in some parts of Australia (GBIF, 2022). In the Iberian Peninsula, it has been found in scattered localities in the northern half (BURGAZ *et al.*, 2020). Our report represents the first record for Galicia.

Sampling collections: 1 (MACB 117791, 117792, 117793, 117794).

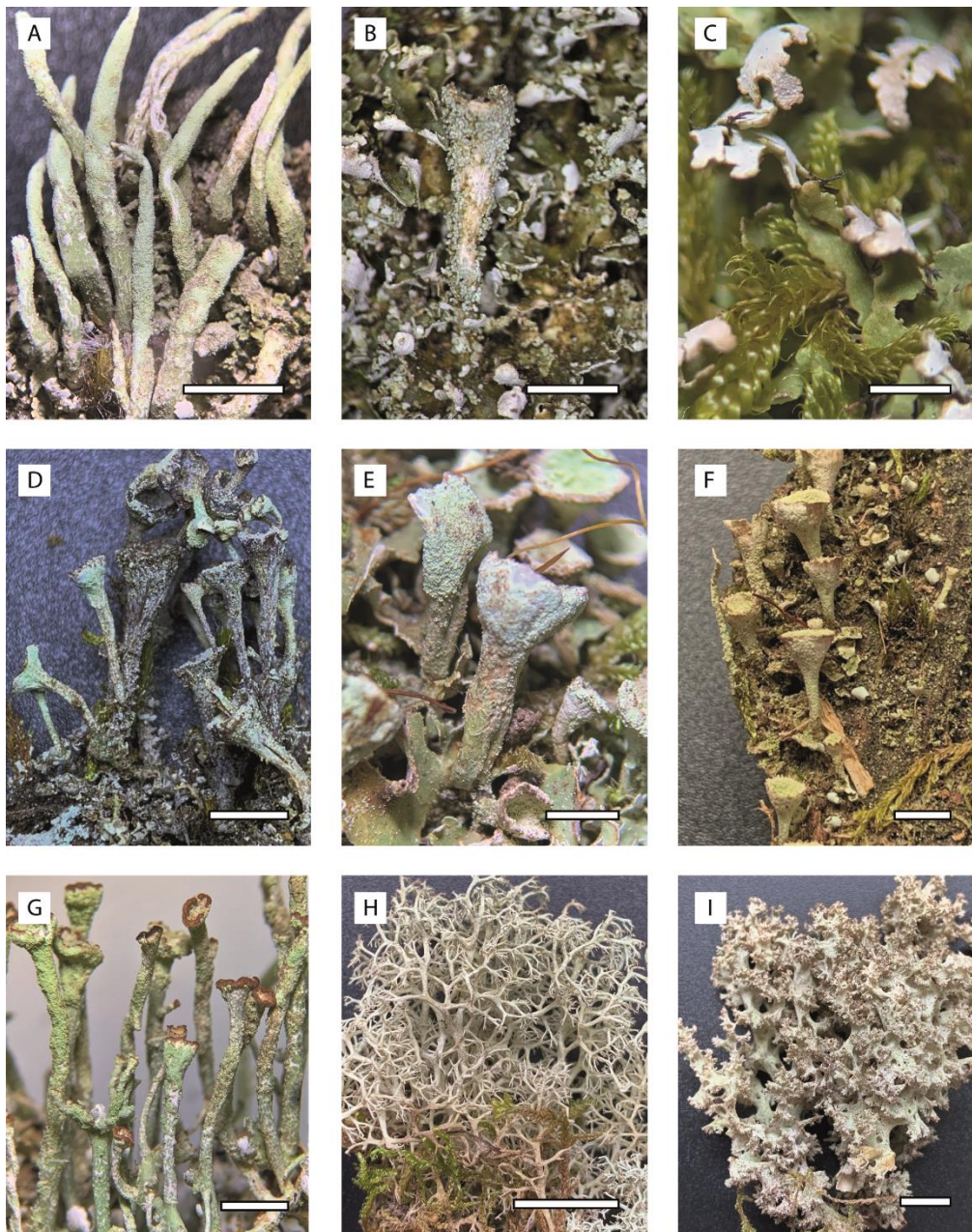
***Cladonia humilis* (With.) J.R. Laundon**

(Fig. 1e-f) The specimens belong to the chemotype: Pd+ (red), K+ (yellow), C-, KC-, UV-, with atranorin and fumarprotocetraric acid. Several specimens were found growing directly on

soil and on various substrates (*Alnus glutinosa*, *Castanea sativa*, *Pinus radiata*, *Quercus robur*, and *Salix atrocinerea*), always among mosses. *C. humilis* has a subcosmopolitan distribution, but it is more widespread in temperate zones (AHTI & STENROOS, 2013; GBIF, 2022). There are numerous records of the species from the Iberian Peninsula (LLIMONA & HLADUN, 2001; BURGAZ *et al.*, 2020).

Sampling collections: 1 (MACB 117704), 3 (MACB 117702), 4 (MACB 117784), 7 (MACB 117698), 8 (MACB 117700, 117701), 10 (MACB 117699), 12 (MACB 117781), 13 (MACB 117703). GenBank accession numbers: OP479907 for MACB 117698, OP479908 for MACB 117703.

Figure 1. A, *Cladonia cornuta*; B, *C. cyathomorpha*; C, *C. cervicornis*; D, *C. homosekikaica*; E-F, *C. humilis*; G, *C. ochrochlora*; H-I, *C. portentosa*. Scales: 5 mm.



***Cladonia macilenta* Hoffm.**

Our specimens are: Pd+ (yellow), K+ (yellow-orange), C-, KC-, UV-, containing barbatic, thamnolic and didymic acids. In some specimens, only thamnolic acid has been detected. It is a very abundant species in the area. It grows on soil surrounded by mosses, on stumps and on bark of *Pinus pinaster* and *P. radiata*. The species has a wide distribution in both hemispheres, as reported by GBIF (2022). It is found in hills and mountainous zones of the Eurosiberian Region, as well as wet areas of the Mediterranean Region (BURGAZ *et al.*, 2020). The majority of records from the Iberian Peninsula are concentrated in the north and northwestern quadrant (LLIMONA & HLADUN, 2001; BURGAZ *et al.*, 2020).

Sampling collections: 1 (MACB 117737), 10 (MACB 117732), 11 (MACB 117735), 12 (MACB 117733), 13 (MACB 117734), 14 (MACB 117736).

***Cladonia mediterranea* P.A. Duvign. & Abbayes**

Our specimen is: Pd-, K-, C-, KC+ (yellow), UV+ (light blue), containing usnic and perlatolic acids. It grows on soil and among mosses. The species is frequently found in humid and warm territories of the hills and montane levels of the Eurosiberian Region. In the Mediterranean basin, its distribution is restricted to pine forests, cork oak groves, and coastal shrub areas (BURGAZ *et al.*, 2020). Its population is currently declining due to the progressive alteration of its habitat. There are numerous records of this species from all over Portugal, the Balearic Islands, Catalonia, and some isolated localities in Galicia (LLIMONA & HLADUN, 2001; CRESPO-PARDO, 2016; BURGAZ *et al.*, 2020).

Sampling collections: 7 (MACB 117738). GenBank accession number: OP479910.

***Cladonia merochlorophaea* Asahina**

Our specimens are: Pd+ (orange-red), K-, C+ (reddish), KC+ (wine red), UV+ (pale blue), containing merochlorophaeic and fumarprotocetraric acids. It was found growing on soil and bases of *Quercus robur*, *Q. suber* and *Pinus pinaster* trunks. The taxon is subcosmopolitan, ranging from the Arctic to the temperate regions of the Northern Hemisphere, as well as scattered territories in Australasia and South America (GBIF, 2022). It has also been reported in various locations in the Iberian Peninsula and other countries in the Mediterranean basin, although its presence is likely underestimated due to identification difficulties (BURGAZ *et al.*, 2020).

Sampling collections: 1 (MACB 117744, 117745), 4 (MACB 117742), 5 (MACB 117741), 7 (MACB 117743), 10 (MACB 117739, 117740), 14 (MACB 117746). GenBank accession numbers: OP479911 for MACB 117739.

*Cladonia portentosa* (Dufour) Coëm.

(Fig. 1h-i) This species is chemically variable. We have detected three chemotypes: 1) Pd-, K-, C-, KC± (pale yellow), UV+ (light blue), containing usnic and perlatolic acids; 2) Pd-, K-, C-, KC± (pale yellow), UV+ (light blue), containing only perlatolic acid; and 3) Pd+ (red), K-, C-, KC± (pale yellow), UV+ (light blue), containing fumarprotocetraric, perlatolic and usnic acids. The first chemotype is more prevalent in the northern half of the Iberian Peninsula, while the second chemotype is limited to a few locations in the western Iberian Peninsula (Galicia,

Portugal, Salamanca), and Navarra (BURGAZ & ATHI, 2009). The third chemotype was previously considered a distinct species, *Cladonia azorica* Ahti, with a Macaronesian distribution. However, PINO-BODAS *et al.* (2016) concluded that it belongs to *C. portentosa*. It is common in the study area, growing on soil accompanied by mosses.

The species has a subcosmopolitan distribution, although it is mainly found in the Northern Hemisphere (GBIF, 2022). In Europe, it is more frequently found in the Eurosiberian Region, while in the Mediterranean Region, it is found at higher altitudes (BURGAZ *et al.*, 2020). There are numerous records of this species in northern Spain and Portugal (LLIMONA & HLADUN, 2001; BURGAZ *et al.*, 2020). The third chemotype is the first record for the Iberian Peninsula and the second for the European continent, as it has also been found in the southwest of France (BURGAZ *et al.*, 2020).

Sampling collections: 1 (MACB 11755), 10 (MACB 117750, 117751, 117752, 117753), 4 (MACB 117754), 7 (MACB 117756), 8 (MACB 117757), 9 (MACB 117760), 15 (MACB 117759). GenBank accession numbers: OP479913 for MACB 117759, OP479914 for MACB 117750, OP479922 for MACB 117751, OP479923 for MACB 117757, OP479924 for MACB 117756.

***Cladonia pyxidata* (L.) Hoffm.**

Our specimens are: Pd+ (red), K-, C-, KC-, UV-, containing fumarprotocetraric acid. The character to differentiate *C. pyxidata* from other related taxa is the presence of large, unclustered, corticated podecia, which gradually open to form greenish-grey scyphi; these are covered with microscumules and flat, peeled plates on the upper part and especially on the inside of the scyphi. Nevertheless, species is highly polymorphic and can be mistaken for both *C. chlorophaea* and some specimens of *C. humilis*. Molecular studies have revealed that it is a polyphyletic taxon (STENROOS *et al.*, 2019), indicating the need for further revision. It was collected on soil and rocks growing in association with mosses. The species has a cosmopolitan distribution, as reported by GBIF in 2022, and has frequently cited in the Iberian Peninsula, according to LLIMONA & HLADUN (2001) and BURGAZ *et al.* (2020).

Sampling collections: 1 (MACB 117780, 117779), 2 (MACB 117782), 7 (MACB 117783), 10 (MACB 117785). GenBank accession numbers: OP479915 for MACB 117782, OP479925 for MACB 117783.

***Cladonia ramulosa* (With.) J.R. Laundon**

The specimens are: Pd + (red), K-, C-, KC-, UV-, containing fumarprotocetraric acid. This species is commonly found in the area and can be found on various substrates such as soil, mosses, rocky substrates, and trunks of *Quercus suber* and *Pinus radiata*. It has a cosmopolitan distribution, ranging from boreal regions to temperate zones (AHTI & STENROOS, 2013), and is frequently found in the Mediterranean Region. There are numerous records of this species in the Iberian Peninsula (LLIMONA & HLADUN, 2001; BURGAZ *et al.*, 2020).

Sampling collections: 1 (MACB 117773), 7 (MACB 117776, 117777), 8 (MACB 117775), 13 (MACB 117774). GenBank accession numbers: OP479916 for MACB 117776, OP479927 for MACB 117775.

***Cladonia rangiformis* Hoffm.**

The specimens are: Pd⁻, K⁺ (yellow), C⁻, KC⁻, UV⁻, containing atranorin and rangiformic acid. It is one of the most abundant species in the area. It grows on soil associated with mosses. *C. rangiformis* has a wide distribution in the Northern Hemisphere, ranging from the Arctic tundra to the Mediterranean Region and Macaronesia (GBIF, 2022). There are numerous records of this species in the Iberian Peninsula (LLIMONA & HLADUN, 2001; BURGAZ *et al.*, 2020).

Sampling collections: 1 (MACB 117770), 7 (MACB 117772, 117771). GenBank accession number: OP479917 for MACB 117772.

***Cladonia squamosa* (Scop.) Hoffm.**

The specimens collected are: Pd⁻, K⁻, C⁻, KC⁻, UV⁺ (white), containing squamatic acid. Molecular phylogenetic studies suggest that it is a polyphyletic taxon (STENROOS *et al.*, 2019). It is relatively abundant in some areas, growing directly on soil and surrounded by mosses, although it has also been found on *Pinus radiata*. Its distribution is subcosmopolitan (GBIF, 2022), and it prefers areas with an oceanic or suboceanic tendency (AHTI & STENROOS, 2013). The species has been reported in numerous localities throughout the Iberian Peninsula, although it appears to be more abundant in the Eurosiberian Region (LLIMONA & HLADUN, 2001; BURGAZ *et al.*, 2020).

Sampling collections: 1 (MACB 117765, 117766, 117767), 7 (MACB 117764), 10 (MACB 117768, 117769).

***Cladonia subcervicornis* (Vain.) Kernst.**

Our specimens are: Pd⁺ (red), K⁺ (yellow), C⁻, KC⁻, UV⁻, containing fumarprotocetraric acid and atranorin. It grows on rocks usually accompanied by mosses. The species is distributed in western regions of Europe, although there is evidence of its presence in some localities near the Mediterranean coast. Most of the records are concentrated in the western third of the Iberian Peninsula, as reported by BURGAZ *et al.* (2020).

Sampling collections: 1 (MACB 117762), 2 (MACB 117763). GenBank accession number: OP479918 for MACB 117762.

***Cladonia subulata* (L.) F.H. Wigg.**

The only specimen collected is: Pd⁺ (red), K⁻, C⁻, KC⁻, UV⁻, containing fumarprotocetraric acid. It was growing on soil among mosses. *C. subulata* has a subcosmopolitan distribution (GBIF, 2022) and is prevalent in continental areas of the Mediterranean Region. In the Iberian Peninsula, records are concentrated in the western half (LLIMONA & HLADUN, 2001; BURGAZ *et al.*, 2020).

Sampling collection: 8 (MACB 117761). GenBank accession number: OP479919.

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

Our specimen is: Pd⁻, K⁻, C⁻, KC⁺ (yellow), medulla UV⁺ (white), containing usnic and squamatic acids. It is very rare in the area. Only one specimen was found growing on soil and

surrounded by mosses. The distribution area of this species covers the European continent and isolated locations in Asia and the eastern coast of North America (GBIF, 2022). In the Mediterranean Region, it is more common than subsp. *uncialis* (BURGAZ *et al.*, 2020). Records in the Iberian Peninsula are concentrated in the northwestern quadrant (LLIMONA & HLADUN, 2001; BURGAZ *et al.*, 2020).

Sampling collection: 7 (MACB 117786). GenBank accession number: OP479921.

The following key is presented to identify the *Cladonia* species in O Rosal:

- | | | | |
|-----|---|----|---------------------------------|
| 1a. | Primary thallus developed, secondary thallus absent or very poorly developed | 2 | |
| 1b. | Primary thallus under developed or absent, secondary thallus with dominant development or equal development of the two parts of the thallus | 4 | |
| 2a. | (1a) Squamules small (<1cm), fragile, with irregular edges | | <i>C. caespiticia</i> |
| 2b. | Squamules bigger, not fragile | 3 | |
| 3a. | (2b) Squamules not pruinose, brownish to greyish blue below, occasionally with some black rizines on the sides of the squamules, usually K- | | <i>C. cervicornis</i> |
| 3b. | Squamules elongate (6-10 mm), erect, base at lower side blackened, K+ yellow to red | | <i>C. subcervicornis</i> |
| 4a. | (1b) Primary thallus absent or very reduced, podetia developed, narrow, generally branched, ascyphose | 5 | |
| 4b. | Primary and secondary thallus present, podetia scyphose or not, simple or little branched | 21 | |
| 5a. | (4a) Podetia without cortex, surface arachnoid (use hand lens!), without soredia, richly branched | 6 | |
| 5b. | Podetia with cortex, smooth, sorediate or squamulose, simple, little branched or richly branched | 9 | |
| 6a. | (5a) Podetia Pd+ red and K- | 7 | |
| 6b. | Podetia Pd- | 8 | |
| 7a. | (6a) Podetia greyish, with apical ramifications dichotomous, deflexed in several directions | | <i>C. ciliata</i> |
| 7b. | Podetia with ultimate branchlets tri-, tetra- or pentachotomous, generally erect or very little deflexed | | <i>C. portentosa</i> |
| 8a. | (6b) Podetial surface with continuous algal layer, thallus forming characteristic subglobose heads, podetia with ultimate branchlets uniformly dichotomous | | <i>C. mediterranea</i> |
| 8b. | Podetial surface without continuous algal layer, thallus not forming characteristic subglobose heads, podetia with ultimate branchlets tri-, tetra- or pentachotomous, somewhat curved and podetia with curly appearance in general | | <i>C. portentosa</i> |

9a.	(5b) Podetia yellowish	<i>C. uncalis</i> subsp. <i>biuncialis</i>	
9b.	Podetia not yellowish		10
10a.	(9b) Podetia corticate over the entire surface, with few squamules, not sorediate		11
10b.	Podetia corticate at the base or in the lower half, with numerous squamules or with soredia		13
11a.	(10a) Podetia Pd ⁻ , with algal layer discontinuous, richly branched, sometimes with squamules, tips closed, UV ⁻	<i>C. rangiformis</i>	
11b.	Podetia Pd ⁺ red or Pd ⁺ yellow		12
12a.	(11b) Podetia K ⁻ , with uniform colour, with continuous algal layer	<i>C. furcata</i>	
12b.	Podetia K ⁺ yellow, usually appearing pale or variegated in part, areolate, with discontinuous algal layer	<i>C. rangiformis</i>	
13a.	(10b) Podetia squamulose, with a variable number of squamules, but distributed throughout the podetia		14
13b.	Podetia with squamules only in the lower half, or without squamules or sorediate		16
14a.	(13a) Podetia Pd ⁻ , UV ⁺ white	<i>C. squamosa</i>	
14b.	Podetia Pd ⁺ red or Pd ⁺ yellow, UV ⁻		15
15a.	(14b) Pd ⁺ yellow	<i>C. squamosa</i>	
15b.	Pd ⁺ red, podetial squamules > 1 mm long, podetial tips always dichotomously branched, axils generally open	<i>C. furcata</i>	
16a.	(13b) Podetia Pd ⁺ red, Pd ⁺ yellow or yellow later red		17
16b.	Podetia Pd ⁻		21
17a.	(16a) Podetia Pd ⁺ yellow, with red apothecia		20
17b.	Podetia Pd ⁻		18
18a.	(17b) Podetia simple or dichotomously branched, or forming scyphi 1-3 mm wide, frequently with long marginal subulate proliferation	<i>C. subulata</i>	
18b.	Podetia unbranched and subulate, sometimes with narrow scyphi (< 1mm), without proliferations		19

19a.	(18b) Podetia not corticate or cortex restricted to podetial base, never in scyphi	<i>C. coniocraea</i>
19b.	Podetia clearly corticate at the base and cortical plates often extending throughout, also to the scyphus	<i>C. coniocraea</i> morph <i>ochrochlora</i>
20a.	(17a) Podetia finely soresdiate, with whitish appearance	<i>C. macilenta</i>
20b.	Podetia not finely soresdiate, usually granular, without whitish appearance	<i>C. floerkeana</i>
21a.	(16b) Podetia without red apothecia, with a lateral groove that looks like a depression, surface soresdiate	<i>C. glauca</i>
21b.	Podetia with red apothecia	22
22a.	(21b) Podetia finely soresdiate, whitish	<i>C. macilenta</i>
22b.	Podetia not finely soresdiate, granulose, greyish brown	<i>C. floerkeana</i>
23a.	(4b) Podetia not widened in scyphi, often very narrow	24
23b.	Podetia widened in scyphi	32
24a.	(23a) Podetia squamulose	<i>C. squamosa</i>
24b.	Podetia not squamulose, soresdiate or granulose	25
25a.	(24b) Apothecia red	26
25b.	Apothecia of different colour or without apothecia	28
26a.	(25a) Primary thallus with small squamules (1-2 mm long)	27
26b.	Primary thallus with large squamules (3-12 mm long), squamules rounded, with shallow lobulations, margin finely soresdiate	<i>C. digitata</i>
27a.	(26a) Podetia finely soresdiate	<i>C. macilenta</i>
27b.	Podetia not finely soresdiate, granulose	<i>C. floerkeana</i>
28a.	(25b) Podetia soresdiate	29
28b.	Podetia corticate, not soresdiate	<i>C. ramulosa</i>
29a.	(28a) Podetia only soresdiate in the upper half	<i>C. cornuta</i>
29b.	Podetia soresdiate almost throughout	30

30a.	(29b) Podetia unbranched, tips attenuate	<i>C. coniocraea</i>	
30b.	Podetia somewhat branched, tips slightly widened or not		31
31a.	(30b) Podetia grey, slightly branched, frequently scyphose, completely ecorticate, sorediate	<i>C. subulata</i>	
31b.	Podetia green to grey, unbranched, occasionally narrowly scyphose, corticate at base and often in patches higher up	<i>C. coniocraea</i> morph <i>ochrochlora</i>	
32a.	(23b) Podetia corticate throughout the surface, not sorediate		33
32b.	Podetia sorediate or squamulose, sometimes corticate in the lower half		37
33a.	(32a) Podetia Pd-	<i>C. merochlorophaea</i>	
33b.	Podetia Pd+ red		34
34a.	(33b) Podetia without proliferation		35
34b.	Podetia with central or lateral proliferations		36
35a.	(34a) Podetia K+ yellow, primary thallus with white underside and base blackening	<i>C. subcervicornis</i>	
35b.	Podetia K- and C+ weakly red	<i>C. merochlorophaea</i>	
36a.	(34b) Scyphi with central proliferations with one to several podetia proliferating centrally from scyphi, with 1-4 tiers of scyphi, podetia short (7-19 mm)	<i>C. cervicornis</i>	
36b.	Scyphi with lateral proliferations, podetial base not much darkened, surface not arachnoid	<i>C. ramulosa</i>	
37a.	(32b) Apothecia red		38
37b.	Apothecia of different colour, or without apothecia		40
38a.	(37a) Secondary thallus more developed than primary thallus		39
38b.	Primary thallus more visible than secondary thallus or equal	<i>C. digitata</i>	
39a.	(38a) Podetia corticate, sometimes areolate, with numerous vegetative propagules	<i>C. coccifera</i>	
39b.	Podetia somewhat branched, tips slightly widened or not	<i>C. diversa</i>	
40a.	(37b) Podetia Pd-		41
40b.	Podetia Pd+ red or Pd+ yellow		42

41a.	(40a) Podetia not corticate, sorediate or covered with squamules, without real scyphi and axils perforated	<i>C. squamosa</i>
41b.	Podetia corticate, if sorediate only at the upper part, without verruculose cortex, sorediate at the upper part	<i>C. homosekikaica</i>
42a.	(29b) Podetia not sorediate, covered by numerous vegetative propagules like plates	43
42b.	Podetia sorediate, without vegetative propagules like plates	45
43a.	(42a) Squamules of the primary thallus large, veiny below, or even partially corticate, sometimes margin sorediate	<i>C. cyathomorpha</i>
43b.	Squamules of the primary thallus never partially corticate	44
44a.	(43b) Podetia sorediate or granulate in the margin and inside the scyphi	<i>C. humilis</i>
44b.	Podetia without soredia, with flat vegetative propagules, sometimes with squamules	<i>C. pyxidata</i>
45a.	Squamules of primary thallus big, rounded or elongate	46
45b.	Squamules of primary thallus small (< 3 mm)	47
46a.	(45a) Podetia with very short stalks, covered with farinose soredia, generally corticate in the lower half	<i>C. humilis</i>
46b.	Podetia with longer stalks, covered with granulose soredia, with granulose soredia, squamules of the primary thallus with many schizidia in the margin, underside with well veins marked	<i>C. cyathomorpha</i>
47a.	(45b) Podetia with farinose soredia (30-50) µm diameter	<i>C. fimbriata</i>
47b.	Podetia with larger granular soredia (usually > 80 µm diameter)	48
48a.	(47b) Podetia with rugose cortex, with thick rounded granules on the upper part, UV- or UV+ yellowish	49
48b.	Podetia without verruculose cortex, sorediate on the upper part, UV+ white	<i>C. homosekikaica</i>
49a.	(29b) Podetia UV+ yellowish	<i>C. cryptochlorophaea</i>
49b.	Podetia UV- yellowish, with granular soredia (60-170 µm diameter), pale greenish brown, without rangiformic acid	<i>C. chlorophaea</i>

Acknowledgments

We thank the council of O Rosal, particularly the Department of the Environment, for granting us permission to conduct this study and for their prompt and courteous assistance throughout.

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