Parmelia barrenoae, a new lichen species related to *Parmelia sulcata* (*Parmeliaceae*) based on molecular and morphological data

Pradeep K. DIVAKAR, M. Carmen MOLINA, H. Thorsten LUMBSCH and Ana CRESPO

Abstract: *Parmelia barrenoae* is described as new to science in the *P. sulcata* complex on the basis of morphological and molecular data. The new species is superficially similar to *P. sulcata* but differs in having simple rhizines whereas the other species of the complex have squarrose rhizines. Nuclear ITS rDNA and partial β-tubulin gene sequences have been used as molecular markers. In the phylogenetic analysis, *P. sulcata* falls into four well supported clades, one of them corresponds to the morphotype that is described here as a new taxon. Six samples of the new taxon from different locations on the Iberian Peninsula form a strongly supported monophyletic group.

Key words: Ascomycota, Bayesian inference, lichens, *Parmelia*, *Parmelia barrenoae*, *Parmeliaceae*, rhizines

Introduction

Phylogenetic studies of some widely distributed lichen species in the *Parmeliaceae* have shown that in many cases the morphological species concept does not coincide with a monophyletic unit. Some traditionally circumscribed species have been shown to include several genetically isolated clades, i.e. phylogenetic species (Kroken & Taylor 2001; Thell *et al.* 2002; Crespo *et al.* 2002; Högnabba & Wedin 2003; Molina *et al.* 2004). These monophyletic groups either have been proposed to represent cryptic species or have merely been described as distinct populations within a variable species.

P. K. Divikar & A. Crespo (corresponding author): Departamento de Biología Vegetal II, Facultad de Farmacia, Universidad Complutense, Madrid 28040, Spain.
M. C. Molina: Departamento de Matemáticas y Física Aplicadas y Ciencias Naturales, Escuela Superior de Ciencias Experimentales y Tecnología, Universidad Rey Juan Carlos, Madrid 28933, Spain.
H. T. Lumbsch: Department of Botany, The Field Museum, 1400 S. Lake Shore Drive, Chicago, IL 60605, USA.

Parmelia sulcata is a very well known lichen species (with squarrose rhizines and sorediate upper surface), reported from all continents, including Antarctica. It occurs in numerous ecotypes and is one of the most common species in temperate Europe. In Europe it is one of the principal lichens recolonizing cities where atmospheric pollution has recently decreased (Hawksworth & McManus 1989; Crespo *et al.* 1999). Preliminary studies on a worldwide basis using molecular markers have reported an unexpectedly high genetic variability within the species (Crespo *et al.* 1997).

The molecular variability detected is accompanied by an unusual heterogeneity in rhizine morphology among samples of *P. sulcata*. Hale (1987: 48) described rhizine morphology as one of the key characters circumscribing *P. sulcata*: ''The most important diagnostic characters of *P. sulcata* are the well–developed laminal soralia and richly squarrosely branched rhizines.'' However, other descriptions of this species indicate that it has simple, furcate or squarrose rhizines (e.g. Purvis *et al.* 1992). This may be interpreted as individual variation

due to age or ecological conditions. However, it has been shown that thalli with rhizines of the squarrose type show a perpendicular secondary ramification from early stages of thallus development (Molina *et al.* 2004). On the other hand, the furcate type of ramification does not form perpendicular secondary branches, suggesting that different rhizine-types are involved.

Contrary to their utility in other parmelioid genera, rhizines have not been used as an important taxonomic character in *Parmelia*, with the exception of recent combined molecular and morphological studies (Molina *et al.* 2004). Although the type of rhizines may vary within monophyletic groups of this genus, it may still be taxonomically informative at specific level. The objective of this work is to test the hypothesis that rhizine morphology is a useful diagnostic character at the species level in *Parmelia*, and in particular to examine whether samples of *P. sulcata* with simple rhizines form a phylogenetically distinct group. Fresh material collected from different regions of the Iberian Peninsula and other areas has been studied using two molecular markers (ITS and β-tubulin sequences).

Material and Methods

Taxon sampling

Thirty-one specimens representing 16 species of *Parmelia* s. str., were used in this study and sequence data from a part of the protein coding β-tubulin gene and nuITS rDNA were collected. Details of the material, area of collection, and location of voucher specimens, are presented in Table 1. Specimens were air-dried at room temperature. *Parmelia laevior*, *P. pseudolaevior* and *P. signifera* were used as out-group. The out-group selection was based on the previous work of Molina *et al.* (2004).

DNA extraction and PCR amplification

Small samples prepared from freshly collected and frozen herbarium specimens were ground with sterile glass pestles. Total genomic DNA was extracted using the DNeasy Plant Mini Kit (Qiagen) according to the manufacturer's instructions with the slight modifications described in Crespo *et al.* (2001). Dilutions of the total DNA were used for PCR amplifications of the genes coding for the nuclear ITS rRNA and partial sequence of the protein coding β-tubulin gene. Fungal nuITS rDNA was amplified using the primers ITS1F (Gardes & Bruns 1993), ITS4 (White *et al.* 1990), and the protein coding β-tubulin gene was amplified using the primers Bt3-LM and Bt10-LM (Myllys *et al.* 2001). Amplifications were performed in 50 µl volumes containing a reaction mixture of 5 µl of 10 × DNA polymerase buffer (Biotools) (containing $MgCl_2$ 2 mM, 10 mM Tris-HCl, pH 8·0, 50 mM KCl, 1 mM EDTA, 0·1% Triton X-100), 1 µl of dinucleotide triphosphate (dNTPs), containing 10 mM of each base, 2·5 µl of each primer (10 µM), 1·25 µl of DNA polymerase (1 U µl^{-1}) and 27.5 µl dH_2O. Finally, 40 µl of this mixture was added to 10 µl of DNA of each sample.

The amplifications for ITS rDNA and the β-tubulin gene were carried out in an automatic thermocycler (Techne Progene) and performed using the following programmes: initial denaturation at 94°C for 5 min, and 30 cycles of: 94°C for 1 min, 54°C (ITS rDNA) or 55–58°C (β-tubulin) for 1 min, 72°C for 1·5 min, and a final extension at 72°C for 5 min.

The PCR products were subsequently purified using the Bioclean Columns kit (Biotools) according to the manufacturer's instructions. The purified PCR products were sequenced using the same amplification primers. The ABI Prism® Dye Terminator Cycle Sequencing Ready reaction kit (Applied Biosystems) was used and the following settings were carried out: denaturation for 3 min at 94°C and 25 cycles at: 96°C for 10 sec, 50°C for 5 sec and 60° for 4 min. Sequencing reactions were electrophoresed on a 3730 DNA analyzer (Applied Biosystems). Partial SSU rDNA, sometimes including an intron at the end of the 3′ (SSU) were removed before the alignment. Sequence fragments obtained were assembled with SeqMan 4.03 (DNAStar) and manually adjusted.

Sequence alignments

An alignment procedure employing a linear Hidden Markov Model (HMM) for the alignment, as implemented in the software SAM (Karplus *et al.* 1998), has been used. Sequences of 31 specimens (Table 1) were aligned separately for the two genes. Regions that could not be aligned with statistical confidence were excluded from the phylogenetic analysis.

Phylogenetic analysis

The alignment was analysed using the programs PAUP* 4.0b10 (Swofford 2003) and MrBAYES 3.0 (Huelsenbeck & Ronquist 2001). Character polarity was assessed with outgroup comparison, using *Parmelia laevior*, *P. pseudolaevior* and *P. signifera* as outgroup. The data were analysed using a Bayesian approach (Larget & Simon 1999; Huelsenbeck *et al.* 2000). Posterior probabilities were approximated by sampling trees using a Markov Chain Monte Carlo (MCMC) method. The posterior probabilities of each branch were calculated by counting the frequency of trees

TABLE 1. *Specimens of* Parmelia *used in this study, with location, reference collection detail and GenBank accession numbers*

No.	Species	Locality and collector	Voucher specimens	GenBank no.	
				ITS	β-tubulin
1	*P. squarrosa*	Forge Creek, USA, 628 m, *Cregler*	MAF 7293	AY036977*	AY580308
2	*P. squarrosa*	Parson, USA, 826 m, *Cregler*	MAF 7270	AY036979*	AY580309
3	*P. sulcata*	Oerkened, Sweden	Thell-Marth SK-9921	AF410840*	AF410844*
4	*P. sulcata*	Ventorrillo (Madrid), Spain, 1500 m, *Divakar*	MAF 9899	AY580313	AY580310
5	*P. sulcata*	S. Gredos 1 (Ávila), Spain, 1300 m, *Crespo*	MAF 9904	AY579445	AY579460
6	*P. sulcata*	Ventorrillo (Madrid), Spain, 1500 m, *Divakar*	MAF 9901	AY579447	AY579462
7	*P. sulcata*	Herbeset (Castellón), Spain, 1140 m, *Crespo, Barreno & Divakar*	MAF 10153	AY579452	AY579466
8	*P. sulcata*	Düsseldorf (Germany), *Crespo*	MAF 10159	AY579454	AY579468
9	*P. sulcata*	Düsseldorf (Germany), *Crespo*	MAF 10155	AY579453	AY579467
10	*P. barrenoae*	La Barranca (Madrid), Spain, 1400 m, *Crespo*	MAF 9750	AY295103*	AY295111*
11	*P. barrenoae*	La Barranca (Madrid), Spain, 1400 m, *Crespo & Divakar*	MAF 10151	AY579444	AY579459
12	*P. barrenoae*	S. Gredos 2 (Ávila), Spain, 1300 m, *Crespo*	MAF 9906	AY579446	AY579461
13	*P. barrenoae*	Las Médulas (León), Spain, 850 m, *Divakar*	MAF 9905	AY579448	AY579463
14	*P. barrenoae*	Marvào (Sao Mamede), Portugal, 900 m, *Crespo*	MAF 9900	AY579450	AY579464
15	*P. barrenoae*	Herbeset (Castellón), Spain, 1140 m, *Barreno, Crespo & Divakar*	MAF 10154	AY579451	AY579465
16	*P. sulcata*	Herbeset (Castellón), Spain, 1140 m, *Barreno, Crespo & Divakar*	MAF 10152	AY579455	AY579469
17	*P. sulcata*	Herbes (Castellón), Spain, 1000 m, *Barreno, Crespo & Divakar*	MAF 10157	AY579456	AY579470
18	*P. sulcata*	Caceres, Spain, 280 m, *Crespo* et al.	MAF 9902	AY579449	—
19	*P. fertilis*	Hokkaido (Tokyo), Japan, 700 m, *Hamada*	MAF 7282	AY036982*	AF391143*
20	*P. cochleata*	Hokkaido (Tokyo), Japan, 700 m, *Harada*	MAF 7280	AY036985*	—
21	*P. pinnatifida*	Kola Peninsula, Russia, *Schlensog*	MAF 7274	AY036987*	AF391134*
22	*P. saxatilis*	Kola Peninsula, Russia	MAF 7276	AY036989*	AF391136*
23	*P. serrana*	El Escorial (Madrid), Spain, *Crespo & Sancho*	MAF 6885	AF350040*	AF391142*
24	*P. ernstiae*	Puerto de Corrales (Burgos), Spain, 1020 , *Crespo*	MAF 9749	AY295110*	AY295117*
25	*P. omphalodes*	La Plataforma del Calvitero (Salamanca), Spain, 1800 m, *Crespo* et al.	MAF 7062	AY036998*	AF391131*
26	*P. adaugescens*	Hokkaido (Tokyo), Japan, 600 m, *Hamada*	MAF 7277	AY036991*	AF391146*
27	*P. submontana*	Segura de la Sierra (Madrid), Spain, 1520 m, *Aragón*	MAF 10233	AY579457	AY580311/AY580312
28	*P. discordans*	S. Aberdeen (Scotland), UK, *Hawksworth*	MAF 10232	AY583212	AY583213
29	*P. pseudolaevior*	Hokkaido (Tokyo), Japan, *Hamada*	MAF 7290	AY036994*	AF391147*
30	*P. laevior*	Hokkaido (Tokyo), Japan, 1560 m, *Harada*	MAF 7278	AY036995*	AF391148*
31	*P. signifera*	Molonglo Gorge (ACT), Australia, 550 m, *Elix, Louwhoff & Molina*	MAF 7283	AY037003*	AF391149*

*Sequences obtained from GenBank.

that were visited during the course of the MCMC analysis.

The program MrBAYES was employed to sample the trees. The analysis was performed assuming the general time reversible model (Rodríguez *et al.* 1990) including estimation of invariant sites and assuming a discrete gamma distribution with six rate categories (GTR+I+G) for single-genes and the combined analyses. No molecular clock was assumed. A run with 2 000 000 generations starting with a random tree and employing 12 simultaneous chains was executed. Every 100th tree was saved into a file.

We plotted the log-likelihood scores of sample points against generation time using TRACER 1.0 (http://evolve.zoo.ox.ac.uk/software.html?id=tracer) and determined that equilibrium was achieved when the log-likelihood values of the sample points reached a stable equilibrium value (Huelsenbeck & Ronquist 2001). The initial 1000 trees were discarded as burn-in before equilibrium was reached. Using PAUP⋆, majority-rule consensus trees were calculated from 19 000 trees sampled after reaching likelihood convergence to calculate the posterior probabilities of the tree nodes. Unlike nonparametric bootstrap values (Felsenstein 1985), these are estimated probabilities of the clades under the assumed model (Rannala & Yang 1996) and hence posterior probabilities equal to and above 95% are considered significant supports. Phylogenetic trees were drawn using TREEVIEW (Page 1996).

A Bayesian approach to examine the heterogeneity in phylogenetic signal between the two data partitions (Buckley *et al.* 2002) was used. For the two genes and the concatenated analyses, the set of topologies reaching 0·95 posterior probability was estimated. The combined analysis topology was then compared for conflict with the 0·95 posterior intervals of the single gene analyses. If no conflict was evident, it was assumed that the two data sets were congruent and could be combined. If conflict was evident, the two data sets were interpreted as incongruent and thus the combined analysis might be potentially misleading (Bull *et al.* 1993).

Hypothesis testing

Since the phylogenetic analysis is incongruent with the current concept of *Parmelia sulcata* in suggesting that group A (=*P. barrenoae*) is a different species, we tested whether our data are sufficient to reject an alternative topology of a monophyletic *P. sulcata* s. lat. (i.e. including groups A, B1, B2 and C). Such a topology might be present in suboptimal trees not sampled or not present in the 50% majority-rule consensus tree of the MCMC sampling, which may not be significantly worse than the obtained topology.

For the hypothesis testing two different methods were used: (1) a Bayesian hypothesis testing following Ihlen & Ekman (2002) and Lumbsch *et al.* (2004), and the expected likelihood weighting approach (ELW) by Strimmer & Rambaut (2002). For the Bayesian hypothesis testing a run of 500 000 generations was performed with the same settings as in the estimation of the phylogeny using the combined data set. Four thousand trees at the equilibrium state for the null hypothesis were used from this analysis. The probability of the null hypothesis being correct was calculated by counting the presence of this topology in the MCMC sample (Lewis 2001). The frequency of trees in the MCMC sample agreeing with the null hypothesis was calculated using the filter command in PAUP⋆ with a certain constraint describing the null hypothesis.

However, it may be argued that using posterior probabilities with a flat topology prior may not be appropriate to reject an alternative topology. The sum of probabilities of trees agreeing with the hypothesis is close to zero at the beginning of the analysis. Thus a low posterior probability in the hypothesis testing may either mean that the hypothesis is unlikely (and this is how we interpreted it) or that the data are not sufficient to move the posterior probability from the prior. Although we feel that the latter interpretation is highly unlikely, we performed a second hypothesis using the ELW approach (Silberman *et al.* 2002; Simpson *et al.* 2002; Strimmer & Rambaut 2002; Andersen & Ekman 2004). We have used this instead of parametric bootstrapping that we used previously (e.g. Schmitt *et al.* 2001, Lumbsch *et al.* 2002), since parametric bootstrapping was shown to be overly prone to reject alternative topologies (Simpson *et al.* 2002; Strimmer & Rambaut 2002). The ELW test employs expected likelihood weights as a measure of confidence and is claimed to be relatively robust against model misspecifications. The expected likelihood weights are measured across a sample of trees, averaged over a number of bootstrap replicates. To calculate confidence intervals for the optimal tree, two PERL scripts (elw.pl and calcwts.pl) available from http://hades.biochem. dal.ca/Rogerlab/Software/software.html (Silberman *et al.* 2002) were used in MacPerl in addition to SEQBOOT in the PHYLIP 3.6 software package (Felsenstein 2002) and PAUP⋆ 4.0b10 (Swofford 2003). One thousand replicates were analyzed in PAUP⋆ on a tree sample of 200 unique trees, including the best tree agreeing with the null hypothesis, the unconstrained ML tree, and 198 trees with the highest likelihood in the MCMC tree sample. The 95% confidence interval based on these 200 trees was calculated with substitution model parameters reestimated for each replicate over a Jukes-Cantor corrected neighbourjoining topology.

Morphology

The lower surfaces of specimens were examined to determine the type of rhizines (i.e. squarrose *vs* simple or furcate) using a Leica Wild M8 dissecting microscope. Lobe shape and width were studied under the same microscope, and width measured to the nearest 0·1 mm using a CBS Beck Kassel calibrated 8-fold magnifier; at least ten measurements were made on the various specimens in the *P. sulcata*–*P. barrenoae* complex.

Chemistry

Chemical constituents were identified by thin layer chromatography using standard methods (Culberson 1972; Culberson & Johnson 1982; Elix & Ernst-Russell 1993).

Results

A total of 17 new partial β-tubulin gene, and 16 new nuclear ITS rDNA sequences were generated. Thirty-five sequences were downloaded from Genbank and aligned with the former ones resulting in an alignment of 31 taxa (Table 1). A matrix consisting of 774 unambiguously aligned β-tubulin basepairs and 511 nu ITS positions was produced. Three hundred and sixty-six characters were variable.

The two separate analyses did not show any incongruence at a significance level of $P = 0.95$ and hence only the combined analysis is described here. The likelihood parameters in the sample had the following average values ($\pm$ one standard deviation): likelihood (LnL) $= -4810.242$ (± 0.212), base frequencies $\pi(A) = 0.204$ (± 0.0015), $\pi(C) = 0.283$ (± 0.0017), $\pi(G) = 0.249$ (± 0.0017), $\pi(T) = 0.264$ (± 0.0017), rate matrix $r(AC) = 2.78$ (± 0.047), $r(AG) = 7.777$ (± 0.127), $r(AT) = 3.175$ (± 0.564), $r(CG) = 2.026$ (± 0.384), $r(CT) = 18.19$ (± 0.314), $r(GT) = 1$, the gamma shape parameter alpha $= 1.05$ (± 0.184), and the pinvar $= 0.364$ (± 0.0323).

The combined tree (Fig. 1) showed two main monophyletic clades. The *P. sulcata* clade, made up of all the *P. sulcata* samples (including those with simple rhizines), together with *P. squarrosa* and *P. fertilis*, forms a strongly supported (1.00 pp.) monophyletic group. Within this clade there are three well-supported groups (A–C in Fig. 1). Group A includes all the samples with simple rhizines described here as *P. barrenoae*. Group B includes samples of the North American *P. squarrosa* and seven samples of *P. sulcata*; the latter falling again into two well supported clades (groups B1 & B2). The relationship of *P. fertilis* is unresolved. Three samples of *P. sulcata* (group C) are basal within the *P. sulcata* clade.

The second major clade includes the *P. saxatilis* group as circumscribed by Molina et al. 2004 (including *P. adaugescens*, *P. discordans*, *P. ernstiae*, *P. omphalodes*, *P. pinnatifida*, *P. saxatilis*, *P. serrana* and *P. submontana*). This group is strongly supported (1.00 pp.). However, the position of *P. cochleata* appears as uncertain in the analysis; it is basal to the *P. saxatilis* group, but this relationship lacks support.

Two hypothesis tests were performed to determine whether a clade of groups A, B1, B2 and C is monophyletic. This null hypothesis was rejected by the Bayesian hypothesis testing at $P \leq 0.001$. In the ELW test the best tree agreeing with a monophyletic *P. sulcata* was found to be outside the 95% confidence set. Consequently, both tests rejected a monophyly of *P. sulcata* s. lat.

Discussion

In our analysis the distinction of the *P. saxatilis* and *P. sulcata* groups as proposed by Molina et al. (2004) is strongly supported. The species in the *P. sulcata* group are morphologically defined by the presence of squarrose rhizines with the notable exception of *P. barrenoae* (Group A) with simple or furcate rhizines.

The samples of group A, in addition to their deviating rhizine-type also share further morphological similarities. They possess more superficial soralia and longer and revolute older lobes compared with *P. sulcata*. In well-developed specimens the lower surface is turned over and can be easily seen from the upper side. This revolute lobe morphology is different from the convoluted or contorted lobes of *P. submontana*, belonging to the *P. saxatilis* clade, which also has simple rhizines. Moreover, *P. submontana* has pustulate orbicular soralia (dactyls *sensu* Elix 1994). Consequently, we interpret clade A of the *P. sulcata* complex, as a separate species, which is described below.

The samples with simple rhizines were collected from relatively humid localities (800–1500 m altitude, Table 1) in different Mediterranean regions of the Iberian Penin-

FIG. 1. 95% majority rule consensus tree of 19 000 trees visited during a B/MCMC tree sampling procedure. Numbers at nodes are posterior probabilities values above or equal to 95%.

sula. In most of the localities these samples were sympatric with other *P. sulcata* populations. Although the distributional area of the new taxon remains poorly known, we suggest that material should be checked for its presence in the south of Great Britain and also in other Mediterranean or sub-Mediterranean regions of Europe and

Africa. It is interesting to note that most Australian and North American authors mention the squarrose type of rhizines as one descriptive feature of *P. sulcata* (Hale, 1987; Hale & Cole, 1988; Elix 1994; Brodo *et al.* 2001), whereas most European lichen floras (e.g Harmand, 1909; Ozenda & Clauzade 1970) do not mention the rhizines as a significant character for the species differentiation. This suggests that *P. barrenoae* may be absent in Australia and America.

Three additional monophyletic groups are present among samples referred to as *P. sulcata* (groups B1, B2 and C). All of these groups have squarrose rhizines. Morphologically these three groups are very similar but include collections from geographically distant regions. We refrain from describing these groups here as distinct species until further molecular data from more samples throughout the distribution area of *P. sulcata* are available. One molecular character that may distinguish group B2 is the presence of a group I intron at position 1516 of the SSU nuclear ribosomal DNA in the three specimens studied. This intron was absent in the other specimens of *P. sulcata*.

The presence of several phylogenetically distinct clades in widely distributed species (Grube & Kroken 2000) and more precisely in *Parmeliaceae*, for example in *Letharia* (Kroken & Taylor 2001) and in *P. saxatilis* (Crespo *et al.* 2002; Molina *et al.* 2004) has been previously reported. Moreover, Crespo *et al.* (1997, 1999) reported genetic variability in *P. sulcata* by comparing lengths of PCR products of the ITS (plus SSU 3′ end) region in over 200 samples from different parts of the world. Our study agrees with the previous studies in showing that *P. sulcata* as circumscribed by morphology is a species complex including several cryptic species. Further data from a large number of populations are necessary to circumscribe these cryptic species. However, the population with simple rhizines (group A, Fig. 1) can hardly be considered cryptic but is a well-defined and recognizable species that is described below.
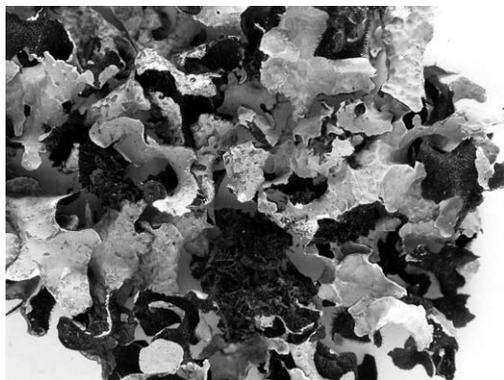

Fig. 2. *Parmelia barrenoae* Divakar, M. C. Molina & A. Crespo, holotype (MAF 9906).

Taxonomy

Parmelia barrenoae Divakar, M. C. Molina & A. Crespo sp. nov.

Similis *Parmeliis sulcatae* sed differt in rhizinae simplices et lobis revolutis et in sequences moleculares ITS et β-tubulin.

Typus: Spain, Ávila, Sierra de Gredos, Navalperal de Tormes, Cruz del Gallo, 1300 m, on *Quercus pyrenaica*. 6 September 2003, *A. Crespo* (MAF 9906—holotypus; BM, GZU, UPS—isotypi)

(Fig. 2)

Thallus adnate to loosely adnate, 5–10 cm diam., lobes contiguous to overlapping and imbricate, apically rounded to sublinear, short, 2–7 mm wide, older lobes becoming revolute. Upper surface glaucous grey to whitish grey, finely foveolatae, becoming reticulately cracked. *Pseudocyphellae* laminal and marginal effigurate, numerous, marginal pseudocyphellae subcontinuous, laminal pseudocyphellae linear to irregular shaped mainly on ridges, separate in the centre but forming a network near periphery. *Soralia* sparse, laminal, developed from old cracked pseudocyphellae, linear to irregular in shaped, soredia granular. *Medulla* white. *Rhizines* on black lower surface moderately abundant, simple to furcately branched, not squarrose, 1–2 mm long.

Apothecia and *pycnidia* not seen.

Chemistry. Cortex K+ yellow; medulla K+ yellow turning red, C−, PD+ red-

orange; containing atranorin and salazinic acid.

Etymology. Parmelia barrenoae is named in honour of the Spanish lichenologist Eva Barreno, in recognition of her numerous contributions to lichenology and of her important contribution in developing Spanish lichenology.

Ecology. The species is widely distributed on oak bark, conifers and occasionally on rocks mostly in open and sunny places. The species occurs in Mediterranean areas at moderately high altitude between 800–1800 m, and is frequent in meso- and supra-Mediterranean belts (Rivas-Martínez *et al.* 2002), especially on *Quercus ilex* ssp. *ballota*, *Q. pyrenaica* and *P. sylvestris*; usually it is associated with *P. serrana*.

Distribution. So far, the species is known from several regions in the centre of the Iberian Peninsula, where it is locally common.

Observations. Parmelia barrenoae is characterized by simple to furcate rhizines, scarce laminal soralia, revolute older lobes, and the presence of salazinic acid. The presence of laminal soralia and salazinic acid are reminiscent of *Parmelia sulcata*. However, *Parmelia sulcata* differs in having richly branched, squarrose rhizines. In addition, older lobes of *P. barrenoae* are revolute and soralia are only laminar and less developed than those of *P. sulcata* (pseudocyphellae soon forming marginal and laminar soralia). *Parmelia barrenoae* could also be confused with *P. submontana* since both are sorediate and have simple to furcate rhizines. However, the soralia of *P. submontana* differ in having a dactylate, orbicular shape (isidioid or pustular soralia) and long, separate, convolute lobes (Hale 1987; Elix 1994). The two species are not closely related as shown by the molecular analysis (Fig. 1).

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