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The species concept in *Bryoria* sect. *Implexae*: when sequence data and morphology do not match can microsatellites help

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Introduction

In fungi, genetic isolation may precede morphological differentiation, leading to cryptic species. Yet the converse may also exist, with little molecular but high morphological divergence. Preliminary studies suggest this may be the case in *Bryoria* sect. *Implexae* in Europe, which traditionally includes seven species. Previous studies (Myllys *et al*. 2011) showed a three-loci phylogeny with *Bryoria glabra* clearly separated, but with, *B. chalybeiformis*, *B. capillaris* , *B. fuscescens*, *B. implexa*, *B. lanestris*, and *B. subcana* as molecularly conspecific. However, *B. fuscescens* and *B. capillaris* (Fig.1 B & C) are differentiated morphologically and chemically and often grow mixed. This suggests that factors others than environmental are involved.

Objectives

To study the population structure of the species complex across Europe.

To identify the putative genetic lineages or genepools.

To delimit the species of *Bryoria* sect. *Implexae* in Europe.

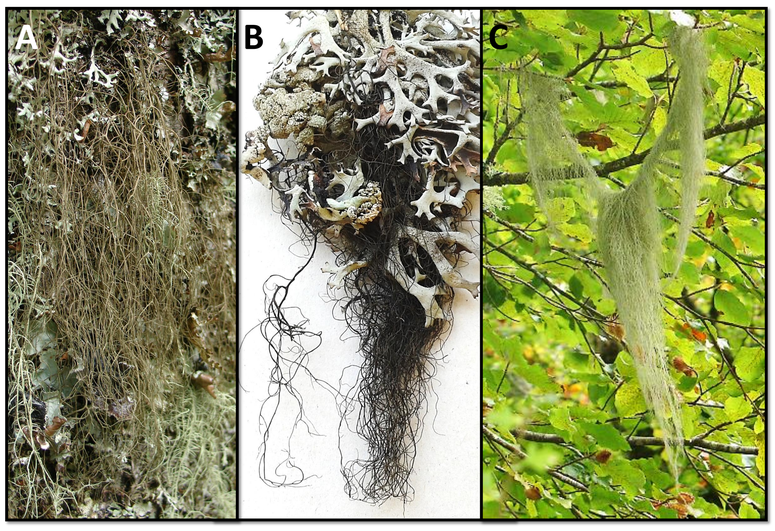


Figure 1: Traditionally recognized species: **A.** *B. implexa* (Asturias, Spain), **B.** *B. fuscescens* (Morocco), **C.** *B. capillaris* (Pyrenees, Spain).

Materials and Methods

Sixteen European and Mediterranean populations with an average of 20 specimens were collected (Fig. 2). To study the genetic structure 18 fungal microsatellites and 5 *Implexae* section fungal specific highly variable phylogenetic markers were developed. Some morphological characters (Tables 1 &2) were analyzed in order to determine correlations.

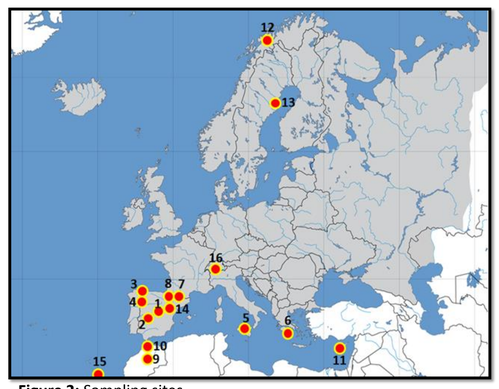


Figure 2: Sampling sites.

Characters
Chemotype
General colour
Basal colour
Main branches aspect
Branching angles
Presence and type of soralia
Presence of pseudocyphellae
Presence of apothecia

Table 1: Characters checked for each individual.

Morphospecies	Individuals
B. capillaris	59
B. fuscescens	128
B. implexa	32
B. lanestris	1
B. subcana	6
B. fus/imp	102
Total	**328**

Table 2: Species and number of individuals collected.
B. fus/imp. = intermediate morphs

Results

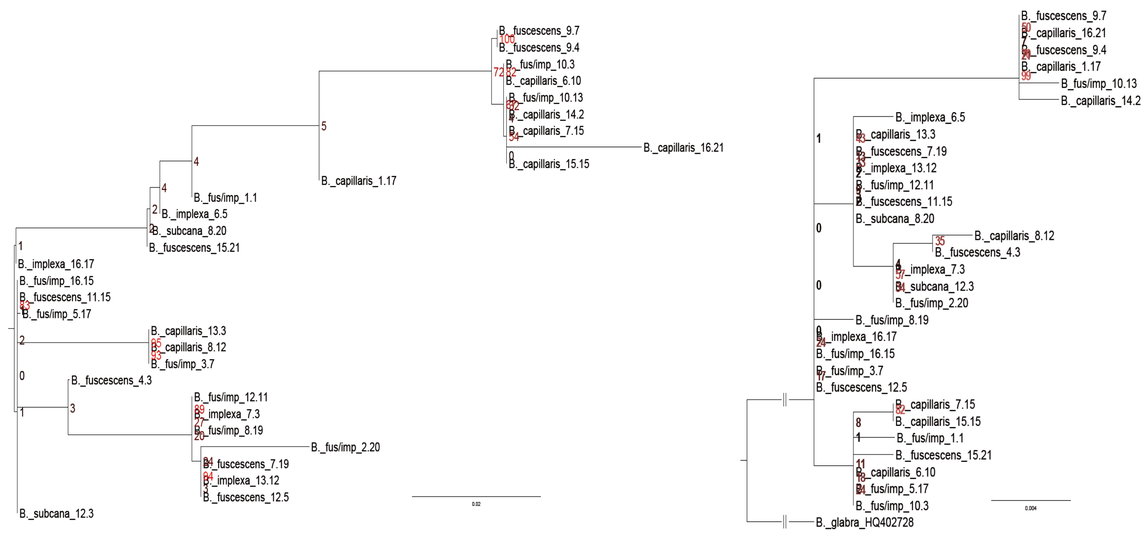


Figure 3: ML tree of 30 selected specimens representing between them the maximum geographical, chemical and morphological variability in the study area **A.** Consensus of the five newly developed intergenic *Bryoria* sect. *Implexae* specific highly variable markers. **B.** With ITSrDNA and *B. glabra* as aoutgroup.

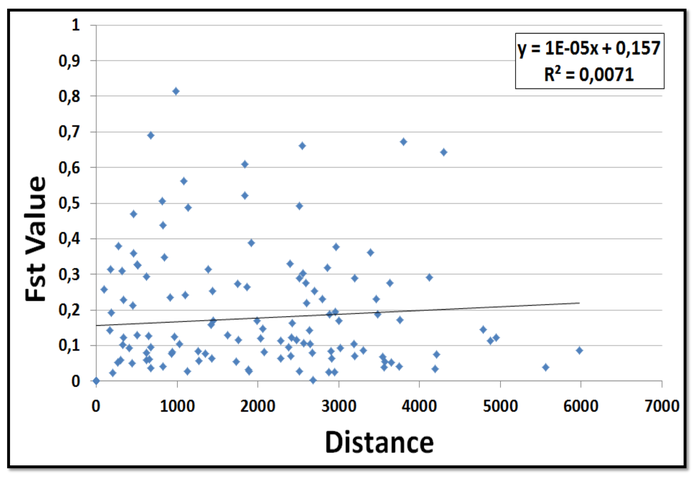


Figure 4: Fst values obtained from 15 microsatelites against geographical distance for each pair of populations. Fst= 0 indicates complete panmixis among each pair, while Fst=1 indicates complete isolation. Note that geographical distance is not acting as a barrier among populations.

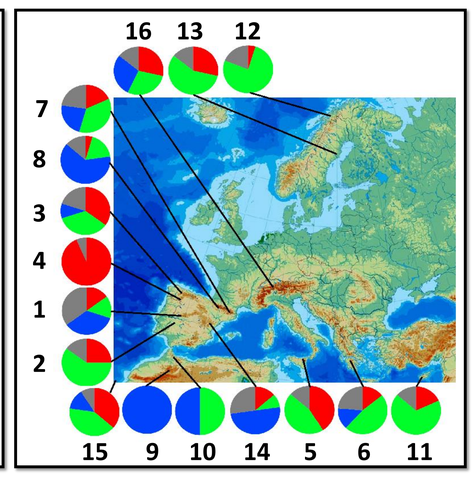


Figure 5: Distribution of the STRUCTURE software gene-pools under the K=3 model (Fig. 6B). Numbers indicate the population and colours are as in Figure 6 but with intermediate specimens represented in grey. Population 4 pattern is due to the high level of clonality, while population 9, is composed of a unique genepool and not high levels of clonality.

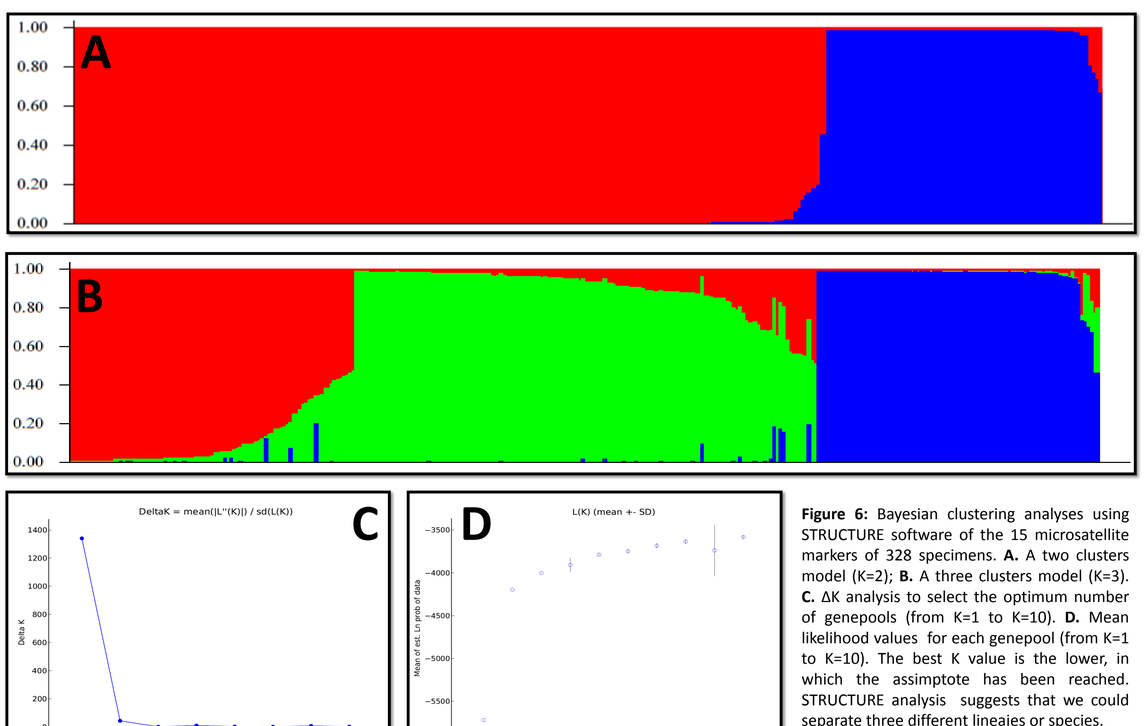


Figure 6: Bayesian clustering analyses using STRUCTURE software of the 15 microsatellite markers of 328 specimens. **A.** A two clusters model (K=2); **B.** A three clusters model (K=3). **C.** ΔK analysis to select the optimum number of genepools (from K=1 to K=10). **D.** Mean likelihood values for each genepool (from K=1 to K=10). The best K value is the lower, in which the assimptote has been reached. STRUCTURE analysis suggests that we could separate three different lineajes or species.

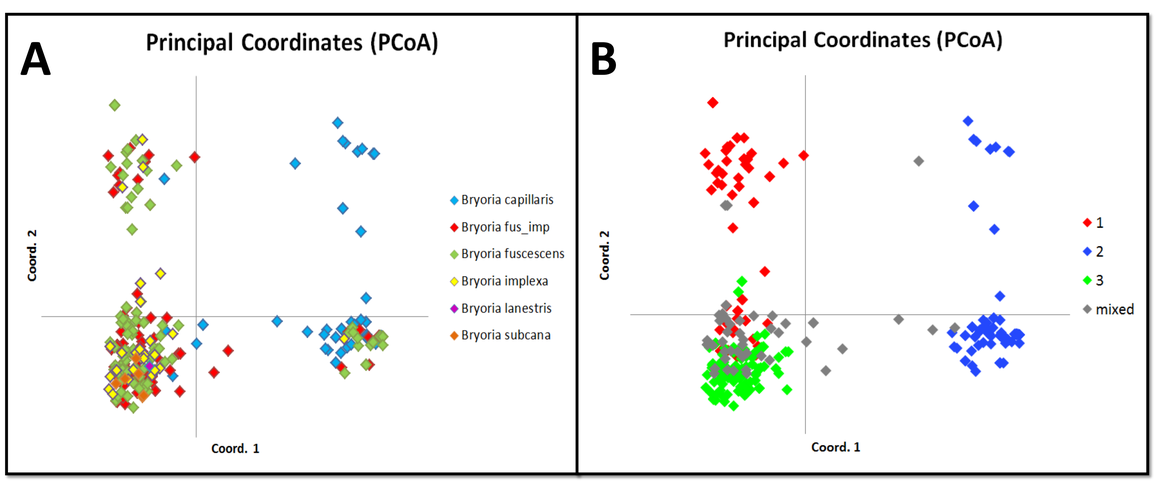
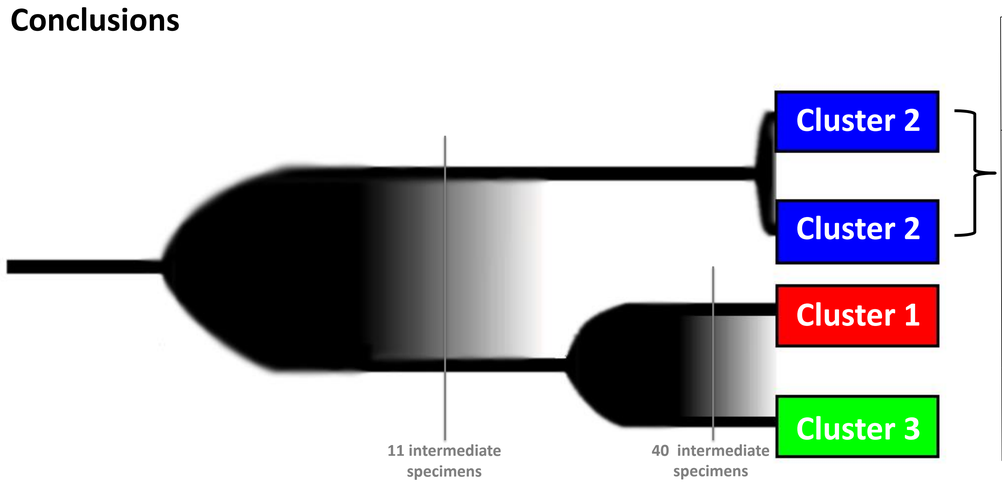


Figure 7: Principal coordinate analysis (PCoA) of the 15 microsatellites. **A.** With traditional species names. **B.** Genepools from Fig. 6 B. using the same colours and with the unassignable specimens in grey. In the first case no clearly isolated groups for any species is evident. In the second, the blue cluster appears isolated, but the red and the green are partially mixed as seen Fig. 6 B. This PCoA analysis shows that these data could be grouped into four clusters not three (Fig. 6).

Population analysis show that *Bryoria* sect. *Implexae* lacks a well defined geographical genetic structure (Fig.4 & Fig. 5), suggesting an ability to migrate either at present or in the recent past. Migration could be facilitated by animals, such as birds, because this complex hardly ever produces ascospores and some genepools lack soralia. The clustering analysis indicates that the material could be grouped into three (Fig. 6) or four (Fig. 7) main genepools which could be in the process of speciation and which are more or less evenly distributed across Europe and the Mediterranean area (Fig.5).

Conclusions



	B. capillaris	*B. fuscescens*	*B. implexa*	*B. fus/imp*	*B. subcana*	*B. lanestris*	Total	Fum. acid	Soralia	Apothecia
Cluster 2	47	23	1	13	0	0	84	0%	0%	0%
	57%	27%	1%	15%	0%	0%	100%			
Cluster 1	2	40	8	20	1	0	72	57%	78%	3%
	3%	57%	11%	28%	1%	0%	100%			
Cluster 3	2	44	17	52	5	1	121	67%	76%	6%
	2%	36%	14%	43%	4%	1%	100%			

Figure 8: Interpretation of the clustering analysis results compared with the traditional species and most relevant morphological and chemical characters that define the clusters. Numbers in the table indicate the number of specimens with each character state. Fum. acid = Fumarprotocetraric acid.

Traditional species concepts in the European members of *Bryoria* sect. *Implexae* are not supported by molecular data, except for *B. glabra*. The lichens named as *B. capillaris*, *B. fuscescens*, *B. implexae*, *B. subcana*, *B. lanestris,* and perhaps *B. chalybeiformis* (not represented), could be as conspecific at least in the study. The microsatellite population analysis revealed three clusters which could correspond to three speciation processes. Clusters 1 and 3 are almost completely composed by *B. fuscescens* and *B. implexa* morphospecies and have a wide morphological and chemical plasticity. Cluster 2 is mainly composed by the *B. capillaris* morphospecie (Fig. 1C), although many of the *B. fuscescens* morphotypes from Morocco (populations 9 and 10, Fig.1B) appear nested here. This cluster can be characterized by the abscence of fumarprotocetraric acid, soralia and apothecia. The abscence of soredia, isidia or spores in cluster 2 does not seem to have been an impediment to dispersal across Europe, in the Mediterranean region, and in the Canary Islands.

The determinants of one specimen expressing a different morphology or chemistry to another growing in physical contact remain unresolved. In order to answer this question and improve the knowledge of the populational structure of this group of lichens, more populations and additional analysis are being performed.

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