Species delimitation and phylogeography of the *Pectenia* species-complex: a misunderstood case of species-pairs in lichenized fungi, where reproduction mode does not delimit lineages.

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Abstract

Species have traditionally been identified and described using morphological characters. Recently, analyses of sequence data have revealed that traditional species delimitation frequently underestimates the diversity of organisms. The main goal of this work was to study species boundaries in the genus *Pectenia* and elucidate the biogeographic history of the four currently accepted species. To accomplish this, we included 92 specimens across the range of *Pectenia* in Europe and northern Africa. We used three nuclear loci (nuITS, RPB1 and RPB2) and assessed species circumscription using two Bayesian coalescent-based methods (*BEAST and BEST) and the Bayes Factor approach. We reviewed the value of reproductive mode and other morphological features as predictors of monophyletic groups. Our results suggest that the production of asexual propagules and sexual structures are not characterizing monophyletic groups. The genus includes three lineages with a case of cryptic speciation explained by a biogeographic pattern. Two species are morphologically well-characterized *Pectenia plumbea* which correspond to a single clade and *P. atlantica* which includes two paraphyletic clades. The results of the biogeographic analysis indicate that the Mediterranean basin is the most likely ancestral distribution area of *P. plumbea*, whereas *P. atlantica* probably originated in Macaronesia, although this result was not well supported.

Keywords: biogeography, phylogeny, synonymy, species boundaries, integrative taxonomy, species complex,

1. Introduction

Understanding the patterns and processes associated with geographical variation in population genetic structure across species' geographic ranges has implications for many questions in ecology, evolution, conservation biology, as well as in taxonomy. These patterns and processes can be understood through an integrative phylogeographical approach (Avise, 2000) that simultaneously entails species delimitation. Species have traditionally been delimited and characterized using morphological characters (Hawksworth 2001; Bickford et al. 2007); however, morphological characters are often affected by convergent evolution, making the recognition of species very difficult. This is especially true for organisms with relative simple morphologies such as lichenized fungi, which have relatively few and simple phenotypic characters (Kroken and Taylor, 2001; Otálora et al. 2008; Crespo and Pérez-Ortega 2009). Recently, DNA sequence data of lichenized fungi have provided valuable information about speciation and species delimitation (Wedin et al. 2009; Parnmen et al. 2012), and this type of studies has showed several inconsistencies between traditional fungal species boundaries and phylogenetic trees (Crespo and Pérez-Ortega 2009). Often times the analyses of sequence data have revealed that traditional species delimitation underestimates the diversity of these organisms. Thus, several studies have reported the presence of distinct lineages within currently accepted species showing that characters suitable for species delimitation had been overlooked (Molina et al. 2004; Argüello et al. 2007; Otálora et al. 2008; Crespo and Pérez-Ortega 2009; Wedin et al. 2009; Leavitt et al. 2011a,b; Spribille et al. 2011; Amo et al. 2012; Parnmen et al. 2012; Leavitt et al. 2013, Lücking et al. 2014), or reporting several cases of cryptic speciation, where morphological features to define species are absent or subtle (Crespo and Pérez-Ortega

2009; Singh et al. 2015, Schneider et al 2016). However, cases with the opposite pattern has seldom been reported; where well-characterized morphological species are not supported by sequence data and thus form a single monophyletic group instead of two, as would have been expected. This last pattern has mainly been reported in closely related species that primarily differ in their reproductive modes ("species pairs"; see Tehler 1982), where one taxon reproduces sexually and the other vegetatively by means of soredia (Myllys et al. 1999 2001; Buschbom and Mueller 2006). However, to our knowledge no studies have been conducted on species pairs where isidia are the vegetative propagules.

Here we studied the species of the lichen genus *Pectenia* P. M. Jørg., L. Lindblom, Wedin & S. Ekman; which belongs to Peltigerales, a large lineage of lichen-forming fungi diverging in the late (Prieto and Wedin 2013) and often associated with cyanobacteria of the genus *Nostoc* Vaucher ex Bornet & Flahault. *Pectenia* was recently described for the species previously included in the *Degelia plumbea* group (Ekman et al. 2014). The genus has an oceanic and sub-oceanic distribution, where populations can be found in Europe from Macaronesian islands to Scandinavia and Mediterranean basin, and in adjacent areas of Africa (Fig.1). In addition, a couple of records of one species, *Pectenia plumbea* (Lightf.) P.M. Jørg., L. Lindblom, Wedin & S. Ekman, are from a restricted region of North-East America (Blom and Lindblom 2010; Richardson et al. 2010). The *Pectenia* species are considered threatened throughout their distribution range (Martínez et al. 2003; Diederich et al. 2008), and are indicator species of habitat health (Martínez et al. 2003). Previous classifications have recognized between two and four species based on different interpretation of morphological characters, habitat requirement and distribution. Two putative cases of "species pairs" have being reported (Jørgensen and James, 1990; Blom

and Lindblom 2010; Carballal et al. 2010). The current classification recognizes four species (Ekman et al. 2014). *Pectenia cyanoloma* (Schaer.) P.M. Jørg., L. Lindblom, Wedin & S. Ekman and *P. plumbea* typically produce numerous sexual structures (apothecia), but no vegetative propagules, while the other two species rarely produce apothecia but are characterized by the presence of abundant isidia in *P. atlantica* (P.M. Jørg.,) L. Lindblom, Wedin & S. Ekman or ligulae in *P. ligulata* (P.M. Jørg. & P. James) P.M. Jørg., L. Lindblom, Wedin & S. Ekman. According to the reproduction mode the two putative "species-pairs" would be *P. atlantica - P. cyanoloma* and *P. plumbea - P. ligulata.* The lobe segmentation and surface are similar in *P. atlantica* and *P. cyanoloma*, as they are composed of concave, successive trough-shaped segments each ending in a transverse curved ridge-like. The lobes in *P. plumbea* and *P. ligulata* lacks the concave transversal segmentation, and the lobe surfaces have instead a irregular network of narrow whitish discoloration (Blom and Lindblom 2010). The four putative species differ somewhat in ecology and distribution. All are mainly oceanic in their distribution (Blom and Lindblom 2010), and occur in areas of high rainfall, or else in sites with locally high humidity. The least oceanic species is *P. plumbea*, with populations in the Mediterranean and Baltic basins, and further inland in southern Europe and Scandinavia (Jørgensen 2007). Records of atypical specimens of *P. atlantica*, having lobes with the network whitish discoloration of *P. plumbea*, has been reported in mediterranean areas of southern Europe (Jørgensen 1978; Martínez et al. 2003; Jørgensen2007; Carballal et al. 2010; Otálora et al. 2013). In a previous study in central Spain (Otálora et al. 2013), we found high levels of genetic similarity between *P. plumbea* and the Mediterranean morphotype of *P. atlantica*; suggesting that probably *P. atlantica* and *P. plumbea* do not correspond to two distinct species and an urgent study of species boundaries of this species complex was needed.

Just as many mosses, fungal species including lichens usually have wider distributions than vascular plants (Lücking 2003; Shaw et al. 2003), and very few endemics are reported for island systems as Macaronesia, compared to what has been observed in vascular plants (Vandeproorten et al. 2008; Laenen et al. 2011). The distribution patterns of *Pectenia* species are similar to many other cryptogamic groups. This may reflect a dynamic exchange and gene flow between Macaronesia and Europe and North Africa, but so far there is no evidence supporting this lack of genetic isolation. As cryptic species have increasingly been documented in lichens (Crespo and Pérez-Ortega 2009; Crespo and Lumbsch 2010), it is not possible to discard cryptic speciation, where island lineages undergo a diversification after a historical colonization event. In this sense, a detailed phylogeographic study would allow us to understand the processes explaining the geographical distribution of species in *Pectenia*, and it would also contribute to the knowledge of the biogeographic relationship between Macaronesia and Europe.

The primary goal of this work is to study species boundaries in *Pectenia* and subsequently to elucidate the biogeographic history of the species. To reach these objectives, we conducted a comprehensive sampling across the geographic range of *Pectenia* in Europe and neighboring parts of Africa. We generated a DNA sequence data set consisting of three loci: the internal transcribe spacer of the nuclear ribosomal gene complex (ITS), and fragments of the nuclear gene coding for the first and second largest subunit of the DNA-dependent RNA polymerase II (RPB1 and RPB2). In order to infer species delimitation within *Pectenia*, we compared the results obtained from several approaches and methods using different criteria (Ané et al. 2007; Liu et al. 2009; Kubatko et al. 2009; Heled and Drummond 2010; Millanes et al. 2014), each with different strengths

and limitations (Yang and Rannala 2010; Zhang et al. 2011). We first analyzed the data within a phylogenetic framework in which clades present in the majority of single locus genealogies are considered distinct lineages (Dettman et al. 2003). Subsequently, we analyzed our data within a coalescence framework using two different approaches. For each of the delimited species we reevaluated their ecology and morphological characters, particularly the value of reproductive mode as predictor of monophyletic groups. With the purpose of investigate the origin and expansion of the species and understand the present day diversity and distribution, we reconstructed the ancestral area by a Bayesian approach .

2. Materials and Methods

In order to cover the entirety of the *Pectenia* species distribution and phylogenetic diversity, we included specimens collected by our working group in Spain, Canary Islands, Portugal and Scotland, and by other researchers in Scotland, Sweden, Azores and Madeira. Additionally, recently collected specimens deposited in European herbaria (BM, O, UPS, B, DBN, TNF) were also included. A total of 50 specimens of *P. plumbea*, 23 of *P. atlantica*, 15 of *P. cyanoloma* and 4 of *P. ligulata* where included (Table 1). Two species of the genus *Parmeliella* were used as outgroup, as this genus is one of the closest relatives to *Pectenia* (Ekman et al. 2014).

2.1. DNA sequencing

Total genomic DNA was extracted from a small section of thallus material using the DNeasy Plant Mini Kit (Qiagen), according to the manufacturer's instructions. Following

Otálora et al. (2013), ITS rDNA (internal transcribed spacer region), RPB1 (RNA polymerase II subunit 1) and RPB2 (RNA polymerase II subunit 2) were selected as molecular markers. The total DNA was used to PCR amplify the ITS rDNA region (including the 5.8S gene), the partial sequence of the protein coding RPB1 (spanning region B-F) and RPB2 (regions 5-7). ITS rDNA was amplified using the primer pair ITS5-ITS4 (White et al. 1990) or ITS5dg (5′-CCGAGAGCGAAGCCTGGC-3')-ITS4, according to a published method (Otálora et al. 2010). The protein coding RPB1 was amplified in most of the cases using RPB1-891F- RPB1-2450R (Otálora et al. 2013) following the published PCR conditions. For some herbarium collections the RPB1 was amplified in two short fragments using primers pairs RPB1-891F - RPB1-DGA456R (5′-CATCATCGCGAGCTTTGTTAAGTG-3') -and RPB1-2450R- DGA403F (5'TGCTACAGCGAACGAGCTTGAG-3'). The RPB2 fragment was amplified with the primers fRPB2-5F and fRPB2-7R (Liu et al. 1999, Matheny 2005) using PCR conditions described in Otálora el al. (2013). For RPB2, internally nested PCR reactions were performed using 1 µL of the PCR product from the first reaction and the internal specific primer RPB2-957R (Otálora et al. 2013). PCR amplifications were performed using Illustra^{TM} Hot Start PCR beads, according to the manufacturer's instructions. Before sequencing PCR products were subsequently purified using the enzymatic method ExoSAP-IT (USB Corporation, Cleveland, OH). DNA sequences were compared with sequences in the GenBank database using the BLAST algorithm (Altschul et al. 1990) to verify the amplification of the correct regions. The sequences were aligned manually using MacClade 4.01 (Maddison and Maddison 2001). Sequences were collapsed into haplotypes using the tool COLLAPSE in the program ALTER (Glez-Peña et al. 2010). One sequence

from each haplotype was submitted to Genbank (Table 1, S1). Evidence for linkage disequilibrium between pairs of loci was assessed using the Recombination Detection Program v. 3.44 (RDP3) (Martin et al. 2010).

2.2. Phylogenetic analysis

Phylogenetic relationships were inferred using maximum likelihood (ML) and Bayesian analysis (MCMC). The combinability of the single locus data sets was assessed by visual inspection of the individual bootstrap values (Mason-Gamer and Kellogg, 1996). A conflict was considered significant when two data partitions supported conflicting monophyletic group with ML bootstrap values ≥ 70% in both trees. Because no significant conflicts were detected, the two data sets were considered congruent and could be combined. Maximum likelihood (ML) analyses were carried out in RAxML-HPC2 (Stamatakis 2006) for the individual datasets, as well as the concatenated dataset. Parameters for the analyses incorporated the GTRGAMMA model of evolution based on 1000 random addition sequence replicates. Branch support values were computed via 1000 non-parametric bootstrap replicates. The ML analyses were performed on the Cipres Web Portal (Miller et al. 2010). The evolutionary models for Bayesian analyses were selected using the Bayesian Information Criteria (BIC) as implemented in JModeltest 3.06 (Posada 2008). The K80+G model was used for the protein coding partitions, and HKY was selected for ITS. The Bayesian inference was performed using MrBayes 3.2.0 (Ronquist et al. 2012). Two runs of 2 million generations starting from an initial random tree and employing four simultaneous chains were executed. A tree was saved every 100th generations. To ensure that stationary was reached and the runs converged at the same log-likelihood levels, we plotted the log-likelihood scores of sample points against generation

time using Tracer 1.0. The first 5000 trees from each run were discarded as "burn-in". For the remaining trees in each analysis, a majority rule consensus tree was assembled using the sumt option of MrBayes. The .con file created by MrBayes was visualized in FigTree V.1.2.2 (Rambaut 2009).

In order to further elucidate the relationships between closely related haplotypes three haplotype networks were constructed by statistical parsimony using the computer program TCS 1.21, with a 95% confidence level. Haplotypes were connected by one connecting branch (Castelloe and Templeton 1994).

2.3. Delimitation of Species Boundaries

The delimitation of species boundaries was assessed using two Bayesian coalescent-based methods. We used the programs *BEAST (Bayesian Evolutionary Analysis Sampling Trees; Heled and Drummond 2010) and BEST (Bayesian Estimation of Species Trees; Edwards et al. 2007) to generate species trees from the concatenated dataset (excluding the outgroup in *BEAST) for the species scenarios suggested by the phylogenetic analyses. *BEAST estimates the species tree directly from the sequence data, and incorporates the coalescent process, uncertainty associated with gene trees, and nucleotide substitution model parameters (Heled and Drummond 2010). We run four independent MCMC analyses of 100 million generations with a burn-in of 1 million generation of each run. We selected the same optimal models of molecular evolution for each locus used in the MrBayes analyses, the Yule process model, constant population size for species tree priors and the strict clock as the molecular clock model. We assessed convergence by comparing the topologies and support values of all independent *BEAST

runs, and then we combined the results after burn-in from all runs using LogCombiner v1.6.1 (Drummond and Rambaut 2007) and examined the effective sample sized (ESS) in TRACER v1.5 (Drummond and Rambaut 2007). Posterior probabilities of nodes were computed from sampled trees after burn-in. The results from the species scenarios were compared with a Bayes Factor (BF) approach. BF calculates the ratio of the marginal likelihood of two models, which has the advantage of taking into account priors used in Bayesian analyses (Xie et al. 2011). The marginal likelihood values of the two competing scenarios were estimated using path sampling (PS) and stepping-stone sampling (SS) as suggested by Baele et al. 2012a and Beale and Lemey using BEAST v 1.8.3.

BEST is a Bayesian approach that approximates the posterior distribution of species trees from the posterior distributions of gene trees and therefore estimates branch lengths of both the species trees and the gene trees (Edwards et al. 2007). To estimate the species tree using BEST we ran four independent analyses, each with two chains, for 20 million generations and sampled trees every 1000 generations. We used same substitution model as MrBayes, and used the default BEST settings for "thetapr" and "genemupr" parameters. We assessed convergence by visually comparing the topologies of the four independent BEST analyses (Linnen and Farrell 2008).

2.4. Biogeographical reconstruction

For the biogeographical reconstruction, specimens where assigned to biogeographical regions following the bioclimatic classification of the European Environment Agency (http://www.eea.europa.eu/legal/copyright) with some modidications following Ortega et al. (2012) (Fig.1, Table 1). We reconstructed the ancestral area

following an indirect Bayesian approach to character state reconstruction using SIMMAP v1.5 (Huelsenbeck et al. 2003; Bollback 2006). SIMMAP implements a post tree analysis for the stochastic mapping of characters to infer character evolution. It summarizes the character maps by calculating posterior predictive P-values from posterior probabilities (PP). The option "multiple mapping" was used over the last 10,000 trees resulting from Bayesian analysis. For each Bayesian tree sampled with SIMMAP during the ancestral area reconstruction, 1000 draws were carried out, with outgroup lineage excluded for the analyses. Four states were used for the area distribution character (according to Fig. 1): 0 = Macaronesia, 1= Atlantic, 2= Mediterranean, 3= North America.

3. Results

3.1. Phylogenetic analyses

The three-locus data set contained 1947 unambiguously aligned sites of which 442, 740 and 765 corresponded to nuITS rDNA partition, RPB1 and RPB2 respectively. A total of 235 sites were variable of which 71 belonged to the nuITS , 70 to RPB1 and 85 to RPB2. Excluding the outgroup taxa the variable sites were 21, 14 and 26 for ITS, RPB1 and RPB2 respectively. The software RPD3 did not detected recombination events among the three molecular markers.

Maximum Likelihood analysis resulted in a single most likely tree with a ln-likelihood of -2201.01. The majority rule consensus tree from the Bayesian analysis was based on 30000 credible trees from two runs. The harmonic mean ln-likelihood was -

2267.48. The tree topologies obtained by the maximum likelihood and a Bayesian approaches did not show any supported conflict and therefore only the 50% majority rule consensus tree from Bayesian analysis is shown, with both bootstrap support and posterior probabilities (Fig. 2). The traditional four species classification was not recovered by any of the two analyses. Three putative lineages can be delimited. Clade I, recovered as monophyletic in both analyses, includes all samples of *P. plumbea*, the atypical inland morphotype samples of *P. atlantica*, and some individuals of *P. ligulata*. Clade II and III together form an unsupported lineage that include individuals of *P. atlantica* s. str. and *P. cyanoloma*. Unlike the poorly supported clade II that includes specimens from almost the entire distribution area of *P. atlantica* s. str and *P. cyanoloma* excluding Canary islands, the well-supported clade III includes specimens only from Canary Islands, Madeira and Portugal.

3.1.1. Haplotype network

The dataset excluding the two outgroup samples comprises 22, 12 and 15 unique haplotypes for ITS, RPB1 and RPB2 respectively. Single-locus parsimony analyses produced three single networks connecting all haplotypes with 95% probability (Fig. 3). A general concordance is observed between the haplotype networks and the topology obtained from phylogenetic analyses. The three putative lineages were recovered in the ITS haplotype networks while RPB1 and RPB2 only recovered two lineages; clade I and clade II+III. Three steps mutation is the largest distance among groups in ITS, five in RPB1, and 11 in RPB2.

3.1.2. Species delimitation

The Bayes factor (BF) comparisons, of the *BEAST species trees scenarios, slightly favour the three species scenario over the two as reflected by the positive BF values (Table 2). Similarly BEST supports the existence of the three putative species with 0.96 of posterior probability on the node splitting clade II and III (Fig. 4).

By contrast our morphological characterization of the studied samples supports a two species model. Lobe segmentation characterizes monophyletic groups; where the lineage formed by clades II and III have lobes that are transversally strongly segmented, while clade I lack this characteristic.

3.3. Biogeographical reconstruction

The distribution range is wider for Clade I, where the representatives are found in the Mediterranean, Atlantic and Macaronesian regions, with rare records in North America. The samples of the lineage formed by clades II and III are exclusively distributed in the Macaronesian and Europa-Atlantic biogeographic regions, with a clear north-south pattern. Clade II exhibits a northern distribution (Scandinavia, British Islands, Portugal, Madeira and Azores). Clade III with a more south distribution includes only specimens from Madeira, Canary Islands and Portugal. The results of the ancestral range reconstruction analysis are summarized in Fig. 4. The Mediterranean basin was recovered as the most likely ancestral distribution area of *P. plumbea* (PP 0.97). Although without support, our analysis suggests a Macaronesian origin of the lineage formed by *clade* II & III (PP 0.57).

4. Discussion

Accurate species delimitations are of critical importance in many areas of biology as evolutionary and conservation biology (Bickford et al. 2007). Different DNA species delimitation methods can support or contradict traditional species boundaries (de Queiroz 2007; Reeves and Richards 2011). Several approaches and methods, using different criteria, have been developed to infer species delimitation (Heled and Drummond 2010), each with different strengths and limitations (Yang and Rannala 2010, 2014; Zhang et al. 2011). Due to multiple evolutionary processes within and between populations, the developed methods sometimes fail to delimit species boundaries properly or give conflicting results (Zhang et al. 2011). Therefore, it is necessary to evaluate different approaches with the same dataset, compare the results and adopt the criteria that better fix either morphology or ecology, or distribution (de Queiroz 2007; Reeves and Richards 2011). The two Bayesian coalescent-based methods suggest that *Pectenia* may be interpreted as comprising three species instead four. The resulted three species scenario is less diverse than and also incongruent with the current phenotypically-based species circumscriptions, which use reproduction system as diagnostic trait to differentiate species. Nevertheless, a three species scenario has no apparent support from morphology, as we were unable to find any morphological differences between clade II and III. No conclusive differences were found between individuals from clades II and III further than a geographic pattern. Both clades include indistinctly representatives of the putative species P. atlantica (isidia) and P. cyanoloma (apothecia) being both type localities in clade II only. The split between clade II and III reveals a case of phenotypically cryptic speciation with an evident north – south parapatric geographic pattern. Clade II has a more northern distribution (Norway, Britisch Islands, Azores and Madeira) compared to the clade III (Madeira, Portugal and Canary Islands).

Reproductive isolation in oceanic Islands and the posterior speciation between parapatric populations is not that common process in the evolution cryptogams including lichens, which have a high capacity for long-distance dispersal (Otalora et al 2010, Patiño & Vanderpoorten 2005), nevertheless some cases of island endemism have been found, where local adaptation contribute to the reproductive isolation and the posterior speciation. These two contrasting patter are occurring in the case the putative species P. atlantica where a partial island endemism (Clade III) is found, with some dispersal events (Madeira and Portugal) and the wide distributed Clade II. The fact that we do not detect phenotypic differences between clade II and III (cryptic species) suggest a recent speciation.

Morphologically we recognize here only two species: *P. plumbea* (clade I) and *P. atlantica* (including lineages II and III*)*, each characterized by having a specific lobes type in terms of texture and shape. The lobes in *P. atlantica* are composed of concave, successive trough-shaped segments each ending in a transverse curved structure, additionally the lobe surface is finely to strongly longitudinally striate. *Pectenia plumbea*, by contrast, lacks the concave transversal segmentation, and the lobe surfaces produce a very typical irregular network of narrow whitish discoloration. Both species may produce isidia, but the occurrence and abundance is higher in *P. atlantica* than *P. plumbea*. Although both species are oceanic, the distribution of *P. plumbea* extends to the Mediterranean, Atlantic and also Boreal and Continental biogeographic regions. The Mediterranean morphotype of *"P. atlantica"* (a distinct morphotype from *P. atlantica* s. str. according to Jørgensen 1978) fits well in *P. plumbea* as expressed by the lobe characteristics, ecology and distribution. The presence of ligulae is a rare, homoplastic character, present in both the *P. atlantica* and *P. plumbea*

lineages, and clearly does not characterize a separate species. The lobes features of the type specimens of *P. ligulata* (BM 0734845, BM 0734920) coincide with the typical of *P. plumbea*. Other "*P. ligulata*" specimens can easily be identified to the correct species by their lobe characteristics.

Neither the phylogenetic (ML and Bayesian analyses) nor the coalescent-based methods showed a correlation between reproductive mode and lineages. This agrees with many previous studies of "species pairs" in lichenized fungi, and suggests that reproductive mode is not a diagnostic character for species delimitation among the *Plectenia* species. However, it partly contradicts the results of a local population genetics study in central Spain, where a weak isolation and weak differentiation between isidiate and apothecia-producing individuals was detected (Otálora et al. 2013). When extending the sampling to the entire geographic distribution of *P. plumbea* (Clade I), we found that isidiate and apotheciate specimens share ITS and RPB1 haplotypes (Fig. 1). This suggests that it is very important to cover the entire distribution of the species in studies of this type.

Taxonomy

Pectenia atlantica (Degel.) P. M. Jørg., L. Lindblom, Wedin & S. Ekman, Lichenologist 46: 652. 2014; type: Ireland, Killarney, near Muckross lake, 1933, Degelius (UPS, holotype).

= *Pectenia cyanoloma* (Schaer.) P. M. Jørg., L. Lindblom, Wedin & S. Ekman, Lichenologist 46: 652. 2014; type: France, Normandie, in sylva Briquebec, Delise (G-lectotype, Jørgensen 1978).

Pectenia plumbea (Lightf.) P.M. Jørg., L. Lindblom, Wedin & S. Ekman, Lichenologist 46: 652. 2014; type: Great Britain (OXF-DILL179: 73a lectotype, fide Jørgensen 1978).
= *Pectenia ligulata* (P.M. Jørg. & P. James) P.M. Jørg., L. Lindblom, Wedin & S. Ekman, in Ekman, Wedin, Lindblom & Jørgensen, Lichenologist 46(5): 652 (2014); type: Azores, on mossy earth bank, 1979, James (BM, holotype)

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Figures captions

Figure 1. Sampling distribution of *Pectenia* species according to the current species concept, blue circles (*P. atlantica*), red circles (*P. plumbea*), brown circles (*P. ligulata*) and purple circles (*P. cyanoloma*)

Figure 2. Phylogenetic relationships of the *Pectenia* species. Tree corresponds to the 50% majority rule consensus from Bayesian analysis. Significant Bayes posterior probabilities/ML bootstrap values are indicated on each branch. Branches with posterior

probabilities equal or above 0.95 and ML bootstrap values equal or above 70% are in bold. Type of propagules of each specimen are showed with colour circles.

Figure 3. Unrooted statistical pasimony haplotype networks at 95% probability of ITS, RPB1 and RPB2 loci. Each geographical region is designed by different color.

Figure 4. Species delimitation scenarios through the different methodologies and criteria including morphology. Lobes morphology, reproduction system and geographic origin are indicated as well as results of ancestral origin reconstruction.