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Ecología y conservación del liquen *Lobarina scrobiculata* (Scop.) Nyl. ex Cromb. en la Península Ibérica

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TESIS DOCTORAL

ECOLOGÍA Y CONSERVACIÓN DEL LIQUEN *LOBARINA SCROBICULATA* (SCOP.) NYL. EX CROMB. EN LA PENÍNSULA IBÉRICA

ECOLOGY AND CONSERVATION OF THE LICHEN *LOBARINA SCROBICULATA* (SCOP.) NYL. EX CROMB. IN THE IBERIAN PENINSULA

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Dra. Isabel Martínez Moreno y Dr. Gregorio Aragón Rubio, ambos Profesores Titulares del Departamento de Biología y Geología, Física y Química Inorgánica de la Universidad Rey Juan Carlos,

Certifican:

Que los trabajos de investigación desarrollados en la memoria de tesis doctoral: “Ecología y conservación del liquen *Lobarina scrobiculata* (Scop.) Nyl. ex Cromb. en la Península Ibérica” son aptos para ser presentados por la Lda. Sonia Merinero Mesa ante el tribunal que en su día se consigne, para aspirar al Grado de Doctor en Conservación de Recursos Naturales por la Universidad Rey Juan Carlos de Madrid.

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Dr. Gregorio Aragón Rubio

A todas las personas que han dedicado y
dedican su vida a hacer ciencia “básica”, porque
sin ellas no habría y no habrá futuro

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RESUMEN ABREVIADO

Reducir la constante pérdida de biodiversidad constituye uno de los principales retos a los que se enfrenta la sociedad del siglo XXI. Consecuentemente, los científicos deben prestar atención a la situación de las especies amenazadas, diseñando estrategias efectivas para garantizar su persistencia. El éxito de estas estrategias reside en el conocimiento exhaustivo de la ecología y los procesos vitales de las especies en cuestión. La mayoría de los esfuerzos dirigidos a la conservación de la biodiversidad han tenido como foco grupos de organismos emblemáticos como mamíferos y plantas. Sin embargo, el conocimiento sobre la ecología y la conservación de organismos menos carismáticos es muy limitado. En este caso, los líquenes constituyen un claro ejemplo de estos organismos. Por este motivo, el objetivo general de esta tesis doctoral es contribuir a mejorar el conocimiento sobre la ecología del cianoliquen epífito *Lobarina scrobiculata* en la Península Ibérica, mediante la evaluación integrada de la biología de sus poblaciones y el desempeño de los individuos a distintas escalas espacio-temporales.

A escala regional, se muestrearon 22 poblaciones de esta especie a lo largo de la Península Ibérica y se caracterizaron sus requerimientos ecológicos y ambientales. Además, se examinó la variación de la estrategia reproductiva de *L. scrobiculata* a lo largo de dicho gradiente. A escala local, llevamos a cabo un experimento factorial de trasplantes para evaluar los principales factores ambientales y específicos de las especies, que afectan al crecimiento de *L. scrobiculata* y *Lobria pulmonaria* en bosques mediterráneos. Además, nos centramos en el estudio de dos poblaciones del centro peninsular donde estudiamos la estructura demográfica, la variación de la estrategia reproductiva y las tasas de crecimiento de *L. scrobiculata* durante dos años en dos hábitats estructuralmente diferentes dentro del bosque. Finalmente, se evaluó la relación existente entre el contenido en metabolitos secundarios y la presencia del hongo parásito *Plectocarpon scrobiculatae*.

Lobarina scrobiculata habita preferentemente en robledales mediterráneos en la Península Ibérica. Es una especie capaz de colonizar bosques con un amplio rango de precipitaciones, aunque su abundancia se ve favorecida a elevados niveles de precipitación. A pesar de la aceptada asunción de que se trata de una especie asociada a bosques antiguos y comunidades epífitas climácicas, en la Península Ibérica es más frecuente y abundante en bosques con alta densidad de árboles de pequeño diámetro (indicadores de bosque joven y/o manejado). La estrategia reproductiva de esta especie varía con la precipitación. En las localidades más húmedas comienza a reproducirse a mayor tamaño y su asignación reproductora asexual es menor que en las localidades más secas, probablemente debido a diferencias en las tasas de crecimiento, que se traducen en edades diferentes. *L. scrobiculata* es una especie menos flexible que *L. pulmonaria*, ya que esta última crece y se aclimata a nuevas condiciones ambientales mucho más rápido. Ambas especies crecieron más rápido en las bases de los árboles y mostraron tasas de crecimiento similares a las registradas en climas más húmedos, sugiriendo que las condiciones del clima mediterráneo son favorables para el desarrollo de estas especies. Asimismo, en los árboles, *L. scrobiculata* crece más despacio, comienza a reproducirse a menor tamaño y muestra mayor asignación reproductora que cuando crece sobre rocas musgosas. Por tanto, la heterogeneidad de hábitats a escala local determina la demografía y la ecología de esta especie. Finalmente, los individuos de *L. scrobiculata* infectados por *Plectocarpon* contienen la mitad de metabolitos secundarios que los individuos sin signos de infección, indicando que los rasgos químicos del hospedador influyen fuertemente en este tipo de relaciones tan específicas.

En conclusión, las amenazas más directas sobre *L. scrobiculata* están relacionadas con el deterioro de la calidad del hábitat, por lo que es necesario preservar la estructura forestal de los bosques en los que habita esta especie y de los fragmentos de bosque donde potencialmente pudiera expandirse, así como mantener la heterogeneidad de hábitats dentro de los bosques. El conjunto de estos resultados, junto con datos de diversidad genética de las poblaciones que serán obtenidos en un futuro, nos permitirá evaluar el estado

de conservación de *L. scrobiculata* y diseñar estrategias eficaces para su conservación.

SUMMARY

The increasing loss of biodiversity is one of the major challenges faced by the society in the XXI century. Consequently, scientists may pay attention to threatened species to design effective conservation strategies guaranteeing their persistence. Successful conservation strategies rely on the exhaustive knowledge of the life processes and ecology of target species. Most conservation efforts have been directed toward emblematic organisms such as mammals and plants. However, knowledge on the ecology and conservation of less charismatic organisms is strongly limited. Lichens are a good example of these organisms. For this reason, the general aim of this PhD thesis is to contribute to improve the knowledge on the ecology of the epiphytic cyanolichen *Lobarina scrobiculata* in the Iberian Peninsula, using an integrative evaluation of its population biology and individual performance at different spatio-temporal scales.

At the regional scale, we sampled 22 populations of this species along the Iberian Peninsula, and its ecological and environmental requirements were characterized. Moreover, we examined the variation of the reproductive strategy of *L. scrobiculata* along this gradient. At the local scale, we carried out a factorial transplant experiment to assess the main environmental and species-specific factors affecting the growth rates of *L. scrobiculata* and *Lobaria pulmonaria* in Mediterranean forests. Besides, we focused on two populations in central Spain, where we studied the demographic structure, the variation of the reproductive strategy and the growth rates of *L. scrobiculata* for two years in two structurally contrasting habitats within forests. Finally, we studied the relationship between the secondary metabolites contents of *L. scrobiculata* and the presence of the parasitic fungus *Plectocarpon scrobiculatae*.

Lobarina scrobiculata mainly thrives in Mediterranean oak forests along the Iberian Peninsula. This species is able to colonize a wide range of locations differing in annual precipitation, although its abundance is enhanced in forests receiving higher levels of precipitation. Despite the widely accepted assumption of its old-forest dependency and its categorization as a late-successional species of epiphytic communities, in the Iberian Peninsula this cyanolichen is more frequent and abundant in forests with high density of small trees (indicators of young and/or managed forests). The reproductive strategy of this species varied with precipitation. In more humid locations it started to reproduce at larger sizes and showed lower reproductive allocation than in drier populations, probably due to differences in the growth rates that are translated into different individual ages. *L. scrobiculata* is less flexible tan *L. pulmonaria*, because the latter grew and acclimated faster to new environmental conditions than the former. Both species grew faster on the tree basis and showed similar growth rates than those documented in more humid climates, suggesting that the Mediterranean climatic conditions are favourable for the development of these species. Likewise, on trees, *L. scrobiculata* grew slower, started to reproduce at smaller sizes and showed higher reproductive allocation than when it grew on mossy rocks. Therefore, habitat heterogeneity at the local scale determined the demography and the ecology of this species. Finally, individuals visibly infected by *Plectocarpon*, had half of the secondary metabolites contents than their non-visibly infected counterparts, indicating the chemical traits of the lichen host strongly influence this type of highly specific relationships.

In conclusion, the main threat factors over *L. scrobiculata* are related to the habitat quality deterioration. Consequently, it is required to maintaining the forest structure where *L. scrobiculata* thrives, as well as the forests fragments that the species may potentially colonize and preserving the habitat heterogeneity within the forests. Overall, these results, together with future data on the population genetic diversity, will allow us to assess the conservation status of *L. scrobiculata* in the Iberian Peninsula and designing effective conservation strategies.

ANTECEDENTES

La actual pérdida de biodiversidad es uno de los mayores retos a los que se enfrenta la sociedad en el siglo XXI. Por tanto, los científicos deben prestar especial atención a las especies amenazadas y diseñar planes de conservación para intentar garantizar su supervivencia (Balmford et al. 2005a,b). Las estrategias efectivas para la conservación de especies amenazadas dependen del conocimiento exhaustivo de sus procesos vitales y su ecología (Schemske et al. 1994; Iriondo et al. 2009). La viabilidad de las poblaciones depende de tres factores que, en conjunto, marcarán la tendencia demográfica de la población: (1) el tamaño de la población; (2) el desempeño de los individuos (i.e., crecimiento, reproducción y supervivencia); y (3) las relaciones interespecíficas (i.e., competencia, facilitación, parasitismo o mutualismo). Estos factores normalmente se encuentran asociados a la calidad del hábitat ya que, generalmente, las poblaciones grandes y con dinámicas de población positivas se encuentran en ambientes favorables (Soulé 1987; Schemske et al. 1994; Iriondo et al. 2009). Identificar los factores ambientales que afectan a las poblaciones de las especies amenazadas implica considerar diferentes escalas espaciales porque los procesos ecológicos operan a diferentes niveles, pudiendo generar diversos patrones que pueden explicar la distribución, abundancia y desempeño de las especies (Wiens 1989; McGill 2010).

La mayor parte de los esfuerzos dirigidos a entender la ecología de especies amenazadas con el fin de su conservación se han centrado en grupos de organismos emblemáticos y ampliamente estudiados como mamíferos, aves y plantas vasculares. Sin embargo, desafortunadamente, aún tenemos un conocimiento muy limitado de la ecología y la conservación de organismos menos carismáticos y consecuentemente más desconocidos, como por ejemplo, los líquenes (Hylander & Jonsson 2007).

La conservación de especies de líquenes

Los líquenes son organismos fruto de millones de años de evolución (Prieto & Wedin 2013) y representan una asociación simbiótica entre un hongo (micobionte) y uno o dos organismos fotoautótrofos unicelulares (fotobionte). Estos productores primarios incluidos en el Reino Fungi constituyen un componente ubicuo en los ecosistemas terrestres, y desempeñan un importante papel en el funcionamiento de dichos ecosistemas, ya que participan en los ciclos biogeoquímicos, especialmente en el ciclo del nitrógeno, y sirven de recurso trófico y de refugio para la fauna (ver revisiones por Seaward 2008; Ellis 2012). Sin embargo, por razones históricas relacionadas con su pequeño tamaño, los líquenes pasan desapercibidos para el público general y normalmente no resultan suficientemente atractivos ni emblemáticos para los gestores de la conservación de la biodiversidad (Scheidegger & Goward 2002). De hecho, convenios internacionales sobre diversidad biológica, por ejemplo la “Convención de la Diversidad Biológica” (CBD Secretariat 2005) o la “Convención sobre el Comercio Internacional de Especies Amenazadas de Fauna y Flora Silvestres” (CITES) (http://www.cites.org), hacen escasa o nula referencia a especies del reino Fungi (Dahlberg et al. 2010).

No obstante, a partir de 1970 en países con una arraigada tradición botánica y naturalista, como por ejemplo Reino Unido o Escandinavia, se comenzó a alertar del declive de ciertas especies de líquenes y, por tanto, a demandar medidas para su conservación (Ferry et al. 1973; Gilbert 1977; Esseen et al. 1981; During & Willems 1986; Hallinbäck 1989; Gauslaa 1995). En Europa, Séruxiaux (1989) se convirtió en un referente al publicar la primera lista roja de macrolíquenes epífitos amenazados en el continente. A partir de aquí, el número de listas rojas y otras herramientas para asesorar sobre el grado de amenaza de líquenes fue aumentando progresivamente, como por ejemplo en Alemania (Wirth et al. 2011), Suiza (Scheidegger et al. 2002), Suecia (Thor et al. 2010), Noruega (Timdal et al. 2006) o Canadá (COSEWIC Species Database; http://www.cosewic.gc.ca). Además, dos especies de líquenes, *Erioderma pedicellatum* (Hue) P. M. Jørg. y *Cladonia perforata* A. Evans, se incluyeron en la Lista Roja de Especies Amenazadas de la Unión Internacional para la Conservación de la Naturaleza (UICN) en la categoría de “En Peligro Crítico” (Scheidegger 2003; Yahr 2003) gracias a detallados estudios cuantitativos ecológicos y demográficos. Fruto de estos trabajos se implementaron medidas de conservación como (1) la prohibición del aprovechamiento forestal en los bosques donde habita *E. pedicellatum* y donde potencialmente puede dispersarse en Canadá (Environment Canada 2007; Cameron et al. 2013; Stehn et al. 2013), y (2) la protección del hábitat y reintroducción de especímenes de *C. perforata* (Yahr 2003). No obstante, la dificultad de encajar las características biológicas de los líquenes y sus poblaciones en los criterios de la UICN (diseñados inicialmente para mamíferos, aves y plantas vasculares) complica la inclusión de más especies liquénicas en estas listas rojas (Scheidegger & Goward 2002).

El principal reto al que se enfrentan los científicos a la hora de diseñar estrategias para conservar especies de líquenes está relacionado con la falta de conocimiento sobre la distribución y la ecología de las especies. No obstante, gracias a recientes estudios sobre la ecología de líquenes epífitos, abordados desde diferentes aproximaciones y escalas, comenzamos a comprender qué factores ambientales son relevantes para estos organismos. A escala regional, las condiciones climáticas y la estructura del paisaje influyen en la distribución y funcionamiento de las especies, mientras que los requerimientos de calidad del hábitat son determinados a escalas local (Barkman 1958; Johansson & Ehrlén 2003; Scheidegger & Werth 2009; Marini et al. 2011). Cuando los requerimientos a gran escala favorecen, o al menos no limitan la presencia de determinadas especies, la estructura del bosque constituye el principal factor determinante de la calidad del hábitat para estos organismos a escala local (Barkman 1958; Ellis 2013). En este sentido, numerosos trabajos en diferentes climas han puesto de manifiesto la importancia de mantener la estructura de bosques antiguos y promover un manejo forestal sostenible que preserve la diversidad liquénica (Sillet et al. 2000; Aragón et al. 2010; Ellis 2012). Por ejemplo, se proponen medidas dirigidas a mantener la diversidad de especies arbóreas, conservar árboles de diferentes edades que generen la estructura de un dosel apropiado, e intentar introducir estas características en bosques ya manejados (Hedenås & Hedström 2007; Vuidot et al. 2011; Ellis 2012). Finalmente, a microescala, las características de los árboles determinan la heterogeneidad y disponibilidad de microhabitats y microclimas adecuados que condicionan la distribución y abundancia de los líquenes epífitos (Ranius et al. 2008; Belinchón et al. 2011; Vuidot et al. 2011; Rubio-Salcedo et al. 2015). Las características de los árboles más relevantes son la edad y el tamaño de los árboles, el pH y la rugosidad de la corteza, o la inclinación del tronco (Barkman 1958; Ranius et al. 2008). Estos factores, junto con gradientes de luz y humedad a lo largo del dosel (Geiger 1950), determinan el establecimiento de los líquenes y la configuración y composición de las comunidades epífitas.

Por tanto, a la hora de diseñar estrategias para conservar especies de líquenes epífitos es necesario, en primer lugar, identificar los factores climáticos y ambientales que determinan la presencia y el tamaño de las poblaciones en un territorio determinado, considerando todo el rango de hábitats en los que aparece. En segundo lugar, es necesario estimar la dinámica de las poblaciones a escala local, mediante la evaluación del crecimiento, la reproducción y la supervivencia de los individuos dentro de las poblaciones, variables que a su vez dependen de los factores a escala de microhábitat.

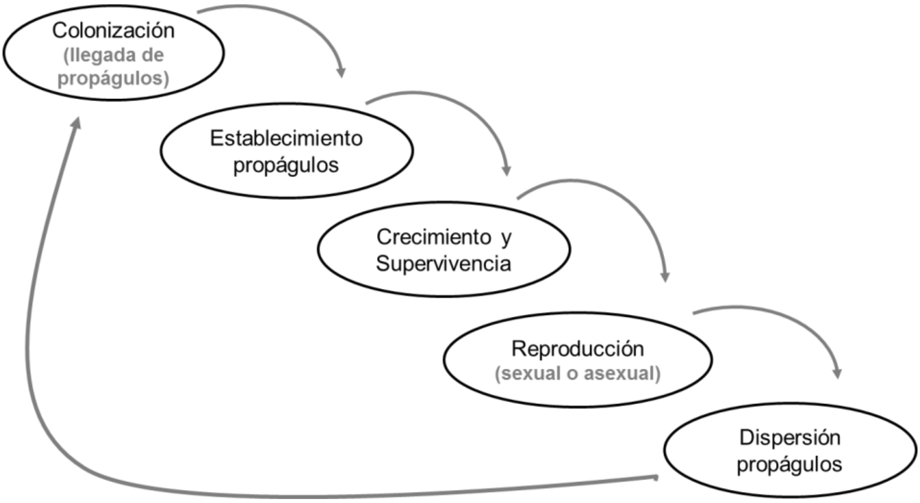
Características biológicasy ecológicas de los líquenes

Los líquenes son organismos poiquilohídricos, es decir, carecen de mecanismos para regular la captación y pérdida de agua y, adquieren los nutrientes de fuentes atmosféricas (Nash 2008). Consecuentemente, estos organismos fotosintéticos dependen estrechamente de las condiciones ambientales en las que se encuentren, concretamente del equilibrio entre la disponibilidad de agua, luz y nutrientes, lo que les hace ser especialmente sensibles a cambios en el microclima (Palmqvist 2000; Nash 2008; Aragón et al. 2010). En concreto, el tipo de fotobionte de la simbiosis condiciona los requerimientos hídricos de cada especie. Los líquenes con cianobacteria (i.e., cianolíquenes) son fijadores de nitrógeno inorgánico atmosférico y requieren agua líquida para poder activar la fotosíntesis, mientras que los líquenes con alga verde (clorolíquenes) se activan con la humedad ambiental (Lange et al. 1986, 1993; Nash 2008). Por otro lado, la mayoría de las especies son sésiles, lo que supone una alta dependencia del tipo y calidad del sustrato sobre el que habitan (e.g., árboles, rocas, suelo, musgos, hojas o madera muerta). En el caso de los líquenes epífitos esta dependencia se acentúa aún más, ya que el destino del liquen se encuentra íntimamente ligado a la esperanza de vida de su árbol hospedador (Scheidegger & Werth 2009; Shriver et al. 2012).

Como se mencionaba anteriormente, la viabilidad de una población dependerá, entre otros factores, del desempeño de los individuos que la componen (supervivencia, crecimiento, reproducción) (Iriondo et al. 2009). Para valorar el desempeño de los individuos dentro de una población es necesario establecer clases de edad o tamaño, fundamentadas en el conocimiento de las etapas del ciclo vital de las especies para que estas clases tengan sentido biológico. Sin embargo, en el caso de los líquenes el establecimiento de estas clases es extremadamente complicado, pues no conocemos los factores responsables de los cambios entre las etapas del ciclo de vida de estos organismos (Fig.1). Por otra parte, uno de los aspectos más desconocidos y a la vez fundamentales a la hora de evaluar la viabilidad de una población, es conocer qué factores determinan la reproducción.

Los líquenes pueden reproducirse de manera sexual, mediante pequeñas esporas del hongo que se generan en unas estructuras llamadas apotecios, y/o de manera asexual, normalmente mediante propágulos simbióticos más grandes que las esporas (e.g. isidios o soredios), donde los componentes de la simbiosis se dispersan juntos (Bowler & Rundel 1975). El tipo de reproducción es crucial porque condiciona la capacidad de dispersión de las especies, ya que presumiblemente la reproducción sexual favorece la dispersión a larga distancia, aunque con la desventaja de tener que encontrar el fotobionte adecuado para restablecer la simbiosis (Bowler & Rundel 1975; Scheidegger & Werth 2009). Por el contrario, la reproducción mediante propágulos simbiontes asexuales supone una menor distancia de dispersión, pero una mayor ventaja a la hora de establecerse ya que ambos simbiontes se dispersan juntos. No obstante, no ha de confundirse la capacidad de dispersión con la capacidad de colonización y establecimiento de los propágulos (e.g., Werth et al. 2006) (Fig. 1), ya que el establecimiento de las diásporas suele requerir unas condiciones ambientales y de calidad del hábitat muy específicas (e.g., Topham 1977; Hilmo & Ott 2002; Lättman et al. 2009). Considerando toda esta serie de dificultades, sólo unos pocos trabajos han intentado abordar la ecología y la dinámica poblacional de líquenes epífitos desde diferentes aproximaciones demográficas (e.g. Rhoades 1983; Goudie et al. 2011; Shriver et al. 2012; Eaton & Ellis 2014).

Figura 1. Principales etapas del ciclo de vida de los líquenes epífitos (adaptado de Scheidegger & Werth 2009).



Por último, otro aspecto importante que influye en la dinámica poblacional de muchos organismos son las interacciones interespecíficas, como por ejemplo las relaciones de parasitismo (Begon et al. 1996; Loreau et al. 2005). Los líquenes constituyen un hábitat esencial para más de 1.500 especies de hongos que normalmente se consideran parásitos, aunque varían en virulencia y especificidad por su hospedador (Hawksworth 1982; Lawrey & Diederich 2003). Sin embargo, el conocimiento sobre estas relaciones parásito-hospedador es sorprendentemente muy escaso (Lawrey & Diederich 2003). Por tanto, es necesario comprender el funcionamiento básico de estas relaciones y evaluar los efectos del parasitismo en estos organismos simbiontes para, posteriormente, considerar sus posibles efectos en la dinámica poblacional de los líquenes hospedadores.

Principales retos para la conservación de los líquenes en la Península Ibérica

Considerando los diversos rasgos de historia de vida de los líquenes, como su naturaleza poiquilohídrica y generalmente sésil, o la diversidad de mecanismos de reproducción y dispersión, las principales amenazas para estos organismos son la contaminación atmosférica, la destrucción y fragmentación de los hábitats en los que habitan y el cambio climático (Scheidegger & Werth 2009; Aragón et al. 2010; Marini et al. 2011; Ellis 2012, 2013). La incidencia de estas amenazas, especialmente la destrucción y fragmentación de los hábitats y el cambio climático, se agravan aún más en ecosistemas terrestres mediterráneos. Estos ecosistemas muestran una heterogeneidad ambiental muy elevada a diferentes escalas debido principalmente a la alta variabilidad climática interanual y estacional, consecuencia de la situación intermedia entre regiones áridas y templadas, y a las intensas perturbaciones de carácter natural y antropogénico (Lavorel et al. 1998; Rodó & Comín 2001; Giorgi 2006; Blondel et al. 2010).

En concreto, en regiones como la Cuenca Mediterránea, una larga historia de cambio en los usos del suelo basada en la destrucción y degradación de los hábitats naturales, especialmente de los bosques (Scarascia-Mugnozza et al. 2000; Blanco et al. 2005), ha dado lugar a la actual configuración de un paisaje altamente fragmentado (Mesa 2002; Blondel et al. 2010). Por otra parte, en la Cuenca Mediterránea todos los modelos propuestos por el IPCC (2007) sugieren un aumento en la temperatura media anual y una fuerte reducción de las precipitaciones, que causará una sequía estival aún más prolongada. Por tanto, debido a la interacción entre estos dos factores de cambio, el clima y la fragmentación, es esperable, entre muchas otras consecuencias, que haya cambios en la distribución y abundancia de las especies (Forman 1995; Blondel et al. 2010).

La Península Ibérica es un enclave de alta diversidad biológica gracias a, entre otros factores, una elevada heterogeneidad geológica, geomorfológica y climática (Williams et al. 2000; Blanco et al. 2005), ya que confluyen tres regiones biogeográficas bien diferenciadas: la región Atlántica, que ocupa la franja norte, la región Alpina que ocupa una pequeña área en sistemas montañosos del noreste, y la región Mediterránea, que se extiende ampliamente por el resto del territorio (EEA 2009). En el caso de los líquenes se trata de un área muy interesante desde un punto de vista biogeográfico porque, por ejemplo, numerosas especies de líquenes epífitos de distribución (sub)oceánica aparecen tanto en bosques atlánticos del norte, como en bosques mediterráneos continentales (Rose 1988; Burgaz et al. 1994). Entre estas especies oceánicas destaca la presencia de numerosos cianolíquenes como *Collema* flaccidum (Ach.) Ach., *C. fasciculare* (L.) F.H.Wigg., *Degelia atlantica* (Degel.) M.Jørg. & P.James , *D. plumbea* (Lightf.) M.Jørg. & P.James, *Fuscopannaria ignobilis* (Anzi) M.Jørg. , *Leptogium saturninum* (Dicks.) Nyl. , *Lobarina scrobiculata* Scop. Nyl. ex Cromb, *Nephroma parile* (Ach.) Ach., *N. resupinatum* (L.) Ach., *Pannaria* conoplea (Ach.) Bory , o *Sticta* fuliginosa (Hoffm.) Ach. Las poblaciones de estas especies en la región mediterránea persisten en pequeñas manchas de bosques fragmentados y han de resistir la intensa sequía estival que caracteriza este clima tan variable. Presumiblemente, estas especies son capaces de persistir y mantenerse bajo estas condiciones climáticas subóptimas gracias al efecto conjunto de factores microclimáticos originados por la orogorafía del terreno (e.g. altitud, orientación de las laderas, circulación de los vientos), y las características del hábitat (por ejemplo un denso dosel arbóreo), que son capaces de compensar las condiciones macroclimáticas más adversas creando un microclima apto para la existencia de estas especies (Barkman 1958; Marini et al. 2011; Ellis 2013).

Los estudios poblacionales dirigidos a la conservación de líquenes en la Cuenca Mediterránea, principalmente en la Península Ibérica e Italia, se han centrado en la ecología, biología y genética de las poblaciones de la especie epífita modelo *Lobaria pulmonaria* (L.) Hoffm. (cefaloliquen) (Gaio-Oliveira et al. 2004; Belinchón et al. 2009, 2011; Nascimbene et al. 2010; Martínez et al. 2012; Otálora et al. 2011, 2015; Brunialti et al. 2015; Rubio-Salcedo et al. 2015) y, en menor medida en otras especies menos estudiadas (Martínez et al. 2014; Giordani et al. 2015; Núñez-Zapata et al. 2015). Los resultados de estos trabajos, junto con de los de estudios desarrollados en climas templados y boreales (e.g. Hallingbäck 1989; Werth et al. 2006; Öckinger & Nilsson 2010; Hilmo et al. 2011a, 2013; Jüriado et al. 2011; Scheidegger et al. 2012), nos han permitido tener un conocimiento preliminar sobre los tipos de respuesta de los líquenes epífitos a las condiciones de los bosques mediterráneos. No obstante, a pesar de estos valiosos esfuerzos, aún nos encontramos lejos de llegar a comprender la ecología y funcionamiento de los líquenes en estos bosques, por lo que su conservación en la Cuenca Mediterránea supone un gran reto y a la vez una tarea urgente. En concreto, en España, la elaboración de una lista roja siguiendo los criterios de la UICN constituye una tarea realmente difícil debido al gran desconocimiento que tenemos sobre la distribución, ecología, tamaños de las poblaciones, número de individuos maduros o la dinámica poblacional de las especies de líquenes amenazados.

Por todos estos motivos, son necesarios estudios ecológicos detallados de especies poco frecuentes y en las que se alerta un declive para evaluar su estado de conservación en un rango geográfico amplio (e.g. Martínez et al. 2003; Nascimbene et al. 2013). Estos estudios a nivel de especie deben abordarse desde diferentes escalas espaciales y temporales, y abordando aspectos corológicos, ecológicos, genéticos y demográficos, prestando especial atención al desempeño de los individuos dentro de las poblaciones y, concretamente, su crecimiento, reproducción y relaciones de parasitismo. El conjunto de toda esta minuciosa y valiosa información permitirá realizar un diagnóstico acertado sobre el estado de conservación de las especies liquénicas, permitiendo finalmente el diseño e implantación de estrategias efectivas para su conservación en la Península Ibérica.

¿Es el cianoliquen Lobarina scrobiculata *una especie amenazada en la Península Ibérica?*

Lobarina scrobiculata es un cianoliquen folioso con una distribución circumpolar incompleta, ya que es mucho más frecuente en la costas este y oeste de Norte América y Europa. En el hemisferio norte su rango de distribución se extiende desde regiones oceánicas boreales hasta regiones mediterráneas (e.g., Península Ibérica, Marruecos, Italia, Grecia, Túnez y Turquía) y, en el hemisferio sur aparece, en menor medida, en las zonas con influencia oceánica de África, Australia, sur de América y Nueva Zelanda (Nimis 1993; John 1996; Seaward 1996; Spribille et al. 2006; Smith et al. 2009; http://data.gbif.org). Esta especie epífita se establece en diferentes especies arbóreas, principalmente sobre los troncos de planifolios (e.g., robles, arces, hayas, álamos) y, con menor frecuencia en coníferas como piceas o abetos. También se ha descrito su presencia sobre taludes sombríos y rocas musgosas (Burgaz & Martínez 1999). Tradicionalmente, esta especie se ha asociado a las comunidades epífitas climácicas, conocidas como *Lobarion* (Barkman 1958; James et al. 1977; Rose 1988; Burgaz et al. 1994). Hace ya varias décadas numerosos estudios en Europa alertaron sobre el declive del Lobarion, y específicamente de *L. scrobiculata*, como consecuencia de la contaminación atmosférica (especialmente de la lluvia ácida), la destrucción de los bosques y el inadecuado aprovechamiento forestal (Rose 1988; Hallingbäck 1989; Burgaz et al. 1994; Gauslaa 1995). Por tanto, a pesar de su amplia distribución, *L.*

scrobiculata ha sufrido un acusado declive en Estados Unidos y Europa, ya que se estima una pérdida del 10 – 30% de localidades desde hace pocas décadas (Sérusiaux 1989; NatureServe 2014). Como consecuencia de este progresivo declive, *L. scrobiculata* ha sido incluida en varias listas rojas en América y Europa bajo diferentes categorías de amenaza, desde “vulnerable” a “extinta” (e.g., Serusiaux 1989; Scheidegger et al. 2002; Liška et al. 2008; Randlane et al. 2008; Thor et al. 2010; NatureServe 2014).

En la Península Ibérica *L. scrobiculata* aparece en bosques montanos situados en zonas con elevada humedad atmosférica, siendo frecuente en el Sistema Central, común en zonas con influencia atlántica en el noroeste y progresivamente más rara en el resto del territorio (Burgaz & Martínez 1999). En la región Mediterránea, Burgaz et al. (1994) sugirieron que debería catalogarse como especie “en peligro” dada la persistencia de sus principales amenazas: la destrucción y degradación de los bosques por el aprovechamiento forestal y la expansión de la agricultura intensiva. Una década más tarde, Martínez et al. (2003) basándose en algunos criterios de la UICN, la clasificaron como “vulnerable” en el centro peninsular. No obstante, carecemos de datos demográficos exhaustivos para esta especie, como por ejemplo el tamaño de sus poblaciones, la estructura de tamaños o etapas de vida de las mismas, su diversidad genética y los factores que afectan al crecimiento y a la reproducción de sus individuos. Por tanto, es necesario recabar este tipo de información con el fin de evaluar el estado de conservación de *L. scrobiculata* en su actual rango geográfico.

Dada su rareza e importancia ecológica principalmente por su capacidad de fijar nitrógeno atmosférico, *L. scrobiculata* ha sido empleada como especie modelo en varios estudios ecológicos y funcionales desarrollados en bosques boreales y templados (e.g., Hallingbäck 1989; Hilmo & Ott 2002; Asplund et al. 2010a, 2010b; Larsson & Gauslaa 2011; Hilmo et al. 2011a, 2011b, 2013; Larsson et al. 2012; Gauslaa et al. 2013). Sin embargo, las condiciones climáticas y ambientales y la historia de los bosques en el norte de Europa y América difieren considerablemente del sur de Europa, especialmente de los bosques de la Región Mediterránea, donde la ecología, los requerimientos de calidad del hábitat, el funcionamiento y, en definitiva, el estado de conservación de las poblaciones de *L. scrobiculata* son totalmente desconocidas.

OBJETIVOS Y ESTRUCTURA DE LA TESIS

El objetivo general de esta tesis es mejorar el conocimiento sobre la ecología del cianoliquen epífito *Lobarina scrobiculata* en la Península Ibérica, mediante la evaluación integrada de la biología de sus poblaciones y el desempeño de los individuos a distintas escalas espacio-temporales, prestando especial atención a su funcionamiento en los bosques mediterráneos. La posterior compilación de toda esta información, junto con datos de diversidad genética, nos permitirá en un futuro evaluar el estado de conservación de esta especie y diseñar estrategias de conservación eficaces, basadas en medidas fundamentadas, dirigidas a prevenir o minimizar los factores de amenaza. Para la consecución de este objetivo general se desarrollaron los siguientes objetivos específicos:

1. Conocer los requerimientos ambientales y ecológicos del cianoliquen *L. scrobiculata* a escala regional a lo largo de un gradiente latitudinal en la Península Ibérica, evaluando los factores que afectan a su presencia y abundancia a escala local (bosque) y microescala (árbol).

2. Estudiar la estrategia reproductiva de *L. scrobiculata* a lo largo de un gradiente climático en la Península Ibérica (escala regional). ¿Varía el patrón de la asignación de recursos a la reproducción en función del clima?

3. Evaluar las tasas de crecimiento en área y biomasa de *L. scrobiculata* y su pariente próximo *L. pulmonaria* (cefaloliquen) mediante trasplantes recíprocos durante un año en dos bosques mediterráneos cercanos donde habitan ambas especies. Además, se considerará el efecto del estado reproductivo de los individuos y la altura en el árbol sobre las tasas de crecimiento.

4. Comparar la estructura demográfica, tasas de crecimiento y patrón en la asignación de recursos a la reproducción de *L. scrobiculata* en dos hábitats estructuralmente diferentes en el interior de robledales mediterráneos (árboles *vs* rocas).

5. Relacionar los rasgos químicos de *L. scrobiculata* con la presencia de *Plectocarpon scrobiculatae*, un hongo liquenícola que exclusivamente coloniza esta especie. ¿Qué papel cumplen los metabolitos secundarios del liquen en esta relación tan específica?

Estos objetivos se desarrollan, en este mismo orden, en cada uno de los cinco capítulos que integran esta memoria de tesis doctoral.

LISTA DE MANUSCRITOS

Esta tesis está basada en cinco artículos escritos en inglés para su publicación en revistas científicas de ámbito internacional. A continuación se detalla el título, la lista de coautores y el estado de publicación de cada capítulo.

Capítulo I	Merinero, S., Rubio-Salcedo, M., Aragón, G. & Martínez, I. 2014. Environmental factors that drive the distribution and abundance of a threatened cyanolichen in Southern Europe: A multi-scale approach. *American Journal of Botany* 101: 1876–1885.
Capítulo II	Merinero, S., Aragón, G., Méndez, M. & Martínez, I. Size-dependent reproductive probability and allocation of an epiphyte changes along a climatic gradient in Southern Europe. Manuscrito inédito.
Capítulo III	Merinero, S., Martínez, I., Rubio-Salcedo, M. & Gauslaa, Y. 2015. Epiphytic lichen growth in Mediterranean forests: Effects of proximity to the ground and reproductive stage. *Basic and Applied Ecology* 16: 220–230.
Capítulo IV	Merinero, S., Aragón, G. & Martínez, I. Demography, growth and reproductive allocation of an epiphyte vary in contrasting habitats. Manuscrito inédito.
Capítulo V	Merinero, S., Bidussi, M. & Gauslaa, Y. 2015. Do lichen secondary compounds play a role in highly specific fungal parasitism? *Fungal Ecology* 14: 125–129.

METODOLOGÍA GENERAL

En este apartado se describen las especies y área de estudio, así como el esquema general de muestreo a diferentes escalas, así como los análisis estadísticos empleados. No obstante, la metodología más específica para la consecución de cada objetivo se describe detalladamente en el apartado de “Material y Métodos” de cada capítulo.

1. Especies objeto de estudio

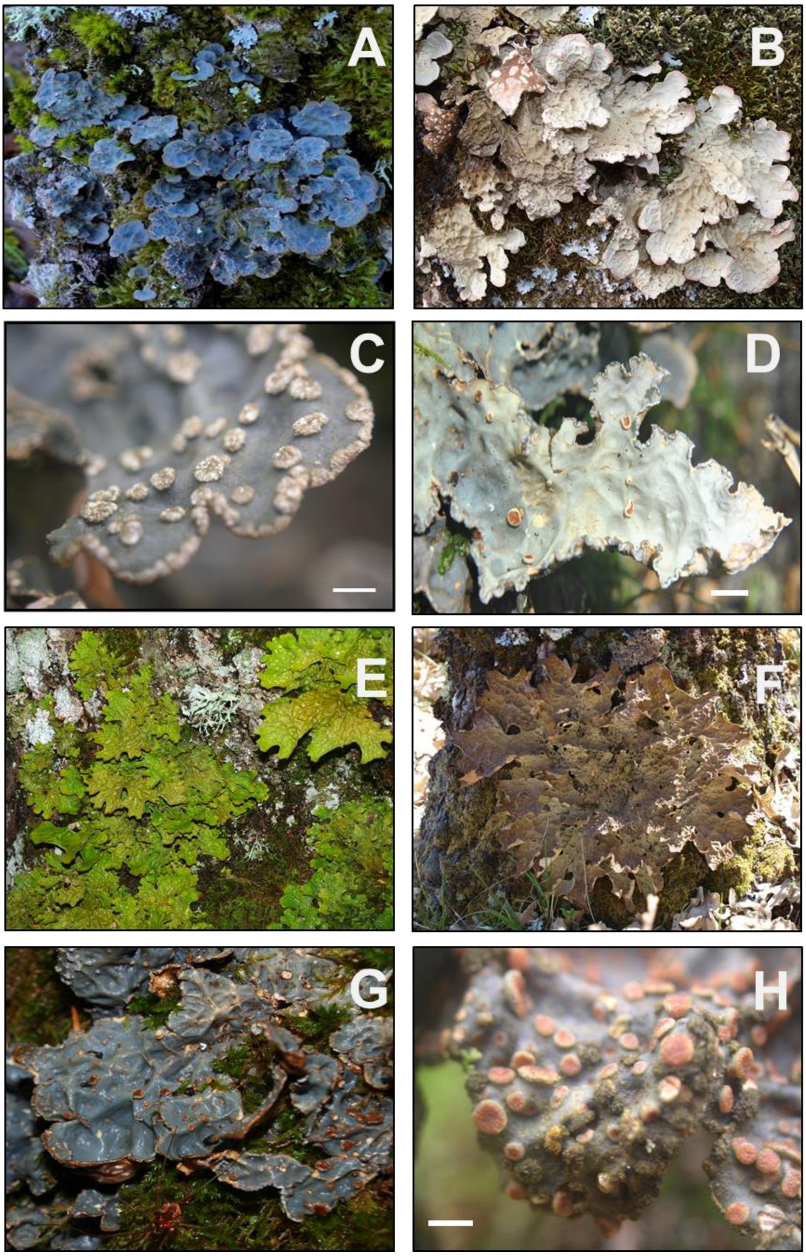
Las especies consideradas fueron *Lobarina scrobiculata* (todos los capítulos), *Lobaria pulmonaria* (capítulo III) y *Plectocarpon scrobiculatae* (capítulo V) y se describen a continuación:

Lobarina scrobiculata (Scop.) Nyl. ex Cromb. (Peltigerales, Lobariaceae) pertenecía al género *Lobaria*, pero recientes análisis filogenéticos sitúan a esta especie dentro del género *Lobarina* (Moncada et al. 2013). Tal y como se mencionaba en el apartado de introducción, se trata de un cianoliquen folioso cuyo único fotobionte es la cianobacteria *Nostoc* (Fig. 2A, B). Se reproduce frecuentemente de manera asexual mediante soredios, que son propágulos donde ambos simbiontes se dispersan conjuntamente. Los soredios se producen en unas estructuras definidas de tamaño y forma variables llamadas soralios, que pueden ser laminares y marginales (Fig. 2C). Con mucha menor frecuencia se reproduce de manera sexual mediante esporas generadas en unas estructuras denominadas apotecios (Burgaz & Martínez 1999; Smith et al. 2009) (Fig. 2D). El tamaño del individuo parece ser el principal factor que determina el inicio de la reproducción asexual en bosques boreales (Hilmo et al. 2011a, 2013; Larsson & Gauslaa 2011). Sin embargo, los factores que inducen la reproducción sexual aún no se conocen, aunque podrían estar relacionados con factores ambientales y/o características específicas de la especie.

Lobaria pulmonaria (L.) Hoffm. es una especie emparentada con *L. scrobiculata*, cuya biología y ecología se conocen mucho mejor porque ha sido frecuentemente utilizada como especie modelo en muchos trabajos (Fig. 2E, F). En la Península Ibérica tiene una distribución más amplia que *L. scrobiculata*, pero también se considera amenazada en la Región Mediterránea (Burgaz et al. 1994; Burgaz & Martínez 1999, Martínez et al. 2003). A diferencia de *L. scrobiculata*, el principal fotobionte de esta especie es un alga verde, *Dictyochloropsis reticulata* Tschermak-Woess, y presenta además colonias de la cianobacteria *Nostoc* en cefalodios internos y dispersos por el talo. Estas diferencias en el fotobionte principal entre estas especies se traducen en diferencias fisiológicas, ya que los cianolíquenes necesitan agua líquida para activar la fotosíntesis (Lange et al. 1986, 1993), suelen tener mayor capacidad de retención de agua (Honneger et al. 1996; Gauslaa & Coxson 2011; Merinero et al. 2014) y resisten mejor la intensidad de la luz en estado de desecación (Gauslaa et al. 2012) que los cefalolíquenes. Esta diferencia en los rasgos de hidratación entre dos especies emparentadas, junto con el mayor conocimiento que existe sobre *L. pulmonaria* fueron los principales motivos que nos llevaron a incluir esta especie en el capítulo III, donde se compara el crecimiento de ambas especies.

En el último capítulo nos centramos en la relación parásito-hospedador integrada por el hongo liquenícola *Plectocarpon scrobiculatae* Diederich & Etayo (Ascomycota, Roccellaceae), que exclusivamente infecta a *L. scrobiculata* (Fig. 2G). *Plectocarpon scrobiculatae* induce la formación de agallas basalmente constreñidas en la superficie del talo liquénico, situándose el ascoma del parásito superficialmente sobre la agalla (Ertz et al. 2005; Fig. 2H). En el interior del ascoma se encuentran las estructuras de reproducción sexual (ascos con ascosporas) y asexual (picnidios con conidios) (Ertz et al. 2005). Se asume que se trata de una relación comensalista porque el parásito aparentemente sólo deforma el talo por la inducción de estas agallas, aunque se desconoce el mecanismo subyacente a la aparición de estas agallas, así como los posibles efectos del parásito en la eficacia biológica del liquen (Hawksworth 1982; Ertz et al. 2005).

Figura 2. (A) *Lobarina scrobiculata* hidratada; (B) *L. scrobiculata* en estado de desecación; (C) soredios en soralios laminares y marginales (escala 3 mm); (D) apotecios (escala 6 mm); (E) *Lobaria pulmonaria* hidratada; (F) *L. pulmonaria* en estado de desecación; (G). *L. scrobiculata* infectada por *Plectocarpon scrobiculatae*; (H) detalle de ascomas y agallas inducidas por *P. scrobiculatae* (escala 4 mm). Fotografías de Gilfernando Giménez, Juan L. Hidalgo y Sonia Merinero.



2. *Áreas de estudio*

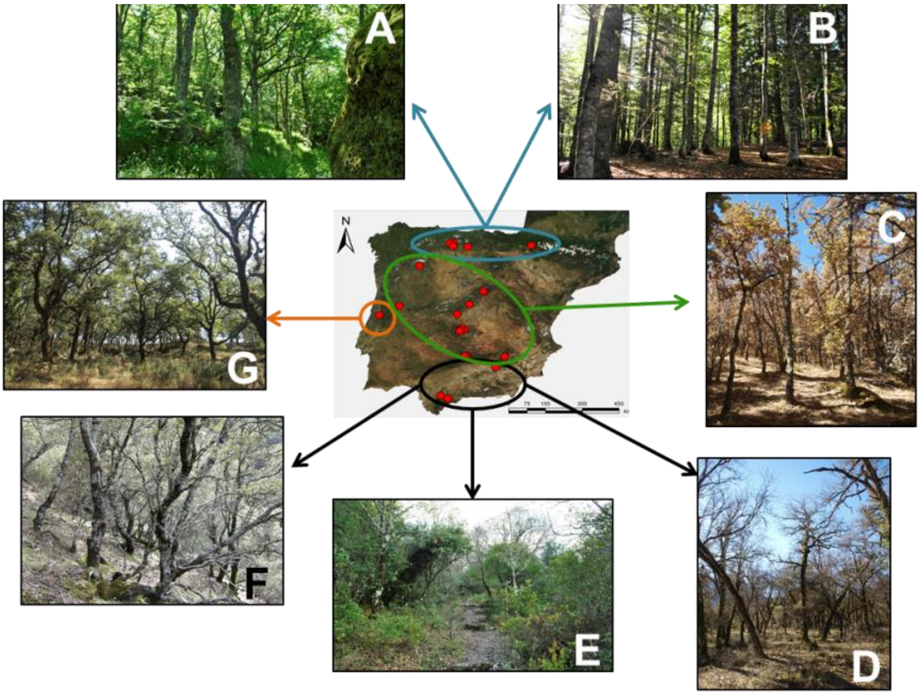
Dado que el objetivo general de esta tesis es profundizar en el conocimiento de la ecología y funcionamiento de un liquen epífito amenazado desde una aproximación multi-escalar, las zonas de estudio varían. Los capítulos I y II se desarrollaron a lo largo de un gradiente latitudinal en la Península Ibérica. El capítulo III se desarrolló en dos localidades muy cercanas en las inmediaciones de Montejo de la Sierra (Madrid) y El Cardoso de la Sierra (Guadalajara), España. El capítulo IV comprende dos localidades en el centro peninsular, la localidad de El Cardoso de la Sierra y El Escorial (Madrid). Finalmente, el capítulo V se desarrolló con muestras de *L. scrobiculata* procedentes de una población en Namsos, Nord-Trøndelag (noreste de Noruega).

Las principales características de los sitios de estudio son las siguientes:

2.1. Gradiente latitudinal en la Península Ibérica (Capítulos I y II)

La Península Ibérica comprende tres regiones biogeográficas bien diferenciadas, la región Mediterránea que ocupa la mayor extensión y en menor proporción las regiones Atlántica y Alpina (EEA 2009). A lo largo de un gradiente latitudinal prospectamos poblaciones representativas de todos los tipos de bosque donde *L. scrobiculata* puede desarrollarse (Fig. 3). En la región Atlántica los principales tipo de bosque fueron robledales (*Quercus robur* L. y *Q. petraea* (Matt.) Liebl.) y hayedos (*Fagus sylvatica* L.) (Fig. 3A, B). En la región Mediterránea predominaron los robledales mediterráneos (*Q. pyrenaica* Willd.) (Fig. 3C) y, en menor proporción, quejigares y encinares (*Q. faginea* Lam. y *Q. ilex* L.) (Fig. 3D, F, G) y bosques mixtos de quejigar-pinsapar (*Q. canariensis* Willd., *Abies pinsapo* Boiss., *Phillyrea latifolia* L.) (Fig. 3E). En el capítulo I se incluyeron 22 poblaciones, mientras que en el capítulo II el número se redujo a 18 por cuestiones estadísticas relacionadas con el tamaño muestral y el ajuste de los modelos.

Figura 3. Poblaciones de *Lobarina scrobiculata* estudiadas a lo largo de un gradiente latitudinal en la Península Ibérica (*n* = 22). Se presentan los bosques más representativos que ocupa la especie. (A) Robledales atlánticos (*Quercus robur* y *Q. petraea*); (B) hayedos (*Fagus sylvatica*); (C) robledales mediterráneos (*Q. pyrenaica*); (D) quejigar mixto con arces y encinas (*Q. faginea*, *Acer monspessulanum* y *Q. ilex* subsp. *ballota*); (E) quejigar–pinsapar (*Q. canariensis*, *Abies pinsapo*, *Q. ilex* subsp. *ballota* y *Phyllirea latifolia*); (F) encinares (*Q. ilex* subsp. *ballota*) y; (G) quejigares (*Q. faginea*). Fotografías de Juan L. Hidalgo, Gregorio Aragón y Sonia Merinero.



2.2. Poblaciones del centro peninsular: robledales mediterráneos (Capítulos III y IV)

Seleccionamos tres poblaciones de *L. scrobiculata* en el centro peninsular en las cercanías de los municipios de Montejo de la Sierra, El Escorial (provincia de Madrid) y El Cardoso de la Sierra (provincia de Guadalajara). Estas poblaciones se encuentran en espacios protegidos bajo diferentes figuras de protección. La población de Montejo y El Cardoso se encuentran situadas en Reserva de la Biosfera y Parque Natural Sierra Norte de

Guadalajara, respectivamente, mientras que el bosque de El Escorial, conocido como el Bosque de la Herrería, pertenece a Patrimonio Nacional y se encuentra bajo la categoría de Paisjae Pintoresco. Las tres poblaciones se desarrollan en robledales de *Quercus pyrenaica* y, como consecuencia de la diferente historia de su aprovechamiento forestal, la estructura del bosque difiere entre ellas. En el robledal de El Cardoso (también conocido como Santuy) la densidad de árboles es la más alta, aunque el diámetro medio de los árboles es muy pequeño. Por el contrario, el robledal de Montejo presenta una baja densidad de árboles, pero alberga árboles de gran tamaño. En el Escorial, tanto la densidad como el tamaño de los árboles presentan valores intermedios entre las otras dos localidades.

2.3. Población noruega: bosque boreal de Picea abies (Capítulo V).

Para alcanzar el objetivo del capítulo V, en el que se relacionan los rasgos químicos de *L. scrobiculata* con la presencia de *P. scrobiculatae* se utilizó material de *L. scrobiculata* procedente de Noruega que se recolectó con otros propósitos (ver Hilmo et al. 2013; Merinero et al. 2014). La población de *L. scrobiculata* procede de un bosque boreal de *Picea abies* (L.) Karst. del centro de Noruega, en el condado de Nord-Trøndelag, a una altitud menor de 100 m y precipitación media de 1375 mm (Fig. 4). En estos bosques perennifolios, nuestras especies de estudio crecen principalmente sobre las ramas de los árboles porque es donde reciben las condiciones adecuadas de luz y humedad.

3. Diseño experimental

El diseño experimental que nos planteamos en esta tesis tiene un enfoque multi-escalar porque examinamos la respuesta de *L. scrobiculata* a tres escalas espaciales diferentes: escala regional (gradiente latitudinal en la Península Ibérica), escala local (nivel de bosque) y microescala (nivel de árbol). A cada escala se consideran diferentes variables respuesta (Tabla 1) y explicativas que se detallan a continuación:

A escala regional, se consideraron variables climáticas (e.g. precipitación anual, temperatura media anual) y de estructura de bosque (el diámetro medio de los árboles, las especies arbóreas y la densidad de árboles). En los capítulos I y II estas variables se estimaron en parcelas aleatorias de 30 x 100 m en el interior de los bosques para evaluar la respuesta de *L. scrobiculata* a nivel regional. Todas las variables climáticas fueron estimadas a partir del modelo climático para la Península Ibérica CLIMOEST (Sánchez–Palomares et al. 1999). A escala local se caracterizó la estructura del bosque en parcelas permanentes de 50 x 50 m para evaluar la respuesta de *L. scrobiculata* a la heterogeneidad ambiental dentro del bosque.

Figura 4. Aspecto de un antiguo bosque boreal de *Picea abies* en el centro de Noruega (Overhalla, Namsos, Nord-Trøndelag). Fotografía de Sonia Merinero.

A microescala se consideraron características relacionadas con el árbol, como la especie de árbol, el diámetro medido a la altura del pecho (DBH), la inclinación del tronco, el grosor de la corteza y la cobertura de briófitos (Capítulos I y IV). Además, en cada árbol se contó el número de individuos de *L. scrobiculata*, se midió su tamaño con un calibre digital (estimado como el diámetro mayor del liquen), y se estimó la capacidad reproductora (i.e., cobertura de soredios, %) (Capítulos I, II y IV). Estas variables también son variables respuesta a nivel de individuo (Tabla 1). Para valorar los efectos del microclima en la respuesta de *L. scrobiculata*, se consideró la posición de cada individuo (la altura en el árbol y la orientación), la luz que reciben, y el sustrato sobre el que crecen (Capítulos I, III y IV).

Finalmente, evaluamos la relación entre la presencia de agallas de *Plectocarpon* con el tamaño y contenido en metabolitos secundarios de cada individuo de *L. scrobiculata* mediante cromatografía líquida de alta resolución (HPLC) (Capítulo V).

Todos los capítulos de esta tesis se basan en un diseño experimental observacional, excepto el capítulo III, consistente en un diseño factorial de trasplantes recíprocos de *L. scrobiculata* y *L. pulmonaria* con el fin de evaluar el crecimiento y la variación de los rasgos funcionales “masa específica del talo (STM)” y “capacidad de retención de agua (WHC)” de estas especies.

4. Análisis estadísticos

Los estudios ecológicos de poblaciones de líquenes epífitos presentan normalmente una estructura espacial anidada de individuos en árboles, y árboles dentro de bosque (o parcela), por lo que los datos presentan una estructura de no independencia. Por tanto, el análisis de datos a escala de individuo o árbol debe realizarse mediante modelos mixtos, donde se incluye el efecto aleatorio del factor árbol o bosque, dependiendo de la escala en cuestión

(Bolker et al. 2009; Zuur et al. 2009). Se emplearon modelos lineales mixtos (LMMs) si la variable respuesta era continua y el error normal. En el caso de variables respuesta con errores binomial o Poisson se emplearon modelos mixtos lineales generalizados (GLMMs). Estos análisis se emplearon en la mayor parte de los capítulos (Capítulos I, III, IV y V) con el objetivo de explicar la relación directa entre diferentes variables dependientes (continuas y no continuas) y sus variables explicativas.

Tabla 1. Descripción de las variables respuesta utilizadas en cada capítulo (en números romanos) y medidas y/o analizadas a cada escala.

		Escala	
Variable respuesta	Regional	Local	Microescala
Presencia de *L. scrobiculata*		I	I
Abundancia de *L. scrobiculata*	I	IV	I
Proporción de árboles ocupados (%)	I	IV	
Tasa relativa de crecimiento en área (mm^2 cm^{-2} $día^{-1}$)		II y IV	III y IV
Tasa relativa de crecimiento en biomasa (mg g^{-1} $día^{-1}$)		III	III
Cambio en masa específica del talo (% STM; mg cm^{-2})		III	III
Cambio en capacidad de retención de agua (% WHC; mg H_20 cm^{-2})		III	III
Esfuerzo reproductivo (cobertura de soredios, %)	II	IV	
Probabilidad de reproducción	II	IV	
Tamaño de los individuos de *L. scrobiculata* (mm)		IV	IV
Contenido en metabolitos secundarios (mg m^{-2})			V
Número de agallas de *P. scrobiculatae*			V

Para el análisis de la alometría reproductiva de *L. scrobiculata* (i.e., la relación entre el tamaño del liquen y su esfuerzo reproductivo) se emplearon regresiones de tipo II, también conocidas como *standardised major axis* (SMA) (Warton et al. 2006, 2012). Este tipo de regresiones son más apropiadas cuando la cuestión de interés reside en estimar el valor de la pendiente entre dos variables medidas con error, y no en utilizar los parámetros de la regresión para realizar predicciones de un modelo lineal, para lo que sí sería correcto utilizar regresiones típicas de mínimos cuadrados (Niklas 2004; Warton et al. 2006). De hecho, el análisis de SMA está más relacionado con análisis multivariantes que con regresiones típicas por la manera en la que se estiman los parámetros y se calculan los errores (Warton et al. 2006). En los capítulos II y IV empleamos este tipo de análisis para evaluar los patrones en la asignación de recursos a la reproducción de *L. scrobiculata*, mediante la estimación de la superficie somática de los individuos (S) y la superficie ocupada por estructuras de reproducción (R).

Todos los análisis estadísticos se han realizado en el entorno y lenguaje de programación R (R Foundation for Statistical Computing, Vienna, Austria). Los principales paquetes que se han empleado son nlme (Pinheiro et al. 2008), lme4 (Bates et al. 2013) y smatr (Warton et al. 2012).

CONCLUSIONES

Integrando los resultados de los cinco capítulos de esta tesis se extraen las siguientes conclusiones:

1 El cianoliquen *Lobarina scrobiculata* es una especie higrófila poco frecuente en la Península Ibérica, pero es localmente abundante en robledales que reciben elevada precipitación. A pesar de la aceptada asunción de que se trata de una especie asociada a bosques antiguos y comunidades epífitas climácicas, en la Península Ibérica es más frecuente y abundante en bosques con alta densidad de árboles de pequeño diámetro (indicadores de bosque joven y/o manejado).

2 El inicio de la reproducción asexual de *L. scrobiculata* depende del tamaño del individuo. A escala regional, *L. scrobiculata* comienza a reproducirse a mayor tamaño en las localidades más húmedas, donde presumiblemente las tasas de crecimiento son mayores. A escala local se confirma que los individuos con crecimiento más rápido comienzan a reproducirse a mayor tamaño que los de crecimiento más lento. Esto sugiere que la duración del ciclo de vida de la especie puede variar a escalas local y regional, aunque sería necesario comprobar que el inicio temprano de la reproducción se relaciona con una mayor tasa de mortalidad.

3 Por primera vez se ha estudiado el patrón en la asignación reproductora asexual de un organismo fotosintético a lo largo de un gradiente ambiental amplio. Las respuestas a este gradiente son similares a los patrones descritos en plantas vasculares con reproducción sexual. Estos resultados generalizan la teoría desarrollada para plantas y, mejoran enormemente el conocimiento sobre la diversidad de las historias de vida.

4 El patrón en la asignación de recursos a la reproducción asexual en *L. scrobiculata* es alométrico: los individuos grandes invierten desproporcionalmente más recursos en reproducirse que en crecer. A escala regional, este patrón depende de la precipitación, ya que a igualdad de tamaño, los individuos de las poblaciones más secas aumentan significativamente la asignación reproductora. A escala local, se confirma que los individuos que crecen más lentamente muestran una mayor asignación reproductora, sugiriendo que la edad del individuo es el mecanismo subyacente al patrón de asignación reproductora a ambas escalas.

5 Las tasas relativas de crecimiento de *L. scrobiculata* y *L. pulmonaria* en bosques mediterráneos son similares e incluso superiores a las documentadas en bosques boreales en climas más húmedos, por lo que el clima mediterráneo no reduce necesariamente las tasas de crecimiento de individuos ya establecidos.

6 Las tasas relativas de crecimiento de *L. scrobiculata* varían fuertemente a escala local (bosque) y microescala (árbol). Este cianoliquen crece mucho más rápido en las bases de los árboles y en orientaciones norte, donde la disponibilidad hídrica es mayor. Además, los individuos tienden a alcanzar mayores tamaños cuando crecen sobre briófito. Asimismo, los individuos juveniles crecen más rápido que los individuos reproductores, indicando que existe un compromiso en la asignación de recursos a la reproducción y al crecimiento.

7 La heterogeneidad de hábitat a escala lcoal (bosque) es un aspecto crucial que determina la ecología y demografía de *L. scrobiculata*. En las rocas musgosas, *L. scrobiculata* crece más rápido, comienza a reproducirse a tamaños mayores y muestra menor asignación reproductora que en árboles. No obstante, se requieren estudios a largo plazo que evalúen las tasas de reclutamiento y la supervivencia para identificar las etapas más sensibles en el ciclo de vida de esta especie.

8 Los individuos de *L. scrobiculata* infectados por *Plectocarpon scrobiculatae* contienen la mitad de metabolitos secundarios que los individuos sin signos de infección. Estos resultados sugieren dos posibles explicaciones: (1) el parásito podría colonizar individuos con baja concentración de metabolitos que actúan como defensa; (2) el parásito reduce el contenido de metabolitos secundarios. Se necesitan estudios experimentales que profundicen en la base química de esta relación y los efectos que tiene *Plectocarpon* en la eficacia biológica de *L. scrobiculata.*

9 Desde una perspectiva conservacionista, proponemos que es necesario mantener la estructura de los bosques donde aparece *L. scrobiculata*, la heterogeneidad de hábitats dentro de los mismos, y la conectividad entre fragmentos de bosque que pueden permitan la expansión de esta especie. Además, es urgente mantener la variabilidad de microhábitats mediante el mantenimiento de robles de diferentes tamaños, sin menospreciar el importante papel que cumplen los árboles pequeños (jóvenes) en la persistencia de esta especie en bosques mediterráneos.

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CAPÍTULOS / CHAPTERS

CAPÍTULO I / CHAPTER I

ENVIRONMENTAL FACTORS THAT DRIVE THE DISTRIBUTION AND ABUNDANCE OF A THREATENED CYANOLICHEN IN SOUTHERN EUROPE: A MULTI-SCALE APPROACH

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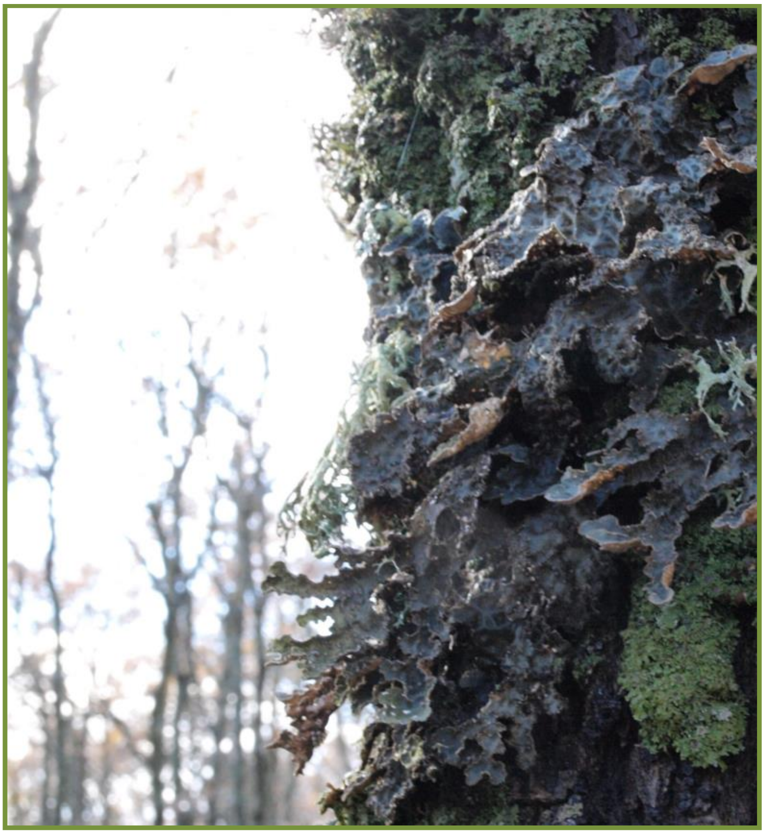


ABSTRACT

Premise of the study: High-quality information about threatened species is required to prevent current global biodiversity losses. Lichens are important components of forest biodiversity, which help to maintain ecosystem functioning. The epiphytic cyanolichen *Lobarina scrobiculata* is red-listed in Europe and North America, but knowledge on its ecology and distribution in Southern Europe is scarce.

Methods: We used a multi-spatial scale design to investigate the effects of macroclimate, forest structure, tree features, and microhabitat on the occurrence, abundance, spatial distribution, and performance of *L. scrobiculata* in the Iberian Peninsula at plot and tree scales. Generalized linear models and mixed models were used for analysis.

Key results: We recorded ca 14000 individuals of the threatened species *L. scrobiculata* from 22 populations in the Iberian Peninsula. Our results suggest that *L. scrobiculata* thrives mainly in oak forests with highly variable annual precipitation levels. At the plot scale, the *L. scrobiculata* abundance increased with annual precipitation and tree density (habitat quantity). At the tree scale, our models highlighted the importance of tree size and bark roughness (habitat quality) as the main drivers of species occurrence and abundance. We detected a marked spatial pattern on tree trunks, i.e., *L. scrobiculata* occurred preferentially on north-facing surfaces and close to the ground, where humidity is higher.

Conclusions: By integrating multi-scale modeling, we analyzed a unique large dataset and these results are essential for understanding the ecology of this threatened cyanolichen. There is an urgent need to preserve the forests this species currently inhabits and potential colonization sites.

Key words:

Atlantic forest; conservation status; epiphytic lichens; *Lobaria scrobiculata*; *Lobarina scrobiculata*, Mediterranean forest; occupation rate; population size; precipitation.

INTRODUCTION

The reduction of current biodiversity losses is one of the major challenges faced by society in the current century and it is the role of scientists to design conservation plans, with a special focus on threatened species (Balmford et al., 2005). Effective conservation strategies rely on a thorough knowledge of the life processes and ecology of threatened species (Hilmo and Ott, 2002; Iriondo et al., 2009; Scheidegger and Werth, 2009). The viability of a population depends on two main factors: population size and individual performance, which are usually associated with the habitat quality (Soulé, 1987; Hilmo and Ott, 2002; Iriondo et al., 2009; Belinchón et al., 2011). At a large scale, climatic conditions may influence the life cycle and performance of species, thereby shifting the species habitat quality requirements at finer scales (Scheidegger and Werth, 2009). Therefore, it is essential to identify the environmental factors that determine the occurrence, abundance, and performance of threatened species in a wide range of suitable habitats and at different spatial scales.

Few previous studies of lichens have utilized extensive quantitative and demographic datasets over a wide geographical range to obtain critical information about the habitat requirements, performance, and main threats for a species (but see, for example, Scheidegger and Werth, 2009; Hilmo et al., 2011a; Schei et al., 2012). Based on this type of study, however, two lichen species were included as Critically Endangered in the International Union for the Conservation of Nature's World Red List of Threatened Species because of their limited distribution and declining population, i.e., the epiphytic cyanolichen *Erioderma pedicellatum* (Hue) P.M. Jørg and the terrestrial *Cladonia perforata* A. Evans (Scheidegger, 2003; Yahr, 2003). For example, the accurate population analysis, demographic monitoring, and identification of the causes of the decline of *E. pedicellatum* (commercial forestry and acid rain) (Cameron et al., 2013; Stehn et al., 2013) led to the implementation of conservation strategies to facilitate its recovery and maintenance in Canada, including the avoidance of forestry in occupied and potential suitable habitats (Environment Canada, 2007).

Similarly, detailed knowledge of the environmental requirements of the endemic species *C. perforata* also led to the establishment of habitat protection measures and reintroductions (Yahr, 2003).

Lichens represent an important component of the terrestrial biodiversity, especially in forests where they are among the dominant epiphytic organisms (Wein and Speer, 1975; Aragón et al., 2010) and they have critical roles in ecosystem functioning, i.e., water and mineral cycles, shelter for micro-fauna, and food webs (reviewed by Ellis, 2012). Lichens are poikilohydric organisms i.e. they lack specialized mechanisms for regulating their water content, which is controlled passively by the water potential of the environment (Nash, 2008). Thus, the distribution and performance of lichens are determined greatly by the macro- and microclimate conditions, especially water and light availability, and their ranges of tolerance for these conditions, as well as other factors (i.e., suitable habitat availability, nutrient availability, and/or air pollution) (Nash, 2008; Ellis et al., 2009; Ellis, 2012; Marini et al., 2011). Therefore, changes in microclimate conditions, such as those induced by forest management, affect the diversity of epiphytic lichens, especially cyanolichens (i.e., lichens with cyanobacteria as the main photobiont) (Gauslaa et al., 2001; Aragón et al., 2010; Ellis, 2012). Cyanolichens are particularly sensitive to humidity because they require liquid water to activate photosynthesis (Lange et al., 1986), thus their distribution is generally controlled by the annual precipitation (see Marini et al., 2011). Cyanolichens play significant roles in the nutrient cycles in forests because they fix atmospheric nitrogen (Nash, 2008; Ellis, 2012). Thus, given the importance of cyanolichens for ecosystem functioning, species-specific studies are required to better understand their functioning and distribution at different spatial scales in the regions where they occur. These studies need to address different spatial scales because ecological processes are scale-dependent and they may generate different patterns at different spatial scales, which may explain the distribution, abundance, and assembly of species (Wiens, 1989; McGill, 2010; Nascimbene et al., 2013).

In the present study, we focused on the cyanolichen species *Lobarina scrobiculata*, which is generally associated with late successional epiphytic communities, traditionally referred as to *Lobarion* (Barkman, 1958; James et al., 1977; Burgaz et al., 1994). In general, *L. scrobiculata* has an incomplete circumboreal distribution, where it is more frequent on the west and east coasts of North America and Europe (http://data.gbif.org). However, a wider distribution does not necessarily correlate with its local abundance because this species has declined greatly in the USA in recent decades, and similar declines are likely in Europe (mean estimates of 10–30% based on the loss of locations) (Sérusiaux, 1989; NatureServe, 2014). The main causes of these declines are air pollution and habitat loss (Hallingbäck, 1989; Sérusiaux, 1989; Nimis, 1993; Smith et al., 2009). According to previous studies, *L. scrobiculata* is now a rare species in Europe and in some parts of North America. A growing awareness of its ongoing decline and increasing rareness has resulted in the inclusion of *L. scrobiculata* in numerous American and European Red Lists in different threatened categories, ranging from vulnerable to extinct (e.g., Sérusiaux, 1989; Randlane et al., 2008; Gärdenfors, 2010; NaturServe, 2014), based mainly on the loss of locations and/or occupancy area contraction. In Mediterranean Spain, Burgaz et al. (1994) suggested that it should be classified as “endangered” based on the persistence of the main threats to this species: forestry and agriculture practices. Based on the IUCN criteria, Martínez et al. (2003) classified it as “vulnerable” at a smaller regional scale (Central Spain). However, there is still a lack of exhaustive demographic data about this species, such as its population size and/or population structure, thereby suggesting that there is a need for new evidence to reassess the conservation status of this species in its current geographic range.

As a consequence of its rarity and ecological importance, *L. scrobiculata* has been a target species in many ecological and population studies in boreal forests (e.g., Hallingbäck, 1989; Hilmo, 2002; Hilmo et al., 2011a, 2011b; Asplund et al., 2010; Larsson and Gauslaa, 2011). However, the macroenvironmental conditions and forest history in North America and Northern Europe differ from those in Southern Europe, where the ecology and habitat requirements of this species are poorly characterized. Thus, the main aim of the present study was to elucidate the environmental factors that modulate the occurrence, abundance, spatial distribution, and performance of *L. scrobiculata* in the Iberian Peninsula at multiple scales. We examined representative *L. scrobiculata* populations throughout the Iberian Peninsula to identify differences in the performance of this species between the Atlantic and Mediterranean biogeographical regions, with a specific focus on the latter. In the Mediterranean Basin, the climatic conditions may be particularly harsh for *L. scrobiculata* due to the physiological stress caused by low humidity during the long summer drought (Valladares et al., 2004), and these conditions might become even more extreme as a consequence of global warming (Giménez-Benavides et al., 2007). We hypothesized that the abundance of *L. scrobiculata* would increase with annual precipitation, which is consistent with the requirements of cyanolichens. Therefore, we also expected that *L. scrobiculata* would perform better in the Atlantic region in terms of abundance and occupation rate because, in addition to the higher macroenvironmental humidity, the forests are older and better preserved (Blanco et al., 2005), thereby providing a more suitable environment for this old-forest cyanolichen. We also discuss the implications of our results for the conservation status of *L. scrobiculata* in the study region.

MATERIALS AND METHODS

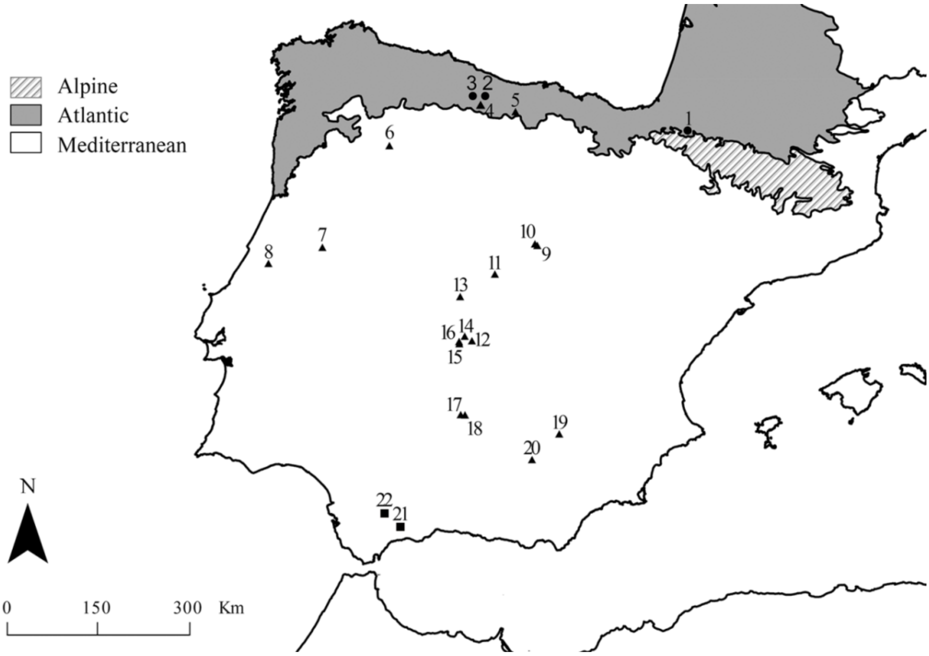
Target species

Lobarina scrobiculata (Scop.) Nyl. ex Cromb. (Lobariaceae) was long considered to belong to the genus *Lobaria* but recent phylogenetic analyses have placed it clearly in the smaller genus *Lobarina* (Moncada et al., 2013). However, in the present study we refer to *Lobaria* sensu lato for convenience to place our results in a broader ecological context. *Lobarina scrobiculata* is a foliose cyanolichen, which contains the cyanobacterium *Nostoc* as its main photobiont. This species occurs in the Northern Hemisphere and in oceanic areas of South America, Australia, New Zealand, and Africa (Smith et al., 2009; Nimis, 1993). In the Iberian Peninsula, it usually inhabits forests in mountain areas and it is relatively frequent in Central Spain, common in oceanic areas of the northwest, and increasingly rarer elsewhere, because it only occurs in forests with high relative air humidity (Burgaz and Martínez, 1999). This species is preferentially epiphytic and grows mainly on deciduous trees, but sometimes on mossy siliceous rocks (Hallingbäck, 1989; Burgaz and Martínez, 1999). The most frequent mode of reproduction is asexual via soredia (asexual diaspores that contain both symbionts), whereas sexual reproduction via apothecia (containing fungal spores) is rather infrequent (Burgaz and Martínez, 1999; Smith et al., 2009). The size of the individual appears to be the main factor that drives the onset of asexual reproduction in boreal forests (e.g., Larsson and Gauslaa, 2011), but the factors that promote sexual reproduction are not known yet, although they may include other species-specific and/or environmental factors.

Study area

We selected 22 populations distributed throughout the Iberian Peninsula, which were representative of the habitats and regions where *Lobarina scrobiculata* occurs (Fig. 1; see Burgaz and Martínez, 1999). We defined a population as at least 10 individuals inhabiting a forest. The latitudinal gradient where *L. scrobiculata* occurs in the Iberian Peninsula includes two biogeographical regions with very different climatic conditions: the Atlantic region (northern fringe of the Iberian Peninsula) with an oceanic climate and the Mediterranean region (the rest of the Iberian Peninsula), which is characterized by a summer drought period that can be quite variable in length (EEA, 2009) (Fig. 1; Table 1). This latitudinal gradient and the topography result in high environmental heterogeneity, thereby leading to high forest diversity. In the Atlantic region, the forests are dominated by broadleaved and deciduous species (e.g., *Fagus sylvatica* L., *Quercus petraea* (Matt.) Lieb., and *Q. robur* L.), whereas perennial or semi-deciduous species (e.g., *Q. ilex* L. and *Q. pyrenaica* Willd.) are the dominant forest species in the Mediterranean region (Blanco et al., 2005) (Fig. 1). Iberian forests have been managed for centuries, especially in the Mediterranean region (i.e., for timber, firewood extraction, and livestock) (Valladares et al., 2004; Blanco et al., 2005). Our study plots within the Mediterranean region exhibited different signs of low intensity management in the past and current livestock activity. However, the plots sampled in the Atlantic region had not been subjected to any forestry practices for more than 50 years.

Figure 1. Locations of the 22 populations of *Lobarina scrobiculata* sampled throughout the Iberian Peninsula. The biogeographical regions are shown according to the EEA (2009). The details of the corresponding localities and plots are shown in Table 1. Triangles: oak forests; circles: beech forests; squares: mixed species forests.



Experimental design and sampling

The field data were collected between October 2009 and July 2012. We approached this study using a multi-scale design, i.e., plot and tree scales. We established a random 3000 m^2 plot inside a forest where the target species was detected. We investigated all living trees present in this area and recorded the occurrence and number of *L. scrobiculata* individuals at a height of up to 2 m on the tree trunks, because *L. scrobiculata* occurs rarely above this height on the trunks and branches in the study region (e.g., Belinchón et al., 2009, 2011; S. Merinero et al., personal observation). We considered “individuals” to be all spatially distinguishable lichen entities that were easily differentiated from others. This definition is also used elsewhere (e.g. Hilmo et al., 2011a; Schei et al., 2012)

At the plot scale, we considered the following climatic variables (Table 1): (1) annual precipitation (mm); (2) winter precipitation (mm); (3) summer precipitation (mm); and (4) mean annual temperature (°C), which were derived using the climate simulation model CLIMOEST (developed for the Iberian Peninsula) by inputting the altitude, geographic coordinates, and hydrographic basin (Sánchez-Palomares et al., 1999). The longitude, latitude, and altitude were recorded in situ using a GPS device (GPSmap 60CSx, Garmin GPS). We also measured the following forest structure variables: (1) the forest type based on the dominant tree species (oak, beech, or mixed); (2) the number of trees per plot (i.e., tree density); and (3) the mean tree diameter at breast height based on all trees within the plot (DBH, cm) (Table 2). At the tree scale, we recorded the tree species, DBH (cm), bark roughness (mm), which was measured using digital calipers as the depth of a representative crevice at approximately 50 cm above ground, and the trunk slope respect to the horizontal (°), which was measured using a clinometer. We also measured the position of each *Lobarina scrobiculata* individual on the trunk in terms of the height above ground (cm) and orientation (°).

Statistical analyses

To avoid problems of multicollinearity, only uncorrelated variables (Pearson’s correlation coefficient < 0.7 and *P* > 0.05) were included in the models. For two correlated variables, the models included the variable that had a stronger biological influence on the response of the lichen because it explained more variation. Thus, from the original 14 explanatory variables, only four were used in the models to examine their effects on the response variables at the two scales. The plot-scale explanatory variables were annual precipitation and tree density, whereas the tree-scale variables were DBH and bark roughness. The response variables at the plot scale were the abundance of *Lobarina scrobiculata* and the proportion of occupied trees (i.e., occupation rate), while the response variables at the tree scale were the probability of occurrence and the abundance of *L. scrobiculata*.

To test the effects of annual precipitation and tree density on the abundance and occupation rate of *Lobarina scrobiculata* at the plot scale we fitted generalized linear models (GLMs) (McCullagh and Nelder, 1989), where Poisson and binomial errors were applied, respectively. At the tree scale, the effects of the explanatory variables on the occurrence and abundance of *L. scrobiculata* were modeled by fitting generalized linear mixed models (GLMMs) with a Laplace approximation (Zuur et al., 2009). Binomial and Poisson errors were applied to the occurrence and abundance models, respectively. We selected this approach because our data had an unbalanced and hierarchical structure with trees nested within the plots.

To overcome this problem, the data were analyzed using a multi-level approach, where we considered the variable “plot” as a random factor in the tree-scale model and the explanatory variables were introduced at the plot and tree scales as fixed factors (i.e., annual precipitation, tree density, DBH, and bark roughness) (Zuur et al., 2009). Further details of the GLM and mixed effects modeling approaches can be found in Zuur et al. (2009). The fixed factors were examined using Wald Z-statistic tests. Significant values with both procedures were estimated using a deviance test (Guisan et al., 2002) and variables were excluded if $P > 0.05$. Models using the complete data set i.e., gathered data from the Atlantic and Mediterranean regions, are referred as to global data/scale models.

Table 1. Summary of the geographic, topographic, and climatic variables in the areas of the study populations (plots). Atl: Atlantic Biogeographical Region; Medit: Mediterranean Biogeographical Region; Coord: coordinates in UTM WGS84; Alt: Altitude (m a.s.l.) P: Precipitation (mm); Mean T: Mean annual temperature (°C).

Plot	Locality	Province	Region	Coord X	Coord Y	Alt	Annual P	Winter P	Summer P	Mean T
1	Irati	Navarra	Atl	30T654582	4761165	965	1615	589	149	8.9
2	Pontón	León	Atl	30T335853	4774195	1328	1721	552	229	7.8
3	Burón	León	Atl	30T321674	4771356	1353	1371	368	135	7.5
4	Riaño	León	Atl	30T335246	4758497	1150	1156	295	117	8.8
5	Brañosera	Palencia	Atl	30T394739	4754232	1260	946	326	131	8.1
6	Sanabria	Zamora	Medit	30T198543	4667428	950	1054	384	95	10.3
7	Estrela	Guarda	Medit	30T116951	4484577	978	2050	865	85	13.5
8	Sicó	Coimbra	Medit	30T133915	4442826	290	1178	612	41	18
9	Santuy	Guadalajara	Medit	30T462970	4549459	1235	801	252	92	9.7
10	Montejo	Madrid	Medit	30T458649	4551345	1263	818	258	92	9.5
11	Escorial	Madrid	Medit	30T402181	4491369	1066	796	272	85	11.2
12	San Pablo	Toledo	Medit	30T381950	4377721	908	621	239	48	12.8
13	San Vicente	Toledo	Medit	30T352060	4445771	1239	947	334	68	10.5
14	Risco Paradas	Ciudad Real	Medit	30T369194	4382836	1165	756	286	54	11.2
15	Cigüeñelas	Ciudad Real	Medit	30T361629	4372486	797	638	246	45	13.6
16	Gargantilla	Ciudad Real	Medit	30T362757	4369364	749	656	235	42	13.8
17	Fuencaliente	Ciudad Real	Medit	30T389768	4256034	881	729	224	49	13.9
18	Robledo	Ciudad Real	Medit	30T382652	4255571	1030	777	248	54	13.2
19	Torca Malojos	Albacete	Medit	30T545875	4252003	1406	1023	396	77	11.6
20	Cazorla	Jaén	Medit	30T508204	4202997	738	790	254	42	15.6
21	Sierra Nieves	Málaga	Medit	30T314483	4059400	1064	1155	517	23	14
22	Grazalema	Cádiz	Medit	30T284707	4073443	775	1753	734	32	15.4

We performed similar GLMs and GLMMs separately for the Atlantic and the Mediterranean regions to compare the performance of the species between regions. The number of populations examined in each region reflected the area of each biogeographical region in the Iberian Peninsula, i.e., 5/22 populations were located in the Atlantic region and 17/22 in the Mediterranean region (Fig. 1). The lower sample size in the Atlantic region meant that we only included tree density as an explanatory variable for the GLMs at the plot level in this region because it explained more variation than the annual precipitation.Finally, all of the models were checked for residual patterns and nonlinear effects. The statistical analyses were performed using R version 2.15.2 (R Foundation for Statistical Computing, Vienna, Austria). We used the packages nlme (Pinheiro et al., 2008) and lme4 (Bates et al., 2013) for the mixed effect model analyses. The mean values ± SD are reported, unless stated otherwise.

RESULTS

Population characteristics (plot scale)

Tables 1 and 2 summarize the environmental and forest structure variables for each plot. The study area comprised a wide range of different mountain systems and there was high environmental heterogeneity. There were very high variations in the altitude (290 to 1406 m a.s.l.), annual precipitation (621 to 2050 mm), and mean temperature (7.5°C to 18°C) (Table 1). As the latitude decreased, the mean annual temperature increased and the summer precipitation declined (Table 1). However, the differences in the annual precipitation were not consistent with the latitudinal gradient because some southern populations received extremely high precipitation levels due to topographic factors (e.g., plots 21 and 22) (Table 1).

In the 22 study populations, a total of 14 tree species were recorded, which were distributed mainly among the following three forest types: 17 oak deciduous forests (*Quercus faginea* Lam., *Q. petraea*, and/or *Q. pyrenaica*), three beech forests (dominated by *Fagus sylvatica*), and two forests that comprised other mainly perennial species (i.e., *Quercus ilex* + *Abies pinsapo*

Boiss. + *Phillyrea latifolia* L.) (Table 2). Minor proportions of other tree species were present in the three forest categories: *Abies alba* Mill. and *Ilex aquifolium* L. in beech forests; *Castanea sativa* Miller., *Coryllus avellana* L., *Olea europaea* L., and *Sorbus sp.* in oak forests; and *Crataegus monogyna* Jacq. in oak and other forests (Table 2).

In addition to the tree species heterogeneity, all of the forests differed greatly in structure where the tree density varied from 46 to 390 trees per plot (Table 2). This variable had a strong negative correlation with the mean DBH (Pearson's correlation coefficient = –0.7, $P < 0.001$), thus a higher tree density indicated a greater number of thinner trees. The mean DBH was 17.5 cm, but it was highly variable among plots (Table 2). Plot 14 had the thinnest trees, whereas plot 19 contained four times the number of larger trees (Table 2). In the Mediterranean region, the mean DBH was significantly less than that in the Atlantic region (Table 2; Kruskal-Wallis, $\chi^2 = 164.95$, df = 1, $P < 0.001$). The bark roughness was also highly variable among plots (Table 2), where it was significantly lower in beech forests and higher in oak forests (Kruskal-Wallis, $\chi^2 = 1805.906$, df = 2, $P < 0.001$).

Among the 22 populations, we recorded 13808 *Lobarina scrobiculata* individuals on 1282 trees (from 4963 trees sampled). The abundance was highly variable, i.e., from 11 to 4063 individuals per plot. *Lobarina scrobiculata* was found mainly in oak forests, which had the largest populations (≥ 1000 individuals) (Table 2). The occupation rate varied from 2.4% to 86.8% but it was not significantly correlated with abundance (Table 2). The occupation rate was >50% in five populations and >70% in two (Table 2). There were clear differences in the *L. scrobiculata* abundance and occupation rates between the two biogeographical regions (Table 2). The mean population size was similar in both regions (Mann-Whitney $W= 54$, d.f. = 1, $P = 0.38$), although the variability was much higher in the Mediterranean (612.7 ± 997.3 vs 678.4 ± 567.4 individuals in the Atlantic). The mean occupation rate was significantly higher in the Mediterranean region than the Atlantic region (Table 2; Mann-Whitney $W=$ 25, d.f. = 1, $P < 0.01$).

The global GLMs showed that the species abundance was higher with increasing annual precipitation and with a greater tree density (i.e., thinner trees) (Table 3). By contrast, the occupation rate was independent of precipitation but it was significantly higher in plots with a lower tree density. According to the regional GLMs, in the Mediterranean region the species abundance increased with tree density, whereas in the Atlantic region tree density did not affect abundance (Table 3). The occupation rate increased with the annual precipitation in the Mediterranean region only (Table 3).

Table 2. Summary of the forest structure variables and tree features at the plot scale. Absolute and mean ± SD values are shown for each plot, all plots, and each of the biogeographical region plots. Plots marked with asterisks (*) belong to the Atlantic biogeographical region, whereas the remainder belong to the Mediterranean region. Substrate: soil type, C, calcareous; S, siliceous. Forest type: main dominant species (*Fs*: *Fagus sylvatica*, Aa: *Abies alba*, *Qpet*: *Quercus petraea*, *Qpyr*: *Quercus pyrenaica*, *Qfag*: *Quercus faginea*, *Qilex*: *Quercus ilex*, *Apin*: *Abies pinsapo*, *Plat*: *Phillyrea latifolia*). No. tree: tree density (number of trees per plot); DBH: diameter at breast height; abundance: number of *Lobarina scrobiculata* individuals; Occup rate: proportion of colonized trees by *L. scrobiculata*.

Plot	Substrate	Forest type	No. tree	Mean DBH (cm)	Mean Bark (mm)	Mean Slope (°)	Abundance	Occup rate (%)
1*	C	*Fs + Aa*	197	28.2 ± 13.7	1.5 ± 1.3	85.1 ± 5.4	129	6.6
2*	C	*Fs*	176	21.6 ± 10.9	1.4 ± 0.7	74.6 ± 15.4	201	21.6
3*	C	*Fs + Qpet*	245	18.7 ± 21.5	1.5 ± 2.0	74.1 ± 14.3	988	6.1
4*	C	*Qpet*	170	20.2 ± 12.8	4.3 ± 2.5	77.6 ± 10.5	1487	19.4
5*	S	*Qpet+Qpyr*	251	22.7 ± 14.0	5.3 ± 3.7	78.5 ± 10	587	8.0
6	S	*Qpyr*	375	17.0 ± 9.8	6.1 ± 2.3	81.3 ± 8.8	1091	8.0
7	S	*Qpyr*	385	12.1± 4.8	5.0 ± 2.7	80.7 ± 10.2	4063	55.1
8	S	*Qfag*	137	18.0 ± 13.4	6.3 ± 2.9	75.2 ± 10.9	158	24.8
9	S	*Qpyr*	355	14.2 ± 7.9	6.0 ± 2.4	84.7 ± 4.9	220	16.9
10	S	*Qpyr*	207	23.2 ± 12.8	7.2 ± 5.0	81.8 ± 9.0	491	2.4
11	S	*Qpyr*	299	14.2 ± 7.4	8.3 ± 3.1	79.9 ± 9.0	1758	70.2
12	S	*Qpyr*	245	18.8 ± 10.3	9.3 ± 2.3	82.7 ± 4.3	11	2.9
13	S	*Qpyr*	135	24.4 ± 11.0	9.9 ± 2.3	84.3 ± 6.8	273	40
14	S	*Qpyr*	390	10.3 ± 4.5	9.7 ± 2.2	79.4 ± 7.4	169	17.9
15	S	*Qpyr*	197	18.8 ± 12.2	7.6 ± 3.5	83.2 ± 4.9	510	65.5
16	S	*Qpyr*	224	16.1 ± 6.6	9.6 ± 2.2	84.4 ± 4.1	203	37.5
17	S	*Qpyr*	169	13.8 ± 7.7	7.6 ± 2.7	83.4 ± 4.6	725	63.3
18	S	*Qpyr*	76	21.1 ± 12.4	11.9 ± 4.9	84.8 ± 4.3	491	86.8
19	C	*Qpyr*	46	49.1 ± 19.7	13.1 ± 1.5	84.8 ± 1.8	49	41.3
20	C	*Qfag*	144	25.0 ± 11.3	9.2 ± 4.8	77.8 ± 8.8	20	4.9
21	C	*Qilex*	275	12.5 ± 5.5	4.0 ± 1.5	76.3 ± 9.9	82	10.2
22	S	*Qfag + Apin + Plat+ Qilex*	265	14.6 ± 16.8	3.2 ± 2.3	74.8 ± 15.7	102	15.5
Atlantic			1039	22.2 ± 15.8	2.8 ± 2.9	77.9 ± 12.3	3392	11.4
Mediterranean			3924	16.3 ± 11.1	7.9 ± 3.0	81.8 ±7.0	10416	29.6
Total			4963	17.5 ± 12.5	6.3 ± 3.9	80.2 ± 9.9	13808	25.8

Table 3. Results of the generalized linear models used to examine the effects of environmental variables on the abundance and occupation rate (%) at the plot scale (global data; n = 22) and by region (Atlantic, n = 5; Mediterranean, n = 17). P: Annual precipitation (mm). No. tree:

tree density (number of trees per plot). The data comprise the Wald-type χ^2-statistic (Z value), degrees of freedom (df), coefficient of the variable in the model (Estimator), standard error of the estimator (SE), and significance value (*P*).

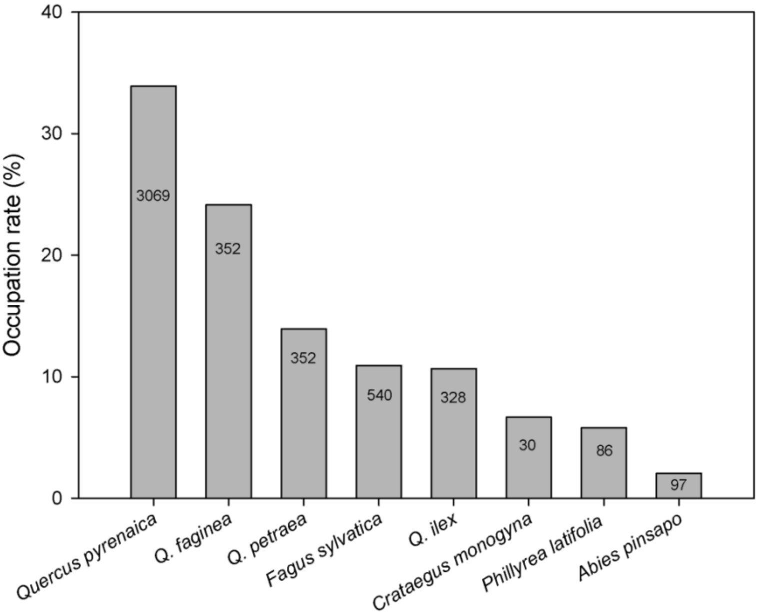
		Global				**Atlantic Region**				**Mediterranean Region**			
Response	Effect	df	Estimator (SE)	Z	*P*	df	Estimator (SE)	Z	*P*	df	Estimator (SE)	Z	*P*
Abundance	P	21	9×10^{-4} (1.77×10^{-5})	50.71	***					16	1.07×10^{-3} $(1.98 \times 10^{-5)}$	54.07	***
	No. tree	21	5.2×10^{-3} (9.60×10^{-5})	53.83	***	4	–0.32 (8.60)	0.41	n.s.	16	5.16×10^{-3} $(1.18 \times 10^{-4)}$	43.63	***
Occupation rate (%)	P	21	2.7×10^{-5} (7.70×10^{-5})	0.36	n.s.					16	4.64×10^{-4} (8.36×10^{-5})	5.55	***
	No. tree	21	-1.1×10^{-3} (3.70×10^{-4})	–2.99	**	4	-0.02 (3.30×10^{-3})	–5.07	***	16	-2.94×10^{-3} (3.99×10^{-4})	–7.36	***

Significance of *P*-values: n.s.: non-significant, * $P < 0.5$, ** $P < 0.01$, and *** $P < 0.001$

Occurrence, abundance and performance of Lobarina scrobiculata (tree scale)

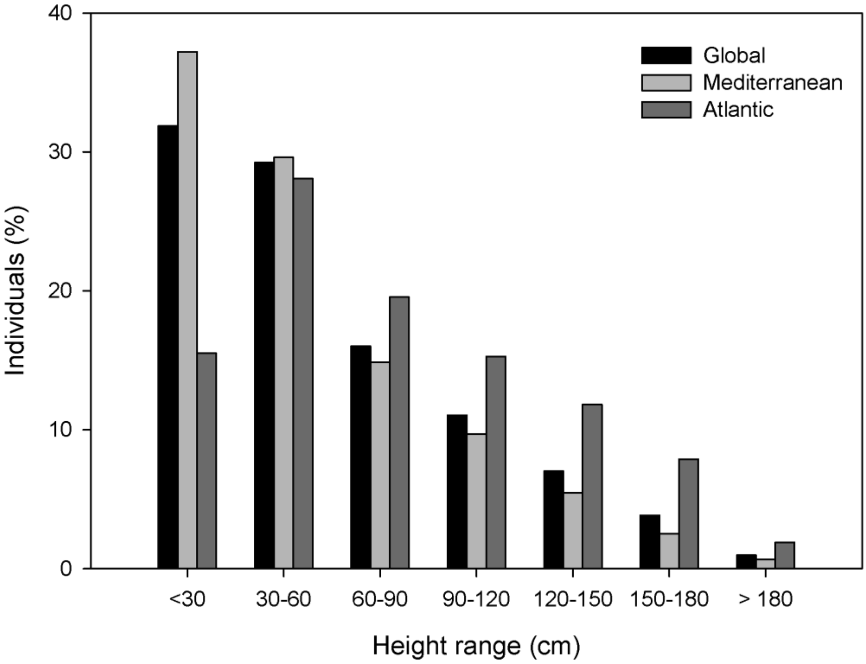
Lobarina scrobiculata was common on oaks followed by beech and other tree species (1177, 59, and 46 trees, respectively, among the 1282 occupied trees) (Fig. 2). Its occurrence was lower on *Abies pinsapo*, *Phillyrea latifolia,* and *Crataegus monogyna* (Fig. 2). Only one *Castanea sativa* tree (n = 2) and one *Olea europaea* tree (n = 2) hosted *L. scrobiculata* individuals. No individuals were recorded on *Abies alba* (n = 51), *Ilex aquifolium* (n = 48), *Sorbus* sp. (n = 16), or *Coryllus avellana* (n = 5). The bark roughness and DBH were not highly correlated when we evaluated all of the trees present in both regions, as well as trees from the Atlantic and Mediterranean regions separately, and the three more common tree species separately (deciduous *Quercus* sp., *Fagus sylvatica*, and *Q. ilex*) (Pearson's correlation coefficients < 0.3, *P* < 0.001).

Figure 2. Occupation rate (%) of *Lobarina scrobiculata* on the tree species where it occurred most frequently in the Iberian Peninsula. The total numbers of trees sampled from each species are shown inside the bars.



The global GLMMs results (Table 4) showed that certain tree features, such as large trunks and rough bark, significantly favored the probability of *Lobarina scrobiculata* occurrence and its abundance. The tree density and annual precipitation did not enhance the occurrence or abundance of *L. scrobiculata* at the global scale (Table 4). According to the regional GLMMs, the pattern obtained for the global dataset was similar in the Mediterranean region, although only the bark roughness increased the probability of occurrence in this region, where larger trees were likely to host more *L. scrobiculata* individuals (Table 4). In the Atlantic region, the probability of occurrence was also increased by a reduced density of trees, while the abundance of *L. scrobiculata* per tree increased significantly with decreasing annual precipitation (Table 4).

Figure 3. Height distribution of *Lobarina scrobiculata* individuals (%) on the tree trunks based on the global data (all plots) (n = 13808), Atlantic region plots (n = 3392), and Mediterranean region plots (n = 10416). The height ranges were measured from the ground.



We observed different distribution patterns of *Lobarina scrobiculata* individuals (13808) along the height and orientation ranges (Figs 3 and 4). The abundance of individuals decreased gradually with increasing height from the ground (Fig. 3). More than half of the individuals grew at <60 cm and <5% at >150 cm. This trend was much stronger in the Mediterranean region where nearly 40% of the individuals were located at the base of the trunk and only 3.5% grew at >150 cm (Fig. 3). The pattern differed greatly in the Atlantic region, where <15% of the individuals grew at the base of the trunk and a higher proportion of individuals were found at >150 cm (10%). Almost 65% of the individuals in this region were located 30–120 cm from the ground (Fig. 3).

The distributions were also imbalanced in terms of their orientations (Fig. 4). Among all of the individuals, the largest portion (37%) had a northerly orientation, followed by westerly and easterly, whereas a much lower number had a southerly orientation (16%) (Fig. 4). This trend was most evident in the Mediterranean plots, where half of the individuals had a northerly orientation but <7% had a southerly orientation. In contrast to the Mediterranean pattern, the proportion of individuals growing on each orientation was more balanced in the Atlantic plots (ca 25% for each cardinal aspect), with no major differences between north and south (Fig. 4).

Figure 4. Proportion of *Lobarina scrobiculata* individuals (%) distributed on the tree trunks in each orientation range: (1) north (311°–45°); (2) south (136°–225°); (3) east (46°–135°); and (4) west (226°–310°). The results are shown for the global data (all plots) (n = 13808), Atlantic region plots (n = 3392), and Mediterranean region plots (n = 10416).

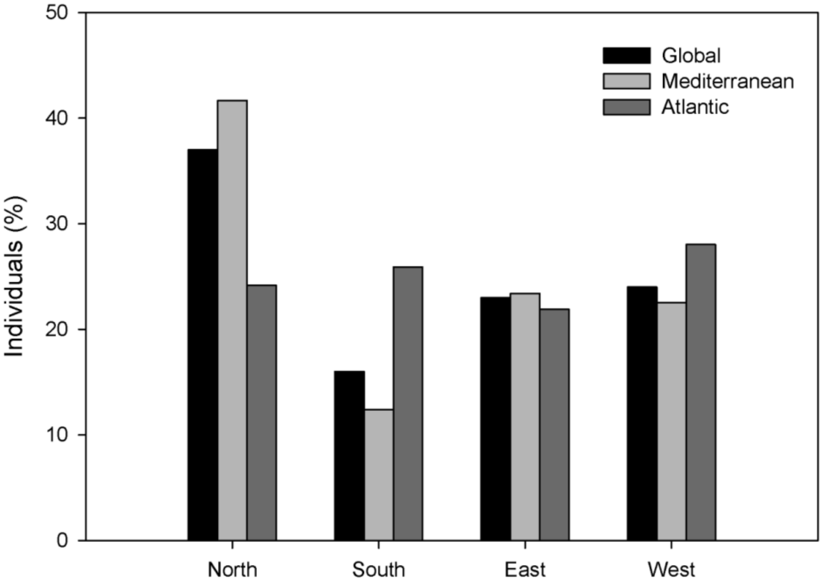


Table 4. Summary of the generalized linear mixed models fit by a Laplace approximation, which were used to examine the effects of environmental factors on the occurrence and abundance of Lobarina scrobiculata at the tree scale in all of the plots sampled in the Iberian Peninsula (global), Atlantic regions plots, and Mediterranean region plots. P: annual precipitation (mm), No. tree: number of trees per plot (tree density), DBH: tree diameter at breast height (cm), Bark: bark roughness (mm). The data comprise the Wald-type Z-statistic value (Z), coefficient of the variable in the model (Estimator), standard error of the estimator (SE), significance value (P), and random effects standard deviation (SD).

		Global			**Atlantic Region**			**Mediterranean Region**		
Response		Estimator (SE)	Z	*P*	Estimator (SE)	Z	*P*	Estimator (SE)	Z	*P*
Occurrence										
	Fixed effects									
	P	0.005 (0.008)	0.64	n.s.	0.002 (0.009)	0.24	n.s.	0.009 (0.009)	0.90	n.s.
	No. tree	–0.001 (0.003)	–0.38	n.s.	–0.020 (0.007)	–2.80	**	–0.002 (0.003)	–0.66	n.s.
	DBH	0.051 (0.008)	6.41	***	0.051 (0.008)	6.41	***	–0.005 (0.005)	–1.09	n.s.
	Bark	0.136 (0.050)	2.69	***	0.136 (0.050)	2.69	***	1.161 (0.018)	9.02	***
	Random effects			SD			SD			SD
	Plot			2.06			0.15			2.27
	Residual			1.44			0.38			1.51
Abundance		Estimator (SE)	Z	*P*	Estimator (SE)	Z	*P*	Estimator (SE)	Z	*P*
	Fixed effects									
	P	0.006 (0.008)	0.73	n.s.	–0.022 (0.002)	–8.84	***	0.007 (0.009)	0.80	n.s.
	No. tree	0.002 (0.003)	0.78	n.s.	–0.028 (0.002)	–14.07	***	0.002 (0.003)	0.67	n.s.
	DBH	0.041 (0.006)	76.67	***	0.035 (0.001)	39.63	***	0.036 (0.001)	40.41	***
	Bark	0.067 (0.002)	35.21	***	1.199 (0.007)	28.00	***	0.056 (0.002)	25.41	***
	Random effects			SD			SD			SD
	Plot			2.03			0.01			2.12
	Residual			1.42			0.12			1.46

Significance of *P*-values: n.s., non-significant, * $P < 0.5$, ** $P < 0.01$, and *** $P < 0.001$

DISCUSSION

The need to reduce current losses of biodiversity demands high-quality information on threatened species to facilitate their preservation. We performed the first multi-scale evaluation of the distribution and population size of *L. scrobiculata* in Southern Europe, and our results highlight key environmental factors that determine its occurrence, abundance and performance at two different spatial scales in this heterogeneous region.

Plot-scale: occurrence, distribution, and abundance

According to our results, *Lobarina scrobiculata* colonizes a wide range of localities, which differ greatly in terms of their annual precipitation, mean temperature, altitude, forest type, and host tree species. However, *L. scrobiculata* is not a frequent species in the Iberian Peninsula (Burgaz et al., 1994; Burgaz and Martínez, 1999), although it can be locally abundant in specific areas (e.g., plots 7 and 11). Thus, according to the rarity patterns described by Rabinowitz (1981), i.e., wide distribution with narrow habitat-specificity and sometimes locally-abundant, *L. scrobiculata* can be classified as a rare species in our study area. The high differences in abundance among populations (up to two orders of magnitude) suggest that different factors and processes might operate at the macro- and micro-scales. Indeed, this situation may also have been affected by historical anthropogenic management, which has caused changes in the landscape configuration and forest structure.

Our results show that *Lobarina scrobiculata* tends to be more abundant in localities that receive more rain. A higher annual precipitation provides a humid environment, which is suitable for satisfying the liquid water demands of cyanolichens (Lange et al., 1986), thus humidity at the macroenvironmental scale facilitates the persistence and population development of this species. However, there was a surprisingly high frequency of *L. scrobiculata* in relatively dry locations (e.g., Central Spain, plots 9–18; Fig. 1). According to Burgaz and Martínez (1999), *L. scrobiculata* is generally considered to belong to the species of *Lobaria* s.l., which have greater tolerance of the xeric conditions in the

Mediterranean climate. A plausible explanation for the occurrence of this cyanolichen in relatively dry forests is that the specific habitat features may compensate for suboptimal climatic conditions (e.g., Ellis et al., 2009). The deciduous forests canopies where *L. scrobiculata* is mainly found are relatively dense in late spring and summer, which probably maintains the humidity and provides the light conditions that this species requires. However, during the defoliated seasons, other species-specific traits may be more important for species persistence, i.e., *L. scrobiculata* has a high thallus water-holding capacity (Merinero et al., 2014) and it contains usnic acid, which is a sun-screening secondary compound (Culberson, 1969; McEvoy et al., 2007). Thus, the habitat conditions and these species-specific features may enhance the performance of this lichen in dry conditions. Indeed, Gauslaa et al. (2012) demonstrated that some old-forest cyanolichens (including *L. scrobiculata*) had better resistance to higher irradiance when desiccated compared with sympatric cephalo- and chlorolichens, thereby suggesting that cyanolichens might be better adapted to long hydration-desiccation cycles.

Lobarina scrobiculata was more abundant in forests with a high tree density and relatively thinner trees. In this forest structure, which is typical of relatively young/managed forests, there is probably more potential habitat available for lichen colonization and propagation at the plot scale. Thus, the higher frequency of *L. scrobiculata* in forests with relatively thin trees instead of large trees suggests that *L. scrobiculata* exhibits slightly different performance compared with *Lobaria pulmonaria* in our study area (e.g., Belinchón et al., 2009, 2011). Both are considered to be old-forest lichen species that are highly dependent on large trees and the microenvironments created by old-forest canopies (Barkman, 1958; James et al., 1977). However, recent studies in boreal forests also suggest that *L. scrobiculata* performs well in young forests, as well as old forests (Hilmo, 2002; Hilmo and Ott, 2002; Hilmo et al., 2011a, 2011b). Our results agree with these findings because *L. scrobiculata* was more abundant in the Mediterranean region, where the tree densities are generally higher but thinner trees are present, which is probably a consequence of the forest management history (Blanco et al., 2005).

This result was unexpected because the climatic conditions are more favorable in the Atlantic region (constant macroenviromental high humidity), where the forests are also older and less intensively managed (Valladares et al., 2004; Blanco et al., 2005). However, the old-forest dependency may vary along ecological gradients (Scheidegger and Werth, 2009). Thus, *Lobarina scrobiculata* occurred in old forests but its abundance appeared to be enhanced in relatively young oak forests in the studied area. It is important to note that population size has been used as an indicator of environmental quality for threatened species (e.g., Carrascal and Seoane, 2009; Belinchón et al., 2011; Stehn et al., 2013). However, other factors such as the genetic diversity or population structure should be assessed to confirm this relationship in our case because a high abundance need not correlate with the optimum environmental conditions. For example, Larsson and Gauslaa (2011) suggested that lichens often experience suboptimal environmental field conditions even where the species are abundant. Nevertheless, the risk of local population extinction increases with decreasing population size (Soulé, 1987; Iriondo et al., 2009), thus special attention must be paid to very small populations (e.g., plots 12, 19, and 20; Table 2).

In addition to the population size, the spatial distribution of individuals within the plot, i.e. occupation rate, is a factor that may determine population persistence. The likelihood of local extinction is probably lower with a high occupation rate (Öckinger and Nilsson, 2010) because stochastic events (e.g., climatic extreme events, cattle activity, or tree falls) are less likely to cause major variations in the population size. The mean occupation rate in the study populations was close to 30%, but most of the populations had a strikingly low occupation rate (Table 2), thereby implying a highly aggregated spatial pattern, which may be a consequence of the following two factors. First, the populations might be young and a result of relatively recent colonization events, so these populations may expand over time (e.g., Kalwij et al., 2005). This may be consistent with our results, which showed that the occupation rate decreased with increasing tree density, and higher tree densities may be related to relatively young forests. Second, they could be declining populations that might become extinct (see Rolstad et al., 2001; Belinchón et al., 2011; Schei et al., 2012). However, the fate of these populations is dependent on the colonization-extinction dynamics because the successful colonization of new trees relies on the dispersal of propagules and the success of establishment. This process is probably constrained by: a) reproductive limitations due to the low production of diaspores (e.g., a lack of reproductive individuals) (Scheidegger, 1995); b) unsuccessful establishment of new individuals due to the low availability of suitable microhabitats (Hedenås et al., 2003); or c) the microenvironments are suitable for establishment but they limit the growth rate (Hilmo et al., 2011b).

The dispersal efficiency of lichens has been shown to be affected by the propagule size, which depends on the type of reproduction, where asexual symbiotic diaspores are much heavier and usually reach shorter distances than sexual fungal spores (Johansson and Ehrlén, 2003; Hedenås et al., 2003; but see Schei et al., 2012). According to Hedenås et al. (2003), asexual dispersal is efficient at a local scale (150 m × 150 m plots) where it results in a homogeneous distribution of epiphytes. Therefore, a high number of colonized trees may indicate effective dispersal within the plot (Johansson and Ehrlén, 2003; Schei et al., 2012). In the populations of *Lobarina scrobiculata* that we studied, soredia production did not appear to be a limitation (Burgaz and Martínez, 1999; S. Merinero et al., unpublished data). Thus, assuming that *L. scrobiculata* disperses effectively at the local scale (but not at the landscape scale; see Hilmo, 2002), we suggest that the high number of non-colonized trees we found might be explained by: a) limited time for colonization and population expansion within the plot and/or, b) the lack of a suitable quality microhabitat for the local establishment of *L. scrobiculata.* The latter reason agrees with other studies at a similar spatial scale, which reported the limited distribution and abundance of epiphytic lichens due to environmental filtering, usually in combination with limitations on local dispersal (Öckinger et al., 2005; Belinchón et al., 2011; Schei et al., 2012; Nascimbene et al., 2013). However, biotic interactions that operate at the tree scale (e.g., competition with other epiphytes) might also influence the distribution and abundance of *L. scrobiculata* at the plot scale.

Tree-scale: occurrence, abundance, and distribution of individuals

The abundance of *Lobarina scrobiculata* was higher at the plot scale where the trees had a relatively thin DBH but, at this finer scale, both the probability of occurrence and abundance increased with the tree DBH. This result agrees with numerous studies (e.g., Hedenås et al., 2003; Ranius et al., 2008; Belinchón et al., 2011) because the tree diameter is generally correlated with tree age (Ranius et al., 2008; Öckinger and Nilsson, 2010) and larger trees have a greater surface area that has been available for colonization for a longer time, while they generally provide a more suitable microhabitat for propagule establishment (i.e., the bark chemistry and roughness may be more suitable) (Barkman, 1958; Belinchón et al., 2011). Our results also highlight the importance of bark roughness, independent of the tree size, as an essential microhabitat quality factor that increases the probability of occurrence and abundance for *L. scrobiculata*. Bark fissures with a greater depth and thickness enhance bark moisture retention (Barkman, 1958; Ranius et al., 2008), which may provide suitable conditions for lichen propagule establishment and development, especially in the dry macroclimatic conditions of the Mediterranean region (Barkman, 1958; Hilmo and Ott, 2002; Belinchón et al., 2009, 2011). The strong effect of bark roughness is probably due to the fact that *L. scrobiculata* mostly colonizes oak trees, which have rough bark even when young (Fig. 2; Belinchón et al., 2009, 2011). In the Atlantic region, the probability of *L. scrobiculata* being abundant on each tree was also higher in forests that received less precipitation and with lower tree densities, which could have been related to the type of forest, because oak forests are found in slightly drier locations and they have a relatively more open canopy than beech forests. Therefore, our results suggest that the distribution of *L. scrobiculata* throughout the Iberian Peninsula is highly dependent on the availability of suitable host trees in favorable oak forests. However, *L. scrobiculata* occurs on different types of broadleaf and coniferous species in other regions (Hallingbäck, 1989; Rolstad et al., 2001; Hilmo et al., 2011a; Smith et al., 2009; Schei et al., 2012).

Lobarina scrobiculata was more frequent on lower parts of the trunks with northerly orientation (Figs 3 and 4). This suggests that its distribution is related to the gradients of light, temperature, and water availability along the trunk. The humidity increases with greater proximity to the ground (Geiger, 1950) and the light availability varies with the aspect on the tree (Gauslaa et al., 2001). Therefore, the pattern detected is consistent with a strong humidity demand at the microhabitat scale, which avoids extreme direct sunlight and wind exposure (i.e., higher desiccation). This trend was stronger in the Mediterranean region where the desiccation risk is higher. By contrast, the individual distribution was not orientation-dependent in the Atlantic region where all sides of the trunk appeared to be equally favorable for *L. scrobiculata* establishment and growth. In addition, it is interesting to note that the proportion of individuals growing close to the ground was much lower in this region compared with the Mediterranean region (Fig. 3). This could be attributable to factors other than humidity and light, which may influence the height distribution on the trunk. For example, we did not evaluate the effect of gastropod grazing but previous studies suggest that the proximity to the ground increases the risk and severity of herbivory for *L. scrobiculata* (Asplund et al., 2010). This factor is probably important in the Atlantic region, which is a very humid and generally calcareous habitat, thus the activity of gastropods may be favored (Gärdenfors et al., 1995).

Conclusions

This study contributes greatly to defining the ecological niche of the threatened species *Lobarina scrobiculata* in two contrasting biogeographical regions of the Iberian Peninsula. *Lobarina scrobiculata* is a rare species in this area but it was locally abundant in rainy locations. However, the occurrence of *L. scrobiculata* in numerous forests with relatively low water availability might be explained by compensating factors, such as forest structural features and/or its high thallus water-holding capacity. Given the low occupation rates in most of the populations, we consider that the status of *L. scrobiculata* remains uncertain in the Iberian Peninsula because the extinction risk increases with a low occupation rate. Our hypothesis that *L. scrobiculata* would perform better in the

Atlantic region was not supported because the abundance and occupation rates were generally lower than those in the Mediterranean region.

The abundance was higher in locations with more but thinner trees. Thus, although it is widely accepted that *Lobarina scrobiculata* belongs to mature epiphytic communities, relatively young and/or slightly managed forests hosted more abundant and vital populations of this species. Therefore, conservation strategies should be directed toward the preservation of known populations, as well as uncolonized and nearby forests, which may be potential areas for population expansion.

Multi-scale field measurements are required because environmental variables may affect the performance of a species in different ways depending on the scale (e.g., the differences in the importance of DBH or annual precipitation at the plot and tree scales). Our findings are important for extending the knowledge of the environmental requirements of *Lobarina scrobiculata*, which are critical for implementing conservation measures. Many questions are still unanswered but new hypotheses arise from these interesting results. For example, the fine-scale demographic population structure related to forest management, long-term population monitoring, and genetic population structure have not been determined but they are needed to assess the current conservation status of *L. scrobiculata* in this heterogeneous region.

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CAPÍTULO II / CHAPTER II

SIZE-DEPENDENT REPRODUCTIVE PROBABILITY AND ALLOCATION OF AN EPIPHYTE CHANGES ALONG A CLIMATIC GRADIENT IN SOUTHERN EUROPE

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Manuscript in preparation

ABSTRACT

Since many species persist in different environments, intraspecific variation in reproductive traits among populations in heterogeneous environments can be expected. Yet, no studies on reproductive strategies along environmental gradients have targeted primary producers such as lichens. We assessed the reproductive strategy of the threatened epiphytic lichen *Lobarina scrobiculata* among 18 populations along the Iberian Peninsula. We hypothesized that this asexually reproducing species reproduces at larger sizes and more efficiently in the favourable environments (e.g. rainy locations), and vice versa in adverse conditions. Using GLMs and SMA regressions we evaluated the reproductive threshold size and reproductive efficiency (defined by the allometric slope between somatic and reproductive surfaces) among populations. Results showed that reproduction of *L. scrobiculata* (n = 9665) was size-dependent, but the reproductive threshold size differed among populations. Most populations showed positive allometry between somatic and reproductive surface, indicating that *L. scrobiculata* efficiently converts somatic tissue into reproductive structures. As expected, *L. scrobiculata* started to reproduce at larger sizes in rainy locations. However, the allometric slopes were greater in drier locations, suggesting a strategy of increased propagules' pressure to enhance colonization in adverse environments, although at the expense of low genetic diversity. The efficient and flexible reproductive strategy of *L. scrobiculata* may influence its population structure and dynamics, ultimately affecting the community functioning. This study provides novel insights on the variation of reproductive strategies due to environmental heterogeneity in lichens. A broad taxa base is needed to substantially widen our general understanding of the evolution of reproductive strategies.

Key words:

Allometry, asexual reproduction, epiphytic lichen, life-history traits, *Lobarina scrobiculata*, precipitation gradient, size-dependency, reproductive ecology, reproductive allocation, soredia.

INTRODUCTION

Life-history theory has emphasized on the patterns of resource allocation to reproduction of species because they reflect strategies that have evolved under natural selection and other ecological constrains (e.g., Williams 1966; Stearns and Koella 1986; Kozlowski 1992; Stearns 1992). Because many species persist in a wide range of environmental conditions, significant intraspecific variation in reproductive allocation patterns among populations in heterogeneous environments can be expected (e.g. Ohlson 1988; Wesselingh et al. 1993, 1997; Guo et al. 2012). The onset of reproduction and the flexibility in the reproductive allocation patterns define the reproductive strategy of a species. Both traits are key aspects in the life history of most organisms because they significantly influence population dynamics and individual fitness, allowing the development of adaptive strategies across environments (Stearns 1992; Bonser & Aarssen 2009; Weiner et al. 2009; Kooijman & Lika 2014). Thereby, understanding the variation of reproductive allocation patterns within and across taxonomic groups has been a frequent ecological research topic. However, despite this interest, literature on variation in reproductive allocation patterns due to e.g. environmental variation have mainly targeted animals (e.g. Wootton 1979; Boggs 2009; Kooijman & Lika 2014) and vascular plants (e.g. Thomas 1996; Méndez & Karlsson 2004; Weiner et al. 2009), with far scarce attention to other taxonomic groups such as fungi, including lichens. Advancing of our understanding of life history variations and their environmental drivers requires the consideration of broader taxonomic groups, especially those with physiological characteristics and environmental constrains different from the more frequently studied taxa within the life-history theory frame.

Lichens are symbiotic organisms formed by a fungus (mycobiont) and a photosynthetic partner that can be a green algae and/or cyanobacteria (photobiont). These primary producers constitute a great portion of biomass on most terrestrial ecosystems and play significant roles in ecosystem functioning, especially in nutrient cycling (Seaward 2008). For example, many lichens with cyanobacteria (e.g. cyanolichens) fix atmospheric nitrogen that is readily available for the rest of the community (e.g. plants) through leaching and decomposition (Nash 2008; Porada et al. 2014), and they may account for a high percentage of the total N input in some forest ecosystems (Denison 1979; Nash 2008). Lichens are perennial, usually long-lived, relatively slow-growing and their life-cycle stages are yet poorly known. They lack mechanisms to regulate water and nutrient uptake, so their growth and functioning are narrowly linked to environmental water, light and nutrient availability (Palmqvist 2000).

Similarly to most organisms, size is an important component of fitness in lichens, because for instance larger individuals (1) have higher water holding capacity and thus longer photosynthetic active periods (Gauslaa and Solhaug 1998; Merinero et al. 2014a), (2) contain higher investment of defence secondary compounds (Asplund and Gauslaa 2007), and (3) invest more resources in reproduction (e.g. Ramstad & Hestmark 2001; Martínez et al. 2012) than smaller individuals. Main lichen modes of reproduction are either asexual (normally via symbiotic diaspores such as soredia and isidia), sexual (via fungal spores produced in reproductive structures like apothecia), or rarely both (Bowler & Rundel 1975). Several studies found a threshold size for the onset of both types of reproduction in various species from different environments (e.g. Jahns & Frey 1982; Hestmark 1992; Ramstad & Hestmark 2001; Pringle et al. 2003; Martínez et al. 2012; Hilmo et al. 2011, 2013). Nevertheless, intraspecific variation of the threshold size for reproduction among populations in contrasting environments has rarely been accounted for (see Hilmo et al. 2013).

Much less information is currently available on the reproductive allometry of lichens. In plants, the reproductive effort usually relates to the vegetative biomass through a power equation which is fitted in a log-transformed relationship. The slope of this relationship (i.e. allometric slope) is interpreted as the plant efficiency in converting vegetative biomass into reproductive biomass (Niklas 2004; Weiner et al. 2009). An allometric slope significantly higher than 1 indicates a high reproductive efficiency (i.e., positive allometry), while an allometric slope non-significantly different from 1 indicates an isometric relationship between the vegetative biomass and the reproductive effort (Weiner et al. 2009). Few studies on lichens, using different methodologies, attempted to describe the shape of the relationship between the reproductive effort and the lichen size. Some species were suggested to show isometric relationships (e.g. Ramstad and Hestmark 2001; Gregersen et al. 2006) while others seemed to show positive allometry (e.g. Pringle et al. 2003; Shimizu and Kubo 2009). Unfortunately, none of these studies evaluated the allometric slopes between the reproductive effort and the lichen surface, limiting the reliability and comparison of these findings.

Life-history theory predicts a negative correlation between the threshold size for reproduction and the allometric slope that often relate with environmental variation (Schmid & Weiner 1993), i.e. plants in favourable environments start reproduction at larger sizes to maximize their fitness during its longer lifetime (Stearns and Koella 1986; Wesselingh et al. 1997) and then should be more efficient in their reproduction at larger sizes (i.e. allometric slope >1) due to greater environmental resource availability promoting larger vegetative size of plants (Kozlowski 1992; Wesselingh et al. 1997; Bonser & Aarssen 2009; Guo et al. 2012; Wang et al. 2014). Conversely, harsh environments typically selects smaller reproductive sizes due to a reduced lifetime, and thus high resource allocation to reproduction at smaller sizes should be an advantage because mortality at larger sizes can be high (Stearns 1992, Kozlowski 1992; Wesselingh et al. 1997; Bonser & Aarssen 2009).From a plant developmental perspective, this can be related to the constraints that the environment pose on the plant growth rates because plants in favourable environments might grow faster than plants in harsh environments. Thereby, populations of the same species adapted to harsh environments will show lower reproductive size and allometric slopes than those in favourable environments. Nevertheless, regardless of the mechanism underlying the changes in the reproductive allocation relationships, the reproductive effort – vegetative size relationship measured in the field is the more important factor influencing the plant populations' dynamics and evolution (Wang et al 2014). Variation in the reproductive allocation patterns can help us to understand how a species adapt to its environment over a wide range of conditions. Yet variations of the reproductive allocation in lichens along environmental gradients have not been documented.

In this study we used the epiphytic cyanolichen *Lobarina scrobiculata* as a model species to assess patterns of reproductive allocations among 18 populations along a climatic gradient in the Iberian Peninsula (Southern Europe). This species is widely distributed but declining and red-listed in many countries in the Northern Hemisphere (Sérusiaux 1989; NatureServe 2014). In our study area the abundance of this rare species increases with increasing precipitation (Merinero et al. 2014b). In boreal forests the onset of reproduction is size-dependent (Larsson and Gauslaa, 2011; Hilmo et al. 2011; 2013), and some studies documented a trade-off between growth and reproduction (Larsson and Gauslaa 2011; Merinero et al. 2015). Following predictions of life-history theory, we hypothesized that *L. scrobiculata* reproduces at larger sizes and more efficiently (allometric slopes >1) in the favourable environments (e.g. higher summer and/or annual precipitation) and vice versa in adverse environments. The specific objectives addressed were: (1) To assess intraspecific differences of threshold size for reproduction among populations in relation with environmental heterogeneity; (2) to study whether the relationships between reproductive (R) and somatic (S) lichen surfaces were allometric in each population; and (3) to examine whether the log R–log S relationships differed among populations and changed with environmental heterogeneity.

MATERIAL AND METHODS

Study species

Lobarina scrobiculata (Scop.) Nyl. ex Cromb. (Lobariaceae) is an epiphytic foliose cyanolichen, containing *Nostoc* as its main photobiont. Although it is widespread, it is more frequent in the Northern hemisphere but progressively declining (Smith et al. 2009; NatureServe 2014; Merinero et al. 2014b). In the Iberian Peninsula is rare and usually inhabits mountain forests with high differences in humidity and forest structure (Merinero et al. 2014b). The most frequent mode of reproduction is by large asexual symbiotic diaspores (soredia) where both mycobiont and photobiont disperse simultaneously. Soredia occur in distinct laminar and marginal areas called soralia variable in size that remain permanently on the thallus surface. Soredia are typically dispersed by wind, rain and animals (Bowler & Rundel 1975). Yet the species-specific and/or environmental factors that stimulate sexual reproduction are unknown, as the occurrence of apothecia is sparse (Smith et al. 2009; Merinero et al. personal observation)

Study area and sampling method

We selected 18 populations of *L. scrobiculata* along a wide climatic gradient that included two biogeographical regions, the Atlantic region (populations 1-5) and Mediterranean region (populations 6-18) in the Iberian Peninsula (Table 1). The sites were representative of the forests where this species occurs, mainly *Quercus* sp. forests (populations 4-18) and *Fagus sylvatica* forests (populations 1-3) (Table 1) (see Merinero et al. 2014b for further details). Field surveys were done from October 2009 to July 2012. We randomly established a 100 x 30 m plot inside a forest where *L. scrobiculata* occurred. For each plot we registered the climatic variables annual precipitation (mm), summer and winter precipitation (mm), and mean annual temperature (ºC) (extracted from CLIMOEST; Sánchez-Palomares et al., 1999) (Table 1). The latitude, longitude and altitude were recorded in situ using a GPS device (GPSmap 60CSx, Garmin GPS) (Table 1).

All *L. scrobiculata* individuals on living trees were counted at a height of up to 2 m because it occurs rarely above this height in the study region (Merinero et al. 2014b). We considered “individuals” to be all spatially distinguishable lichen entities that were easily differentiated from others (e.g. Martínez et al. 2012; Merinero et al. 2014b). We measured the largest diameter of each individual (mm) (hereafter referred as to size) and estimated the surface of the individual covered by soredia (%), i.e. reproductive effort. We evaluated the relationship between lichen area (mm^2) and largest diameter (mm) from photographs of 200 randomly chosen individuals in the population 9 with largest diameters ranging from 5.6 to 171 mm. The fitted power regression for this relationship was: Lichen area= 0.6188 x Largest diameter$^{1.8422}$ (R^2adj = 0.95; $P < 0.001$). Based on this strong relationship, the somatic lichen surface (S) and the area covered by soredia, i.e., reproductive surface (R) were calculated for reproductive individuals. We used the term “somatic” (S) instead of “vegetative” (V), which is commonly used for plants, because the former usually refers to asexual reproduction in the fungal literature.

Table 1. Summary of the geographic and climatic variables in 18 populations of *Lobarina scrobiculata* along the Iberian Peninsula. Pop.: Population; Precipitation (mm); Mean T: Mean annual temperature (ºC). Populations ordered by decreasing latitude.

Pop.	Locality	Province	Latitude	Longitude	Altitude (m a.s.l.)	Annual P	Summer P	Winter P	Mean T
1	Pontón	León	43°06'10"N	05°01'02"W	1328	1721	229	552	7.8
2	Burón	León	43°04'26"N	05°11'25"W	1353	1371	135	368	7.5
3	Irati	Navarra	42°59'15"N	01°06'14"W	965	1615	149	589	8.9
4	Riaño	León	42°57'41"N	05°01'12"W	1150	1156	117	295	8.8
5	Brañosera	Palencia	42°56'00"N	04°17'24"W	1260	946	131	326	8.1
6	Sanabria	Zamora	42°06'04"N	06°38'43"W	950	1054	95	384	10.3
7	Montejo	Madrid	41°06'44"N	03°29'33"W	1263	818	92	258	9.5
8	Santuy	Guadalajara	41°05'44"N	03°26'27"W	1235	801	92	252	9.7
9	Escorial	Madrid	40°34'02"N	04°09'20"W	1066	796	85	272	11.2
10	San Vicente	Toledo	40°08'57"N	04°44'13"W	1239	947	68	334	10.5
11	Sicó	Coimbra	40°00'25"N	08°27'25"W	290	1178	41	612	18.0
12	Risco Paradas	Ciudad Real	39°35'07"N	04°31'23"W	1165	756	54	286	11.2
13	Cigüeñelas	Ciudad Real	39°29'27"N	04°36'33"W	797	638	45	246	13.6
14	Gargantilla	Ciudad Real	39°27'46"N	04°35'43"W	749	656	42	235	13.8
15	Fuencaliente	Ciudad Real	38°26'45"N	04°15'47"W	881	729	49	224	13.9
16	Robledo Hoyas	Ciudad Real	38°26'27"N	04°20'41"W	1030	777	54	248	13.2
17	Grazalema	Cádiz	36°45'56"N	05°24'45"W	775	1753	32	734	15.4
18	Sierra Nieves	Málaga	36°39'44"N	05°04'33"W	1064	1155	23	517	14.0

Statistical analyses

We run Generalized Linear Models (GLMs) with binomial error (McCullagh and Nelder, 1989) to examine the relationship between reproductive status and individual size for each population. The parameters of these logistic models were used to estimate probability (P) for reproduction in relation with individual size (x) according to the formula $P = 1/\ (1+e^{(\mu+\alpha x)})$, where parameters μ and α determine the intercept with the X-axis and the slope of the curve, respectively (Wesselingh et al. 1993; 1997). These parameters can be related to the threshold size for reproduction (Wesselingh et al. 1993; Méndez and Karlsson 2004), which was estimated at 0.5 and 0.95 probabilities in each population.

To estimate the reproductive allocation of *Lobarina scrobiculata* in each population juveniles were removed from the data set prior to analyses. We analysed the reproductive surface (R) –somatic lichen surface (S) relationship using the classical allometric model used for plants that relates the reproductive output (R) with the vegetative biomass/size (V) as: $R= aV^b$, usually fit as log-log relationship ($\log R= \log a + b \log V$ (Klinkhamer et al. 1992; Niklas 2004). The intercept (parameter a) is referred to as the “allometric coefficient” and the slope (parameter b) the “allometric exponent”. A slope significantly different from 1.0 indicates a non-isometric relationship. We used model II regressions (standardized major axis; SMA) because this method is more appropriate than traditional least squares regression for fitting allometric data and estimation of parameters (e.g. Niklas 2004; Warton et al. 2006). We tested whether the slope of each population statistically differed from 1.0, and we compared the slopes of the regressions among populations using multiple *post hoc* comparisons. All the SMA analyses were computed with the smatr R package (Warton et al. 2012). Residuals vs predicted values were checked for normality.

Finally, we investigated the relationships between the estimated parameters (threshold sizes for reproduction and allometric slopes) and the climatic variables with parametric and non-parametric correlations. Least square regression were run for significant relationships (Pearson’s and or Spearman’s correlations coefficient $r \geq 0.6$, $P<0.05$). All statistical analyses were done in R version 3.1.1 (R Foundation for Statistical Computing, Vienna, Austria). Mean values ± 1SE are reported in the text and the significance level for all analyses was $P < 0.05$.

RESULTS

Environmental heterogeneity and populations' characteristics

The studied populations comprised a wide range of localities highly varying in topographic and climatic factors (Table 1). For example, altitude varied from 290 to 1353 m a.s.l., and the corresponding negatively correlated mean annual temperature ranged from 18 to 7.5 ºC, and also increased with decreasing latitude (Pearson's correlation coefficient r =-0.75, P <0.001; Table 1). Annual precipitation varied in more than 1000 mm (638 to 1753 mm), but it was not correlated with latitude because the southernmost localities (e.g. populations 17 and 18) were extremely rainy due to topographic factors (Table 1). However, the summer precipitation declined as the latitude decreased (Pearson's correlation coefficient r =0.72, P <0.001) and it was especially scarce in the Mediterranean region (populations 6-18) (Table 1).

Among the 18 populations, 9665 individuals of *L. scrobiculata* were recorded, from which 3048 were reproductive (Table 2). However, the proportion of juvenile-reproductive individuals highly varied among populations (Table 2). Population 18 hosted the highest proportion of reproductive individuals, whilst population 6 had the lowest (Table 2). Mean individual size also varied among populations (ANOVA, df =17, F= 100.35, P < 0.001), being largest in population 10 and smallest in populations 6 and 7 (Table 2). Mean size of reproductive individuals was greater than of juvenile individuals in all populations (ANOVAs, data not shown, all tests P < 0.001). The smallest reproductive individual was 6 mm (population 8) while the largest juvenile was 200 mm (population 7). Mean size of reproductive individuals among populations differed (ANOVA, df= 17, F= 28.88, P<0.001), but it was uncorrelated with environmental variables.

Table 2. Population structure, individual size (Min=minimum, Max = maximum), size of reproductive individuals, reproductive effort (soredia cover) and threshold size for reproduction at 0.5 and 0.95 probabilities of *Lobarina scrobiculata* along the Iberian Peninsula. Absolute and mean ± 1SE values are shown. Pop.: population code; abund.: number of individuals.

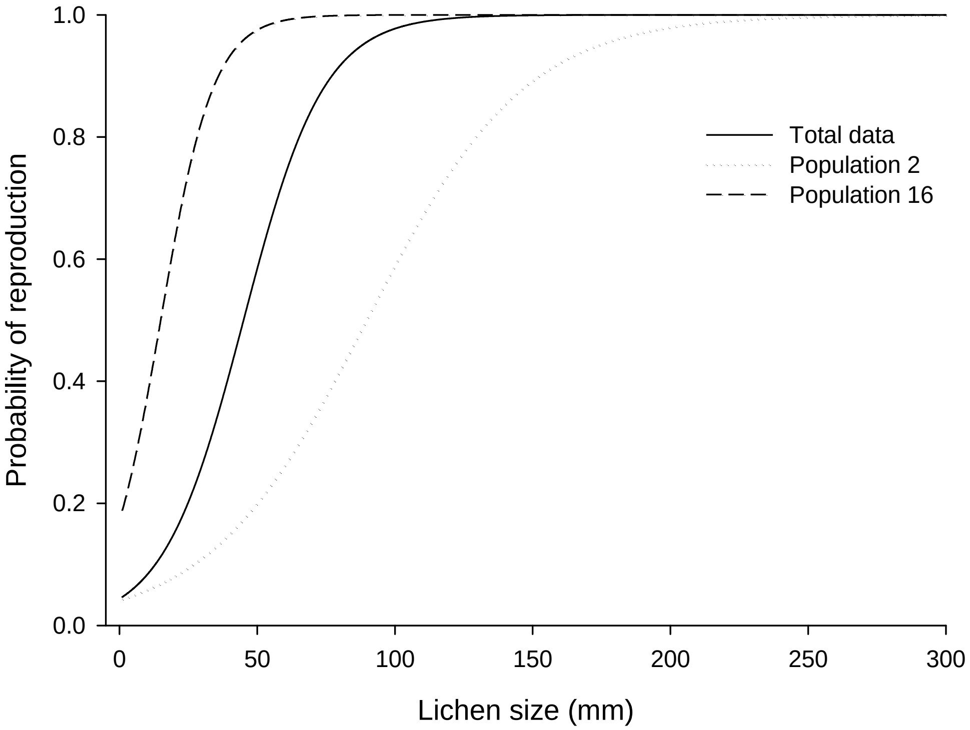
Pop.	Abund.	Juvenile (%)	Reproductive (%)	Min-Max size (mm)	Size (mm)	Reproductive size (mm)	Threshold 0.5 (mm)	Soredia cover (%)
1	201	83.6	16.4	2.5-220.0	36.1±2.4	74.6±7.6	89.7	5.4±0.9
2	988	85.6	14.4	3.0-133.2	23.6±0.6	46.8±1.9	53.6	6.7±0.6
3	129	88.4	11.6	3.5-115.2	29.8±2.0	59.9 ±6.3	76.8	2.1±0.3
4	1487	87.4	12.6	1.0-230.0	21.2±0.7	67.7±3.1	55.9	11.9±0.8
5	587	63.9	36.1	1.7-160.0	32.3±1.1	56.5±2.1	37.8	10.4±0.7
6	1091	93.6	6.4	2.3-270.0	17.5±0.8	85.9±5.9	69.8	9.6±1.5
7	491	90.8	9.2	3.3-410.0	17.6±1.6	97.4±11.4	41.1	12.6±1.5
8	220	67.7	32.3	2.8-460.0	49.3±4.3	107.9±10.0	49.1	22.1±2.7
9	1758	48.2	51.8	5.0-400.0	52.4±1.0	70.9±1.7	43.8	11.9±0.5
10	273	41.8	58.2	10.2-350.0	59.4±2.7	72.3±4.0	52.1	11.0±0.9
11	158	60.1	39.9	3.0-154.4	35.7±2.6	61.5±4.5	37	14.2±1.5
12	169	58	42	6.7-236.1	48.0±3.1	81.5±4.8	47	22.8±1.8
13	510	69.4	30.6	4.7-138.7	36.3±1.0	52.2±2.0	53.7	6.8±0.2
14	203	48.8	51.2	10.5-114.2	45.1±1.6	57.4±2.2	42.2	7.0±0.4
15	725	54.8	45.2	3.6-155.8	27.3±0.6	33.3±1.0	29.5	3.8±0.3
16	491	14.7	85.3	7.2-255.0	41.3±1.1	44.1±1.2	15	6.9±0.3
17	102	66.7	33.3	1.3-162.4	41.0±3.6	75.4±6.2	53.1	7.4±1.1
18	82	10.2	89.8	4.5-121.0	31.9±2.5	47.5±5.2	44	4.5±0.8
Total	9665	68.5	31.5	1.0-460.0	29.2±0.3	61.0±0.8	45	9.8±0.2

Population 8 hosted larger reproductive individuals and population 15 smaller ones (Table 2). The reproductive effort within individuals ranged from 1 to 90% of soredia cover, and mean values per population were below 25% and significantly different among them (Table 2; ANOVA, df= 17; F=25.80, *P*<0.001). Minimum mean soredia cover was recorded in population 3 and maximum in population 12 (Table 2).

Threshold size for reproduction and climatic correlations

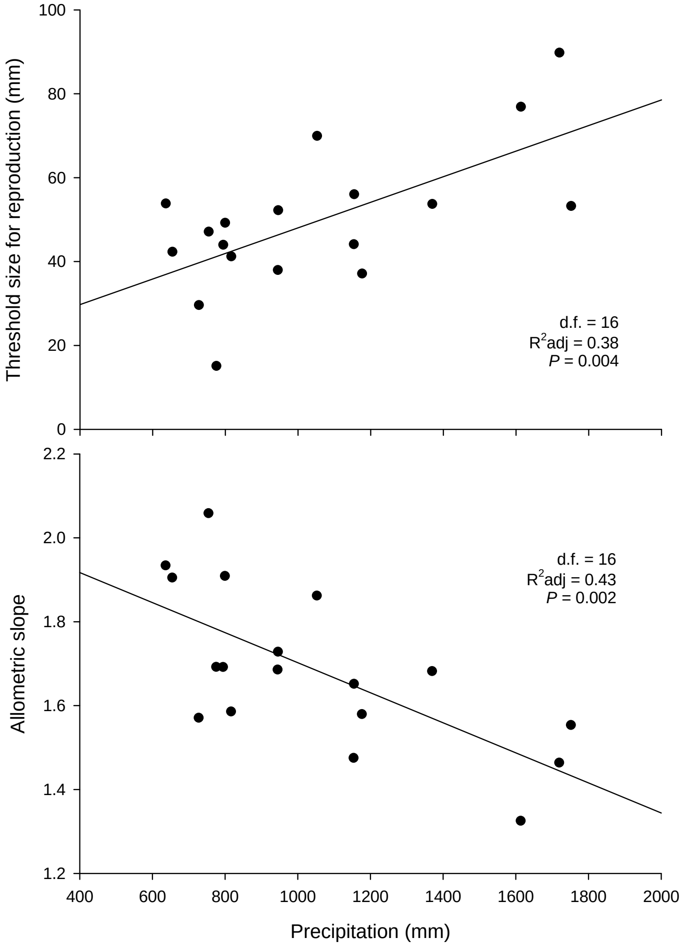
The probability of reproduction increased with increasing individual size in all populations (GLMs, *P*<0.001; data not shown). The threshold size for the onset of asexual reproduction with a probability of P =0.5 highly varied among populations (Table 2). The lowest threshold size for reproduction was estimated in population 16, whilst the highest was in population 1, with a 6-fold and 4-fold greater threshold size for probabilities of 0.5 and 0.95, respectively (Table 2; Fig. 1). A total of 7.7% of juveniles had the required threshold size for reproduction at P=0.5. This proportion was highest in population 16 with 83.1% of juvenile individuals above the threshold size for reproduction and lowest in population 6 with 1.1% of juvenile individuals above the threshold size for reproduction.

Figure 1. Size-dependent asexual reproduction probability in 18 populations of *Lobarina scrobiculata* along the Iberian Peninsula (n= 9665; total data). Populations with minimum and maximum threshold size for reproduction are shown. Identity of populations is given in Table 1.



Regarding the relationships with the climatic variables, the threshold size for reproduction was only highly and significantly correlated with the annual precipitation (Pearson's correlation coefficient *r* = 0.64, *P* = 0.004). Threshold size for reproduction significantly increased with increasing annual precipitation (Fig. 2a).

Figure 2. Relationships of the (a) threshold size for reproduction (estimated at a probability of 0.5) and (b) the allometric slopes for the log R- logS regressions of *Lobarina scrobiculata* populations along a precipitation gradient in the Iberian Peninsula (n=18).



Log R – Log S relationships and environmental effects

In all populations the allometric relationships between log reproductive surface (R) and log somatic lichen surface (S) were positive and significantly correlated (Fig 1 supplementary data). All populations had slopes significantly >1, except population 3 (*n*= 21) that had a slope non-significantly different from 1 (Table 1 supplementary material). The *post hoc* multiple comparisons among populations showed significant differences of slopes among them (Table 3). The estimated allometric slopes were only significantly correlated with annual precipitation (Pearson's coefficient *r* = -0.68, *P* =0.002), and they did not correlate with the threshold size for reproduction (Pearson's coefficient *r* = 0.37, *P* =0.383). The slopes of the logR-logS relationships significantly decreased with increasing annual precipitation (Fig. 2b).

Table 3. Post-hoc multiple comparisons of allometric slopes among 18 populations of *Lobarina scrobiculata* along the Iberian Peninsula. Identity of populations is given in Table 1.The pair-wise significant matrix for *P* < 0.05 *, *P* < 0.01 ** and *P*<0.001 ***.

Slope	Population	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1.46	1	0							*				**		*				
1.68	2		0										**						
1.32	3			0					*				**		*				
1.65	4				0								**						
1.68	5					0							**						
1.86	6						0									*			*
1.58	7							0	*				**		*				
1.91	8	*		*				*	0			*		*		**			*
1.69	9									0			***						
1.73	10										0		*						
1.58	11								*			0	**		*				
2.06	12	**	**	*	**	**		**		***	*	**	0	***		***	***	*	**
1.93	13								*				***	0	*				
1.9	14	*		*				*				*		*	0	**			*
1.57	15						*		**				***		**	0			
1.67	16																0		
1.55	17												*					0	
1.47	18						*		*				**		**				0

DISCUSSION

Evaluating the reproductive allocation patterns has been a major topic of research in decades. It has been motivated by their implication in the adaptive response of species to environmental changes and thus their meaning in the life-history of different organisms (e.g., Weiner et al. 2009; Santos-del-Blanco et al. 2013). Yet, no studies on reproductive allocation patterns along environmental gradients have targeted photosynthetic symbiotic organisms such as lichens. Our results showed important and consistent relationships of annual precipitation with the threshold size for reproduction and the positive allometric reproductive allocation variation of an epiphytic cyanolichen along a climatic gradient in Southern Europe.

Threshold size of size-dependent reproduction

In agreement with previous results in boreal forests (Hilmo et al. 2011; 2013; Larsson and Gauslaa 2011), asexual reproduction in *L. scrobiculata* was size-dependent i.e., larger thalli are more likely to become reproductive than smaller ones. The threshold size for reproduction for *L. scrobiculata* estimated in the Iberian Peninsula was 45 mm, fairly similar to the estimated by Hilmo et al. (2011) in a spruce plantation in Norway (49.9 mm), but almost twice than in a neighbour old-spruce forest (28.5 mm) (Hilmo et al. 2013). Hilmo et al (2013) did not test the effects of environmental factors on the reproductive threshold size, but suggested that habitat heterogeneity could account for such difference. Nevertheless no study on lichens has related threshold size for reproduction variation with environmental heterogeneity before. However, we found that the threshold size for reproduction strongly varied among 18 populations of *L. scrobiculata* (Table 2; Fig. 1), and this variation was significantly related with annual precipitation along a latitudinal gradient.

As we expected, the threshold size for reproduction significantly increased with increasing precipitation (favourable habitat) and decreased in drier locations (adverse habitat) (Fig. 2a) i.e., *L. scrobiculata* starts to reproduce at smaller sizes in stressful locations, consistent with qualitative predictions of life history theory (Stearns & Koella 1986). Similarly, Santos-del-Blanco et al. (2013) documented a significant smaller threshold size for reproduction in the driest populations of a Mediterranean pine, suggesting that this pattern is the product of an adaptive response to drought stress. In our case, it is reasonable to attribute the high variability in the threshold size for reproduction to the variability in the relative growth rates likely caused by precipitation differences. For instance, Wesselingh et al. (1997) found that the populations of an herbaceous plant with faster relative growth rates had greater threshold sizes for reproduction than those with slower growth. In our study region relative growth rates are probably higher in the more rainy locations because the abundance of *L. scrobiculata* at the forest scale increases with precipitation (Merinero et al. 2014b), probably because cyanolichens require liquid water to activate photosynthesis and thus to grow (Lange et al. 1986). Nevertheless, we should not disregard that this variability might be also the product of adaptive responses maintained by natural selection (Bonser & Aarssen 2009; Weiner et al. 2009; Santos-del-Blanco et al. 2013).

There has been a long debate on the implications of reproduction at smaller sizes in the individual life-trajectory in plants. In monocarpic species, current reproduction compromises the future reproduction (demographic costs), because the reproductive effort increases with vegetative size, and plants that reproduce at small sizes should have less potential to reproduce in the future at their final development than larger plants because reproduction reduce the amount of resources allocated to growth (Williams 1966; Stearns 1992; Obeso 2002). However, in polycarpic species (like asexually reproducing lichens) the onset of reproduction at small sizes does not necessarily constrain or drive the relative allocation to reproduction at final development because the developmental trajectories of plants are not fixed and the investment in maintenance of similar-sized mature individuals might differ along their lives (Bonser & Aarssen 2009).

An intriguing result is the relatively high proportion of *L. scrobiculata* individuals that remained juvenile when their size exceeded the threshold size for reproduction (e.g. population 16), indicating that the threshold size also vary within populations. Various studies on lichens documented a similar trend (Gregersen et al. 2006; Pringle et al. 2003; Martínez et al. 2012) and this often occurs in plant populations (e.g. Ohlson 1988; Méndez & Karlsson 2004; Santos-del-Blanco et al. 2013). This pattern could be due to genetic variation among individuals, serving for example as a bet-hedging diversification strategy in which genetically determined individuals start reproduction at larger sizes and others at smaller, because in the case of a disturbance not all the future reproductive output of the population is compromised (Simons 2007), and increasing allocation to growth may also increase survival probability and thus fitness in plants (Stearns and Koella1986; Stearns 1992; Obeso 2002). However, and nonexclusively, this situation can be due to resource availability heterogeneity (e.g., water, nutrients and light) within a population, because individuals within a population usually experience different environmental conditions and thus have different developmental rates influencing reproductive and vegetative size (Bonser & Aarseen 2009). For example, lichen growth rates among individuals within a population vary at the local spatial scale due to microclimatic factors such as the heterogeneity of light and water availability along the canopy and trees (e.g. Shriver et al. 2012; Larsson et al. 2014; Merinero et al. 2015). However, the mechanisms underlying the reproductive threshold size variability of lichens within populations deserve more attention to understand the role of genotypes and their interaction with the environment.

Log reproductive – Log somatic surfaces relationships and environmental effects

From an allocation perspective in connection with reproductive strategies in plants, the slope of the R- S biomass relationship reveals how efficiently resources stored or acquired are converted into reproductive structures across plant sizes (Weiner et al. 2009; Guo et al. 2012). Many studies in organisms with indeterminate growth documented a disproportional increase in resource allocation to reproduction through the organism development after a vegetative growth period (e.g. Thomas 1996, Wesselingh et al. 1993 1997; Bonser & Aarseen 2009). In agreement, our results showed that the allometric slopes of the log R-log S surfaces relationships were significantly higher than one in all populations of *L. scrobiculata*, excepting population 3 probably due to small population size and/or narrow lichen size range. Thus, we interpret that *L. scrobiculata* efficiently transforms somatic surface into reproductive structures, because individuals allocate more resources to reproduction than the expected proportionally to the corresponding size. Such pattern has rarely been documented in annual plants (but see Guo et al. 2012) because reproductive and vegetative biomass relationships are usually proportional (Weiner et al. 2009). By contrast, this high efficient pattern occurs in tropical trees (e.g. Thomas 1996). However, given the high diversity of methodologies and surrogates for reproductive and vegetative size or biomass to estimate the R-S relationships in plants, and the lack of studies appropriately testing allometric reproductive allocation in lichens, comparisons among taxa cannot be extended.

Furthermore, we found significant heterogeneity of the allometric slopes among populations (Table 3), suggesting that the amount of resources allocated to reproduction disproportionally vary among populations. Intraspecific variation in the R-S allometric slope among populations has been documented in various plant studies that found changes in slopes in relation to environmental heterogeneity (e.g. Ohlson 1988; Sugiyama & Bazzaz 1998; Guo et al. 2012). Theory and evidence on plants suggest that individuals in favourable environments show higher reproductive output because plants generally improve their reproductive efficiency where they have more resources to reach larger sizes (Schmid and Weiner 1993; Bonser & Aarseen 2009; Weiner et al. 2009; Guo et al. 2012). Interestingly, and contrary to our expectation based on theory, the allocation of resources to reproduction significantly increased with increasing dryness, i.e. higher reproductive efficiency in adverse environments (Fig. 2b). However, this pattern would be consistent with a higher investment in reproduction in dry locations due to higher adult mortality in adverse environments (Stearns 1992; Welham & Setter 1998). Nevertheless, here we assess asexual reproduction, and life-history theory predictions are based on sexual reproduction, which is presumably more costly (Bowler & Rundel 1975; Hestmark 1992). Despite we lack data on mortality rates, it is possible that *L. scrobiculata* enhances its probability of survival by increasing the propagules' pressure in dry locations because in these habitats the requirements for its establishment are narrower than in the rainy locations, as it preferentially occurs on the northern exposure and on the base of the trees in the dry locations, while this pattern is faded in the humid locations (Merinero et al. 2014b). Although it is not a common trend, various plant species show a higher investment in reproduction as a plastic/adaptive response to stress (e.g. review by Chiarello & Gulmon 1991; Santos-del-Blanco et al. 2013), although this response highly varied among taxa and the source of stress (Chiarello & Gulmon 1991).

Despite the allometric slopes were steeper in dry locations (Fig 2b), it is noteworthy that the effect of precipitation varied across the range of lichen sizes among some populations, reflected by the crossing slopes that indicate an interaction between precipitation and lichen size (Table 3). Consequently, small individuals from relatively rainy locations allocated resources more efficiently to reproduction than their counterparts in drier locations (i.e. disproportionally produced more soredia), whilst large individuals in dry locations were more efficient than their counterparts in rainy locations. A similar relationship between plant size and altitude was found in herbaceous plants along an altitudinal gradient (Guo et al. 2012), suggesting that the effect of the environmental constrains vary depending on the individual reproductive size. Nevertheless, and similarly with the threshold size variation previously discussed, we cannot disregard that genetic factors allowing adaptive responses to environmental variability and/or phenotypic plasticity (e.g. growth and/or reproductive plasticity) influence the heterogeneity of slopes among populations (Schmid and Weiner 1993; Sugiyama & Bazzaz 1998; Santos-del-Blanco et al 2013). Nevertheless, plasticity of reproductive allometry for a given genotype in herbaceous plants is considered uncommon (Weiner 2004; Weiner et al., 2009), whereas plasticity in the growth and developmental rates tend to be more frequent, so intraspecific variation may be more influenced by the direct effect of the environment on the allometric growth genotypes (Bonser & Aarseen 2009; Weiner et al. 2009). We lack knowledge on the diversity of genotypes and phenotypic plasticity of *L. scrobiculata* in the studied region and thus on its ability to adapt to varying environments, but it is plausible that plasticity in the relative growth rates of *L. scrobiculata* means a stronger mechanism influencing its reproductive allometry rather than genetic based constrains along the studied populations. To gain better insight into this uncertainty, data on genotypes and relative growth rates of individuals within and among populations in the long term would be needed.

Implications and conclusions

Lobarina scrobiculata showed a high variable reproductive threshold size for reproduction, and had a high reproductive efficiency among populations. There was not a negative correlation between threshold size for reproduction and reproductive efficiency predicted by life-history (Schmid & Weiner 1993). However, both reproductive traits changed with precipitation, indicating that *L. scrobiculata* initiates reproduction at smaller sizes but highly efficient in drier locations. Clonal reproduction, which is frequent in lichens, has been identified as an optimal strategy for local colonization of stable environments (Bowler & Rundel 1975; Caswell 1985; Hestmark 1992). The small reproductive size of *L. scrobiculata* in the drier locations together with the disproportionally high reproduction in most populations likely cause a high density of propagules released into the environment, increasing the probability of local colonization and thus contributing to the population growth and maintenance, although at the expense of low genetic diversity. This pattern points to a strategy typical of expanding populations (or colonising species), where an early and higher investment in reproduction is advantageous because survival possibilities may decrease (Williams 1966; Stearns 1992; Welham and Setter 1998; Obeso 2002). Nevertheless, deeper insight into this question requires long-term demographic studies to relate lichen reproductive strategies with survival rates along a range of environmental heterogeneity. The efficient and flexible reproductive strategy of *L. scrobiculata* may determine its population structure (e.g. reproductive vs juvenile individuals) and dynamics, ultimately affecting the epiphytic community composition and functioning.

In conclusion, our study provides for the first time evidence of the variation in the reproductive allocation patterns of a symbiotic organism along a precipitation gradient. Plasticity in the growth rates in response to environmental constrains, likely underlie the heterogeneous pattern of the reproductive size and allocation. The small threshold sizes for reproduction together with the high reproductive efficiency in drier locations suggest a reproductive strategy based on early and intense reproduction, typical from early colonizers species. To substantially widen our general understanding on the life- history evolution a comprehensive (broad) taxa base in needed. Data on single species help to understand intra- and interspecific differences, providing insights. This study improves our understanding on patterns of reproductive allocation in overlooked taxa like lichens, providing insights on the evolved life-history traits of these photosynthetic symbiotic organisms. Because *L.scrobiculata* is a nitrogen-fixing species, it potentially plays a relevant role in the ecosystem functioning where it is abundant. Under a predicted climate change scenario of reduced precipitation in the Iberian Peninsula (IPCC 2007), it is tempting to expect changes (1) in the nitrogen-fixation rates because moisture is a requirement for nitrogen-fixation in lichens (Nash 2008), and (2) on the reproductive strategy of *L. scrobiculata*, predicting smaller reproductive sizes leading to smaller mature sizes, impacting its populations dynamics and ultimately the ecosystem functioning through a reduced input of available forms of nitrogen for e.g. plants. However, the potential adaptive response or phenotypic plasticity of reproductive traits of lichens to increasing dryness remains unknown.

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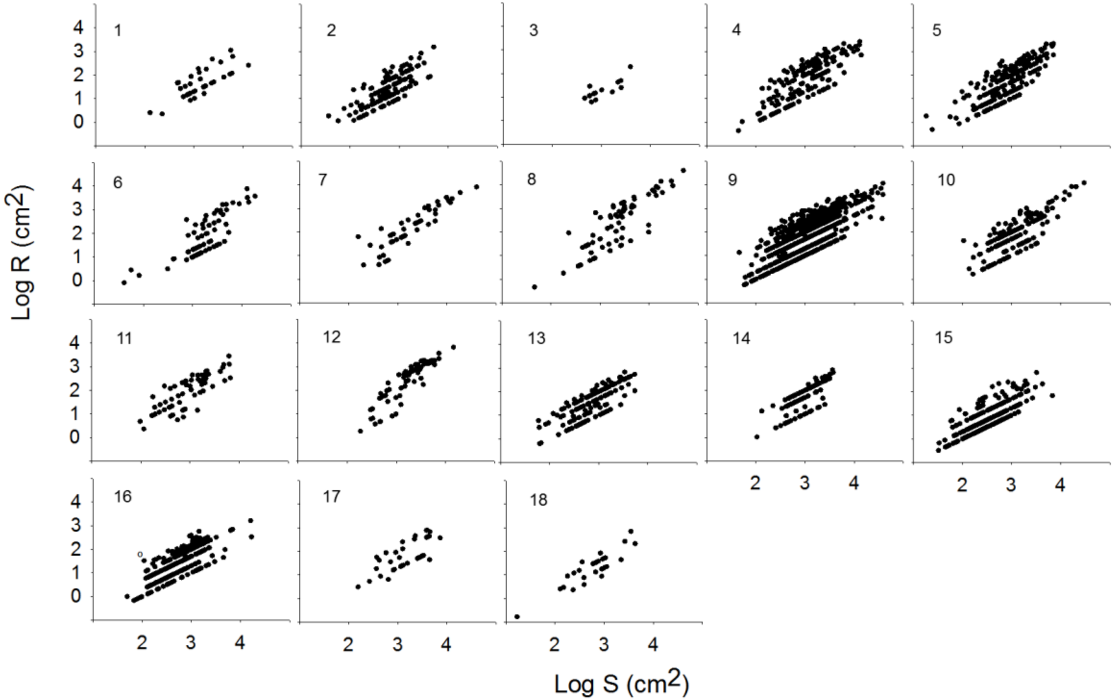
SUPPLEMENTARY MATERIAL

Table S1. Summary of the Standarised Major Axis regression scaling exponents (slopes), allometric constant (intercept), and their respective 95% confidence intervals (CI) for the allometric model: log (reproductive surface) = log a + b log (somatic surface) in 18 populations of *Lobarina scrobiculata* along the Iberian Peninsula. Results of zero hypothesis test: slope = 1. N = number of reproductive individuals. Boldfaces slopes are statistically significant different from 1 at *P* <0.05 level. Identity of populations is given in Table 1.

								H0: Slope = 1		
Population	N	Slope	Low CI	Upp CI	Intercept	Low CI	Upp CI	df	r	P value
1	33	**1.46**	1.16	1.84	-2.90	-3.96	-1.83	31	0.53	**0.002**
2	142	**1.68**	1.52	1.86	-3.26	-3.75	-2.78	140	0.66	**0.000**
3	15	1.32	0.94	1.86	-2.72	-4.12	-1.32	13	0.44	0.102
4	187	**1.65**	1.51	1.80	-3.13	-3.57	-2.69	185	0.66	**0.000**
5	212	**1.68**	1.55	1.83	-3.20	-3.60	-2.80	210	0.64	**0.000**
6	70	**1.86**	1.62	2.14	-4.15	-5.00	-3.31	68	0.75	**0.000**
7	45	**1.58**	1.38	1.81	-2.99	-3.70	-2.28	43	0.73	**0.000**
8	71	**1.91**	1.68	2.16	-4.06	-4.87	-3.24	69	0.80	**0.000**
9	911	**1.69**	1.62	1.76	-3.31	-3.52	-3.10	909	0.66	**0.000**
10	159	**1.73**	1.58	1.89	-3.43	-3.92	-2.94	157	0.71	**0.000**
11	63	**1.58**	1.37	1.82	-2.73	-3.41	-2.06	61	0.64	**0.000**
12	71	**2.06**	1.86	2.27	-4.23	-4.90	-3.56	69	0.88	**0.000**
13	156	**1.93**	1.67	2.23	-4.24	-5.05	-3.42	154	0.61	**0.000**
14	104	**1.90**	1.69	2.14	-3.98	-4.65	-3.30	102	0.75	**0.000**
15	328	**1.57**	1.43	1.73	-2.95	-3.40	-2.51	154	0.61	**0.000**
16	419	**1.67**	1.58	1.77	-3.15	-3.41	-2.89	418	0.67	**0.000**
17	34	**1.55**	1.23	1.96	-3.07	-4.22	-1.91	32	0.57	**0.000**
18	27	**1.47**	1.23	1.76	-2.80	-3.54	-2.05	25	0.67	**0.000**

Figure S1 Bivariate plots of log reproductive surface vs. log somatic surface in 18 populations of *Lobarina scrobiculata* along the Iberian Peninsula. The number corresponds to the population (see Table 1).



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CAPÍTULO III / CHAPTER III

EPIPHYTIC LICHEN GROWTH IN MEDITERRANEAN FORESTS: EFFECTS OF PROXIMITY TO THE GROUND AND REPRODUCTIVE STAGE

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ABSTRACT

Reasonable lichen growth rates are required to maintain the important ecosystem functions provided by epiphytic lichen populations. Previously, lichen growth has mainly been quantified in boreal forests. Thus, there is a need for comparable studies from Mediterranean forests. We used reciprocal transplantation to compare relative biomass (RGR) and thallus area (RT_AGR) growth rates of the cephalolichen *Lobaria pulmonaria* and the cyanolichen *Lobarina scrobiculata* (*n* = 720 thalli) in two Mediterranean oak forests. Both juvenile and reproductive thalli were transplanted for 334 days at the base and at breast height of random trees in both forests. We measured functional traits, specific thallus mass (STM) and water holding capacity (WHC), to search for adaptation/acclimation patterns between regions and between local stands. Linear Mixed Models were used to assess the effects of (1) forest, (2) height on the trunk and (3) reproductive stage on RGR, RT_AGR, and functional traits. *L. pulmonaria* grew faster than *L. scrobiculata*, consistent with its more flexible hydration traits. Growth rates in both species were fastest in juveniles at the trunk bases, consistent with trade-offs between 1: growth and reproduction and 2: humidity availability and distance from the ground. In *L. pulmonaria,* STM increased with increasing evaporative demands, consistent with acclimation mechanisms. Fundamental and realized niches differed in *L. scrobiculata*, indicating that high abundance does not necessarily coincide with optimal growth habitat. Both species grew as fast in drier and warmer Mediterranean forests as reported from boreal forests, presumably because local conditions during hydration periods differ less between macroclimate regions than average climatic parameters, and/or because dew formation is frequent, particularly near the ground. Furthermore, STM and WHC of lichens in Mediterranean forests were high, thus partly compensating for the drier conditions. This study improves our understanding of mechanisms underlying epiphytic lichen growth in Mediterranean climates.

Key words: Functional traits, *Lobaria pulmonaria*, *Lobarina scrobiculata*, *Quercus* forest, relative growth rate, Spain, specific thallus mass, vertical gradient, water holding capacity.

INTRODUCTION

Epiphytic lichens often represent a highly diverse biodiversity component in forests and play important functional roles in ecosystem functioning (reviewed by Ellis 2012). Previously, lichens were considered slow-growing (Grime 1979), but recent studies reported fast growth (e.g. Gaio-Oliveira, Dahlman, Máguas & Palmqvist 2004; Larsson, Solhaug & Gauslaa 2012;2014; Bidussi, Gauslaa & Solhaug 2013). However, epiphytic lichen growth is mainly quantified in boreal and temperate climates (e.g. Hilmo 2002; Antoine & McCune 2004; Larsson, Solhaug & Gauslaa 2012; Shriver, Cutler & Doak 2012) and/but? rarely in Mediterranean climates (see Gaio-Oliveira, Dahlman, Máguas & Palmqvist 2004) with summer drought (Valladares, Camarero, Pulido & Gil-Pelgrín 2004). Interestingly, these dry and sunny areas share various epiphytic lichens with temperate and boreal regions. To improve our understanding of lichen functioning across contrasting ecosystems and environmental conditions, there is a need to quantify growth rates and functional traits also in Mediterranean forests.

One reason to identify growth-regulating factors is that reproduction and fitness shaping population viability partly depend on growth (e.g. Martínez et al. 2012; Shriver, Cutler & Doak 2012). Although environmental factors determine lichen growth (e.g. Palmqvist 2000; Bidussi, Gauslaa & Solhaug 2013), internal factors such as reproductive effort also influence lichen growth by a trade-off between growth and reproduction (e. g. Gauslaa 2006). Lichens grow three-dimensionally: Area expansion enhances the interception surface for light, water, and nutrients; while dry mass gain per area unit e.g. improves water holding capacity (Larsson, Solhaug& Gauslaa 2012; Merinero, Hilmo & Gauslaa 2014). The specific thallus mass (STM = dry mass per area) is an important functional trait controlled by light exposure (Snelgar & Green 1981; Larsson, Solhaug & Gauslaa 2012) and/or nutrient availability (Asplund, Sandling & Wardle 2012). STM changes when area and dry mass do not increase concurrently, meaning that these processes are regulated differently. Photosynthetic carbon gain shapes dry mass investments (Palmqvist 2000; Larsson, Solhaug & Gauslaa

2012), while fungal hyphae expansion drives area growth (Gaio-Oliveira, Dahlman, Máguas & Palmqvist 2004; Larsson, Solhaug& Gauslaa 2012). STM is a driver of the water holding capacity (WHC); thick (Gauslaa & Coxson 2011) and large (Merinero, Hilmo & Gauslaa 2014) thalli retain more water. STM, and thus WHC, increase with increasing evaporative demands (e.g. Hilmo 2002; Gauslaa & Coxson 2011; Larsson, Solhaug & Gauslaa 2012; Merinero, Hilmo & Gauslaa 2014). Comparisons of WHC (and STM) in epiphytic lichens between open, dry Mediterranean sites (Valladares, Camarero, Pulido & Gil-Pelgrín 2004) and wet boreal sites including closed rain forests (e.g. Coxson & Stevenson 2007; Larsson, Solhaug & Gauslaa 2012) may thus give clues to understand links between site-specific hydration sources and lichen adaptation/acclimation traits.

The vertical distribution of lichens on tree trunks is influenced by e.g. solar radiation and humidity (reviewed by Ellis 2012). Air humidity increases with proximity to the ground, whereas light and wind exposure enhancing evaporation often increase with height (Geiger 1950). In Mediterranean sites, moisture availability is usually a limiting factor causing vertical gradients of lichens on tree trunks (Burgaz, Fuertes & Escudero 1994). The height on the trunk affects lichen performance, evidenced by height-dependent gradients in thallus size in lichen populations (e.g. Martínez et al. 2012).

We studied the cephalolichen *Lobaria pulmonaria* L. (Hoffm.) and the cyanolichen *Lobarina scrobiculata* (Scop.) Nyl. ex Cromb. They are widely distributed globally, but declining and red-listed in many European countries, including Spain (Martínez et al. 2003). In our study area, they inhabited two Mediterranean oak forests spaced by 7 km, although in contrasting proportions. We established reciprocal transplants to compare relative growth rates between species and between sites. In boreal canopies, both species acclimate to higher evaporative demand at greater heights by increasing their WHC (Merinero, Hilmo & Gauslaa 2014). To search for height-dependent growth and acclimation, we transplanted lichens at trunk bases and breast height. By using juvenile and reproductive specimens, we tested whether the trade-off between growth and reproduction is consistent across regions and species. Furthermore, cyano- and cephalolichens have different hydration traits; the former requires liquid water to activate photosynthesis, whereas cephalolichens additionally benefit from humid air (Lange, Kilian& Ziegler 1986). A comparison of such functional groups in Mediterranean and boreal climates (e.g. Hilmo 2002; Larsson, Solhaug & Gauslaa 2012; 2014) may improve our understanding of their functioning. We hypothesize that (1) relative growth rates in Mediterranean populations are lower than those reported in boreal ones due to drier climate; (2) *L. pulmonaria* with flexible hydration traits grows faster than *L. scrobiculata*; (3) both species grow faster near the trunk base than at the breast height; (4) juvenile specimens grow faster than reproductive ones; (5) each species maximize their in the forests with the highest respective abundance, consistent with similar fundamental and realized niches.

MATERIALS AND METHODS

Study area

The field experiment was done in two oak forests in central Spain with similar macroclimate, but yet different proportions of our target species (Table 1). The distance between the stands was 7 km. The Montejo stand (6.5 ha) is in Madrid (41º8’46’’N, 3º29’33’’W), and the Santuy stand (21 ha) in Guadalajara (41º5’44’’N, 3º26’27’’W). The oak forests are discontinuous, located in a matrix of pine plantations and grasslands. The climate (Table 1) is Mediterranean, characterized by 1.8 months of drought during summer, annual precipitation ≈800 mm, and mean annual temperature 9.5-9.7 ºC (CLIMOEST; Sánchez-Palomares, Sánchez-Serrano & Carretero-Carrero 1999). Both forests were *Quercus pyrenaica*-dominated, accompanied by *Crataegus monogyna*, *Cytisus scoparius*, and *Rubus ulmifolius.* In Montejo, atlantic species like *Ilex aquifolium* and *Erica arborea* also occurred. Montejo is a northern slope facing the Jarama river, while Santuy is on flatter land far from permanent water courses. Forest structure differed between the sites, mainly due to contrasting management in the past; the Montejo forest hosted larger but fewer trees than the Santuy forest (Table 1). Contrasts in orography and history may lead to the reported differences in microclimate and stand characteristics (Table 1). Many old-forest epiphytes occurred in Montejo (i.e. the *Lobarion*), whilst the pioneer epiphytic community *Parmelion* was common in Santuy.

Study species

We studied 1: the cephalolichen *Lobaria pulmonaria with* the green alga *Dictyochloropsis reticulata* as its primary photobiont and the cyanobacterium *Nostoc* in scatterd internal cephalodia and 2: the cyanolichen *Lobarina scrobiculata* with *Nostoc* as its only photobiont. The abundance of both species (Table 1) had been recorded in two 50 x 50 m plots within both sites (unpublished data). Three-hundred-sixty *L. pulmonaria* lobes were randomly collected in Montejo, and the same number of *L. scrobiculata* was taken in Santuy on 4 May 2012. One-hundred-and-eighty lobes were juvenile and another 180 reproductive with 10-30% cover of soredia and/or isidia. Thalli were air-dried in the laboratory on the collection day and kept at room temperature protected from high light. They were cleaned from debris and randomly labelled. Mean (±SD) lobe area for *L. pulmonaria* and *L. scrobiculata* were 13.0±4.1 and 8.6±4.0 cm^2, respectively.

Experimental design

The transplantation unit, a 20x20 cm nylon net, had four lobes, one juvenile and one reproductive of each species (see Appendix A: Fig. 1). These lobes were randomly assigned to each net, fastened with polyester thread in a way allowing the lobes to curl (Barták, Solhaug, Vrábíková & Gauslaa 2006). Each net was randomly assigned to forest and tree trunk; 45 trees in each forest were randomly chosen. We stapled two nets per tree, one at the base of the trunk (25 cm height), the other at breast height (150 cm). We placed all nets on the north side of the trunks. A total of 90 nets (360 lobes) were placed in each forest. In Santuy five nets were lost, so we collected 85 nets in each forest at the end of the experiment. The transplants stayed in the field from 24 May 2012 to 22 April 2013.

Table 1. Descriptive absolute or mean ± 1SE forest variables for each study site. P values from ANOVA are given. Summer= 21st June-20th September; Winter= 21st December-20th March.

Bold

Variable	Montejo	Santuy	*P*
Climate and orography			
Annual precipitation (mm)	818	801	
Mean annual temperature (°C)	9.5	9.7	
Altitude (m a.s.l.)	1263	1235	
Microclimate			
Mean temperature (T, °C)	9.2 ± 0.1	10.2 ± 0.1	**0.000**
Mean T in summer (°C)	17.4 ± 0.1	19.5 ± 0.1	**0.000**
Mean T in winter (°C)	2.3 ± 0.1	2.7 ± 0.1	**0.001**
Mean relative humidity (RH, %)	74.1 ± 0.2	69.6 ± 0.3	**0.000**
n hours (days) RH ≥ 96.5% in total	1026 (100)	759 (88)	
n hours (days) RH ≥ 96.5% in summer	14 (2)	35 (5)	
n hours (days) RH ≥ 96.5% in winter	425 (45)	214 (29)	
Forest structure			
Tree density (trees in 1/4 ha)	158	720	
Tree DBH (cm)	22.9 ± 1.1	12.3 ± 0.1	**0.000**
Tree base diameter (cm)	66.0± 1.7	18.1 ± 0.2	**0.000**
Trunk slope (º)	81.5 ± 0.6	82.0 ± 0.2	0.487
Bark roughness (mm)	8.6 ± 0.4	8.4 ± 0.1	0.555
Bark pH	6.2 ± 0.1	5.8 ± 0.1	**0.002**
Bryophyte cover (%) <1m	20.5 ± 1.3	21.1 ± 0.5	0.587
Bryophyte cover (%)1-2 m	11.9 ± 1.0	6.8 ± 0.4	**0.000**
Canopy openness (%)	29.1 ± 0.1	30.6 ± 0.1	0.150
Total light (%)	27.1 ± 0.1	29.7 ± 0.1	**0.046**
Study species			
n individuals of *Lobaria pulmonaria*	2 385	15	
n individuals of *Lobarina scrobiculata*	8	10 546	

values indicate significant effects at $P < 0.05$.

Lichen area, mass, growth, and fluorescence measurements

Before and after transplantation, we fully hydrated each lobe by deionised water, and flattened them under a piece of glass before taking photographs (Canon 550D equipped with a Tanrom AF-18-200 mm lens). Lobe area (A) was assessed in Photoshop 6.0 (Adobe Systems, San Jose, CA). Immediately thereafter, thalli were gently blotted with filter paper before measuring wet mass (WM) to the nearest mg. While hydrated, the maximum photochemical efficiency of photosystem II (F_V/F_M) was measured with a fluorometer (Plant Efficiency Analyser; Handsatech, King's Lynn, Norfolk, UK) after 15 min dark adaptation and after 1 h hydration. Then, lichens were air-dried 48 h before recording air-dry mass (DM). Ten control thalli similarly treated, were repeatedly weighed together with the transplants and no changes in mass were detected. These controls were oven-dried (60 ºC for 72 h) before their DM was determined, and oven-dry DM of transplants was computed by using the weight reduction factor of these sacrificed thalli. Specific thallus mass was calculated as STM (mg DM cm^{-2}) = DM/A; the water holding capacity (WHC in mg H_2O cm^{-2}) = (WM-DM)/A. Growth during the transplant period (Δt = 334 days) was quantified as follows (c.f. Evans 1972):

Relative growth rate (RGR) = $((\ln(DM_{end}/DM_{start})) \times 1000/\Delta t$ ($mg \cdot g^{-1} \cdot day^{-1}$)

Relative thallus area growth rate (RT_AGR) = $(\ln(A_{end}/A_{start})) \times 100/\Delta t$ ($mm^2 \cdot cm^{-2} \cdot day^{-1}$)

Percent changes in STM and WHC were quantified as:

$\Delta STM (\%) = (STM_{end}-STM_{start})x100/STM_{start}$

$\Delta WHC (\%) = (WHC_{end}-WHC_{start})x100/WHC_{start}$

Besides the lost nets, 49 transplants were lost (22 *L. pulmonaria* and 27 *L. scrobiculata*) from mainly the base of trunks, likely due to detachment by cattle or wildlife. The loss was similar in the two sites. Furthermore, lobes with area and/or dry mass reductions > 10% were excluded (*L. pulmonaria*: 3.9%; *L. scrobiculata*: 10.3% lost or fragmented). Thus, the final sample size for statistical analyses comprised 326 *L. pulmonaria* and 303 *L. scrobiculata.*

Canopy and microclimate measurements

Canopy cover and light exposure at each net were estimated from hemispherical photographs (Englund, O'Brien & Clark 2000). Digital photographs were taken from a horizontal position close to each transplantation net, at breast height and at base, using a horizontally levelled digital camera (Nikon Coolpix 4500, Nikon, Japan) aimed at the zenith, using the fish-eye lens

FCE8, Nikon. Photographs were analysed using Gap Light Analyser (GLA) software ver. 2.0. (Frazer, Canham & Lertzman 2000).

Air temperature and relative humidity 1.60 m above the ground were recorded every hour in both forests with four HOBO ® H8 Pro Series data loggers (Onset, USA) along one transect within each forest and during the transplant period (Table 1). Liquid water likely occurred at relative air humidity > 96.5%, because humidity sensors had an accuracy of < ±3.5% (Onset Corporation 2009). Because climatic data were not replicated at the tree scale, they were not used in statistical models.

Bark pH measurement

Bark from 14 trees in each forest was taken. Three to four bark pieces from each tree constituted one sample. Bark samples were ground in a coffee mill; pH measurements (CRISON GLP21 pH meter; Alella, Barcelona, Spain) followed Kricke (2002).

Statistical analyses

Linear Mixed Models (LMMs) with a Laplace approximation (Bolker et al. 2009) and Gaussian error were fitted for each species to test the effect of site (Montejo-Santuy), height on the trunk (base-breast), the interaction site x height, reproductive stage (juvenile-reproductive), and the total light (%) on RGR, RT_AGR, and changes in STM and WHC. We used total light (%) because it highly correlated with canopy openness (Fig. 1, Table 1). Thallus area at start (A_{start}) was included as a covariate because growth is size-dependent (e.g. Larsson & Gauslaa 2011). To disentangle the correlation between A_{start} and reproductive stage, we run a Generalized Linear Model (GLM) with reproductive stage as the response variable and A_{start} as a predictor. Residuals of these models were included in the LMMs as the reproductive stage variable (Legendre & Legendre 1998). We used mixed effects analyses because our data had an unbalanced and hierarchical structure with “lobes” nested within “tree” as nets on the same tree might be spatially dependent. Thus, data were analysed from a multi-level approach, considering “tree” as random factor and introducing the explanatory variables as fixed factors (Bolker et al. 2009). Fixed factors were examined by Wald *F*-statistic tests. Models were finally checked for residuals normality and homoscedasticity. Statistical analyses were done in R version 2.15.2 (R Foundation for Statistical Computing, Vienna, Austria). We used the package nlme (Pinheiro, Bates, DebRoy & Sarkar 2008) for mixed effect models. Mean values ± SD are reported in the text.

RESULTS

Forest characteristics: microclimate, canopy openness, bark pH

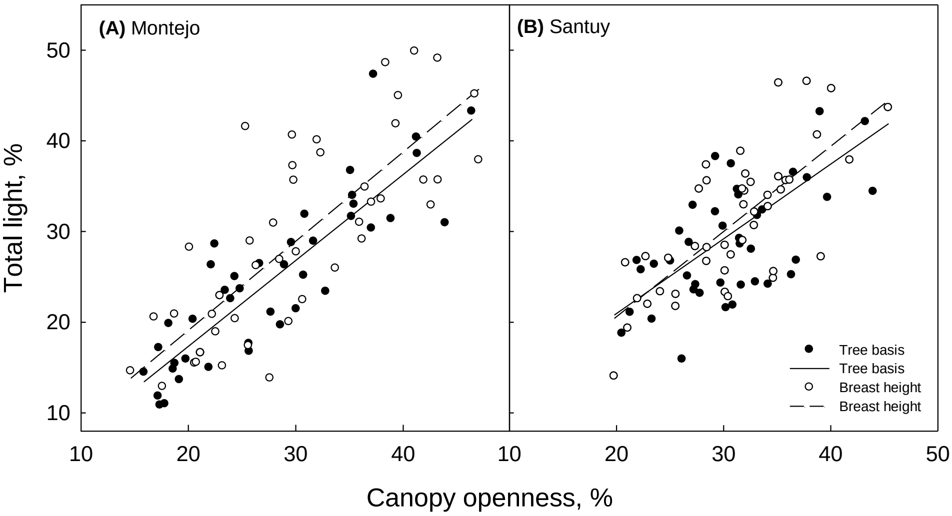
Macroclimate was similar in both sites (Table 1). However, microclimate differed significantly because Montejo had higher relative humidity and lower temperature than Santuy (Table 1). In total, Montejo had more hours with liquid water than Santuy, except in summer, although humid days were few in both sites (Table 1). Santuy had 4.5 times more trees per area than Montejo with more heterogeneous canopy openness (15-48%; Fig. 1). Nevertheless, the canopy openness did not differ significantly between sites. Neither did canopy openness differ between heights on the trunk (data not shown). Total light was slightly, but significantly higher in Santuy (Table 1; Fig. 1), and slightly higher at breast height (29.2±1.6% of above canopy light) than at the tree base (25±1.3%; t-test; $P = 0.045$) in Montejo (Fig. 1). Besides, mean bark pH was higher in Montejo than in Santuy (Table 1).

Growth rates

Lobaria pulmonaria grew significantly faster than *L. scrobiculata* across sites, heights and lichen life stages (Fig. 2A-D; ANOVAs, $P < 0.001$). Both species grew significantly faster in Montejo, and on tree bases in both forests (Fig. 2A-D, Table 2). In Montejo, the RGR of *L. pulmonaria* was 0.99±0.37 *vs* 0.79±0.33 mg g^{-1} day^{-1} in Santuy. RT_AGR was also significantly higher in Montejo than in Santuy (0.07±0.03 and 0.06±0.03 mm^2 cm^{-2} day^{-1}, respectively).

Lobarina scrobiculata grew significantly faster (RGR = 0.50±0.37 mg g^{-1} day^{-1}; RT_AGR = 0.05±0.03) in Montejo where it was rare, than in Santuy where it was abundant (RGR = 0.32±0.31 mg g^{-1} day^{-1}; RT_AGR = 0.04±0.03 mm^2 cm^{-2} day^{-1}; Table 2, Fig. 2B,D). Height on the trunk and reproductive stage strongly influenced growth rates (Table 2, Fig. 2A-D). In both species and sites, the highest growth rates occurred in juveniles at the trunk bases (Table 2, Fig. 2A-D). Reproductive *L. pulmonaria* thalli grew significantly slower than juvenile ones (Table 2, Fig. 2A,C). By contrast, the reproductive structures on *L. scrobiculata* significantly reduced the area expansion, but not the dry mass gain (Table 2, Fig. 2B,D).

Figure 1. Correlation between canopy openness and total light in Montejo and Santuy forests for each transplanted net. Overall Pearson correlation coefficient r = 0.82, *P* <0.001 in Montejo and r = 0.69, *P* <0.001 in Santuy.



The STM_{start} was similar for *L. pulmonaria* (12.9±1.6 mg cm^{-2}) and *L. scrobiculata* (12.9±1.9 mg cm^{-2}), but reproductive lobes had significantly higher STM_{start} than juvenile ones in both species (Fig. 2K-L; ANOVAs, $P < 0.001$). Nevertheless, area and dry mass investments significantly differed between species (Fig. 3A-B). In *L. pulmonaria*, the dry mass gain increased more than the area expansion, resulting in a mean STM increase of ~10%. The increased STM was mainly attributable to the effect of height on the trunk and reproduction (Figs. 2E and 3C, Table 3); STM became significantly lower in the reproductive lobes at breast height due to slow growth (Fig. 2E-F, Tables 2 and 3). In *L. scrobiculata* there were no changes in STM due to balanced area and dry mass gains (Figs. 2F and 3D), and no explanatory variables influenced STM change (data not shown).

The WHC_{start} was significantly higher in *L. scrobiculata* than in *L. pulmonaria* (27.9±7.5 *vs* 18.2±3.0 mg H_2O cm^{-2}; ANOVA, $P < 0.001$). However, the WHC of *L. pulmonaria* significantly increased in Santuy, particularly in juvenile lobes (Fig. 2I; Table 3). By contrast, the WHC of *L. scrobiculata* responded less (Fig. 2J) without consistent patterns and/or significant effects of tested variables (data not shown). Change in STM was significantly coupled to change in WHC in both species, particularly in *L. scrobiculata* (see Appendix A: Fig. 2).

Figure 2. Relative growth rates (RGR); relative thallus area growth rates (RT_AGR); changes in specific thallus mass (STM) and water holding capacity (WHC); and start values of STM and WHC (mean ± SE) in *Lobaria pulmonaria* (n = 326) and *Lobarina scrobiculata* (n= 303) across two forest stands (white columns: Santuy; grey columns: Montejo), and two heights on the trunk (base and breast height). Hatched columns represent reproductive lobes; open columns: juveniles.

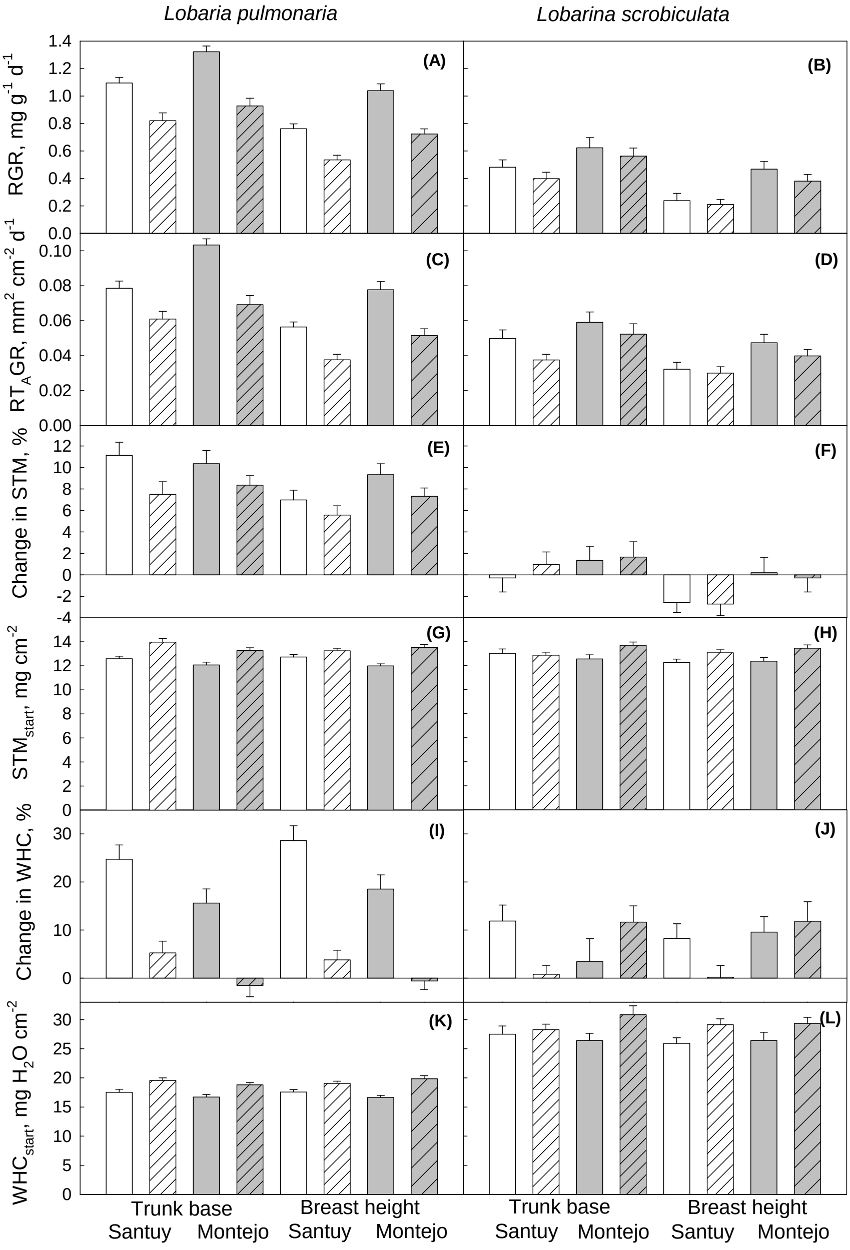


Figure 3. Relationships between dry matter gain and (A) area gain, and (B) change in specific thallus mass (STM) for *Lobaria pulmonaria* and *Lobarina scrobiculata*. Differences between reproductive stage and height on the trunk are shown. BH= breast height.

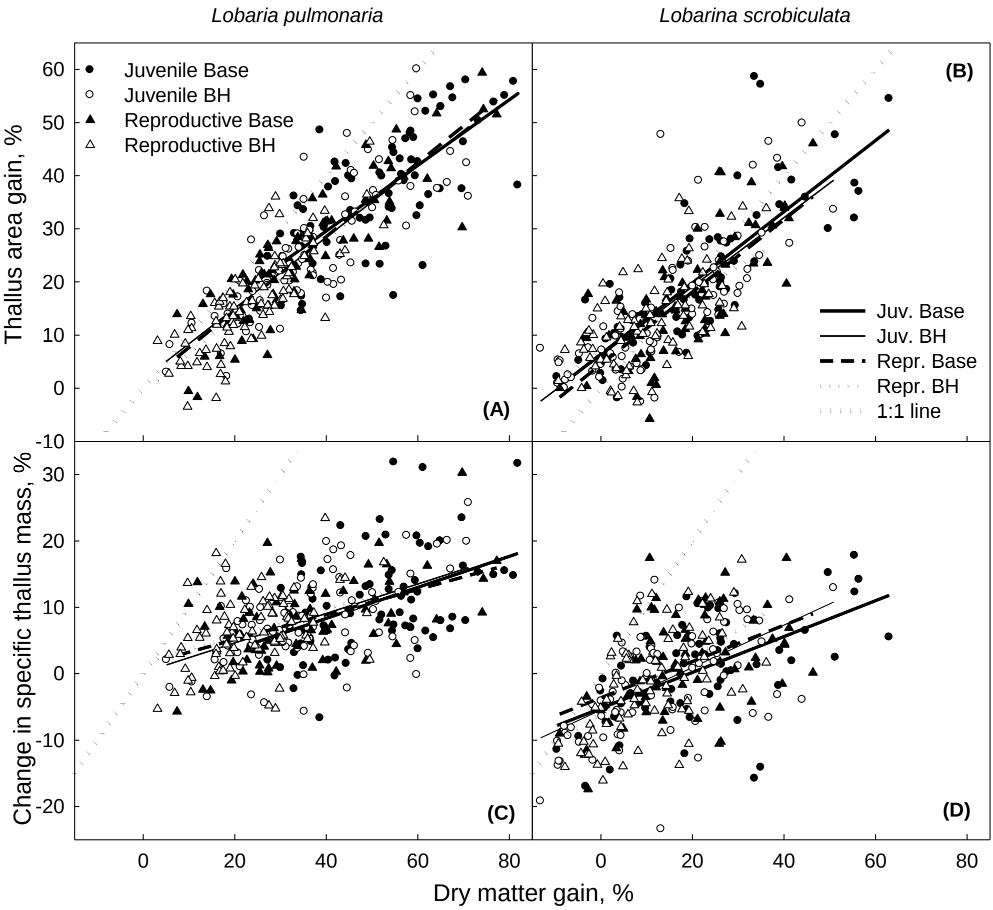


Table 2. Summary of the linear mixed models fit by a Laplace approximation used to examine the effects of environmental and intrinsic variables on the relative growth rates of *Lobaria pulmonaria* and *Lobarina scrobiculata* transplanted in two Mediterranean sites. The data comprise the Wald-type F-statistic value (*F*), degrees of freedom (df), coefficient of the variable in the model (Estimate), standard error of the estimator (SE), significance value (*P*), and random effects standard deviation (SD).

		RGR[a]			RT$_A$GR[b]		
	df	Estimate (SE)	*F*	*P*	Estimate (SE)	*F*	*P*
Lobaria pulmonaria							
Fixed effects							
Site (S)							
Santuy	88	-0.16 (0.05)	29.9	**0.000**	-0.02 (0.004)	29.6	**0.000**
Height (H)							
Breast	231	-0.25 (0.04)	89.4	**0.000**	-0.02 (0.004)	63.2	**0.000**
Life stage							
Reproductive	231	-0.07 (0.007)	98.1	**0.000**	-0.006 (6x10^{-4})	69.6	**0.000**
A$_{start}$[c]	231	-0.01 (0.004)	9.04	**0.003**	-7x10^{-4} (3x10^{-4})	4.7	**0.031**
Total light	231	-3x10^{-4}(0.002)	0	0.950	-9x10^{-5} (2x10^{-4})	0.3	0.598
S x H	231	-0.06 (0.06)	0.9	0.340	-0.001 (0.006)	0	0.849
Random effects				SD			SD
Tree				0.11			0.01
Residuals				0.26			0.02
Lobarina scrobiculata							
Fixed effects							
Site (S)							
Santuy	88	-0.167 (0.062)	15.2	**0.000**	-0.013 (0.005)	10.5	**0.002**
Height (H)							
Breast	208	-0.178 (0.051)	34.9	**0.000**	-0.012 (0.004)	18.1	**0.000**
Life stage							
Reproductive	208	-0.013 (0.007)	3.2	0.070	-1.6x10^{-3}(6x10^{-4})	6.4	**0.012**
A$_{start}$[c]	208	-0.001 (0.005)	0.1	0.740	-4x10^{-5} (4x10^{-4})	0.0	0.922
Total light	208	-0.001 (0.002)	0.2	0.630	2x10^{-5} (2x10^{-4})	0.0	0.840
S x H	208	-0.032 (0.068)	0.2	0.640	2x10^{-5} (0.006)	0.0	0.905
Random effects				SD			SD
Tree				0.16			0.01
Residuals				0.29			0.02

[a] RGR= Relative growth rate (mg g^{-1} day^{-1}), [b] RT$_A$GR= Relative thallus area growth rate (mm^2 cm^{-2} day^{-1}), [c] A$_{start}$= Thallus initial size (mm^2). Bold values indicate significant effects at $P < 0.05$.

The mean $F_V/F_{M\ start}$ was 0.71±0.03 for *L. pulmonaria* and 0.48±0.08 for *L. scrobiculata*. Both species had slight higher F_V/F_M after the transplant period in both sites; F_V/F_M of *L. pulmonaria* did not differ between forests (0.72±0.05), but *L. scrobiculata* showed slightly higher means in Santuy (0.57±0.04) than in Montejo (0.55±0.05) (ANOVA; $P = 0.003$).

Table 3. Results of the linear mixed models used to examine the effects of environmental and intrinsic variables on the changes in specific thallus mass (STM) and water holding capacity (WHC) (%) of *Lobaria pulmonaria* transplanted in two Mediterranean forests. The data comprise the Wald-type *F*-statistic value (*F*), degrees of freedom (df), coefficient of the variable in the model (Estimate), standard error of the estimator (SE), significance value (*P*), and random effects standard deviation (SD).

		Lobaria pulmonaria					
		Change in STM (%)			Change in WHC (%)		
Fixed effects	df	Estimate (SE)	*F*	*P*	Estimate (SE)	*F*	*P*
Site (S)							
Santuy	88	-0.08 (1.07)	2.2	0.141	7.75 (2.75)	18.6	**0.000**
Height (H)							
Breast	231	-1.43 (1.01)	8.8	**0.003**	1.08 (2.68)	0.72	0.398
Life stage							
Reproductive	231	-0.52 (0.17)	9.3	**0.003**	-4.8 (0.45)	112.2	**0.000**
^{a}A$_{start}$	231	-0.16 (0.09)	3.6	0.058	-0.67 (0.23)	8.61	**0.004**
Total light	231	0.03 (0.04)	0.8	0.371	0.08 (0.11)	0.59	0.444
S x H	231	-1.78 (1.41)	1.6	0.206	-0.12 (3.72)	0.001	0.975
Random effects				SD			SD
Tree				1.24			0.002
Residuals				6.27			16.65

^{a}A$_{start}$= Thallus initial size (mm^2). Bold values indicate significant effects at $P < 0.05$.

DISCUSSION

This is the first experimental study that compares annual growth rates of the threatened epiphytic lichens *L. pulmonaria* and *L. scrobiculata* in Mediterranean forests and quantifies height-on-trunk-effects on growth and performance of juvenile and reproductive stages. Contrary to our hypothesis, both species can grow as fast in dry Mediterranean climates as in moist boreal sites (e.g. Hilmo 2002; Coxson & Stevenson 2007; Larsson, Solhaug & Gauslaa 2012; 2014). However, *L. pulmonaria* grew slower than in inland British Columbia (Gauslaa & Goward 2012) and in Portugal (Gaio-Oliveira, Dahlman, Máguas & Palmqvist 2004), and much slower than juveniles in boreal rain forests (Larsson & Gauslaa 2011). There are fewer studies on *L. scrobiculata*, but our specimens gained area three times faster than in boreal clearcuts (Larsson, Solhaug & Gauslaa 2014), and at similar rates as in suboceanic Norwegian spruce forests (Hilmo 2002), whereas juveniles in boreal rain forests grew three times faster than our juveniles (Larsson & Gauslaa 2011). Moreover, our lichens showed adaptation/acclimation mechanisms with respect to STM and WHC. STM is higher in *L. pulmonaria* from Spain than from Norway and British Columbia, whereas *L. scrobiculata* varies little between dry and wet forests (Table 4). Both species have higher WHC in Mediterranean forests (Table 4).

Light availability recorded at a lichen site level did not influence lichen growth. Thus, water availability is likely a more important limiting factor than light for lichens in Mediterranean forests (Burgaz, Fuertes & Escudero 1994). By contrast, light scarcity strongly limits lichen growth in moist boreal forests (e.g. Gauslaa, Lie, Solhaug & Ohlson 2006). Mediterranean deciduous forests are open (e.g. Gaio-Oliveira, Dahlman, Máguas & Palmqvist 2004), providing sufficient light for lichen growth in all seasons, especially in winter when trees are leafless. Recent studies showed that lichen growth may be limited by nutrient availability (reviewed by Palmqvist 2000). However, such mechanism might be less important for the growth rates of our lichens in our forests with relatively high bark-pH.

Table 4. A comparison of available data (means±SE) for specific thallus mass (STM) and water holding capacity (WHC) in *Lobaria pulmonaria* and *Lobarina scrobiculata* taken from: 1)Bidussi, Gauslaa, & Solhaug (2013), 2)Larsson, Solhaug, & Gauslaa (2012), 3)Gauslaa & Coxson (2011).

	L. pulmonaria	*L. scrobiculata*
STM (mg cm^{-2})		
Mediterranean oak forest, Spain, at start (n=303-326)	12.90±0.09	12.88±0.11
Mediterranean oak forest, Spain, at end (n=303-326)	13.94±0.09	12.82±0.11
Boreal rain forest, Norway (n=194-197)[1]	9.10±0.10	11.67±0.10
Boreal rain forest, Norway (n=480)[2]	9.75±0.06	13.22±0.08
Inland rain forest, British Columbia (n=120-122)[3]	8.50±0.13	10.73±0.22
WHC (mg H_2O cm^{-2})		
Mediterranean oak forest Spain, at start (n=303-326)	18.20±0.17	27.92±0.43
Mediterranean oak forest Spain, at end (n=303-326)	20.06±0.19	29.13±0.39
Inland rain forest, British Columbia (n=120-122)[3]	13.12±0.18	20.90±0.36

Our results highlight the flexibility of *L. pulmonaria* with faster growth than *L. scrobiculata* in Mediterranean (Fig. 2A-D) as in boreal forests (Larsson & Gauslaa 2011; Larsson, Solhaug & Gauslaa 2012). This higher flexibility is probably facilitated by its green algal photobiont that can utilize also humid air to activate photosynthesis (Lange, Kilian & Ziegler 1986). Máguas, Griffiths and Broadmeadow (1995) showed that at 90% of relative humidity, *L. pulmonaria* efficiently assimilated carbon, whereas *L. scrobiculata* released carbon, indicating inactive photosynthesis and active respiration. On average, *L. pulmonaria* gained dry mass faster than area, whereas in *L. scrobiculata* these processes were similar (Figs 3A and B), consistent with the view that area and dry mass gains are regulated differently and in species-specific ways (Larsson, Solhaug & Gauslaa 2012).

An important finding is the faster growth rates at trunk bases than at 1.5 m, consistent with the much higher abundance of these lichens at stem bases in Spain (Martínez et al. 2012; Merinero, Rubio-Salcedo, Aragón & Martínez 2014).

Studies in North America either did not find significant effects of height on lichen growth rates along a vertical gradient of 3 m (Bidussi & Gauslaa in press), or did so, but along a larger gradient (3–43 m; Antoine & McCune 2004). Thus, the effect of height on lichen growth rates depends on climate and forest types. In open Mediterranean forests, the fast growth near the ground is likely boosted by nocturnal cooling forming strong temperature profiles and, thus, humidity gradients (Geiger 1950). Moreover, the base is less vertical than the stem itself, and likely catches more rain/dew. Despite lacking height-dependent differences in canopy openness, light was slightly higher at breast height than at the base in Montejo, probably increasing desiccation rates and reducing growth. Excess light during defoliated months (≈October-March) and low humidity in summer presumably exclude our study species from the upper canopy (Merinero, Rubio-Salcedo, Aragón & Martínez 2014; M. Rubio-Salcedo, unpublished data). To compensate for scarcity of water, we expected both species to increase their STM and WHC at breast height and in Santuy. However, only *L. pulmonaria* significantly increased its WHC in Santuy, despite higher STM gain at the breast height in both forests. Acclimation was strongest in juvenile *L. pulmonaria*, because reproductive specimens already had high WHC. Neither forest site nor height on the trunk influenced STM or WHC in *L. scrobiculata*, consistent with low cyanolichen flexibility.

The slower area growth in reproductive lobes than in juvenile ones is consistent with a trade-off between growth and reproduction in both species. Indeed, STM_{start} was significantly higher for the reproductive lobes than for the juvenile ones, suggesting that reproduction may increase with increasing thallus thickness and presumably with ageing, or that reproduction competes with growth for carbon (see Gauslaa 2006).

Contrary to our hypothesis, *L. scrobiculata* grew faster in Montejo where it was rare. The high cyanolichen growth is likely explained by the slightly higher liquid water availability in Montejo (Table 1), located in a northern slope near a river. A valley position, such as in Montejo, likely causes cold air-ponding from elevated position and favours dew formation during nocturnal cooling (Oke 1987).

Discrepancy between abundance and growth rates of *L. scrobiculata*, suggests that realized and fundamental niches do not coincide. The paradox with highest cyanolichen growth rates in Montejo where it rarely occurs naturally is intriguing and calls for explanations. Mixed effects of dispersal limitation (low propagule pressure) and environmental filtering in the establishment phase can be underlying mechanisms. By contrast, realized and fundamental niches of *L. pulmonaria* coincided. Small *L. scrobiculata* thalli have significantly higher WHC than those of *L. pulmonaria* (Merinero, Hilmo & Gauslaa 2014), a trait that probably allows *L. scrobiculata* to cope with drier conditions and compensate its inability to be activated by humid air.

CONCLUSIONS

Overall growth rates of *L. pulmonaria* and *L. scrobiculata* were fairly similar in Mediterranean and boreal climates, showing that dry macroclimate does not necessarily reduce lichen growth rates of established thalli. High STM and WHC of Spanish lichens, consistent with adaptation/acclimation mechanisms to drier conditions, may have contributed to the similar growth rates across climatic regions. However, growth-stimulating effects of increasing proximity to the ground highlight the importance of microhabitat differentiation. Forestry practices should be addressed to maintain the forest structure that allows the required microhabitat and microclimate differentiation for our lichen species. Finally, our results emphasize the higher flexibility of *L. pulmonaria* than of *L. scrobiculata* due to species-specific hydration traits, and the strong trade-off between reproductive efforts and growth.

ACKNOWLEDGEMENTS

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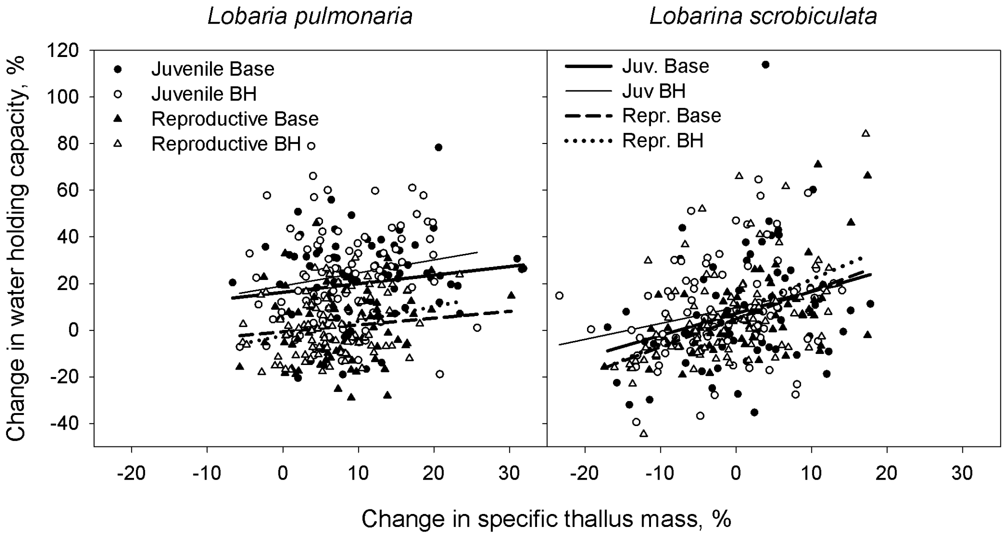
APPENDIX A. SUPPLEMENTARY DATA

Figure 1. A transplantation net with one juvenile and one reproductive transplant of *Lobaria pulmonaria* (upper right and lower left) and *Lobarina scrobiculata* (lower right and upper left), respectively, stapled to the northern side at the breast height of an oak trunk in the Santuy stand. Photo by Sonia Merinero.

Figure 2. The relationship between change in specific thallus mass (STM) and water holding capacity (WHC) in *Lobaria pulmonaria* and *Lobarina scrobiculata* transplanted in Mediterranean forests. Legends (symbols and lines) shown as inserts are valid for both plots. Filled symbols: thalli at the trunk base; open symbols: thalli at the breast height (BH). Circles refer to juvenile thalli, triangles to reproductive thalli.



CAPÍTULO IV / CHAPTER IV

DEMOGRAPHIC STRUCTURE, GROWTH AND REPRODUCTIVE ALLOCATION OF A THREATENED EPIPHYTE DIFFER IN CONTRASTING HABITATS

Sonia Merinero, Gregorio Aragón & Isabel Martínez

Manuscript in preparation

ABSTRACT

Fine-scale demographic studies in natural habitats are essential to understand the life-histories and the mechanisms through which populations of threatened species persist. Contrasting habitats can be selective environments providing different life history patterns. Yet scarce literature has been devoted to the understanding of the demographic structure, growth, start of reproduction and reproductive allocation patterns in contrasting habitats of fungi, including lichens. However, advancing our understanding on life history patterns in contrasting habitats require the inclusion of physiologically distinct taxonomic groups such as lichens. We used the epiphytic cyanolichen *Lobarina scrobiculata* as a model because it can colonize trees and mossy rocks inside forests. We selected two populations within Mediterranean forests in central Spain, and compared the life stage structure, growth (monitored for 2 yr), threshold size for reproduction and reproductive allocation patterns between rocks and trees. Linear mixed models and standardised major axis regressions (SMA) were used. The population life stage structure of *L. scrobiculata* in both habitats was strongly skewed toward small juvenile individuals (ca. 75%), although the proportion of recruits (< 5 mm) was higher on rocks. Individuals grew faster on rocks and consistently with life history theory, started reproduction at larger sizes than on trees. The moss cover and the leaning surface of rocks probably accounted for such differences. By contrast, individuals on trees, with smaller threshold size for reproduction exhibited higher reproductive allocation than their counterparts on rocks, thereby increasing the propagules density within the populations. This is the first study that evaluates the demographic structure and life history traits of lichens in structurally contrasting habitats. Our results suggest that both trees and mossy rocks provide habitat heterogeneity that may facilitate the persistence of these cyanolichen populations under the unpredictable conditions of the Mediterranean climate. Conservation and management strategies should be addressed to preserve the moss cover on rocks together with the maintenance of the current forest structure. The habitat-specific differences we detected imply that ecological studies and conservation plans addressed to preserve threatened species need to consider the effect of fine-scale habitat heterogeneity on species demography and life history.

Keywords: Cyanolichen; lichen demography; *Lobarina scrobiculata*; population structure; relative growth rate; reproductive allocation.

INTRODUCTION

Detailed demographic studies in natural habitats are required to understand the life-histories and the mechanisms through which populations of threatened species persist (Soulé 1987; Schemske et al. 1994; García et al. 2002). The demographic structure (i.e. life-stage population structure) of a species is highly relevant since changes in this population structure may affect the functional composition of communities, ultimately impacting on the ecosystem processes (Rudolf & Ramussen 2013). In addition, understanding the growth and reproductive patterns in response to environmental heterogeneity at fine temporal and spatial scales is also essential to develop appropriate conservation and management strategies for threatened species (Fahrig & Merriam 1994; Schemske et al. 1994).

Habitat features directly influence individual growth and reproduction, thus affecting the population responses such as the population size, dynamics and genetic structure (Loveless & Hamrick 1984; Lin & Batzli 2001; García et al. 2002; Bruna et al. 2009). Thus, predictions of population persistence rely on the understanding of how plant population demography differs between habitat types (e.g., Thomas 1996; Kephart & Paladino 1997; Albert et al. 2001). Many species can persist in heterogeneous habitats, so intraspecific variation in demographic structure, individual growth and reproductive allocation patterns can be expected as a response to contrasting habitats providing environmental heterogeneity. Life history theory predicts that plants in favourable habitats should grow faster, exhibit larger threshold size for reproduction, and consequently should have higher reproductive allocation than plants in adverse environments (Stearns & Koella 1986; Stearns 1992, Kozlowski 1992). For instance, various fine-scale plant studies showed that the habitat type influenced the plant demography by affecting the plant growth and thereby the reproductive allocation and the population dynamics (e.g. Doust 1981; Thomas 1996; Kephart & Paladino 1997;

Albert et al. 2001). It has been evidenced that environmental heterogeneity influences plant reproductive allocation patterns (e.g. Ohlson 1988; Sugiyama & Bazzaz 1998; Albert et al. 2001; Méndez & Karlsson 2004; Guo et al. 2012). Yet, patterns of reproductive allocation in relation to plant growth or threshold size for reproduction are contrasting (Wesselingh et al. 1997; Sugiyama & Bazzaz 1998; Weiner et al. 2009; Santos-del-Blanco et al. 2013).

So far, literature assessing demographic variability in contrasting habitats has focused on vascular plants (e.g. Doust 1981; Thomas 1996; Kephart & Paladino 1997; Albert et al. 2001) with far less attention to the third major pluricelular kingdom, the fungi, including lichens. Advancing of our understanding of life histories and the mechanisms for population persistence in contrasting habitats requires the inclusion of this physiologically distinct group. Lichens are sensitive to habitat heterogeneity because variation in abiotic factors at a fine scale such as microclimate, substrate and nutrient availability usually affect most stages of lichen development, from propagules establishment to growth and maturity (Scheidegger & Werth 2009). Thus, fine-scale habitat features that affect lichen establishment, growth, and reproductive allocation need to be understood.

Many studies have described the population structure of species based on lichen size or life-stage, which is usually skewed toward small and juvenile individuals (e.g. Rhoades 1983; Hestmark 1992; Hilmo et al. 2013; Shriver et al. 2012; McDonald & Coxson 2013; Rubio-Salcedo et al. 2015). More literature has been devoted to quantify and understand lichen growth, which mainly depends on a water-light and nutrient availability balance (Palmqvist 2000). Because different (micro) habitats may provide contrasting microclimatic conditions, patterns of lichen growth can differ (e.g. Sillet & McCune 1998; Larsson et al. 2014; Merinero et al. 2015). Growth is important because the onset of reproduction and the reproductive allocation are size-dependent (e.g. Hestmark 2001; Pringle et al. 2003; Hilmo et al. 2013; Martínez et al. 2012; Rubio-Salcedo et al. 2015) and may also vary with climate (Merinero et al. in prep). Some studies found substantial differences in the population size structure, growth and reproductive capacity of an epiphytic lichen between different tree species (MacDonald & Coxson 2013; Rubio-Salcedo et al. 2015). However, fine-scale demographic studies, including the population life stage structure, individual establishment, growth and reproductive allocation, are largely missing for lichens (but see Shriver et al. 2012). Besides, to our knowledge, no study has targeted lichen populations on structurally different habitats, which could act as selective environments resulting in different life history patterns (Shriver et al. 2012). Such data become even more needed to understand and analyse the declining of threatened species.

We focused on the widespread forest lichen species *Lobarina scrobiculata*, which is frequent in the Northern Hemisphere (http://data.gbif.org), but given its progressive declining (mean estimates of 10–30% of lost locations; NatureServe, 2014) it has been included in numerous American and European red lists (e.g., Sérusiaux, 1989; Randlane et al., 2008; NaturServe, 2014). In our Mediterranean study area (central Spain) it was classified as “vulnerable” (Martínez et al. 2003). This epiphytic cyanolichen grows on the bark of many tree species and on mossy siliceous rocks (Hallingbäck 1989; Burgaz & Martínez 1999; Smith et al. 2009; Merinero et al. 2014a). The occupation of these contrasting habitats may become a strategy to persist, especially under an unpredictable climatic scenario such as the Mediterranean climate (Cowling et al., 1996; Rodó & Comín, 2001). Moreover, in a previous large-scale study Merinero et al. (in prep) documented that the reproductive allocation of *L. scrobiculata* increased with decreasing rainfall, suggesting that the species produces more asexual propagules in adverse environments to improve the persistence probabilities. Therefore, this epiphyte provides an excellent opportunity to evaluate the fine-scale demographic responses in two contrasting but interspersed habitats within forests.

Here, we evaluated the demographic structure, relative growth rates, threshold size for reproduction and reproductive allocation patterns of *L. scrobiculata* in two contrasting habitats (trees and rocks) within two Mediterranean oak forests in Central Spain. We hypothesized that *L. scrobiculata* had habitat-specific patterns of establishment, growth and reproductive allocation, regardless the site. Our specific objects were: (1) To assess the population life-stage structure between habitats; (2) to compare the relative annual growth rates (n = 800) during two years between habitats; (3) to analyse which microhabitat factors influence growth and lichen size in each habitat; (4) to compare the threshold size for reproduction and reproductive allocation patterns between habitats; (5) to assess the relationships between growth and reproduction patterns and: (6) evaluate the implications of habitat variability for the management and conservation of the species.

MATERIAL AND METHODS

Species and study area

Lobarina scrobiculata (Scop.) Nyl. ex Cromb. (Lobariaceae) is an epiphytic foliose cyanolichen. It is widespread, but more frequent in the Northern hemisphere where it is progressively declining (Sérusiaux 1989; Smith et al. 2009; NatureServe 2014). In Mediterranean forests usually inhabits mountain areas differing in humidity and forest structure (Burgaz & Martínez 1999; Merinero et al. 2014a). The most frequent mode of reproduction is asexual by large (50-100 µm) symbiotic propagules (soredia) where both mycobiont and photobiont disperse together. Soredia are released from simple structures called soralia, which are distinct laminar and marginal areas variable in size that remain permanently on the thallus surface and are presumably functional until the lichen die. Soredia are typically dispersed by wind, rain and animals (Bowler & Rundel 1975).

We selected two distant (~100 km) Mediterranean oak forests (*Quercus pyrenaica*) with siliceous mossy rocks where *L. scrobiculata* occurs in central Spain. The Escorial forest, in Madrid (40°34'06"N, 04°09'23"W) is in a northern slope, at 1066 m a.s.l. By contrast, the Cardoso forest, in Guadalajara (41°05'33"N, 03°26'22"W) is in a northwest facing slope at 1235 m. The climate is Mediterranean, characterized by 1.8 months of drought during summer. Mean annual precipitation and temperature were 796 mm and 11.2ºC in the Escorial forest, and 801 mm, 9.7ºC respectively in the Cardoso forest (data from CLIMOEST; Sánchez-Palomares et al. 1999). The type and morphology of rocks differed between sites. The Escorial site is granitic, mainly composed of small rounded stones and numerous large boulders with granular texture, whereas panel-like rocks and sparser small boulders of gneiss rocks (metamorphic granitic rock) with foliated and finer texture, dominate in the Cardoso forest. In both sites mossy rocks and trees were homogeneously interspersed within the plots. However, mossy rocks were usually located under the canopy whereas bare boulders were in the middle of open areas. Both forests have not been subjected to tree felling since approximately 1950. The Escorial forest has no specific forest management plans, and given its proximity to Madrid city (40 km) it is a popular recreational area with abundant visitors whose number is not regulated. By contrast, the Cardoso forest belongs to the Sierra del Rincón Natural Park, where forest management and biodiversity conservation plans are developed, and the number of visitors is limited and controlled.

Lobarina scrobiculata is the dominant cyanolichen in both habitats and sites, although the macro-epiphytic communities differed between habitats. Trees hosted higher diversity of foliose and fruticose lichen species and acrocarpic mosses, whereas rocks hosted more bryophytes, mainly continuous carpets of pleurocarpic mosses, and crustose lichens. The Escorial forest hosted more lichen richness and biomass than the Cardoso forest (Merinero, Aragón & Martínez personal observation).

Experimental design and sampling

In each forest we randomly established a 50m x 50m plot representative of the forest structure of each site, and tagged all trees and rocks (habitat scale). For each tree we recorded (1) the tree species, (2) tree diameter at breast height (DBH, cm); (3) bark roughness (mm), measured with a digital caliper as the depth of a representative crevice at approximately 50 cm above ground; (4) the slope of the trunk respect to the horizontal (º) using a clinometer; and (5) the bryophyte cover estimated as a percentage of the trunk surface. For each rock we measured the (1) height of the rock from the ground (cm); (2) the slope of all sides; and (3) the bryophyte cover (%). To estimate the abundance we sampled *L. scrobiculata* individuals at least 2 m from the ground in both habitats because it is the height range where this species usually occurs on trees in Mediterranean forests (Merinero et al. 2014a). We considered “individuals” to be all spatially distinguishable lichen entities that were differentiated from others. This concept is often used elsewhere (Martínez et al. 2012; Shriver et al. 2012; Hilmo et al. 2013; Merinero et al. 2014a). At the individual scale, we recorded at the central point of each individual the (1) height from the ground (cm), (2) the orientation (º), and (3) the substrate where the lichen mainly attached (bryophyte, lichen, bark or rock).

Besides, we measured with a digital calliper (4) the largest diameter of each individual (mm) (hereafter referred as to size), and (2) estimated the soredia cover (%), equivalent to the reproductive allocation. We evaluated the relationship between lichen area (mm^2) and largest diameter (mm) from photographs of 200 randomly chosen individuals used to estimate growth rates in the trees of the Escorial forest (see below). The fitted power regression for this relationship was: Lichen area = 0.6188 x Largest diameter$^{1.8422}$ ($r^2 = 0.95$; $P < 0.001$). Based on this strong relationship, the somatic lichen surface (S) and the area covered by soredia, i.e., reproductive surface (R) were calculated. The sum of all somatic surfaces was used to estimate the total lichen cover (cm^2) in each habitat. For the analyses of population structure we established three life stages categories following Hilmo et al. (2013): reproductive, juvenile and recruit, i.e., juveniles of ≤ 5 mm. The field data were collected between April 2011 and September 2013.

Lichen growth measurements

In 2011, 200 randomly selected individuals covering the whole lichen size range (5 – 360 mm) in each habitat were tagged (n = 800 individuals). We hydrated the lichens by spraying deionised water and took photographs of each individual at the beginning and end of the monitoring period, which were slightly different between sites due to logistic constrains: from 26th April 2011 to 14th November 2013, and 16th May 2011 to 14th November 2013 for individuals on the trees and on the rocks, respectively, in the Escorial forest; and 1st December 2011 to 7th November 2013, and 28th November 2011 to 7th November 2013 for individuals on the trees and on the rocks, respectively, in the Cardoso forest. Lichen area (A) was calculated in Photoshop 6.0 (Adobe Systems, San Jose, CA). The relative thallus area growth rate during the monitoring period was quantified as (c.f. Evans 1972): RT_AGR (mm^2 cm^{-2} day^{-1}) = ln (A_{end}/A_{start}) x 100/ Δt. The monitoring period (Δt) comprised 932 days for tree and 912 days for rock in Escorial, respectively; 676 days for tree and 709 days for rocks in Cardoso, respectively. Dead and shrank individuals were not included in further analyses, reducing the sample size from 800 to 562 individuals.

Statistical analyses

We performed Linear Mixed Models (LMMs) to test the influence of interaction habitat x site on the lichen size and relative growth rates using the total data set, including “replicate” as a random factor because our lichens may not be independent (Bolker et al. 2009). If these models were significant, lichen size and growth LMMs for each habitat separately were run. Prior to modelling, Pearson correlation analyses were carried out among the predictors to avoid multicolinearity, so only uncorrelated variables were included in the models. For each habitat model we tested the influence of site (Escorial vs Cardoso), habitat-scale variables (bryophyte cover, trunk/rock slope) and individual-scale variables (i.e. height, orientation, substrate, and soredia cover). In the tree models we also included the tree DBH, and in both growth models the thallus area at start (A_{start}) was included as a covariate because growth is usually size-dependent (e.g. Larsson & Gauslaa 2011; Merinero et al. 2015). To obtain normality and homoscedasticity of residuals the response variables thallus size and relative growth rate were log10 transformed and raised to the power of 0.4 following the Box-Cox transformation, respectively. We further calculated a marginal R^2 value for all models, providing an estimate of the variance explained by the fixed effects (Nakagawa & Schielzeth 2013). We used the package nlme (Pinheiro et al., 2008) for mixed effect model analyses. Pairwise post-hoc comparisons within significant variables with more than two levels were carried out using the lsmeans package (Lenth 2013).

To examine the relationship between reproductive stage and individual size we run Generalized Linear Models (GLMs) with binomial error (Bolker et al. 2009) including all life stages for each habitat in each site. The parameters of these logistic models were used to estimate probability (P) for reproduction in relation with individual size (x) according to the formula $P = 1/\ (1+e^{(\mu+\alpha x)})$, where parameters μ and α determine the intercept with the X-axis and the slope of the curve, respectively (Wesselingh et al. 1993; 1997). These parameters were used to estimate the threshold size for reproduction at 0.5 probabilities (Wesselingh et al. 1993; Méndez & Karlsson 2004; Merinero et al. in prep.), in each habitat in each site. To test whether the probability of reproduction differed between habitats in each site, a pooled logistic model of reproduction depending on size was compared with another model with the additional term “habitat-site” to refer to each habitat and site. A *post hoc* Tukey test was performed if the term “habitat-site” was significant.

Finally, to estimate the reproductive allocation of *L. scrobiculata* in each habitat only reproductives were included in the analyses. We analysed the reproductive surface (R) –somatic lichen surface (S) relationship using the classical allometric model used for plants: $R = aS^b$, usually fit as log-log relationship ($\log R = \log a + b \log S$ (Klinkhamer et al. 1992; Niklas 2004). The intercept (parameter a) is referred to as the “allometric coefficient” and the slope (parameter b) the “allometric exponent”. A slope significantly different from 1.0 indicates a non-isometric relationship. A positive reproductive allometry (slope significantly > 1) is interpreted in terms of high reproductive allocation and high efficiency of individuals in transforming somatic biomass/surface into reproductive biomass/ surface (Weiner et al. 2009; Merinero et al. in prep.). We used model II regressions (standardized major axis; SMA) because this method is more appropriate than traditional least squares regression for fitting allometric data and estimation of parameters (e.g. Niklas 2004; Warton et al. 2006). We tested whether the slope of each habitat and site statistically differed from 1.0, and we compared the slopes and intercepts of the regressions among them using multiple *post hoc* comparisons. All the SMA analyses were computed with the smatr R package (Warton et al. 2012). Residuals were checked for normality. All analyses were done in R version 3.0.2 (R Foundation for Statistical Computing, Vienna, Austria). Mean values ± 1SE are reported and statistical significance was set at $P < 0.05$.

RESULTS

Population size, structure and habitat variability

Within the two plots we recorded 20418 individuals of *Lobarina scrobiculata*, the 25.5% in the Escorial forest and 74.5% in the Cardoso forest. The abundance was much higher on trees than on rocks (62% and

69.4% of individuals in Escorial and Cardoso, respectively), although *L. scrobiculata* cover (%) varied with site (Table 1). The individual size population structure was skewed toward small individuals in both habitats, with more than 60% of individuals below 30 mm (Fig. 1). Accordantly, the stage structure was similar between habitats, with a clear dominance of juvenile individuals, followed by reproductive and recruits (Table 1). In Cardoso, the population structure between habitats slightly differed, with 7% more reproductive individuals on trees than on rocks (Table 1). The bryophyte cover was significantly higher on rocks than on trees (Table 1).

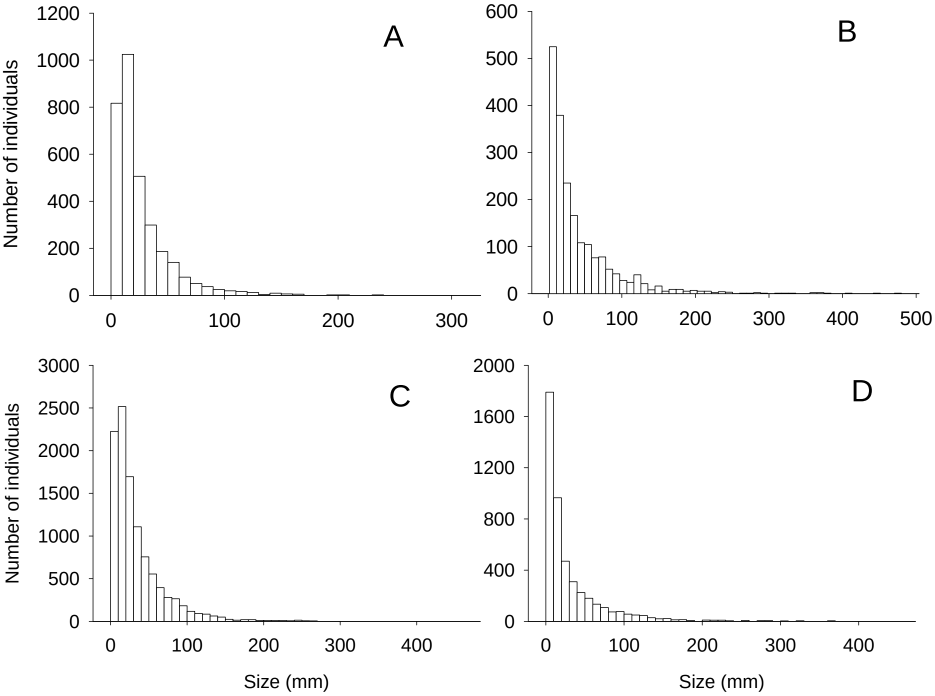
The distribution patterns of *Lobarina scrobiculata* individuals along the height gradient did not strongly differ between habitats because the number of individuals decreased gradually with increasing height from the ground (Fig. S1a supplementary material). Despite the slight difference in the distribution of individuals between sites, the ≈ 80% of the populations in both microhabitats were at < 90 cm (Fig. S1a supplementary material). By contrast, the distribution pattern along the orientation gradient differed between habitats (Fig. S1b supplementary material). In trees, individuals were spread along the whole orientation range, although the largest proportion of individuals had a northerly orientation, followed by easterly and westerly, whereas a much lower proportion had a southerly orientation (Fig. S1b supplementary material). In rocks, the distribution pattern varied between sites. In the Escorial forest, with rounded rocks, more than 70% of individuals grew at northerly orientation; whereas a similar proportion in Cardoso, with panel-like rocks, had easterly orientation (Fig. S1b supplementary material).

Table 1. Descriptive absolute or mean ± 1SE forest variables and *Lobarina scrobiculata* population features on different habitats (trees and rocks) in two Mediterranean forests in central Spain. Occupied refers to the proportion of trees/rocks hosting *L. scrobiculata*. P- values from ANOVA are given.

Variable	Trees	Rocks	*P*
Escorial forest			
Density (*n* in1/4 ha)	343	257	
Tree DBH / Rock height (cm)	17.8 ± 0.6	86.8 ± 5.4	
Slope (º)	77.0 ± 0.6	72.8 ± 0.3	**0.000**
Bryophyte cover (%)	17.4 ± 1.0	60.8 ± 1.8	**0.000**
n individuals of *L. scrobiculata*	3 241	1 972	
Occupied (%)	53.6	33.1	
L. scrobiculata cover (m^2)	138.2	243.8	
Reproductive (%)	25.4	27.2	
Juvenile (%)	70.9	67.2	
Recruits (%)	3.8	5.6	
Cardoso forest			
Density (*n* in 1/4 ha)	720	136	
Tree DBH / Rock height (cm)	12.3 ± 3.7	65.1 ± 4.2	
Slope (º)	82.0 ± 6.7	71.0 ± 0.2	**0.000**
Bryophyte cover (%)	27.9 ± 0.7	57.4 ± 2.2	**0.000**
n individuals of *L. scrobiculata*	10 547	4 658	
Occupied (%)	48.8	72.8	
L. scrobiculata cover (m^2)	799.1	418.3	
Reproductive (%)	24.9	18.3	
Juvenile (%)	70.5	67.1	
Recruits (%)	4.6	14.5	

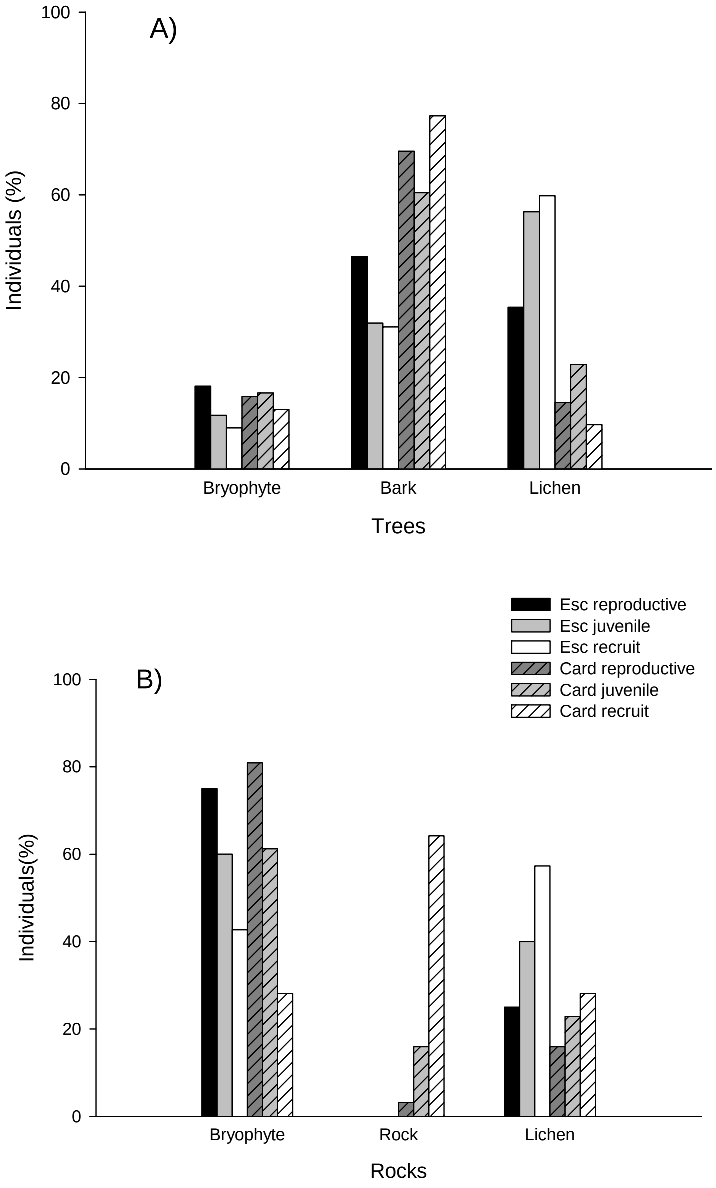
Bold values indicate significant differences at $P < 0.05$

Figure 1. Size distributions of populations of *Lobarina scrobiculata* on trees and rocks in two Mediterranean forests in central Spain. (A) Trees Escorial, (B) rocks Escorial, (C) trees Cardoso and (D) rocks Cardoso.



Regarding the main substrate where *L. scrobiculata* grows, more than the half of the individuals grew on bryophytes on rocks, whereas the bark and other lichens were the most frequent substrates on trees (Fig.S2 supplementary material). Nevertheless, this trend varied among life stages (Fig. 2). For example, on trees there were more reproductives growing on bark, and the proportion of juvenile and recruits were higher on other lichens and bark in Escorial and Cardoso, respectively (Fig. 2a). On rocks most reproductives grew on bryophyte in both sites, whereas the proportion of recruits was extremely higher on bare rock and other lichens in Cardoso and Escorial, respectively (Fig. 2b).

Figure 2. Distribution of the *Lobarina scrobiculata* life stages: recruits, juvenile and reproductive individuals (%), growing on different substrates in contrasting habitats: (A) trees and (B) rocks in two Mediterranean forests in central Spain (Esc: Escorial; Card: Cardoso).



Lichen growth and individual size

Over the initial 800 monitored individuals, 562 with positive relative growth rates were included in the models. Relative growth was significantly faster on rocks than on trees regardless the site (Table 2). *Lobarina scrobiculata* grew significantly faster in the rocks of Cardoso (0.097 ± 0.005 mm^2 cm^{-2} day^{-1}), followed by the rocks in Escorial (0.075±0.005 mm^2 cm^{-2} day^{-1}), the trees in Cardoso (0.053±0.003 mm^2 cm^{-2} day^{-1}) and the trees in Escorial (0.039±0.002 mm^2 cm^{-2} day^{-1}). The *post hoc* Tukey test showed significant differences between habitats in both sites ($P < 0.001$). Relative growth rate was size-dependent in both habitats and higher in Cardoso (Table 3). On rocks growth was faster in individuals with less soredia, at northern orientation and on leaning rocks, whereas effects of environmental variables on trees were not detected (Table 3). On trees the effect of substrate was marginally significant, but the *post hoc* comparison did not discriminate the effect of each substrate (Table 3).

Table 2. Summary of the linear mixed models fit by a Laplace approximation used to examine the effects of habitat, site, and their interaction on the relative growth rates (mm^2 cm^{-2} day^{-1}) and individual size (mm) of *Lobarina scrobiculata* in two contrasting habitats (trees vs rocks) in two Mediterranean forests. The data comprise the Wald-type F-statistic value (*F*), degrees of freedom (df), coefficient of the variable in the model (Estimate), standard error of the estimator (SE), significance value (*P*), marginal R^2 (R^2m) and random effects standard deviation (SD).

	RT$_A$GR					**Individual size**				
	df	Estimate (SE)	*F*	*P*	R^2m	*df*	Estimate (SE)	*F*	*P*	R^2m
Fixed effects										
Intercept	419	0.326 (0.011)	3809.12	**0.000**		19833	1.469 (0.018)	26383.34	**0.000**	
Forest (F)										
Cardoso	139	0.054 (0.015)	24.05	**0.000**		19833	-0.092 (0.022)	0.23	0.633	
Habitat (H)										
Tree	139	-0.072 (0.015)	60.62	**0.000**	0.20	19833	-0.144 (0.024)	1.716	0.190	0.30
F x H	139	-0.013 (0.020)	0.42	0.516		19833	0.190 (0.031)	38.83	**0.000**	
Random effects				SD					SD	
Intercept				0.04					0.16	
Residual				0.09					0.39	

Mean lichen size significantly varied between habitats but the pattern was opposite between sites (Table 3). In Escorial, individuals were larger on rocks than on trees (42.2±1.1 vs 26.0±0.4 mm), whereas in Cardoso individuals on trees were larger than on rocks (34.6±0.3 vs 32.1±0.7 mm, respectively; Tukey *post hoc* comparisons, *P* < 0.001). The results of the separated LMMs showed that in both habitats greater height from the ground favoured larger individual size (Table 3). On trees, individuals tended to be larger on thinner trunks and when growing on bryophyte, followed by bark and lichens (Tukey *post hoc* comparisons, *P* < 0.001) (Table 3). On rocks, individual size tended to increase on leaning rocks with higher bryophyte cover, at northerly orientation, and growing on bryophyte (followed by lichen and bare rock) (Tukey *post hoc* comparisons, *P* < 0.001) (Table 3).

Probability of reproduction and reproductive allocation

The probability of reproduction increased with increasing individual size in both habitats and sites (Fig. 3; GLMs, *P* < 0.001; data not shown). The threshold size for the onset of asexual reproduction with a probability of P = 0.5 was significantly smaller on trees than on rocks in both sites (Fig. 3). The allometric relationships between log reproductive surface (R) and log somatic lichen surface (S) were positive and significantly correlated (data not shown). All the estimated allometric slopes were significantly higher than one, indicating a positive allometric pattern. In Escorial the slope on trees was significantly greater than on rocks (1.81 and 1.65, respectively; pairwise comparison, *P* < 0.001), indicating that individuals on trees disproportionally allocated more resources to reproduction than on rocks. The slope between habitats in Cardoso did not differed significantly (1.79 and 1.74, in trees and rocks respectively; pairwise comparison, *P* >0.05). However, the y-intercept was significantly greater in trees than on rocks (-3.49 and -3.69, respectively), indicating that similar-sized individuals on trees had higher reproductive allocation than on rocks.

Figure 3. The relationship between lichen size and asexual reproduction probability of *Lobarina scrobiculata* on trees and rocks in two Mediterranean forests (Escorial and Cardoso). Probability curves sharing the same letters are not statistically different from each other (all pairwise multiple comparisons: *post hoc* Tukey test; $P < 0.05$).

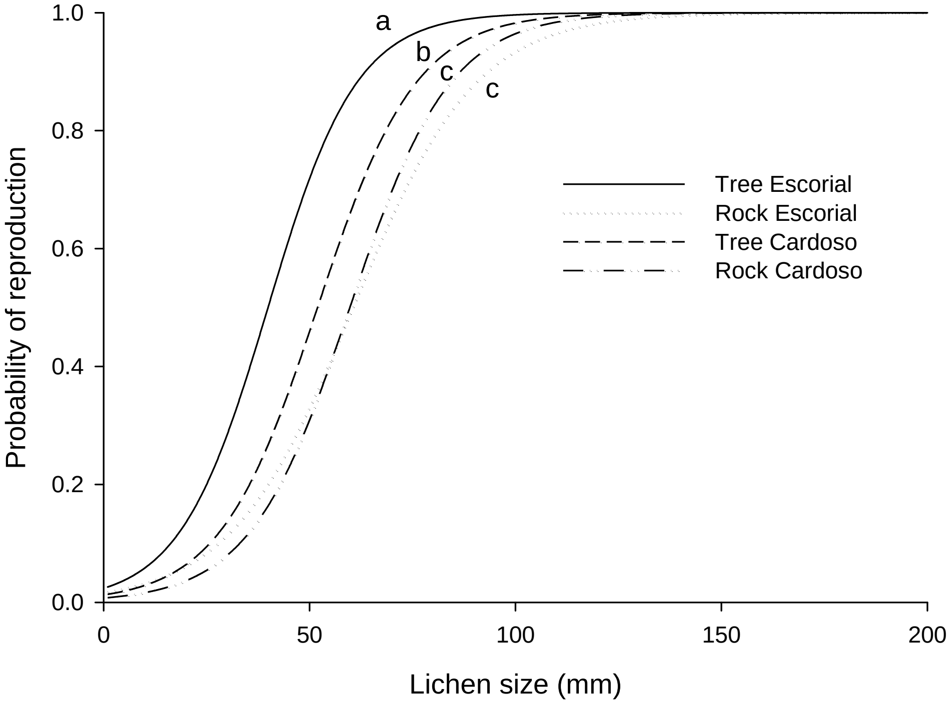


Table 3. Summary of the linear mixed models fit by a Laplace approximation used to examine the effects of site, habitat and individual variables on the relative growth rates (units) and individual size (mm) of *Lobarina scrobiculata* in two contrasting habitats (trees vs rocks) in two Mediterranean forests. The data comprise the Wald-type F-statistic value (*F*), degrees of freedom (df), coefficient of the variable in the model (Estimate), standard error of the estimator (SE), significance value (*P*), marginal R^2 (R^2m) and random effects standard deviation (SD). Only significant effects are shown.

	RT_AGR					**Individual size**				
	df	Estimate (SE)	*F*	*P*	*R^2m*	df	Estimate (SE)	*F*	*P*	*R^2m*
Trees										
Fixed effects					0.14					0.30
Intercept	201	0.248 (0.081)	2020.4	0.000		13249	1.408 (0.075)	37803.3	0.000	
Site (Cardoso)	67	0.032 (0.016)	10.73	**0**		529	0.060 (0.016)	57.67	**0.000**	
DBH	67	-0.001 (6.8×10^{-4})	3.82	0.06		529	-0.007 (0.001)	12.71	**0.000**	
Height	201	-5.8×10^{-5} (1.6×10^{-4})	1.84	0.18		13249	0.001 (0.000)	194.27	**0.000**	
Substrate	201	Br=Bark=Li	3.13	**0.05**		13249	Br>Bark>Li	265.6	**0.000**	
Soredia cover	201	-4.5×10^{-4} (5×10^{-3})	1.17	0.28		13249	0.020 (0.000)	5283.86	**0.000**	
Area start	201	-5.1×10^{-4} (2.5×10^{-4})	6.88	**0.01**						
Random effects				SD					SD	
Intercept				0.03					0.12	
Residual				0.08					0.30	
Rocks										
Fixed effects					0.23					0.36
Intercept	205	0.398 (0.043)	2475.5	0		6438	1.416 (0.051)	10373.1	0.000	
Site (Cardoso)	69	0.091 (0.018)	14.01	**0**		181	-0.055 (0.029)	22.61	**0.000**	
Bryophyte cover	69	-5×10^{-4} (3×10^{-4})	1.36	0.25		181	0.002 (0.001)	17.53	**0.001**	
Slope	205	-9×10^{-4} (4×10^{-4})	4.97	**0.03**		6438	-0.002 (0.000)	108.89	**0.002**	
Orientation	205	0.046 (0.014)	10	**0**		6438	-0.007 (0.012)	5.636	**0.018**	
Height	205	2×10^{-4} (2×10^{-4})	1.39	0.24		6438	0.000 (0.000)	23.21	**0.000**	
Substrate	205	Br=Li=R	1.85	0.16		6438	Br>Li>R	546.25	**0.000**	
Soredia cover	205	-0.003 (7×10^{-4})	11.98	**0.000**		6438	0.031 (0.001)	2206.52	**0.000**	
Area start	205	-5×10^{-4} (2×10^{-4})	26.54	**0.000**						
Random effects				SD					SD	
Intercept				0.04					0.14	
Residual				0.08					0.36	

DISCUSSION

In the present study we have accomplished, for the first time, a comparative fine-scale demographic analysis of an epiphytic lichen (*Lobarina scrobiculata*) in two contrasting habitats within two Mediterranean forests. Despite the different forest structure and characteristics of each site, the effect of habitat (trees and rocks) significantly influenced the species performance at the individual and population scales. We found different patterns of abundance, establishment, individual growth, threshold size for reproduction and reproductive allocation between trees and rocks. We found larger populations on trees, suggesting that the establishment and colonization were favoured on this habitat. However, individuals grew faster on rocks, where they became reproductive at larger sizes and allocated less resources to reproduction than their counterparts on trees. Thereby, both habitats complementarily contribute to the persistence of the *L. scrobiculata* populations in Mediterranean forests

Population size and structure

The population size, although very different between the two forests, was higher on trees than on rocks. However, the population size and life-stage structure was similar in both habitats, where small juveniles (including recruits) comprised ca 75% of established lichens (Table 1; Fig. 1).This life-stage and size distribution patterns have been found for various lichen species in different climates (e.g. Rhoades 1983; Hestmark 1992; Hilmo et al. 2013; Rubio-Salcedo et al. 2015) and it presumable reflects that populations are expanding and they have not saturated the local habitats yet (Hestmark 1992). Nevertheless, only by means of a long-term monitoring study that evaluates population vital rates (i.e. survival, growth and recruitment) we could understand the population dynamics and predicting the population viability.

The chemical, biological and structural features of the substrate, i.e. the bare bark or rock, and the covers of other organisms such as bryophytes, lichens or fungi, may significantly influence the attachment and development of lichen propagules (Barkman 1958; Ott 1987; Hilmo et al. 2011). Within each habitat the variability of microenvironments was high, as it was reflected in the life-stage distribution pattern of adhesion to the substrate. On trees, the life-stage pattern was not surprising, as most of recruits, juvenile and reproductive individuals were similarly growing on bark (in Cardoso forest) and other lichens (in Escorial forest) (Fig. 2a). The discrepancy between forests is likely explained by a higher lichen epiphytic biomass in the latter forest, limiting the bark availability for *L. scrobiculata* establishment. However, the pattern on rocks was striking, because most recruits grew on other lichens (Escorial) or directly on the bare rock (Santuy), whereas most reproductive and juvenile individuals were found on bryophytes (Fig. 2b). Thus, the apparently less favourable substrate for recruitment turned out to be the best for lichen survival and growth on rocks. The small juvenile stages are particularly sensitive to environmental variation (Ott 1987; Hilmo & Ott 2002), while large thalli have broader ecological amplitude due to their higher physiological resistance to these variations (e.g. Hestmark 1992; Merinero et al. 2014b).

Although some studies suggest that bryophytes exclude lichens by competition (reviewed by During & Van Tooren 1990), our results suggest a positive effect of the bryophyte cover on the *L. scrobiculata* persistence on rocks, because it is where individuals reached larger size and become reproductive (Table 3). The establishment of soredia within the moss leaves might be difficult (Jahns 1982). However, once they attached, the thick moss mats absorbing and retaining water (Veneklaas et al. 1990; Colesie et al. 2012), may enable associated lichens to remain hydrated and thus metabolically active for extended periods than if they grew on bare bark/rock or other lichens, especially under Mediterranean climatic conditions (Sillet & McCune 1998; Colesie et al. 2012). Thereby, suitable substrates for colonization of new propagules like the moss covers are essential for the maintenance of the *L. scrobiculata* populations.

Lichen growth and size

Lobarina scrobiculata showed similar and even higher growth rates in both habitats compared to transplants annual growth rates documented in a nearby Mediterranean forest (Merinero et al. 2015). Within forests, individuals growing on rocks grew much faster than on trees. According to our models, the significant faster growth of *L. scrobiculata* on mossy rocks was due to the lean surface offered by rocks, likely retaining water for longer periods than the vertical tree trunks. The effects of the bryophyte cover or substrate were not significant for lichen growth, but they strongly influenced the individual lichen size in both habitats. Maybe, a longer monitoring period including more individuals with positive growth could result in differences in growth due to substrate or bryophyte cover. For example, some experimental studies documented positive effects of bryophyte cover on cephalolichens growth (Sillet & McCune 1998; Colesie et al. 2012).

Probability of reproduction and reproductive allocation patterns

As other studies had shown before, asexual reproduction in *L. scrobiculata* was size-dependent and a threshold size to start reproduction was required (Hilmo et al. 2013; Merinero et al. in prep). Interestingly, our results at this fine-scale showed that the threshold size for reproduction was habitat-dependent and thus varied within populations (Fig. 2). *Lobarina scrobiculata* had smaller threshold sizes for reproduction on trees than on rocks. According to Bonser & Aarssen (2009) this difference in reproductive size is probably explained by different developmental trajectories of individuals which are selected by environment, because environmental adversity selects for reproduction at smaller sizes. However, this does not mean that trees are an adverse environment for this epiphyte, but instead that rocks are more favourable. In agreement with our results, Wesselingh et al. (1997) showed that a biennial plant in contrasting populations had a larger threshold size for reproduction where it grew faster. Various intraspecific lichen studies at different spatial scales found differences in the threshold size for reproduction, and they all suggested that differences in relative growth rates may underlie such discrepancy (e.g. Hilmo et al. 2013; Rubio-Salcedo et al. 2015; Merinero et al. in prep). In this study we evidenced that individuals on trees grew much slowly than on rocks and, in agreement with theory and evidence on plants (Stearns & Koella 1986; Stearns 1992; Wesselingh et al. 1997; Weiner et al. 2009; Santos-del-Blanco 2013), had smaller threshold size for reproduction. Nevertheless, these results should be taken cautiously, because both traits reflect different processes at different time scales. Growth data correspond to a certain period of time and it can vary among years (e.g. Shriver et al. 2012), whereas the threshold size for reproduction may reflect a longer and population historical process (Wesselingh et al. 1997).

The reproductive allocation in both habitats and sites showed a significant positive allometry, i.e. large individuals allocate disproportionally more resources to reproduction than their small counterparts (Weiner et al. 2009). However, the patterns of reproductive allocation significantly varied between trees and rocks. In Escorial, the individuals growing on trees had significantly higher slope than those on rocks. This implies that individuals on trees were more efficient in transforming somatic biomass into reproductive structures than their counterparts on rocks. In Cardoso forest, the allometric pattern did not differ between habitats (the allometric slopes were not significantly different) but, the y-intercept was significantly higher in trees than on rocks, implying that individuals with similar size had higher reproductive allocation on trees than on rocks. Thus, it is remarkably that individuals on trees, with slower growth rates and smaller reproductive size, invested more resources to reproduction than their counterparts on rocks. These results are not consistent with the theoretical predictions that state that plants in favourable environments (e.g. with high resources availability, low competition, high plant survival) should grow fast, show larger threshold size for reproduction and exhibit higher reproductive allocation than their counterparts in adverse environments (e.g. with low resources availability, high competition, and high mortality) (Stearns & Koella 1986; Kozlowski 1992; Stearns 1992; Bonser & Aarssen 2009).

However, these results are consistent with the reproductive allocation pattern documented along climatic gradients of a Mediterranean pine and *L. scrobiculata* populations that, saving the organism-specific differences, exhibited higher reproductive allocation on dry locations (adverse environment) than on rainy locations (favourable environment) (Santos-del-Blanco et al. 2013; Merinero et al. in prep). Thus, we hypothesized that the higher reproductive allocation in “adverse” environments might be a response to stress, so the species produces more propagules where its persistence can be reduced. It is probably that epiphytic competition for space on trees together with the reduced bryophyte cover and verticality of trees limit the growth rates and survival of *L. scrobiculata* individuals, favouring the initiation of reproduction at relatively small sizes (Bonser & Aarssen 2009). However, during the ca. two years monitoring period we did not detect higher mortality on trees, so survival rates during a longer period of time are essential to test the prediction that slow growth rate together with small reproductive size relates to lower survival, and vice versa. Thus, the fact that mossy rocks are more favourable for lichen growth does not mean that trees are adverse environments for this epiphyte. Precisely, the efficient reproductive individuals on trees are presumably increasing the propagule density in the local environment, favouring the population expansion. Similarly, plant studies reported differences in the sexual reproductive success due to differences in two microhabitats, although each contrasting microhabitat was “favourable” for reproduction depending on the weather of each year (e.g. Thomas 1996; Kephart & Paladino 1997; Albert et al. 2001). In our case, it is unknown whether lichen asexual reproduction depends on climate, but this is of less importance as we measured the total reproductive allocation of each individual at one time.

Because in both populations asexual symbiotic reproduction is the only mode of propagation in the present, these abundant populations are likely constituted by *L. scrobiculata* genetic clones (reviewed by Scheidegger & Werth 2009). Thereby, the differences in growth, threshold size for reproduction and reproductive allocation between trees and rocks are likely the product of phenotypic plasticity rather than selected/adaptive species traits between habitats (see Weiner et al. 2009).

Conservation and management implications

Results indicated that fine-scale demography, growth and reproduction of *L. scrobiculata* are determined by the type of habitat within Mediterranean forests. On trees, *L. scrobiculata* grew slower, started reproduction at smaller sizes and had higher reproductive allocation than on rocks. The observed differences in threshold size for reproduction of *L. scrobiculata* inhabiting two different habitats are probably associated with the differences in the relative growth rates between the two habitats, qualitatively confirming part of the life-history theory predictions. However, the reproductive allocation pattern was not consistent with the predicted higher reproductive allocation in the habitats with faster reproductive growth rates. Regardless this difference, it is evident that individuals in both habitats increased the density of asexual propagules released in to the local environment, contributing then to the population persistence.

Growth of established individuals cannot be an indicator of population survival and recruitment, which may be decisive factors for lichen population dynamics. Therefore, long-term detailed studies of recruitment and survival, including also propagule dispersal may contribute to identify the most sensitive stage in the life cycle of this lichen. Our results also reflect that including more than one site in detailed demographic studies comparing habitats is essential, because for instance, patterns *of L. scrobiculata* cover and mean individual size were opposite between habitats depending on the forest.

An important matter from a conservation perspective is to know which characteristics of each habitat play a role in the species performance. On rocks, the moss cover is an essential requirement for the persistence of *L. scrobiculata* because it likely supplies a niche of improved water and surface available for colonization, growth and maintenance of *L. scrobiculata*. By contrast, trees do not need to be covered by any organisms, as the bare bark is an appropriate substrate sustaining all *L. scrobiculata* life-stages. Despite we studied two abundant populations of this threatened species, it is important to highlight that protection strategies need to be developed, emphasizing in the areas where no forest management is planned, as it happens in the Escorial forest. From our results, it seems crucial to preserve the large moss covers on rocks and the maintenance of the current forest structure that provides such high lichen relative growth rates and population sizes. Living under the unpredictable Mediterranean climatic conditions, the habitat diversity within Mediterranean forests may greatly contribute to the viability of *L. scrobiculata* populations. Therefore, the maintenance of habitat heterogeneity together with the consideration of demography at the habitat scale are crucial parameters to develop conservation plans.

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SUPPLEMENTARY MATERIAL

Figure S1. Distribution of *Lobarina scrobiculata* individuals (%) on trees and rocks in two Mediterranean forests in central Spain (A) along a height gradient measured from the ground; and B) in each orientation range: (1) north (311º-45º); (2) south (136º-225º); (3) east (46º-135º); and (4) west (226º-310º).

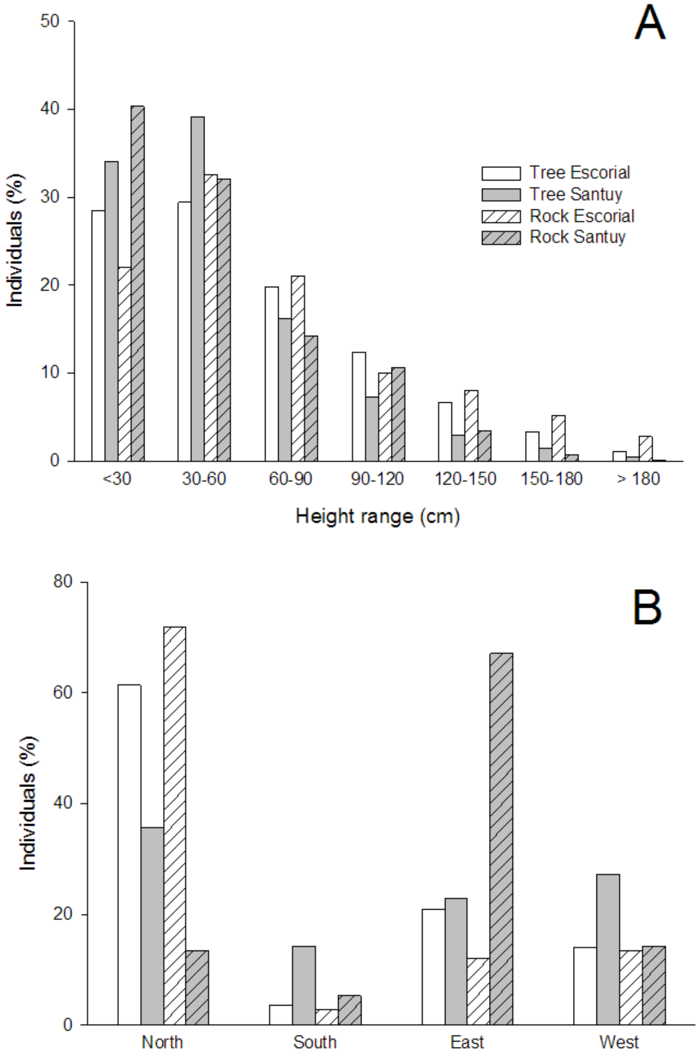
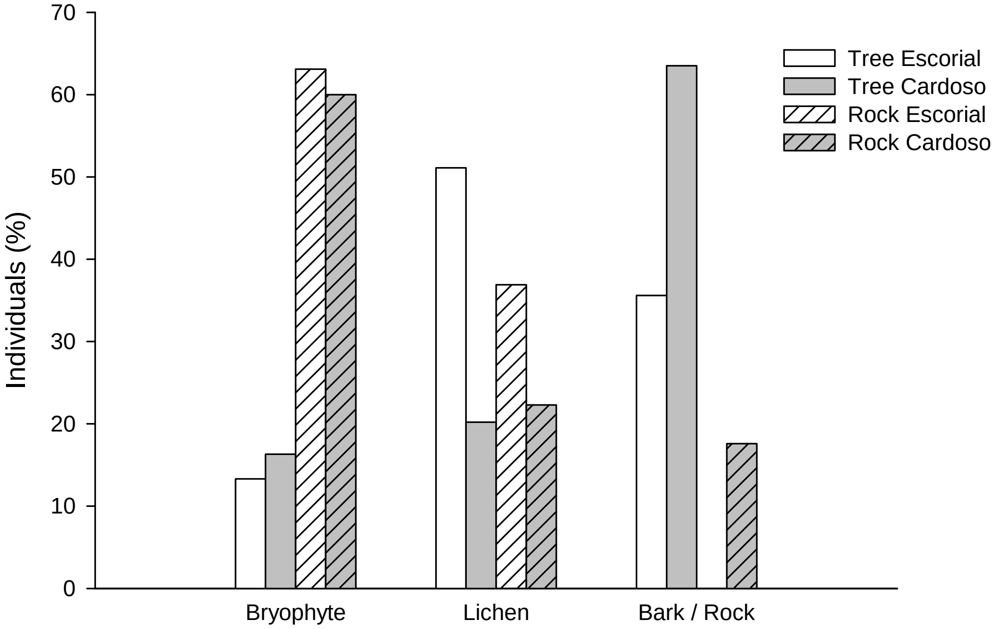


Figure S2. Distribution of the *Lobarina scrobiculata* individuals (%) growing on different substrates in trees and rocks in two Mediterranean forests in central Spain (Esc: Escorial; Card: Cardoso).



CAPÍTULO V / CHAPTER V

DO LICHEN SECONDARY COMPOUNDS PLAY A ROLE IN HIGHLY SPECIFIC FUNGAL PARASITISM?

Sonia Merinero, Massimo Bidussi & Yngvar Gauslaa

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ABSTRACT

Chemical interactions between highly host-specific lichenicolous fungi and their lichen hosts are poorly studied. In an allometric study, we quantified carbon based secondary compounds (CBSCs) in a mixed natural *Lobarina scrobiculata* population (N=147) of the normal and the stictic acid-deficient chemotypes, both with and without galls of *Plectocarpon scrobiculatae*. We assessed the correlation between the presence/abundance of parasite galls and the lichen CBSCs contents, and quantified size-dependent contents of CBSCs. The parasite produced galls similarly in both chemotypes, indicating that the stictic acid complex do not deter *Plectocarpon.* Within both chemotypes, thalli with *Plectocarpon* had half the contents of all individual CBSCs as those without galls*.* There was a significant size-dependent increase in CBSC contents in thalli without galls, but not in those with. This study shows that lichen chemistry is involved in highly host-specific fungal parasitism, and widens our knowledge on specialized biotrophic fungal interactions.

Keywords: Allometry, biotrophy, chemical defense, lichenicolous fungi, *Lobarina scrobiculata*, parasitism, *Plectocarpon scrobiculatae*, secondary compounds, symbiosis.

INTRODUCTION

Lichenicolous fungi, inhabiting lichens, comprise ≈1500 parasites differing in specialization and virulence (Hawksworth, 1982; Lawrey & Diederich, 2003). Low-virulent, highly specialized lichenicolous fungi are biotrophic mycoparasites because their ecological niche is restricted to healthy specific hosts for long periods (Mendgen & Hahn, 2002; Lawrey & Diederich, 2003). However, relationships between highly specialized lichenicolous fungi and their hosts are rarely studied. Mechanisms regulating host specificity could be related to chemical traits, but we do not know to what extent secondary chemistry regulate these associations (Lawrey & Diederich, 2003).

Most lichens produce carbon-based secondary compounds (CBSCs; Huneck & Yoshimura, 1996). Medullary CBSCs often deter grazers (e.g. Asplund et al., 2010; Solhaug & Gauslaa, 2012), whereas cortical ones mainly serve as sunscreens (Solhaug & Gauslaa, 2012). CBSCs may also deter microbes and lichenicolous fungi (Lawrey, 1989). In some cases, lichenicolous fungi cannot overcome the lichen’s defense, and they only degrade lichen tissues after CBSC extraction (Lawrey, 1993, 2000). Moreover, some lichens inhibit growth of relatively specialized lichenicolous fungi (Lawrey, 1993). However, CBSC may not necessarily deter lichenicolous fungi because virulent generalist parasites degrade CBSCs (e.g. lecanoric acid), facilitating other parasites (see Torzilli et al., 1999; Lawrey, 2000). Specialized lichenicolous fungi may enhance their growth when inoculated on their frequent host (Lawrey, 1993), suggesting that specialized lichen parasites tolerate and/or overcome the defense of their main host. Thus, lichenicolous fungi with different degrees of specialization vary in their responses to CBSCs. Yet, relationships between highly host-specific lichenicolous fungi and their hosts’ CBSCs are poorly known.

We focus on the parasite-host system comprising the cyanolichen *Lobarina scrobiculata* (Scop.) Nyl. ex Cromb (Lobariaceae, Ascomycota) and its highly specialized lichenicolous fungus *Plectocarpon scrobiculatae* Diederich & Etayo (Roccellaceae, Ascomycota; Fig. S1). The host occurs across the North Hemisphere and in oceanic parts of Africa, Australia, New Zealand and South

America (Smith et al., 2009), whereas its parasite only occurs in Europe, Macaronesia and North America (Ertz et al., 2005). *Plectocarpon* forms basally constricted galls resembling lichen apothecia and reproduces mainly sexually (Ertz et al., 2005; Fig. S1). The relationship is likely commensalistic, because the parasite does not cause visual damage apart from local deformation due to gall induction (Hawksworth, 1982; Ertz et al., 2005). Its host produces the sun-screening cortical usnic acid (Solhaug & Gauslaa, 2012), the herbivore-deterrent medullary stictic acid complex and meta-scrobiculin (Gauslaa, 2008; Asplund et al., 2010). However, a chemotype lacking the stictic acid complex occurs in Alaska and Norway (Holien & Hilmo, 1991).

Here we compare CBSCs contents in two *L. scrobiculata* chemotypes with and without *P. scrobiculatae* galls. Because lichen CBSC contents in lichens may increase with size (Asplund & Gauslaa, 2007), we sampled a range of sizes of thalli with and without galls. Given the high specialization of *Plectocarpon* for one host, we hypothesize that lichen CBSCs do not constitute a defense against *Plectocarpon*. Our specific hypotheses are: (1) CBSC contents are similar and size-dependent in thalli with and without *Plectocarpon* galls; (2) the parasite forms galls in both chemotypes; (3) the abundance of parasite galls, a surrogate of the parasite reproductive effort, does not correlate with the lichen CBSC contents.

LICHEN MATERIAL

Lobarina scrobiculata was collected on *Picea abies* in boreal rainforests at Foss, Nord-Trøndelag, Norway (see Hilmo et al., 2013 for details on the study area and sampling). We used lichens from 29 intact branches (2–13 m from the ground) from 21 trees randomly collected in June 2011, the season with peak values of CBSCs in *L. scrobiculata* (Gauslaa et al., 2013). Branches with air-dried lichens were stored at -20 °C until extraction of CBSCs. We gathered all *L. scrobiculata* thalli with *P. scrobiculatae* galls from these branches. For each thallus with galls, we randomly sampled one thallus of similar size without *P. scrobiculatae* galls from the same tree. Mean height on the tree for both categories did not differ (7.0±3.6 and 6.8±4.4 m for the thalli without galls and the ones with galls, respectively; ANOVA; *P* >0.05). Size varied from 0.23 to 124.5 cm^2 with no difference between thalli with and without galls (ANOVA, *P*=0.95). We measured the size of each thallus, extracted the CBSCs and quantified the CBSCs with HPLC (Supplementary data Methods S1). We used Linear Mixed Models to assess effects of thallus size on the CBSCs content and number of galls (Supplementary data Methods S1).

RESULTS

Sixteen thalli with galls and fifteen without (in total 21% of all thalli) lacked detectable amounts of the stictic acid complex (Table 1), including stictic, constictic, norstictic, cryptostictic and methyl-norstictic acids. These stictic acid-deficient thalli occurred on 15 of the 21 sampled trees. In these trees the normal and the deficient chemotypes co-occurred. Usnic acid, the only cortical compound, and meta-scrobiculin were present in all studied thalli of both chemotypes (Table 1). Total CBSC concentrations in all *L. scrobiculata* without galls varied from 0.1 to 13.8% of DM (4.8±3.3%; mean ± SD; n=69), corresponding to a content of 0.1-21.7 g m^{-2} (6.1 ± 4.0 gm^{-2}).

The proportion of the individual CBSCs was similar in thalli with and without galls for each chemotype (ANOVAs and t-tests, *P* >0.05). For the normal chemotype (n=116), usnic acid represented 20.6±11.3 and 19.9±11.0% of the total compounds content in thalli with and without galls, respectively. Thereby, medullary compounds accounted for nearly 80% of the total CBSC pool. Meta-scrobiculin comprised 19.8±23.7 and 25.4±23.5% of the CBSC pool in thalli with and without galls, respectively; the corresponding figures for the stictic acid complex were 59.6±19.1 and 54.7±19.8%. For the stictic-deficient chemotype (n=31) the proportion of usnic acid was 33.8±16.1 and 38.32±17.3% in thalli with and without galls, respectively; whereas the proportion of meta-scrobiculin was 66.2±16.1 and 61.7±17.3% in thalli with and without galls, respectively.

The number of *P. scrobiculatae* galls per thallus ranged from 1 to 85 (8.9±14.4; mean ±SD). Twenty-seven percent of the thalli hosted only one gall, 51.3% had

≤3 galls, 81% ≤10, and only 2.6% had >50 galls. The number of galls significantly increased with thallus size (Fig. S2). However, gall number did not correlate with the content of any CBSCs (Table 2).

Apart from the minor norstictic and methyl norstictic acid, thalli with galls had on average 50% significantly lower total CBSC content than thalli without galls (Table 1). The presence of *P. scrobiculatae* was significantly related with the lower individual and total CBSCs contents (LMMs, $P<0.001$; data not shown). Allometric relationships for CBSCs contents differed between thalli with and without *P. scrobiculatae* galls (Fig. 1). In thalli without galls, all three main groups of CBSCs significantly increased with increasing size, whereas in those with galls, CBSCs did not increased with size and all had the concentration typical for small thalli even at large sizes (Fig. 1; Table 2).

Table 1. Mean lichen size and carbon based secondary compound (CBSC) contents (± 1SE) in *Lobarina scrobiculata* with and without galls of *Plectocarpon scrobiculatae* for the normal and stictic deficient-chemotypes. Sample size for each category in brackets.

Lichen parameter	*Normal chemotype*			*Deficient chemotype*		
	without galls (54)	with galls (62)	*P*	without galls (15)	with galls (16)	*P*
Thallus area (cm^2)	13.99 ± 2.65	12.86 ± 2.55	0.759	11.57 ± 3.69	19.14 ± 5.90	0.287
Thallus dry mass (mg)	204.2 ± 42.2	200.0 ± 49.6	0.949	146.6 ± 52.1	236.7 ± 75.69	0.336
Specific thallus mass (mg cm^{-2})	12.08 ± 0.42	12.20 ± 0.37	0.825	11.26 ± 0.66	11.93 ± 0.37	0.388
CBSC (g m^{-2}):						
Usnic acid (cortical compound)	1.17 ± 0.12	0.64 ± 0.06	**0.000**	1.35 ± 0.28	0.49 ± 0.06	**0.009**
Meta-scrobiculin	1.64 ± 0.23	0.67 ± 0.09	**0.000**	2.21 ± 0.41	1.15 ± 0.18	**0.031**
Stictic acid complex, in total	4.05 ± 0.52	1.96 ± 0.19	**0.000**			
Constictic acid	1.01 ± 0.14	0.43 ± 0.05	**0.000**			
Cryptostictic acid	0.34 ± 0.04	0.17 ± 0.02	**0.000**			
Methyl norstictic acid	0.02 ± 0.00	0.01 ± 0.00	**0.042**			
Norstictic acid	0.06 ± 0.01	0.04 ± 0.01	0.277			
Stictic acid	2.63 ± 0.34	1.31 ± 0.13	**0.000**			
Total medullary compounds	5.69 ± 0.62	2.64 ± 0.25	**0.000**			
Total compounds	6.86 ± 0.72	3.28 ± 0.28	**0.000**	3.55 ± 0.58	1.65 ± 0.19	**0.006**

Significant *P*-values (*P*<0.05) for differences between the two groups in bold (ANOVA and t- test)

Figure 1. The relationship between thallus size ($\log_{10}$ transformed) and content of secondary compounds in *Lobarina scrobiculata*. Filled dots and solid lines: thalli with *Plectocarpon* galls. Open dots and broken lines: thalli without *Plectocarpon* galls. The stictic acid complex plot only includes the stictic acid chemotype.

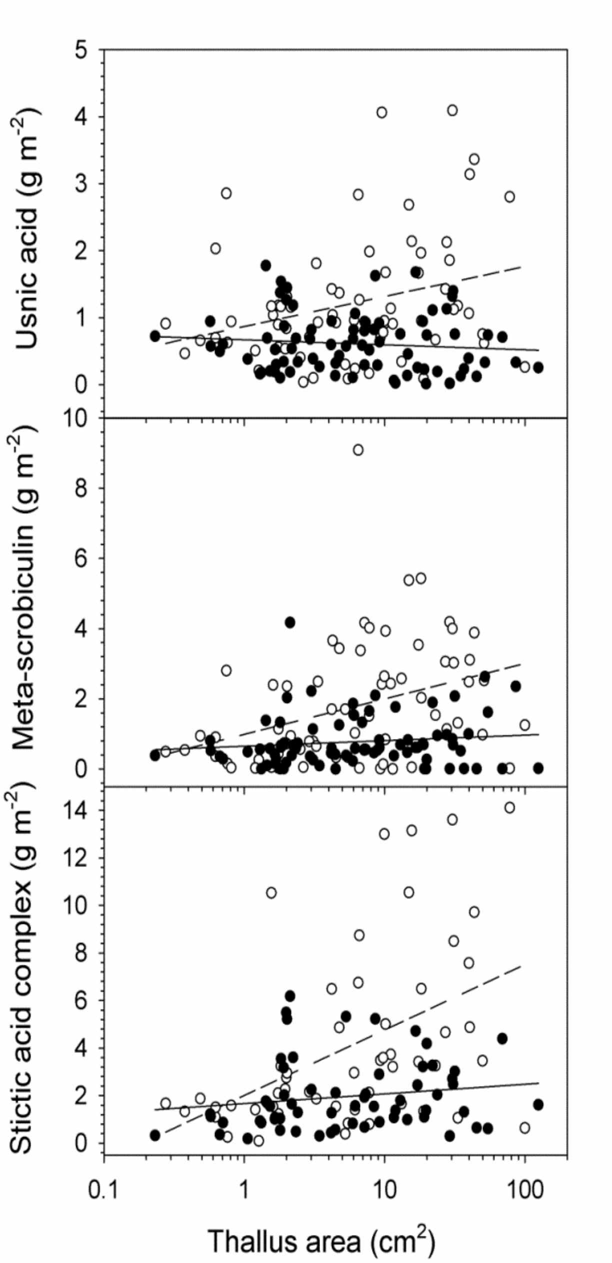

Table 2. Summary of the Linear Mixed Models examining the effects of thallus size on contents of secondary compounds of *Lobarina scrobiculata* without *Plectocarpon scrobiculatae* galls (n=69) and with parasite galls (n=78). The effect of the number of parasite galls (No. galls) was tested for the parasitized thalli.

	Usnic acid					Meta-scrobiculin				Stictic acid complex					Total compounds			
	df	Coeff±SE	*F*	*P*	R^2m	Coeff±SE	*F*	*P*	R^2m	df	Coeff±SE	*F*	*P*	R^2m	Coeff±SE	*F*	*P*	R^2m
Without galls																		
Fixed effects																		
Intercept	52	0.74 (0.24)	73.93	<0.001		0.92 (0.48)	37.51	<0.001		39	0.72 (1.01)	48.88	<0.001		2.18 (1.39)	70.16	<0.001	
Log (Size+1)	52	0.24 (0.09)	6.67	**0.013**	0.08	0.48 (0.16)	8.8	**0.005**	0.09	39	1.62 (0.4)	16	**<0.001**	0.23	2.35 (0.54)	19.13	**<0.001**	0.25
Random effects				SD				SD					SD				SD	
Intercept				0.37				0.98					1.26				2.05	
Residual				0.79				1.38					3.17				4.14	
With galls																		
Fixed effects																		
Intercept	57	0.67 (0.12)		<0.001		0.58 (0.20)	55.22	<0.001		43	1.46 (0.43)	56.61	<0.001		2.86 (0.62)	79.57	<0.001	
Log (Size+1)	57	-0.03 (0.05)		n.s.		0.10 (0.08)	1.58	n.s.		43	0.29 (0.19)	1.81	n.s.		0.29 (0.27)	0.48	n.s.	
No. Galls	57	$2\cdot10^{-3}$ (0.03)		n.s.		$2\cdot10^{-4}$ ($6\cdot10^{-3}$)	0.001	n.s.		43	-0.02 (0.02)	0.62	n.s.		-0.04 (0.03)	1.21	n.s.	
Random effects				SD				SD					SD				SD	
Intercept				0.18				0.22					0.60				0.78	
Residual				0.40				0.72					1.38				2.07	

The data are the Wald-type F-statistic (*F* value) and degrees of freedom (df), coefficient of the variable in the model (Coeff) with the standard error (SE) and the significance value (*P*). R^2m = marginal R^2. Random effects standard deviation (SD)
Significant *P*-values ($P<0.05$) of the explanatory variables in bold.

DISCUSSION

Contrary to our hypotheses, CBSC contents in thalli with *Plectocarpon* galls were substantially lower than in those without (Tables 1 and 2). This result is consistent with traditional chemical defense theory: The parasite forms galls on lichens with naturally lower CBSC contents only, i.e. thalli with parasitic galls had too weak CBSC-defense to deter *Plectocarpon*, as reported in other parasitic fungal-plant interactions (e.g. Kalia & Sharma, 1988).

However, parasite galls occurred as frequent in the normal as in the stictic acid-deficient *L. scrobiculata* chemotype. Thus, the stictic acid complex, with well-documented herbivore-deterrent role (Gauslaa, 2008), is not a potent inhibitor for the highly specialized *Plectocarpon.* Thereby, the reported deterring effects of CBSCs on studied lichenicolous fungi (Lawrey, 1989; Torzilli et al., 1999) do not readily apply to the *Plectocarpon*/stictic acid relationship. The lack of (1) significant relationships between CBSC content and number of *P. scrobiculatae* galls, and (2) the lack of CBSC contents size-dependency in thalli with galls may lead to the hypothesis that *P. scrobiculatae* has overcome the CBSC defense of its host. This interpretation is consistent with laboratory experiments (Lawrey, 1993) showing that two other specialized lichenicolous fungi tolerated, overcame and exploited the CBSCs in their lichen hosts. The significantly lower CBSC contents in thalli with galls could be due to the suppression of the host’s defense, consistent with biotrophic fungal-plants interactions (see Panstruga 2003).

Our results highlight the negative correlation between the presence of galls of the lichenicolous fungus *P. scrobiculatae* and the CBSC contents in its host *L. scrobiculata*. Yet, mechanisms underlying this host-parasite interaction and factors influencing the formation of parasite galls are unknown. We do not know whether the lower CBSC contents promote formation of galls or if *Plectocarpon* galls reduce CBSCs. Nevertheless, *P. scrobiculatae* did not discriminate between the normal and the stictic acid-containing chemotype, showing that the stictic acid complex does not form a potent parasite defense. These findings call for new experiments in which an active role of the parasite needs to be tested.

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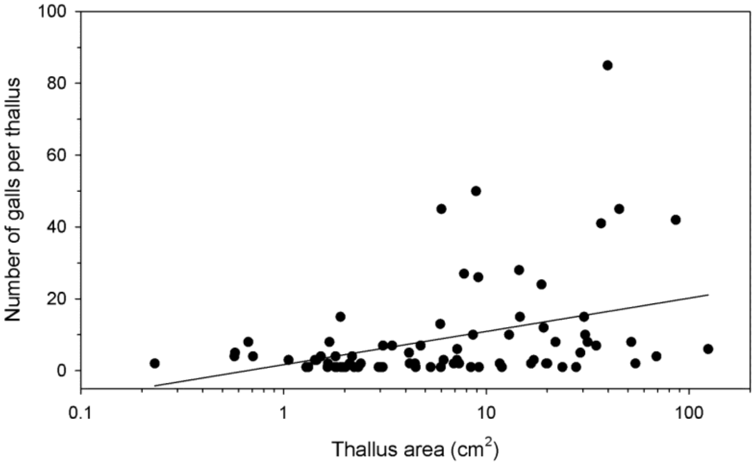
SUPPLEMENTARY MATERIAL

Figure S1. *Lobarina scrobiculata,* with galls of *Plectocarpon scrobiculatae,* growing on an oak trunk (central Spain). *Plectocarpon scrobiculatae* galls are the many round and brown disc resembling lichen apothecia. Photo by Gilfernando Giménez.

Figure S2. Relation between *Lobarina scrobiculata* thallus area ($\log_{10}$ transformed) and number of *Plectocarpon scrobiculatae* galls (N= 78). Adj. r^2= 0.14, *P* <0.001.



METHODS S1.

a) Assessment of thallus size and parasite abundance

We rinsed lichens from debris and hydrated with deionised water before taking photographs of fully hydrated thalli (Nikon D300 camera; Nikon AF-S Micro Nikkor 105 mm lens) placed on top of a white light transilluminator (TW-43 White light, UVP, Upland, CA 91786 USA) and flattened below a piece of glass. Area (A) was recorded by analyzing photographs in ImageJ 1.46f version (Wayne Rasband, National Institutes of Health, USA). After 2 h of air-drying at room temperature, thalli were oven-dried for 24 h at 70 °C and reweighed to obtain dry mass (DM). Mass was measured to the nearest 10 µg, or 0.1 mg, depending on thallus size. Based on A and DM values, specific thallus mass (STM) was calculated as STM = DM/A. Number of *P. scrobiculatae* galls on each thallus was counted.

b) Extraction of secondary compounds

Possible effects of oven drying on secondary compounds (see Culberson et al., 1977) were examined by taking 20 additional thalli with the same size range as the experimental thalli, cutting each of them in two pieces, placing one piece of each pair in the drying oven at 70 °C and keeping the other piece at room temperature. The 24 hrs oven drying had no significant effect (P=0.53; pairwise t-test) on total amounts of CBSCs (24.5 ± 13.0 mg$\cdot$g^{-1}) relative to those stored 24 hrs at room temperature (21.0 ± 10.5 mg$\cdot$g^{-1}).

Thalli were ground using a mixer mill (MN 301, Retsch GmBH). 15 mg of each thallus were put into a vial with 500 µl of 100% acetone for the extraction. After 10 min extraction, they were centrifuged for 3 min at 15000 rpm and acetone was then poured into 10 ml tube. The procedure was repeated 3 times and all extracts were combined. Acetone was then left to evaporate from the tubes using a Concentrator plus / Vacufuge™ plus (Eppendorf, Hamburg, Germany) and the dried material was stored at -18 °C for 5 days until analysis.

c) HPLC analysis

Dried material was dissolved in 500 µl of 100% acetone and the carbon based secondary compounds (CBSCs) were measured by HPLC on a Hewlett-Packard (Palo Alto, CA, USA) 1100 series chromatograph (Agilent Technologies, Waldbronn, Germany) utilizing an ODS Hypersil column (50 × 4.6 mm).The solvents for the mobile phase at 2 mL min^{-1} were 0.25% orthophosphoric acid and 1.5% tetrahydrofuran in Millipore (Millipore, Billerica, MA, USA) water (solution A) and 100% methanol (solution B); the UV detection was at 245 nm. The injection volume for the analysis was 10 µl. The run started with 30% solution B, increased then to 70% after 15 min and to 100% after another 15 min. The condition was held for 5 min and then solution B was reduced to 30% within 1 min. Before the next analysis, a 10 min post run with 30% solution B was performed. The identification of CBSCs was based on retention time, online UV spectra and co-chromatography of commercial standards. Content per unit area of each compound was calculated as Content (g m^{-2}) = (Concentration (mg g^{-1})/1000) x STM (g m^{-2}).

d) Statistical Analysis

We performed Linear Mixed Models (LMMs) to test the influence of the presence of parasite galls on the individual and total CBSCs contents, including “tree” as a random factor because our samples may not be independent (Pinheiro & Bates 2000). If these models were significant, separate LMMs for thalli with and without galls were run to test the influence of thallus size on the CBSCs contents in both categories. Because the correlation between thallus size and number of galls was relatively weak (Spearman correlation = 0.38, $P < 0.001$) both variables were included in the models for specimens with galls. The explanatory variable thallus size was $\log_{10}(x+1)$ transformed in all models to obtain approximate residuals normal distributions. Residuals were checked for normality and homoscedasticity (Pinheiro & Bates 2000). We further calculated a marginal R^2 value for all models, providing an estimate of the variance explained by the fixed effects (Nakagawa &Schielzeth 2013). We used the package nlme (Pinheiro et al., 2008) for mixed effect model analyses. All analyses were done in R version 2.15.2 (R Foundation for Statistical Computing, Vienna, Austria). Unless otherwise stated, mean values ± SD are reported. Statistical significance was set at $P < 0.05$.

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DISCUSIÓN GENERAL

La necesidad de frenar la pérdida de biodiversidad actual requiere información detallada y de calidad sobre las especies amenazadas que permita el desarrollo de estrategias de conservación efectivas (Balmford et al. 2005a, b). En la Península Ibérica se ha detectado la presencia de especies de líquenes amenazadas incluidas en las Listas Rojas de diferentes países Europeos. Sin embargo, debido al desconocimiento sobre el estado de conservación de las poblaciones de estas especies, no se pueden diseñar planes específicos para su conservación. Por este motivo, en esta tesis nos planteamos realizar un estudio corológico, ecológico y funcional de las poblaciones del cianoliquen epífito *Lobarina scrobiculata* abordado desde diferentes escalas.

A escala regional nos centramos en los factores climáticos y ambientales que condicionan la presencia, distribución, tamaño de las poblaciones y estrategia reproductiva a lo largo de un gradiente latitudinal en la Península Ibérica, aunque teniendo también en cuenta los factores ambientales que condicionan estas respuestas a nivel de bosque (escala local) y árbol (microescala). Dado que el conocimiento sobre esta especie es especialmente limitado en la región mediterránea, a escala local nos centramos en el estudio demográfico detallado de poblaciones del centro peninsular. Así, evaluamos los parámetros que nos permitirían conocer la dinámica poblacional de la especie, como por ejemplo la estructura demográfica de las poblaciones, el crecimiento y la reproducción de los individuos y los factores que les influyen en los mismos, la probabilidad de reproducción junto con la asignación reproductora y las relaciones con otras especies. Esta tesis doctoral amplía de manera significativa el escaso conocimiento que tenemos hasta ahora sobre la ecología y funcionamiento de los líquenes, un grupo de organismos muy diverso que cumple un importante papel en el funcionamiento de los ecosistemas (Ellis 2012).

Requerimientos ambientales y respuesta funcional de *L. scrobiculata* a escalas regional y local: el papel de la precipitación, la estructura forestal, la heterogeneidad de hábitats dentro del bosque y las relaciones interespecíficas

Lobarina scrobiculata es una especie de cianoliquen epífito de distribución mayoritariamente oceánica (Degelius 1935; Goward & Spribille 2005; Ellis et al. 2009). Sin embargo, su rango de distribución en Europa se extiende desde regiones oceánicas boreales hasta regiones mediterráneas, donde las condiciones climáticas en principio resultan adversas para los cianolíquenes debido, sobre todo, a la falta de disponibilidad hídrica durante el verano (Burgaz et al. 1994; Goward & Spribille 2005). Por este motivo, el primer objetivo de esta tesis fue identificar los factores climáticos y los relacionados con la estructura forestal que determinan la presencia, el tamaño de las poblaciones y el desempeño de este cianoliquen en las regiones Mediterránea y Atlántica de la Península Ibérica.

Respecto a los factores climáticos, nuestros resultados indican que la precipitación anual es el factor climático más determinante para la abundancia y el funcionamiento de la especie (Capítulos I y II). *Lobarina scrobiculata* ocupa un amplio rango de localidades con diferentes regímenes de precipitación anual, desde 621 mm en el centro peninsular hasta 2050 mm en bosques mediterráneos con influencia oceánica situados en Portugal (Capítulo I). Sin embargo, el tamaño de las poblaciones (i.e., el número de individuos) es mayor en localidades con precipitación anual más elevada (Capítulo I). Se trata de un resultado esperable ya que la presencia y riqueza de cianolíquenes dependen estrechamente de la precipitación (Jovan & McCune 2004; Goward & Spribille 2005; Marini et al. 2011), ya que sus fotobiontes necesitan agua en estado líquido para activar la fotosíntesis (Lange et al. 1986, 1993). No obstante, es llamativo el hecho de que esta especie sea capaz de persistir en localidades con bajas precipitaciones, soportando la intensa sequía estival del clima mediterráneo. Fisiológicamente, *L. scrobiculata* podría tener una estrategia de tolerancia al estrés hídrico y lumínico. Según Gauslaa et al. (2012) este cianoliquen presenta una resistencia a la luz intensa en estado deshidratado mucho mayor que otros cloro- y cefalolíquenes, probablemente porque sus ciclos de hidratación-desecación son muy largos, pasando gran parte del tiempo en estado deshidratado, al contrario que los líquenes con alga verde como fotobionte. Además, *L. scrobiculata* contiene ácido úsnico en su córtex, un metabolito secundario fotoprotector cuya producción es inducida al aumentar los niveles de radiación (McEvoy et al. 2006; Solhaug & Gauslaa 2012). Asimismo, *L. scrobiculata* es un cianoliquen que posee una alta capacidad de retención de agua (Gauslaa & Coxson 2011; Merinero et al. 2014), lo que seguramente le permite aprovechar de forma más eficaz los escasos recursos hídricos en forma de agua líquida (lluvia y rocío). No obstante, a pesar de este comportamiento fisiológico de tolerancia al estrés, es probable que otros factores ambientales como la estructura forestal favorezcan la persistencia de *L. scrobiculata* en ambientes mediterráneos. La estructura del bosque es un factor relevante porque puede compensar las condiciones macroclimáticas subóptimas o adversas generando un microclima apropiado para esta especie (Ellis et al. 2009, 2013).

Numerosos trabajos sugieren que una estructura forestal típica de bosques antiguos y/o poco manejados (i.e., árboles de gran diámetro, continuidad de un dosel arbóreo denso y la diversidad de especies arbóreas y microhábitats) favorecen la presencia de *Lobaria pulmonaria* y otras especies de cianolíquenes en condiciones climáticas subóptimas (Nascimbene et al. 2007; Belinchón et al. 2009; Ellis et al. 2009, 2013; Aragón et al. 2010; Brunialti et al. 2015). Sin embargo, nuestros resultados indican que la estructura forestal que podría estar compensando los efectos adversos del clima mediterráneo para *L. scrobiculata* es una estructura típica de bosques jóvenes y/o manejados, donde numerosos robles finos forman un dosel que genera las condiciones microclimáticas que favorecen la abundancia de esta especie (Capítulo I). Esta estructura forestal puede estar favoreciendo la abundancia de *L. scrobiculata*, probablemente por dos motivos: (1) una alta densidad de árboles puede suponer mayor cantidad de hábitat disponible (Schei et al. 2013) y, (2) durante los periodos lluviosos, los troncos finos de los árboles jóvenes podrían humedecerse más rápidamente que los troncos de árboles gruesos ya que el dosel de los bosques jóvenes es más abierto, permitiendo una mayor incidencia de la lluvia sobre la corteza de los árboles pequeños, en comparación con la menor cantidad de agua líquida que pueden recibir y drenar los árboles más gruesos. Consecuentemente, nuestros resultados demuestran que *L. scrobiculata* no es una especie necesariamente ligada a bosques antiguos ni a etapas climácicas de las comunidades epífitas. En consonancia con este resultado, algunos trabajos experimentales desarrollados en bosques boreales de coníferas sugieren que *L. scrobiculata* puede crecer y establecerse con el mismo éxito en plantaciones (donde no aparece naturalmente) que en los bosques antiguos donde es frecuente y abundante, estableciéndose mejor en árboles pequeños (Hilmo & Såstad 2001; Hilmo 2002; Hilmo et al. 2011a, b). Por tanto, aunque *L. scrobiculata* sí puede depender exclusivamente de bosques antiguos muy húmedos y poco manejados en algunas regiones, (Barkman 1958; James et al. 1977; Rose 1988), hemos de ser cautelosos a la hora de extrapolar estos patrones ecológicos en diferentes regiones climáticas y tipos de bosque.

Por otra parte, nuestros resultados demuestran que la precipitación influye significativamente en la estrategia reproductiva de *L. scrobiculata* (Capítulo II). El inicio de la reproducción asexual en *L. scrobiculata* depende del tamaño del individuo (Larsson & Gauslaa 2011; Hilmo et al. 2013; Capítulo II) y, precisamente, en las poblaciones con menor precipitación los individuos comienzan a reproducirse a tamaños más pequeños pero de manera más eficiente. En otras palabras, *L. scrobiculata* inicia la reproducción a tamaños mayores donde llueve más, pero la asignación reproductora (i.e., inversión de recursos a la reproducción) es menor que en las poblaciones más secas. Probablemente, en las localidades más secas, los individuos crecen más lentamente, comenzando a producir soredios a menor tamaño y, al ser más antiguos que individuos de igual tamaño en localidades lluviosas, su asignación reproductora es mayor por una cuestión de edad. No obstante, otra posible explicación a este marcado gradiente en la asignación reproductora podría estar relacionada con una respuesta plástica o adaptativa de la especie, ya que los resultados sugieren que *L. scrobiculata* aumenta la presión de propágulos en ambientes más estresantes. El establecimiento de los soredios de *L. scrobiculata* requiere condiciones microambientales muy específicas (Hilmo & Såstad 2001; Hilmo & Ott 2002; Hilmo et al 2011b). Por este motivo, la intensificación en la producción de propágulos asexuales en condiciones más adversas para el establecimiento y/o crecimiento podría constituir una respuesta al estrés hídrico basada en aumentar la probabilidad de colonización y establecimiento de *L. scrobiculata* a una escala local. De hecho, en las poblaciones mediterráneas el establecimiento de los individuos es espacialmente más limitado que en las localidades atlánticas, ya que los individuos se concentran en las orientaciones norte del tronco de los árboles y cerca del suelo, situaciones donde la humedad es mayor (Capítulos I, IV).

A pesar de que la teoría (Roff 1992; Stearns 1992) y algunas evidencias en plantas con reproducción sexual (Wesselingh et al. 1993, 1997; Weiner et al. 2009) sugieren un mayor tamaño para el inicio de la reproducción y una mayor asignación reproductora en ambientes favorables, otros estudios indican que las plantas adaptadas o aclimatadas a ambientes estresantes (i.e. constante déficit hídrico o de nutrientes) pueden comenzar a reproducirse a tamaños más pequeños para asignar los escasos recursos a reproducirse en lugar de crecer, con el fin de generar descendencia y asegurarse la permanencia de la especie en el tiempo (Chiarello & Gulmon 1991; Kozlowski 1992; Santos-del-Blanco et al. 2013). Para comprender a qué tipo de compromisos responde *L. scrobiculata* a lo largo de este gradiente climático evaluamos su respuesta funcional (e.g. tasas de crecimiento, supervivencia, asignación reproductora y las relaciones entre éstas) a factores ambientales como la heterogeneidad de hábitats y microhábitat a nivel de población (escala local).

Gracias a estos trabajos a nivel de población, pudimos comprobar que el patrón en la asignación de recursos a la reproducción asexual no solo responde a un gradiente climático, sino que también varía a escala local como respuesta a la heterogeneidad de hábitats dentro del bosque (Capítulo IV). Debido al bajo número de poblaciones estudiadas no pudimos establecer una relación estadística entre el crecimiento, el tamaño umbral de reproducción y la asignación reproductora. Sin embargo, las relaciones entre las respuestas de *L. scrobiculata* entre rocas y árboles se mantuvieron constantes entre sitios: *L. scrobiculata* crece mucho más rápido en las rocas y comienza a reproducirse a menor tamaño en los árboles, donde la asignación reproductora es mayor. Parte de estos resultados están de acuerdo con la teoría y evidencias en plantas vasculares que establecen que las plantas que crecen más rápido comienzan a reproducirse a mayor tamaño para asegurarse un mayor éxito reproductivo (Stearns & Koella 1986; Stearns 1992; Wesselingh et al. 1997; Weiner et al. 2009; Santos-del-Blanco 2013).

Sin embargo, en contra de lo esperado, pero en concordancia con la hipótesis a escala regional, la asignación reproductora es mayor donde crecen más despacio. Dadas las diferencias de crecimiento, es razonable pensar que los talos de similar tamaño en árboles y rocas difieran en su edad y, por tanto, los talos con mayor asignación reproductora en árboles puedan llevar mucho más tiempo originando soredios que talos de mayor tamaño, pero más jóvenes, en rocas. Consecuentemente, nuestros resultados indican que las rocas constituyen un ambiente favorable para el crecimiento, mientras que la reproducción es más eficaz en los árboles. Por tanto, en un intento de caracterizar la “adecuación” de cada hábitat, no es posible concluir que existen diferencias en la adversidad entre hábitats, ya que ambos favorecen diferentes funciones de la especie. Para ahondar en esta caracterización, sería conveniente evaluar las tasas de reclutamiento y supervivencia a largo plazo, ya que cada hábitat puede ser más fundamental para *L. scrobiculata* dependiendo de la fase del ciclo vital o respuesta funcional que se examine. Además, esto permitiría evaluar si existe un compromiso entre el tamaño de inicio de la reproducción y la mortalidad en cada hábitat.

Respecto a los factores que influyen en el crecimiento de *L. scrobiculata* en ambientes mediterráneos, nuestros resultados a escala de microhabitat (microescala) indican de nuevo el carácter higrófilo de este cianoliquen descrito con anterioridad a escala regional. *Lobarina scrobiculata* crece mucho más rápido en la base de los árboles y en orientaciones norte, situaciones donde la humedad es mayor ya que ésta aumenta con la cercanía al suelo (Geiger 1950). En concordancia, *L. scrobiculata* es siempre más abundante en estas situaciones (Capítulos I y IV). A pesar de que los principales factores que condicionan el crecimiento de los líquenes son factores abióticos como la luz y la disponibilidad de agua, otras características intrínsecas de la especie y de los individuos también influyen en el crecimiento. De este modo, nuestros resultados indican que el tamaño del individuo es un factor relevante, ya que el crecimiento depende del tamaño: los individuos pequeños crecen proporcionalmente más que los grandes, tal y como ya han demostrado otros estudios con ésta y otras especies (Gauslaa et al. 2009; Larsson & Gauslaa 2011).

Además, *L. scrobiculata* presenta un compromiso entre la reproducción asexual y el crecimiento (Capítulos III y IV), ya que los individuos con mayor asignación reproductora crecen más lentamente. Estos resultados están en consonancia con los demostrados por Gauslaa (2006) para *L. pulmonaria*, sugiriendo dos posibles mecanismos subyacentes a este compromiso: (1) la formación de soredios marginales implica la inactivación del crecimiento apical responsable de la expansión del área del liquen (Giordani & Brunialti 2002), y (2) los soredios y otras estructuras de reproducción son menos eficaces fotosintéticamente (Tretiach & Campanelli 1992; Del-Prado et al. 2001), por lo que la fijación de carbono del liquen podría disminuir a medida que transforma su superficie somática en superficie reproductora. No obstante, e independiente del mecanismo responsable de este compromiso, el hecho de que los individuos comiencen a reproducirse a tamaños pequeños en ciertos ambientes puede implicar que el tamaño final de los individuos adultos sea pequeño, lo que potencialmente supone una desventaja en un contexto de competencia por el espacio con otros epífitos. Estos resultados ponen de manifiesto la importancia que tienen los factores ambientales a diferentes escalas en la reproducción y en el funcionamiento de *L. scrobiculata* y, potencialmente, en la dinámica de sus poblaciones.

Otros factores que podrían estar influyendo en el crecimiento y la dinámica poblacional de *L. scrobiculata* son aquellos relacionados con las interacciones bióticas. Por ejemplo, Asplund et al. (2010a) sugieren que en Noruega la distribución de *L. scrobiculata* se encuentra condicionada por la distribución de gasterópodos, ya que se trata de una especie muy palatable. En nuestro área de estudio, más seca y principalmente sobre suelos ácidos, no consideramos que este tipo de interacciones tengan una fuerte influencia en la distribución y el desempeño de *L. scrobiculata*. Sin embargo, en varias poblaciones detectamos la presencia de *Plectocarpon scrobiculatae*, un hongo liquenícola que exclusivamente crece sobre *L. scrobiculata*. Las relaciones de parasitismo pueden influir significativamente en la dinámica poblacional de los hospedadores y, consecuentemente, en el funcionamiento de los ecosistemas (Preston & Johnson 2010). Sin embargo, a pesar de su relevancia, las consecuencias del parasitismo en líquenes son totalmente desconocidas. Por este motivo, nos planteamos intentar desvelar algunos factores que influyen en la relación *Plectocarpon–Lobarina*. Nuestros resultados demuestran que las características del metabolismo secundario de *L. scrobiculata* se relacionan con la presencia de *Plectocarpon scrobiculatae*, confirmando que los rasgos químicos del hospedador son relevantes en estas interacciones (Lawrey 1989; Lawrey & Diederich 2003). Los especímenes parasitados contienen la mitad de metabolitos secundarios que los líquenes visiblemente sin parásito (Capítulo V). Estos metabolitos secundarios suponen una defensa para el liquen ante el exceso de luz (ácido úsnico) y frente a herbívoros (complejo de ácidos estícticos y meta-escrobiculina) (Asplund et al. 2010 a, b; Solhaug & Gauslaa 2012). Sin embargo, dado que nuestro estudio es correlativo, no podemos discernir si la presencia del parásito es causa o efecto de la baja concentración de metabolitos secundarios. En principio, es lógico pensar que el parásito coloniza individuos con niveles de defensas bajos pero, algunos estudios en plantas han demostrado que los parásitos fúngicos muy específicos por su hospedador pueden reducir la producción de metabolitos de defensa de sus hospedadores (Mendgen & Hahn 2002; Panstruga 2003).

Implicaciones de los resultados obtenidos en la conservación de *Lobarina scrobiculata* en la Península Ibérica

Gracias a los resultados obtenidos hemos podido comprender algunos aspectos relacionados con el nicho ecológico de *L. scrobiculata* en la Península Ibérica, incluyendo su estrategia reproductiva y las relaciones con factores ambientales y bióticos a diferentes escalas. Además, los resultados de esta tesis nos pueden permitir realizar una evaluación preliminar del estado de conservación de *L. scrobiculata* en una región ambientalmente muy heterogénea. A pesar de la amplia distribución de *L. scrobiculata* en el hemisferio norte, su distribución en nuestro área de estudio se restringe a pocas localidades (Burgaz & Martínez 1999). Además, la amplitud de sus requerimientos ambientales hacen que predecir su presencia sea más complejo que la de otras especies cercanas con nichos ecológicos más restringidos (por ejemplo *Lobaria pulmonaria*, *L. amplissima* o *L. virens*). A pesar de que *L. scrobiculata* puede ser localmente abundante en ciertas localidades con elevada precipitación, en general, el tamaño de sus poblaciones es pequeño, y la estructura de etapas del ciclo vida varían considerablemente (Capítulo II, Tabla 2). En algunas poblaciones el número de individuos reproductores es muy bajo, mientras que en otras la falta de reclutamiento podría suponer el cuello de botella para la viabilidad de la población (Capítulo II, Tabla 2).

Lobarina scrobiculata se reproduce asexualmente mediante soredios en todas las poblaciones. En principio, este tipo de reproducción es ventajoso para colonizar el hábitat a escala local porque ambos simbiontes se dispersan juntos, esquivando la necesidad de restablecer la simbiosis que exige la reproducción sexual mediante pequeñas esporas fúngicas (Bowler & Rundel 1975; Scheidegger & Werth 2009). No obstante, los propágulos asexuales simbiontes, al ser más pesados que las esporas fúngicas, en principio limitan la dispersión a larga distancia de la especie (escala regional) (Hedenås et al. 2003; Werth et al.

2006; Lättman et al. 2009). Además, la reproducción mediante propágulos asexuales supone una reducción de la diversidad genética de la población que limita el potencial adaptativo de la especie a cambios ambientales (Scheidegger & Werth 2099). En principio *L. scrobiculata* puede reproducirse sexualmente mediante la producción de apotecios, pero la frecuencia de los mismos es muy escasa. *Lobarina scrobiculata* podría ser una especie heterotálica, por lo que, la formación de apotecios dependería de la fusión de dos talos con signo sexual diferente ("*mating type*") y compatibles, tal y como Singh et al. (2012) demostraron en *Lobaria pulmonaria*. Por lo tanto, es esperable que las poblaciones de *L. scrobiculata* en nuestro área de estudio presenten una diversidad genética baja y una alta estructuración de las poblaciones porque teóricamente el flujo génico entre ellas es muy bajo. No obstante, según Bowler & Rundel (1975) los líquenes pueden reproducirse tanto sexual como asexualmente, pero normalmente se acaba seleccionando uno de los tipos de reproducción porque les confiere más ventajas en el ambiente en el que se encuentran.

Un aspecto clave para la conservación de especies amenazadas es identificar los principales factores de amenaza para sus poblaciones, con el fin de mitigarlos, reducirlos o eliminarlos (Iriondo et al. 2009). Según nuestros resultados, las amenazas más directas sobre *L. scrobiculata* están relacionadas con el deterioro de la calidad del hábitat de los bosques. Una aproximación basada en la conservación del hábitat suele ser efectiva para la conservación de especies de líquenes, pues normalmente no hay recursos suficientes para desarrollar planes de conservación dirigidos a especies de amplia distribución (Hallingbäck 1997; Scheidegger & Werth 2009). Por lo tanto, proponemos la necesidad de preservar la estructura forestal de los bosques en los que habita este cianoliquen, así como de los fragmentos de los bosques y su conectividad que puedan permitir la posible expansión de *L. scrobiculata*, enfatizando en la importancia que tienen los robledales jóvenes para esta especie. Además, es urgente mantener la variabilidad de microhábitats mediante el mantenimiento de de árboles de diferentes tamaños, sin menospreciar el valor que tienen los árboles jóvenes (pequeños) en la persistencia de esta especie en bosques mediterráneos. Asimismo, se debe mantener la heterogeneidad de hábitats en el interior del bosque, especialmente la preservación de rocas musgosas. En nuestro caso, concretamente, la población de El Escorial merece especial atención, ya que se trata de un área no protegida que recibe un ingente número de visitantes y, donde la frecuente extracción de la cobertura musgosa de las rocas para facilitar la escalada o para usarla como decoración navideña, debería impedirse.

¿Qué otros aspectos necesitamos conocer? Líneas futuras de investigación

A pesar de la valiosa información que aporta esta tesis para evaluar el estado de conservación de *L. scrobiculata*, es necesario conocer otros aspectos de la especie que nos permitan priorizar la conservación de determinadas poblaciones y elaborar planes de manejo de la especie adecuadamente fundamentados.

Por ejemplo, la pérdida de diversidad genética podría contribuir a aumentar el riesgo de extinción de las poblaciones, ya que los análisis de viabilidad podrían no ser adecuados si los factores genéticos son ignorados (Frankham 2003). Por lo tanto, creemos que es imprescindible evaluar la diversidad y estructura genética de las poblaciones de *L. scrobiculata*, y su relación con factores ambientales, con el fin de conocer si existe flujo génico entre ellas y valorar el potencial adaptativo de dichas poblaciones a perturbaciones en el ambiente a escalas local y regional (Werth et al. 2006; Lättman et al. 2009; Hilmo et al. 2012; Otálora et al. 2013, 2015). Además, otra posible causa del declive poblacional de *L. scrobiculata* podría estar relacionada con la limitación en la disponibilidad del fotobionte en el medio o una alta especificidad por el fotobionte (Yahr et al. 2004; Myllis et al. 2007; Otálora et al. 2010). Consecuentemente, la diversidad y estructura genética del fotobionte *Nostoc* y su relación con los factores ambientales también deberían ser evaluadas.

A escala regional, los efectos del cambio climático en las poblaciones mediterráneas deberían evaluarse, por ejemplo, mediante experimentos que simulen las condiciones de reducción de las precipitaciones y aumento de las temperaturas predichas por el IPCC (e.g. Pirintsos et al. 2011; Delgado-Baquerizo et al. 2014). Los resultados de dichos experimentos nos permitirían comprender la respuesta plástica de esta especie que, en principio, tolera el estrés hídrico y lumínico, y así poder predecir posibles cambios en su distribución y el desempeño de los individuos.

Otros trabajos futuros deberían dirigirse a realizar el seguimiento de individuos a largo plazo para evaluar las tasas de crecimiento, supervivencia y reclutamiento con el fin de estudiar la dinámica poblacional de la especie y predecir la tendencia demográfica de sus poblaciones mediante análisis de la viabilidad de poblaciones (PVA) (e.g., Shriver et al. 2012). En esta línea, es necesario realizar estudios que ahonden en los efectos que tiene el parásito *P. scrobiculatae* en la eficacia biológica de su hospedador (p. ej. en la tasas de crecimiento), con el fin de incluir estos efectos en la dinámica poblacional de *L. scrobiculata*.

Finalmente, los resultados de los experimentos de trasplantes no sólo nos ayudan a comprender aspectos del funcionamiento de la especie (Capítulo III), sino que además pueden suponer una exitosa herramienta para la conservación de líquenes epífitos. Dado el éxito de la técnica que empleamos, este tipo de trasplantes podrían utilizarse como medida de refuerzo o reintroducción de individuos en poblaciones donde la viabilidad de la población de *L. scrobiculata* pueda verse comprometida por la escasez de individuos reproductores o por dificultades en el establecimiento de nuevos individuos. En esta línea, Lidén et al. (2004) demostraron que los trasplantes de fragmentos de líquenes amenazados en ambientes boreales son exitosos porque es una técnica económicamente eficiente y los resultados son altamente satisfactorios. Además, varios estudios ratifican la supervivencia y crecimiento de trasplantes de fragmentos de *Lobaria amplissima* y *L. pulmonaria* durante más de 14 años (Gilbert 2002; Gustafsson et al. 2013).

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CONCLUSIONS

Integrating the results from the five chapters composing this PhD thesis we can extract the main following conclusions:

1 The epiphytic cyanolichen *Lobarina scrobiculata* is an higrophylous species unfrequent in the Iberian Peninsula, but locally abudnant in oak forests receiving high precipitation levles. Despite the widely accepted assumption of its old-forest dependency and its clasiffication as a late-successional species in epiphytic communities, in the Iberian Peninsula this cyanolichen is more frequent and abundant in forests with high density of small trees (indicators of young and/or managed forests.

2 The onset of asexual reproduction of *L. scrobiculata* is size-dependent. At the regional scale, *L. scrobiculata* starts to reproduce at larger sizes in humid locations, where the growth rates are presumably higher. The results at the local scale confirm that individuals with faster growth rates start to reproduce at larger sizes than individuals with slower growth. This result suggest that the spcies life cycle may vary at the local and regional scales, although it is a requirement to test wheter an earlier onset of reproduction correlates with higher mortality rates.

3 For the first time, the asexual reproductive pattern of a photosynthetic organism along a wide environmental gradient has been assessed. The response to this gradient were similar than the patterns reported in sexually reproducing plants. Thereby, these results extend the theory formerly developed for plants, and greatly improve the knowledge on life histories diversity.

4 The asexual reproductive allocation pattern of *L. scrobiculata* is allometric: individuals disproportionally invest more resources to reproduction than to growth. At the regional scale, this pattern depends upon precipitation, because at a similar size, individuals from drier populations signficantly increase their reproductive allocation. At the local scale, our results confirm that slow-growing individuals had higher reproductive allocation, suggesting that the individuals's age might be the underlying mechanism of the reproductive allocation pattern found at both scales.

5 The relative growth rates of *L. scrobiculata* and *L. pulmonaria* in Mediterranean forests are similar and even higher than those documented in boreal forests in more humid climates, so the drier Mediterranean climatic conditions do not necessarily reduce the growth rates of established individuals.

6 The relative growth rates of *L. scrobiculata* strongly vary at the local scale (forest) and the microscale (tree). This cyanolichen grow much faster on the tree basis and at northern orientation, where water availability is higher. Moreover, individuals become larger when they gro won bryophytes. Juvenile individuals grow faster than reproductive individuals, indicating that there is a trade-off between growth and reproduction.

7 Habitat heterogeneity at the local scale (forest) means a crucial factor determining the ecology and demography of *L. scrobiculata*. On mossy rocks, *L. scrobiculata* grow faster, start reproduction at larger sizes and show less reproductive allocation than their counterparts on tres. Nevertheless, long-term studies evaluation the recruitment and survival rates are needed in order to identify the most sensitive life-cycle stages of this species.

8 Individuals of *L. scrobiculata* infected by *Plectocarpon scrobiculatae* contain the half of secondary metabolites than individuals without infection signs. These results suggest two possible explanations: (1) the parasite could colonize individuals with naturally low secondary metabolites contents that act as a defense; (2) the parasite reduces the lichen's secondary metabolites content. Further experimental studies deeping in the chemical bases of this relationships and the effects on the host fitness are needed.

9 From a conservacionist perspective, it is necessary to maintain the forest structure where *L. scrobiculata* occurs and the neighbour forest fragments that the species may potentially colonize, as well as preserving the habitat heterogeneity within forests. It is urgent to favour the microhabitat variability by maintaining diferent-sized oak trees, without disregarding the important role that small (young) trees play on the persistence of *L. scrobiculata* in Mediterranean forests.

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