BIOLOGÍA Y CONSERVACIÓN DE LAS ESPECIES DE *Sonchus* SECT. *Pustulati* (ASTERÁCEAS): ENDEMISMOS RUPÍCOLAS DEL COMPLEJO BÉTICO-RIFEÑO (MEDITERRÁNEO OCCIDENTAL)

BIOLOGY AND CONSERVATION OF *Sonchus* SECT. *Pustulati* (ASTERACEAE): CLIFF-DWELLING SPECIES ENDEMIC TO THE BAETIC-RIFAN COMPLEX (WESTERN MEDITERRANEAN)

Tesis Doctoral

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Biología y conservación de las especies de *Sonchus* sect. *Pustulati* (Asteráceas): endemismos rupícolas del complejo Bético-Rifeño (Mediterráneo occidental)

Memoria que José Luis Silva Hernández de Santaolalla, licenciado en Biología, presenta para optar al grado de Doctor por la Universidad de Sevilla a 20 de junio de 2014.

El Dr. **José Antonio Mejías Gimeno**, Profesor Titular del área de Botánica del Departamento de Biología Vegetal y Ecología de la Universidad de Sevilla, CERTIFICA:

Que esta memoria ha sido realizada bajo su dirección en el Departamento de Biología Vegetal y Ecología por José Luis Silva Hernández de Santaolalla. Ante lo cual, considerando que tiene la suficiente entidad para constituir un trabajo de Tesis Doctoral, autoriza su presentación ante el Consejo de Departamento y la Comisión de Doctorado.

Y para que conste a efectos legales, firma el presente documento en Sevilla a 20 de junio de 2014.

Jose A.

Fdo.: José Antonio Mejías Gimeno

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A mis queridísimos padre y madre…

ÍNDICE

RESUMEN GENERAL

La sección *Pustulati* del género *Sonchus* (Asteraceae) está constituida únicamente por tres especies de hábitat rupícola: *S. pustulatus* Willk., *S. fragilis* Ball y *S. masguindalii* Pau & Font Quer, cuyas poblaciones se encuentran restringidas a áreas muy reducidas no superpuestas dentro de la región Bético-Rifeña (Mediterráneo occidental). Las tres especies aparecen en el rango Rifeño (noroeste de África) y solamente *S. pustulatus* se encuentra en el rango Bético (sureste de la Península Ibérica). Los dos objetivos principales de esta Tesis Doctoral han sido (i) desentrañar las causas que han llevado a estas especies tan desconocidas a tal estenoicidad y disyunción a ambos lados del mar de Alborán, y (ii) diagnosticar el estado de conservación de sus poblaciones y analizar los factores determinantes de su persistencia. Para abordar estos objetivos se ha usado un enfoque multidisciplinar basado en el estudio en detalle de las características demográficas, ecológicas, filogenéticas y reproductivas de estas especies, abarcando prácticamente todas las poblaciones conocidas. A partir de la aplicación de un reloj molecular se ha estimado que el origen y diversificación de estas especies ocurrieron durante la crisis del Messiniense y la subsecuente reinundación progresiva de la cuenca Mediterránea en la edad Zancliense, de manera previa al establecimiento del clima mediterráneo, cuando el ambiente era suave y más húmedo en la región. La reinundación Zancliense, el posterior establecimiento del clima mediterráneo y las glaciaciones del Cuaternario debieron suponer fuertes constricciones en la distribución geográfica de las tres especies, las cuales serían responsables de su rareza actual y la condición de relictas. Las características biológicas detectadas en el grupo sugieren que la disyunción geográfica actual de *S. pustulatus* se debe a un proceso de vicarianza mediada por la reinundación de mar Mediterráneo. No obstante, estas especies

desarrollaron una fuerte resiliencia en determinados roquedos que les han servido como refugio esencialmente microclimático. La orientación norte, el sustrato rocoso, la proximidad al mar y la baja altitud de estos roquedos mantienen niveles suficientes de humedad ambiental, la cual tampona las temperaturas extremas altas y bajas propias del clima mediterráneo y de las glaciaciones del Cuaternario, respectivamente. Se ha averiguado que, mientras la persistencia de los taxones en el rango Rifeño parece depender básicamente de la protección de sus hábitats, el taxón *S. pustulatus* en el rango Bético se encuentra seriamente amenazado y, por lo tanto, requiere mayores esfuerzos para su conservación. En este último rango, la especie parece estar en el límite de su amplitud ecológica, tal y como sugieren, entre otras características, el declive demográfico, el área de ocupación reducida, el pequeño tamaño de sus poblaciones y las bajas diversidades genéticas neutra y de alelos *S* (responsables de la auto-incompatibilidad). Por otro lado, se ha demostrado la presencia de un control genético esporofítico de los sistemas de auto-incompatibilidad, el cual no siempre es totalmente efectivo a nivel individual. También se ha detectado la presencia de un sistema sexual potencialmente mixto en una población de *S. fragilis*, el cual supone un material excepcional para estudios futuros sobre la evolución de los sistemas de reproducción en plantas a partir de una especie con poblaciones demográficamente estables y una esperanza de vida relativamente alta.

GENERAL ABSTRACT

The section *Pustulati* of the genus *Sonchus* (Asteraceae) is only constituted by three species of cliff habitat: *S. pustulatus* Willk., *S. fragilis* Ball and *S. masguindalii* Pau & Font Quer, whose populations are restricted to very reduced non overlapped areas within the Baetic-Rifan region (Western Mediterranean). The three species are in the Rifan range (northwestern Africa) and only *S. pustulatus* is in the Baetic range (southeastern Iberian Peninsula). The two main goals of this PhD thesis have been (i) to unravel the causes that have led to these much unknown species to such stenoicity and disjunction on both sides of the Alboran Sea, and (ii) to diagnose the state of conservation of their populations and to analyze the factors that determine their persistence. To address these objectives, it has been used a multidisciplinary approach based on a detailed study of the demographical, ecological, phylogenetic and reproductive characteristics of these species, covering virtually all known populations. From the application of a molecular dating it has been estimated that the origin and diversification of these species happened during the Messinian Salinity Crisis and the subsequent Zanclean reflooding of the Mediterranean basin previously to the establishment of the Mediterranean climate, when the environment was mild and wetter in the region. The Zanclean reflooding, the posterior establishment of the Mediterranean climate and the Quaternary glaciations should suppose strong constrictions of the geographical distribution of the three species, which would be responsible for their current rarity and the condition of relict. The biological features detected in these species suggest that the current geographical disjunction of *S. pustulatus* is due to a vicariance process mediated by the reflooding of the Mediterranean Sea. Nonetheless, these species developed a strong resilience in certain

cliffs which have served them as essentially microclimatic refuge. The north orientation, rocky substratum, proximity to the sea and low altitude of these cliffs maintain enough levels of air humidity, which buffer the extreme high and low temperatures characteristic of the Mediterranean climate and the Quaternary glaciations, respectively. It has been found out that, while the persistence of the taxa in the Rifan range it seems to basically depend on the conservation of their habitats, *S. pustulatus* in the Baetic range is seriously threatened, and it requires therefore greater conservation efforts. In the latter range, the species seems to be on the limit of its ecological amplitude as it is suggested, among other features, by the demographical decline, the reduced area of occupancy, the small size of its populations and the low neutral and *S* allele (responsible for the self-incompatibility) genetic diversities. On the other hand, it has been demonstrated the presence of a sporophytic genetic control of the self-incompatibility systems, which is not always totally effective at individual level. It has been also highlighted the presence of a potentially mixed mating system in a population of *S. fragilis*, that supposes a outstanding material for future studies about the evolution of plant reproductive systems in a species with demographically stable populations and a relatively high lifespan.

Capítulo 1:

INTRODUCCIÓN GENERAL

GENERAL INTRODUCTION

En la presente memoria se estudian las características demográficas, ecológicas, filogenéticas y reproductivas de las poblaciones del género *Sonchus* L. subgénero *Sonchus* Boulos sección *Pustulati* Boulos (Asteraceae). Esta sección está constituida por un pequeño grupo de taxones rupícolas endémicos del Mediterráneo occidental que presentan una distribución muy restringida y a su vez disyunta. Se hace especial hincapié en desentrañar los patrones biogeográficos de estos taxones, particularmente interesantes en el marco histórico de la región Bético-Rifeña, así como en la incidencia que los aspectos estudiados pueden tener sobre la conservación del grupo. Los análisis demográficos, la investigación de los procesos reproductivos y los estudios de diversidad genética en las poblaciones son tres enfoques claves para conocer la abundancia y viabilidad de las poblaciones de las especies raras y para diagnosticar su estado de conservación (Schemske *et al.*, 1994; Bañares, 2002). La combinación de estos cuerpos de conocimiento con una determinación taxonómica precisa de las especies permite asignar grados de amenaza adecuados y programar actuaciones de conservación realmente efectivas sobre listas de taxones ajustadas a la realidad.

1. REGIÓN MEDITERRÁNEA Y COMPLEJO BÉTICO-RIFEÑO

La cuenca Mediterránea es reconocida como un punto caliente de biodiversidad mundial (Médail & Quézel, 1997). De las aproximadamente 22.500 especies nativas que alberga esta región casi el 60% son endémicas (Greuter, 1991), y la mayoría de ellas están confinadas a una región determinada dentro de la cuenca (Médail & Quézel, 1997). Tres factores han sido históricamente determinantes de la diversidad de plantas en la región Mediterránea: una paleogeografía compleja, el establecimiento del clima mediterráneo (3.8–2.8 Ma; Suc, 1984), que impone un efecto de sequía estival en las plantas, y una larga historia de presencia y actividad humana (Médail & Quézel, 1997; Thompson, 2005). Si bien el mar Mediterráneo ha constituido en mayor o menor medida un factor de separación y aislamiento entre diferentes regiones de la cuenca, las variaciones históricas del nivel de este mar parecen haber propiciado niveles de intercambio superiores a las actuales (Thompson, 2005; Ortiz *et al.*, 2007). Uno de los eventos más dramáticos e importantes en este sentido fue la casi desaparición del mar Mediterráneo durante la crisis del Messiniense (5.96–5.33 Ma) y la posterior reinundación Zancliense (5.33–3.6 Ma). Estos episodios pudieron suponer una intermitencia en la presencia de barreras marinas para la expansión de muchas plantas, especialmente para aquellas con baja capacidad de dispersión, lo cual debió facilitar la diversificación de especies mediante la disyunción creada en sus distribuciones. Por otra parte, la evolución climática en la región Mediterránea ha hecho que entre los componentes contemporáneos de su flora actual se encuentren especies que se han originado en dicha región con el clima tal cual lo conocemos hoy, especies que se hicieron mediterráneas tras colonizar la región una vez el clima mediterráneo fue establecido, y especies que persistieron y evolucionaron *in situ* conforme se estableció el clima mediterráneo (Herrera, 1992; Thompson, 2005). Por último, la antiquísima actividad humana en la cuenca Mediterránea ha supuesto un importantísimo impacto en la flora mediante su influencia sobre la ecología de los hábitats y la configuración espacial del paisaje, normalmente aumentando los niveles de fragmentación de los sistemas naturales y alterando así los procesos de diferenciación poblacional (Thompson, 2005).

Uno de los puntos calientes de mayor biodiversidad y nivel de endemismos dentro de la cuenca Mediterránea es el complejo Bético-Rifeño (Médail & Quézel, 1997), localizado en su extremo occidental. Este complejo incluye dos rangos, ibérico (Bético) y norteafricano (Rifeño), separadas por el mar de Alborán y el estrecho de Gibraltar. A pesar de esta separación, las dos áreas muestran un alto porcentaje de especies en común (aproximadamente el 75%; Valdés, 1991). El complejo Bético-Rifeño simboliza un intrincado escenario desde una perspectiva biogeográfica (Valdés, 1999; Molina-Venegas *et al.*, 2013), y sus endemismos se consideran un material fascinante para estudios de filogeografía (Thompson, 2005).

2. PALEOGEOGRAFÍA DE LA REGIÓN BÉTICO-RIFEÑA (MEDITERRÁNEO OCCIDENTAL)

Las Zonas Internas de los orógenos Bético y Rifeño (Figura 1b) se originaron a partir de un bloque cortical o *terrane* común localizado entre la Península Ibérica y el sur de Francia durante el Oligoceno, hace aproximadamente 30 Ma. Durante el Mioceno, este bloque sufrió un proceso de deriva, fragmentación y rotación, dando lugar a las actuales regiones del complejo Bético-Rifeño, Cabilia, Islas Baleares, Córcega, Cerdeña y Calabria (Lonergan & White, 1997; Rosenbaum *et al.*, 2002). Cuando la microplaca Bético-Rifeña alcanzó el suroeste del Mediterráneo (21–18 Ma), ésta sufrió esencialmente una traslación hacia el oeste. Permaneció separada de la Península Ibérica por el corredor Bético y de África por el corredor Rifeño, mientras un cinturón arqueado de pliegues y cabalgamientos que conectaba las ramas orogénicas norte (Béticas) y sur (Rif) constituía su límite occidental. Esta zona frontal occidental del orógeno Bético-Rifeño migró hacia el oeste mientras la microplaca Bético-Rifeña se trasladaba. La corteza continental superior de la zona este se extendió, adelgazó y colapsó, dando lugar al mar de Alborán (Lonnergan & White, 1997; Rosembaun *et al.*, 2002).

Esta configuración tectónica dio lugar a una evolución paleogeográfica compleja. El relieve positivo asociado al cinturón frontal de pliegues y cabalgamientos dio lugar a una franja de tierra que, hacia el Mioceno medio (15 Ma), probablemente unió el norte de África y el sur de la Península Ibérica en una posición en el centro del actual mar de Alborán (Rosembaun *et al.*, 2002: Fig. 15; Capítulo 4: Fig. 4b). A medida que el cinturón frontal migraba y el dominio de Alborán experimentaba un progresivo colapso, dicho puente de tierra migró también hacia el oeste hasta alcanzar la actual posición del arco de Gibraltar (Mioceno tardío, 10 Ma). El cierre de los corredores Bético y Rifeño acompañó este proceso, dando lugar finalmente a la separación de los mares Mediterráneo y Atlántico durante el Messiniense (6 Ma; Capítulo 4: Fig. 4c-d). Esto promovió la desecación casi completa del mar Mediterráneo (crisis de salinidad del Messiniense, 5.96–5.33 Ma; Krijgsman *et al.*, 1999; Fauquette *et al.*, 2006; Capítulo 4: Fig. 4e) y debió facilitar la expansión intercontinental de las especies de plantas hasta la posterior apertura del estrecho de Gibraltar (5.33 Ma) (p. ej., Caujapé-Castells & Jansen, 2003; Cano-Maqueda *et al.*, 2008) y reinundación de la cuenca Mediterránea (edad Zancliense; 5.33–3.60 Ma; Capítulo 4: Fig. 4f). A partir de entonces, la barrera marina hizo probablemente que la expansión intercontinental de las plantas pasase a ser altamente dependiente de la dispersión de semillas a larga distancia (Capítulo 4: Fig. 4g).

3. HÁBITATS RUPÍCOLAS EN LA REGIÓN MEDITERRÁNEA

Una de las mayores consecuencias de la bajada del nivel del mar Mediterráneo durante el período Messiniense fue la fuerte reincisión de los ríos que fluían hacia este mar, lo cual resultó en la formación de cañones profundos por la erosión en toda la región Mediterránea, incluyendo el área del actual mar de Alborán (Loget & Van Den Driessche, 2006). Esto, junto con la intrincada orografía a la que llevó la compleja paleogeografía de la región Bético-Rifeña, favoreció una relativa abundancia de roquedos en la región, aunque muchos de ellos quedaron cubiertos por el mar de Alborán desde el final de la gran reinundación Zancliense del mar Mediterráneo (3.6 Ma).

En general, los acantilados y sistemas rocosos representan uno de los principales hábitats de muchas especies de plantas raras y en peligro de extinción, ya que ofrecen un refugio antropogénico y ambiental (Polunin, 1980; Albert *et al.*, 2001; García, 2003; Lavergne *et al.*, 2004; Thompson, 2005), y además existe un gran número de plantas endémicas en estos hábitats (Davis, 1951; García, 2003; Thompson, 2005; Bragazza, 2009; Pérez-García *et al.*, 2012). Por tanto, los ecosistemas rupícolas contribuyen generalmente más a la biodiversidad de una región de lo que su cobertura superficial pudiese sugerir. Ya que esta circunstancia es muy notable en la región Mediterránea (Bragazza, 2009; Pérez-García *et al.*, 2012), resulta deseable que las asociaciones de plantas vasculares en estos hábitats fuesen estudiadas más extensamente. Dada la condición relicta de muchos taxones rupícolas en la cuenca Mediterránea, dicho análisis podría dar pistas muy valiosas sobre la historia del clima y la flora de la región (Davis, 1951). A pesar de ello, el conocimiento de la biogeografía y situación actual de muchas especies rupícolas en la región Mediterránea es bastante reducido, especialmente en países que han sido históricamente menos accesibles para los investigadores en general, como los norteafricanos Argelia y Marruecos.

4. GÉNERO *SONCHUS*, SUBGÉNERO *SONCHUS*, SECCIÓN *PUSTULATI*

El género *Sonchus* L. (subtribu Hyosseridinae Less.) presenta una amplia distribución mundial, incluyendo muchas especies ruderales o arvenses (p. ej., *S. oleraceus* L., *S tenerrimus* L., *S. asper* L. (Hill), *S. arvensis* L.). Además, el género también cuenta con numerosos endemismos de distribución restringida, entre los cuales se encuentran las especies que conforman la alianza de los *Sonchus* leñosos de las islas macaronésicas (Lee *et al.*, 2005) y los taxones incluidos por Boulos (1973) en la sección *Pustulati*, endémicos del Mediterráneo occidental.

Dentro de *Sonchus* L. subgénero *Sonchus* Boulos sección *Pustulati* Boulos, este último autor reconoció cuatro especies restringidas a la región Bético-Rifeña: *S. pustulatus* Willkomm in Willk. & Lange, Prodr. Fl. Hisp. 2: 242 (1865), *S. fragilis* Ball in J. Bot. 11: 372 (1873), *S. masguindalii* Pau & Font Quer in Font Quer, Iter Marocc. nº 732 (1927) y *S. briquietianus* Gandoger in Bull. Soc. Bot. France 55: 657 (1908). Las tres primeras especies han sido citadas con posterioridad, existiendo diversos pliegos en herbarios institucionales. Por el contrario, la última especie, *S. briquetianus*, solo está representada por el material tipo (Castro, 1988) procedente de las islas Chafarinas que, al menos en el caso del herbario de Ginebra (G8602/1!, G8602/2!), incluye individuos en flor y sin frutos desarrollados. Siendo los caracteres carpológicos básicos en la separación y reconocimiento de la sección, este material no muestra evidencias suficientes para la segregación de un nuevo taxón con categoría de especie. En palabras de Gandoger (1908), estas plantas muestran mucha afinidad con *S. tenerrimus* y *S. pectinatus* («*Nulli descripti vere affinis quamvis prope S. pectinatus et S. tenerrimus*…»). Por lo tanto, se trata posiblemente de representantes de un ecotipo costero de *S. tenerrimus* (Mejías, 1988), como Jahandiez y Maire (1934) ya consideraron. Blanco (1988) exploró recientemente de forma exhaustiva las islas Chafarinas y no encontró ningún material distinto de *S. tenerrimus*, por lo que concluyó que en caso de ser efectivamente una especie estaría extinta. El material aportado por dicho autor (conservado en MA) fue revisado en el presente trabajo sin encontrarse indicios de la existencia de tal taxón.

Morfológicamente, las tres especies reconocidas de la sección *Pustulati* (*S. pustulatus*, *S. fragilis* y *S. masguindalii*) son relativamente semejantes a algunos ecotipos de *S. tenerrimus*, no en vano existen combinaciones nomenclaturales subordinadas a *S. tenerrimus* en los tres casos. A diferencia de *S. tenerrimus*, un taxón no específicamente rupícola con números cromosómicos n = 7, 2n = 14 (Mejías & Andrés, 2004), las especies de la sección *Pustulati*, son de hábitat rupícola y con números cromosómicos n = 9 y 2n = 18 (Mejías & Andrés, 2004; Vogt & Oberprieler, 2008).

En esta Tesis se consideran como únicos componentes de la sección *Pustulati* las especies rupícolas *S. pustulatus* Wilk., *S. fragilis* Ball y *S. masguindalii* Pau & Font Quer. Estas tres especies presentan ciertas características morfológicas que se consideran ancestrales dentro del género *Sonchus* (Boulos, 1973). Además, parecen constituir un grupo monofilético, tal como sugiere la filogenia molecular basada en la secuenciación del marcador ITS de ADN nuclear (Kim *et al.*, 2007). Dentro de esta filogenia, *S.*

pustulatus aparece como clado hermano de *S. fragilis*, y *S. masguindalii* es a su vez hermano del clado *S. pustulatus*–*S. fragilis* (Kim *et al.*, 2007). Por el contrario, análisis filogenéticos basados en el marcador *mat*k de ADN cloroplástico mostraron evidencia de un origen híbrido de *S. pustulatus*, a partir de *S. fragilis* y un donador aún desconocido perteneciente a un linaje tal vez extinto de las secciones hermanas *Sonchus* o *Asperi* (Kim *et al.*, 2007, 2008).

De acuerdo con la bibliografía consultada (Boulos, 1973; Deil & Galán de Mera, 1996; Deil & Hammoumi, 1997; Cueto *et al.*, 2003; Mota *et al.*, 2005) y la revisión de diversos herbarios institucionales (BC, BM, COI, G, HUAL, MA, MPU, SEV), las especies de la sección *Pustulati* están restringidas a cinco áreas disyuntas (A-E) localizadas geográficamente dentro de los dominios geológicos de Alborán y Zona Interna de la región Bético-Rifeña (Figura 1). El área **A** se sitúa en la provincia de Almería (sureste de la Península Ibérica), donde está presente *S. pustulatus*, catalogada como taxón “en peligro crítico de extinción” (CR), con un registro de poco más de 50 individuos censados (Cueto *et al.*, 2003). El área **B** se localiza en el Rif occidental (Marruecos), donde *S. pustulatus* cuenta con numerosas pequeñas poblaciones en valles fluviales encajados de los ríos Oued Laou y Oued Al-Kannar. En el área **C**, en el Rif noroccidental (Marruecos), está representado *S. fragilis*, con poblaciones restringidas a algunos roquedos de los montes Gorghiz y Djebel Dersa en el entorno de la ciudad de Tetuán (Fennane & Ibn Tattou, 1998). El área **D** se localiza en el Rif central (Marruecos), donde *S. masguindalii* se asienta en roquedos marinos o cercanos al mar, a lo largo de los montes Bokkoya (Fennane & Ibn Tattou, 1998). Por último, el área **E**, en el entorno de Ghazaouet (antigua Nemours, Noroeste de Argelia), es la menos explorada y parece limitarse a roquedos costeros al norte del Atlas Telliano occidental. En esta región la

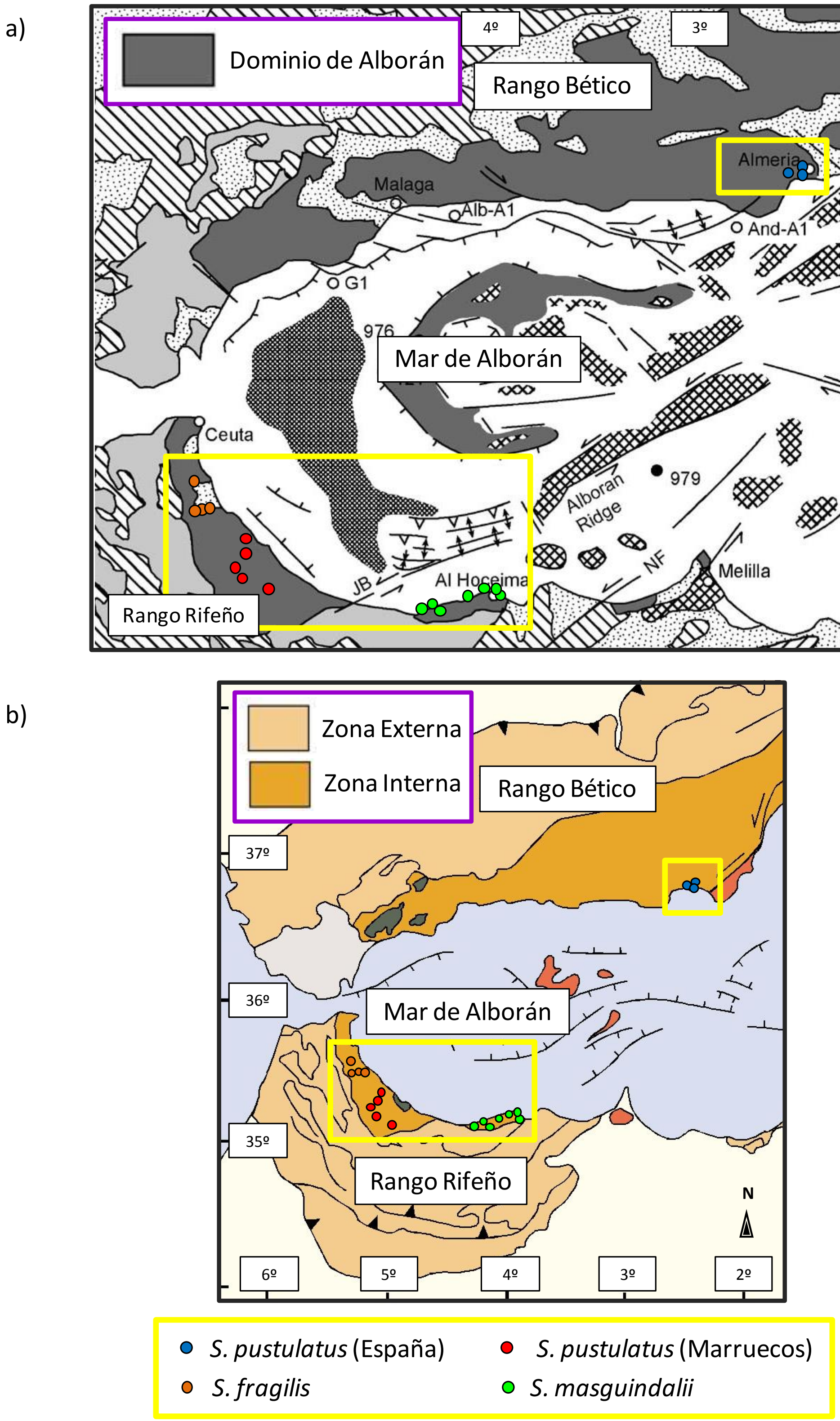

Figura 1 Localización geológica de las poblaciones del grupo *Sonchus* sección *Pustulati* en el extremo occidental de la cuenca Mediterránea, en a) el dominio de Alborán y b) la Zona Interna del complejo Bético-Rifeño. Imágenes tomadas de: a) Loget & Van Den Driessche (2006), y b) Rosenbaum *et al.* (2002).

especie citada es *S. pustulatus*, a partir de pliegos recolectados en el siglo XIX por E. Bourgeau y conservados en los herbarios de los Jardines Botánicos de Ginebra (G) y Coimbra (COI). La dificultad de acceso a la zona por motivos de seguridad no ha permitido explorar la persistencia de esta especie en la región y ha determinado que no dispongamos de ningún material de estudio de sus posibles poblaciones.

5. CARACTERÍSTICAS GENERALES DE LAS ESPECIES DEL GRUPO *SONCHUS* SECCIÓN *PUSTULATI*

Sonchus masguindalii, *S. fragilis* y *S. pustulatus* son caméfitos, perennifolios, de hábitat rupícola y con frecuencia procumbentes (Figura 2). En conjunto, los individuos adultos pueden variar enormemente desde unos cinco centímetros cuadrados a unos dos metros cuadrados en superficie, y alcanzar hasta los 45 cm de altura, presentando con frecuencia ramificación intrincada. *S. pustulatus* y *S. masguindalii* muestran ramas moderadamente largas, que pueden alcanzar hasta 50 cm y 1 m, respectivamente, llegando a estar completamente lignificadas. En *S. fragilis*, las ramas son habitualmente más cortas, generalmente menores de 20 cm, y la lignificación es basal, salvo en ramas muy viejas. Las tres especies presentan hojas pinnatisectas hasta de 12,5 × 4 cm con pinnas ovadas, elípticas, triangulares o redondeadas, a veces arriñonadas en *S. masguindalii*. La suculencia de dichas hojas es particularmente notable en esta última especie.

Aunque parecen ser especies relativamente longevas (Capítulo 2), los individuos suelen alcanzar la madurez sexual durante el primer año de vida. Florecen principalmente en

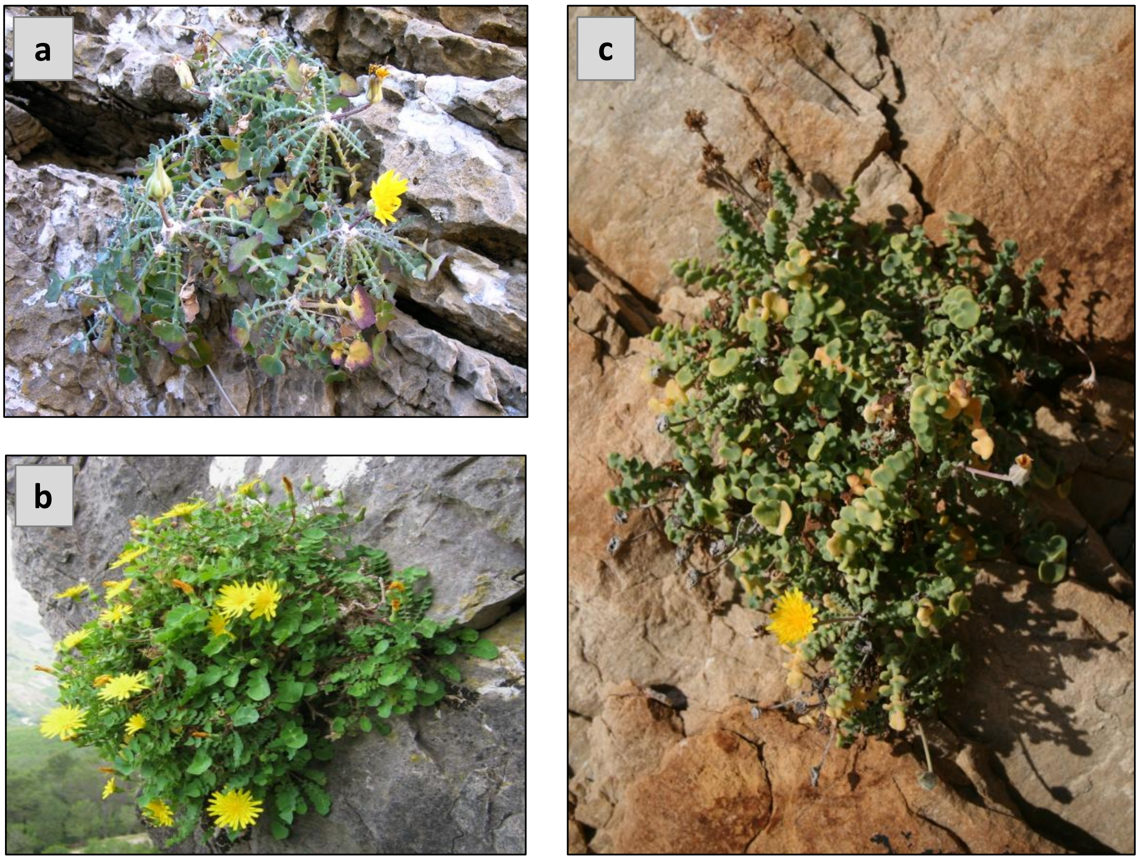

Figura 2 Ejemplares adultos de las tres especies de *Sonchus* sección *Pustulati*: *S. pustulatus* (a), *S. fragilis* (b) y *S. masguindalii* (c).

primavera, aunque se pueden observar algunos individuos en flor en otoño. Los capítulos son terminales, solitarios o reunidos en grupos de hasta cuatro capítulos por tallo florífero. Todas las flores son hermafroditas, protándricas, liguladas y de un color amarillo intenso. Sus tamaños son relativamente grandes dentro del género (Tabla 1), lo que determina diámetros del capítulo elevados durante la antesis. Esto unido a la coloración intensa de las flores y la presencia de anteras relativamente grandes con abundancia de polen permite suponer que se trata de especies básicamente alógamas (Mejías 1992). Los visitantes florales de *Sonchus* sección *Pustulati* son insectos generalistas (J.L. Silva, observaciones personales; Apéndice: Figura 4).

Los frutos son aquenios cuya morfología constituye el carácter taxonómico que mejor permite reconocer la sección. Son liberados básicamente entre finales de primavera y principio de verano y están adaptados primariamente a la dispersión por anemocoria mediante un vilano de pelos marcadamente caducos. En el caso de *S. pustulatus* y *S. fragilis*, estos frutos presentan el mayor tamaño de entre los representantes mediterráneos del género (Silva & Mejías, no publicado) y se encuentran entre los mayores a nivel mundial de *Sonchus* s.l. (ver Boulos, 1973; 1974a; 1974b), siendo por tanto relativamente pesados (Tabla 1). En *S. masguindalii* el tamaño es menor pero presenta valores por encima de la media en el género. Además, los frutos son escasamente comprimidos, por lo que no hay diseño aerodinámico que facilite su dispersión por el viento. Por todo ello, se postula que la capacidad de dispersión a larga distancia por semillas debe ser muy limitada en estas especies. Es posible que, como en otras plantas rupícolas, puedan contar con dispersión secundaria por hormigas (García *et al.*, 2011) pero nuestras observaciones no han permitido por el momento comprobar esta hipótesis.

Los individuos de las especies de *Sonchus* sección *Pustulati* colonizan oquedades en paredones y cortados de rocas generalmente calizas. El desarrollo de los individuos debe depender de la constitución del sustrato y si éste lo permite, se produce el desarrollo de numerosas ramas. La capacidad de enraizamiento de las ramas permite previsiblemente el desarrollo de cierta multiplicación vegetativa en el entorno cercano de cada individuo. Este carácter ha sido aprovechado en la presente Tesis para desarrollar estudios de biología de la reproducción utilizando esquejes georreferenciados. Con la excepción de *S. masguindalii*, estas especies pueden florecer a lo largo de casi todo el año en invernaderos bajo condiciones de iluminación de ciclo largo y temperatura entre 18–22ºC.

Tabla 1 Caracteres morfológico-reproductores de las especies de *Sonchus* sección *Pustulati* Boulos (Silva & Mejías, no publicado). L, longitud; DC máx., diámetro máximo de los capítulos durante la antesis. Decimales indicados con comas.

	S. pustulatus	*S. fragilis*	*S. masguindallii*
FLORES			
No. flores/capítulo	15–100	20–86	63–283
L tubo (mm)	3,8–7,2(–12,4)	4,0–7,4	4,0–7,2
L lígula externa (mm)	9,2–15,2	(8,3–)8,9–13,1	11,8–15,0
L anteras (mm)	3,2–5,5	2,8–4,6	3,2–4,1
DC máx. (mm)	29,4–48,7	30,1–47,5	23,5–52,9
FRUTOS			
Morfología	Elípticas a rómbicas, a veces curvadas	Elípticas, a veces curvadas	Elípticos a obovados
Costillas	1 central gruesa y 2-4 secundarias	1 central gruesa y 2-4 secundarias	1 central y 4 secundarias
Márgenes	Gruesos	Gruesos	Engrosados
Tamaño (mm)	4,0–5,9 × 0,8–1,6	2,9–4,2 × 0,7–1,1	2,6–3,6 × 0,7–0,9
Peso (mg)	0,58–1,08	(0,43–)0,51–1,19	0,35–0,46

6. OBJETIVOS Y ESTRUCTURA DE LA TESIS

Los dos objetivos generales de esta Tesis Doctoral han sido:

1- Desentrañar los patrones biogeográficos y las relaciones filogenéticas y ecológicas entre los taxones que constituyen el género *Sonchus* L. subgénero *Sonchus* Boulos sección *Pustulati* Boulos, con distribución disyunta a ambos lados del mar de Alborán dentro del complejo Bético-Rifeño (sureste de España y norte de Marruecos).

2- Describir el estado de conservación actual de estos taxones tan poco conocidos y con distribución tan restringida y analizar los factores determinantes de su persistencia.

Los dos objetivos generales han sido abordados usando un enfoque multidisciplinar, con cuatro grandes bloques de trabajo correspondientes con los cuatros siguientes capítulos de la Tesis. Casi todas las poblaciones de *Sonchus* sección *Pustulati*, que se encontraron o cuya existencia se confirmó en la exploración descrita en el Capítulo 2, fueron muestreadas en cada uno de los siguientes capítulos (Figura 3).

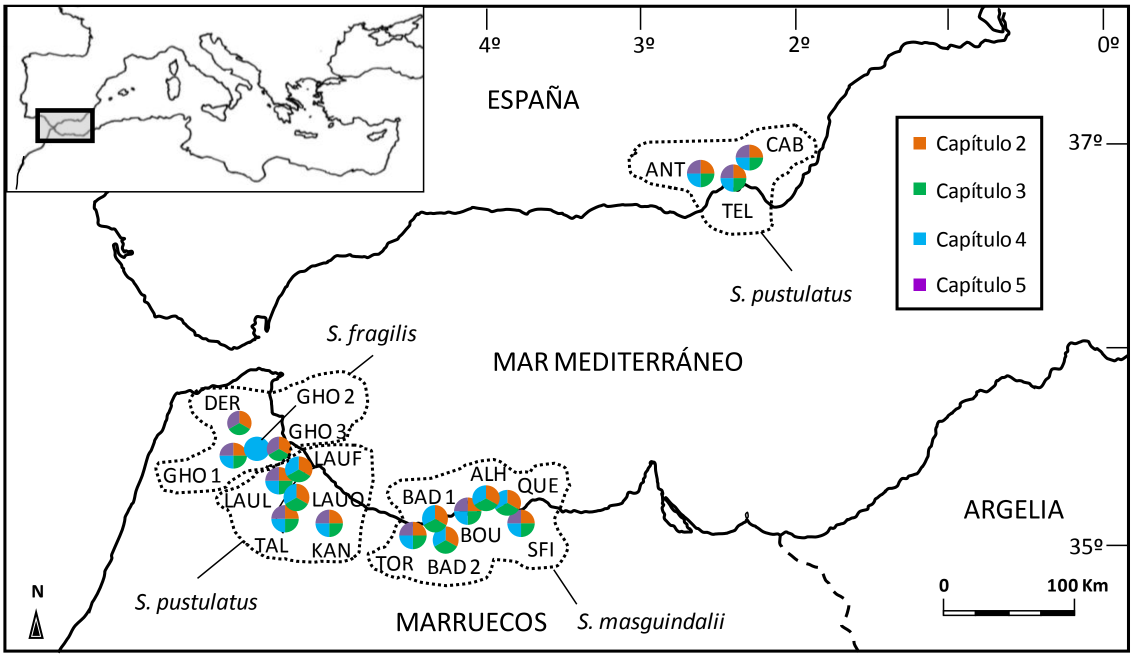

Figura 3 Localización geográfica en el Mediterráneo occidental de las poblaciones de *Sonchus* sección *Pustulati* detectadas y muestreadas en cada uno de los siguientes capítulos de la tesis.

En el Capítulo 2, "Vulnerabilidad demográfica de unas plantas rupícolas endémicas del Mediterráneo occidental (*Sonchus* sección *Pustulati*)", se caracterizaron demográficamente las poblaciones para evaluar la rareza y vulnerabilidad de los taxones. Para ello, se estimaron los tamaños poblacionales, las estructuras demográficas y el éxito reproductivo de prácticamente todas las poblaciones conocidas de la sección. Además se analizaron las tendencias poblacionales y la viabilidad de *S. pustulatus* en el rango español, donde está actualmente catalogada como “en peligro crítico de extinción”. En concreto, en las tres poblaciones españolas detectadas se estimaron las tasas de crecimiento poblacional correspondientes y en una de ellas se llevó a cabo un seguimiento demográfico detallado durante un período de seis años.

En el Capítulo 3, “Caracterización ecológica del hábitat asociado a las especies de *Sonchus* sección *Pustulati*, un pequeño grupo de endemismos rupícolas del Mediterráneo occidental”, se estudiaron los factores ecológicos determinantes de la distribución tan restringida de las especies que conforman este grupo. Además, en base a datos ecológicos, se analizó si la distribución disyunta actual de *S. pustulatus* podría estar asociada con un proceso de vicarianza por reducción de área de distribución, más que con eventos de dispersión a larga distancia. Para ello, se estudiaron los rangos fisiográfico y edáfico, junto con las características del tipo de hábitat y corología de las plantas acompañantes a lo largo de todas las poblaciones conocidas. También se analizaron las diversidades alfa y beta de los conjuntos de plantas en los que se integran las especies de la sección *Pustulati*.

En el Capítulo 4, “Desentrañando los patrones filogenéticos y filogeográficos de un pequeño grupo de especies rupícolas del Mediterráneo occidental (*Sonchus* sección *Pustulati*, Asteraceae)”, se investigó si los eventos climáticos y geológicos más importantes de la región Mediterránea occidental moldearon los patrones filogenéticos y filogeográficos en el conjunto de especies endémicas de *Sonchus* sección *Pustulati*. Para ello se determinaron de manera independiente las relaciones filogenéticas de las poblaciones con tres tipos de marcadores moleculares distintos: polimorfismos en la longitud de fragmentos amplificados (AFLP), espaciadores internos y externos transcritos del ADN ribosomal nuclear (ITS/ETS) y regiones no codificantes de ADN cloroplástico (3’*trn*L–*ndh*J/*psa*I–*acc*D). También se aplicó un reloj molecular bayesiano para datar los tiempos de divergencia de los linajes y se correlacionaron éstos con los eventos climáticos y geológicos más importantes de la región Mediterránea occidental.

En el Capítulo 5, “Incidencia de la auto-incompatibilidad en las especies de *Sonchus* sección *Pustulati* (Asteraceae), un pequeño grupo de endemismos rupícolas del Mediterráneo occidental”, se estudió la incidencia del sistema de autoincompatibilidad esporofítico (SSI) y su relación con la persistencia de las poblaciones. Para ello, se investigó la eficiencia del SSI y la diversidad de alelos *S*, responsables del SSI, en diferentes poblaciones representativas de los taxones del grupo mediante un estudio genético detallado basado en un intensivo programa de polinizaciones manuales. También se comparó la morfología floral y el *fitness* reproductivo entre individuos con distintas capacidades de auto-fertilización en una población de *S. fragilis*. Por último, se analizó la distribución espacial de los alelos *S* y de los posibles individuos con capacidad de auto-fertilización dentro de las poblaciones.

Finalmente, en el Capítulo 6 se discuten todos los resultados de los anteriores capítulos de una manera conjunta e integrando los mismos se procura cubrir los dos objetivos generales de la tesis.

La originalidad de esta Tesis Doctoral radica fundamentalmente en los siguientes aspectos. Por un lado, se ha estudiado la biogeografía de un pequeño grupo de plantas restringidas a unas cuantas áreas pequeñas y no superpuestas dentro de la región Bético-Rifeña, apareciendo en ambas vertientes de la misma, pero lejos del estrecho de Gibraltar, al menos en el rango Bético. La alta especificidad por los hábitats rupícolas, normalmente no alterados por el hombre, la alta resiliencia ecológica, el carácter antiguo (Capítulos 2–5) y la presumiblemente baja capacidad de dispersión de estas plantas han hecho de ellas un material fascinante para probar la efectividad del mar de Alborán como barrera geográfica a la expansión de estas plantas (Capítulos 3 y 4). Por otro lado, la facilidad de multiplicar estas plantas mediante esquejes, el ciclo de vida perenne de las mismas y el análisis de las características demográficas y ecológicas de sus poblaciones (Capítulos 2 y 3) han permitido desarrollar aproximaciones muy valiosas sobre los sistemas sexuales de estas plantas y los patrones espaciales de éstos en sus poblacionales. Entre ellas destacan las estimaciones llevadas a cabo de la incidencia de la auto-incompatibilidad a niveles individual y poblacional, y de la diversidad poblacional de alelos *S*, responsables de la respuesta de auto-incompatibilidad (Capítulo 5).

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APÉNDICE

Figura 4 Diversos insectos polinizadores observados en las especies de *Sonchus* sección *Pustulati*: Dípteros (columna izquierda) y Coleópteros (columna derecha).

Capítulo 2:

VULNERABILIDAD DEMOGRÁFICA DE UNAS PLANTAS RUPÍCOLAS ENDÉMICAS DEL MEDITERRÁNEO OCCIDENTAL (*SONCHUS* SECCIÓN *PUSTULATI*)

DEMOGRAPHIC VULNERABILITY IN CLIFF-DWELLING PLANTS ENDEMIC TO THE WESTERN MEDITERRANEAN (*SONCHUS* SECTION *PUSTULATI*)

Submitted to “BASIC AND APPLIED ECOLOGY”

Authors: José L. Silva, José A. Mejías & María B. García

RESUMEN

Objetivos Caracterizar demográficamente la rareza y vulnerabilidad de las especies de *Sonchus* sección *Pustulati* (*Sonchus masguindalii*, *S. fragilis* y *S. pustulatus*) y estimar las tendencias poblacionales y viabilidad de la especie *S. pustulatus* en su rango Bético, donde está catalogada como “en peligro crítico de extinción”.

Ubicación Las especies de *Sonchus* sección *Pustulati* constituyen un grupo de plantas rupícolas endémicas en la región Mediterránea, restringidas a áreas reducidas no superpuestas dentro del punto caliente de biodiversidad Bético-Rifeño. *S. pustulatus* se encuentra tanto en el sureste de la Península Ibérica como en el norte África, mientras que las otras son exclusivas del norte de África.

Métodos Caracterizamos demográficamente todas las poblaciones existentes de este clado en 2008, registrando tamaños poblacionales, estructuras demográficas y éxito reproductivo. Además, estimamos las tendencias poblacionales de *S. pustulatus* en su rango español mediante censos repetidos en 2013. También calculamos la tasa de crecimiento poblacional estocástico (λ_S) y modelamos la viabilidad futura (PVA) de una de las poblaciones españolas mediante el uso de modelos matriciales derivados de un seguimiento demográfico detallado durante un período de seis años, desde 2003 hasta 2008.

Resultados A pesar de la gran variabilidad detectada en los tamaños poblacionales, todas ellas exhibieron estructuras poblacionales bastante similares (baja abundancia de plántulas y juveniles), sugiriendo bajo reclutamiento y alta supervivencia de los individuos adultos bien establecidos. Las poblaciones españolas crecieron a una tasa de entre 0.91 y 1.01, y el modelo matricial mostró un declive significativo en una de ellas ($\lambda_S = 0.904$; 95% CI: 0.9041–0.9043). El PVA proyectó que esta población quedaría reducida a unos pocos individuos en aproximadamente 40 años bajo las mismas condiciones presentes.

Conclusiones principales Las relaciones negativas observadas entre la dinámica poblacional total y la temperatura máxima y sequía sugieren que el calentamiento climático global y el régimen de precipitaciones pronosticados en el rango Bético pondrán en peligro la futura viabilidad de la población. Esta vulnerabilidad podría estar relacionada y acentuada por la posición geográfica periférica.

Palabras claves Área de ocupación; Estructura poblacional; Éxito reproductivo; Plantas raras; PVA; Tasa de crecimiento poblacional estocástico; Tendencias climáticas.

ABSTRACT

Aims We attempted to demographically characterise the rarity and vulnerability of the species of *Sonchus* section *Pustulati* (*Sonchus masguindalii*, *S. fragilis* and *S. pustulatus*) and to estimate the population trends and viability for the “critically endangered” Spanish *S. pustulatus*.

Location The species of *Sonchus* section *Pustulati* constitute a group of endemic cliff plants in the Mediterranean region, restricted to narrow non-overlapping areas within the Baetic-Rifan hotspot. *S. pustulatus* occurs both in SE Iberian Peninsula and N Africa, whereas the others are exclusive to N Africa.

Methods We demographically characterized all the extant populations of this clade in 2008, by recording population sizes, demographic structures and reproductive success, In addition, we estimated the population trends for the critically endangered Spanish *S. pustulatus* by repeating censuses in 2013. We also calculated the stochastic population growth rate (λ_S) and modelled future viability (PVA) of one of the Spanish populations by using matrix models derived from a detailed demographic monitoring over a six-year period, from 2003 to 2008.

Results Despite the high variability detected in population size, they all exhibited quite similar demographic structures (low abundances of seedlings and juveniles), suggesting low recruitment and high survival of well-established adults. The Spanish populations grew at a ratio of between 0.91 and 1.01, and the matrix model showed a significant decline in one of them (λ_S = 0.904; 95% CI: 0.9041–0.9043). The PVA projected that this population would be reduced to a few individuals in approximately forty years under present conditions.

Main conclusions The negative relationships between the overall population dynamic and maximum temperature and drought suggest that the forecasted global warming and precipitation regime in the Baetic range will jeopardize future viability. Such vulnerability might be related and accentuated by the peripheral geographical position.

Keywords Area of occupancy; Climatic trends; Population structure; PVA; Reproductive success; Rare plants; Stochastic population growth rate.

1. INTRODUCTION

For the conservation of rare or endangered species, a demographic approach to species management is of critical importance (Schemske *et al.*, 1994). Detailed demographic characterisation in terms of population number, size and structure across the species distribution provides us with an overall picture of the current status of the species. Population monitoring can reveal both temporal trends and the most critical life stages for the population growth rate (Horvitz & Schemske, 1995), but this is more time and resource consuming, and this is therefore often restricted to the most critically endangered species (Heywood & Iriondo, 2003). Both static and dynamic approaches are key in assessing both potential and actual threats, allowing an appropriate management strategy to be defined.

Long-term monitoring programmes are uncommon because of the need to maintain structures and funding in the long run. This becomes even more difficult in the case of cliff-dwelling species due to their difficult access. Consequently, our knowledge about the conservation and biology of cliff plants is greatly limited (Larsson *et al.*, 2005), despite the fact that rocky habitats harbour many rare and endangered plants with narrow distributions; particularly in the Mediterranean region (Thompson, 2005). Cliffs are highly fragmented and offer very particular ecological environments that make life difficult, such as scarce microsite availability for plant establishment. However, competition in these habitats is also lower, and adapted organisms have a lower probability of suffering disturbances than in common substrates. Although limited, studies on the populations and dynamics of cliff-dwelling plants suggest that local persistence is high (Lavergne *et al.*, 2004; Thompson, 2005), they are extraordinarily long-lived (Larson *et al.*, 2000; García *et al.*, 2002), and their populations sizes are unusually stable for this habitat (Morris & Doak, 1998; Picó & Riba, 2002; García, 2003).

In this study, we examine the demography of a small group of related plants that constitute the subgenus *Sonchus* section *Pustulati* (Asteraceae): *Sonchus pustulatus* Willk., *S. fragilis* Ball and *S. masguindalii* Pau and Font Quer (Boulos, 1973; Kim *et al.*, 2007). These narrow endemics are restricted to small areas of the Western Mediterranean Basin (Boulos, 1973) on both sides of the Alboran Sea (Figure 1) and tend to be on localized to rocky cliffs at low altitudes. All three species are found in North Africa, where they are considered to be very rare (Fennane & Ibn Tatoo, 1998). *S. pustulatus* also occurs in the SE Iberian Peninsula, where it is categorized as "critically endangered" (Cueto *et al.*, 2003). Phylogenetic and phylogeographic data, coupled with the peripheral locations of the Spanish populations, suggest that these taxa are relict of the late Tertiary (Chapter 4). Their restricted and disjunct distribution seems to be related to old geological events of large biogeographic impact: the Messinian Salinity Crisis and the subsequent Zanclean reflooding of the Mediterranean Sea.

The principal goal of this study was to assess the current conservation status and future risk of this clade of narrowly distributed cliff-dwelling plants, as well as discussing possible implications of its vulnerability in relation to the ongoing climatic change. For that purpose, we undertook extensive fieldwork to describe the overall demographic situation of the clade, and modelled the dynamics of the Iberian populations. We focused on the following specific objectives: (1) to accurately define the species range boundaries after visiting all known populations and potentially suitable habitats; (2) to estimate the extent of occurrence of each taxon and the occupancy areas, population sizes, structures and reproductive success in all the extant populations; (3) to project the demographic trend of the three Spanish populations of *S. pustulatus* by means of count-based models, and to model the viability (PVA) of the *a priori* most endangered population by matrix models. Our results will help in assessing the conservation status and vulnerability of a small group of cliff-dwelling species of high biogeographic and conservation value.

2. MATERIAL AND METHODS

2.1 Plant species

The subgenus *Sonchus* section *Pustulati* includes three species: *Sonchus pustulatus*, *S. fragilis* and *S. masguindalii*. They are restricted to four narrow non-overlapping small areas within the Baetic-Rifan complex hotspot (Western Mediterranean; Figure 1, A-D), always occurring on low cliffs located on the coast or less than 20 km from the sea. The Spanish populations of *S. pustulatus* are located in the environs of Almeria city (Figure 1, A, southeastern Spain), whereas the Moroccan ones colonize cliffs in the Oued Laou valley and some neighbouring valleys (Figure 1, B, western Rif). *S. fragilis* is exclusive to the mountains surrounding the city of Tetouan (Figure 1, C, northwestern Rif). Finally, *S. masguindalii* is distributed along the coast in the Bokkoya Mountains and on some sea cliffs in the environs of Al-Hoceima city (Figure 1, D, central Rif). Some herbarium specimens of *S. pustulatus* collected in the 19th century (e.g. herbarium COI) indicate that the species was also present in rocky places on the oceanfront near Ghazaouet

(Figure 1, E, northwestern Algeria), but we were unable to visit this area and their presence could not be therefore confirmed.

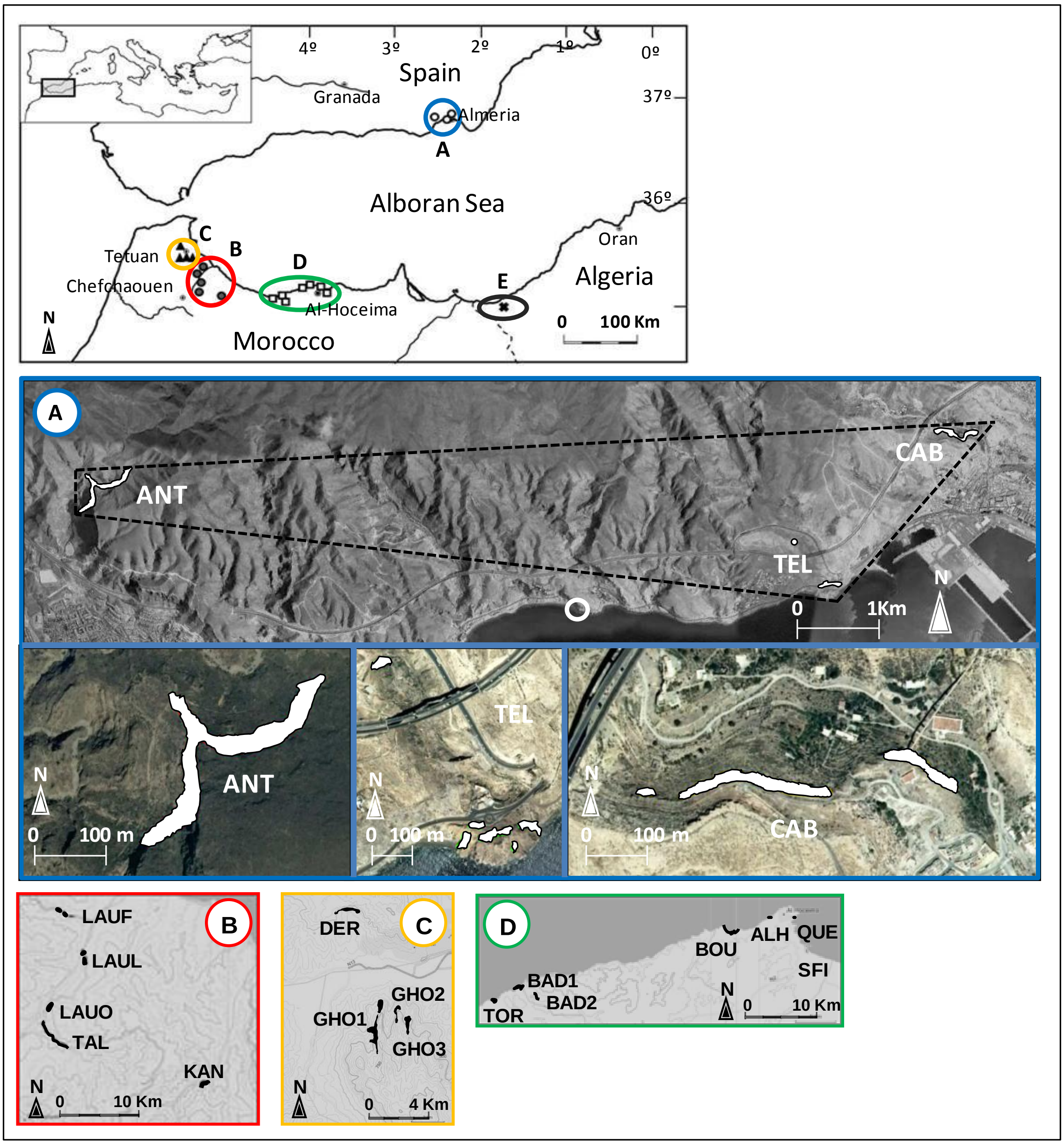

Figure 1 Overall geographical distribution of the species of *Sonchus* section *Pustulati* and areas of occupancy of populations in the Spanish Baetic (A) and Moroccan Rifan ranges (B-C-D). A and B, *S. pustulatus*; C, *S. fragilis*; D, *S. masguindalii*. Algerian range (E), *S. pustulatus* unconfirmed. In the Spanish area (A), the extent of occurrence is marked off by a dashed line and an empty white circle indicates the location of the extinguished population (Playa El Palmer).

The *Sonchus* section *Pustulati* includes suffrutescent chamaephyte plants, with flower heads comprising approximately 30–120 florets in the case of *S. pustulatus* and *S. fragilis*, and 60–250 florets in *S. masguindalii*. They bloom in the spring and are visited by many species of Diptera and Coleoptera (Chapter 1). The incidence of the self-incompatibility is high in *S. pustulatus* and *S. masguindalii*, and very low in *S. fragilis* (Chapter 5). Fruits are achenes with a very low persistent pappus, released during late spring and early summer.

2.2 Distribution area, extent of occurrence and area of occupancy

We conducted an intensive field work campaign from spring to early summer in 2007 and 2008. We visited all the locations for each of the three species recorded in different herbariums (BC, BM, COI, G, HUAL, MA, MPU, RNG and SEV) and bibliographic references (Boulos, 1973; Deil & Galán de Mera, 1996; Deil & Hammoumi, 1997; Cueto *et al.*, 2003; Mota *et al.*, 2005), plus potential habitats nearby. According to the IUCN guidelines (2001), we estimated the area of occupancy for each located population as “the occupied area within the extent of occurrence, excluding cases of vagrancy” and the extent of occurrence for each taxon as “the area contained within the shortest continuous imaginary boundary encompassing all known sites in which the taxon is present”. For the area of occupancy, we used the population longitudinal ranges and the average distance between the highest and lowest heights where individuals occurred (measured at the vertical projection of each sampling plot; see sampling design below). For the extent of taxa occurrence, we used orthophotos (obtained from REDIAM, 2008, to Spain), maps (GPS GARMIN, to Morocco) and AUTOCAD software (v.2006).

2.3 Population size and structure

Population size was estimated from the area of occupancy and plant density. Density was obtained during May and June 2008 using a variable number of homogeneously distributed sampling plots (2×2 m), in which we recorded the number of individuals. In order to adapt the sampling strategy to the fragmented and clumped nature of cliff-dwelling plants, and to their population sizes, the number of plots (6–30, mean 21.5; Table 1) and distances between plots varied. Sampling plots were placed along the cliffs alternately at low (0–2 m from the cliff base), medium (2–4 m) or high (>4 m) transects. When plots were inaccessible we used binoculars to record the number of individuals. Most of the spatial range of plant occurrence was covered with this sampling system, because either the cliffs were not higher than this or the individuals did not occur on the higher parts. When populations occurred in a defile (KAN) or in a narrow valley (LAUF) and plants were settled on both wall sides, plots were alternatively established in two parallel transects; one on each cliff. In order to test the accuracy of this method for estimating population size, the Spanish populations were also recorded by directly counting all of the individuals.

We also used the sampling plots to estimate population structures (frequency of plant states) as most recorded individuals were sufficiently accessible to be classified first into three categories: seedlings (less than 1 year old, usually with cotyledons), juveniles (one or, rarely, two herbaceous stems bearing mature leaves, no flowering stalks and no evidence of past flowering events) and adults (plants with lignification, well-developed leaves, and usually with one or more flowering stems). The two largest perpendicular axes were then measured for each plant to calculate their elliptic surface, and individuals were classified into one of the following size classes (see Appendix: Figure 3): *Seedlings*; *Juveniles*; *Class-I* [0–50] cm^2; *Class-II* (50–200] cm^2; *Class-III* (200–500] cm^2; *Class-IV* (500–1000] cm^2; *Class-V* (1000–3000] cm^2; and *Class-VI* >3000 cm^2. One Spanish population (CAB, Table 1) was monitored over six consecutive years (2003–2008), and the average demographic structure across years was used in this case.

2.4 Reproductive success

For each population, we estimated seed set (seed/ovule ratio) in 2008 from one to three mature non-dispersed flower heads in 20 randomly selected individuals. We performed one way ANOVA and Tamhane post hoc multiple comparisons tests to compare differences in mean seed number per flower head among taxa, after checking normality and homocedasticity. These statistical analyses were performed with SPSS software (SPSS for Windows, ver.15.0.1, 2006, Chicago: Inc.). For each population, we also counted the number of flower heads in all accessible adults using the sampling plots.

The density of released seeds was considered as an accurate estimation of pre-emergent population reproductive success (Wiens *et al.*, 1987), and it was calculated from the number of flower heads per individual, seeds per flower head, number of reproductive plants and area of occupancy. In addition, in order to estimate the efficiency in the conversion of the released seeds on seedlings, juveniles and adults in the populations we calculated the ratio between the density of each of the three last phenophases and the density of released seeds. The density of seedlings, juveniles and adults was estimated from the area of occupancy and number of individuals counted on the sampling plots.

2.5 Population trends and viability of the Spanish populations of S. pustulatus

Direct censuses performed in 2008 in the Spanish populations of *S. pustulatus* were repeated in 2013, and population growth rates were estimated as the 5th-root of the ratio between the number of plants in 2013 and 2008.

In the CAB population, a detailed individual-based monitoring study was performed from May 2003 to May 2008. All accessible individuals on the cliffs (approximately 75% of the total population) were mapped and their positions precisely recorded in the first year. We annually recorded individual persistence or death, and estimated plant size with same method as before, but in this case adults were separated into generative (one or more flowering stems) and vegetative (just a few leaves with no evidence of flowering during the current year) (see Appendix: Figure 3). All new individuals appearing within the sampling area were also measured, mapped and incorporated into the monitoring study.

Stage-based population projection matrix models were used to explore the demographic population trend of CAB under current conditions. Here, we grouped some of the classes used for the population structures in order to consider a reasonable minimum sample size and thus to obtain reliable estimates of vital rates. *Seedlings, Juveniles* and *Class-I* were joined in a unique category (*Class-S&J&I*), as well as the individuals of the *Class-V* and the *Class-VI* (*Class-V&VI*) since the individuals greater than 3000 cm^2 are very rare in this population. We constructed a total of five 5×5 projection matrices for each pair of consecutive years (from 2003–2004, till 2007–2008), according to the standard procedure of transition probabilities of the life-cycle graph (Caswell, 2001). We calculated the dominant eigenvalue of each projection matrix, which represents the deterministic population growth rate (λ) of the species present in a particular environment and at a particular time. We also calculated, by simulation, the stochastic population growth rate (λ_s) and the 95% Confidence Interval for each population, using 50000 iterations where each matrix had the same probability of occurrence. These were calculated using the 'Stoch_log_lam' routine of Morris and Doak (2002). In addition, we calculated the conditional total life span of plants in this population (mean age at death, conditional on reaching a given stage) from the average matrix over years and the algorithm published by Cochran and Ellner (1992).

Finally, we estimated the probability of population extinction over the next 100 years using the 'simex' routine of Morris and Doak (2002) with MATLAB R2010. For this population viability analysis (PVA), we ran 2000 simulations with the 5 transitions presenting the same probability of occurrence and using 10 plants as the quasi-extinction threshold. A minimum of 10 plants was used to ensure successful crosses for this highly self-incompatible species, whose individuals usually start reproducing in their first year of life (Silva & Mejías, unpublished).

2.6 Population dynamics and climate

In the CAB population we tested the relationship between λ and climatic variables by means of Pearson tests. We compiled the following monthly information from a weather station located 9 km from the population (Almeria Airport Weather Station): mean temperature, average of minimum and maximum temperatures, and total precipitation. We also recorded the total number of days per year with rainfall or light precipitation. Climatic variables were arranged for climatic years (from September to the following August), both including and excluding the dry and warm stressful summer for most Mediterranean plants. We also tested the relationship between demographic trend and climate considering only the spring period of expected highest plant growth (from March to June).

3. RESULTS

3.1 Demographic status

A total of 19 populations were registered after our extensive inspections in 2008 (Table 1, Figure 1). The Spanish populations of *S. pustulatus* showed the lowest extent of occurrence, area of occupancy and population size within the section *Pustulati* (Table 1). Furthermore, one population (Playa El Palmer; Mota *et al.*, 2005), visited in 2008, has been considered to be extinct (Figure 1), suggesting a reduction in the extent of occurrence during the last few years. The Moroccan populations of *S. pustulatus* comprised a considerable number of plants, accounting for the greatest extent of occurrence in the section (Table 1), and the largest population (KAN) was included within the Talassemtane National Park. *S. fragilis* showed an exceptionally large population (GHO1), but a small extent of occurrence (Table 1). Finally, *S. masguindalii* showed the largest total size (Table 1), and most of the area was included within the Al-Hoceima National Park.

As expected for cliff habitats, perturbations were rare (Table 1). However, diverse anthropogenic factors can still be listed, some of them providing evidence of negative effects on the populations. A small area of the eastern end of the Spanish population CAB of *S. pustulatus* (approximately 20% of the area of occupancy) is being used for domestic waste disposal. Goat grazing has been observed in the Spanish populations ANT and TEL of *S. pustulatus*, as well as in populations GHO1, GHO2 and GHO3 of *S. fragilis*. Several individual groups on the low cliffs of population SFI of *S. masguindalii* occur in the vicinity of beach bars, whereas some groups in the BAD1 population of the same species are very close to small dwellings. The restoration of a lighthouse in the TEL population also resulted in the loss of some plants. Finally, we have recently (2013) detected numerous rock-climbing routes that cross the ANT population.

Most populations exhibited similar demographic structures (Figure 2). Adult plants dominate, particularly of the *Class-II*, followed by those of the *Class-I* (in populations of *S. fragilis*) or *Class-III* (in the remaining). The frequency of seedlings and juveniles was always below 15%. In the Spanish population of *S. pustulatus* (CAB), generative adults were clearly the most abundant plants (63.0–89.0%, depending on years), while vegetative adults only accounted for a very small fraction of the population (3.2–26.1%).

Table 1 Populations of the taxa of *Sonchus* section *Pustulati* confirmed for this study. Nuclei means groups of individuals spatially well delimited within a population. N: population size (* in parentheses by direct counting of individuals). Plots refer to the number of plots used in the demographic sampling for density and population size estimates.

Species, populations, altitude	Acronym	Coordinates (N/W)	Protected area/ Anthropogenic disturbance factor	Extent of occurrence (Km^2)	Area of occupancy (m^2)	Nuclei	N (*)	Reproductive plants	Density (plants/ 100 m^2)	Plots
***Sonchus pustulatus* - Spain, southeast**				**6.7**	**3505**		**1058 (879)**	**799**		
Aguadulce, Bco. San Antonio, 300m	ANT	36º49.8’ / 2º34.4’	No/Yes		1972	2	707 (484)	426	34	30
Almeria, Faro de San Telmo, 22m	TEL	36º49.7’ / 2º29.0’	No/Yes		546	6	121 (112)	104	22	18
Almeria, Bco. del Caballar, 100m	CAB	36º50.6’ / 2º28.7’	No/Yes		987	3	230 (292)	269	26	30
***S. pustulatus* - Morocco, western Rif**				**242.9**	**15785**		**9991**	**8403**		
Oued Laud valley, 120m	LAUF	35º23.3’ / 5º12.6’	No/No		1800	3	1062	945	46	25
Oued Laud valley, 153m	LAUL	35º20.8’ / 5º11.0’	No/No		1038	2	540	427	42	25
Oued Laud valley, 150m	LAUO	35º17.4’ / 5º13.8’	No/No		840	1	221	190	25	20
Oued Laud valley, 235m	TAL	35º16.0’ / 5º13.8’	No/No		3008	5	1980	1643	64	30
Oued Al-Kannar gorge, 244m	KAN	35º13.0’ / 5º01.2’	Yes/No		9100	1	6188	5198	72	25
***S. fragilis* - Morocco, northwestern Rif**				**16.1**	**41336**		**33927**	**27409**		
Tetouan, Montes Ghorghiz, 550m	GHO1	35º32.1’ / 5º23.5’	No/Yes		28912	2	26985	21858	92	30
Tetouan, Montes Ghorghiz, 550m	GHO2	35º32.8’ / 5º22.6’	No/Yes		-	1	-	-	-	-
Tetouan, Montes Ghorghiz, 550m	GHO3	35º32.3’ / 5º22.3’	No/Yes		2760	2	661	588	21	24
Tetouan, Montes Dersa, 275m	DER	35º35.9’ / 5º24.6’	No/No		9664	1	6282	4963	47	15
***S. masguindalii* - Morocco, central Rif**				**107.0**	**40001**		**42269**	**33281**		
Torres de Alcalá beach, sea level	TOR	35º09.4’ / 4º19.7’	Yes/No		3568	1	6244	5495	90	10
Bades beach - Peñon de Vélez, s.l.	BAD1	35º10.2’ / 4º17.9’	Yes/Yes		6038	2	6444	5542	106	26
Valley to Bades beach, 60 m	BAD2	35º09.6’ / 4º16.9’	Yes/No		486	2	638	555	145	20
Boumahdi beach, s.l.	BOU	35º14.0’ / 4º00.7’	Yes/No		16536	2	19513	14635	129	25
Cebadilla beach, Al-Hoceimas, s.l.	ALH	35º14.6’ / 3º58.0’	Yes/No		1100	1	688	688	63	6
Quemado beach, Al-Hoceimas, s.l.	QUE	35º14.5’ / 3º55.5’	No/No		10063	1	6708	4494	88	6
Sfiha beach, Al-hoceimas, s.l.	SFI	35º12.8’ / 3º54.3’	No/Yes		2211	3	2035	1872	92	22

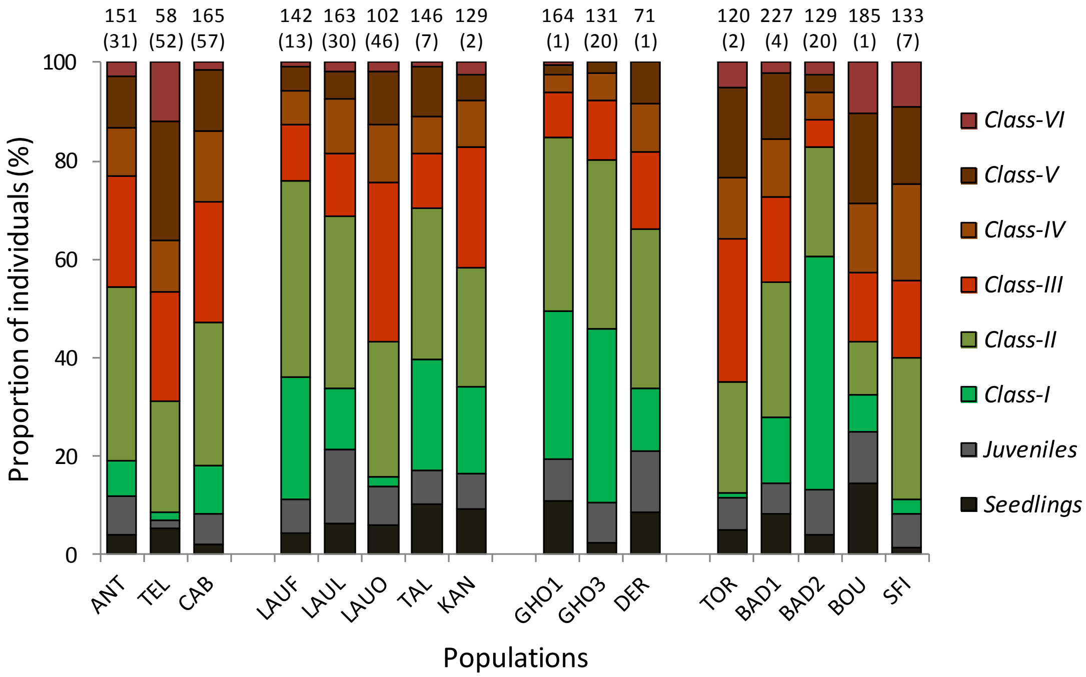

Figure 2 Demographic structures of populations based on individual phenophases and size classes. Numbers above bars are sampled individuals, and their proportion of the total estimated population size is in brackets.

3.2 Reproductive success

S. masguindalii produced significantly more seeds per flower head than the other taxa (*F* = 56.17, *P* = 0.000; Tamhane post hoc, *P* < 0.001), as well as higher densities of released seeds (see Appendix: Table 3). The population of highest pre-emergent success was BOU, which was also the one with the highest values of flower head production and seeds per flower head (see Appendix: Table 3). The high density of released seeds in the populations (between 53–5742/m^2) contrasted with the low density of seedlings, juveniles and adults recorded in the 2008 sampling (between 0–15/100 m^2, 0–12/100 m^2 and 19–130/100 m^2, respectively); and the ratios between the density of seedlings, juveniles or adults and the density of released seeds in the populations were therefore very low (always below 0.007; see Appendix: Table 3). Since individual plants were monitored in the CAB population, we could estimate the proportion of seedlings and juveniles per year, which ranged between 5–8% and 1–4% across years, respectively. The high pre-emergent success also contrasted with the low annual survival of seedlings (0.13 ± 0.13), which showed a considerable increase during the juvenile state (0.71 ± 0.07), and particularly at the adult stage (ranging from 0.77 ± 0.06 to 0.96 ± 0.01, depending on size class).

3.3 Population trends and viability of the Spanish populations of S. pustulatus

The number of individuals in the CAB population dropped from 292 in 2008 to 188 in 2013 (λ =0.916). A similar decline was estimated in TEL during the same period, population size decreasing from 112 to 94 individuals (λ = 0.966). Unlike these two populations, the ANT population increased slightly from 484 to 505 individuals (λ = 1.009).

All five yearly matrices of the CAB population produced λ-values below 1 (ranging from 0.868 to 0.946; see also Table 2), and the stochastic lambda indicated a significant rate of decline over the study period of 9.6% (λ_S = 0.904; 95% CI: [0.9041–0.9043]). This negative trend is similar to the one estimated by the count-based model, and it was only in part due to the contraction of the area of occupancy. The population viability analysis (PVA) projected a sharp decline of the population, with 100% probability of quasiextinction (N=10) in the next 100 years. Should the present conditions persist, the population is projected to decrease up to 10% of current population size (below 30 individuals) in only 23 years. The conditional total life span of the largest class (average age at death for seedlings getting to that class) was estimated at 22 years.

Table 2 Mean transition matrix corresponding to the CAB population of *S. pustulatus* over the 2003–2008 period. Column and row labels represent the first (origin) and second (destination) year for the transition probabilities (mean ± SE) between size categories, respectively. *Class-S&J&I*: seedlings, juveniles and adults between [0–50] cm^2; *Class-II*: adults between (50–200] cm^2; *Class-III*: (200–500] cm^2; *Class-IV*: (500–1000] cm^2; and *Class-V&VI*: >1000 cm^2.

	Class-S&J&I	*Class-II*	*Class-III*	*Class-IV*	*Class-V&VI*
Class-S&J&I	0.500 ± 0.041	0.100 ± 0.027	0.050 ± 0.010	0.056 ± 0.011	0.069 ± 0.023
Class-II	0.189 ± 0.048	0.550 ± 0.035	0.183 ± 0.021	0.022 ± 0.022	0.015 ± 0.009
Class-III	0.047 ± 0.014	0.162 ± 0.010	0.584 ± 0.018	0.198 ± 0.036	0.026 ± 0.011
Class-IV	0.011 ± 0.007	0.028 ± 0.010	0.095 ± 0.028	0.468 ± 0.041	0.195 ± 0.028
Class-V&VI	0.006 ± 0.006	0.012 ± 0.008	0.025 ± 0.004	0.215 ± 0.033	0.682 ± 0.038

Lambda was found to have a significant negative correlation with monthly mean maximum temperature during the growing period (summer months excluded; r = -0.901, P = 0.037), and a positive correlation with number of days with rainfall or light precipitation during the spring months (r = 0.896, P = 0.040).

4. DISCUSSION

Our study showed and compared demographic variables related to the rarity and vulnerability of a whole clade of three narrow endemic species of *Sonchus* section *Pustulati* across two continents separated by the Alboran Sea. The information gathered served to update the conservation status of the three taxa according to the categories and criteria of the IUCN Red List (IUCN, 2012). The Spanish *S. pustulatus* would move from "critically endangered" (CR) to "endangered" (EN) but, based on the sharp decline projected for CAB population, they will revert to the CR category by the end of the century (see Appendix: Conservation status). The remaining taxa should be considered as "deficient data" until we have more information on gain or loss of extent of occurrence, area of distribution, size and/or number of populations (see Appendix: Conservation status and Table 4). Consequently, the situation of these taxa cannot be considered dramatic or irreversible right now. We will now discuss how their demographic properties and population dynamics might help us to assess their vulnerability under the ongoing climatic change.

The demographic structure of most populations produced an overall similar pattern, with adult plants being dominant and low proportion of seedlings and juveniles. This is a typical structure in rocky habitats (García *et al.*, 2002; Larson *et al.*, 2005) and not necessarily an evidence of "population senescence". A combination of factors such as difficulty for seed germination and seedling establishment in the few appropriate microsites available on cliffs, along with early reproductive onset (Silva & Mejías, unpublished) and longevity of individual plants (over 20 years for the largest ones in the case of one Spanish population) may explain such a pattern.

Nevertheless, two of the three Spanish populations of *S. pustulatus* are in decline. The few existing studies on population dynamics of cliff-dwelling species suggested an unusual demographic stability and resilience of cliff plants (Pico & Riba, 2002; García, 2003) due to the much higher importance of the survival of existing individuals than recruitment of new ones for the maintenance of populations (Larson *et al.*, 2000;

García *et al.*, 2008). High survival rates translate into high individual longevity. However, our estimation of longevity in *S. pustulatus* is notoriously lower than for other cliff-dwelling plants (Ehrlén & Lehtilä, 2002, Forbis & Doak, 2004; García *et al.*, 2008). This fact could be species-specific but may also be related to the lower performance of plants in a peripheral position regarding the geographical distribution of the taxa of *Sonchus* section *Pustulati*, as found for *Silene ciliata* (Giménez-Benavides *et al.*, 2011). In any case, the cliff-dwelling *Sonchus* studied, at least in the Baetic range, might not be as resilient to global changes as generally assumed for plants of very stable habitats such as cliffs.

Due to the short distance between CAB and the other two populations, TEL and ANT (1.9 and 8.2 km, respectively), similar demographic trends were expected under the assumption of similar environmental factors acting in neighbouring populations (Buckley *et al.*, 2010). However, this was not the case, as TEL declined whereas ANT slightly increased. Different anthropogenic disturbances at low scale and/or different relationships between population dynamics and environmental factors may have promoted divergent trends. *Pustulati* species have been considered as part of the pre-Mediterranean flora (Chapter 4), a previously much wetter climatic period, and the ANT population is located on high cliffs protected from the sun and with many points where water often oozes. Such conditions are presumably more similar to the typical habitats during species diversification, suggesting that climatic refuges play an important role in the persistence of old floristic elements.

Given that our results demonstrated the high influence of some climatic variables on population performance in the Spanish CAB population of *S. pustulatus*, forecasting how the climatic trends could affect the future of this Mediterranean clade seems an interesting exercise. Global warming and the increase in the number of days with no rainfall forecasted for the southern Spanish Mediterranean (Ruiz-Sinoga *et al.*, 2012) are expected to have a negative effect on the population performance. This is because we have found that annual λ were negatively related to the mean maximum temperature during the growing period (i.e. the summer months excluded) and the number of spring days with no rainfall.

North Africa (Rif) represents the main distribution area for the *Pustulati* taxa (Figure 1) and constitutes their most plausible origin and diversification centre (Chapter 4). Although we do not know about population trends in Morocco, which prevents us from comparing these with the Spanish ones, we found that the latter tended to present the lowest extent of occurrence, occupancy areas and densities, and to contain less individuals. Although both the Spanish and Moroccan areas of *S. pustulatus* experience similar dry and warm summers, the Moroccan area is considerably wetter: with up to three times higher annual rainfall than in the Spanish area (Hijmans *et al.*, 2005). Consequently, the peripheral position of the Spanish populations regarding the whole *Pustulati* taxa distribution, currently at the limit of the optimal ecological amplitude, might reinforce the vulnerability of these populations from a demographic point of view.

Although all populations of *Sonchus* section *Pustulati* show a similar structure and limitation for recruitment resulting from their habitat stenoicity, the larger Moroccan Rifan populations do not appear to be as threatened as the ones from the Spanish Baetic range, for which there is some evidence of extinction in the past, population decline in the present time, and less suitable future climatic conditions for population performance. Nevertheless, only through long term monitoring programs we will be able to objectively prove such forecasted vulnerability.

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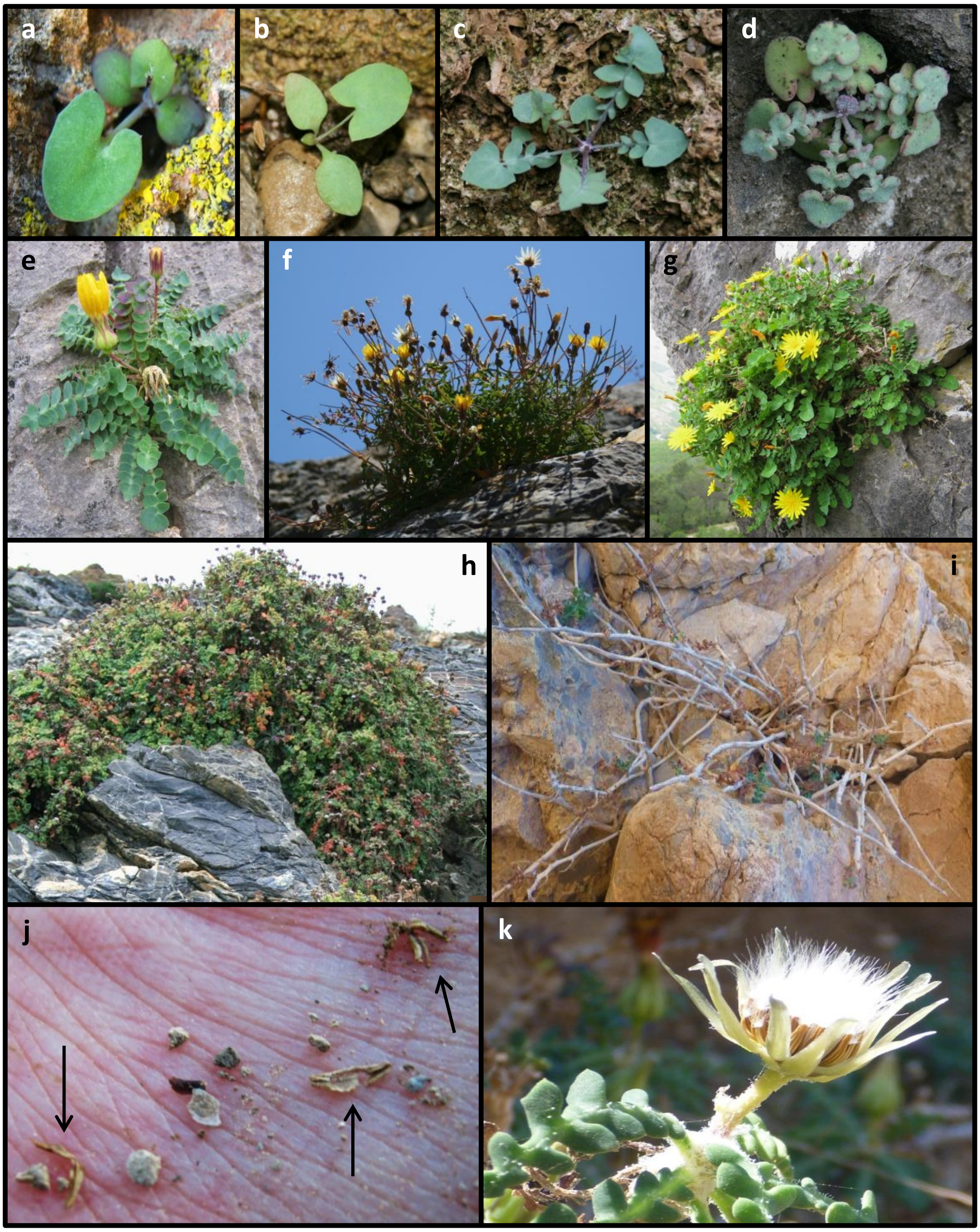
APPENDICES



Appendix: Figure 3 Phenophases and size classes of the individuals of *Sonchus* section *Pustulati*. a-b, seedlings of *S. pustulatus* (Spanish ANT and Moroccan KAN populations, respectively). c-d, juveniles of Moroccan *S. pustulatus* (KAN) and *S. masguindalii* (BOU), respectively, with herbaceous stems. Adult plants: e, *Class-I*, of *S. fragilis* (GHO3); f, *Class-III*, of Moroccan *S. pustulatus* (LAUL); g, *Class-IV*, of *S. fragilis* (GHO1); and h, *Class-VI*, of *S. masguindalii* (BOU). i, vegetative adult classified within *Class-I* of Spanish *S. pustulatus* (CAB). j, devoured seeds of Spanish *S. pustulatus* (ANT). k, flower head of a Spanish *S. pustulatus* individual (CAB) about to disperse its seeds.

Appendix: Table 3 Reproductive parameters and success of *Sonchus* section *Pustulati* in 2008. Mean ± SE of flower head production per adult plant, seeds produced per flower head, and seed set. Density of released seeds was calculated per square meter, and density of seedlings, juveniles and adults per 100 square meters. Ratio refers to relationships between the density of seedlings, juveniles or adults and the density of released seeds.

				Density of				Ratio (*10^{-2})		
Population	Flower head	Seeds per flower head	Seed set	released seeds (/m^2)	seedlings (/100m^2)	juveniles (/100m^2)	adults (/100m^2)	seedlings / seeds	juveniles / seeds	adults / seeds
S. pustulatus (Spain)										
ANT	5 ± 0.9	55 ± 2.8	0.66 ± 0.030	59	2	5	28	0.03	0.08	0.47
TEL	11 ± 1.2	32 ± 4.9	0.46 ± 0.069	68	0	1	21	<0.01	0.02	0.31
CAB	8 ± 0.9	51 ± 3.8	0.58 ± 0.034	109	0	7	19	<0.01	0.06	0.18
S. pustulatus (Morocco)										
LAUF	3 ± 0.5	36 ± 3.7	0.66 ± 0.053	66	1	3	43	<0.01	0.05	0.65
LAUL	6 ± 1.2	46 ± 3.4	0.77 ± 0.042	126	5	4	33	0.04	0.03	0.26
LAUO	10 ± 1.4	40 ± 3.8	0.71 ± 0.050	99	5	5	15	0.05	0.05	0.15
TAL	6 ± 1.0	48 ± 2.6	0.78 ± 0.031	170	5	4	56	0.02	0.02	0.33
KAN	4 ± 0.6	44 ± 5.8	0.76 ± 0.048	112	12	5	61	0.05	0.04	0.54
S. fragilis										
GHO1	8 ± 1.6	36 ± 3.4	0.63 ± 0.058	216	13	8	72	0.05	0.04	0.33
GHO3	5 ± 0.8	45 ± 2.2	0.76 ± 0.021	53	1	1	19	0.02	0.02	0.35
DER	17 ± 2.9	42 ± 2.7	0.85 ± 0.028	377	3	7	38	<0.01	0.02	0.10
S. masguindalii										
TOR	8 ± 1.3	106 ± 4.4	0.94 ± 0.017	1242	3	3	88	<0.01	<0.01	0.07
BAD1	7 ± 1.0	64 ± 6.4	0.64 ± 0.035	413	9	6	91	0.02	0.01	0.22
BAD2	7 ± 2.2	59 ± 18.6	0.47 ± 0.117	426	4	11	130	0.01	0.03	0.31
BOU	50 ± 10.8	125 ± 9.2	0.84 ± 0.033	5742	15	12	100	<0.01	<0.01	0.02
ALH	-	63 ± 8.6	0.60 ± 0.079	-	0	0	63	-	-	-
QUE	-	102 ± 9.5	0.73 ± 0.046	-	13	8	67	-	-	-
SFI	24 ± 10.0	67 ± 10.8	0.54 ± 0.072	1500	2	7	83	<0.01	<0.01	0.06

Appendix: Conservation status. According to the categories and criteria of the IUCN Red List (IUCN, 2012), the Spanish *S. pustulatus* would move from “critically endangered” (CR) to “endangered” [EN B1ab(i,ii,iv,v)+2ab(i,ii,iv,v); C1], despite the decline inferred for the CAB population, the extinguished population (Playa El Palmer; Mota *et al.*, 2005), and the very low extent of occurrence and area of occupancy. This counterintuitive situation is due to the existence of a large population (ANT, approximately 500 individuals). However, a number of reasons not considered in the IUCN criteria suggest that the Spanish populations are in a critical situation: effective population sizes are the lowest within the group studied, populations are not located within protected areas, and different negative factors of unknown effects are acting upon these populations. Furthermore, if our demographic predictions for the CAB and TEL populations hold up, and no conservation actions are taken, ANT could become the only population extant in a few decades, a fact that would return the taxon to the CR category. The remaining taxa should be considered as “deficient data” according to the IUCN criteria, as we do not avail of information relating to possible reduction or increase episodes in the distribution area or in population sizes. Nevertheless, we provide criteria and subcriteria (detected and discarded) for future assessments and estimations of population trends (see Appendix: Table 4). Due to their rarity, these taxa should be considered closer to some of the threatened categories rather than simply “not evaluated”.

Given the scattered nature of populations, restricted to small non-fertile areas, it seems clear that the main conservation effort for the *Sonchus* section *Pustulati* populations must involve protection of their habitats to avoid anthropogenic disturbance. Microreserves constitute an essential tool for effective protection of diverse flora in the Western Mediterranean region (Laguna *et al.*, 2004). As for the Spanish populations (particularly CAB and TEL), conservation efforts should be primarily aimed at removing negative factors (e.g. fencing off the area of CAB used as a dump) and enhancing the establishment of new individuals by means of controlled seed sowing.

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Appendix: Table 4 Classification of the taxa of *Sonchus* section *Pustulati* according to the categories and criteria of the IUCN Red List (IUCN 2012). Categories: EN, endangered; VU, vulnerable; DD, deficient data.

Taxa	IUCN category and criteria	Confirmed IUCN subcriteria	Discarded criteria
Spanish *S. pustulatus*	EN B1ab(i,ii,iv,v)+2ab(i,ii,iv,v); C1	-	-
Moroccan *S. pustulatus*	DD	EN B1a+2a; VU B1a+2a	D
S. fragilis	DD	EN B1a+2a; VU B1a+2a	C, D
S. masguindalii	DD	VU B1a+2a	C, D

Capítulo 3:

CARACTERIZACIÓN ECOLÓGICA DEL HÁBITAT ASOCIADO A LAS ESPECIES DE *SONCHUS* SECCIÓN *PUSTULATI*, UN PEQUEÑO GRUPO DE ENDEMISMOS RUPÍCOLAS DEL MEDITERRÁNEO OCCIDENTAL

ECOLOGICAL CHARACTERIZATION OF THE HABITAT ASSOCIATED WITH *SONCHUS* SECTION *PUSTULATI*, A SMALL GROUP OF CLIFF-DWELLING SPECIES ENDEMIC TO THE WESTERN MEDITERRANEAN

Authors: José L. Silva & José A. Mejías

RESUMEN

Objetivos 1) Estudiar los factores ecológicos que determinan la distribución tan restringida de las especies rupícolas de *Sonchus* sección *Pustulati*; y 2) determinar si, basándonos en datos ecológicos, la distribución actual disyunta en la región Bético-Rifeña de *S. pustulatus* encaja con un proceso de vicarianza más que con eventos de dispersión a larga distancia.

Ubicación Región Bético-Rifeña (Mediterráneo occidental). *Sonchus masguindalii* y *S. fragilis* están confinadas a áreas pequeñas no superpuestas del norte de Marruecos (Rif central y noroccidental, respectivamente). *S. pustulatus* es un taxón vicariante que ocurre en el norte de Marruecos (Rif occidental) y en el sureste de España (oeste de la ciudad de Almería).

Métodos Muestreamos prácticamente todas las poblaciones conocidas de la sección. Un número de parcelas regularmente distribuidas a lo largo de los roquedos nos permitió registrar los componentes de los conjuntos de plantas y los rangos fisiográfico y edáfico. Los especies acompañantes fueron clasificadas según los tipos de hábitat y corológico. Calculamos una serie de índices de diversidad alfa y analizamos la diversidad beta por medio de análisis de correspondencia sin tendencia (DCAs).

Resultados Las especies de *Sonchus* sección *Pustulati* muestraron un rango ecológico bastante estrecho. Acompañando a estas especies, registramos un total de 278 taxones de plantas, siendo las especies del grupo *Pustulati* las dominantes con la excepción de *S. pustulatus* en su rango español. Prácticamente en todos los sitios muestreados, el grupo de especies exclusivas de hábitat rupícola mostró la riqueza de especies más baja y la abundancia más alta, y el grupo de especies típicas de otros hábitats exhibió la riqueza de especies más alta y la abundancia más baja. El grupo de especies Mediterráneas mostró la mayor riqueza de especies en casi todos los conjuntos de plantas, seguidos por el grupo de plantas Ibéricas y/o del norte de África. Los DCAs no separaron claramente los sitios españoles del resto únicamente cuando usamos la categoría de género.

Conclusiones principales Los acantilados donde se asientan las especies de *Sonchus* sección *Pustulati* suponen un refugio microclimático contra la fuerte estacionalidad del actual régimen mediterráneo. Las numerosas afinidades ecológicas (abióticas y florísticas) detectadas entre el área española de *S. pustulatus* y las áreas marroquíes de los tres taxones apoyan que su actual distribución podría ser el resultado de una restricción demográfica de un grupo de plantas que alguna vez prosperaron en áreas más extensas o conectadas entre sí en el arco de Gibraltar durante los tiempos del Mioceno tardío-Plioceno.

Palabras claves Amplitud ecológica; Arco de Gibraltar; Biogeografía; Conjunto de plantas; Distribución disyunta; Diversidad de hábitat y colorógica; Ecología rupícola; Refugio; Región Bético-Rifeña.

ABSTRACT

Aims 1) To study the ecological factors determining the very restricted distribution of the cliff-dwelling species of *Sonchus* section *Pustulati*; and 2) to assess whether, based on ecological data, the current disjunct distribution in the Baetic-Rifan region of *S. pustulatus* fits with a vicariance process rather than long-dispersal events.

Location Baetic-Rifan region (Western Mediterranean). *Sonchus masguindalii* and *S. fragilis* are confined to non-overlapped small areas in northern Morocco (central and northwestern Rif, respectively). *S. pustulatus* is a vicariant taxon occurring both in northern Morocco (western Rif) and in southeastern Spain (west of Almeria city).

Methods We virtually sampled all known populations of the section. A number of plots regularly distributed along cliffs enabled us to record the physiographic and edaphic ranges and the components of the plant assemblages. The latter were classified according to habitat and chorological types. We calculated a series of α-diversity indexes and we analyzed the β-diversity by means of detrended correspondence analyses (DCAs).

Results The species of *Sonchus* section *Pustulati* showed a quite narrow ecological range. In total, we recorded 278 plant taxa in their plant assemblages, the species of the *Pustulati* group being the dominant with the exception of *S. pustulatus* in its Spanish range. Virtually in all sampled sites, the species exclusive from cliff habitat showed the lowest species richness and the highest abundance, and the species typical from other habitats exhibited the highest species richness and the lowest abundance. The Mediterranean species group showed the highest species richness in almost all plant assemblages, followed by the Iberian and/or North African ones. DCA did not clearly separate Spanish sites from the remaining only when we used the generic taxonomic category.

Main conclusions The cliffs where the species of *Sonchus* section *Pustulati* are settled suppose a microclimatic refuge against the strong seasonality of the present-day Mediterranean regime. The many ecological affinities (abiotic and floristic) detected between the Spanish area of *S. pustulatus* and the Moroccan areas of the three taxa support that their current distribution might be the result of a demographical restriction of a plant group that once prospered in larger and more connected areas among them in the Gibraltar arc in the late Miocene-Pliocene times.

Keywords Baetic-Rifan region; Biogeography; Cliff ecology; Disjunct distribution; Ecological amplitude; Gibraltar arc; Habitat and chorological diversity; Plant assemblages; Refuge.

1. INTRODUCTION

The Mediterranean rocky slopes and cliffs are the main habitat of many rare and endangered plant species since they offer an environmental and anthropogenic refuge (Polunin, 1980; Albert *et al.*, 2001; Lavergne *et al.*, 2004; Thompson, 2005) despite constitute a harsh habitat, generally unsuitable for most plants (Larson, 2005). Consequently, cliff ecosystems generally contribute more to the biodiversity of the region than their surface coverage would indicate. Within a biological time scale, cliffs provide very stable, predictable and secure environments where many endemic and rare species can persist (Larson, 2005). The relictual and/or restricted nature of most Mediterranean cliff plants is usually tied to a high habitat specificity and low dispersal ability (e.g. García *et al.*, 2002; Picó & Riba, 2002).

Cliff plants and plant assemblages have been in general poorly investigated, probably due to their little economic interest, although they are receiving increasing attention in recent years (Larson *et al.*, 2005). The most widely used approaches have been phylogenetic and demographic analyses. Phylogenetic surveys have been used to explain species origin and range movements (e.g. Torres *et al.*, 2003; Hardy *et al.*, 2004; Guzmán & Vargas, 2009; Vargas *et al.*, 2009; Chapter 4). Demographic studies have shown examples of very high longevity and the difficulties for individual recruitment in the habitat (e.g. Picó & Riba, 2002; García, 2003; Matthes & Larson, 2006; García *et al.*, 2012; Chapter 2). The integration of phylogeographical and demographic analyses with the following additional approaches may result quite helpful in the understanding of the biogeographical significance of plant communities (e.g. Ojeda *et al.*, 1996; Mejías *et al.*, 2007): 1º) the analysis of the ecological limitations (Wiens & Graham, 2005); and 2º) the analysis of the diversity patterns of plant assemblages (Davis, 1951; Molina-Venegas *et al.*, 2013). Due to the high incidence of endemic species on the cliff habitats these approaches may be especially informative.

Sonchus pustulatus Willk., *S. fragilis* Ball and *S. masguindalii* Pau & Font Quer (Asteraceae, Cichorieae) constitue a group of rare cliff-dwelling taxa, which comprise a highly supported clade within the genus (*Sonchus* subgenus, *Sonchus* section *Pustulati* Boulos; Kim *et al.*, 2007). They are restricted to the Baetic-Rifan region (Western Mediterranean Basin) occurring on quite localized cliffs of low altitude (from the sea level to approximately 550m). All the three species are distributed in northwestern Africa, where they have been considered as very rare (Fennane & Ibn Tatoo, 1998), and *S. pustulatus* is the only one localized to the southeastern Iberian Peninsula, where it is categorized as critically endangered (Cueto *et al.*, 2003). The group has received little attention despite their interest as endemic species exhibiting disjunct distribution and as part of the plant assemblages of cliffs.

The entire evolutionary history of the group *Pustulati*, i.e. the origin and diversification on species, seems to have taken place on the Baetic-Rifan region (Chapter 4). During the process, the species could have covered a wider distribution being likely settled on the land bridge connecting the Iberian Peninsula and Africa from the late Miocene to the early Pliocene (7.2–3.6 Ma) and thus occupying near or overlapped areas on adjacent cliffs, or, at least, more connected than they do at present days. This proposal is supported by several facts. First, the distribution of the species is fully restricted to small areas within the Alboran domain (Loget & Van Den Driessche, 2006), just in the

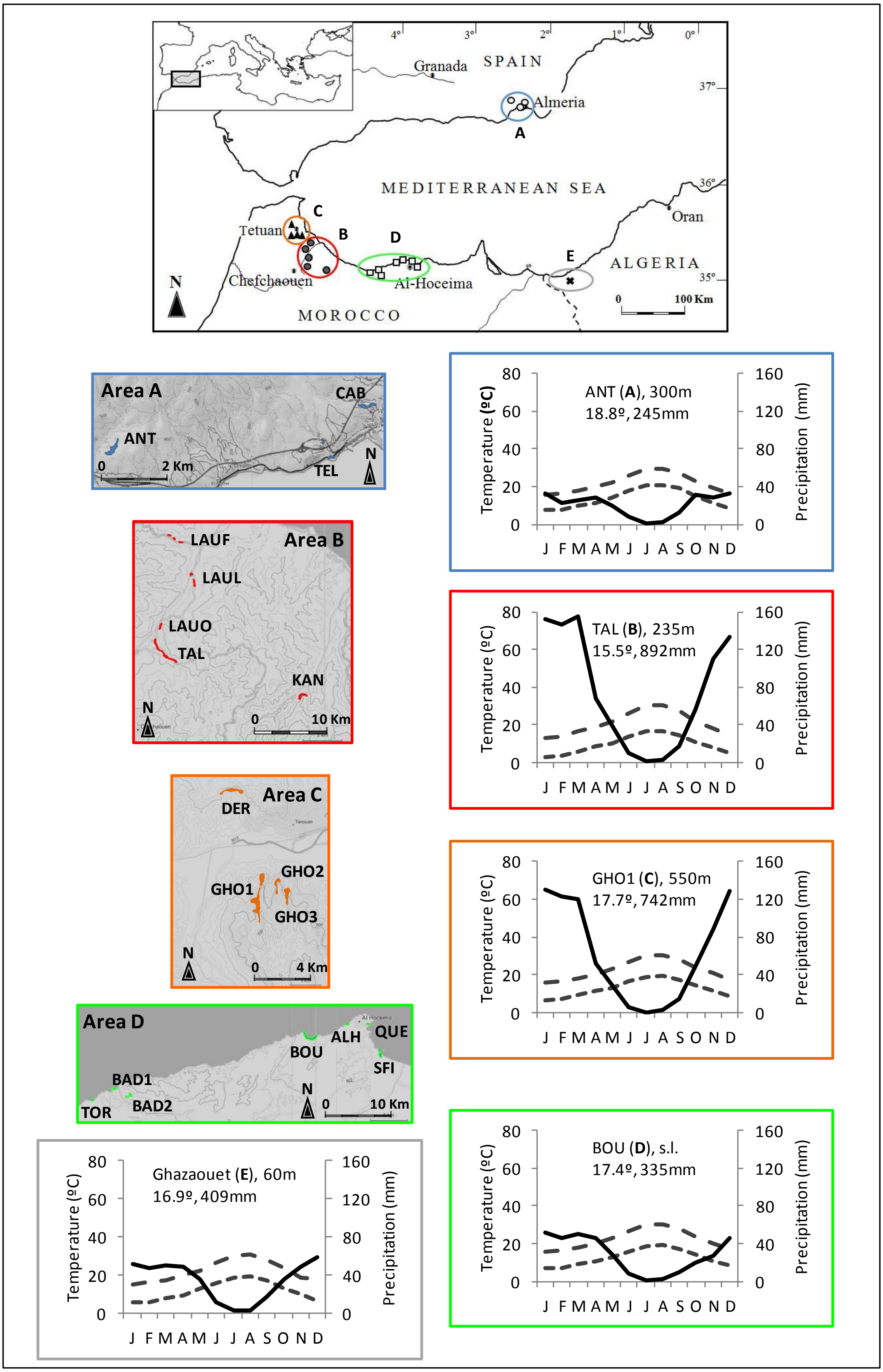
Baetic-Rifan Internal Zone (Rosenbaum *et al.*, 2002; Chapter 1 and 2). Secondly, the origin and diversification of the species of *Sonchus* section *Pustulati* presumably took place 5.5–3.9 Ma ago (Chapter 4), from when the Iberian Peninsula and Africa were connected by a more or less continuous stretch of land (the Gibraltar arc; 5.96–5.33 Ma ago) to when this latter was partially submerged (5.33–3.60 Ma ago). Thirdly, the relatively heavy seeds and the markedly deciduous pappus lead us to presume low seed dispersal ability. Assuming that the geographical expansion of these species is basically due to the movement of their *terrane* (Chapter 1), rather than long-distance dispersion, the current occupied areas may therefore show some footprint of their comoon past in their abiotic and biotic traits (plant assemblages).

Here we analyzed the ecological range of the taxa comprising the section *Pustulati* throughout their distribution in order to understand factors determining their restricted distribution and to assess the level of similarity among the plants assemblages associated with these species. We focused on the following particular objectives: (1) to determine the ecological range of the taxa of *Sonchus* section *Pustulati* studying the physiographic and edaphic traits of their habitats, under the hypothesis that this ecological amplitude should be quite low due to their rarity; (2) to describe the habitat specificity and the chorology diversity of the plant components comprising assemblages associated with *Sonchus* section *Pustulati*; and (3) to study the β-diversity between plant assemblages by multivariate ordination techniques. The last two points aim to determine whether plant assemblages (especially by comparison of the Spanish and Moroccan areas) are similar enough to be an indication of past geographical connection or proximity among the current occupied areas, supporting the relict condition of the species.

2. MATERIAL AND METHODS

2.1 Plant species and distribution areas

The section *Pustulati* of the genus *Sonchus* (Asteraceae) only includes three species: *Sonchus pustulatus* Willk., *S. fragilis* Ball and *S. masguindalii* Pau & Font Quer. They are restricted to several narrow distribution areas within the Baetic-Rifan complex hotspot (Western Mediterranean Basin; Figure 1), mainly on crevices of limestone rocky cliffs near or facing the sea. The rare Spanish populations of *S. pustulatus* occur in the environs of Almeria city (Figure 1, area A, southeastern Spain), whereas Moroccan populations mainly colonize rocky cliffs in the Oued Laou valley, and some neighboring valleys (Figure 1, area B, western Rif). *S. fragilis* is exclusive to the mountains surrounding the city of Tetouan (Figure 1, area C, northwestern Rif). Moreover, *S. masguindalii* is distributed along the coast in the Bokkoya Mountains and some sea cliffs in the environs of Al-Hoceima city (Figure 1, area D, central Rif). Some herbarium specimens of *S. pustulatus* collected in the 19th century (e.g. herbarium COI) indicate that the species was also present in rocky places on the oceanfront near Ghazaouet (formerly Nemours; Figure 1, E, northwestern Algeria), but we were unable to visit this area and their presence therefore remained unconfirmed.


4°
3°
2°
1°
0°
Granada
SPAIN
Almeria
A
37°
MEDITERRANEAN SEA
36°
Oran
C
Tetuan
B
D
E
ALGERIA
35°
N
Chefchaouen
Al-Hoceima
0
100 Km
MOROCCO
Area A
CAB
ANT
0
2 Km
TEL
N
Area B
LAUF
LAUL
LAUO
TAL
KAN
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0
10 Km
Area C
DER
GHO2
GHO1
GHO3
N
0
4 Km
Area D
ALH
QUE
BOU
SFI
BAD1
BAD2
TOR
N
0
10 Km
ANT (A), 300m
18.8º, 245mm
TAL (B), 235m
15.5º, 892mm
GHO1 (C), 550m
17.7º, 742mm
BOU (D), s.l.
17.4º, 335mm
Ghazaouet (E), 60m
16.9º, 409mm
Temperature (ºC)
Precipitation (mm)
0
20
40
60
80
0
40
80
120
160
J F M A M J J A S O N D

Figure 1 Geographical distribution areas (A-E) of the taxa of *Sonchus* section *Pustulati*, areas of occupancy of populations and ombrothermal diagrams. Area A, Spanish *S. pustulatus*; Area B, Moroccan *S. pustulatus*; Area C, *S. fragilis*; Area D, *S. masguindalii*; and Area E, Algerian *S. pustulatus* (current presence not confirmed).

These cliff-dwelling species are long-lived suffrutescent chamaephytic plants, usually procumbent, which develop moderate-long branches, particularly lignified in *S. masguindalii*. Depending on species, individuals range approximately from only 5 cm^2 to 2 m^2 in surface and can reach up to 45 cm in height, with somewhat intricate branching. Fruits are achenes that bear a highly deciduous pappus and are mainly released during late spring and early summer.

2.2 Climate in the distribution areas

Even though the group of studied plants colonizes areas of Mediterranean climate, important differences can be pointed out between them, mainly on the precipitation (Figure 1). The climatic data were obtained from Hijmans *et al.* (2005) for each geographical distribution area and the consequent classification in climatic types was based on Peel *et al.* (2007). Area **A** (southeastern Spain) has an arid steppe cold climate type (*Bsk*), with a total annual precipitation (PREC) of 249 mm and an annual mean temperature (TEMP) of 17.6ºC. The areas **D** (central Rif) and **E** (northwestern Algeria) show the typical Mediterranean climate (*Csa*), with similar temperature (TEMP = 17.3 and 16.9ºC, respectively), although somewhat wetter (PREC = 346 and 409 mm respectively). By contrast, both area **B** (western Rif) and the adjacent area **C** (northwestern Rif) are included in a rainier region where PREC reach 844 mm and 742 mm, respectively (TEMP = 15.5ºC and 17.7ºC), despite also show a Mediterranean climate type (*Csa*).

2.3 Study of plant assemblages and abiotic traits of cliffs

The sampling of the plant assemblages associated with the populations of *Sonchus* section *Pustulati* and the physiographic traits of their habitats was conducted using homogeneously distributed sampling plots (2×2 m) during May and June of 2008. In order to adapt the sampling strategy to the fragmented and clumped nature of cliff-dwelling plants and to the *Pustulati* population sizes, the number of plots (6–30, mean 21.5; Table 1) and distances between plots varied. Sampling plots were placed along the cliffs alternately at low (0–2 m from the cliff base), medium (2–4 m) or high (>4 m) transects. When plots were inaccessible we used binoculars to the sampling. Most of the spatial range of plant occurrence was covered with this sampling system because either the cliffs were not higher than this or the individuals did not occur on the higher parts. When populations occurred in a defile (KAN; Appendix: Figure 8) or in a narrow valley (LAUF) and plants were settled on both cliff sides, plots were alternatively established in two parallel transects; one on each cliff.

In each plot we inventoried all plant species including ferns, and we took plant samples for subsequent taxonomic identification in the laboratory. This end was performed by means of Valdés *et al.* (2002) and Blanca *et al.* (2011). We updated and fitted the nomenclature with the following sources according to this order of priority: Euro+Med

(2006–2012), Greuter *et al.* (1984, 1986, 1989), Mateos & Valdés (2009, 2010a, 2010b), Valdés *et al.* (2002) and Blanca *et al.* (2011).

We also recorded the following physiographic traits of the habitats in each plot: geographical coordinates, cliff geographical orientation (with the GPS GARMIN ETREX VISTA HCX), slope (with a protractor) and height of the cliff (by visual estimation). The conservation state of the cliff in each plot was assessed using a semi-quantitative scale: 3, highly conserved; 2, conserved but with some scree; 1, deteriorated cliff with large scree; and 0, with debris and/or some garbage dumps. We also registered the highest and lowest heights where individuals of *Sonchus* section *Pustulati* occurred at the vertical projection of each sampling plot.

In addition, in each sampled population area (site hereafter), we collected 2–3 samples of soil from different crevices and mixed into a single one, reaching at least 250g by site, which were sent to Agrama Labs (www.laboratorioagrama.com) for chemical analyses. The following parameters were obtained: pH (at 25ºC 1/5), electric conductivity (mS/cm, at 25ºC 1/5), granulometry (% of clay, silt and sand), carbonates (%), active lime (%), oxidizable organic matter (%), concentrations of macronutrients (mg/Kg of N-Kjeldahl, and Olsen P) and micronutrients (mg/Kg of Cu, Fe, Mn, Zn and B), assimilable cations (meq/100g of Ca, Mg, K and Na) and Ca/Mg, K/Mg and C/N ratios.

Table 1 Coordinates and geological substrate of the sampled sites where the species of *Sonchus* section *Pustulati* are settled. No. plots, number of sampled plots by site. * Information was taken from: Cartografía geológica MAGNA (1:50.000) from the Instituto Geológico y Minero de España, sheets 1045, 1058 and 1059 (1976–1981); Carte Géologique du Maroc 1:1.000.000, Service Géologique du Maroc (1985); New geological and mining guidebooks of Morocco (Volume 5; 2011).

Species, sites	Altitude (msnm)	Coordinates (North /West)	Era*	Period*	Geologic unit*	Lithology*	Population size	No. plots
SPAIN, southeast								
Sonchus pustulatus							898	
ANT	300	36º49.8’ / 2º34.4’	Mesozoic	Middle Triassic-Upper Triassic		Calc-schists, dolostones and limestones	484	30
TEL	22	36º49.7’ / 2º29.0’	Mesozoic	Middle Triassic-Upper Triassic		Calc-schists, dolostones and limestones	112	18
CAB	100	36º50.6’ / 2º28.7’	Mesozoic	Middle Triassic (Ladiniense)		Dolostones and limestones	292	30
MOROCCO								
S. pustulatus - W Rif							9991	
LAUF	120	35º23.3’ / 5º12.6’	Palaeozoic	Ordovician-Lower Silurian	Akaili thrust-sheet	Schists and sandy slates	1062	25
LAUL	153	35º20.8’ / 5º11.0’	Mesozoic	Middle Triassic-Lower Jurassic	Calcareous ridge	Dolostones and limestones	540	25
LAUO	150	35º17.4’ / 5º13.8’	Mesozoic	Upper Triassic-Lower Jurassic	Calcareous ridge	Dolostones and limestones	221	20
TAL	235	35º16.0’ / 5º13.8’	Mesozoic	Upper Triassic-Lower Jurassic	Calcareous ridge	Dolostones and limestones	1980	30
KAN	244	35º13.0’ / 5º01.2’	Mesozoic	Upper Triassic-Lower Jurassic	Calcareous ridge	Dolostones and limestones	6188	25
S. fragilis - NW Rif							33927	
GHO1	550	35º32.1’ / 5º23.5’	Mesozoic	Middle Triassic-Lower Jurassic	Calcareous ridge	Dolostones and limestones	26985	30
GHO2	550	35º32.8’ / 5º22.6’	Mesozoic	Middle Triassic-Lower Jurassic	Calcareous ridge	Dolostones and limestones	-	-
GHO3	550	35º32.3’ / 5º22.3’	Mesozoic	Middle Triassic-Lower Jurassic	Calcareous ridge	Dolostones and limestones	661	24
DER	275	35º35.9’ / 5º24.6’	Mesozoic	Upper Triassic-Lower Jurassic	Calcareous ridge	Dolostones and limestones	6282	15
S. masguindalii - central Rif							42269	
TOR	0	35º09.4’ / 4º19.7’	Mesozoic	Upper Triassic-Lower Jurassic	Calcareous ridge	Dolostones and limestones	6244	10
BAD1	0	35º10.2’ / 4º17.9’	Mesozoic-Cenozoic	Jurassic-Upper Cretaceous-Eocene	Calcareous ridge	Limestones and marls	6444	26
BAD2	60	35º09.6’ / 4º16.9’	Mesozoic-Cenozoic	Jurassic-Upper Cretaceous-Eocene	Calcareous ridge	Limestones and marls	638	20
BOU	0	35º14.0’ / 4º00.7’	Palaeozoic	Silurian-Devonian	Akaili thrust-sheet	Limestones and slates	19513	25
ALH	0	35º14.6’ / 3º58.0’	Mesozoic	Middle Triassic-Jurassic	Calcareous ridge	Dolostones, limestones and clays	688	6
QUE	0	35º14.5’ / 3º55.5’	Mesozoic	Middle Triassic-Lower Jurassic	Calcareous ridge	Dolostones and limestones	6708	6
SFI	0	35º12.8’ / 3º54.3’	Mesozoic & Quaternary	Triassic-Lower Jurassic & Quaternary	Calcareous ridge	Limestones and calcareous conglomerates	2035	22

2.4 Analyses of the α-diversity of plant assemblages

The following parameters were calculated to assess the α-diversity in the plant assemblages (i.e. diversity within sites): species richness, family richness, plant abundance, Evenness index (Pielou, 1969), Simpson's Index of Diversity (Lande, 1996), endemic species level (*section 2.5*) and taxonomic singularity. The species richness and family richness were calculated as the number of species and families registered in each site, respectively. The abundance for every single species was estimated as the proportion of the number of plots where a particular species was recorded by site (weighted frequency). We obtained the Evenness index (focused on rare species abundance) and the Simpson's Index of Diversity (strongly influenced by the importance of the dominant species; Magurran, 1988) with the statistical software VEGAN package v.2.0–4 (Oksanen *et al.*, 2012), implemented in the R software environment version 2.15.0 (R Core Development Team, 2012).

The taxonomic singularity of the plant assemblages was calculated as the inverse of the infra-generic diversity (e.g. Ojeda *et al.*, 1996). The number of co-generic species at the Mediterranean level was recorded independently for each single registered species in the sites. Then, the average of these values for all those species detected in each site was considered the infra-generic diversity for each site. Co-generic species information were extracted from Euro+Med (2006–2012) and Greuter *et al.* (1984, 1986, 1989) except for the genera *Centranthus*, *Fedia*, *Lycium*, *Ruta*, *Theligonium*, *Viola* and *Withania*, whose information was taken from Post & Dinsmore (1932–1933), Ozenda (1958), Quézel & Santa (1962–1963), Tutin *et al.* (1964–1980), Davis (1965–1984), Muschler (1970), Ali & Jafri (1977), and Jafri & El Gadi (1977).

2.5 Ecological and biogeographical analyses of plant assemblages

In order to analyze the ecological and biogeographical diversity patterns of the plant assemblages, we classified the species according to the following criteria. First, we separated three groups according to the habitat type: species exclusive from rocky habitats (*R*), species with broader habitat amplitude, non-exclusive from rocky habitat (*R+*), and species typical from other types of habitats (*Not R*). To this end, we took information from Mateos & Valdés (2009, 2010a, 2010b), Blanca *et al.* (2011) and herbarium specimens from SEV (e.g. for the Moroccan Poaceae species). Secondly, we arranged the plant species into six chorological types: Introduced (IN), Cosmopolitan (C), Mediterranean-Eurosiberian (ME), Circum-Mediterranean (M), Western Mediterranean (WM) and Iberian and/or North African (IBNA). To this end, we took information regarding species distribution from Euro+Med (2006–2012) but other sources were also consulted when it was necessary: Greuter *et al.* (1984, 1986, 1989), Mateos & Valdés (2009, 2010a, 2010b), Valdés *et al.* (2002) and Blanca *et al.* (2011). These areas approximately correspond with floristic units used in phytogeography (e.g. Takhtajan & Crovello, 1986). For simplicity, we considered the Macaronesian range as a part of the Mediterranean. Three levels of endemic species were considered for the species with a narrow distribution: *Ibero-North African*, *Iberian* and *North African*.

2.6 Statistical analyses

We performed the statistical analyses with SPSS software (SPSS for Windows, ver.15.0.1, 2006, Chicago: Inc.) with the exception of the canonical analyses. To detect the general ecological range across 18 cliff sites of the 19 ones where the species of the section *Pustulati* have been found (Capítulo 2), the site data were organized into: a) a single 5 physiographic traits × 387 sampled plots matrix; and b) a single 23 edaphic traits × 18 sites matrix. Each matrix was submitted to a Principal Component Analysis (PCA) based on the correlation matrix of variables. For a), geographic orientation was transformed to north deviation in order to better manage the general north facing of the sampled cliffs. Moreover, Kruskal Wallis tests (between groups) and Mann Whitney tests (between pairs of groups) were performed to compare: i) the weighted frequency of all registered species in each site according to their different habitat types; ii) the taxonomic singularity of all registered species in the whole sampling according to their different habitat types; and iii) taxonomic singularity of all registered species in each site according to their geographical distribution areas. Unless indicated, the null hypothesis was rejected at a significance level of at least 0.05. Means ± SE are indicated in the text.

For examination of the β-diversity among the plant assemblages associated with the species of *Sonchus* section *Pustulati* (i.e. with their sites), four detrended correspondence analyses (DCA) were performed to examine the gradients in the floristic composition at genus and species levels with the software CANOCO ver.4.52 (Ter Braak & Smilauer, 2002). For each DCA, down-weight was applied to rare species and detrending was performed with second order polynomials due to the unequivocal unimodal response along the gradient (the gradient length, expressed in standard deviation units, was over 4 SD). The first DCA included all registered species and the second one, all registered genera. In addition, we also performed two DCAs with the same configuration taking into account first only *R* and *R+* species, and secondly, only *R* species, removing in both cases the *No R* species, with high species richness but usually with very low abundances.

3. RESULTS

3.1 Environmental range of the habitats of Sonchus section Pustulati

The three species as a whole exhibited a narrow ecological range (Table 2; Appendix: Table 5; Appendix: Table 6). They generally colonized quite well conserved cliffs with a steep slope (77.0 ± 0.88%; Appendix: Figure 8) and mainly facing to the north (356.3 ± 3.68º; Figure 2). Exceptionally, plants from the population DER of *S. fragilis* grow on SE-S-facing cliffs (165 ± 11.3º), and those from LAUF (in a narrow valley) and KAN (in a defile) of *S. pustulatus* are settled on north (LAUF-a: 26 ± 6.8º, KAN-a: 19 ± 6.3º) and south (LAUF-b: 237 ± 18.2º, KAN-b: 223 ± 9.9º) facing cliffs. The plants of *Sonchus* section *Pustulati* use to colonize cliffs from the base (3.0 ± 0.43 m) to a mean maximum height of 11.7 ± 1.47 m. The *S. masguindalii* individuals generally reached the highest levels (17.7 ± 1.27 m) together with those of *S. fragilis* in DER (21.2 ± 3.80 m) and GHO1 (18.6 ± 3.8 m). With regard to the edaphic traits, all sites showed quite alkaline soils (pH 8.3 ± 0.01) with variable salinity (conductivity 1.1 ± 0.26), generally higher in the sites closest to the sea; particularly in those of *S. masguindalii*. Soils also exhibited a variable granulometry, mainly with loam texture (43.0 ± 0.77% of sand and 24.2 ± 0.51% of clay), commonly rich in assimilable Ca (34.9 ± 1.11 meq/100g), optimal organic matter (5.2 ± 0.15%) and poor in the assimilable K (0.9 ± 0.02%).

Table 2 Environmental range and mean values for the physiographic and edaphic traits of the habitats of *Sonchus* section *Pustulati* (n = 18). CV, coefficient of variation.

Environmental features	Mean	Min–max	CV (%)
Physiographic			
Geographic orientation (º)	356.3	181–180	20
Slope (º)	77.0	9–110	22
State (0–3)	2.5	0–3	26
Height (m)	23.9	1–150	105
Plant cover (%)	24.0	0–100	84
Edaphic			
pH (25ºC)	8.3	8.0–8.8	3
Conductivity (mS/cm)	1.1	0.2–6.6	130
Granulometry			
Clay (%)	24.2	10–47	41
Silt (%)	31.8	12–54	33
Sand (%)	43.0	16–68	34
Carbonates (%)	13.1	0.5–33.7	81
Active lime (%)	3.8	1.5–12.1	72
OOM (%)	5.2	0.9–11.1	57
[Macronutrients] (mg/Kg)			
N Kjeldahl	3 105.1	626–6 244	54
P Olsen	27.1	5–78	75
Ca	34.9	5.8–80.3	62
Mg	8.0	1.5–13.8	51
K	0.9	0.2–1.8	44
Na	4.9	0.1–31.2	162
[Micronutrients] (mg/Kg)			
Cu	1.1	0.2–33.4	68
Fe	14.1	2.4–27.0	61
Mn	18.1	1.8–44.2	62
Zn	5.6	3.1–32.6	105
B	1.1	0.4–2.7	57
C/N	5.8	3.1–11.5	44
Ca/Mg	5.7	0.9–17.2	81
K/Mg	0.1	0.1–0.4	67

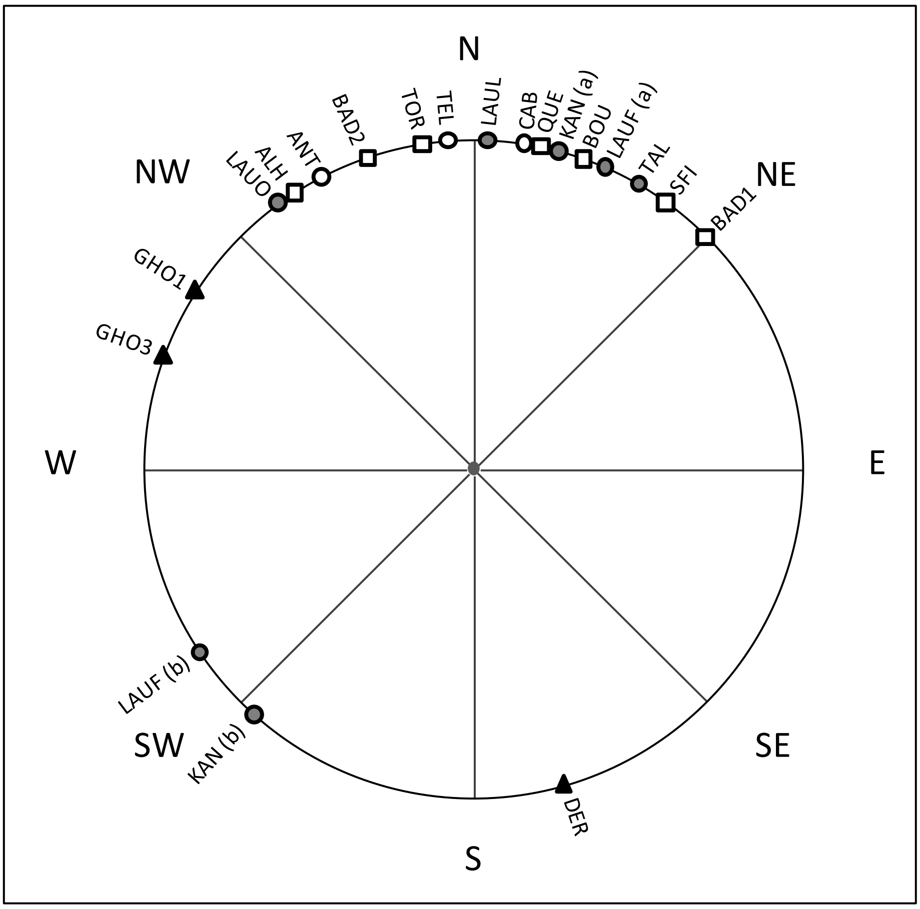

Figure 2 Mean geographic orientation of the cliffs where the populations of *Sonchus* section *Pustulati* are settled: Spanish *S. pustulatus* (white circles), Moroccan *S. pustulatus* (grey circles), *S. fragilis* (solid triangles) and *S. masguindalii* (white squares). For populations located in opposite cliffs, orientation was represented separately for each side (a, b) to avoid misinterpretation.

In the PCA ordination based on physiographic traits, most plots were quite concentrated and mixed whatever their origin (Figure 3a). The most spread plots were those from the Moroccan sites of *S. pustulatus*, indicating greater physiographic amplitude. In the PCA ordination based on edaphic traits, sites were mixed without any clear pattern with regard to their distribution areas (Figure 3b). Only, *S. masguindalii* sites showed an edaphic range slightly wider than the remaining.

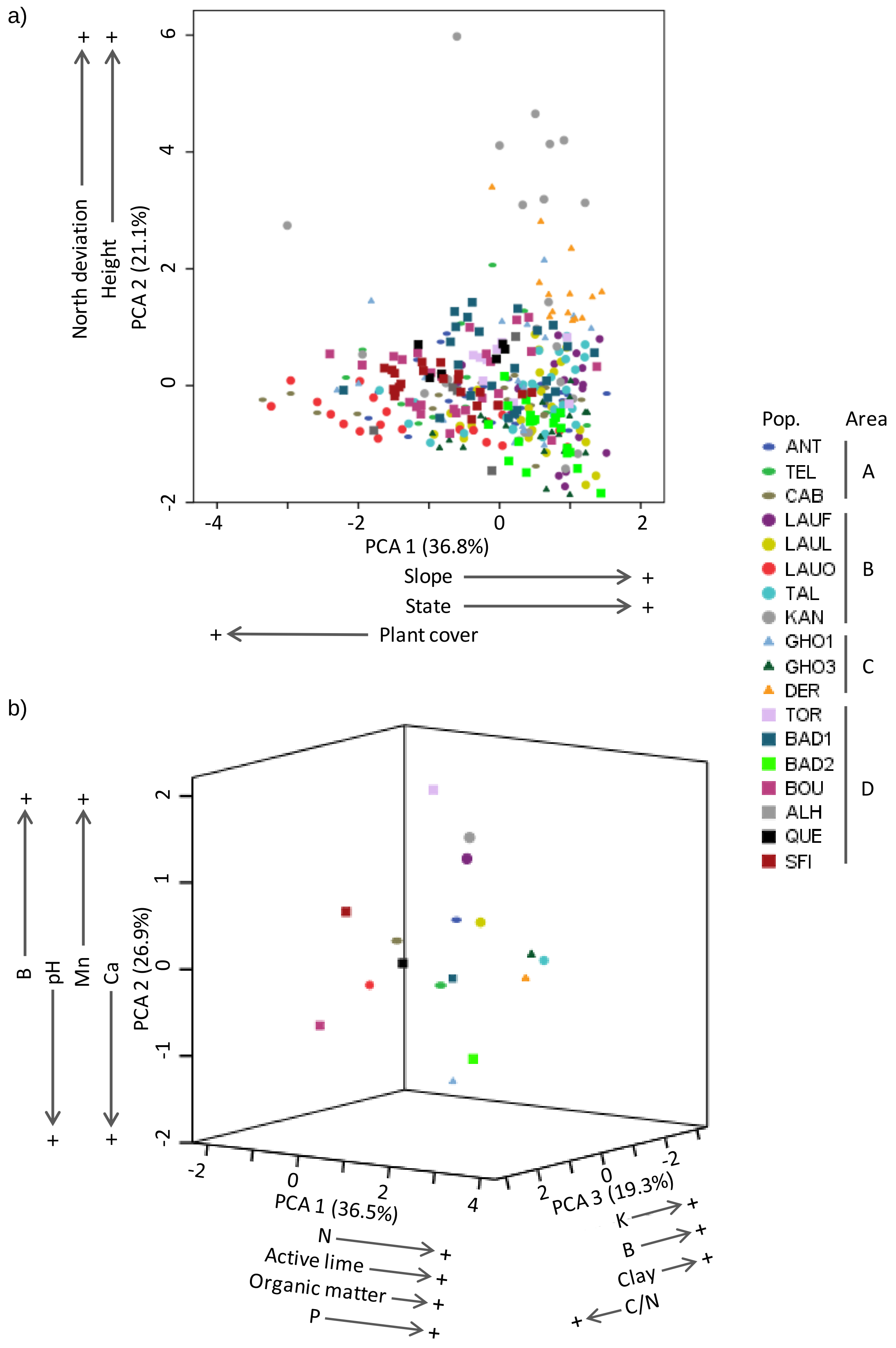

Figure 3 PCA ordinations of: a) the 387 sampled plots from the 18 sampled sites of the species of *Sonchus* section *Pustulati* based on five physiographic traits; and b) the 18

sampled sites based on 23 edaphic traits. Labels display traits with the highest eigenvector scores on PCA axes, with the label with the highest score presented nearest to the axis. Values in parentheses are variance explained by each axis. See Materials and Methods section for details of traits and Figure 1 (A–D) for the geographical areas.

3.2 α-diversity of plant assemblages

A total collection of 278 different plant species (196 genera and 72 families) were recorded in the 18 sites sampled (Appendix: Table 7). The mean number of species per site was 42.9 ± 5.34, and it was exceptionally high in GHO1 (104 species; Table 3). Many species presented very low abundance: the 61% of them exhibited weighted frequencies lower than 10%, the 34% ranged between 10–50% and exclusively the 5% of species showed values higher than 50%. Family richness was quite similar between distribution areas (mean 21.9 ± 1.96; Table 3). Asteraceae, Poaceae and Lamiaceae were the most represented families (Figure 4).

The taxa of *Sonchus* section *Pustulati* showed the following mean weighted frequencies: the Spanish *S. pustulatus* 58.1 ± 1.48%, the Moroccan *S. pustulatus* 71.1 ± 7.38%, *S. fragilis* 65.6 ± 10.94% and *S. masguindalii* 85.5 ± 3.23%. They were always the dominant species in the Moroccan plant assemblages, particularly in the case of the sites of *S. masguindalii* (77–100%) and with exception of the LAUO site of *S. pustulatus* where the most common taxon was *Lavandula dentata* (65%). The dominant species in Spanish sites were: *Teucrium rotundifolium* (77%) in ANT, *Teucrium intricatum* in TEL (72%) and *Pallenis maritima* and *Melica minuta* subsp *latifolia* in CAB (93% and 63%, respectively). Species with high weighted frequency values in Moroccan sites were

Table 3 α-diversity of the plant assemblages associated with the species of *Sonchus* section *Pustulati*. Sites are labelled as in Table 1. *Pustulati* abundance, weighted frequency of the species of *Sonchus* section *Pustulati*; E, Evenness index; 1–D, Simpson's Index of Diversity.

Localities	Family richness	Species richness	Taxonomic singularity (Mean ± SE)	Species abundance (Mean % ± SE)	*Pustulati* abundance (%)	E	1–D
S. pustulatus - Spain, southeast							
ANT	24	54	0.18 ± 0.037	14.0 ± 2.03	57	0.63	0.04
TEL	26	43	0.16 ± 0.038	19.0 ± 2.79	61	0.60	0.04
CAB	27	57	0.13 ± 0.025	16.8 ± 2.33	57	0.64	0.04
S. pustulatus - Morocco, western Rif							
LAUF	28	53	0.20 ± 0.040	10.6 ± 1.96	88	0.62	0.05
LAUL	30	61	0.21 ± 0.041	10.3 ± 1.44	76	0.66	0.04
LAUO	17	44	0.12 ± 0.028	19.7 ± 2.61	45	0.61	0.04
TAL	32	64	0.16 ± 0.032	8.9 ± 1.30	67	0.66	0.04
KAN	26	47	0.27 ± 0.046	10.7 ± 1.93	80	0.61	0.05
S. fragilis - Morocco, northwestern Rif							
GHO1	40	104	0.15 ± 0.026	11.7 ± 1.35	87	0.75	0.02
GHO3	25	56	0.17 ± 0.040	10.6 ± 1.44	50	0.65	0.04
DER	18	25	0.20 ± 0.055	14.7 ± 2.34	60	0.53	0.06
S. masguindalii - Morocco, central Rif							
TOR	10	14	0.21 ± 0,092	22.1 ± 6.31	100	0.41	0.15
BAD1	22	40	0.24 ± 0.051	15.9 ± 2.76	77	0.58	0.05
BAD2	19	35	0.21 ± 0.050	17.1 ± 3.05	75	0.56	0.06
BOU	13	23	0.21 ± 0.050	16.5 ± 3.85	88	0.48	0.10
ALH	8	11	0.20 ± 0.087	51.5 ± 7.60	83	0.40	0.11
QUE	13	15	0.13 ± 0.066	37.8 ± 6.40	83	0.45	0.09
SFI	17	28	0.15 ± 0.043	17.9 ± 3.84	91	0.51	0.08

Crithmum maritimum in ALH (83%), *Sonchus tenerrimus* in QUE (83%) and *Erica multiflora* in GHO1 (80%). The following species were recorded in 11 sites: *Pallenis maritima* (in the geographical areas of Spanish *S. pustulatus* and *S. masguindalii*), *Sedum sediforme* (in the four distribution areas) and *Pistacia lentiscus* (absent in Spanish plant assemblages), whose frequencies ranged between 15–93%, 4–40% and 5–37%, respectively.

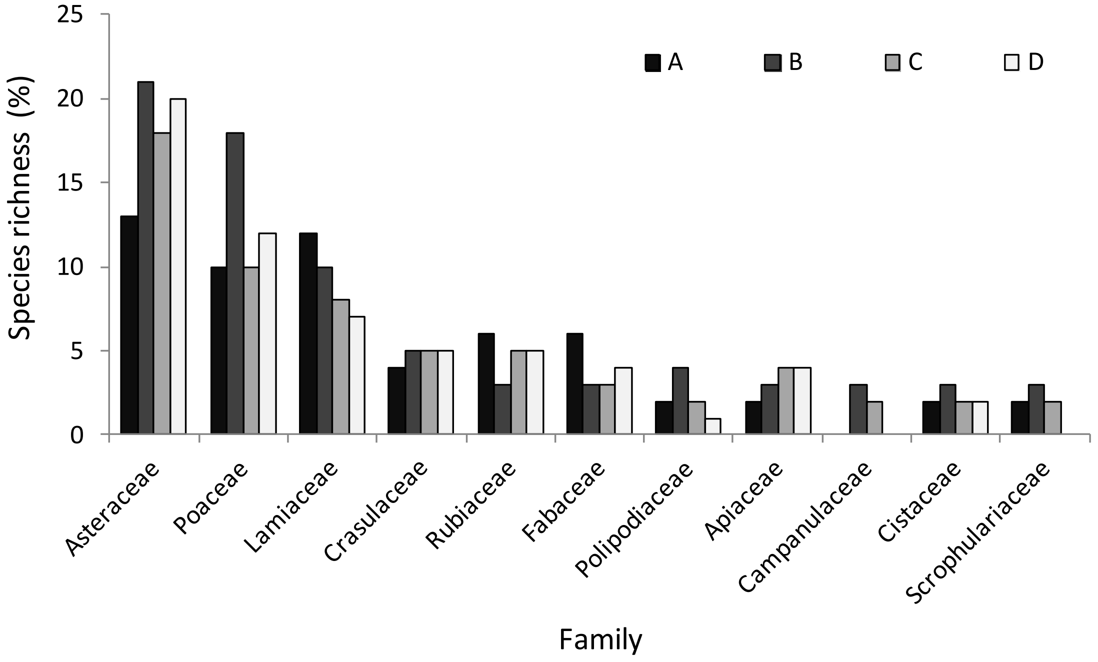

Figure 4 Species richness by distribution areas (A-D, Figure 1) of the families most represented in the plant assemblages associated with *Sonchus* section *Pustulati*.

The Evenness index always showed relatively high values (min-max 0.40–0.75, Table 3), being lower in *S. masguindalii* sites (mean 0.48 ± 0.026). On the contrary, the Simpson’s Index of Diversity, was quite low in all sites (min-max 0.02–0.15), being generally higher in those of *S. masguindalii* (Table 3). The high number of species with very low abundance registered and the low number of species with high abundances led to these differences.

3.3 Habitat type diversity of components within plant assemblages

Among the whole set of 278 plant species recorded in the 18 sites, 48 of them were exclusive from rocky habitats (*R*), another 60 were not exclusive from rocky habitat but usually common in others (*R+*) and the remaining 170 species were typical from no rocky habitats (*Not R*). The species richness by each habitat type was shockingly very similar in the four distribution areas (Figure 5a). The *Not R* species group, in general, showed the highest species richness by site (mean 49.7 ± 1.71%) followed by the *R+* ones (mean 32.0 ± 2.52%). The *R* species group commonly had the lowest number of species (mean 18.3 ± 1.76%), but exceptionally in some sites of *S. masguindalii* (ALH, QUE and BAD1) it was the most diverse group (46, 47 and 50%, respectively). Moreover, only in the three plant assemblages associated with *S. fragilis* the proportions of the *R* species (24–36%) were higher than those of the *R+* species (12–21%).

The very similar proportions of species richness by each habitat type across the four distribution areas were not reflected in terms of abundance. The mean weighted frequencies of species by each habitat types significantly differed in nine sites from the four distribution areas: ANT, CAB, LAUF, LAUL, KAN, GHO3, BAD1, BOU and SFI (χ^2 = 6.25–11.67, P = 0.003–0.044). Differences depended mainly on the higher abundance of the *R* species compared to the *Not R* ones (U = 1–103, P = 0.001–0.045). The *R* species only showed significantly higher abundance values than the *R+* ones in LAUL (U = 47, P = 0.019), from the Moroccan *S. pustulatus* area. The abundance of the *R+* species was significantly higher than that of the *Not R* ones exclusively in two sites: GHO3 from the area of *S. fragilis* and CAB from the Spanish area of *S. pustulatus* (U = 115 and 95, P = 0.002 and 0.026, respectively).

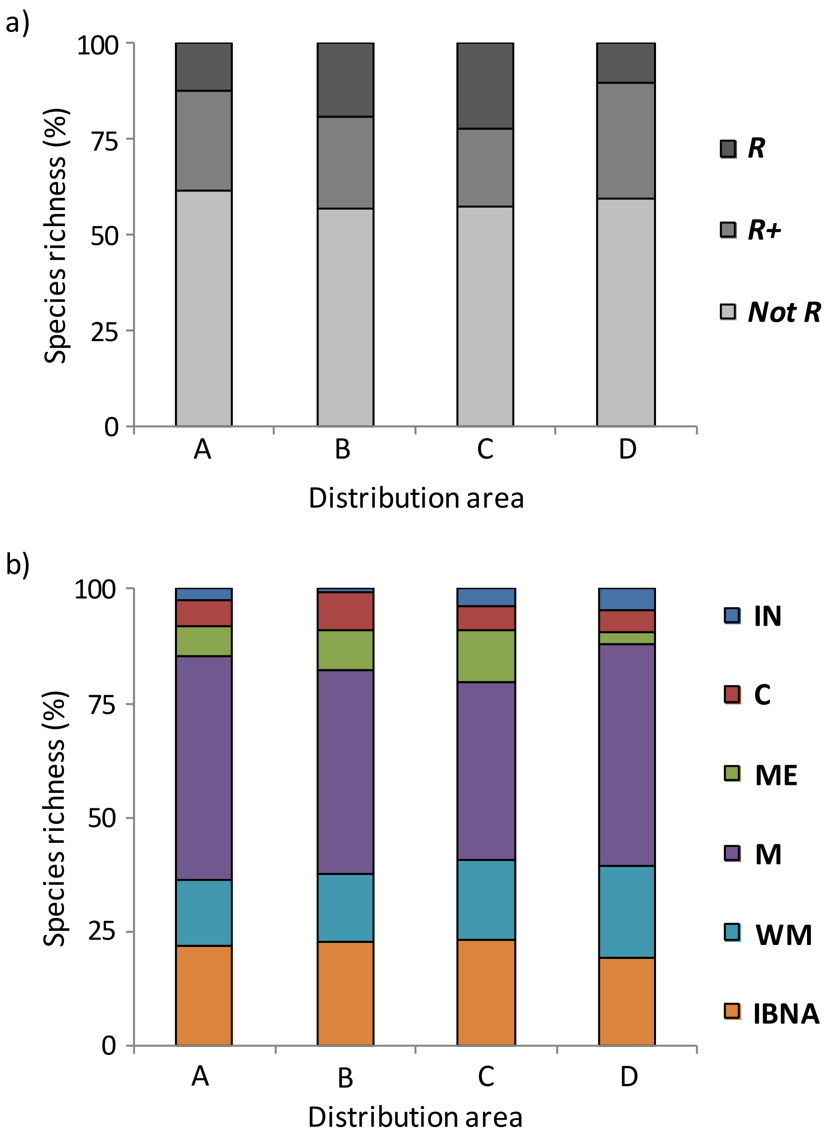

Figure 5 Diversity of habitat (a) and chorological (b) types of the species registered in the plant assemblages associated with the taxa of *Sonchus* section *Pustulati*. These are represented by distribution areas (A-D, Figure 1). See Materials and Methods section for details and full names of the habitat and chorological types.

3.4 Taxonomic singularity

The taxonomic singularity of the plant assemblages ranged from 0.12 ± 0.028 to 0.27 ± 0.046 (LAUO and KAN, respectively, both from the Moroccan area of *S. pustulatus*; Table 3) and did not show significant differences between geographical distribution areas ($X^2 = 3.522$; $p = 0.318$). It was slightly higher for the *Not R* species (0.21 ± 0.023) than for the *R* (0.17 ± 0.44) ($U = 3234$, $P = 0.03$) and *R+* (0.12 ± 0.026) ($U = 3883$, $P = 0.01$) species. In turn, the Ibero-North African and *R* species showed a mean taxonomic singularity (0.07 ± 0.024) significantly lower ($U = 1864$, $P = 0.040$) than *R+* (0.17 ± 0.073) and *Not R* species with wider geographic distributions (0.20 ± 0.021).

3.5 Chorological diversity of components within plant assemblages

On the set of 278 plant species registered in the sampling, 11 of them were Introduced (IN), 16 Cosmopolitan (C), 24 Mediterranean-Eurosiberian (ME), 113 Circum-Mediterranean (M), 43 Western Mediterranean (WM) and 71 Iberian and/or North African plants (IBNA). The latter group (IBNA) comprised narrow endemic species and summed up to 25% of total number of registered species, only exceeded by M species (41%). Specifically, 45 species were endemic to Ibero-African (16%), 20 species were exclusive to North Africa (7%) and the remaining 6 species to the Iberian Peninsula (2%). Among the *R* plants, the most common chorological type was IBNA, which comprised 20 species (42% within the *R* plants). On the contrary, in the *R+* and *Not R* groups, the M type was the most common one (37% and 47%, respectively), although they also showed a substantial amount of endemic species (IBNA: 32% and 19%, respectively). The species richness by chorological types exhibited a consistent pattern among the plant assemblages (represented by distribution areas in Figure 5b). In all cases, the M species showed the highest proportion (mean 48.0 ± 2.36%), generally followed by the IBNA (mean 21.7 ± 1.27%) and WM (mean 15.3 ± 1.70%) species. The ME and C species were rare (mean 6.9 ± 1.20%, mean 4.7 ± 0.79%, respectively); and the IN plants were very poorly represented in all sites (mean 3.3 ± 1.16%), being absent in eight ones.

Regarding species abundance by chorological types, the IBNA plants were the most abundant in Spanish *S. pustulatus* sites and in most of the Moroccan *S. pustulatus* and *S. masguindalii* ones. The IN species were particularly abundant in the Spanish sites TEL and CAB due to the incidence of *Oxalis pes-caprae* (28% and 17%, respectively) which covers large areas in the region, shockingly even invading cliffs.

3.6 Floristic gradients and β-diversity

The proportions of shared species among distribution areas were always low, as well as the proportion of species exclusive to each area (Table 4). The whole of the Moroccan plant assemblages (Figure 1, areas B, C and D) shared the 17% of all registered species with the Spanish ones (area A) but shared up to 31% of all genera. Apart from *S. pustulatus*, only five *R* species were detected both in North African and in SE Spain (2%): *Cheilanthes vellea* (ME), *Polygala rupestris* (WM), *Sarcocapnos enneaphylla* (WM), *Sedum dasyphyllum* (M) and *Teucrium rotundifolium* (IBNA). Moreover, among the species exclusive to the area A (14%), the *R* species were:

Antirrhinum hispanicum subsp. *mollissimum* (IBNA), *Euphorbia squamigera* (WM), *Silene vulgaris* subsp. *commutata* (M), *Teucrium intricatum* (IBNA) and *Umbilicus gaditanus* (WM). All these species belonged to genera detected in both Baetic and Rifan ranges (Appendix: Table 7). Similarly, four of the six *R+* species detected exclusive to the area A also belonged to genera detected in both ranges: *Fumana procumbens* (ME), *Launaea lanifera* (IBNA), *Silene secundiflora* (IBNA), *Thymus vulgaris* L subsp. *vulgaris* (WM).

Table 4 Species richness and genus richness: a) exclusive to each distribution area; and b) shared between areas (Figure 1, A-D). See Materials and Methods section for details of the habitat types.

	Species richness (%)				Genus richness (%)
	R	*R+*	*Not R*	*R*, *R+* and *Not R*	*R*, *R+* and *Not R*
a) Exclusive					
Area A	2	2	10	14	9
Area B	3	3	11	17	13
Area C	4	2	11	17	14
Area D	0	3	7	10	9
b) Shared					
A - [B, C, D]	2	5	10	17	31
Areas A - B	1	4	7	12	21
Areas A - C	1	3	4	8	18
Areas A - D	1	3	5	9	17
Areas B - C	6	6	14	26	34
Areas B - D	1	5	7	13	20
Areas C - D	2	4	7	13	19
Areas A - B - C -D	1	1	2	4	15

The results of the DCA ordination of the 18 sites by only their floristic composition, considering components as species category, showed strong gradients (total variance of 4.7; Figure 6a). Here, the Moroccan sites associated with *S. pustulatus* and *S. fragilis* were grouped on the left side, although LAUO of *S. pustulatus* was isolated from this group; the Spanish sites were separately pooled in the underside and those associated with *S. masguindalii* were basically scattered in the positive area. When the plant components were included in the data matrix as generic category, the DCA showed slightly less strong gradients (total variance of 3.6; Figure 6b). Here, the Spanish sites of *S. pustulatus* and two sites of *S. masguindalii* (BAD1 and BAD2) were closer to those Moroccan ones of *S. pustulatus* (excluding KAN) and *S. fragilis* (excluding DER).

When the *Not R* plant species were removed from the data matrix, the DCA basically provided the same ordination that taking all components as species into account (total variance of 3.9; Figure 7a). When only the *R* species were included in the data matrix, the eigenvalues of its DCA ordination increased while the total variance remained similar (total variance of 4.8; Figure 7b). Here, the groupings were the same but better defined, with the exception of *S. masguindalii* sites which were very separated due to their low number of *R* species. Once again, the Spanish sites were separated from the remaining.

4. DISCUSSION

The stenochoric species of *Sonchus* section *Pustulati* show a considerably narrow ecological range, which is shaped by several physiographic (north facing, steeply sloping and quite conserved cliffs, with little plant cover) and edaphic (limestone cliffs, i.e. quite alkaline substratum, usually with loam texture, optimal organic matter and poor in assimilable K) traits. The high overlapping of the sampled plots detected in the PCA ordination based on physiographic traits also indicates that these traits are very similar and this prevails over climatic differentiations (basically rainfall regime) among geographical areas.

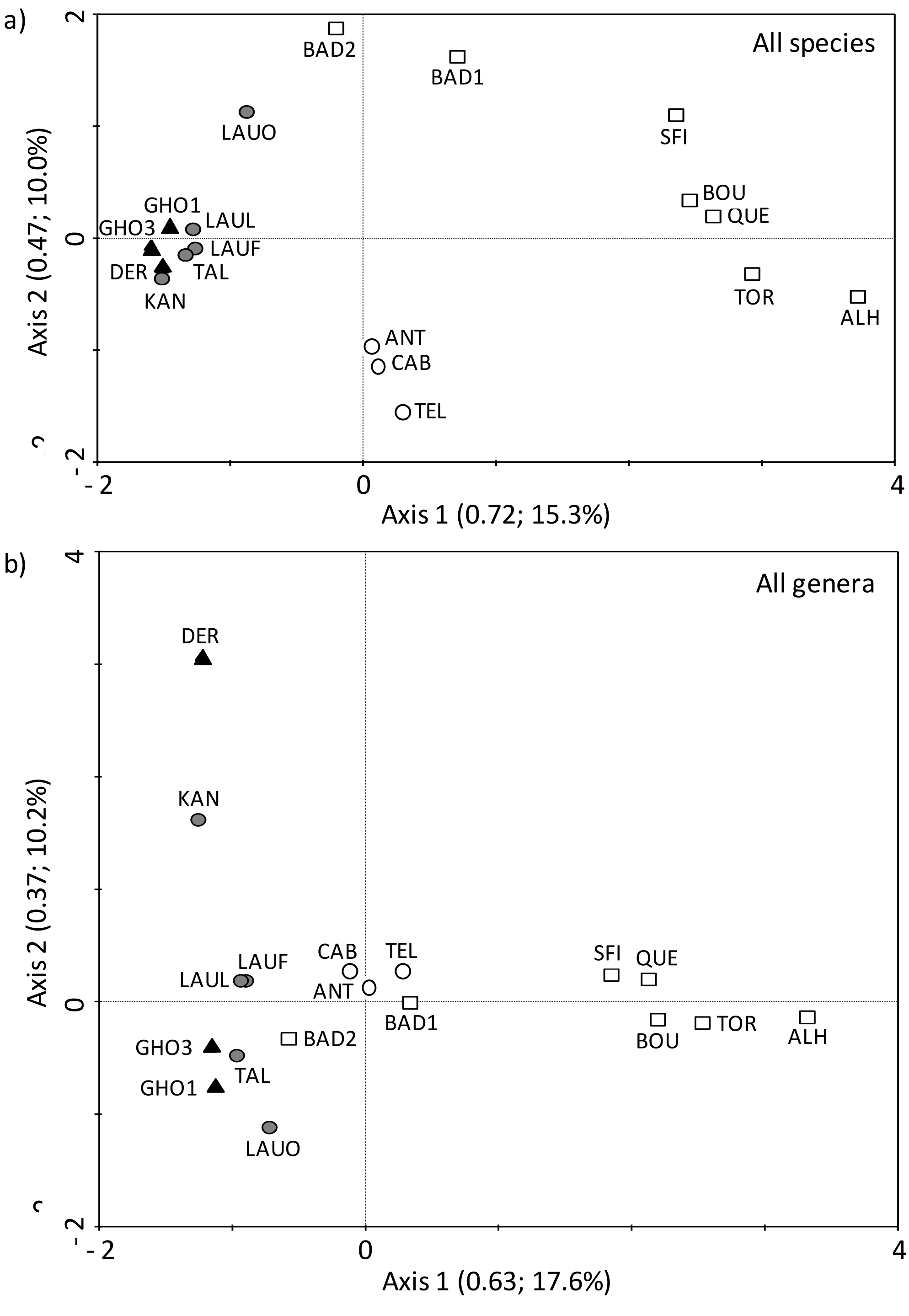

Figure 6 First and second axis of the DCA ordination (bi-plot scaling) for the plant assemblages associated with the sites where the species of *Sonchus* section *Pustulati* are settled (white circles for Spanish *S. pustulatus*, grey circles for Moroccan *S. pustulatus*, solid triangles for *S. fragilis*, and white squares for *S. masguindalii*) incorporating all sampled: a) species; and b) genera. Values in parentheses are eigenvalue and variance explained by each axis.

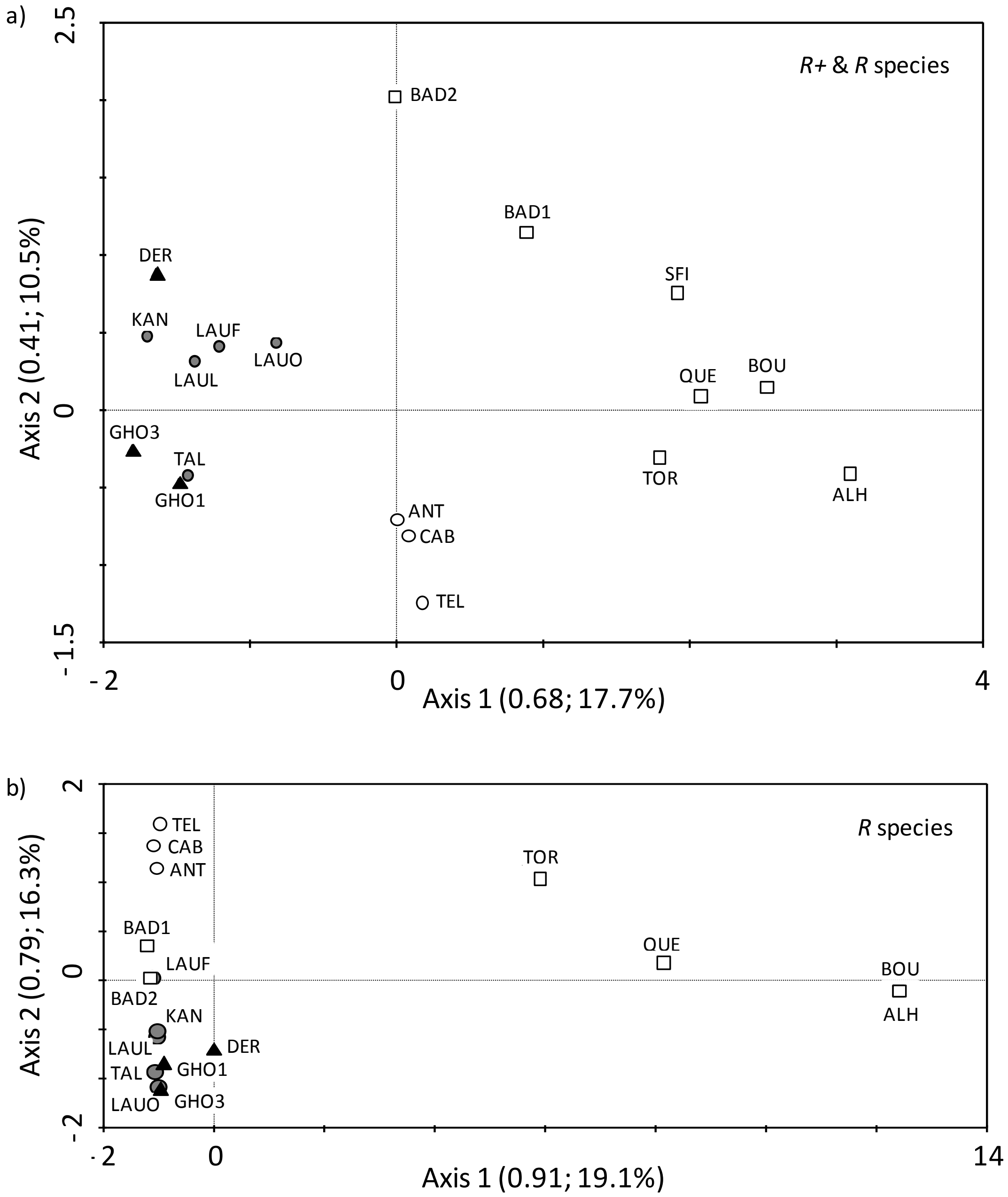

Figure 7 First and second axis of the DCA ordination (bi-plot scaling) for the plant assemblages associated with the sites where the species of *Sonchus* section *Pustulati* are settled (white circles for Spanish *S. pustulatus*, grey circles for Moroccan *S. pustulatus*, solid triangles for *S. fragilis*, and white squares for *S. masguindalii*) incorporating: a) *R+* and *R* species; and b) exclusively *R* species (absent in SFI). Values in parentheses are eigenvalue and variance explained by each axis.

4.1 The role of cliff habitat as refuge for plant species

It has often been considered that cliffs act as environmental and anthropogenic disturbance refuges (e.g. Davis, 1951; Polunin, 1980; Albert *et al.*, 2001; Lavergne *et al.*, 2004; Larson *et al.*, 2005; Thompson, 2005). The cliffs studied here are not only a refuge as an undisturbed, relatively constant and uncompetitive habitat but also as a protection against the strong seasonality of the present-day Mediterranean regime. Several features of the studied cliffs can be pointed out regarding their role as microclimatic refuges for the species of *Sonchus* section *Pustulati* (1–3). 1) The north-facing cliffs avoid the direct solar radiation and, together with proximity to the sea, maintain air humidity at high levels which buffers the high temperatures (e.g. Médail & Quézel, 1999) of the spring and autumn seasons. This favours the control of the plant evapotranspiration; individuals resist the dry-hot summer months as leafless lignified branches. The striking south-facing orientation of the DER cliffs of *S. fragilis* which are located in an area with a relatively high rainfall (Figure 1, area B) should be favoured by the water which usually oozes from several points of its walls. 2) Under dry conditions, rocks can be a more favourable substrate than soil (Larson *et al.*, 2005; Schiwinning, 2010). 3) Low altitude and the proximity to the sea avoid extreme low temperatures, which may have had a particular incidence during past glaciations. In addition, two more points unrelated to climate should be added: 4) invasions of competitive invaders are minimized due to their unsuccessful colonization events (Lavergne *et al.*, 2004); and 5) their usual inaccessibility and little economic interest suppose limited anthropogenic influences (Lavergne *et al.*, 2004; Larson *et al.*, 2005), although some threats has recently been pointed out for *Pustulati* taxa (Chapter 2).

The Spanish populations of *Sonchus pustulatus* appear to be at the limit of the ecological range of the group (Chapter 2). A demographic decline detected in two of the three known populations was correlated with a decreasing of the number of days with rainfall during the spring and with an increasing of the mean maximum temperature during the growing periods, a general tendency in SE Iberian Peninsula (Ruiz-Sinoga *et al.*, 2012). In this area, the habitat can be therefore losing its role as perpetual refuge, probably under the influence of the global climatic change.

4.2 Plant assemblages associated with the species of Sonchus section Pustulati

Plant assemblages colonizing cliff habitats often highlight by their great floristic richness, despite the inhospitable that these habitats seem (e.g. Mota *et al.*, 1991), although the richness of vascular plants use to be lower than in the surrounding environmental (Larson *et al.*, 1989). Those plant assemblages associated with the limestone cliffs, where the species of *Sonchus* section *Pustulati* grow, are characterized by high species richness independently of the cliff size. Moreover, the generally lower family and species richness found in the plant assemblages associated with the marine cliffs of *S. masguindalii* support the idea that plant assemblages associated with these cliffs are always poorly developed compared with those from inland cliffs (Davis, 1951; Malloch & Okusanya, 1979). It has generally been attributed to salt spray which significantly limits the growth of many plants by severe desiccation stress (Larson *et al.*, 2005).

Since we only sampled those plant assemblages associated with the species of *Sonchus* section *Pustulati* along their very restricted distribution (with very low occupancy areas), it was expected that we were not going to assess associations as communities in a strict sense. This results exceptionally unlikely in the studied habitats since cliff-dwelling species appear as a complex mosaic of discrete micro-communities (Thompson, 2005) mixed with many no specifically cliff-dwelling species with very low abundance. According Davis (1951), to observe real associations on cliff ecosystems we should have focused on micro-groups constituted by obligated cliff-dwelling species. Even these micro-associations use to occur mixed with each other since they occupy different zones on cliffs (pavement, step crevice, sloping, vertical or overhanging rocks and/or ledge) which may be partially overlapped among each other.

4.2.1 Habitat specificity in the plant assemblages

The pattern observed in the habitat specificity of the species comprising the plant assemblages associated with the species of *Sonchus* section *Pustulati* migth be common in all plant assemblages associated with cliffs. However, the floristic composition of those species not typical from rocky habitats (*Not R*) in any plant assemblages associated with cliffs might be highly dependent on the vegetation that surrounds it. All plant species capable of retaining some propagules, germinating and growing in small cracks and supporting a minimum weight of their aerial parts against gravity may appear in the plant assemblages, despite being far from the most optimal life conditions. This explains the presence of small-sized individuals observed in many of the sampled *Not R* species compared to their optimal typical sizes (e.g. *Ampelodesmos mauritanicus*, *Daphne gnidium*, *Erica multiflora*, *Foeniculum vulgare*, *Nerium oleander*, *Pinus halepensis*, *Quercus coccifera, Q. rotundifolia*, *Tetraclinis* *articulata*, etc). Nevertheless, some case of *Not R* species might show small populations on cliffs constituted by the most ancient individuals of the species distribution on the region, being they older than those populations located on the more optimal surrounding habitats (e.g. Larson *et al.*, 1999). Cliffs may also provide temporal shelter against large disturbances at level ground, as for instance an intense fire.

4.2.2 Chorological diversity in the plant assemblages

The plant assemblages associated with the cliffs that harbour the species of *Sonchus* section *Pustulati* generally include a high number of species endemic to the Iberian Peninsula and/or North Africa (IBNA; 25% of all registered species). Once detected the high richness of *Not R* species in the assemblages on cliffs, i.e. many species of the surrounding habitats, it was expected that the proportion of endemic species was high because of how the biodiversity is integrated within the Baetic-Rifan hotspot. The high endemic species level therein has been associated with the complex geologic and climatic history which gave rise to highly diverse habitats (Médail & Quézel, 1997; Thompson, 2005). Even so, the thought regarding cliff habitats as refuge for endemic taxa is supported by the high proportion of *R* species that were IBNA in the present study (42%). Moreover, the low abundance of alien exotic invaders (both C and IN species) detected appears to be typical of the vegetation on cliffs (e.g. Crawley *et al.*, 1986; Larson *et al.*, 1989).

4.2.3 The species of Sonchus section Pustulati in their plant assemblages

The species of *Sonchus* section *Pustulati* are strictly cliff plants, mainly located at low and medium levels on cliffs. Only few individuals were detected just on the basis of the cliffs or at level-ground close to it, healthy when they were protected by prickly woody shrubs (e.g. *Lycium intricatum* or *Ulex parviflorus*) or highly grazed and small-sized on unprotected. Despite of their rarity, they were always the dominant species in their plant assemblages except in the Spanish area. *S. masguindalii* has been included in the Moroccan plant groupings of calcareous cliffs named *Sedo wilczekiani–Sonchetum masguindalii* ass. nov. (Deil & Hammoumi, 1997); and similarly, *S. fragilis* in the grouping *Soncho fragilis-Rhodanthemetum lauoense* (Deil, 1994). But the latter assemblages surely referred to Moroccan *S. pustulatus* instead of *S. fragilis*, which could be included in the grouping *Stachydo circinatae-Rhodanthemetum hosmariense,* around Tetouan city (Deil, 1994), since *S. fragilis* is absent in the Oued Laou valley (Chapter 1). Both *Stachys circinata* and *Rhodanthemetum hosmariense* were registered on the area of *S. fragilis*. As for the Spanish *S. pustulatus*, it was associated with *Crithmo-Limonietea* assemblages by Romero & Lopez (1985) but they wrongly considered *S. tenerrimus* as *S. pustulatus* on marine cliffs from Granada where they are not really present (Chapter 2; Mejías, 1988). Based on our study, Spanish *S. pustulatus* was associated with assemblages dominated by *Pallenis maritima*, *Teucrium rotundifolium* and *T. intricatum*, which may act as competing species.

Those species capable of solving the desiccation stress that supposes the continuous spray salt over marine cliffs can find low competition in them (e.g. Goldsmith, 1973). The plants of *Sonchus* section *Pustulati* face this stress developing succulent leaves and lignified branches, particularly those of *S. masguindalii* which are mostly settled on marine cliffs. Moreover, the supposed greater sensitivity to cold of some marine cliff-dwelling species (e.g. Malloch & Okuzanya, 1979) could explain the slightly lower abundance of *S. masguindalii* in the only inland population sampled (BAD2, 75%) than in the remaining of this species, and their smaller sizes (Chapter 2).

4.3 A relict condition of Sonchus section Pustulati

The origin and diversification of the species of *Sonchus* section *Pustulati* have been estimated by molecular dating at 5.5–3.9 Ma (Chapter 4), when the climate was wetter and warmer than today (Mai, 1989; Fauquette *et al.*, 1998; Thompson, 2005). As the Mediterranean climate presumably became established since approximately 3 Ma ago (Suc, 1984), these species have been therefore considered as part of the pre-Mediterranean element (Herrera, 1992) of the Mediterranean flora (Chapter 4). If so, they have been sufficiently resilient to face such present-day Mediterranean climate, taking refuge in certain cliffs with the ecological traits described herein. The extant populations might be therefore the result of a demographical restriction of a plant group that once prospered in somewhat larger and more connected areas in the late Miocene-Pliocene times.

The case of the disjunct distribution of *Sonchus pustulatus* in both sides of the Alborán Sea is one of the most interesting points within the evolutionary history of the group. This species shows the greatest ecological amplitude within the section (Figure 3a) and a current highly disjunct distribution (Figure 1, areas A and B). The presence of a highly deciduous pappus and the marked genetic differentiation detected in the clade between Baetic and Rifan ranges (Chapter 4) does not suggest the occurrence of long-distance dispersal. This and the similarities between plant assemblages from A and B areas (see *section 4.4*) support the following hypotheses. *S. pustulatus* should be more common in the Western Mediterranean during past geological times occupying more extensive areas within the stretch of land (Gibraltar arc) connecting Iberia and North Africa during Messinian and Zanclean periods (Rosenbaum *et al.*, 2002; Chapter 1 and 4). The progressive re-flooding of the Mediterranean Sea to the end of the Zanclean age (5.33–3.6 Ma) was leaving under seawater part of the presumably ancient areas of occupancy. Then, the establishment of the Mediterranean climate (approx. 3 Ma ago) was progressively decreasing the annual rainfall, probably with the exception of the western Baetic-Rifan region (Figure 1), which could progressively give rise to the detected moderate ecological and genetic divergences of the species (Chapter 4) and associated plant assemblages. These climate divergences, together with the Quaternary glaciations which were presumably more accentuated in the Baetic range, could lead to the Spanish *S. pustulatus* to a narrow range compared to the Moroccan *S. pustulatus*, although we cannot underestimate the anthropogenic effects in such asymmetry (Chapter 2). In addition, a historical vicariance process between Moroccan and Spanish plants of *S. pustulatus* may have favoured some of the detected physiological differentiations in plants growing in the greenhouse: the first ones exhibited a clearly higher survival, more developed root system and contrasted resistance to fungal infections (J.L. Silva, personal observations).

4.4 Shifts of the plant assemblages associated with the species of Sonchus section Pustulati

From the analyses of biotic (associated plants) and abiotic (physiographic and edaphic) traits of the plant assemblages we have found a common narrow ecological range for the species of *Sonchus* section *Pustulati* (Table 5) despite the marked differences in the rainfall regime among them. The few results that clearly differed among geographical areas were generally those related to the β-diversity (DCAs). Most of the patterns of divergence involve differences on the floristic composition of plant assemblages, this being particularly common in the Mediterranean cliff habitats (Bragazza, 2009; Pérez-Garcia *et al.*, 2012). Interestingly, the similarity on the floristic composition between areas was higher between B-C (W Rif) and A (SE Spain) than between B-C and D (central Rif). Higher floristic affinities among western Rifan and some Baetic ecoregions than among western Rifan and some nearby Rifan ecoregions have been already detected (Molina-Venegas *et al.*, 2013), although such areas are not coincident in the Baetic range. This latter might be related to the complex paleogeography of the Baetic-Rifan region which generated different biogeographical patterns for instance depending on the ecological specificity and dispersal capacity of the species.

The shared species between the Baetic and Rifan cliffs where the species of *Sonchus* section *Pustulati* are settled (17%) represent an important proportion if we consider that approximately 75% of species, whatever their habitat specificity, are present within the whole Baetic-Rifan region are common to both ranges (Valdés, 1991).

Table 5 Evidences for the ecological similarities between the plant assemblages and habitats associated with the species of *Sonchus* section *Pustulati* by distribution areas (Figure 1, A-D). The area A is in bold because this summary is mainly focused on the comparison between this with the remaining ones (C-D). *Refers to similarity of the shared richness results between the different pairs of areas, not that areas share high richness.

	Similarity	Notes
Floristic composition		
Family richness	High	**A** = B = C ≈ D; D somewhat lower values; Table 3
More common families	High	**A** = B = C = D; Fig. 4
Species richness	High	**A** ≈ B ≈ C > D; GHO1 exceptionally high; Table 3
Species richness by habitat type	High	**A** = B = D ≈ C; Fig. 5a
Species richness by chorological type	High	**A** = B = C = D; Fig. 5b
Taxonomic singularity	High	**A** = B = C = D; Table 3
Exclusive species richness	High*	Slightly lower in D; Table 4
Exclusive genera richness	High*	Slightly lower in **A** & D; Table 4
Shared species richness	High*	Low, less between B-C; Table 4
Shared genera richness	High*	Low, less between B-C & **A**-[B, C, D]; Table 4
Shared *Not R*/*R+*/*R* species richness	High*	Low, somewhat higher between B-C; Table 4
Floristic abundance		
Weight frequencies	High	**A** ≈ B ≈ C < D; but not always significantly
Weight frequencies by habitat type	High	**A** = B ≈ D ≠ C
Weight frequencies by chorological type	Low	In **A**, B & D, IBNA plants were the most abundant
Floristic composition & abundance		
Diversities index (E)	High	**A** ≈ B ≈ C > D; Table 3
Diversities index (1-D)	High	**A** ≈ B ≈ C < D; Table 3
DCA with all species	Low	**A** ≠ B = C ≠ D; Fig. 6a
DCA with all genera	High	**A** ≈ B ≈ C ≠ D; Fig. 6b
DCA without *Not R* species	Low	**A** ≠ B ≈ C ≠ D; Fig. 7a
DCA only *R* species	Low	**A** ≠ B ≈ C ≠ D; very few *R* plants in D; Fig. 7b
Cliff abiotic features		
Physiographic traits (orientation, state, plant cover, slope)	High	**A** = C = D ≈ B; Table 2, Fig. 3a & Appendix: Table 5
Edaphic traits (pH, granulometry, assimilable Ca & K, etc.)	High	**A** = B = C ≈ D; Table 2, Fig. 3b & Appendix: Table 6
Mediterranean climate		
Temperature	High	**A** = D ≈ B = C; Fig. 1
Precipitation	Low	**A** = D ≠ B = C; Fig. 1

Interestingly, at genus level, the richness shared between these ranges (31%) became quite similar to that shared between the geographically closer areas of the Moroccan *S. pustulatus* and *S. fragilis* taxa (34%).

This suggest that many species detected in the plant assemblages which are not shared between geographical areas might be phylogenetically related species (even someone with different genus), particularly between the Spanish (A) and Moroccan (B) areas of *S. pustulatus* or between area A and that of *S. fragilis* (C; Table 4). The ancestors of the possible phylogenetic-related species might be located in areas which were closer and/or more connected by the Gibraltar arc from the late Miocene to the early Pliocene, but we have no evidence for it beyond the similarities detected in the present study (Table 5). According to Deil (1997), the phenomenon of vicariance in the cliff plant taxa from the Western Mediterranean is common. Some of the taxa reported by this author take part of the plant assemblages recorded during our survey, as some Fumarioids (*Sarcocapnos*, *Rupicapnos*) and the genera *Rhodanthemum* and *Campanula* (Appendix: Table 7).

The hypothesis proposed here about past connections among Baetic and Rifan ranges based on ecological similarities should be taken with caution if we consider the general conclusions of Larson *et al.* (2005). They proposed a considerable degree of global ecological, and sometimes of taxonomic, similarity among cliffs worldwide. Its main reason lies in the common ecological control regardless of the location or geological history of the cliff. Although it may be true for many abiotic traits of the habitats of *Sonchus* section *Pustulati*, it does not seem to be so for their biotic traits. For instance, the species richness of the vegetation on cliffs is highly dependent on the vegetation of the surrounding habitats, both on its biodiversity and the capacity of their components to grow on cliffs. Moreover, the pattern detected in the species richness by chorological type through the geographical ranges might be only similar on all the calcareous cliffs located in the Baetic-Rifan region. The chorology of the vegetation from the study cliffs and that from other calcareous Mediterranean ones should basically vary in the composition of the narrow endemic taxa, the groups IBNA and/or WM being replaced by other endemic groups, since cliffs are the main habitat of many rare plant species in such region (Thompson, 2005).

In sum, based on ecological data, the current distribution of the species of *Sonchus* section *Pustulati* appears to be mainly due to the current or similar occupied areas were near, overlapped or at least more connected among each other through the Gibraltar arc from the late Miocene to the early Pliocene (7.2–3.6 Ma), rather than to long dispersal events which are particularly unexpected between the Baetic and Rifan ranges. This ancient land-connection would indicate that current occupied areas likely presented biotic and floristic affinities, which can be partially detectable nowadays.

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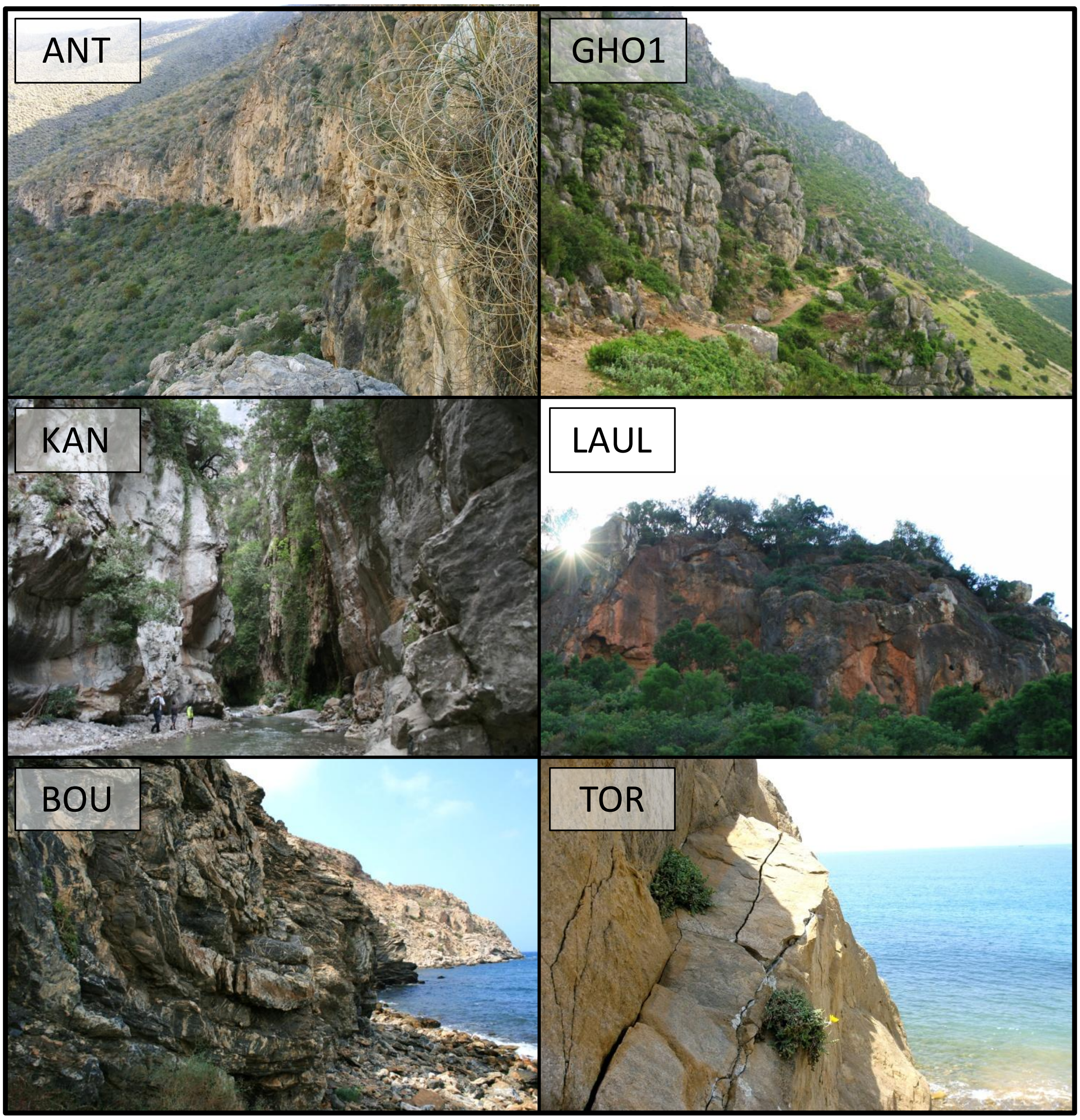
APPENDICES



Appendix: Figure 8 Cliffs where the species of *Sonchus* section *Pustulati* are settled: from Spanish (ANT) and Moroccan (KAN and LAUL) *S. pustulatus*, *S. fragilis* (GHO1) and *S. masguindalii* (BOU and TOR).

Appendix: Table 5 Physiographic traits of the cliffs where the *Sonchus* section *Pustulati* species are settled. See Materials and methods section for details.

Habitats	Geographical orientation (º) Mean ± SE [min - max]	Slope (º) Mean ± SE (min - max)	Conservation state Mean ± SE	Height (m) Mean ± SE (min - max)	Plant cover (%) Mean ± SE (min - max)
S. pustulatus - Spain, southeast					
ANT	334 ± 7.1º NW-N [265º - 55º]	81 ± 2.9 (40 - 110)	2.7 ± 0.1	21 ± 2.2 (4 - 50)	31 ± 3.5 (5 - 70)
TEL	356 ± 10.0º NW-N [320º - 140º]	71 ± 3.3 (50 - 100)	2.4 ± 0.1	25 ± 3.8 (7 - 56)	32 ± 4.4 (5 - 70)
CAB	15 ± 7.0º N-NE [235º - 60º]	74 ± 3.3 (30 - 100)	2.4 ± 0.2	13 ± 0.8 (5 - 20)	33 ± 3.9 (5 - 80)
S. pustulatus - Morocco, western Rif					
LAUF	26 ± 6.8º N-NE [10º - 60º] - 237 ± 18.2º SW-W [200º - 0º]	90 ± 2.3 (65 - 110)	2.8 ± 0.1	18 ± 2.3 (4 - 50)	12 ± 1.9 (0 - 30)
LAUL	4 ± 15.6º NW-N [190º - 140º]	82 ± 2.6 (50 - 105)	2.8 ± 0.1	11 ± 1.4 (4 - 30)	16 ± 2.2 (0 - 40)
LAUO	327 ± 3.7º NW-N [270º - 85º]	63 ± 4.2 (25 - 90)	1.7 ± 0.2	14 ± 1.5 (3 - 30)	39 ± 7.1 (2 - 90)
TAL	34 ± 7.9º N-NE [310º - 125º]	84 ± 1.8 (45 - 95)	2.6 ± 0.1	13 ± 1.7 (1 - 35)	16 ± 3.0 (0 - 70)
KAN	19 ± 6.3º N-NE [0º - 60º] - 223 ± 9.9º S-SW [160º - 280º]	76 ± 3.9 (20 - 100)	2.9 ± 0.1	74 ± 12.0 (10 - 150)	28 ± 5.8 (0 - 100)
S. fragilis - Morocco, north-western Rif					
GHO1	306 ± 9.1º W-NW [210º - 90º]	79 ± 2.6 (45 - 100)	2.7 ± 0.1	37 ± 5.0 (4 - 95)	28 ± 4.1 (5 - 80)
GHO3	289 ± 9.4º W-NW [186º - 20º]	83 ± 2.7 (45 - 100)	2.7 ± 0.1	16 ± 2.1 (1 - 40)	14 ± 3.0 (0 - 50)
DER	165 ± 11.3º SE-S [90º - 236º]	90 ± 2.4 (70 - 110)	2.9 ± 0.1	40 ± 4.0 (25 - 70)	15 ± 4.6 (0 - 70)
S. masguindalii - Morocco, central Rif					
TOR	350 ± 13.3º NW-N [260º - 60º]	75 ± 3.2 (60 - 90)	2.8 ± 0.1	38 ± 2.9 (25 - 60)	24 ± 6.1 (3 - 50)
BAD1	45 ± 11.5º N-NE [260º - 165º]	74 ± 2.9 (40 - 100)	2.4 ± 0.1	25 ± 3.3 (3 - 50)	23 ± 2.5 (3 - 60)
BAD2	327 ± 16.6º NW-N [200º - 120º]	85 ± 2.2 (65 - 110)	3.0 ± 0.1	9 ± 1.1 (5 - 20)	16 ± 2.9 (3 - 40)
BOU	21 ± 6.5º N-NE [300º - 90º]	70 ± 2.4 (50 - 100)	1.9 ± 0.1	26 ± 2.7 (10 - 50)	24 ± 3.5 (0 - 70)
ALH	343 ± 16.1º NW-N [280º - 40º]	63 ± 7.0 (35 - 80)	1.8 ± 0.3	20 ± 8.2 (4 - 50)	24 ± 7.7 (5 - 40)
QUE	15 ± 14.8º N-NE [320º - 60º]	76 ± 4.7 (60 - 90)	2.2 ± 0.2	35 ± 4.2 (25 - 50)	35 ± 7.3 (15 - 60)
SFI	38 ± 8.4º N-NE [330º - 120º]	59 ± 5.8 (9 - 95)	2.3 ± 0.1	13 ± 1.4 (5 - 25)	32 ± 5.2 (3 - 100)

Appendix: Table 6 Edaphic traits of the cliffs where the species of *Sonchus* section *Pustulati* are settled. OOM, oxidizable organic matter.

Habitat	pH (25ºC)	Conductivity (mS/cm)	Granulometry (% clay/silt/sand)	Carbonates (%)	Active lime (%)	OOM (%)	[Macronutrients] (mg/Kg)		[Assimilable cations] (meq/100g)				[Micronutrients] (mg/Kg)					C/N	Ca/Mg	K/Mg
							N kjeldahl	P Olsen	Ca	Mg	K	Na	Cu	Fe	Mn	Zn	B			
S. pustulatus - Spain, southeast																				
ANT	8.8	4.2	13 / 30 / 57	7.0	3.8	5.7	3649	78	22.3	13.8	1.1	9.5	1.4	2.4	16.8	9.1	2.7	4.6	1.6	0.1
TEL	8.6	0.2	20 / 25 / 55	23.9	1.5	5.1	2949	20	64.8	9.6	0.7	1.0	3.4	19.5	16.2	9.8	1.4	3.3	6.8	0.1
CAB	8.2	2.2	13 / 38 / 49	21.5	4.8	3.8	2648	35	32.1	5.7	0.4	5.5	1.5	9.5	5.7	11.3	1.0	5.6	5.5	0.1
S. pustulatus - Morocco, western Rif																				
LAUF	8.4	2.5	20 / 37 / 43	13.1	3.5	6.6	2576	27	22.0	13.7	1.0	8.6	0.7	18.0	24.4	13.4	1.4	7.4	1.6	0.1
LAUL	8.0	0.2	35 / 37 / 38	1.3	3.7	6.5	4242	27	23.9	4.3	0.6	0.2	1.3	16.8	22.0	3.8	0.7	4.5	5.5	0.1
LAUO	8.1	0.5	32 / 30 / 38	33.7	6.0	2.1	1407	10	25.2	1.5	0.4	0.5	0.5	6.5	21.2	0.5	0.6	8.6	17.2	0.3
TAL	8.4	0.2	47 / 17 / 16	18.2	1.5	7.9	4978	19	80.3	4.9	1.8	0.4	1.5	26.0	42.6	4.2	1.8	3.1	16.5	0.4
KAN	8.0	0.2	20 / 41 / 39	1.9	3.7	5.4	3635	18	19.6	4.1	0.5	0.1	0.3	16.8	16.8	14.2	0.5	4.3	4.8	0.1
S. fragilis - Morocco, north-western Rif																				
GHO1	8.4	0.3	15 / 37 / 48	8.6	1.5	7.9	4913	14	70.5	13.3	0.7	0.4	0.6	25.6	13.5	1.0	1.1	3.1	5.3	0.1
GHO3	8.1	0.2	27 / 54 / 19	2.3	1.5	10.2	6244	31	47.2	7.7	0.8	0.6	1.3	27.0	25.0	8.4	0.5	3.2	6.1	0.1
DER	8.3	0.3	30 /37 / 33	6.3	2.1	11.1	4238	38	34.0	13.7	1.0	0.4	1.7	12.0	29.0	4.4	0.6	3.8	2.5	0.1
S. masguindalii - Morocco, central Rif																				
TOR	8.0	0.9	25 / 18 / 57	0.5	6.3	3.1	1951	16	5.8	6.5	1.0	4.9	0.8	22.0	44.2	1.9	1.5	9.2	0.9	0.2
BAD1	8.6	0.6	30 / 31 / 39	4.8	4.2	1.0	792	5	18.8	5.6	1.2	2.9	0.2	3.0	5.6	0.1	1.4	7.0	3.3	0.2
BAD2	8.4	0.7	30 / 38 / 32	31.7	12.1	3.5	2345	61	25.3	11.3	1.1	6.7	1.0	5.0	7.4	1.7	0.7	8.6	2.2	0.1
BOU	8.6	2.3	15 / 20 / 65	8.5	2.0	0.9	626	9	21.8	7.8	0.6	31.2	0.5	11.4	6.7	0.4	0.7	7.9	2.8	0.1
ALH	8.3	1.1	24 / 24 / 52	14.6	6.3	2.0	1326	22	18.0	6.9	0.9	9.8	0.6	9.3	14.5	1.0	1.1	8.8	2.9	0.1
QUE	8.3	1.1	24 / 24 / 52	14.6	6.3	2.0	1326	22	18.0	6.9	0.9	9.8	0.6	9.3	14.5	1.0	1.1	8.8	2.9	0.1
SFI	8.0	1.0	20 / 12 / 68	27.6	7.0	1.8	916	18	18.3	3.4	0.6	3.4	0.6	5.1	8.8	0.9	1.1	11.5	5.4	0.2

Appendix: Table 7 List of species registered in the sampled plant assemblages. Taxonomic species and family names, habitat and chorological types, and distribution areas where the species were sampled (A–D). See Material and methods section for details.

Associated species	**Family**	**Habitat**	**Chorology**	**Areas**
Acanthus mollis L.	Acanthaceae	Not R	IN	B, C
Achyranthes sicula (L.) All.	Amaranthaceae	Not R	IN	C
Adiantum capillus-veneris L.	Polypodiaceae	R	C	B
Aegilops geniculata Roth	Poaceae	Not R	M	D
Agave americana L.	Asparagaceae	Not R	IN	A
Allium pallens L. subsp *pallens* var. *pallens*	Amaryllidaceae	R+	M	B
Allium subvillosum Salzm. ex Schult. & Schult. f.	Amaryllidaceae	R+	WM	A, B, C, D
Ampelodesmos mauritanicus (Poir.) T. Duran & Schinz	Poaceae	Not R	WM	C, D
Anagallis arvensis L.	Primulaceae	Not R	C	A, B
Andryala integrifolia L.	Compositae	Not R	M	B, C
Anisantha rubens (L.) Nevski	Poaceae	Not R	M	A, B, C
Anogramma leptophylla (L.) Link	Polypodiaceae	R	C	B
Anthoxanthum aristatum Boiss.	Poaceae	Not R	WM	B
Antirrhinum hispanicum subsp. *mollissimum* (Rothm.) D. A. Webb	Scrophulariaceae	R	IBNA	A
Antirrhinum majus subsp. *tortuosum* (Lam.) Rouy	Scrophulariaceae	R	M	B, C
Aphyllanthes monspeliensis L.	Asparagaceae	Not R	WM	C
Arisarum simorrhinum Durieu	Araceae	Not R	WM	C
Aristolochia baetica L.	Aristolochiaceae	Not R	IBNA	A, B, C, D
Arthrocnemum macrostachyum (Moric.) K. Kosh	Chenopodiaceae	Not R	M	A, C, D
Asparagus albus L.	Asparagaceae	R+	WM	B
Asparagus horridus L.	Asparagaceae	Not R	M	A, D
Asperula hirsuta Desf.	Rubiaceae	R+	IBNA	B, C
Asphodelus cerasiferus J. Gay	Xanthorrhoeaceae	Not R	WM	A, D
Asplenium adiantum-nigrum aggr.	Polypodiaceae	R	M	C
Asplenium ceterach L.	Polypodiaceae	R	M	B, C
Asplenium obovatum aggr.	Polypodiaceae	R	M	C
Asplenium ruta-muraria L. subsp. *ruta–muraria*	Polypodiaceae	R	M	B
Asplenium trichomanes L. subsp *quadrivalens* D. E. Meyer	Polypodiaceae	R	C	B, C
Asterolinon linum–stellatum (L.) Duby	Primulaceae	Not R	M	A
Atractylis cancellata L.	Compositae	Not R	M	B
Atriplex halimus L.	Chenopodiaceae	Not R	M	D
Avena spp.	Poaceae	Not R	C	A, B, C, D
Ballota hirsuta Benth.	Lamiaceae	R+	IBNA	A, D
Ballota nigra L.	Lamiaceae	Not R	ME	C
Bidens pilosus L.	Compositae	Not R	IN	C
Biscutella baetica Boiss. & Reut.	Brassicaceae	Not R	IBNA	C
Bituminaria bituminosa (L.) C. H. Stirt.	Fabaceae	Not R	M	A, B
Blackstonia perfoliata subsp *grandiflora* (Viv.) Maire	Gentianaceae	Not R	WM	B, D
Brachypodium spp.	Poaceae	R+	ME	A, B, C, D
Brassica barrelieri (L.) Janka	Brassicaceae	Not R	IBNA	A
Brassica fruticulosa Cirillo subsp *mauritanica* (Coss.) Maire	Brassicaceae	R	IBNA	B
Bromus tectorum L.	Poaceae	Not R	ME	C
Bupleurum balansae Boiss. & Reut.	Apiaceae	R+	IBNA	C

Associated species	**Family**	**Habitat**	**Chorology**	**Areas**
Bupleurum fruticescens subsp. *spinosum* (Gouan) O. Bolòs & Vigo	Apiaceae	Not R	IBNA	C
Bupleurum gibraltarium Lam.	Apiaceae	R+	IBNA	B
Buxus balearica Lam.	Buxaceae	R+	WM	B
Calendula arvensis (Vaill.) L.	Compositae	Not R	M	B
Calendula suffruticosa Vahl	Compositae	R+	WM	B, C, D
Campanula dichotoma L.	Campanulaceae	Not R	IBNA	B
Campanula erinus L.	Campanulaceae	Not R	M	B, C
Campanula lusitanica L.	Campanulaceae	Not R	IBNA	B
Campanula mollis L.	Campanulaceae	R	M	C
Carduus bourgeanus Boiss. & Reut.	Compositae	Not R	IBNA	C
Carduus pycnocephalus L.	Compositae	Not R	M	A, C, D
Carex hallerana Asso	Cyperaceae	Not R	M	B
Carlina hispanica Lam.	Compositae	Not R	IBNA	B, C, D
Carpobrotus acinaciformis (L.) L. Bolus	Aizoaceae	Not R	IN	D
Carthamus lanatus L.	Compositae	Not R	M	D
Catapodium marinum (L.) C. E. Hubb.	Poaceae	Not R	M	B
Catapodium rigidum subsp. *hemipoa* (Spreng.) Stace	Poaceae	Not R	WM	B, C
Centaurea clementei DC.	Compositae	R	IBNA	B
Centaurea fragilis Durieu	Compositae	R+	IBNA	C
Centranthus macrosiphon Boiss.	Valerianaceae	Not R	IBNA	B, C
Chamaerops humilis L.	Arecaceae	Not R	WM	B, C
Cheilanthes vellea (Aiton) F. Mueller	Polypodiaceae	R	ME	A, B
Chiliadenus glutinosus (L.) Fourr.	Compositae	R	WM	B, C
Chiliadenus rupestris (Pomel) Brullo	Compositae	R	IBNA	C, D
Cistus albidus L.	Cistaceae	Not R	WM	B, C
Cistus clusii Dunal	Cistaceae	Not R	WM	D
Cistus monspeliensis L.	Cistaceae	Not R	M	B
Cistus salvifolius L.	Cistaceae	Not R	M	B
Cladanthus mixtus (L.) Chevall.	Compositae	Not R	M	C
Clematis flammula L.	Ranunculaceae	Not R	M	C
Clinopodium nepeta (L.) Kuntze	Lamiaceae	Not R	M	B, C
Conopodium bunioides subsp *atlantis* (Humbert & Maire) Molero	Apiaceae	R	IBNA	C
Convolvulus althaeoides L.	Convolvulaceae	Not R	M	C, D
Convolvulus siculus L. subsp siculus	Convolvulaceae	Not R	M	A
Coronilla juncea L.	Fabaceae	Not R	M	A
Crambe filiformis Jacq.	Brassicaceae	R	IBNA	C
Crepis foetida L.	Compositae	Not R	M	B
Crepis tectorum L.	Compositae	Not R	ME	A, B
Crithmum maritimum L.	Apiaceae	R	M	D
Cuscuta approximata Bab.	Convolvulaceae	Not R	M	A
Cuscuta epithymum (L.) L	Convolvulaceae	Not R	ME	C
Cymbalaria muralis P. Gaertn. , B. Mey. & Scherb. subsp. *Muralis*	Scrophulariaceae	R	IN	C
Cynosurus sp.	Poaceae	Not R	M	C
Dactylis glomerata subsp *hackelii* (Asch. & Graebn.) Cif. & Giacom.	Poaceae	Not R	M	D
Dactylis glomerata subsp *hispanica* (Roth) Nyman	Poaceae	Not R	M	B
Dactylis glomerata subsp *juncinella* (Bory) Stebbins & Zohary	Poaceae	Not R	IBNA	B
Daphne gnidium L.	Tymelaeaceae	Not R	M	B, D
Dianthus lusitanus Brot.	Caryophyllaceae	R	IBNA	C
Digitalis obscura subsp. *laciniata* (Lindl.) Maire	Scrophulariaceae	Not R	IBNA	B

Associated species	**Family**	**Habitat**	**Chorology**	**Areas**
Dipcadi serotinum (L.) Medik.	Asparagaceae	Not R	WM	A
Dittrichia viscosa (L.) Greuter	Compositae	Not R	M	A, B, C, D
Drimia maritima (L.) Stearn	Asparagaceae	Not R	M	A, B, C
Echinops spinosissimus subsp *fontqueri* (Pau) Greuter	Compositae	Not R	IBNA	D
Echium vulgare L. subsp. *pustulatum* (Sm.) E. Schmind & Gams	Boraginaceae	Not R	M	A
Elaeoselinum foetidum (L.) Boiss.	Apiaceae	Not R	IBNA	C
Equisetum ramosissimum Desf.	Equisetaceae	Not R	C	B
Erica multiflora L.	Ericaceae	Not R	M	B, C, D
Erodium chium (L.) Willd.	Geraniaceae	Not R	M	B, C
Erodium malacoides (L.) L'Hér.	Geraniaceae	Not R	M	A, B, C
Eryngium bovei Boiss.	Apiaceae	Not R	IBNA	B
Eryngium campestre L.	Apiaceae	Not R	ME	A
Eupatorium cannabinum L.	Compositae	Not R	ME	B
Euphorbia exigua L. subsp *exigua*	Euphorbiaceae	Not R	ME	B, C
Euphorbia squamigera Loisel.	Euphorbiaceae	R	WM	A
Fagonia cretica L.	Zygophyllaceae	Not R	M	A, C, D
Fedia cornucopiae (L.) Gaertn.	Valerianaceae	Not R	IBNA	B
Ficus carica L.	Moraceae	R+	M	B, C, D
Foeniculum vulgare Mill.	Apiaceae	Not R	M	D
Frankenia corymbosa Desf.	Frankeniaceae	Not R	IBNA	D
Frankenia hirsuta L.	Frankeniaceae	Not R	M	A
Fumana laevipes (L.) Spach	Cistaceae	R+	M	B, C, D
Fumana procumbens (Dunal) Gren. & Godron	Cistaceae	R+	ME	A
Galium album P.Mill	Rubiaceae	R+	ME	C
Galium divaritacum Pour. ex Lam.	Rubiaceae	Not R	M	C
Galium ephedroides Willk	Rubiaceae	R+	IBNA	A, D
Galium setaceum Lam.	Rubiaceae	R+	M	B, C
Galium verrucosum Hudson	Rubiaceae	R+	M	A, C
Galium viscosum Vahl	Rubiaceae	Not R	IBNA	C
Gastridium phleoides (Nees & Meyen) C. E. Hubb	Poaceae	Not R	M	D
Genista spartioides Spach	Fabaceae	Not R	IBNA	A
Geranium spp.	Geraniaceae	R+	ME	B, C
Gladiolus illyricus W. D. J. Koch	Iridaceae	Not R	M	A
Glaucium flavum Crantz	Papaveraceae	Not R	M	D
Hedera maroccana McAllister	Araliaceae	R+	IBNA	B, C
Hedypnois rhagadioloides (L.) F.W. Schmidt	Compositae	Not R	M	A, B, D
Helianthemum hirtum (L.) Miller	Cistaceae	Not R	WM	A
Hyoseris radiata L.	Compositae	R	M	B, C
Hyoseris scabra L.	Compositae	Not R	M	A
Hyparrhenia hirta (L.) Stapf	Poaceae	Not R	M	B, C, D
Hypochaeris achyrophorus L.	Compositae	R+	M	B
Hypochaeris laevigata (L.) Ces., Pass. & Gibelli	Compositae	R	WM	B, C
Hypochaeris radicata L.	Compositae	R+	ME	C
Juniperus sabina L.	Cupressaceae	Not R	M	C
Lactuca tenerrima Pourr.	Compositae	R	WM	B, C
Lagurus ovatus L.	Poaceae	Not R	M	A, C
Lamium maculatum (L.) L.	Lamiaceae	Not R	ME	C
Lapiedra martinezii Lag.	Amaryllidaceae	R+	IBNA	A
Launaea arborescens (batt.) Murb	Compositae	Not R	IBNA	D
Launaea lanifera Pau	Compositae	R+	IBNA	A
Laurus nobilis L.	Lauraceae	Not R	M	B
Lavandula dentata L.	Lamiaceae	R+	M	A, C, D

Associated species	**Family**	**Habitat**	**Chorology**	**Areas**
Lavandula multifida L.	Lamiaceae	R+	M	A, B
Leontodon saxatilis subsp. *rothii* Maire	Compositae	Not R	M	A, B
Limonium ovalifolium (Poir.) Kuntze	Plumbaginaceae	R+	WM	D
Linaria tristis (L.) Mill. subsp *pectinata* (Pau & Font Quer) Maire	Scrophulariaceae	R	IBNA	B, C
Linum strictum L.	Linaceae	Not R	M	A, B
Lithodora maroccana I.M. Johnston	Boraginaceae	R+	IBNA	B
Lobularia maritima (L.) Desv. subsp *maritima*	Brassicaceae	Not R	M	A
Lonicera implexa Acton	Caprifoliaceae	Not R	M	C
Lotus corniculatus L.	Fabaceae	R+	ME	B
Lotus preslii Ten.	Fabaceae	R+	M	D
Lycium intricatum Boiss	Solanaceae	Not R	M	A, D
Macrochloa tenacissima (L.) Kunth	Poaceae	Not R	M	A, D
Malva hispanica L.	Malvaceae	Not R	IBNA	C
Malva subovata (DC.) Molero & J. M. Monts.	Malvaceae	R+	WM	D
Mauranthemum paludosum (Poir.) Vogt & Oberprieler	Compositae	Not R	IBNA	B
Maytenus senegalensis (Lam.) Exell	Celastraceae	Not R	IBNA	A
Melica ciliata subsp. *magnolii* (Gren. & Godr.) K. Richt.	Poaceae	R+	WM	B, D
Melica minuta L. subsp. *Minuta*	Poaceae	Not R	WM	C
Melica minuta subsp. *latifolia* (Coss.) W. Hempel	Poaceae	R+	M	A, B, C
Melica uniflora Retz	Poaceae	R+	ME	D
Mentha aquatica L.	Lamiaceae	Not R	C	B
Mercurialis annua L.	Euphorbiaceae	Not R	C	A, B, C, D
Mesembryanthemum crystallinum L.	Aizoaceae	Not R	IN	D
Micromeria inodora (Desf.) Benth.	Lamiaceae	R+	IBNA	B
Myrtus communis L.	Myrtaceae	Not R	M	B
Neoschischkinia reuteri (Boiss.) Valdés & H. Scholz	Poaceae	Not R	IBNA	B
Nerium oleander L.	Apocynaceae	Not R	M	B, D
Nothoscordum gracile (Aiton) Stearn	Amaryllidaceae	Not R	IN	D
Olea europaea L.	Oleaceae	Not R	M	B, C
Ononis pusilla L. subsp *saxicola* (Boiss. & Reut) Malag.	Fabaceae	R+	IBNA	C, D
Opuntia maxima Mill	Cactaceae	Not R	IN	C
Origanum elongatum (Bonnet) Emb. & Maire	Lamiaceae	Not R	IBNA	B
Orobanche sp.	Orobanchaceae	Not R	C	A
Osyris alba L.	Santalaceae	Not R	M	B, C
Osyris lanceolata Hochst & Stend	Santalaceae	Not R	IBNA	A, B
Oxalis pes-caprae L.	Oxalidaceae	Not R	IN	A
Pallenis maritima (L.) Greuter	Compositae	R+	M	A, B, D
Pallenis spinosa (L.) Cass	Compositae	Not R	M	B, C, D
Parietaria judaica L.	Urticaceae	R	M	C, D
Paronychia suffruticosa (L.) DC. subsp *suffruticosa*	Caryophyllaceae	R+	IBNA	A
Phagnalon rupestre (L.) DC.	Compositae	R+	M	D
Phagnalon saxatile (L.) Cass.	Compositae	R+	WM	A, B, C
Phalaris paradoxa L.	Poaceae	Not R	M	B, C
Phillyrea latifolia L.	Oleaceae	Not R	M	B, C
*Phlomis purpure*a subsp *caballeroi* (Pau) Rivas Mart.	Lamiaceae	Not R	IBNA	A
Pinus halepensis Mill	Pinaceae	Not R	M	B, C
Piptatherum coerulescens (Desf.) P. Beauv.	Poaceae	R+	M	A, B, D
Piptatherum miliaceum (L.) Cossou.	Poaceae	Not R	M	A, B, C
Pistacia lentiscus L.	Anacardiaceae	Not R	M	B, C, D
Pistacia terebinthus L.	Anacardiaceae	Not R	M	C
Plantago weldenii Rchb.	Plantaginaceae	Not R	M	D
Polygala rupestris Pourr.	Polygalaceae	R	WM	A, B, C, D

Associated species	**Family**	**Habitat**	**Chorology**	**Areas**
Prasium majus L.	Lamiaceae	R+	M	B, C, D
Ptilostemon rhiphaeus (Pau & Font Quer) Greuter	Compositae	R+	IBNA	B, C
Putoria brevifolia Coss. & Durieu	Rubiaceae	R	IBNA	B, D
Quercus coccifera L.	Fagaceae	Not R	WM	C
Quercus rotundifolia Lam.	Fagaceae	Not R	M	C
Reichardia picroides (L.) Roth	Compositae	R+	M	B, D
Reichardia tingitana (L.) Roth	Compositae	Not R	M	A
Rhamnus lycioides subsp *oleoides* (L.) Jahandiez & Maire	Rhamnaceae	Not R	WM	D
Rhodanthemum hosmariense (Ball) B. H. Wilcox & al.	Compositae	R	IBNA	C
Rhodanthemum laouense Vogt	Compositae	R	IBNA	B
Rosa sempervirens L.	Rosaceae	Not R	M	B
Rosmarinus officinalis L.	Lamiaceae	Not R	M	A, B
Rosmarinus tomentosus Hub.-Mor. & Maire	Lamiaceae	R	IBNA	B
Rostraria cristata (L.) Tzvelev	Poaceae	Not R	C	C
Rubia peregrina L.	Rubiaceae	R+	WM	D
Rubus ulmifolius Schott	Rosaceae	Not R	ME	B
Rumex bucephalophorus L.	Polygonaceae	Not R	M	C
Rupicapnos africana subsp *decipiens* (Pugsley) Maire	Papaveraceae	R	IBNA	B, C
Ruscus hypophyllum L.	Asparagaceae	Not R	WM	B, C
Russelia equisetiformis Schltdl. & Cham.	Scrophulariaceae	Not R	IN	D
Ruta angustifolia Pers.	Rutaceae	R+	WM	B, C
Ruta chalepensis L.	Rutaceae	Not R	M	A
Salsola oppositifolia Desf.	Chenopodiaceae	Not R	WM	A
Salvia interrupta subsp. *paui* (Maire) Maire	Lamiaceae	R+	IBNA	C
Sanguisorba ancistroides (Desf.) Ces.	Rosaceae	R	IBNA	C
Sarcocapnos enneaphylla (L.) DC	Papaveraceae	R	WM	A, D
Satureja obovata Lag.	Lamiaceae	Not R	IBNA	A
Scabiosa columbaria L.	Dipsacaceae	R+	M	C
Schoenus nigricans L.	Poaceae	Not R	C	B
Sedum album L.	Crassulaceae	R	ME	B, C
Sedum brevifolium DC	Crassulaceae	R+	WM	B, C, D
Sedum dasyphyllum L.	Crassulaceae	R	M	A, B, C, D
Sedum forsterianum Sm.	Crassulaceae	R	WM	B, C, D
Sedum mucizonia (Ortega) Raym.-Hamet	Crassulaceae	R	IBNA	B, C
Sedum sediforme (Jacq.) Pau	Crassulaceae	R+	M	A, B, C, D
Selaginella sp.	Selaginellaceae	R+	M	C
Sherardia arvensis L.	Rubiaceae	Not R	ME	C
Sideritis romana L.	Lamiaceae	Not R	M	B
Silene obtusifolia Willd.	Caryophyllaceae	R+	IBNA	D
Silene secundiflora Otth	Caryophyllaceae	R+	IBNA	A
Silene vulgaris subsp *commutata* (Guss.) Hayek	Caryophyllaceae	R	M	A
Smilax aspera L.	Smilacaceae	Not R	M	B, C, D
Sonchus asper (L.) Hill	Compositae	Not R	C	B
Sonchus oleraceus L.	Compositae	Not R	C	A, B
Sonchus tenerrimus L.	Compositae	R+	M	A, B, D
Spergularia marina (L.) Griseb.	Caryophyllaceae	Not R	C	D
Stachys circinata L'Hér	Lamiaceae	R	IBNA	C
Stachys fontqueri Pau	Lamiaceae	R	IBNA	C
Stellaria sp.	Caryophyllaceae	Not R	ME	B
Tetraclinis articulata (Vahl) Masters	Cupressaceae	Not R	IBNA	B, C, D
Teucrium dunense Sennen	Lamiaceae	Not R	WM	A
Teucrium fruticans L.	Lamiaceae	Not R	WM	B, C

Associated species	**Family**	**Habitat**	**Chorology**	**Areas**
Teucrium intricatum Lange	Lamiaceae	R	IBNA	A
Teucrium polium L. subsp. polium	Lamiaceae	Not R	WM	C
Teucrium rotundifolium Schreb	Lamiaceae	R	IBNA	A, B
Thapsia villosa L.	Apiaceae	R+	IBNA	A, B
Theligonum cynocrambe L.	Theligonaceae	R	M	B
Thymbra capitata (L.) Cav.	Lamiaceae	Not R	M	D
Thymus munbyanus subsp *coloratus* (Boiss. & Reut.) Greuter & Burdet	Lamiaceae	Not R	IBNA	B, C, D
Thymus vulgaris L subsp. *vulgaris*	Lamiaceae	R+	WM	A
Torilis nodosa (L.) Gaertn	Apiaceae	Not R	ME	B, C
Trachelium caeruleum L.	Campanulaceae	R	WM	B, C
Trifolium campestre Schreb.	Fabaceae	Not R	ME	B, C
Trifolium scabrum L.	Fabaceae	Not R	ME	A
Trifolium stellatum L.	Fabaceae	Not R	M	A, B
Ulex parviflorus Pourr. subsp *parviflorus*	Fabaceae	Not R	IBNA	A
Ulex parviflorus subsp *africanus* (Webb) Greuter	Fabaceae	Not R	IBNA	D
Umbilicus gaditanus Boiss	Crassulaceae	R	WM	A
Umbilicus rupestris (Salisb.) Dandy	Crassulaceae	R	ME	B, C
Urospermum picroides (L.) F. W. Schmidt	Compositae	Not R	M	A, B, C, D
Urtica urens L.	Urticaceae	Not R	C	C
Valantia hispida L.	Rubiaceae	R+	M	A, B
Valantia muralis L.	Rubiaceae	R+	M	A, B, C
Viburnum tinus L.	Caprifoliaceae	Not R	M	B
Viola arborescens L.	Violaceae	Not R	WM	C, D
Vulpia geniculata (L.) Link subsp geniculata	Poaceae	Not R	WM	B, C
Vulpia geniculata subsp *pauana* (Font Quer) Maire	Poaceae	Not R	IBNA	B, C
Vulpia myuros (L.) C.C. Gmel.	Poaceae	Not R	C	B, C, D
Withania frutescens (L.) Pauquy	Solanaceae	R+	IBNA	D
Withania somnifera (L.) Duncal.	Solanaceae	Not R	M	A

Capítulo 4:

DESENTRAÑANDO LOS PATRONES FILOGENÉTICOS Y FILOGEOGRÁFICOS DE UN PEQUEÑO GRUPO DE ESPECIES RUPÍCOLAS DEL MEDITERRÁNEO OCCIDENTAL (*SONCHUS* SECCIÓN *PUSTULATI*, ASTERACEAE)

UNRAVELING PHYLOGENETIC AND PHYLOGEOGRAPHIC PATTERNS OF A SMALL GROUP OF CLIFF-DWELLING SPECIES FROM THE WESTERN MEDITERRANEAN (*SONCHUS* SECTION *PUSTULATI*, ASTERACEAE)

Submitted to: “ANNALS OF BOTANY”

Authors: Jose L. Silva, So-Yeon Lim, Seung-Chul Kim and José A. Mejías

RESUMEN

Objetivos Investigar si eventos geológicos y climáticos ocurridos en la región Mediterránea occidental dieron forma a los patrones filogenéticos y filogeográficos de las especies de *Sonchus* sección *Pustulati*.

Ubicación Las especies de *Sonchus* sección *Pustulati* (*S. masguindalii*, *S. fragilis* y *S. pustulatus*) están restringidas a varias áreas pequeñas no superpuestas dentro del punto caliente de biodiversidad Bético-Rifeño (Mediterráneo occidental). *S. pustulatus* es la única especie que aparece tanto en el suroeste de Europa como en el norte de África, mientras que las otras dos están restringidas al norte de África.

Métodos De manera independiente, estimamos la variación genética y las relaciones filogenéticas de las poblaciones, basándonos en polimorfismos de longitudes de fragmentos amplificados (AFLP) y secuencias de regiones no codificantes de ADN nuclear (ITS/ETS) y cloroplástico (3'*trn*L–*ndh*J). Aplicamos un reloj molecular mediante un método Bayesiano para la datación de los tiempos de divergencia de los linajes principales y para correlacionarlos con los principales eventos geológicos y climáticos.

Resultados Un alto grado de concordancia fue encontrado entre los datos de ITS/ETS y AFLP, los cuales identificaron cinco grupos principales correspondientes con sus distribuciones geográficas. Dos grupos de poblaciones de *S. masguindalii* constituyeron el conjunto más aislado. Por el contrario, los datos de ADNcp separaron claramente las poblaciones de *S. pustulatus* de aquellas de *S. masguindalii* y *S. fragilis*. La divergencia entre *S. masguindalii* y el clado *S. fragilis*-*S. pustulatus* fue datada en 5.48 Ma, entre *S. fragilis* y *S. pustulatus* en 3.89 Ma, y entre *S. pustulatus* de España y de Marruecos en 1.18 Ma. Dentro de cada una de las áreas geográficas se detectó una estructuración genética relativamente alta y una diversidad genética baja-moderada, estando la última particularmente empobrecida en las poblaciones españolas.

Conclusiones principales Nuestros resultados confirmaron la monofilia de la sección *Pustulati* del género *Sonchus* y el origen híbrido de *S. pustulatus* a partir de *S. fragilis* y una especie desconocida como donador materno. El origen y diversificación de los linajes parecen haber ocurrido en el arco de Gibraltar durante la crisis del Messiniense (5.96–5.33 Ma) y la subsecuente inundación Zancliense, la cual fue rellenando progresivamente el mar Mediterráneo (5.33–3.60 Ma). Las poblaciones españolas de *S. pustulatus* se originaron lo más probablemente a partir de las marroquíes, ya fuese mediante una dispersión a larga distancia relativamente reciente o mediante un proceso de vicarianza antiguo.

Palabras claves Arco de Gibraltar; Dispersión a larga distancia; Distribución disyunta; Ecología rupícola; Edades Messiniense-Zancliense; Región Bético-Rifeña; Vicarianza.

ABSTRACT

Aim To investigate whether past major geologic and climatic events in the Western Mediterranean region shaped the phylogenetic and phylogeographic patterns of the species of *Sonchus* section *Pustulati*.

Location The species of *Sonchus* section *Pustulati* (*S. masguindalii*, *S. fragilis* and *S. pustulatus*) are restricted to several narrow non-overlapping areas within the Baetic-Rifan hotspot (Western Mediterranean). *S. pustulatus* is the only species occurring both in SW Europe and N Africa, whereas the other two are restricted to N Africa.

Methods We independently estimated the genetic variation and phylogenetic relationships of populations, based on amplified fragment length polymorphisms (AFLPs) and sequences of nuclear (ITS/ETS) and cloroplastic (3’*trn*L–*ndh*J/*psa*I–*acc*D) noncoding DNA regions. We performed a relaxed Bayesian molecular clock dating to date divergence times for major lineages and to correlate them with major geologic/climatic events.

Results A high degree of concordance was found between the ITS/ETS and AFLP datasets, which identified five major groups corresponding to their geographical distributions. Two population groups of *S. masguindalii* were found to constitute the most isolated set. By contrast, the cpDNA data clearly separated the *S. pustulatus* populations from those of *S. masguindalii* and *S. fragilis*. The divergence between *S. masguindalii* and the clade *S. fragilis*-*S. pustulatus* was dated at 5.48 Ma, between *S. fragilis* and *S. pustulatus* at 3.89 Ma, and between the Spanish and Moroccan *S. pustulatus* at 1.18 Ma. Within each of the geographical areas it was detected a relatively high genetic structuring and a low-moderate genetic diversity, the latter being particularly impoverished in the Spanish populations.

Main conclusions Our results confirmed the monophyly of *Sonchus* section *Pustulati* and the hybrid origin of *S. pustulatus* from *S. fragilis* and an unknown species as the maternal donor. The origin and diversification of lineages appear to have occurred in the Gibraltar arc during the Messinian Salinity Crisis (5.96–5.33 Ma) and the subsequent Zanclean flood that progressively refilled the Mediterranean Sea (5.33–3.60 Ma). The Spanish populations of *S. pustulatus* most likely originated from the Moroccan ones, either via recent long-distance dispersal or ancient vicariance.

Keywords Baetic-Rifan region; Cliff ecology; Disjunct distribution; Gibraltar arc; Long distance dispersal; Messinian-Zanclean ages; Vicariance**.**

1. INTRODUCTION

Mediterranean endemic plant species provide a fascinating material for the study of phylogeography (Thompson, 2005). One of the most interesting regions is the plant biodiversity Baetic-Rifan hotspot (Médail & Quézel, 1997), which includes European and African areas separated by the Mediterranean Sea and the Strait of Gibraltar. The high biodiversity therein is explained by a complex geologic and climatic history that gave rise to highly diverse habitats (Médail & Quézel, 1997; Thompson, 2005). However, this region is very consistent from a floristic point of view, which is reflected by the high percentage of species common to the European and African ranges (about 75%; Valdés, 1991). Thus, the Baetic-Rifan hotspot symbolizes quite an intricate scenario from a biogeographical perspective (Molina-Venegas *et al.*, 2013).

The Internal Zones of the Baetic and Rifan orogens (Chapter 1: Fig. 1b) originated from a common terrain located between the Iberian Peninsula and southern France during the Oligocene (≈30 Ma), which suffered drift and fragmentation into microplates during the Miocene (Lonergan & White, 1997; Rosenbaum *et al.*, 2002). When the Baetic-Rifan microplate reached the south-western Mediterranean (21–18 Ma), it remained separated from the Iberian Peninsula and Africa by the Baetic and Rifan corridors, respectively. The positive relief associated with the fold-and-thrust belt at the western frontal zone of the microplate gave rise to a stretch of land that, by the Middle Miocene (15 Ma), probably joined Africa and Iberia in a position in the middle of the present-day Alboran Sea (Rosembaun *et al.*, 2002). As the eastwards-dipping subduction zone migrated to the west and the Alboran domain underwent progressive back collapse, this land bridge/stretch migrated westwards until it reached its current position in the Gibraltar Arc (Late Miocene, 10 Ma). Closure of the Rif and Betic corridors accompanied the process, finally leading to the separation of the Mediterranean and Atlantic seas during the Messinian (6 Ma). This promoted the desiccation of the Mediterranean Sea (Messinian Salinity Crisis, 5.96–5.33 Ma; Krijgsman *et al.*, 1999; Fauquette *et al.*, 2006) and facilitated intercontinental expansion of plant distribution until the opening of the Strait of Gibraltar (5.33 Ma; e.g. Caujapé-Castells & Jansen, 2003; Cano-Maqueda *et al.*, 2008) and the Zanclean flood that progressively refilled the Mediterranean sea (5.33–3.60 Ma). Thereinafter, the sea barrier likely made the plant intercontinental expansion highly dependent on long-distance seed dispersal.

Most phylogeographical studies in the Baetic-Rifan region focus upon the role of the Strait of Gibraltar as a bridge or a barrier for species migration and gene flow between the northern (European) and southern (African) ranges (see review of Rodríguez-Sánchez *et al.*, 2008). Apart from addressing the disjunct populations around the Strait of Gibraltar, phylogenetic/phylogeographic investigation of additional plant taxa in the Western Mediterranean Basin provides us with insights into the role of sea barriers as a limiting factor for range expansion. Incorporating time-calibrated phylogenetic analyses of taxa with disjunct distributions is crucial with regard to fully understanding dispersal, colonization and isolation processes in the region (e.g. Caujapé-Castells & Jansen, 2003; Casimiro-Soriguer *et al.*, 2010; Fernández-Mazuecos & Vargas, 2011). In addition, analyses of population genetic structure and diversity enable us to determine the degree of connection between them and the range that potentially acted as the diversification origin centre (e.g. Casimiro-Soriguer *et al.*, 2010).

Sonchus pustulatus Willk., *S. fragilis* Ball and *S. masguindalii* Pau and Font Quer constitute a group of rare cliff-dwelling species (Asteraceae), endemic to narrow areas within the Baetic-Rifan hotspot (Figure 1a; Boulos, 1973). They belong to the well-supported section *Pustulati* within the *Sonchus* subgenus *Sonchus* (Boulos, 1973; Kim *et al.*, 2007). All three species are distributed in the Rifan range, where they have been considered as very rare (Fennane & Ibn Tatoo, 1998) and *S. pustulatus* is the only species present in the Baetic range, where it is categorized as “critically endangered” (Bañares *et al.*, 2004). Due to the highly restricted and disjunct distribution, and the ecological particularities of these cliff plants (Chapter 3), the section *Pustulati* is a good model system for study of the plant intercontinental colonization processes and their relationship with the paleogeography of the Baetic-Rifan region.

In the present study, we attempted to test whether the current distributions of these species are due to historical vicariance events in the Baetic-Rifan region. We conducted phylogenetic and phylogeographic studies in *Sonchus* section *Pustulati* based on noncoding cpDNA and nrDNA (ITS/ETS) sequences and amplified fragment length polymorphism (AFLP). The following specific questions were addressed: (i) how consistent are phylogenetic analyses with the current taxonomic treatment?; (ii) could the origin and diversification of the species be related with the Messinian Salinity Crisis and the subsequent Zanclean flood that progressively refilled the Mediterranean Sea?; (iii) what are the implications of the biological and ecological traits of the species for their phylogeography and population genetic structure?; and (iv) is the narrow range expansion associated with low genetic diversity?

2. MATERIAL AND METHODS

2.1 The species of Sonchus section Pustulati

Based on a previous prospection, 19 populations of *Sonchus* section *Pustulati* were distinguished in five small distribution areas (Chapter 2; Figure 1a) restricted to the Baetic-Rifan Internal Zone. The rare Spanish populations of *S. pustulatus* are located in the environs of Almeria city (Figure 1a, area A, southeastern Spain), whereas the Moroccan ones colonize cliffs in the Oued Laou valley and some neighbouring valleys (Figure 1a, area B, western Rif). *S. fragilis* is exclusive to the mountains surrounding the city of Tetouan (Figure 1a, area C, northwestern Rif). Finally, *S. masguindalii* is distributed along the coast in the Bokkoya Mountains and some sea cliffs in the environs of Al-Hoceima city (Figure 1a, area D, central Rif). Some herbarium specimens of *S. pustulatus* from northwestern Algeria collected in the 19th century (e.g. herbarium COI) indicate that this was also present in rocky places on the oceanfront near Ghazaouet (formerly Nemours; Figure 1a, area E, northwestern Algeria), but its persistence in the area has not been determined.

These endemic species are found in a highly fragmented habitat and are specifically associated with north-facing cliffs at a low-to-medium altitude, close to or facing the sea, and with an alkaline substrate, mainly limestones (Chapter 3). They are polycarpic perennial, mostly self-incompatible, with generalist entomophilous pollination, and exceptionally *S. fragilis* shows a high incidence of the self-compatibility (Chapters 5). Achenes are heavier than the widespread *Sonchus* species and have a markedly deciduous pappus.

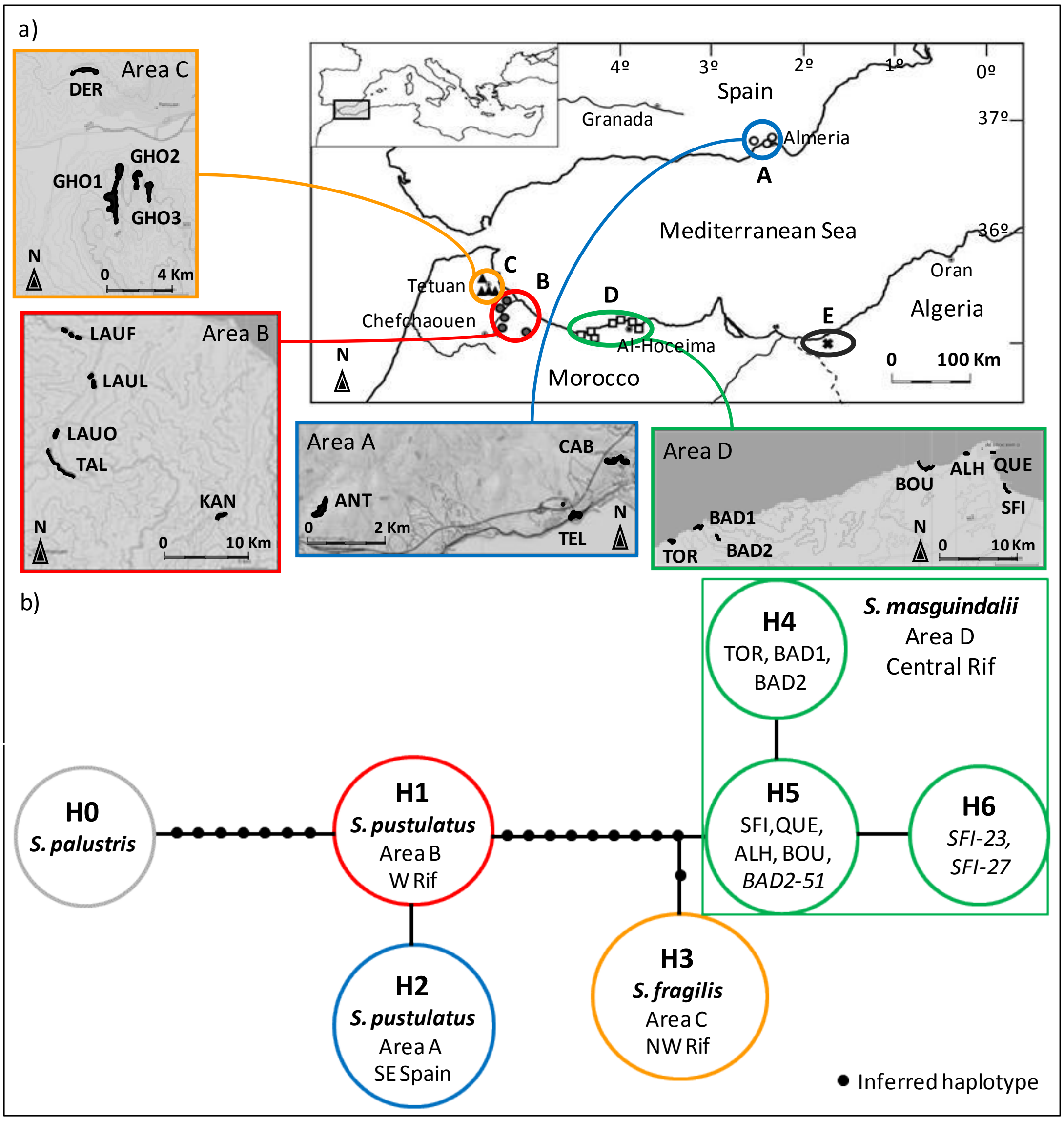


Figure 1 a) Distribution range of the species of *Sonchus* section *Pustulati*, locations of all known populations, and area of occupancy. Area A (blue), Spanish populations of *S. pustulatus*; area B (red), Moroccan populations of *S. pustulatus*; area C (orange), of *S. fragilis*; area D (green), of *S. masguindalii*; Area E, unconfirmed current presence of *S. pustulatus*. b) Statistical parsimony network of the six plastid haplotypes found in the section. Each circle, line and small black dot represents a haplotype, a nucleotide substitution and an inferred intermediate unsampled haplotype, respectively.

2.2 Populations sampled and DNA extraction

Leaf tissue samples were collected from 15 to 20 georeferenced individuals per population (using GPS). In order to obtain representative samples of whole populations and to avoid collecting siblings, we made a linear transect along the cliffs and collected one sample every 10–15 meters. The leaves were immediately dried and stored in silica gel at room temperature until further processing. Voucher specimens were deposited in the University of Seville herbarium (SEV). A total of 316 individuals were used for the molecular analyses. Total genomic DNA was isolated from ca. 1 cm^2 of leaf tissue with the Nucleo Spin Plant II Kit (Macherey-Nagel GmbH and Co. KG, Düren, Germany) according to the manufacturer's protocol. DNA concentration and quality of each sample were checked on 0.9% agarose gels and in a NanoDrop1000 spectrophotometer (ThermoScientific, Wilmington, USA). DNA was diluted to a final concentration of 30 ng/µl.

2.3 DNA sequencing

For cpDNA sequencing, a total of 92 individuals were selected (3–5 per population; Table 1), plus three individuals of the related species *S. palustris*, which was used as the outgroup. To sequence the ITS/ETS region of nrDNA, three individuals from each population were selected, making a total of 60 individuals including three of the same outgroup species. For cpDNA, of the 34 noncoding regions that were initially surveyed (Shaw *et al.*, 2007), the two regions with the highest potentially informative character value were selected, i.e. the *ndh*J–3'*trn*L(UAA) [=Tab E] and *acc*D–*psa*I. PCR amplification of nrDNA ITS and ETS was performed as described by Lee *et al.* (2005), but the ETS region was amplified with the use of the primers *ETS1f* (5'–CTTTTTGTGCATAATGTATATATAGGGGG–3') and *18S*–2L (5'–TGACTACTGGCAGGATCAACCAG–3') designed by Linder *et al.* (2000). The same PCR conditions for two cpDNA regions were applied. PCR products were purified with GENEALL EXPIN PCR SV (GeneAll Biotechnology, Co., Ltd., Seoul, Korea). Sequencing was conducted at the Geno Tech Corporation (Seoul, Korea). Base calling and sequence editing were performed with SEQUENCHER v4.2.2 (Gene Codes, Ann Arbor, Michigan, USA). Sequences were aligned manually with MACCLADE (v.4.06, Maddison & Maddison, 2003).

Table 1 Populations of *Sonchus* section *Pustulati* and AFLP results. N, population size (Chapter 2); n_1, n_2 and n_3, number of analyzed individuals for cpDNA and ITS/ETS nrDNA sequencing and AFLP, respectively. *%P*, percentage of polymorphic loci; Fr_{PRI}, number of private fragments. DW, *rarity index*: *a*, based on all plants analyzed, *b*, based on populations of *S. pustulatus* and *S. fragilis*, and *c*, based on populations of *S. pustulatus*. DW values significantly higher ($^{+}$) or lower ($^{-}$) than those to be expected by chance appear in bold. H_E, average gene diversity.

Species and location	Acronym	Coordinates North / West	N	n_1	n_2	n_3	%P	Fr_{PRI}	DW			H_E ± SE
									a	*b*	*c*	
A) SPAIN, southeast												
Sonchus pustulatus			898	15	9	34						0.109 ± 0.056
Bco. San Antonio, Aguadulce, 300m	ANT	36º49.8’ / 2º34.4’	484	5	3	12	41.9	1	1.8	2.8	3.2	0.088 ± 0.014
Bco. San Telmo, Almeria, 22m	TEL	36º49.7’ / 2º29.0’	112	5	3	11	45.1		1.8	**2.6**$^{-}$	**2.9**$^{-}$	0.101 ± 0.017
Bco. Caballar, Almeria, 100m	CAB	36º50.6’ / 2º28.7’	292	5	3	11	47.8	2	1.9	**2.6**$^{-}$	**2.9**$^{-}$	0.093 ± 0.016
B) MOROCCO, western Rif												
Sonchus pustulatus			9991	24	15	48						0.167 ± 0.082
Oued Laou valley, 120m	LAUF	35º23.3’ / 5º12.6’	1062	5	3	12	50.6		**2.3**$^{+}$	**3.6**$^{+}$	**4.2**$^{+}$	0.160 ± 0.025
Oued Laou valley, 153m	LAUL	35º20.8’ / 5º11.0’	540	5	3	11	52.6		2.0	2.9	3.2	0.138 ± 0.023
Oued Laou valley, 150m	LAUO	35º17.4’ / 5º13.8’	221	4	3	4	44.3		2.0	3.0	**4.3**$^{+}$	0.145 ± 0.048
Oued Laou valley, 235m	TAL	35º16.0’ / 5º13.8’	1980	5	3	10	48.0		-	-	-	0.146 ± 0.025
Oued Al-Kannar defile, 244m	KAN	35º13.0’ / 5º01.2’	6188	5	3	11	52.2		1.8	2.7	3.2	0.155 ± 0.025
C) MOROCCO, northwestern Rif												
Sonchus fragilis			33927	18	12	11						0.138 ± 0.074
Montes Ghorghiz, Tetouan, 550m	GHO1	35º32.1’ / 5º23.1’	26985	5	3	6	45.8	1	**2.6**$^{+}$	**4.5**$^{+}$		0.146 ± 0.036
Montes Ghorghiz, Tetouan, 550m	GHO2	35º32.8’ / 5º22.6’	-	3	3	5	41.9		**2.5**$^{+}$	**4.6**$^{+}$		0.120 ± 0.034
Montes Ghorghiz, Tetouan, 550m	GHO3	35º32.3’ / 5º22.3’	661	5	3	-	-	-	-	-		-
Montes Dersa, Tetouan, 275m	DER	35º35.9’ / 5º24.6’	6282	5	3	-	-	-	-	-		-
D) MOROCCO, central Rif												
Sonchus masguindalii			42269	35	21	74						0.177 ± 0.086
Torres de Alcalá beach, sea level	TOR	35º09.4’ / 4º19.7’	6244	5	3	10	44.3	1	**1.8**$^{-}$			0.146 ± 0.025
Bades beach - Peñón Vélez, s.l.	BAD1	35º10.2’ / 4º17.9’	6444	5	3	10	50.6	2	2.1			0.139 ± 0.024
Valley to Bades beach, 60 m	BAD2	35º09.6’ / 4º16.9’	638	5	3	12	43.5		1.8			0.138 ± 0.021
Boumahdi beach, s.l.	BOU	35º14.0’ / 4º00.7’	19513	5	3	12	45.1		1.6			0.117 ± 0.018
Cebadilla beach, Al-Hoceima, s.l.	ALH	35º14.6’ / 3º58.0’	688	5	3	9	42.3		**1.5**$^{-}$			0.096 ± 0.018
Quemado beach, Al-Hoceima, s.l.	QUE	35º14.5’ / 3º55.5’	6708	5	3	11	52.2	3	**2.2**$^{+}$			0.157 ± 0.025
Sfiha beach, Al-Hoceima, s.l.	SFI	36º12.8’ / 3º54.3’	2035	5	3	10	50.2		2.0			0.169 ± 0.029

2.4 AFLP reaction

For the AFLP we analyzed a total of 167 individuals, ranging from 4 to 12 per population (Table 1). We followed the protocol of Vos *et al.* (1995) with some modifications (Lauterbach *et al.*, 2011). A total of 18 AFLP primer combinations were tested for their capacity to detect polymorphisms and their reproducibility of banding patterns in all the *Sonchus* taxa studied. The three most polymorphic primer combinations were used for selective PCR: *Eco*RI ACA–*Tru*1l CTG; *Eco*RI ACA–*Tru*1l CAG; *Eco*RI ACC–*Tru*1l CAA. One sample from each distribution area (3% of the total) was run on different plates to calculate the average reproducibility of the method (Bonin, 2004). The AFLP fragments were separated on a polyacrylamide gel with an internal size standard (GenomeLab DNA Size Standard Kit 400, Beckman Coulter, Krefeld, Germany) by means of an automated sequencer (CEQ 8000, Beckman Coulter). Data files were imported into GENOGRAPHER software (v1.6.0, J.J. Benham, Montana State University, Bozeman, MT, USA) and fragments from 75 to 450 bp were identified and scored automatically for their presence and absence. A negative control (no DNA) showed no amplification. Finally, fragments were checked individually, without prior information on their origin, by means of the “thumbnail” function, and a presence/absence matrix was exported for further analyses.

2.5 Data analyses

2.5.1 cpDNA and nrDNA ITS/ETS sequences

Three different phylogenetic analyses were conducted for both cpDNA (two combined noncoding regions) and nrDNA (combined ITS and ETS regions) datasets. We first conducted an equally weighted unordered maximum parsimony (MP) approach (Fitch, 1971) implemented in PAUP v4.0 (Swofford, 2002). The MP analyses included a default heuristic search option with TBR branch swapping and MULPARS on. Bootstrap support (BS) was calculated by bootstrap analysis from 1000 replicates with the same heuristic options. Secondly, we conducted maximum likelihood (ML) analyses, in which optimal models of molecular evolution were chosen with the likelihood ratio test (Whelan & Goldman, 1999) implemented in MODELTEST v3.7 (Posada & Crandall, 1998). Model parameters were then imported into PAUP, and a heuristic search (asis sequence addition, TBR branch swapping, and MULPARS option on) was executed. We also conducted ML bootstrap analyses with 1000 replicates to determine support of each clade. Thirdly, we performed Bayesian analyses (BI) using MRBAYES v3.1.2 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). We calculated the likelihood parameters for the Bayesian analysis using MRMODELTEST v2.2 (Nylander, 2004). We ran the Bayesian Markov chain Monte Carlo algorithm for 1000000 generations, with four simultaneous chains (three “cold” and one “heated”), starting from random trees and sampling every 100 generations. A 20% burn-in was removed from the sampled set of trees and a 50% majority-rule consensus tree from the remaining trees was generated. For estimates of the Bayesian clade support, we considered strong support for values of posterior probability (PP) ≥0.95, moderate support for 0.90≤ PP ≥0.94, and weak to no support for PP ≤0.89.

A haplotype network was also constructed from the cpDNA dataset. Due to the absence of homoplasy in this dataset, based on the consistency index (CI) of the MP analysis (see Results), haplotypes were networked manually using the principle of maximum parsimony.

In order to calibrate the phylogeny, we performed a Bayesian relaxed-clock approach using the uncorrelated-rates model implemented in the program BEAST v1.7.3 (Drummond *et al.*, 2006; Drummond & Rambaut, 2007). Our newly generated sequences of *Sonchus* section *Pustulati* were combined with the ITS dataset of the subtribe *Sonchinae* available in Gen Bank, which were generated by Kim *et al.* (2007). We identified the best-fit substitution model for the ITS data (GTR+I+G) with MRMODELTEST v2.2 (Nylander, 2004). The input data were compiled in BEAUTI v1.7.3 (Drummond *et al.*, 2006; Drummond & Rambaut, 2007) with the two prior sets as follows: i) the age for the most recent common ancestor of genus *Dendroseris* in the Juan Fernandez Islands; normal prior distribution with a mean of 3.3 Ma and standard deviation of 0.4 (giving a 95% CI ranging from 2.6–4.0 Ma; Sang *et al.*, 1994); ii) the age for the monophyletic clade of *Reichardia*, early diverged genus within the *Sonchinae*; uniform prior distribution with a mean of 8.1 Ma and standard deviation of 2.8 (giving a 95% CI of 3.6–13.6 Ma; S-C. Kim, unpublished). The clock model was set to relaxed uncorrelated lognormal and the Yule process was chosen as speciation process. Posterior distributions for each parameter were estimated by means of a MCMC run for 40000000 generations with parameters logged every 5000 generations. The output log files were analyzed with TRACER v1.5 (Rambaut & Drummond, 2009) to assess convergence and to confirm that the effective sample sizes (ESS) for all parameters were larger than 200. This ensures that the MCMC chain had run long enough to produce a valid estimate of the parameters. We discarded 10% of burn-in (i.e. 800 trees) trees and produced a maximum credibility tree using TREEANNOTATOR v1.7.3 (Drummond *et al.*, 2006; Drummond & Rambaut, 2007).

2.5.2 AFLP analyses

To assess population genetic diversity from the presence/absence matrix, we calculated the percentage of polymorphic fragments (*%P*) with AFLP-SURV v1.0 (Vekemans, 2002), and the number of private fragments (Fr_{PRI}) with FAMD v1.25 (Schlüter & Harris, 2006). Average gene diversity (H_E) at population level was computed with ARLEQUIN v3.11 (Excoffier *et al.*, 2005). We determined an additional measure of isolation degree, the *Rarity 1 Index* (equivalent to the frequency of down-weighted marker values; i.e. DW sensu Schönswetter & Tribsch, 2005) with R v2.14.1 (R development Core Team, 2011) and AFLPDAT functions (Ehrich, 2006). The values were calculated from 100000 repetitions by means of the R function *Rarity.permut*, which implements a permutation approach to assess whether rarity values for populations are higher or lower than what is to be expected by chance, assuming that all markers are distributed in all populations at random. In order to estimate among-populations relationships, Nei's pairwise genetic distance data and 10000 bootstrap distance matrices were computed with AFLP-SURV v1.0 (Vekemans, 2002). We used these to build a neighbour-joining tree (PHYLIP software; Felsenstein, 1993).

We employed two Bayesian approaches to infer the number of independent genetic clusters (*K*) in our data using STRUCTURE v2.3.1 (Pritchard *et al.*, 2000) and the R package GENELAND v4.0.2 (Guillot *et al.*, 2005; Guillot & Santos, 2010). For STRUCTURE analysis we used the 'admixture' and the 'correlated allele frequency' models. This combination most accurately assigns individuals to closely related groups (Falush *et al.*, 2007). Twenty independent runs for each *K* = 1–18 were performed by means of 75000 iterations of burn-in followed by 500000 iterations to ensure convergence of the Monte Carlo Markov Chains (MCMC). The best estimate for the number of clusters (Delta-*K*, as described by Evanno *et al.*, 2005) was computed with STRUCTURE HARVESTER (Earl & vonHoldt, 2011). The software CLUMPP, v1.1.2 (Jakobsson & Rosenberg, 2007) was utilized with the Greedy algorithm and 10000 random input orders of 20 independent runs to determine the optimal alignment of clusters across individual runs for each *K*. The results from CLUMPP were imported into DISTRUCT, v1.1 (Rosenberg, 2004) for viewing.

For the inference of *K* with GENELAND, we ran 100000 MCMC iterations and 20 replications for each value of *K*, setting the thinning to 100, allowing *K* to vary from 1 to 18, and with all spatial individual coordinates added. We employed the correlated frequency model, which enables even subtle genetic structuring to be detected (Guillot, 2008). A burn-in period of 200 was computed in the post-processing. We then calculated the mean posterior probability distribution of the data for each of the 20 runs, and selected only the 10 runs with the highest posterior distribution to be considered in the analysis. The results of computations were visually checked and were not consistent across runs (10 independent MCMC simulations) in terms of estimated number of population *K*, so the conclusion was based on the run giving the highest average posterior probability, as suggested by the GENELAND software manual.

In order to detect possible patterns of isolation by distance, we performed Mantel tests with 9999 permutations using GENALEX v6.41 (Peakall & Smouse, 2006). To this end, we tested the correlation between individual-by-individual genetic (pairwise Nei's distances) and log-transformed geographic distances for all sampled individuals and for each distribution area. We finally determined the extent of genetic differentiation, measured as F_{ST} (the fixation index), both for pairs of populations (pairwise-F_{ST}) and for geographically-based sets of populations (AMOVA; ARLEQUIN v3.1.1; Excoffier *et al.*, 2005). The confidence intervals of the F_{ST} values were determined through bootstrapping (20000 replicates), as implemented in ARLEQUIN.

3. RESULTS

3.1 cpDNA sequences

The aligned sites of *acc*D–*psa*I and 3'*trn*L(UAA)–*ndh*J intergenic spacers were 775 and 723 base pairs (bp), respectively. Out of 1498 bp of aligned sequences from the two combined intergenic regions, 1475 characters were constant and 23 characters were parsimony informative. For the *acc*D–*psa*I intergenic spacer, three indels were found: (1) a seven-bp insertion and a 17-bp direct repeat insertion shared by *S. fragilis* and *S. masguindalii*; (2) a four-bp direct repeat insertion and an eight-bp direct repeat deletion in all ingroup taxa; (3) a seven-bp insertion shared by *S. pustulatus* and *S.*

masguindalii. As for the 3’*trn*L(UAA)–*ndh*J intergenic spacer, 23-bp and 40-bp deletions were found in *S. fragilis* and *S. pustulatus*, respectively.

The MP analysis for cpDNA data provided more than 100000 equally parsimonious trees with a tree length (TL) of 23, a consistency index (CI) of 1.000 and a retention index (RI) of 1.000. The topology of the resulting MP and ML trees was identical and also consistent with the Bayesian analysis results (Figure 2). These trees showed early divergence of *S. pustulatus* within the section *Pustulati* and a strongly supported branch with *S. masguindalii* and *S. fragilis* as sister taxa (MP:100%BS / ML:100%BS / BI:1.00PP).

In the haplotype network (Figure 1b), two haplotypes were found in *S. pustulatus*, H1 for Moroccan populations (LAUF, LAUL, LAUO, TAL and KAN) and H2 for Spanish populations (ANT, TEL and CAB). *S. fragilis* had only one haplotype (H3) and *S. masguindalii* three: H4 (TOR, BAD1 and BAD2), H5 (BOU, ALH, QUE, SFI and one individual from BAD2), and H6 (two individuals from SFI).

3.2 ITS and ETS nuclear ribosomal DNA sequences

The aligned ITS and ETS regions were 646 and 393 bp, respectively. Out of 1039 aligned sites, 908 were constant, 2 were variable but parsimony uninformative, and 129 were variable and parsimony informative. In the Moroccan populations of *S. pustulatus*, we detected few polymorphic sites in the ITS and ETS sequences, with only four of a total of 393 aligned sites in ETS (1%) and 12 of 495 aligned sites in the ITS 1 and 2 regions

(2.4%). The populations of Spanish *S. pustulatus*, *S. fragilis* and *S. masguindalii* showed no polymorphisms in the ITS and ETS sequences.

The MP search found more than 100000 equally parsimonious trees, with a TL of 144, a CI of 0.9583 and an RI of 0.9946 (trees not shown). The model test analysis found GTR+G as the best model for nucleotide evolution, and the ML search found one single tree (Figure 3). The BI tree (not shown) presented the same tree topology as that of the MP and ML. All three different analyses based on combined ITS/ETS showed that the section *Pustulati* is monophyletic (100%BS/100%BS/1.00PP) and is quite divergent from the chosen outgroup species *S. palustris*. They also clearly demonstrated early divergence of *S. masguindalii* within the section *Pustulati* and a sister relationship between *S. pustulatus* and *S. fragilis* (92%BS/95%BS/1.00PP).

There was no ribotype variation within *S. fragilis*, whereas three ribotypes were found in each of the *S. masguindalii* and *S. pustulatus* species. Within *S. masguindalii*, populations TOR, BAD1 and BAD2 turned out to be sisters to populations BOU and ALH (80%BS/91%BS/0.99PP), and in turn, the latter were sisters to SFI and QUE. In *S. pustulatus*, two and one ribotypes were found in the Moroccan and Spanish populations, respectively.

The most recent common ancestor age of *Sonchus* section *Pustulati* was estimated to be approximately 5.48 Ma (3.171–8.766 Ma 95% CI; Figure 4a and Appendix: Figure 6). Within the section *Pustulati* (Figure 4), the divergence time between *S. pustulatus* and *S. fragilis* was approximately 3.89 Ma (2.04–6.48 Ma 95% CI), and between Spanish and Moroccan populations of *S. pustulatus*, relatively recent, i.e. approximately 1.18

Ma (0.42–2.41 Ma 95% CI). The latter time was similar to the gradual divergence of *S. masguindalii* populations (1.39 Ma; 0.46–2.83 Ma 95% CI).

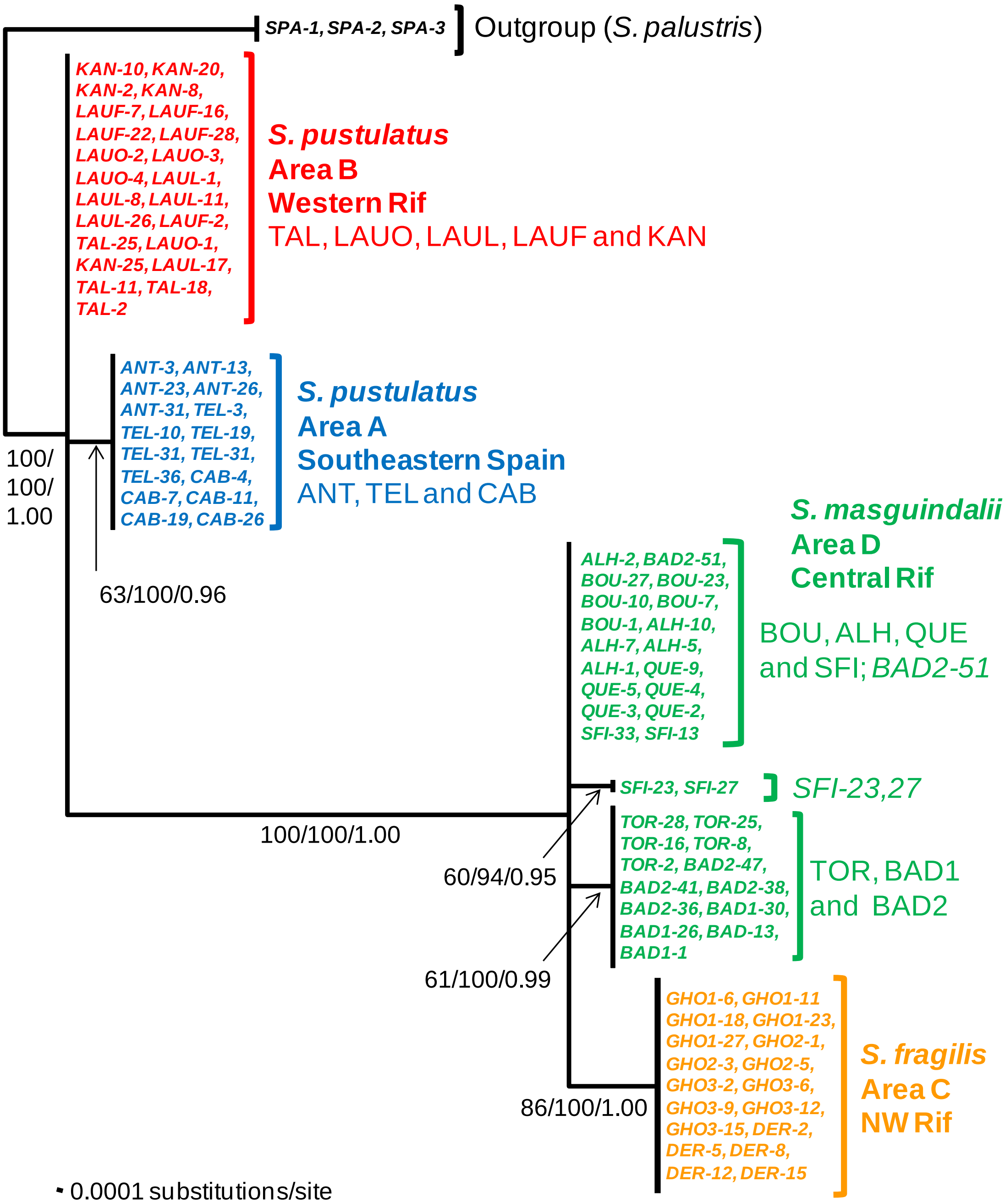


Figure 2 One ML tree from the combined analysis of plastid accD–psaI/3’trnL(UAA)–ndhJ sequences. Numbers of branches are bootstrap values of MP/ML/PP.

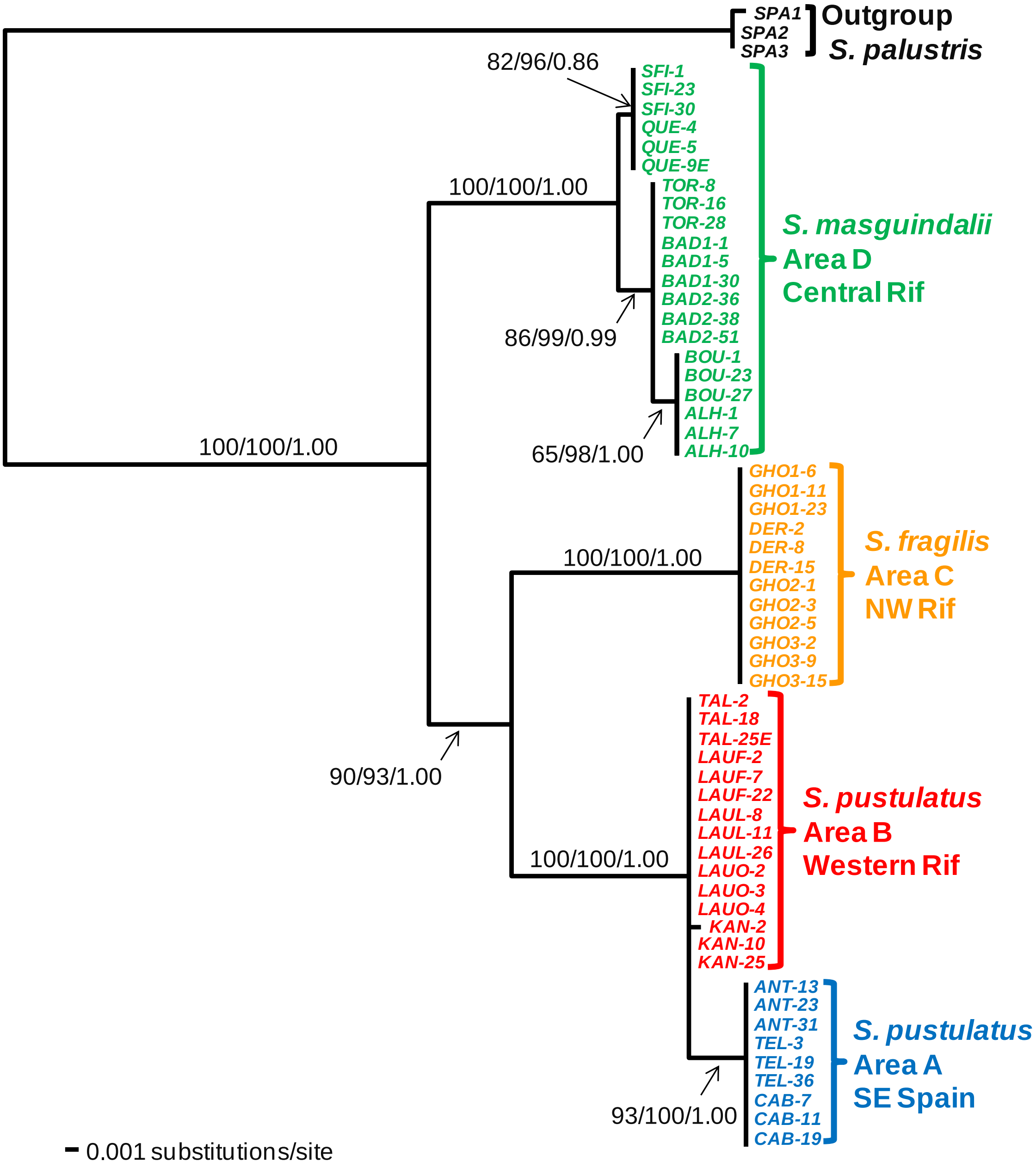


Figure 3 One ML tree from the analysis of nuclear ITS/ETS sequences. Numbers of branches are bootstrap values of MP/ML/PP.

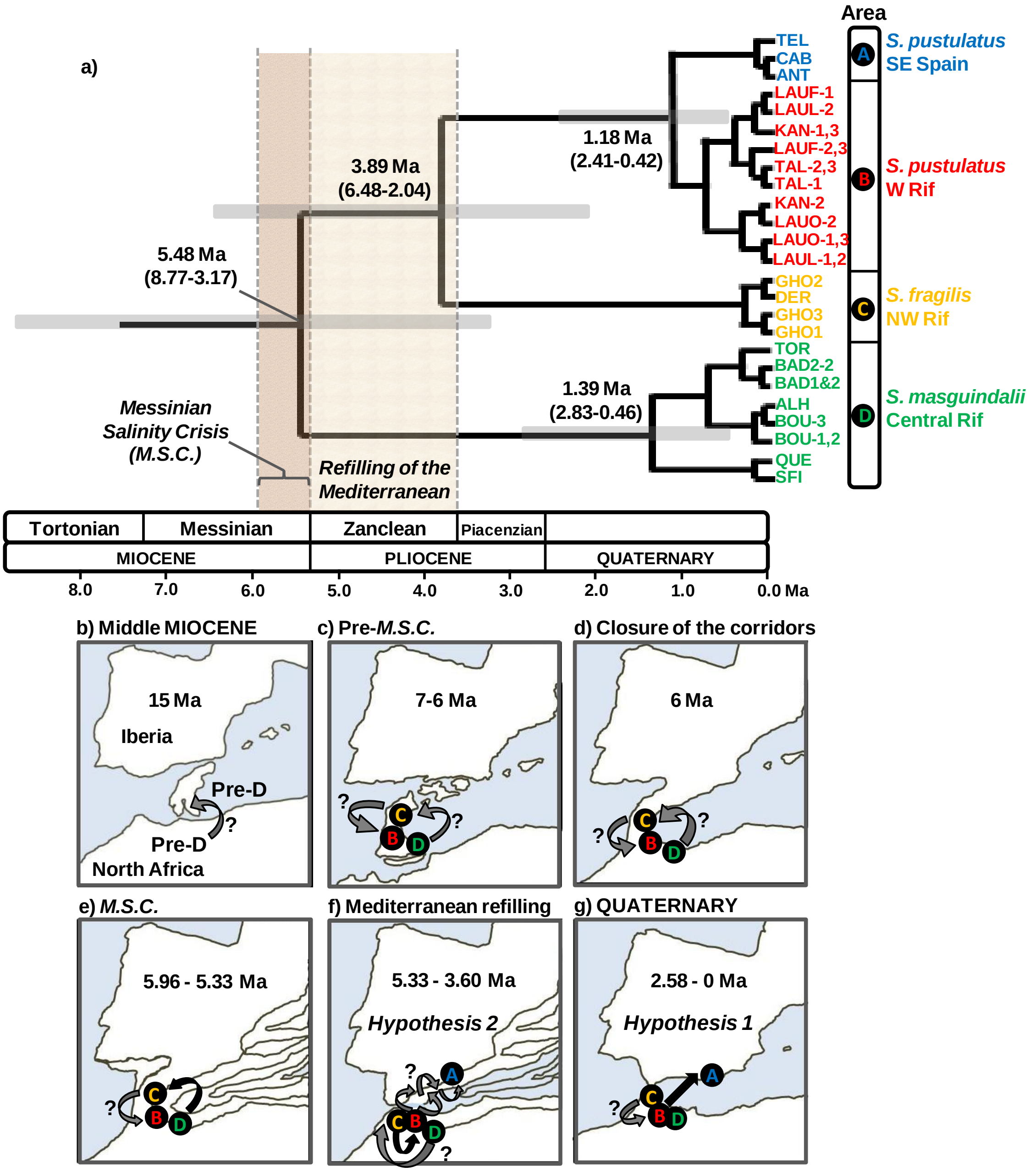


Figure 4 a) Chronograms of the evolution of *Sonchus* section *Pustulati* based on molecular dating from the nuclear ITS/ETS sequences. Capital letters (A-D) represent the distribution areas of taxa as in Figure 1a. Node bars represent the 95% highest posterior density intervals for the divergence time estimates of clades. b)-g) Phylogeographical reconstruction of the lineages across the Western Mediterranean Basin since the Middle Miocene. Pre-D (ancestor) and A-D letters on maps represent the estimated occupied region for the taxa; arrows indicate possible diversification and colonization events. Paleogeographic information was taken from Krijgsman *et al.* (1999), Martín *et al.* (2001), Rosenbaum *et al.* (2002), Fauquette *et al.* (2006) and García-Castellanos *et al.* (2009).

3.3 AFLP marker diversity

The average reproducibility of the AFLP analysis, i.e. the average proportion of correctly reproduced bands over all replicates, was 98.4 ± 1.17 % (mean ± SE). All individuals (167) exhibited different multiloci genotypes. The percentage of polymorphism (*%P*) varied from 41.9 to 52.6% and $Frag_{PRI}$ was zero in most populations (Table 1). The DW values based on all studied plants and those based populations of *S. pustulatus*-*S. fragilis* were significantly higher in the *S. fragilis* populations and, in general, were significantly lower in the Spanish populations (Table 1). The genetic diversity values of the Spanish populations of *S. pustulatus* were low (total H_E = 0.109) in comparison with the remaining distribution areas (total H_E = 0.138–0.177), and with the Moroccan *S. pustulatus* (total H_E = 0.167; Table 1).

3.4 Population genetic structure estimated by AFLP

3.4.1 Genetic distances among populations

Two main groups were recognized within the section *Pustulati* in the neighbour-joining phylogram based on the Nei's genetic distance (Figure 5a). One of them comprised the *S. pustulatus* and *S. fragilis* species and the other comprised *S. masguindalii*. The first group was clearly divided into three clusters. The populations of *S. fragilis* were separated from the whole set of *S. pustulatus* (82% BS); and within this latter, the Spanish and Moroccan populations were also separated (82% BS). The Moroccan populations of *S. pustulatus* from the Oued Laou valley were grouped together excluding LAUO, which was clustered apart with KAN (78%), the most isolated population located in the Oued Al-Kannar valley (to 20 km away, Figure 1a). Within the Spanish cluster, the first node separated population ANT from the others (100%). A remarkable feature within the cluster of *S. masguindalii* was the presence of an independent clade (100% BS) which included the two easternmost populations, SFI and QUE.

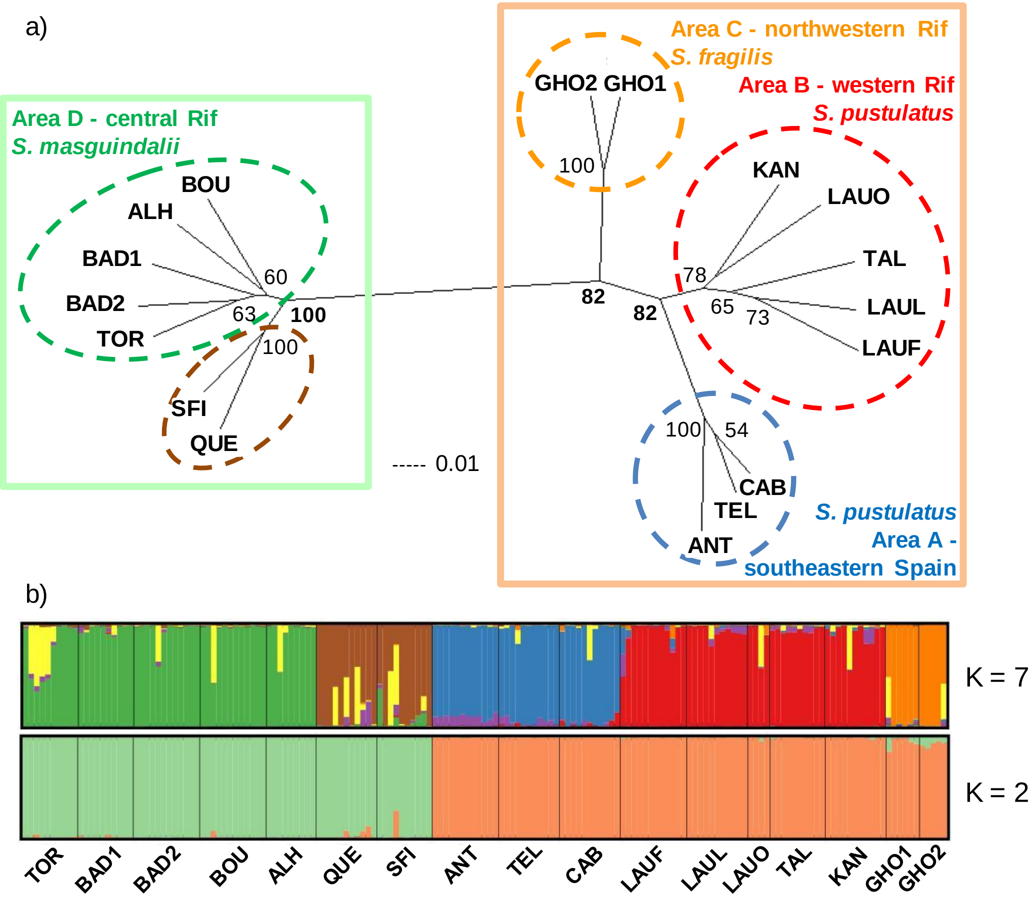


Figure 5 a) Neighbour-joining analysis of 17 populations of *Sonchus* section *Pustulati* based on Nei's genetic distance data. Numbers by nodes are BS >50. Coloured circles denote main genetic groups inferred from Bayesian clustering for *K* = 7 and coloured squares for *K* = 2. b) Genetic structure of populations inferred using the model-based Bayesian algorithm implemented in the software STRUCTURE for *K* = 7 and *K* = 2. Each vertical line represents an individual that is divided into *K* coloured segments depicting their membership in each of the *K* clusters. Populations are separated by vertical black bars.

3.4.2 Bayesian approach: STRUCTURE and GENELAND results

The Bayesian analysis conducted with STRUCTURE showed that K = 2 was the most likely number of clusters in our dataset, followed by K = 7 (Appendix: Figure 7). K = 2 clearly separated all individuals of *S. masguindalii* from the rest (Figure 5b). K = 7 basically separated five groups in which most individuals exhibited very high probabilities to belong to one of them (Figure 5b). All individuals of *S. fragilis* were grouped in the same cluster, and individuals of *S. pustulatus* were separated into two clusters: a Moroccan one and a Spanish one. The individuals from the populations QUE and SFI were also separated from the remaining individuals of *S. masguindalii*, although with a higher level of genetic admixture. The two remaining clusters appear in many individuals of diverse origins and may result from noise produced by the sensibility of the STRUCTURE to variation in sample size (Kalinowski, 2011).

The Bayesian analysis performed with GENELAND found *K* = 6 as the most likely number of clusters in our dataset (Appendix: Figure 7). This analysis also separated individuals according to distribution areas, but giving three (rather than two) clusters for individuals of *S. masguindalii*: those from the west (populations TOR, BAD1 and BAD2), intermediate (ALH and BOU) and east ends (SFI and QUE). The splitting between western and intermediate populations could be a consequence of the over sub-structuring of population nuclei that GENELAND generates in cases of isolation by distance (Cushman *et al.*, 2006), as was detected by the Mantel test for this species (see below).

3.4.3 Partition of genetic variance among individuals and populations

The Mantel test revealed a pattern of isolation by distance on analyzing the whole dataset (r = 0.52, P = 0.000). This was also detected both for the Moroccan individual subsets of *S. masguindalii* (r = 0.39, P = 0.000; Appendix: Figure 8) and *S. pustulatus*, although a weaker correlation was found in the latter case (r = 0.23, P = 0.000). On the contrary, no significant isolation by distance was detected within the Spanish *S. pustulatus* (r = 0.05, P = 0.190) or *S. fragilis* (r = -0.009, P = 0.691) individual subsets.

The AMOVA results (Table 2) generally showed a very high genetic structuring among geographical distribution areas (62% of genetic variation, F_{ST} = 0.70). Nearly half of the genetic variation also occurred among groups both between the Moroccan populations of *S. fragilis* and *S. pustulatus* (47%, F_{ST} = 0.57) and between the Spanish and Moroccan populations of *S. pustulatus* (40%, F_{ST} = 0.51). All pairwise-F_{ST} values between populations from different distribution areas were high (Table 3). Within each distribution area, the AMOVA results always showed a relatively high genetic structuring, especially in *S. masguindalii* (F_{ST} = 0.14–0.25; Table 2). Most geographically close populations showed a low genetic differentiation among each other: Spanish *S. pustulatus* TEL-CAB (pairwise-F_{ST} < 0.01), *S. masguindalii* SFI-QUE (0.02) and BAD1-BAD2 (0.09) and between those of the Moroccan *S. pustulatus* from the Oued Laou valley (0.05–0.09).

Table 2 Results of analyses of molecular variance (AMOVA) based on AFLP analysis of the populations of *Sonchus* section *Pustulati*. Statistics included degrees of freedom (df), variance component (VC), percentage of variation (%) and general fixation index (F_{ST}). *All P values were < 0.001.

Groupings	N	Source of variation	df	VC	%	F_{ST}*	(95% CI)
Among distribution areas							
[*S. pustulatus* MOROCCO];	4	Among groups	3	13.96	62.27	0.70*	(0.675-0.748)
[*S. pus*. SPAIN]; [*S. fragilis*];		Among populations within groups	13	1.68	7.50		
[*S. masguindalii*]		Within populations	150	6.77	30.23		
Area A - Area B	2	Among groups	1	8.51	40.29	0.51*	(0.438-0.568)
[*S. pus*. SPAIN];							
[*S. pus.* MOROCCO]		Among populations within groups	6	2.23	10.54		
		Within populations	74	10.39	49.17		
Area B - Area C	2	Among groups	1	7.55	46.97	0.57*	(0.498-0.631)
[*S. pus*. MOROCCO];		Among populations within groups	5	1.07	6.64		
[*S. fragilis*]		Within populations	52	7.46	46.39		
S. pustulatus SPAIN							
[ANT, TEL, CAB]	1	Among groups	2	0.84	13.58	0.14*	(0.116-0.199)
		Within populations	31	5.33	86.42		
S. pustulatus MOROCCO							
[LAUF, LAUL, LAUO, TAL, KAN]	1	Among groups	4	2.53	17.17	0.17*	(0.128-0.220)
		Within populations	43	12.21	82.83		
[LAUF, LAUL, LAUO, TAL]	1	Among groups	3	1.97	13.63	0.14*	(0.091-0.189)
		Within populations		12.50	86.37		
S. masguindalii							
[TOR, BAD1, BAD2, BOU, ALH, QUE, SFI]	1	Among groups	6	4.31	25.04	0.25*	(0.219-0.312)
		Within populations	67	12.90	74.96		
[TOR, BAD1, BAD2, BOU, ALH]; [QUE, SFI]	2	Among groups	1	2.56	13.78	0.30*	(0.251-0.393)
		Among populations within groups	5	3.09	16.68		
		Within populations	67	12.90	69.54		

Table 3 Pairwise F_{ST} (below diagonal) based on AFLP data and *p values* > 0.001 (above diagonal, non significant after the sequential Bonferroni procedure) in the populations of *Sonchus* section *Pustulati*. F_{ST} values lower than 0.10 are highlighted in bold.

	SPAIN			MOROCCO													
	S. pustulatus (Area A)			*S. pustulatus* (Area B)					*S. fragilis* (Area C)		*S. masguindalii* (Area D)						
Population	ANT	TEL	CAB	LAUF	LAUL	LAUO	TAL	KAN	GHO1	GHO2	TOR	BAD1	BAD2	BOU	ALH	QUE	SFI
ANT	-																
TEL	0.16	-	0.533														
CAB	0.22	**0.00**	-														
LAUF	0.53	0.49	0.48	-		0.062	0.042										
LAUL	0.60	0.57	0.56	**0.07**	-	0.062	0.015										
LAUO	0.63	0.61	0.61	**0.08**	0.12	-	0.048										
TAL	0.59	0.57	0.55	**0.05**	**0.09**	0.10	-										
KAN	0.56	0.53	0.51	0.13	0.22	0.26	0.14	-									
GHO1	0.63	0.61	0.61	0.44	0.53	0.57	0.53	0.50	-	0.034							
GHO2	0.71	0.70	0.70	0.51	0.61	0.68	0.62	0.59	0.13	-							
TOR	0.76	0.76	0.78	0.68	0.72	0.76	0.71	0.71	0.70	0.75	-						
BAD1	0.78	0.78	0.79	0.70	0.74	0.76	0.73	0.72	0.71	0.74	0.32	-					
BAD2	0.74	0.74	0.75	0.66	0.70	0.71	0.68	0.69	0.67	0.70	0.22	**0.09**	-				
BOU	0.77	0.78	0.79	0.68	0.73	0.75	0.71	0.72	0.71	0.74	0.21	0.22	0.20	-			
ALH	0.80	0.81	0.82	0.71	0.77	0.82	0.75	0.75	0.76	0.81	0.36	0.36	0.29	0.28	-		
QUE	0.74	0.75	0.76	0.65	0.70	0.71	0.68	0.68	0.65	0.68	0.27	0.33	0.27	0.24	0.39	-	0.245
SFI	0.73	0.74	0.75	0.64	0.69	0.68	0.67	0.67	0.63	0.66	0.27	0.28	0.24	0.18	0.38	**0.02**	-

4. DISCUSSION

4.1 Phylogeography and evolution of Sonchus section Pustulati

In the present study, we found that the population genetic structure of *Sonchus* section *Pustulati* fits an unequivocal taxonomic and geographical pattern across the Western Mediterranean Basin. Our dating of the molecular divergence indicates an old origin for the group which suggets a palaeo-endemic (Thompson, 2005) and, likely, a relict conditions of the species. The high congruence identified between the ITS/ETS and AFLP analyses supports the monophyletic origin of *Sonchus* section *Pustulati* (Kim *et al.*, 2007 and 2008). Furthermore, the topological incongruence detected between phylogenies based on maternally inherited chloroplast sequences (cpDNA) and biparentally inherited nuclear sequences (ITS/ETS and AFLP) clearly supports the hypothesis of the origin of *S. pustulatus* by interspecific hybridization from *S. fragilis*, as a likely paternal contributor, and an still unknown maternal donor from the root lineage of sections *Sonchus*/*Asperi* (Kim *et al.*, 2008). We can therefore reliably infer the origin and the possible paths and periods of migration of these lineages.

4.1.1 A pre-Mediterranean clade: Late Miocene-Pliocene origin of the species

Within the genus *Sonchus* sensu lato (Kim *et al.*, 2007), the section *Pustulati* appears to be quite old, more ancient than the Macaronesian woody *Sonchus* Alliance [4.23 Ma (6.68–2.29 Ma 95% CI); Appendix: Figure 6] or Juan Fernandez *Dendroseris* radiations [3.3 Ma (2.6–4.0 Ma 95% CI); Sang *et al.*, 1994]. Based on our molecular dating, the early divergence of *S. masguindalii* from the remaining species took place approximately 5.48 Ma ago (8.77–3.17 Ma 95% CI), suggesting that section *Pustulati* was originated between late Miocene and early Pliocene. It most likely occurred during the Messinian age (7.25–5.33 Ma) or the subsequent Zanclean age (5.33–3.60 Ma), when the Mediterranean Sea was progressively reflooded (Figure 4). Within the section, *S. masguindalii* turns out to be the earliest diverged species. The hybridization events that generated the most widespread species *S. pustulatus* probably took place during the Pliocene.

Climate in the Mediterranean area during all these geologic times was generally warmer and wetter (e.g. Mai, 1989; Thompson, 2005) than the present-day typical Mediterranean regime, which seems to have become established since 3.4–2.8 Ma (Suc, 1984). The estimated dates for the origin of *Pustulati* species, together with their current very narrow ecological amplitude (Chapter 3) and restricted distribution suggest that the species have been sufficiently resilient to the present-day Mediterranean climate. We therefore consider the section as part of the Pre-Mediterranean element of Mediterranean flora (Herrera, 1992) and suggest a relict character of current populations.

The current geographic distribution of the species of *Sonchus* section *Pustulati* fully within the Baetic-Rifan Internal Zone, their high ecological specificity and low dispersal ability suggest that their entire evolutionary history took place in the Baetic-Rifan region. Nonetheless, we cannot determine whether the ancestors of these species (Pre-D in Figure 4b) were found in North Africa and/or the Baetic-Rifan microplate. Based on our molecular dating, the origin and diversification of the species of *Sonchus* section *Pustulati* occurred during the development of the Gibraltar arc (Figure 4c-f).

Moreover, the current clear predominance of the species in the Rifan range and the narrow distribution of the oldest species in the central Rif suggest that they diversified in southern areas of the Gibraltar arc (Figure 4c-f).

4.1.2 Divergence of the populations of S. masguindalii

At present, *Sonchus masguindalii* occupies a narrow range (11 km^2) with a common geologic substrate (Triassic and Jurassic limestone and dolostone; Déil & Hammoumi, 1997), probably with a continuous distribution along the coast. Although we do not avail of data relating to changes in their distribution throughout geologic times, the high population genetic structuring indicates long permanence in the area, likely during most of the Pleistocene. Genetic divergence between the easternmost populations (QUE and SFI) and the remaining populations was dated at 1.39 Ma (0.46–2.83 Ma 95% CI). The Al-Hoceima Cape, located between these population groups, surely acted as a semi-permeable but effective physical barrier to gene flow among populations from both sides. This led to long-time isolation, which has probably caused the substantial genetic differentiation detected by most AFLP and ITS analyses.

4.1.3 Divergence of the Spanish and Moroccan populations of S. pustulatus

The disjunct distribution of the populations of *Sonchus pustulatus* involves one of the most striking themes in the phylogeography of the section. Both Baetic and Rifan ranges of this species (areas A and B; Figure 1a) are included in different floristic units (Valdés, 1991) or ecoregions (Molina-Venegas *et al.*, 2013), present significant climatic differences and are located approximately 280 km from each other. Mean annual rainfall in the Spanish (A) and Moroccan (B) areas rises to 250 and 850 mm, respectively (Hijmans, 2005). The disjunction appears to involve an ecological differentiation process which has been capable of promoting the clear genetic differentiation detected by the AFLP (Figure 5) and ITS/ETS (Figure 3) analyses, as well as the presence of different cpDNA haplotypes in both ranges (Figure 1b). However, cryptic speciation has been discarded due to the high interfertility detected among plants from both areas (Silva & Mejías, unpublished).

The geographical pattern of *S. pustulatus* is not common among Baetic-Rifan plants. Most studied plant taxa exhibiting disjunct distribution in the region are currently located close to the Strait of Gibraltar in both Baetic-Rifan ranges (e.g. Burban & Petit, 2003; Ortiz *et al.*, 2007; Arroyo *et al.*, 2008; Escudero *et al.*, 2008) which present similar climates and a transmarine separation of 14 km. To our knowledge, the Colchicaceae *Androcymbium gramineum* (Caujapé-Castells & Jansen, 2003), which colonizes the Atlantic Moroccan coast and southeastern Spain, is the studied taxon with the most similar geographic disjunction, at least in the northern range.

We propose two alternative hypotheses to explain the current disjunction of *S. pustulatus* (Figure 4f-g). One of them suggests that recent long-distance dispersal over the Mediterranean Sea during the Quaternary is responsible for this current disjunction [*hypothesis 1*]. This hypothesis consists of a long dispersal event from North Africa to the southeastern Iberian Peninsula once the Mediterranean Basin had been totally refloofed, via an unknown vector (wind, marine currents, etc.), followed by effective colonization. Another hypothesis is that short-distance dispersal events by the Gibraltar arc, followed by historical isolation, were responsible for the current disjunct distribution [*hypothesis 2*]. This involves a south-north range expansion following a stepping-stone process (Kimura, 1953) during late Messinian or early Zanclean times. At the end of the Mediterranean refilling (5.33–3.60 Ma), a restriction of distribution range may have taken place by direct flooding of their habitats, resulting in an increasingly disjunct distribution. The differences in the rainfall regimen between the two ranges, probably established during the late Pliocene (3–2.8 Ma; Suc, 1984), could have favoured the conditions for genetic differentiation around the estimated divergence age.

The molecular dating of the divergence between the Moroccan and Spanish populations of *S. pustulatus* (1.18 Ma, Figure 4) seems to fit better with *hypothesis 1*. In addition, the latter is also supported by the generally low DW values of the Spanish populations (Table 1), which is to be expected in newer established populations (Schönswetter & Tribsch, 2005). Assuming *hypothesis 1* in the phylogeography of *S. pustulatus*, the ecological and biological traits (heavy seeds with a markedly deciduous pappus, relatively strong self-incompatibility systems, high ecological specificity, low capacity for recruitment, long-life cycle, etc.), generally typical of cliff-dwelling species (e.g. Larson *et al.*, 2005), would not be associated with limited dispersal and colonization abilities. Indeed, long-distance dispersal events are recently being proposed in several taxa without special long-distance dispersal mechanisms (e.g. Guzmán & Vargas, 2009; Fernández-Mazuecos & Vargas, 2011; Vargas *et al.*, 2012; Lavergne *et al.*, 2013), but probably with different demographical structures and dynamics, and wider ecological amplitude than *Pustulati* species.

The most similar scenario to the alternative *Hypothesis 2* has been suggested for the disjunction in *Androcymbium gramineum*, a species also lacking adaptations to long-distance seed-dispersal. This disjunction seems to have originated through range expansion in the region during the Messinian desiccation of the Mediterranean Sea (Caujapé-Castells & Jansen, 2003). This biogeographical scenario has also been proposed for several species disjunctions that are clearly closer to the Strait of Gibraltar, have a current wider distribution and a high ecological range and/or show adaptations to long-distance seed-dispersal (e.g. *Saxifraga*, Vargas *et al.*, 1999; *Frangula*, Hampe *et al.*, 2003; *Campanula broussonetiana*/*C. transtagana* clade, Cano-Maqueda *et al.*, 2008).

All this clearly highlights the need for further study on the phylogeographic relationships between plants from both ranges in the Western Mediterranean Basin. The incorporation of molecular data of *S. pustulatus* samples from Northern Algeria (area D, Figure1), assuming that the species is currently present there, will be crucial with regard to discerning which of the two proposed hypotheses is the more accurate one, or for revealing new possibilities.

4.2. Genetic population structure within areas

As expected for cliff-dwelling species, a relatively high genetic structuring was the rule within each distribution area, especially in *S. masguindalii*. Seed dispersal and gene flow generally appear to be virtually restricted to neighbouring populations (Table 3). This scenario has also been detected in other cliff-dwelling taxa, e.g. in *Centaurea* *horrida* (Mameli *et al.*, 2008) or in *C. borjae* (Lopez & Barriero, 2013). As a strictly entomophilous species, pollen-mediated gene flow is probably spatially limited from a few meters to several kilometres (e.g. Ishihama *et al.*, 2005) and rarely over 10 km (e.g. Fénart *et al.*, 2007). Moreover, seed dispersal is surely limited to short displacements on the same cliff or to nearby ones, most seeds remaining at the cliff base where they do not successfully develop (Chapter 2).

In Spanish *S. pustulatus* (area A), the narrow distribution range with only three small and genetically impoverished populations appears to result from a restriction process from a previous large quasi- or metapopulation, where intermediate populations were present. The clear tendency towards decline detected in two populations and the registered extinction of another one (Chapter 2) support such restriction. By contrast, the Moroccan populations of *S. pustulatus* would seem to constitute a system of metapopulations in which there is likely a moderate gene flow between adjacent or nearby populations from the Oued Laou valley (Table 3). However, neither recurrent population extinctions nor colonization events are expected due to their slow process of individual recruitment and high resilience (Chapter 2 and 3). In the case of the populations of *S. fragilis*, a process similar to area A might have taken place. In this case, the very high levels of self-compatibility detected (Chapters 4), which ensure seed production when conditions for cross-pollination become limited (Kalisz *et al.*, 2004), may help to maintain greater population sizes, thus enhancing their resilience. Finally, the high genetic structuring detected among the populations of *S. masguindalii* may be favoured by a restricted pollination environment, typical of sea cliff habitats (e.g. Gemmill *et al.*, 1998; Cureton, 2006).

4.3. Genetic diversity and conservation considerations

In general, genetic diversity in the taxa of *Sonchus* section *Pustulati* is not depleted, with the exception of the impoverished Spanish populations of *S. pustulatus*. However, all these diversities resulted low in comparison with those estimated in other rare endemic cliff-dwelling species with the same AFLP technique; e.g. in the phylogenetically close *Sonchus gandogeri* (H_E = 0.38; Kim *et al.*, 2005), or in *Centaurea borjae* (H_E = 0.26; Lopez & Barreiro, 2013).

The populations with the most restricted geographical distribution, i.e. those of the Spanish *S. pustulatus*, *S. fragilis* and the easternmost ones of *S. masguindalii* (SFI and QUE), are not located within protected areas. In these cases, conservation efforts may well be crucial with regard to maintaining such species. These conservation measures might consist of creating microreserves, which have become an essential tool for effective protection of diverse flora in the Western Mediterranean region (Laguna *et al.*, 2004). Apart from the creation of seed banks of as many populations as possible from each geographical area, the Spanish populations should be reinforced by manual seed sowing.

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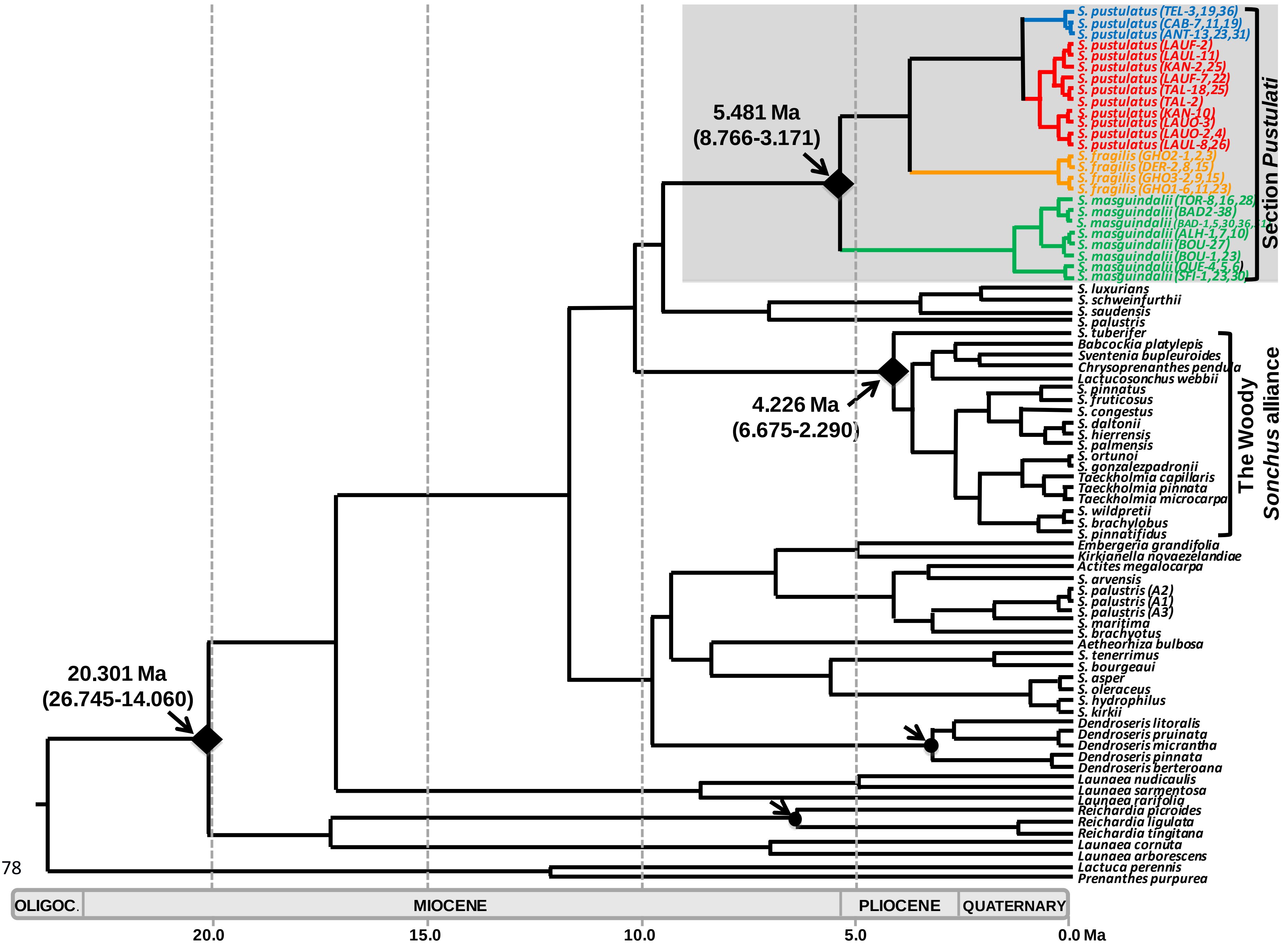
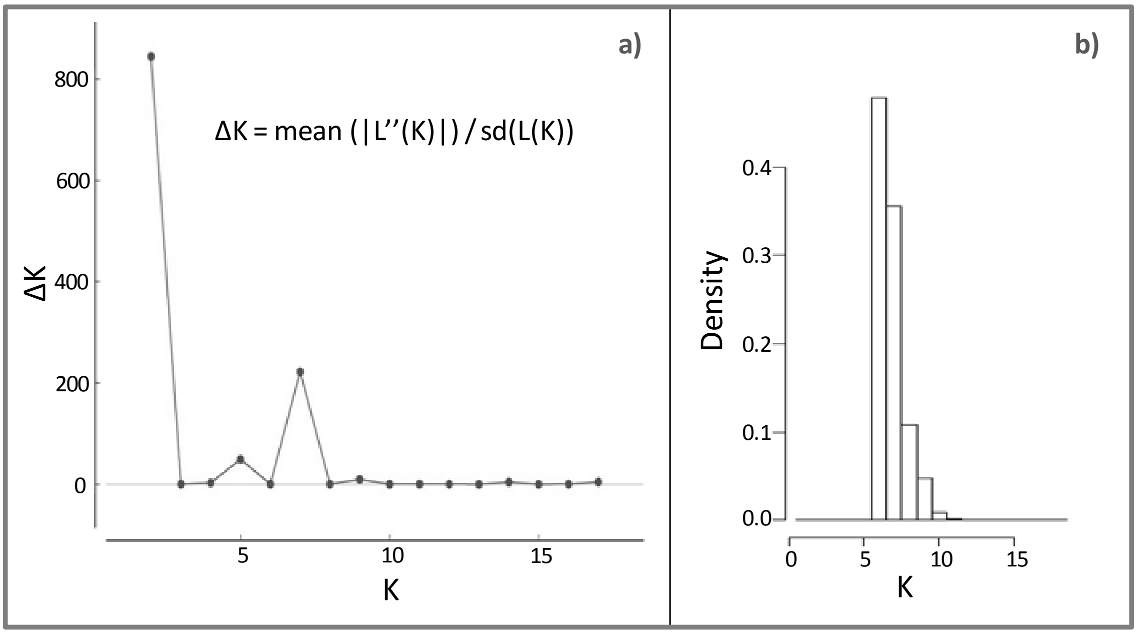
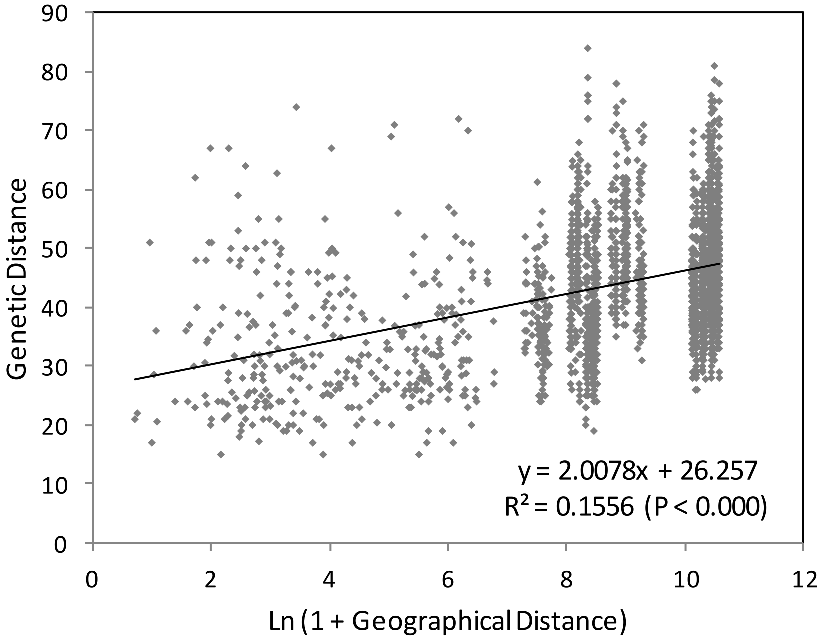
APPENDICES


5.481 Ma
(8.766-3.171)
4.226 Ma
(6.675-2.290)
20.301 Ma
(26.745-14.060)
S. pustulatus (TEL-3,19,36)
S. pustulatus (CAB-7,11,19)
S. pustulatus (ANT-13,23,31)
S. pustulatus (LAUF-2)
S. pustulatus (LAUL-11)
S. pustulatus (KAN-2,25)
S. pustulatus (LAUF-7,22)
S. pustulatus (TAL-18,25)
S. pustulatus (TAL-2)
S. pustulatus (KAN-10)
S. pustulatus (LAUO-3)
S. pustulatus (LAUO-2,4)
S. pustulatus (LAUL-8,26)
S. fragilis (GHO2-1,2,3)
S. fragilis (DER-2,8,15)
S. fragilis (GHO3-2,9,15)
S. fragilis (GHO1-6,11,23)
S. masguindalii (TOR-8,16,28)
S. masguindalii (BAD2-38)
S. masguindalii (BAD-1,5,30,36,
S. masguindalii (ALH-1,7,10)
S. masguindalii (BOU-27)
S. masguindalii (BOU-1,23)
S. masguindalii (QUE-4,5,6)
S. masguindalii (SFI-1,23,30)
Section Pustulati
S. luxurians
S. schweinfurthii
S. saudensis
S. palustris
S. tuberifer
Babcockia platylepis
Sventenia bupleuroides
Chrysoprenanthes pendula
Lactucosonchus webbii
S. pinnatus
S. fruticosus
S. congestus
S. daltonii
S. hierrensis
S. palmensis
S. ortunoi
S. gonzalezpadronii
Taeckholmia capillaris
Taeckholmia pinnata
Taeckholmia microcarpa
S. wildpretii
S. brachylobus
S. pinnatifidus
The Woody Sonchus alliance
Embergeria grandifolia
Kirkianella novaezelandiae
Actites megalocarpa
S. arvensis
S. palustris (A2)
S. palustris (A1)
S. palustris (A3)
S. maritima
S. brachyotus
Aetheorhiza bulbosa
S. tenerrimus
S. bourgeaui
S. asper
S. oleraceus
S. hydrophilus
S. kirkii
Dendroseris litoralis
Dendroseris pruinata
Dendroseris micrantha
Dendroseris pinnata
Dendroseris berteroana
Launaea nudicaulis
Launaea sarmentosa
Launaea rarifolia
Reichardia picroides
Reichardia ligulata
Reichardia tingitana
Launaea cornuta
Launaea arborescens
Lactuca perennis
Prenanthes purpurea
OLIGOC.
MIOCENE
PLIOCENE
QUATERNARY
20.0
15.0
10.0
5.0
0.0 Ma

Appendix: Figure 6 Chronomogram of the subtribe *Sonchinae*. Two calibration points using the genera *Dendroseris* and *Reichardia* are indicated by black circles with arrows.



Appendix: Figure 7 a) Plot of ΔK analysis (Evanno *et al.*, 2005) for detecting the number of clusters (*K*) that best fit the data from the results obtained in the STRUCTURE analysis. b) Plot of the posterior density (distribution) of the number of *K* estimated from the GENELAND analysis results. This run displayed a clear mode at *K* = 6 which is hence the maximum a posteriori estimate of *K*.



Appendix: Figure 8 Graph of Mantel's test results for the relationship between genetic distance (Nei's genetic distances) and geographic distance of the *Sonchus masguindalii* individuals analyzed (N = 74).

Capítulo 5:

INCIDENCIA DE LA AUTO-INCOMPATIBILIDAD EN LAS ESPECIES DE *SONCHUS* SECCIÓN *PUSTULATI* (ASTERACEAE), UN PEQUEÑO GRUPO DE ENDEMISMOS RUPÍCOLAS DEL MEDITERRÁNEO OCCIDENTAL

INCIDENCE OF SELF-INCOMPATIBILITY IN *SONCHUS* SECTION *PUSTULATI* (ASTERACEAE), A SMALL GROUP OF CLIFF-DWELLING SPECIES ENDEMIC TO THE WESTERN MEDITERRANEAN

Authors: José L. Silva & José A. Mejías

RESUMEN

Objetivos 1) Investigar la incidencia de la respuesta de auto-incompatibilidad (SI) en las especies de *Sonchus* sección *Pustulati* (Asteraceae): *S. masguindalii*, *S. fragilis* y *S. pustulatus*; 2) comparar la morfología floral y el fitness entre plantas de *S. fragilis* con índices de auto-incompatibilidad (ISI) contrastados; 3) estimar la diversidad de alelos *S* en poblaciones representativas de *S. pustulatus* y *S. fragilis*; 4) estudiar cómo los alelos *S* y los fenotipos de auto-compatibilidad están espacialmente distribuidos dentro de las poblaciones; y 5) evaluar el significado evolutivo de los rasgos anteriores y sus consecuencias en las poblaciones.

Métodos Para estudiar la incidencia de la auto-incompatibilidad llevamos a cabo un programa de polinizaciones manuales con 368 plantas recolectadas de tres poblaciones de cada área de distribución (12 poblaciones). Los fenotipos de auto-incompatibilidad fueron determinados por el nivel de fructificación y evaluados mediante los valores del ISI (SI; pSI, SI parcial; SC, auto-compatible). La morfología floral y el fitness fueron analizados sobre 87 plantas obtenidas mediante esquejes a partir de una población de *S. fragilis* (GHO1). Además, 11–14 plantas SI de tres poblaciones (de *S. pustulatus* y *S. fragilis*) fueron cruzadas en tres dialelos completos independientes.

Resultados Una alta incidencia de la SI fue lo común entre las plantas de las poblaciones de *S. masguindalii* y *S. pustulatus* (ISI promedio = 0.80–0.90), y de baja a moderada en las de *S. fragilis* (0.11–0.53). Dentro de GHO1, la mayoría de los individuos fueron SI o SC. Las plantas tanto SI como pSI de GHO1 mostraron capítulos florales significativamente más grandes que las plantas SC. El ISI individual y el tamaño de las plantas en esta población mostraron una agregación espacial significativa. El estimador predijo un número bajo de alelos *S* para la población española de *S. pustulatus* (5) y moderado para las poblaciones marroquíes de *S. pustulatus* y *S. fragilis* (12 y 15, respectivamente).

Conclusiones principales Los resultados de los cruces dialélicos apoyan la presencia de un control genético esporofítico de la auto-incompatibilidad en los taxones de *Sonchus* sección *Pustulati*. Cierta incidencia de sistemas sexuales mixtos parece estar presente en las poblaciones SC/pSI (especialmente en GHO1), dada la variabilidad inter-individual observada de capacidades de autofecundación y su dependencia general de polinizadores para ser auto-fertilizadas. Las posibles causas del origen del patrón espacial de ISI detectado en GHO1 son discutidas. Las frecuentes interacciones de dominancia entre alelos *S* y la parcial o completa ruptura de la auto-incompatibilidad detectada en las poblaciones estudiadas deben haber sido cruciales para evitar fuertes efectos Allee-*S* y así reforzar su resiliencia frente a contingencias demográficas.

Palabras claves Cruces dialélicos; Distribución espacial de alelos *S*; Distribución espacial de la capacidad de autofecundación; Diversidad de alelos *S*; Efecto Allee-*S*; Índice de auto-incompatibilidad; Resiliencia; Rotura de la SI; Sistemas de auto-incompatibilidad esporofíticos.

ABSTRACT

Aims 1) To investigate the strength of the self-incompatibility (SI) response across the species of *Sonchus* section *Pustulati* (Asteraceae): *S. masguindalii*, *S. fragilis* and *S. pustulatus*; 2) to compare floral morphology and fitness among plants of *S. fragilis* with contrasting indexes of SI (ISI); 3) to estimate the *S* allele diversity in representative populations of *S. pustulatus* and *S. fragilis*; 4) to study how *S* alleles and selfing phenotypes are spatially distributed within populations; and 5) to evaluate evolutionary significance and consequences in populations of the previous traits.

Methods To study the strength of the SI we performed a program of hand pollinations with 368 plants collected from three populations of each distribution area (12 populations). Incompatibility phenotypes were determined by fruit set and evaluated by ISI values (SI; pSI, partial SI; SC, self-incompatible). Floral morphology and fitness were analyzed across 87 cutting-plants from a population of *S. fragilis* (GHO1). In addition, 11–14 SI plants from three populations (two of *S. pustulatus* and one of *S. fragilis*) were crossed in three independent full diallels.

Results High incidence of the SI was the common across plants from populations of *S. masguindalii* and *S. pustulatus* (average ISI = 0.80–0.90), and from low to moderate across those of *S. fragilis* (0.11–0.53). Within GHO1, most of individuals were SI or SC. Both SI and pSI plants from GHO1 showed significantly larger flower head than the SC plants. The individual ISI and plant size in this population showed a significant spatial aggregation. Estimator predicted a low number of *S* alleles for the entire Spanish population of *S. pustulatus* (5) and moderate for the Moroccan populations of *S. pustulatus* and *S. fragilis* (12 and 15, respectively).

Main conclusions Diallel crosses results support the presence of a sporophytic genetic control of self-incompatibility in the taxa of *Sonchus* section *Pustulati*. Certain incidence of mixed mating systems might be reached in the SC/pSI populations (especially in GHO1), given the observed inter-individual variability of selfing abilities and their general dependence on pollinators to be self-fertilize. Possible causes for the origin of the ISI spatial distribution pattern detected in GHO1 are discussed. The frequent dominance interactions among *S* alleles and the partial or complete breakdown of SI detected across study populations should have been crucial to avoid strong *S* Allee effects and thus reinforce their resilience against demographical contingencies.

Keywords Breakdown of SI; Diallel crosses; Index of self-incompatibility; *S* allele diversity; *S* Allee effect; *S* allele spatial distribution; Selfing ability spatial distribution; Sporophytic self-incompatibility; Resilience.

1. INTRODUCTION

Self-incompatibility (SI) is a genetic barrier to inbreeding broadly distributed among hermaphroditic angiosperms (Busch & Schoen, 2008). Basically, two types of self-incompatibility have been reported regarding to the genetic control of the incompatibility reaction: gametophytic (GSI) and sporophytic (SSI) systems. In both of them, the incompatibility reactions are controlled by a linked cluster of genes collectively known as the '*S* locus' ('*S*' denotes self-sterility), and individual plants that share alleles at this locus are incapable to produce offspring in cross-pollinations (Richards, 1997). In the GSI, the more common type of SI, the incompatibility reaction between pollen and pistil is governed by the genotype of each single pollen grain (haploid). In the SSI, which has been only detected in seven dicotyledonous families (Asteraceae, Betulaceae, Brassicaceae, Caryophyllaceae, Convolvulaceae, Polemoniaceae and Sterculiaceae; Hiscock & Kües, 1999), the incompatibility reaction is genetically controlled by the plant that produces the pollen grains (i.e. the sporophyte) through the pollen grain cover (diploid). In both cases, if the pollen of the donor plant is recognized by the receptor plant as own pollen (i.e. they share one allele, unmasked in case of SSI) the receiver triggers the SI reaction.

Regardless of the type of SI, sexual reproduction in populations of SI species depends on the number and distribution of alleles at the *S* locus (Busch & Schoen, 2008) and, in turn, the number of *S* alleles depends on the population size and the mutation rate of the *S* locus (Wright, 1939; Busch *et al.*, 2014). The *S* allele diversity has therefore important consequences for the population biology, and it has been suggested that in large populations many *S* alleles should be maintained by negative-frequency-dependent selection (Wright, 1939; Schierup *et al.*, 1997; Lawrence, 2000). Meanwhile, in small or highly fragmented populations *S* allele diversity can be lost owing to genetic drift (Wagenius *et al.*, 2007). In this case, SI species suffer from a highly limited reproduction through scarcity of potential mates (*S* Allee effect) and increased inbreeding depression (e.g. Byers & Meagher, 1992; Willi & Fisher, 2005; Glémin *et al.*, 2005; Wagenius *et al.*, 2007; Caujapé-Castell *et al.*, 2008; Young & Pickup, 2010; Leducq *et al.*, 2010), which can lead to the extinction of rare or endemic taxa (Demauro, 1993; Reinartz & Les, 1994; Caujapé-Castells *et al.*, 2008). The demographic consequences of the *S* Allee effect in SSI systems may be especially important in populations with fewer than 10 *S* alleles (Busch *et al.*, 2014), whose spatial distribution within the population can also influence importantly the outcome of its reproductive success.

The reproductive assurance in SI plants subjected to strong mate limitation in declining or new populations is usually expected to involve the selection of self-compatibility (SC) (Reinartz & Les, 1994; Ortiz *et al.*, 2006; Wagenius *et al.*, 2007). The transition from SI to SC seems to be associated with a decrease in size of flowers (e.g. Ornduff, 1969; Gibbs *et al.*, 1975; Oliveira *et al.*, 1992; Gibbs & Talavera, 2001); a fact that has been demonstrated at congeneric level, though the information at intraspecific level is scarce (but see Anderson, 1989; Ortiz *et al.*, 2006). Nevertheless, the selection of SC does not always occur, and some experimental studies on small populations have shown cases of complete maintenance of SI, which use to correspond to colonization events (Carr & Powel, 1986; Kim *et al.*, 1999; Brennan *et al.*, 2002, 2003). Moreover, between these two ends (SI and SC), it has also been reported the presence of sexual systems of intermediate nature that seem to combine the advantages of both selfing and outcrossing, i.e. the partial self-incompatibility (pSI) systems (Levin, 1996). Last evidences and theoretical models have suggested that pSI systems represent a stable condition rather than an evolutionarily transient state between full expression and full dissolution of the SI (e.g. Vallejo-Marín & Uyenoyama, 2004; Goodwillie *et al.*, 2005; Igic & Busch, 2013), as it has been traditionally considered (Lande & Schemske, 1985).

The number of *S* alleles in populations has been estimated from nine to 193 across 21 wild species with GSI and from two to 54 across 15 wild species with SSI (Busch *et al.*, 2014). Within the group of species with SSI, the scarcity of studies is especially shocking in the case of the Asteraceae (Brennan *et al.*, 2002, 2006 and 2013; Young & Pickup, 2010), which is one of the most diverse family in the Angiosperms (over 20000 species) and where the SSI mechanism was discovered more than fifty years ago (Hughes & Babcock 1950; Gerstel, 1950). Since then, the presence of this SI system in this large family has been broadly recorded (e.g. Brauner & Gottlieb, 1987; Mejías, 1992; Reinartz & Less, 1994; Young *et al.*, 2000; Hiscock, 2000; Ortiz *et al.*, 2006; Scheffknecht *et al.*, 2007), together with a relatively high number of cases of pSI (e.g. Hiscock, 2000; Cheptou *et al.*, 2000; Nielsen *et al.*, 2003; Lafuma & Maurice, 2007; Ferrer *et al.*, 2009). An intensive review of the breeding system in 571 species of Asteraceae revealed that 63% of plants in this family were SI, 10% showed pSI, and 27% were SC (Ferrer & Good-Ávila, 2007).

Sonchus pustulatus Willk., *S. fragilis* Ball and *S. masguindalii* Pau & Font Quer constitute the well-supported clade *Sonchus* L. section *Pustulati* Boulos (Asteraceae, Cichorieae; Boulos, 1973; Kim *et al.*, 2007; Chapter 4). These species are narrow endemic to the Western Mediterranean Basin occurring on quite localized cliffs at low altitude (Chapter 2). Preliminary hand pollinations suggested that *S. pustulatus* in Spain is SI (Mejías, 1992). The showy flower heads of all the species in the section could indicate the spread presence of such SI. However, their relict condition, ancient origin and high resilience (Chapter 2 and 3) suggest that either a partial or a complete breakdown of SI could have occurred during the evolution of the species of *Sonchus* section *Pustulati*. Should we consider that these rare taxa had ever suffered from bottleneck events and genetic drift (e.g. Chapter 2 and 4), they would have lost *S* allele diversity and therefore they could have evolved increasing their capacity of selfing or, by contrast, increasing dominance interactions among *S* alleles in the diploid control of the SSI reaction (Brennan *et al.*, 2002).

Here we report a detailed population genetic study of the mating system in the species of *Sonchus* section *Pustulati* based on an intensive program of hand pollinations. Though in recent decades the development of biochemical and molecular techniques in SSI in *Brassica* (e.g. Gaude *et al.*, 1991; Brace *et al.*, 1993) has allowed a faster and less tedious *S* allele estimation than using classical diallel crosses (Glémin *et al.*, 2005), the molecular mechanism of SSI in the Brassicaceae is not shared by the Asteraceae (Allen *et al.*, 2011; Gounthier *et al.*, 2013) and it is difficult to make generalizations about. Fortunately, a recent study with *Senecio* species showed how extensive controlled crossing surveys can be applied to investigate the SSI systems in species of particular ecological or evolutionary interest (Brennan *et al.*, 2013). We attempted: (1) to determine if there is any variation in the strength of SSI within and among populations and species; (2) to study what features of floral morphology and fitness may be related to the outstanding variability detected in the strength of the SI among individuals from a single population of *S. fragilis*, and how are the incompatibility phenotypes spatially distributed within the population; (3) to identify *S* allele diversity in the prior population of *S. fragilis*, one of the Spanish populations of *S. pustulatus* and one of the Moroccan populations of *S. pustulatus*; (4) to study how *S* alleles are spatially distributed within populations; and 5) to evaluate evolutionary significance and consequences in populations of previous traits.

2. MATERIAL AND METHODS

2.1 Plant species

The species *Sonchus pustulatus*, *S. fragilis* and *S. masguindalii* constitute the section *Pustulati* of the subgenus *Sonchus*. They are long-lived suffrutescent chamaephytic cliff plants, usually procumbent, which develop moderate-long branches, particularly lignified in *S. masguindalii*. Depending on the species, individuals range approximately from only 5 cm^2 to 2 m^2 in surface and can reach up to 45 cm in height, with somewhat intricate branching. They commonly attain sexual maturity during the first year of life (Silva & Mejías, unpublished) and mainly flowering in spring. Flower heads are terminal, solitary or in groups from two to four, with bright yellow ligule florets. All florets are hermaphrodites and protandrous, which develop centripetally over a period of 3–6 days. Flower heads comprise 30–120 florets in the case of *S. pustulatus* and *S. fragilis*, and 60–250 florets in *S. masguindalii*. Fruits are achenes with a highly deciduous pappus (aprox. 8 mm in length), released during late spring and early summer. There are only 19 known populations of these species, which are located in four small distribution areas restricted to the Baetic-Rifan complex hotspot (Western Mediterranean Basin; Chapter 2: Fig. 1).

2.2 Plant sampling and pollinations

In May and June 2008, we collected and georeferenced reproductive material from 288 plants (131 as cuttings and 157 as flower heads with mature seeds) from 12 populations (Table 1). Cuttings were dipped into powder rooting hormones and sowed directly on large pots in field prior to transportation. In May 2011, we further sampled 87 additional cuttings with mature flower heads in the population GHO1 of *S. fragilis*, given the high variability detected in the incidence of SI in the individuals from this population (see results). In each case, we established a minimum distance of 20 m between plants to avoid collecting siblings. The distance between individuals varied to a maximum of 100 m in order to obtain a spatially representative sample of each population.

The following procedures and experiments were performed in the greenhouses of the General Services of the University of Seville, under standard light and warm conditions (16 hours of light, 18–22ºC). We separated cuttings in individual pots few days after collection and germinated seeds at a minimum of three months after collection. In each case, plants were grown in plastic nine cm-diameter pots, with a substrate of peat and perlite (3:1 v/v) plus a solid organic fertilizer of slow release (Osmocote 12 months; 3.5 g/L of substratum). We watered and kept them until they reached the reproductive stage (approx. 2–4 months). Then, plants were separated from each other by at least 10 cm and all opened flower heads were removed. Immediately, they were placed in tables covered with canopies of 1.5 m of height made with a tulle mesh with 1.5 mm diameter pore to avoid the presence of pollinators. Additionally, in order to remove possible insect contaminations, we hanged pheromone traps (yellow

Atrapaxon plates) within the boxes. Indiscriminate cross pollinations were therefore prevented. We conserved all plants with similar sizes by manual pruning to avoid possible maternal effects in the seed production level and tangles among long branches of different individuals.

We performed the following pollination treatments: *autonomous self-pollination* (*ASP*), in which the flower head was not hand pollinated neither used as pollen donor; *hand forced geitonogamic pollination* (*FGP*), in which the flower head had one or several flower heads of the same individual as pollen donors; *hand massive crossed pollination* (*MCP*), in which the flower head had several individuals from the same population as pollen donors; and *hand inter-individual crossed pollination* (*ICP*), in which the flower head had exclusively one individual from the same population as pollen donor. Pollinations were manually carried out with small cotton sticks (e.g. Ortiz *et al.*, 2006). We rubbed the end of the stick with the florets of a flower head with available pollen and then we softly spent the charged stick end over the floret stigmas acting as pollen receptors. When the flower heads could approach each other without breaking them, this method was substituted by gently brushing flowering heads (e.g. Brennan *et al.*, 2002). The procedure was repeated three-four times in each flower head during the whole anthesis period to ensure pollination of all florets. After the anthesis, the treated flower heads were individually covered with new clean tea-bags. All these bags were independently collected when flower heads had fully dehisced, approximately one month after flowering.

Fruit set was chosen as a reliable measure of the incompatibility response since incompatible pollinations usually resulted in little or no fruit set and between 0.3–1.0

of fruit set for a compatible cross (e.g. Hiscock, 2000; Brennan *et al.*, 2002; Ortiz *et al.*, 2006). Fruit-set (FR; seed set in Asteraceae) was estimated according to the formula:

FR = [(No. fertile fruits) / (No. fertile fruits + No. sterile fruits)]

Fertile fruits appeared fatter and more pigmented than fruits containing an unfertilized ovule, which were thin and whitish-pale in colour (Appendix: Figure 9). We calculated the average fruit-set of the flower heads with the same pollination treatment for each individual.

2.3 Strength of self-incompatibility

We independently applied the treatments *ASP*, *FGP* and *MCP* for the study of the strength of the SI over three to six flower heads per individual in each of the 12 populations of the *Sonchus* section *Pustulati* sampled. To this end, we used a total of 368 plants obtained both from seeds and cuttings (6–132 individuals per population; mean ± SE = 28.2 ± 4.41; Table 1). The population GHO1 of *S. fragilis* included all cuttings collected in 2011. Approximately 2500 flower heads received the treatments *ASP*, *FGP* or *MCP* (including repeats) among these sampled individuals.

To assess the strength and variation of SI we calculated, for each individual, the most widely reported quantitative measure of SI, the index of self-incompatibility (ISI; Lloyd, 1965; Raduski *et al.*, 2011):

ISI = 1 – relative selfed success / relative outcrossed success, where relative pollination success is defined as the ratio of fruits set to flowers pollinated. According to the definition of ISI, we considered the FR after *FGP*, instead of *ASP*, as the relative selfed success. We had previously observed under the binocular magnifier that spontaneous self-pollination can occur under the *ASP* treatment by means of the nystinastic movements of the flower heads (daily opening and closing) during the anthesis. However, only the *FGP* treatment could ensure the pollen deposit on the stigmatic papilla where the SSI response occurs. Historically, species with ISI values above 0.8 have been classified as self-incompatibles (Bawa, 1974). We arbitrarily classified the breeding system of individuals into three states according to their ISI values, following Raduski *et al.* (2011): self-incompatibility (SI; ISI ≥ 0.8), partial self-incompatibility (pSI; 0.2 < ISI < 0.8), and self-compatibility (SC; ISI ≤ 0.2).

We calculated the proportion of SC and pSI plants in each of the twelve populations. We tested the relationship between the proportion of SC and pSI plants in the populations and a) effective population size and b) density of plants (individuals/100m^2) by means of Pearson correlations. These data were extracted from Chapter 2. We also performed Kruskal-Wallis and Mann-Whitney tests to compare (i) the FR obtained after each pollination treatment within each taxon (i.e. distribution area); and (ii) the differences between the individual FR obtained after *FGP* and *ASP* treatments (FR (*FGP*) – FR (*ASP*)) among taxa.

2.3.1 Floral morphology and fitness in the population GHO1 of S. fragilis

We analyzed the variability in floral morphology and fitness of individuals from the population GHO1 of *S. fragilis* and we related them to the ISI groups (SI, pSI and SC). To this end, we used the 87 cutting-plants with flower heads collected in the field in 2011. From the flower heads collected in the field (1–4 per individual), we measured the FR, fruit weight, number of fruits per flower head and number of florets per flower head. From the cutting-plants grown in the greenhouse, we measured the flower head size (maximum diameter during the anthesis), the length and width of the ligule, as well as the length of the tube, of an outer floret.

We performed one way ANOVAs and Bonferroni post hoc tests to compare the floral morphology and fitness traits among the three ISI groups. When data lacked a normal distribution (i.e. FR after *ASP* and *FGP* treatments) the comparisons were based on Kruskal-Wallis and Mann-Whitney tests. In addition, within the SC group, the means FR after *ASP* and *FGP* treatments were compared between them by Mann-Whitney tests.

2.3.2 Spatial autocorrelation of ISI in the population GHO1 of S. fragilis

In order to determine the existence of spatial autocorrelation in the ISI across individuals in the population GHO1 of *S. fragilis*, we performed a Mantel test with 9999 permutations with Passage v.2 (Rosenberg & Anderson, 2011). The distance matrix for the ISI variable was created by means of the individual-by-individual differences between ISI values; the geographic distances between pair of individuals were log-transformed. The smaller sample size precluded us to perform this analysis in the other populations.

As we detected two sectors with highly contrasted individual selfing capacities between them in the population GHO1 (see results; *SC* and *SI sectors*), we also compared the floral morphology and the fitness traits between these two sectors, with independent two-sample t-tests. In addition, we performed two-sample t-tests to compare also the size of the adult-plants in the field and some physiographic features of the cliffs (geographical orientation, plant cover, slope and height) between the two sectors. These data were taken from Chapter 2 and Chapter 3, respectively; the size of the adult-plants was log-transformed to achieve normality. Mann-Whitney tests were carried out to compare differences between the *SC* and *SI sectors* in the FR after the *ASP* and *FGP* treatments and in the number of flower heads per adult-plant, since these data showed no normal distribution. Data of flower heads per plant data were also taken from Chapter 2.

All previous statistical analyses (*section 2.3*) were performed with SPSS software (SPSS for Windows, ver.15.0.1, 2006, Chicago: Inc.). The null hypothesis was rejected at a significance level of 0.05. Means ± SE are indicated in the text.

2.4 Identification of the incompatibility groups and S allele diversity in natural populations

The *ICP* treatment was applied to estimate the *S* allele diversity in three independent diallels in the Spanish population ANT of *S. pustulatus*, the Moroccan population TAL of *S. pustulatus* and the population GHO1 of *S. fragilis*. To this end, we used 14, 14 and 11 individuals in each diallel, respectively, all obtained from cuttings.

We only chose individuals highly self-incompatible (ISI ≥ 0.8) because the *ICP* treatment could not avoid self-pollination. Each *ICP* cross was reciprocally repeated between individual pairs from two to six times until three independent full cross diallels had been achieved. Approximately 1200 flower heads received the *ICP* treatment, including repeats.

Compatibility phenotypes were scored according to fruits set after the *ICP* inter-individual crosses. Taking into account the rates of self-pollination within the flower heads inferred from the different detected fructifications after the *FGP* and *ASP* treatments (see results), we considered incompatible (-) a cross between two individuals when the FR after the *ICP* treatment was less than 0.10; indeterminate (+/-) when FR ranged from 0.10 to 0.20; and compatible (+) when FR was higher than 0.20. Within each diallel, individuals were grouped according to shared incompatibility interactions to form incompatibility groups corresponding with shared *S* alleles (Brennan *et al.*, 2002). *S* alleles were inferred to be interacting either dominantly or co-dominantly when individuals belonged to one or two incompatibility groups, respectively.

The total number of *S* alleles present within each population (N) was estimated according to Brennan *et al.* (2002, 2013):

$$n = N(1 - (1 - (1/N))^r),$$

where n is the number of *S* alleles identified in a sample and r the number of plants sampled. This maximum-likelihood estimator was modified from that developed for GSI systems (Paxman, 1963) in order to make it suitable for SSI systems. Since the GSI estimator assumed equally frequent *S* phenotypes (isoplethy) within panmictic populations, the SSI estimator has therefore to assume dominance interactions among *S* alleles which would allow isoplethy and thus, these would mask one of the two alleles implied in the diploid control of the SI reaction. In addition, we calculated the repeatability index R (Stevens & Kay, 1989) of our results in order to measure the thoroughness of the study:

$$R = 1 - [(n - 2)/(r - 2)],$$

which ranges from 0 (as many different *S* alleles identified as *S* alleles sampled) to 1 (the minimum number of *S* alleles possible for a SSI system identified in the entire sample).

3. RESULTS

3.1 Strength of SI

A high incidence of SI was the common in *S. masguindalii* and both the Moroccan and Spanish ranges of *S. pustulatus*, and they were classified as SI taxa (Table 1). In contrast, *S. fragilis* showed a lower incidence of SI and was classified as a pSI species (Table 1). At the population level, seven and four populations from the three species were classified as SI and pSI, respectively, and only one population of *S. fragilis* was classified as SC (Table 1). At the intra-population level, the proportion of pSI and SC plants ranged from 0 to 50% and from 0 to 80%, respectively (Figure 1). The proportion of pSI plants in populations had a significant negative correlation with the density of plants (r = -0.59, P = 0.040), but it was not related to the effective population size (P = 0.351; Figure 2). The proportion of SC plants did not correlate with any of these two variables (P >0.670; Figure 2).

Table 1 General reproductive behaviour of plants from representative populations of the species of *Sonchus* section *Pustulati*. FR, fruit (achene)-set after pollinations: *ASP* (autonomous self-pollination), *FGP* (hand forced geitonogamic pollination) and *MCP* (hand massive crossed pollination). ISI, index of self-incompatibility (Lloyd, 1965; Raduski *et al.*, 2011). ISI states of populations or taxa were addressed according to the same criteria as for individuals (see Methods): SC (self-compatibility, mean ISI < 0.2), pSI (partial self-incompatibility, 0.2 ≥ mean ISI ≥ 0.8) and SI (self-incompatibility, mean ISI ≥ 0.8). SE, standard error.

Species and	Mean FR ± SE (N)			Mean ISI ± S.E. (N)	ISI states	Origin
populations	*ASP*	*FGP*	*MCP*			
S. pustulatus, SE Spain				*0.90 ± 0.12 (57)*	*SI*	
ANT	0.00 ± 0.001 (15)	0.07 ± 0.014 (15)	0.54 ± 0.025 (15)	0.87 ± 0.032 (15)	SI	Cuttings
TEL	0.06 ± 0.024 (14)	0.14 ± 0.056 (14)	0.33 ± 0.046 (12)	0.60 ± 0.161 (12)	pSI	Cuttings
CAB	0.04 ± 0.023 (37)	0.08 ± 0.037 (33)	0.47 ± 0.039 (31)	0.84 ± 0.001 (30)	SI	Seeds / Cuttings
S. pustulatus, W Rif, Morocco				*0.90 ± 0.12 (53)*	*SI*	
LAUL	0.00 ± 0.001 (20)	0.08 ± 0.031 (20)	0.68 ± 0.030 (20)	0.87 ± 0.051 (20)	SI	Seeds
TAL	0.00 ± 0.001 (34)	0.01 ± 0.007 (34)	0.55 ± 0.028 (22)	0.96 ± 0.021 (21)	SI	Seeds / Cuttings
KAN	0.00 ± 0.000 (14)	0.11 ± 0.067 (12)	0.59 ± 0.043 (13)	0.84 ± 0.096 (12)	SI	Cuttings
S. fragilis, NW Rif, Morocco				*0.45 ± 0.04 (160)*	*pSI*	
GHO1	0.15 ± 0.018 (131)	0.35 ± 0.030 (132)	0.72 ± 0.013 (120)	0.53 ± 0.043 (120)	pSI	Cuttings
GHO3	0.17 ± 0.036 (20)	0.48 ± 0.057 (20)	0.69 ± 0.047 (20)	0.29 ± 0.079 (20)	pSI	Seeds
DER	0.42 ± 0.059 (20)	0.72 ± 0.055 (20)	0.82 ± 0.046 (20)	0.11 ± 0.059 (20)	SC	Seeds
S. masguindalii, central Rif, Morocco				*0.84 ± 0.13 (43)*	*SI*	
TOR	0.00 ±0.000 (24)	0.00 ± 0.000 (23)	0.65 ± 0.041 (22)	1.00 ± 0.000 (21)	SI	Seeds
BOU	0.00 ± 0.001 (11)	0.02 ± 0.012 (8)	0.41 ± 0.106 (6)	0.96 ± 0.030 (6)	SI	Cuttings
SFI	0.05 ± 0.028 (26)	0.12 ± 0.044 (27)	0.62 ± 0.055 (17)	0.58 ± 0.157 (16)	pSI	Seeds

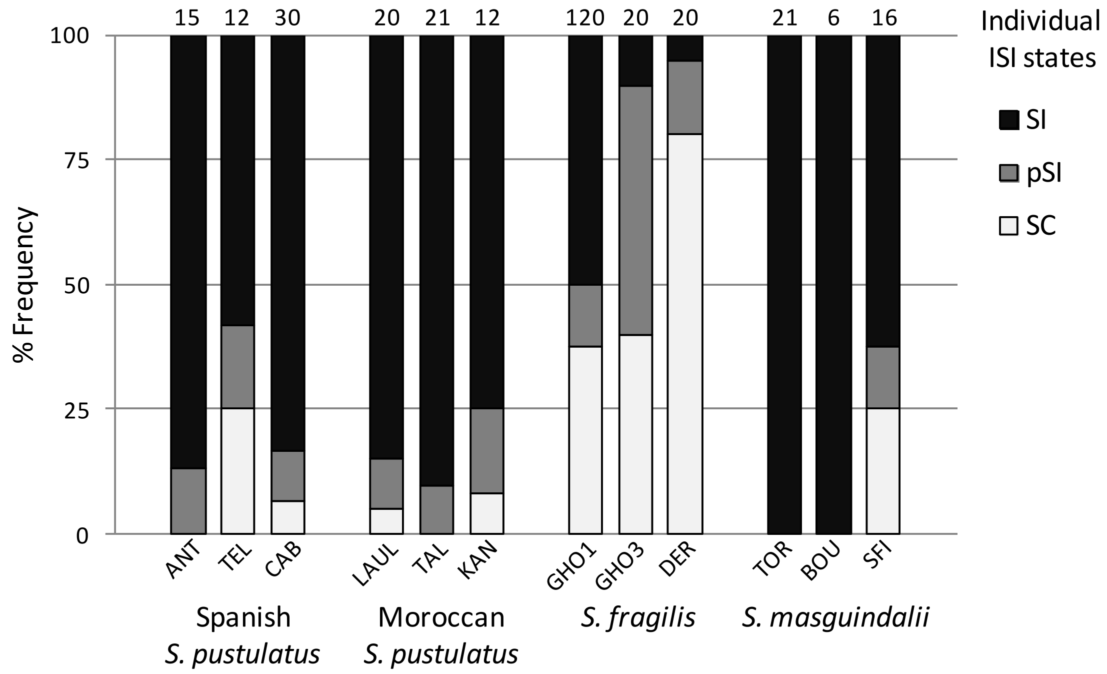
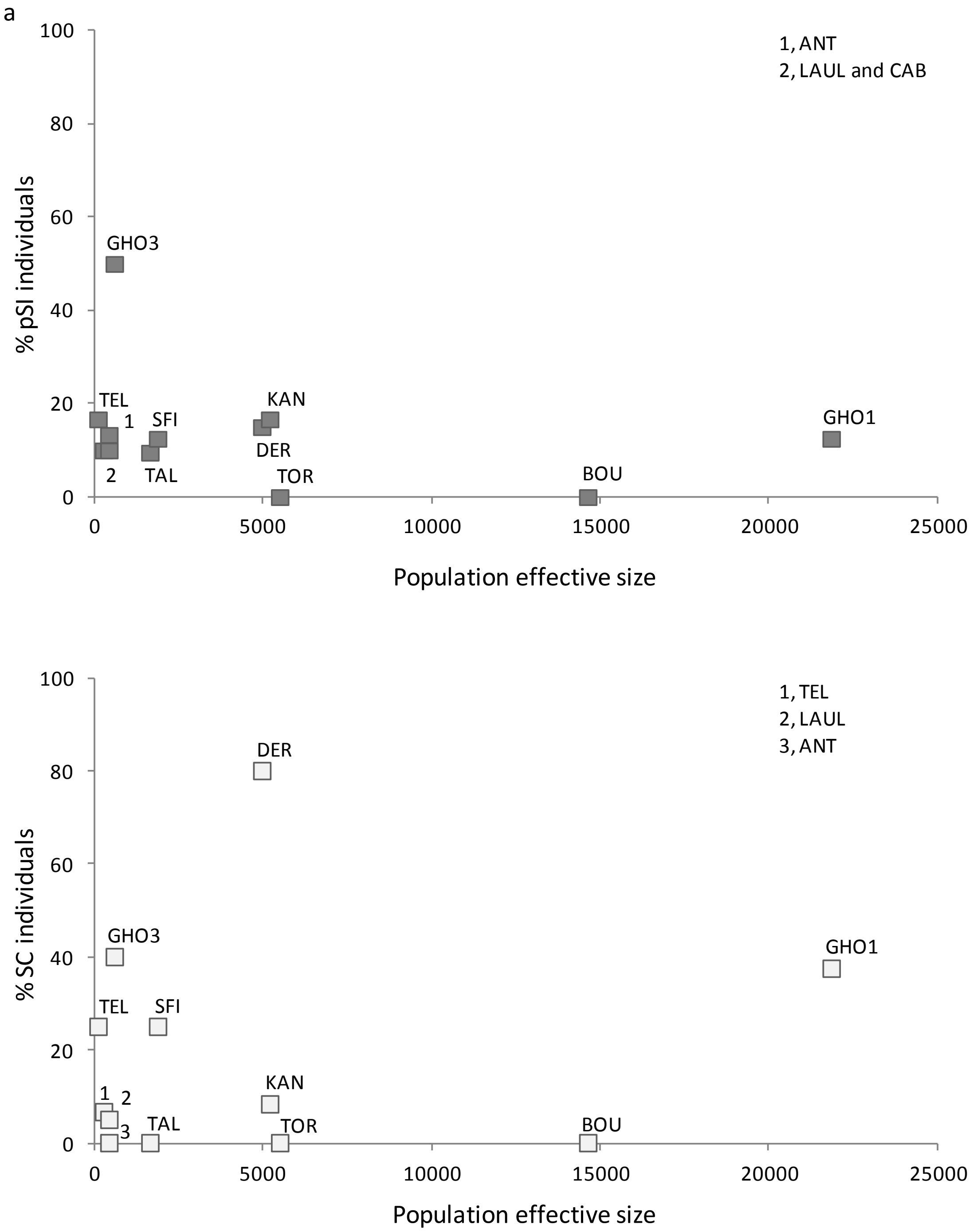


Figure 1 Strength and variation of the self-incompatibility across populations of the species of *Sonchus* section *Pustulati* assessed by the index of self-incompatibility (ISI; Lloyd, 1965; Raduski *et al.*, 2011). Individuals were classified according to the ISI states: self-compatible (SC, ISI < 0.2), partial self-compatible (pSI, 0.2 ≥ ISI ≥ 0.8) and self-incompatible (SI, ISI ≥ 0.8). Numbers above bars are sampled individuals used to calculate frequencies of ISI states.


a
100
80
60
40
20
0
% pSI individuals
1, ANT
2, LAUL and CAB
GHO3
TEL
1
SFI
KAN
DER
2
TAL
TOR
BOU
GHO1
0
5000
10000
15000
20000
25000
Population effective size
100
80
60
40
20
0
% SC individuals
1, TEL
2, LAUL
3, ANT
DER
GHO3
GHO1
TEL
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Population effective size

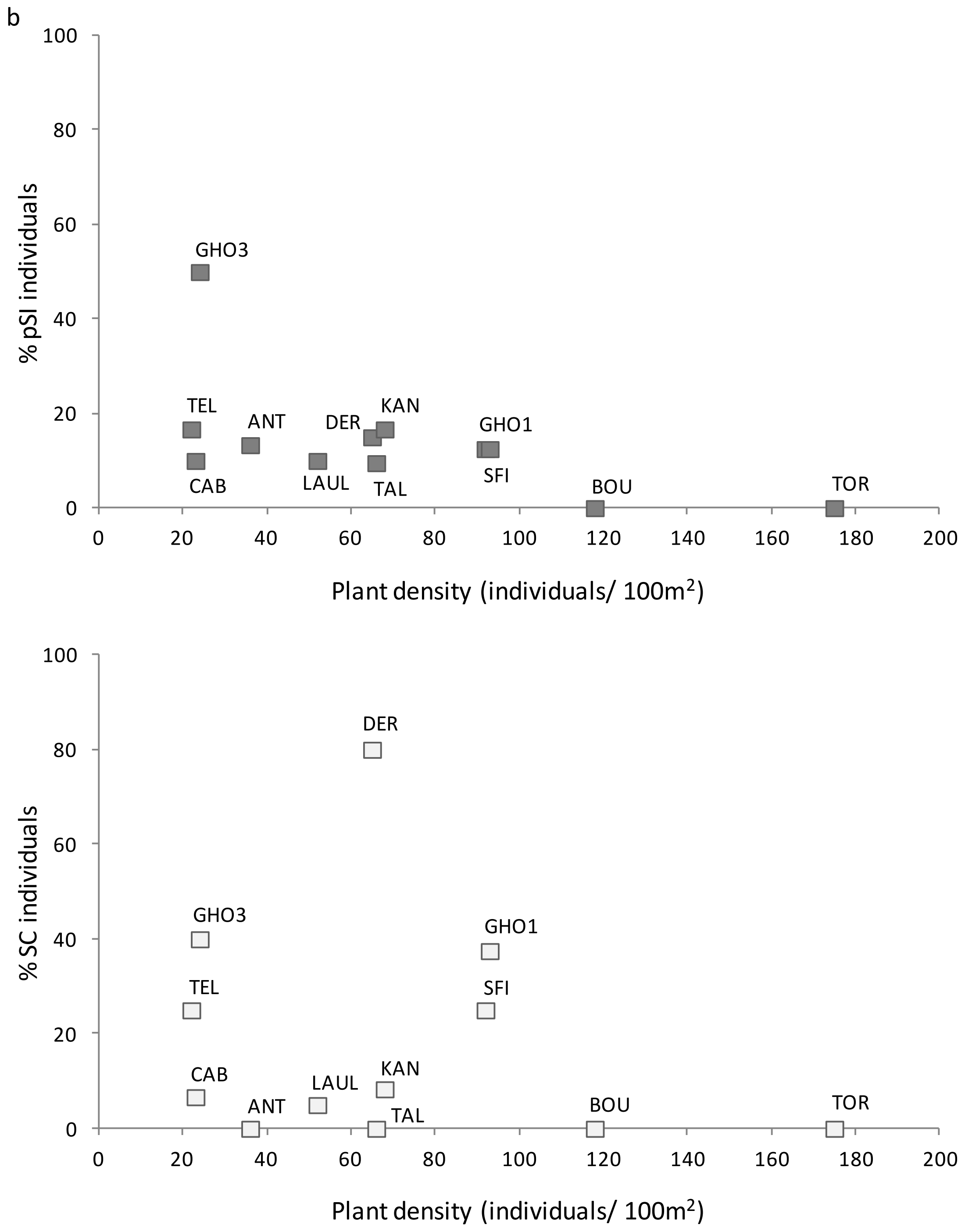


Figure 2 Relationships between population proportion of partial-self-incompatible (0.2 ≥ ISI ≥ 0.8; %pSI) and self-compatible plants (ISI < 0.2; %SC) with a) effective population size and b) population plant density.

The FR values for the *MCP* were significantly higher than those of the *FGP* and *ASP* treatments in every taxon (X^2 >110.80, *P* <0.001; *U* >0.001, *P* <0.001). FR values after *FGP* were higher than after *ASP* in each species (*U* >1673.00, *P* <0.020) excepting *S. masguindalii* (*U* = 1609.50, *P* = 0.117; Figure 3). The difference between the FR obtained after *FGP* and *ASP* pollinations [i.e. FR (*FGP* – *ASP*)] in each individual was 0.22 ± 0.02 in *S. fragilis*, 0.05 ± 0.01 and 0.05 ± 0.02 in the Spanish and Moroccan ranges of *S. pustulatus*, respectively, and 0.03 ± 0.01 in *S. masguindalii* (Figure 4). This difference was significantly higher in *S. fragilis* than in the remaining taxa (X^2 = 71.80, *P* = 0.000; *U* >2165.50, *P* <0.001), in accordance with the lower incidence of SI in the former species. Shockingly, some plants (12%), particularly of *S. fragilis* (9%), showed even a higher fructification after *ASP* than after *FGP*.

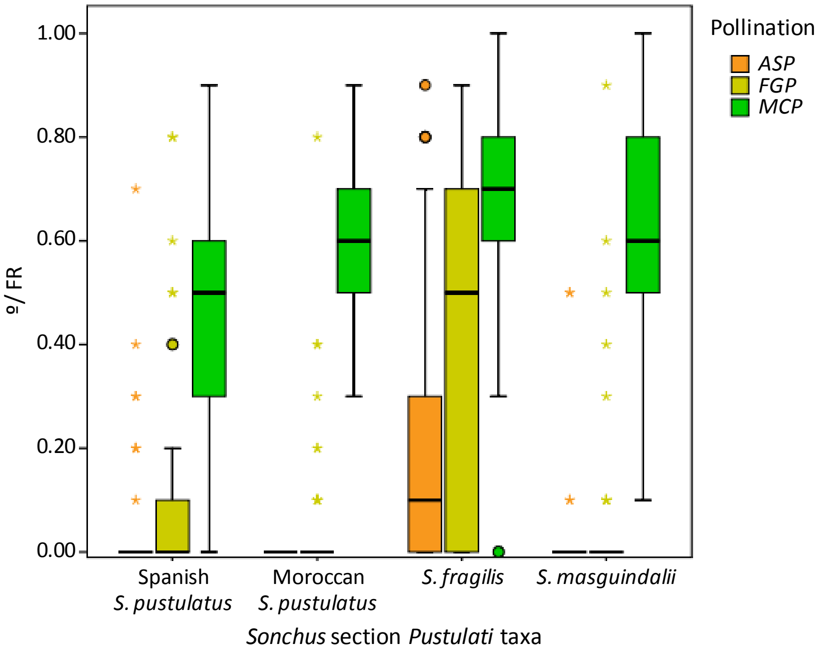


Figure 3 Fruit (achene)-set (º/FR) variation across the species of *Sonchus* section *Pustulati* according to the different types of pollinations applied over sampled individuals: autonomous self-pollination (*ASP*), forced geitonogamic pollination (*FGP*) and massive crossed pollination (*MCP*). Small circles and asterisks indicate atypical and extreme values, respectively.

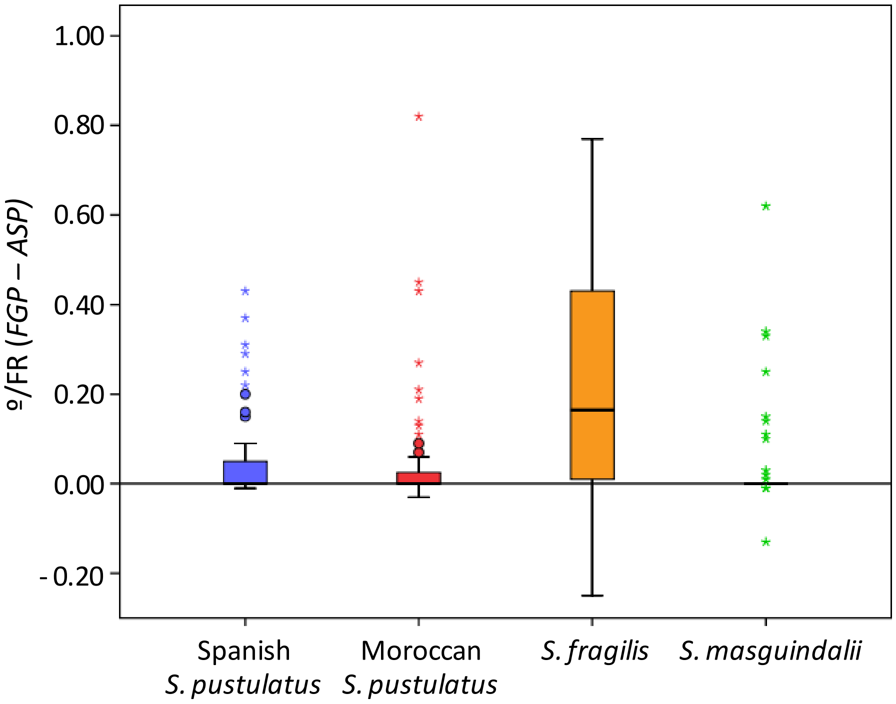


Figure 4 Variation of the individual difference on fruit set (º/FR) obtained after forced geitonogamic (*FGP*) and autonomous self- (*ASP*) pollinations across the *Sonchus* section *Pustulati* taxa. Small circles and asterisks indicate atypical and extreme values, respectively.

3.1.1 The outstanding case of the population GHO1 of S. fragilis

The individuals from the population GHO1 of *S. fragilis* showed an extreme variability in the capacity of self-fecundation. The standard deviation of the FR after *FGP* in this population was 0.345, the highest among the twelve populations analysed (range of standard deviations of the FR after *FGP* in the rest of populations = 0.000–0.275). The distribution of the index of self-incompatibility (ISI) within population GHO1 fitted a clear bimodal pattern (Figure 5). Shockingly, 20 SC plants showed negative ISI values (range (-0.39)–(-0.01)), which meant a higher fruit set by self-pollination (after *FGP*) than by cross-pollination (after *MCP*).

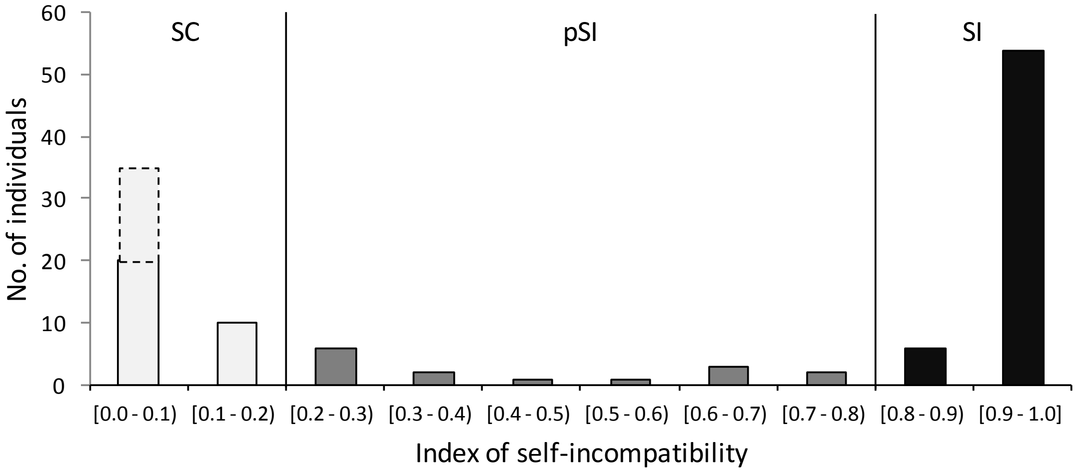


Figure 5 Distribution of the index of self-incompatibility (ISI) in the population GHO1 of *Sonchus fragilis* (N= 120 individuals). ISI measures the strength of SI reaction by comparison of relative selfed and outcrossed success of fruit set per flower after hand pollinations (Lloyd, 1965). Species with negative ISI values were set to zero (area delimited by dashed line). Histograms are positioned of increasing ranges of ISI by column and are coloured according to the ISI states: white, self-compatible; grey, partial self-compatible; and black, self-incompatible.

Within GHO1, the FR after *MCP* was always high and did not show significant differences among SI, pSI and SC plants (*P* = 0.296; Table 2a). This result supports the good performance of the conducted manual technique for the pollinations and the certainty of our estimations. According to our grouping, the FR after *ASP* was significantly the lowest in the SI plant group, intermediate in the pSI group, and the highest in the SC plants (X^2 = 86.27, *P* = 0.000; *U* >82.00, *P* <0.010; Table 2a). Within the SC group, the *ASP* treatment gave a significant lower FR (0.33 ± 0.031) than that obtained after the *FGP* (0.74 ± 0.017; *U* = 113.00, *P* = 0.000) and *MCP* (0.73 ± 0.018; *U* = 120.00, *P* = 0.000) treatments.

Both SI and pSI plants showed significantly bigger flower heads (*F* = 13.23, *P* = 0.000; Bonferroni post hoc *P* <0.030) and longer outer florets (*F* = 11.67, *P* = 0.000; Bonferroni post hoc *P* <0.024) than SC plants (Table 2a). The differences on the outer florets were due to differences in the ligule length (*F* = 15.25, *P* = 0.000; Bonferroni post hoc *P* <0.005; Table 2a). There were no significant differences on fitness in the field among SI groups (*F* > 0.06; *P* >0.195; Table 2a).

Table 2 Means ± SE of the analysed features on fitness and floral morphology in the population GHO1 of *Sonchus fragilis*: a) among the three ISI groups (SC, pSI and SI plants); and b) among the *SI* and *SC sectors*, mainly dominated by SI and SC plants, respectively. Data were taken from plants growing in natural conditions or in the greenhouse. FR, fruit set; *ASP*, autonomous self-pollination; *FGP*, forced geitonogamic pollination; *MCP*, massive crossed pollination. ^{1} refers to those variables measured over cuttings collected both in 2008 (37) and in 2011 (87). * indicates variables with significant differences ($P < 0.05$) among groups and the remaining exponents refers to those groups that have such significant differences.

a)

ISI states	ISI [1]*	In natural conditions			
		FR	Florets per flower head	Fruits per flower head	Weight of 20 fruits (mg)
SC	-0.02 ± 0.021	0.78 ± 0.027	60.4 ± 2.49	48.2 ± 2.08	13.2 ± 0.57
pSI	0.44 ± 0.054	0.74 ± 0.048	66.4 ± 4.07	47.9 ± 4.71	14.2 ± 0.65
SI	0.97 ± 0.006	0.75 ± 0.024	64.7 ± 3.12	51.3 ± 2.34	14.6 ± 0.54

ISI states	In the greenhouse							
	FR after each pollination treatment [1]			Flower head size (mm) *	Outer florets length (mm) *	Outer florets ligule length (mm) *	Outer florets tube length (mm)	Outer florets ligule width (mm)
	ASP *	*FGP* *	*MCP*					
SC	0.34 ± 0.030 $^{\text{pSI, SI}}$	0.74 ± 0.017 $^{\text{pSI, SI}}$	0.73 ± 0.019	29.9 ± 1.01 $^{\text{SI, pSI}}$	16.0 ± 0.42 $^{\text{SI, pSI}}$	10.5 ± 0.33 $^{\text{SI, pSI}}$	5.5 ± 0.18	3.8 ± 0.10
pSI	0.16 ± 0.060 $^{\text{SC, SI}}$	0.37 ± 0.045 $^{\text{SC, SI}}$	0.65 ± 0.033	32.5 ± 1.42 $^{\text{SC}}$	16.7 ± 0.57 $^{\text{SC}}$	11.1 ± 0.38 $^{\text{SC}}$	5.7 ± 0.23	3.8 ± 0.12
SI	0.01 ± 0.017 $^{\text{SC, pSI}}$	0.03 ± 0.020 $^{\text{SC, pSI}}$	0.73 ± 0.026	36.6 ± 0.78 $^{\text{SC}}$	18.4 ± 0.28 $^{\text{SC}}$	12.6 ± 0.21 $^{\text{SC}}$	5.9 ± 0.12	4.1 ± 0.05

b)

Sector	ISI [1]*	In natural conditions				In the greenhouse				
		FR	Florets per flower head	Fruits per flower head	Fruit weight (mg)	Plant size (cm^2) *	Flower head per plant *	FR after each pollination treatment [1]		
								ASP *	*FGP* *	*MCP* *
SC sector	-0.00 ± 0.031	0.71 ± 0.041	59.0 ± 2.73	46.5 ± 2.27	13.8 ± 0.70	81.1 ± 10.86	4.3 ± 0.80	0.26 ± 0.025	0.68 ± 0.025	0.68 ± 0.017
SI sector	0.76 ± 0.004	0.76 ± 0.019	65.1 ± 2.30	51.0 ± 1.90	14.0 ± 0.40	247.9 ± 58.12	6.5 ± 1.73	0.10 ± 0.024	0.19 ± 0.032	0.74 ± 0.016

3.1.2 Spatial distribution of selfing phenotypes within populations

The ISI of individuals showed a spatial aggregation in the population GHO1 of *S. fragilis*, indicated by a significant isolation-by-distance pattern (r = 0.52, P = 0.000; Mantel test). Two sectors within the population could be delimited (Figure 6a). The northern area (*SC sector*, 37 individuals tested) was basically constituted by SC plants (83.8%), which were accompanied by some pSI plants (16.2%). The southern area (*SI sector*, 83 individuals tested) was mainly constituted by SI plants (72.3%), followed by SC and pSI plants (16.9% and 10.8%, respectively). Adult-plants were significantly larger and produced more flower heads per individual in the *SI sector* than in the *SC sector* (N = 132 and 127 individuals for each variable, respectively; t = -2.54, P = 0.012; U = 1507.00, P = 0.042; Table 2b). The FR after *MCP* was significantly higher and the FR after *ASP* and *FGP* were significantly lower in the *SI sector* than in the *SC sector* (U >381.50; t = -2.23; P <0.028 in all cases). However, none of the physiographic features of the cliffs (geographical orientation, plant cover, slope and height) were significantly different between *SC* and *SI* sectors (N = 30 plots; t >(-1.81), P >0.080).

Despite the low number of individuals sampled (N = 12) across the population TEL of *S. pustulatus*, we could depict a similar spatial distribution of ISI to that of the GHO1 population, although at a much lower scale in accordance to the population size. There was a small group of SC and pSI plants (with none SI plant detected among them) that was spatially isolated from the core of the population, which was mainly SI (Figure 6b). In the case of the 20 individuals sampled in each one of the populations GHO3 and DER of *S. fragilis* we did not find any spatial pattern regarding the ISI distribution at individual level, i.e. the rare SI individuals were spatially located among the SC and pSI

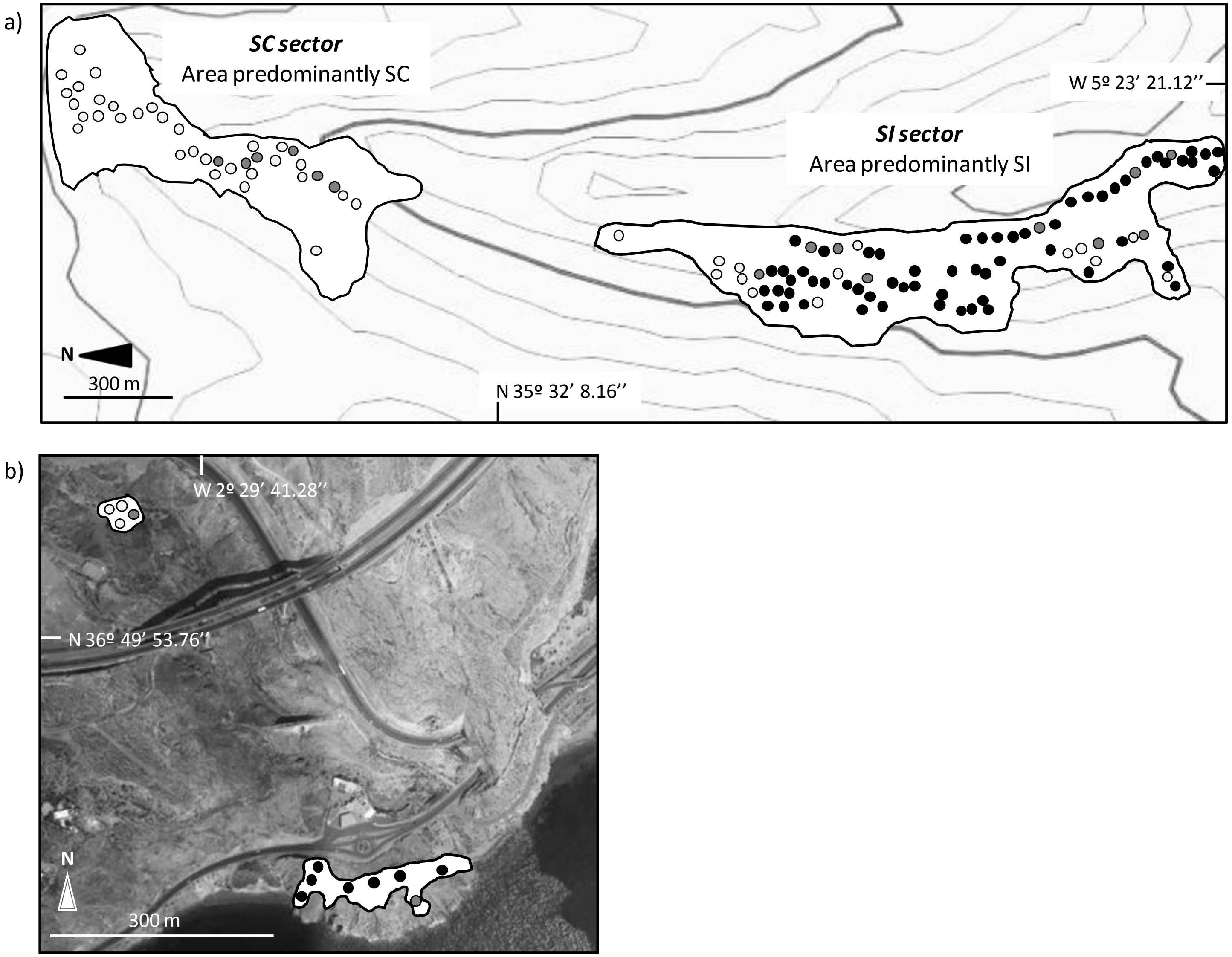
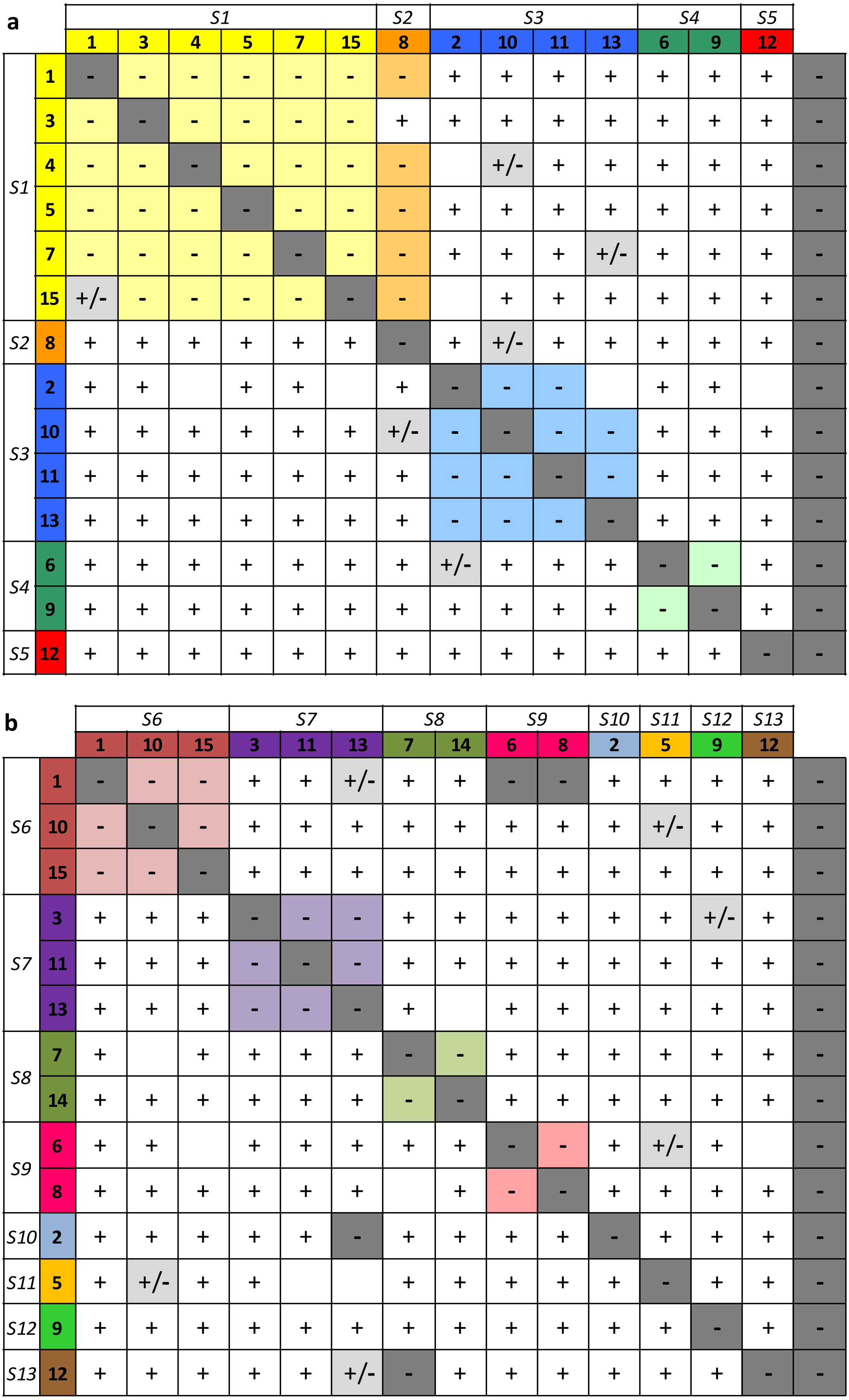

a)
SC sector
Area predominantly SC
SI sector
Area predominantly SI
W 5º 23’ 21.12’’
N
300 m
N 35º 32’ 8.16’’
b)
W 2º 29’ 41.28’’
N 36º 49’ 53.76’’
N
300 m

Figure 6 Spatial distribution of the ISI states at individual level: a) in the population GHO1 of *S. fragilis* (N = 120); b) in the population TEL of *S. pustulatus* (N = 12). Individuals are represented by circles and their ISI states by colours: white, self-compatible (SC); grey, partial self-compatible (pSI); and black, self-incompatible (SI).

plants (data not shown). This also happened with the SC and pSI individuals in the remaining populations of *S. pustulatus* and *S. masguindalii* (data not shown).

3.2 S allele diversity estimation

Our three complete diallels in the populations ANT, TAL and GHO1 of Spanish and Moroccan ranges of *S. pustulatus* and *S. fragilis* allowed the identification of five, eight and eight incompatibility groups, respectively (Figure 7a-c). These were interpreted as the correspondent numbers of different expressed *S* alleles in each population sample (Figure 7a-c and Table 3 and 4). It is worth noting that as the number of individuals used in the crosses was 14, 14 and 11, respectively, the number of potential alleles were 28, 28 and 22. We numbered the *S* alleles found from *S1* to *S21*, although individuals from different populations may share some of these alleles among them. From our results we interpreted dominance relations among alleles. A SSI model of complete dominance among alleles satisfactorily explained the majority of the crossing results in each population (94.3–99.1% of results supported; Table 5). Furthermore, assuming co-dominance of alleles *S1* and *S2* in the pollen of plant 8 from ANT, and co-dominance of alleles *S6* and *S9* in the stigma of plant 1 from TAL (Appendix: Figure 10), the SSI model explained a further 2.8% and 1.1% of the crossing results for each of

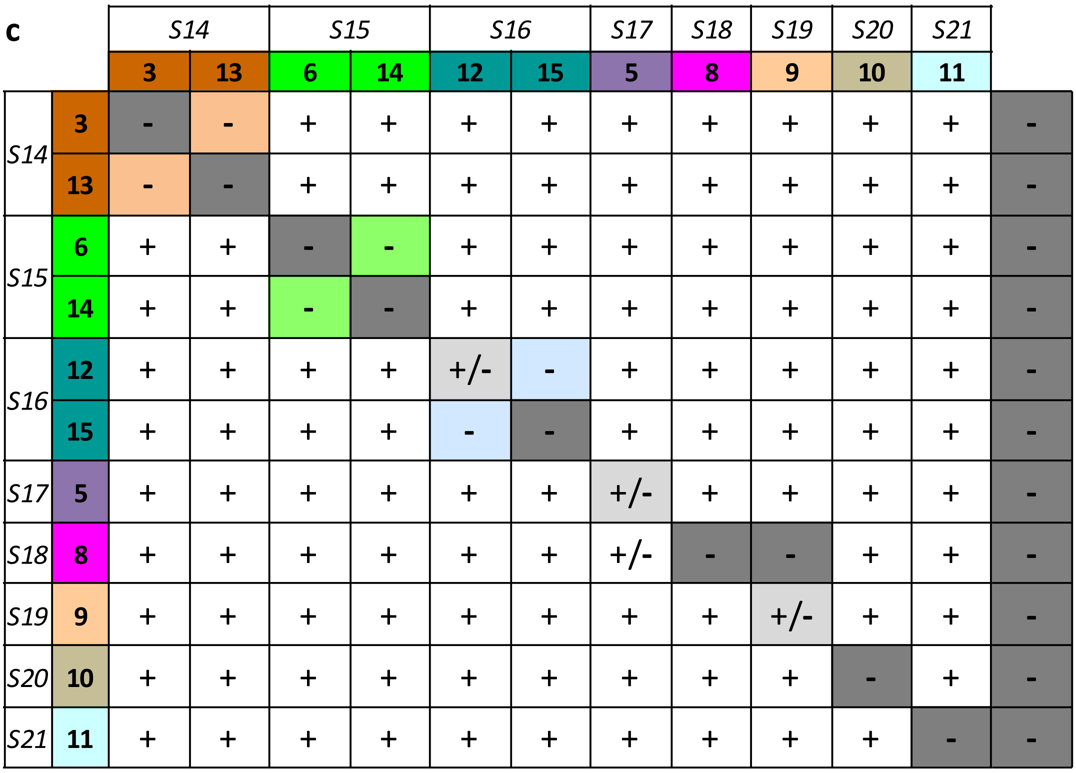


a

		S1						*S2*	*S3*				*S4*		*S5*	
		1	**3**	**4**	**5**	**7**	**15**	**8**	**2**	**10**	**11**	**13**	**6**	**9**	**12**	
S1	**1**	-	-	-	-	-	-	-	+	+	+	+	+	+	+	-
	3	-	-	-	-	-	-	+	+	+	+	+	+	+	+	-
	4	-	-	-	-	-	-	-		+/-	+	+	+	+	+	-
	5	-	-	-	-	-	-	-	+	+	+	+	+	+	+	-
	7	-	-	-	-	-	-	-	+	+	+	+/-	+	+	+	-
	15	+/-	-	-	-	-	-	-		+	+	+	+	+	+	-
S2	**8**	+	+	+	+	+	+	-	+	+/-	+	+	+	+	+	-
S3	**2**	+	+		+	+		+	-	-	-		+	+		-
	10	+	+	+	+	+	+	+/-	-	-	-	-	+	+	+	-
	11	+	+	+	+	+	+	+	-	-	-	-	+	+	+	-
	13	+	+	+	+	+	+	+	-	-	-	-	+	+	+	-
S4	**6**	+	+	+	+	+	+	+	+/-	+	+	+	-	-	+	-
	9	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-
S5	**12**	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-

b

		S6			*S7*			*S8*		*S9*		*S10*	*S11*	*S12*	*S13*	
		1	**10**	**15**	**3**	**11**	**13**	**7**	**14**	**6**	**8**	**2**	**5**	**9**	**12**	
S6	**1**	-	-	-	+	+	+/-	+	+	-	-	+	+	+	+	-
	10	-	-	-	+	+	+	+	+	+	+	+	+/-	+	+	-
	15	-	-	-	+	+	+	+	+	+	+	+	+	+	+	-
S7	**3**	+	+	+	-	-	-	+	+	+	+	+	+	+/-	+	-
	11	+	+	+	-	-	-	+	+	+	+	+	+	+	+	-
	13	+	+	+	-	-	-	+		+	+	+	+	+	+	-
S8	**7**	+		+	+	+	+	-	-	+	+	+	+	+	+	-
	14	+	+	+	+	+	+	-	-	+	+	+	+	+	+	-
S9	**6**	+	+		+	+	+	+	+	-	-	+	+/-	+		-
	8	+	+	+	+	+	+		+	-	-	+	+	+	+	-
S10	**2**	+	+	+	+	+	-	+	+	+	+	-	+	+	+	-
S11	**5**	+	+/-	+	+			+	+	+	+	+	-	+	+	-
S12	**9**	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-
S13	**12**	+	+	+	+	+	+/-	-	+	+	+	+	+	+	-	-



c		*S14*		*S15*		*S16*		*S17*	*S18*	*S19*	*S20*	*S21*	
		3	**13**	**6**	**14**	**12**	**15**	**5**	**8**	**9**	**10**	**11**	
S14	**3**	-	-	+	+	+	+	+	+	+	+	+	-
	13	-	-	+	+	+	+	+	+	+	+	+	-
S15	**6**	+	+	-	-	+	+	+	+	+	+	+	-
	14	+	+	-	-	+	+	+	+	+	+	+	-
S16	**12**	+	+	+	+	+/-	-	+	+	+	+	+	-
	15	+	+	+	+	-	-	+	+	+	+	+	-
S17	**5**	+	+	+	+	+	+	+/-	+	+	+	+	-
S18	**8**	+	+	+	+	+	+	+/-	-	-	+	+	-
S19	**9**	+	+	+	+	+	+	+	+	+/-	+	+	-
S20	**10**	+	+	+	+	+	+	+	+	+	-	+	-
S21	**11**	+	+	+	+	+	+	+	+	+	+	-	-

Figure 7 Analysed diallel of cross results between pairs of individuals from the Spanish population ANT of *Sonchus pustulatus* (a), the Moroccan population TAL of *S. pustulatus* (b) and the population GHO1 of *S. fragilis* (c). The first row and column refer to the dominantly or co-dominantly-expressed *S* alleles that have been identified. Codes of individuals acting as maternal stigma receptor are in the second column, whereas codes of individuals acting as paternal pollen donor are listed in the second row. Symbols indicate compatibility reaction of crosses involving each pair of individuals according to the fruit set (see Methods): compatible (+ and white shading), incompatible (**-** and coloured or dark grey shading) and indeterminate (+/- and pale grey shading). No symbol indicates missing data. The incompatibility groups are differentiated by colours to each identified *S* allele. The last column refers to the compatibility reaction obtained after autonomous self-pollinations.

these populations, respectively (Table 5). Very few crossing anomalies, i.e. those not fitted into the SSI model, were detected across the performed diallels (0.9–4.6%; Table 5). The *S* allele number estimator of Brennan *et al.* (2002) predicted approximately 5, 12, and 15 *S* distinct alleles for the entire populations ANT, TAL and GHO1, respectively (Table 4). According to the *R* parameter, the 75%, 50% and 30% of the total *S* allele diversity within the populations ANT, TAL and GHO1 were likely represented within our samples, respectively (Table 4).

Table 3 *S* allele designations for the individuals of *Sonchus pustulatus* and *S. fragilis* in the three population samples, based on a sporophytic self-incompatible *S* allele dominance interpretation of the analysed diallels presented in Figure 7. Individuals with *S* alleles codominance interactions: (p) codominant in paternal pollen tissue but recessiveness to such allele in the maternal stigma tissue; and (s), when the codominance interactions only occurs in the maternal stigma tissue.

S allele designations	Plant						
Population ANT of *S. pustulatus*, SE Spain							
S1	1	3	4	5	7	15	8(p)
S2	8						
S3	2	10	11	13			
S4	6	9					
S5	12						
Population TAL of *S. pustulatus*, N Morocco							
S6	1	10	15				
S7	3	11	13				
S8	7	14					
S9	6	8	1(s)				
S10	2						
S11	5						
S12	9						
S13	12						
Population GHO1 of *S. fragilis*, N Morocco							
S14	3	13					
S15	6	14					
S16	12	15					
S17	5						
S18	8						
S19	9						
S20	10						
S21	11						

Table 4 Analyses of the *S* allele diversity in the three populations sampled of *Sonchus pustulatus* and *S. fragilis*. *r*, number of sampled plants; *n*, number of *S* alleles identified in a sample, i.e. number of incompatibility groups identified in the sample (Hiscock, 2002); ***N***, estimated number of *S* alleles present in the entire population; *R*, thoroughness of the study. N (pop.), effective population size; Mean FR, average fructification level in natural conditions; H_E, average gene diversity based on AFLP data. * and **, according to Chapters 2 and 4, respectively.

Species	*S. pustulatus*						*S. fragilis*		
Distribution	SE Spain	N Morocco					N Morocco		
Population	ANT	TEL	CAB	TAL	LAUL	KAN	GHO1	GHO3	DER
r	14			14			11		
n	5			8			8		
N	**5**			**12**			**15**		
R	0.75			0.50			0.30		
N (pop.)*	426	104	269	1643	427	5198	21858	588	4963
Density (plants/100m^2)*	32	22	26	64	42	72	92	21	47
Mean FR*	0.66	0.46	0.58	0.78	0.77	0.76	0.63	0.76	0.85
H_E**	0.088	0.101	0.093	0.146	0.138	0.155	0.146	-	-

Table 5 Summary statistics for cross-classifications and the explanatory power of the sporophytic self-incompatibility model of near-complete *S* allele dominance interactions for the analysed diallels of cross results in the three populations sampled of *Sonchus pustulatus* and *S. fragilis*.

Species	*S. pustulatus*		*S. fragilis*
Distribution	SE Spain	N Morocco	N Morocco
Population	ANT	TAL	GHO1
Cross classifications (between pairwise of individuals)			
Crossed classed as incompatible	25.8%	10.9%	6.4%
Crossed classed as compatible	67.6%	82.4%	93.6%
Undetermined crossed	3.3%	3.4%	0.0%
Missing data	3.3%	3.3%	0.0%
Explanatory power of the fitted SSI model			
Crosses explained by designation of dominantly expressed *S* alleles	94.3%	94.3%	99.1%
Crosses explained by designation of tissue-specific co-dominance interactions between *S* alleles in plants 8 (ANT) and 1 (TAL)	2.8%	1.1%	0%
Crosses not explained by *S* allele designations	4.0%	4.6%	0.9%

3.2.1 Spatial S allele distribution within populations

The *S* alleles identified did not show a contagious spatial distribution within the populations. The mean geographical distances between pairs of individuals sharing an *S* allele relative to the maximum distance among the sampled individuals across the populations ANT, TAL and GHO1 were 40.8 ± 6.56%, 44.8 ± 11.95% and 43.6 ± 17.99%, respectively (Figure 8a-c). In populations TAL and GHO1, the low number of individuals sharing an *S* allele and their high effective population sizes (Table 4) made unreliable this estimation. In the population ANT, the individuals of each of the incompatibility groups most represented in the diallels (those assigned with the *S1* and *S3* alleles; Table 3 and Figure 7a) were mainly distributed in each one of the two nuclei of individuals existing within the population (Figure 8a).

4. DISCUSSION

The results and interpretations from our three diallels support the presence of a sporophytic genetic control of self-incompatibility in the species of *Sonchus* section *Pustulati*. The high frequency of reciprocally compatible or incompatible inter-individual crossing results could be explained by *S* allele dominance interactions occurring both in pollen and stigma. Similarly, the very low proportion of only one-way compatible or incompatible inter-individual crossing results could be explained by *S* allele co-dominance interactions. Most of the crossing results could thus be fitted to a sporophytic incompatibility model, and we could assign at least one allele to each of the individuals implied into these crossings. In addition, the presence of more than two incompatibility groups in all the three diallels supports the multiallelic nature of the *S* locus (e.g. Brennan, 2002, 2006 and 2013; Young & Pickup, 2010).

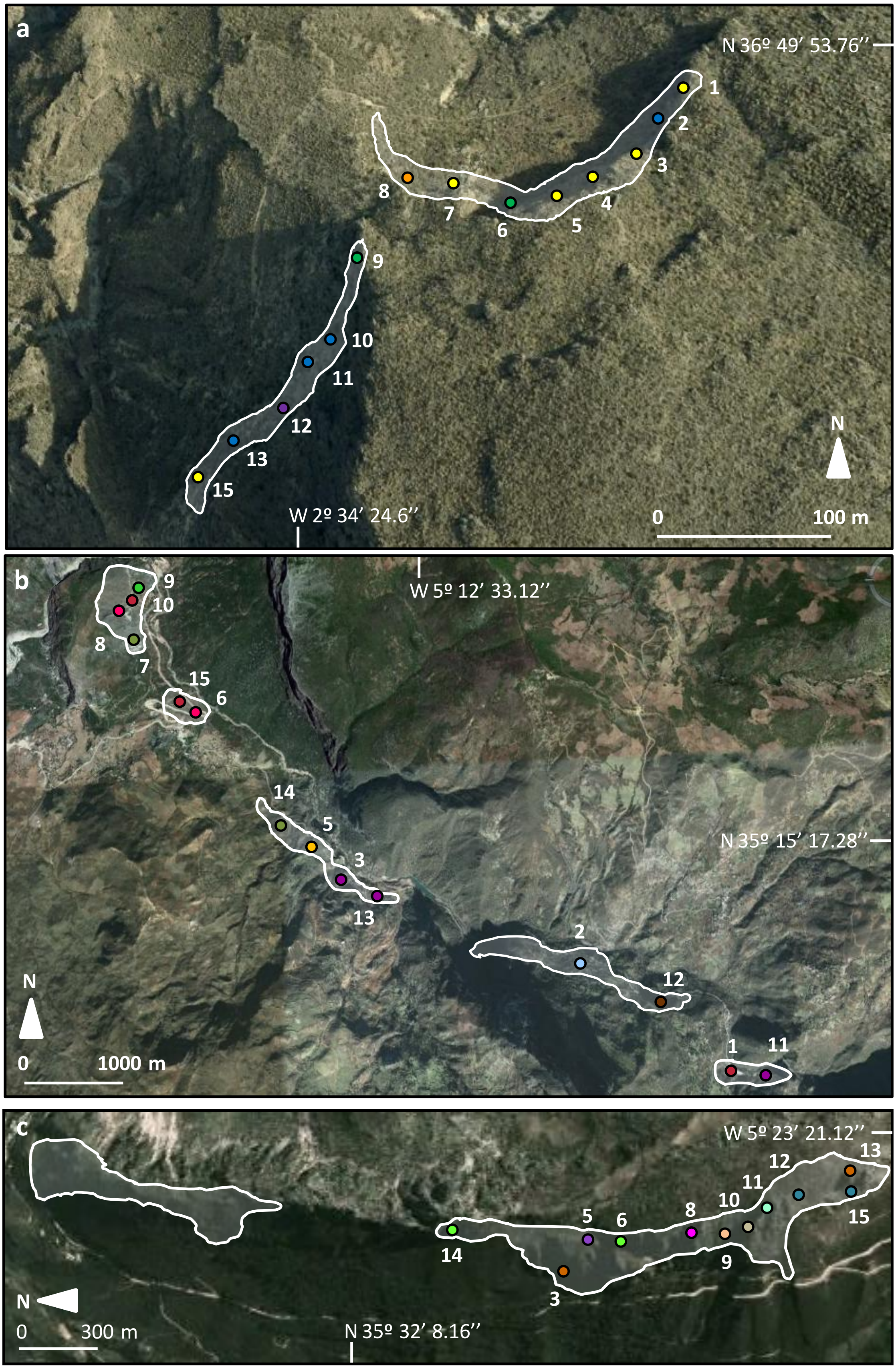

a
N 36º 49’ 53.76’’
1
2
3
4
5
6
7
8
9
10
11
12
13
15
N
0
100 m
W 2º 34’ 24.6’’
b
W 5º 12’ 33.12’’
9
10
8
7
15
6
14
5
3
13
N 35º 15’ 17.28’’
2
12
N
0
1000 m
1
11
c
W 5º 23’ 21.12’’
13
12
11
10
8
5
6
15
14
9
3
N
0
300 m
N 35º 32’ 8.16’’

Figure 8 Spatial distribution of incompatibility groups in the Spanish population ANT of *Sonchus pustulatus* (a), the Moroccan population TAL of *S. pustulatus* (b), and the population GHO1 of *S. fragilis* (c). Circles represent individuals used in diallel crosses and their colours indicate the incompatibility groups which they belong to (see Figure 7). White lines delimit the area of occupancy.

The generally low fruit set observed after self-pollination (particularly autonomous), relative to the large amounts of fruits for outcrossed treatments across individuals indicate that *Sonchus pustulatus* and *S. masguindalii* have a relatively strong SI mechanism. This is not the case of *S. fragilis*, which according to our results has a weak SI system. Nevertheless, virtually all populations of *S. pustulatus* and *S. masguindalii* showed low proportions of SC and pSI individuals, which indicates that the breakdown of SI, although weakly, is always presented in the section. According to the ISI criteria used in the present study, the three *Sonchus* species of the section *Pustulati* would be therefore included in the long list of Asteraceae taxa that have been considered to bear a not-always-100% efficient SSI system (i.e. SI-pSI species; e.g. Brauner & Gottlieb, 1987; Reinartz & Less, 1994; Young *et al.*, 2000; Nielsen *et al.*, 2003; Brennan *et al.*, 2005; Ortiz *et al.*, 2006; Lafuma & Maurice, 2007; Scheffknecht *et al.*, 2007; Ferrer *et al.*, 2009).

4.1 Does the highly pollinator-dependent self-fecundation promote mixed mating system?

One of our findings consisted of the individual differences on seed production detected between the *ASP* and *FGP* treatments, which were especially high in the plants of *S. fragilis* (Figure 4) due to the high incidence of SC therein. Such a difference indicates that, despite of the capacity of self-fertilization, the flowers of these species are mostly unable of autonomously self-pollinate. Therefore, SC and pSI plants likely need pollinators both to be either outcrossed or self-pollinated, and hence the mating system (from inbreeding to outcrossing) in the SC and pSI populations will primarily depend on the pollen load of pollinators and, secondly, on which type of pollen (own or alien) gets more successful fecundations. The higher values of fruit set detected after the crossed pollinations than in the geitonogamic ones suggest that alien pollen seems to dominate fecundations in these species. In the Asteraceae, SC taxa tend to constitute mixed mating systems rather than fully inbreeding populations (e.g. Ellstrand *et al.*, 1978; Sun & Ganders, 1988), as the incidence of mixed mating systems is expected to be higher in cases of attractive bloom as in the present one. The use of molecular techniques would be necessary to address the actual rates of self- and cross-fertilization in the SC and pSI populations of *Sonchus* section *Pustulati*. In any case, the dependence on pollinators for increasing selfing rates discards the limitation of pollination service as a main reason for the breakdown of SI on these species.

4.2 A case of a likely mixed mating system

The existence of populations bearing individuals with an extremely variable capacity of selfing and that are spatially aggregated has been very rarely documented (but see Ortiz *et al.*, 2006; Ferrer *et al.*, 2009). In fact, the bimodal pattern detected in the distribution of the ISI at individual level in the population GHO1 of *Sonchus fragilis* (Figure 5) is surprisingly similar to that obtained when more than a thousand species of

Angiosperms are jointly analysed (see Raduski *et al.*, 2011). The SI system in GHO1 is therefore an exceptional case of potentially mixed mating system. In fact, the significant differences detected in the flower morphology between SI and SC plants and in the plant size between *SI* and *SC sectors*, and the long-lived perennial condition of this species (Chapter 2), suggest a stable and effective mixed mating system. Here, we have shown that the mixed mating system functions both at the individual level, with selfing and outcrossing potentially occurring simultaneously in SC/pSI plants after the visits of pollinators, and at the population level, given the high variability of the capacity of selfing among individuals. If so, the *SI sector* may present an outcrossing mating system and the *SC sector* a high or moderate inbreeding. The population would be therefore constituted of two spatially separated groups with different mating systems based on a genetic polymorphism, situation that is considered to be rare in plants (Stone, 2002).

4.3 The breakdown of the self-incompatibility in S. fragilis

Molecular studies support the origin of the clade *S. fragilis*-*S. pustulatus* from ancestral populations of *S. masguindalii* (Chapter 4). Hence, a strong SI system could be the ancestral condition of *S. fragilis*, probably only represented nowadays in the *SI sector* of population GHO1. This sector has a peripheral position within the whole geographical distribution of this species, as the *SC sector* is located between the *SI sector* and the remaining nearby populations (Chapter 2), which seem to be pSI or SC. Assuming an ancestral SI condition of *S. fragilis*, the origin of GHO1 sectors with such contrasted capacities of selfing might be related, for instance, with founder effects, with pSI or SC plants reaching the *SC sector* from the *SI sector*. Both sectors may have subsequently evolved independently as in a metapopulation structure, which has been proposed to favour the polymorphisms on the capacities of selfing (Good-Ávila *et al.*, 2008). Another hypothesis for the origin of the GHO1 sectors is that, the species being originally SI, possible strong past demographical constrictions less intensive in the *SI* sector might led to strong *S* Allee effects which could selected the SC plants. In addition, and despite of the relatively short spatial distance extant between sectors (approximately 500 meters), gene flow between them seems to be highly restricted. This flow would be especially mediated by pollen dispersal, since the seeds are relatively heavy weight and have a highly deciduous pappus. A moderate-high gene flow would be congruent with the presence of some SC plants in the *SI sector* but not with the lack of SI plants in the *SC sector*.

4.4 Historical demographic constrains and SSI evolutionary trends

The partial or complete breakdown of SI is virtually found across all sampled populations of the species of *Sonchus* section *Pustulati*, and it is not associated with small population sizes in the Moroccan distribution area as it may be expected under the frame of mating assurance (Reinartz & Les, 1994; Wagenius *et al.*, 2007). Historical contingencies which led to major demographical constrains may represent a scenario for the breakdown of SI (Mable & Adam, 2007; Hoebe *et al.*, 2009) that could be appropriate for the species of *Sonchus* section *Pustulati*. A serial of demographic, ecological and genetic features of these species described in the prior chapters (2-4) suggested an ancient origin and diversification, presumably relict condition, high ecological resilience and narrow ecological amplitude within a very restricted geographical distribution of the section *Pustulati*. The persistence of the Moroccan populations could have been reinforced through the partial or complete breakdown of SI, decreasing therefore the *S* Allee threshold, that is, the number of *S* alleles under which a population shows a decline through scarcity of potential mates. In the case of the Spanish populations of *S. pustulatus*, demographical contingences seem to be currently acting.

4.5 Implications of SSI in the demographic trends of the Spanish populations of S. pustulatus

The number of *S* alleles estimated by means of diallel crosses was low in the Spanish population ANT of *S. pustulatus* (5) and intermediate in the Moroccan populations TAL of *S. pustulatus* (12) and GHO1 of *S. fragilis* (15) in relation to those estimated in other species with SSI (range of the number of *S* alleles for other species 2.1–54; mean 16.8; Busch *et al.*, 2014). However, the low roughness of the model obtained for the population GHO1 of *S. fragilis*, and, to a lesser extent, for the population TAL of *S. pustulatus*, indicated that we only sampled a low proportion of their population *S* allele diversities, and higher number of *S* alleles should be expected across these populations.

Dominance interactions among *S* alleles were the common in the three populations sampled by means of diallels. These interactions are thought to lead to a higher number of compatible crosses within a population than in the case of common co-dominant interactions among *S* alleles (Hiscock & Tabah, 2003). In the latter case, any

S allele is unmasked and therefore is detected easier promoting more incompatibility reactions among individuals. When the number of *S* alleles is low, dominance interactions are expected to increase potential mates between individuals sharing recessive *S* alleles, so increase in dominance is predicted to be an evolutionary response to reduced *S* allele frequency (Byers & Meagher, 1992; Brennan *et al.*, 2003). Our results regarding the population ANT of *S. pustulatus* support this idea, which had not been empirically demonstrated yet for any narrow endemic taxa within the large Asteraceae family (but see Brennan *et al.*, 2013).

The presence of pSI and SC plants and the dominance interactions among *S* alleles may have been crucial for the certain demographically stability of the population ANT of *S. pustulatus* (Chapter 2), increasing the *S* Allee threshold. However, in the case of the populations CAB and TEL, which have been found to be in decline (Chapter 2), these compensatory strategies may have been non-sufficient. For instance, the population decline and fragmentation in TEL seem to be so advanced that its isolated small group of individuals probably remains due to their self-fertilisation ability (Figure 6b). The two populations (CAB and TEL) could have surpassed the *S* Allee threshold, although the main factor contributing to the declining could be another. Although the moderate fruit set in natural conditions detected across all studied populations (Table 4; Chapter 2) seems to indicate that they do not suffer from mate limitation (and therefore *S* Allee effects would be absent), the very low capacity of recruitment of the species (Chapter 2) would suppose that slight decreases in the fruit set (see TEL and CAB in Table 4), due for instance to a mate limitation, might be quite negative for the stability of the small populations.

Regardless of the main cause for the population declines, the loss of *S* allele diversity is one of the many density-dependent factors that appear into scene when populations decrease in size (Wagenius *et al.*, 2007). All the three Spanish populations of *S. pustulatus* showed signals of possible inbreeding depression in comparison with the Moroccan populations of the group regarding a serial of demographical, reproductive and genetic features (Table 4 and Chapters 2–4; and extended in Chapter 6). To accurately define in what extent the SSI systems result crucial in the last stages of population quasi-extinctions as well as in the first stages of re-colonization after strong bottleneck events, it would be necessary to provide fine empirical data about the level of inbreeding depression and so to satisfactorily understand these particular evolutionary processes (Goodwillie *et al.*, 2005; Winn *et al.*, 2011).

4.6 Conservation aspects

As habitat areas decrease and fragmentation increases, it becomes increasingly important to consider breeding systems when designing conservation plans. Here, we show that it would be convenient to carry out a genetic rescue among the genetically impoverished Spanish populations of *S. pustulatus* (Chapter 4); at least from the most demographically stable and genetically isolated population ANT to the declining ones, TEL and CAB (Chapters 2 and 4). In a metapopulation where individual populations have lost *S* allele diversity, interpopulation crosses should more often alleviate mate limitation compared to crosses between plants from the same population (Busch & Schoen, 2008). As gene flow among the three Spanish populations has been almost completely discarded (Chapter 4), it would be therefore convenient for their conservation to perform interpopulation crosses in field (e.g. Paschke *et al.*, 2002; Willi & Fischer, 2005; Pickup & Young, 2008); for instance, by means of hand massive pollinations among them.

On the other hand, due to the deficiency that these species show regarding the establishment of new individuals leading to low recruitment rates (Chapter 2) we suggest the use of cuttings for reintroductions on conservation programs rather than seed sowings. To this end, it would be also necessary use individuals with high capacity of selfing (both SC to pSI plants) since they are considered the most adequate for the recuperation of small patches of occupancy. In smaller and older fragments, predominantly SC/pSI plants will likely perform better than SI plants because of possible mate limitation and *S* Allee effect (Wagenius *et al.*, 2007).

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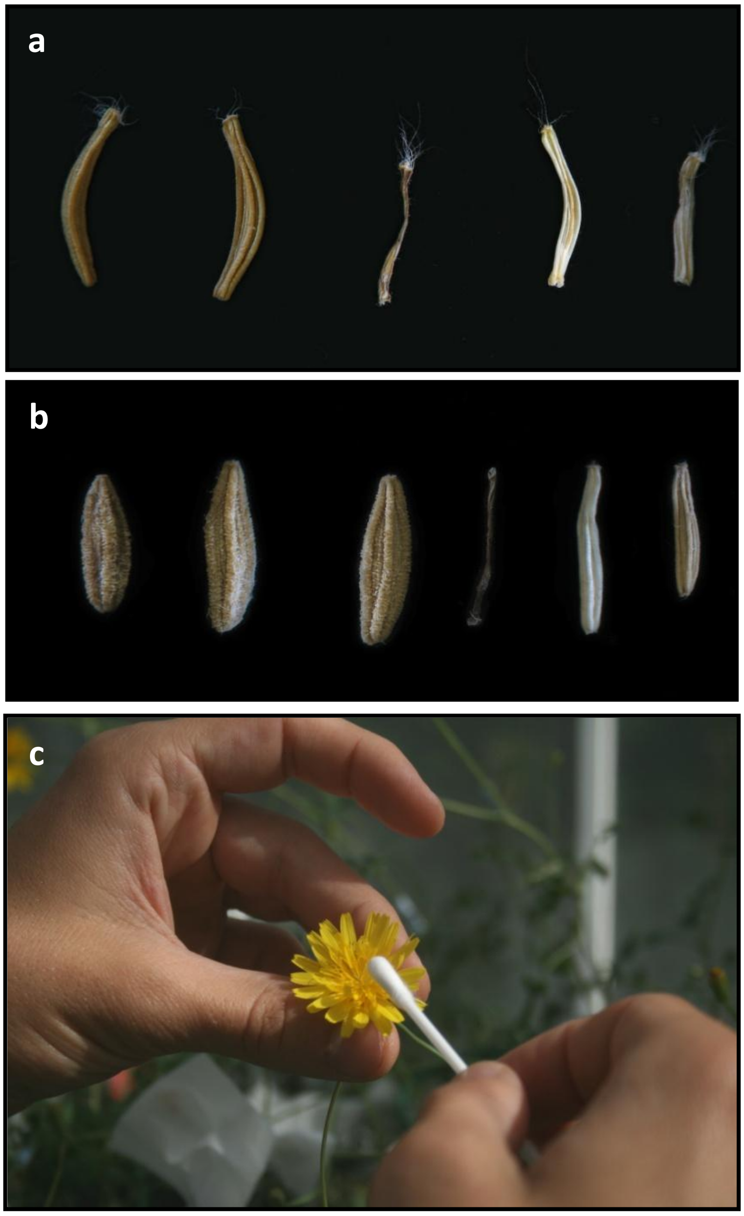
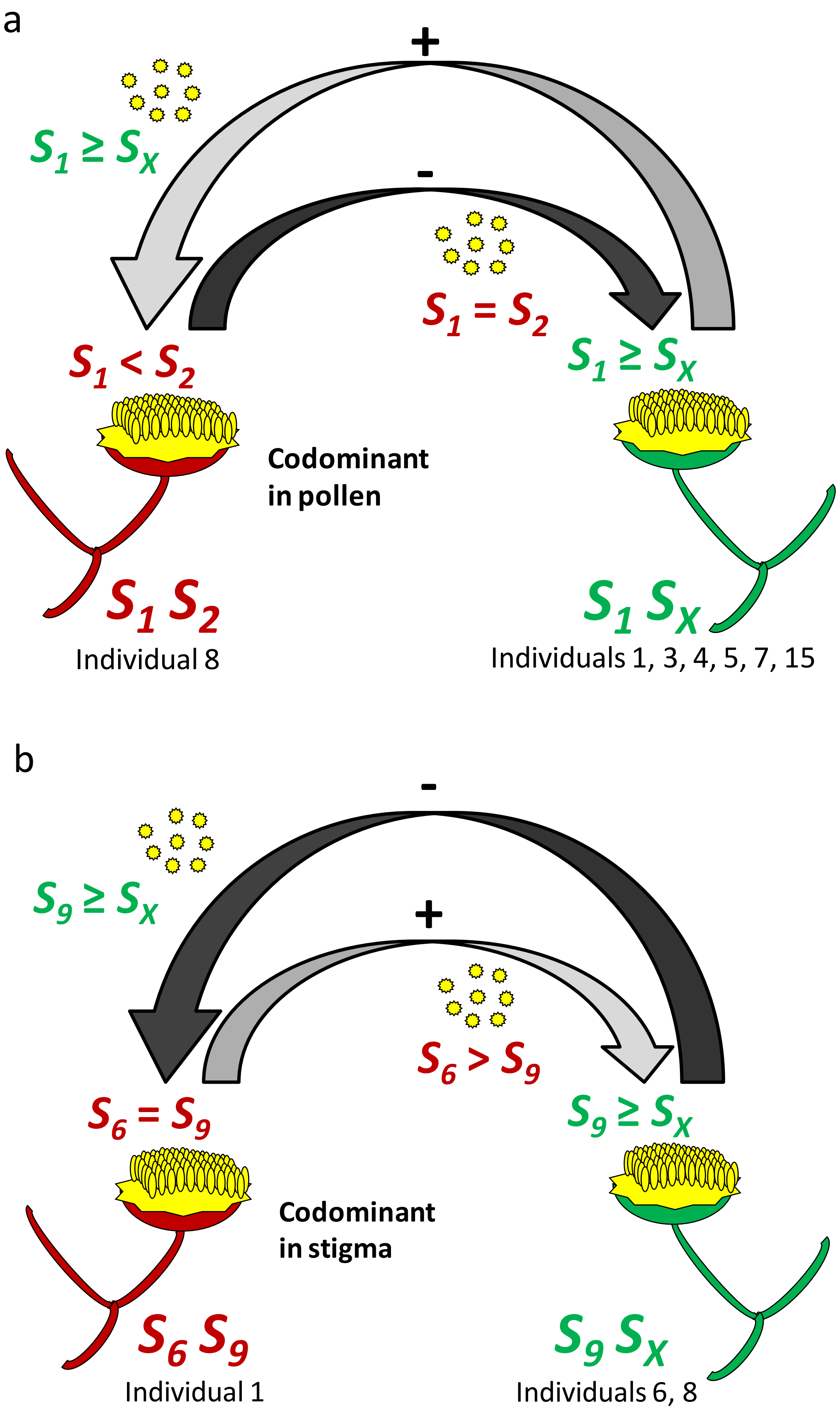
APPENDICES



Appendix: Figure 9 Photos of fertilized (left side) and unfertilized (right side) achenes from the Spanish *S. pustulatus* (a) and *S. fragilis* (b), and hand pollination using cotton swab (c).


a
+
-
$S_1 \geq S_X$
$S_1 = S_2$
$S_1 < S_2$
$S_1 \geq S_X$
Codominant in pollen
$S_1\ S_2$
Individual 8
$S_1\ S_X$
Individuals 1, 3, 4, 5, 7, 15
b
-
+
$S_9 \geq S_X$
$S_6 > S_9$
$S_6 = S_9$
$S_9 \geq S_X$
Codominant in stigma
$S_6\ S_9$
Individual 1
$S_9\ S_X$
Individuals 6, 8

Appendix: Figure 10 Different dominance and co-dominance relationships amog *S* alleles in the stigmas and the pollen grains of some individuals. Co-dominance of *S* alleles in the pollen grains of the individual 8 from the diallel crosses performed for the Spanish population ANT of *Sonchus pustulatus* (alleles *S1* and *S2*; a) and in the stigmas of individual 1 from the diallel completed for the Moroccan population TAL of *S. pustulatus* (alleles *S6* and *S9*; b). This SSI model explained a further 2.8% and 1.1% of the crossing results for each of these populations, respectively.

Capítulo 6:

DISCUSIÓN GENERAL

GENERAL DISCUSSION

En la presente Tesis Doctoral se han estudiado las características demográficas, ecológicas, filogenéticas y reproductivas de las poblaciones de los taxones que constituyen el grupo *Sonchus* L. subgénero *Sonchus* Boulos sección *Pustulati* Boulos, clado constituido por especies rupícolas endémicas de la región Bético-Rifeña. Los estudios han permitido: (i) desentrañar los patrones biogeográficos de estos taxones, aportando información valiosa para la reconstrucción de la biogeografía de la flora en dicha región, (ii) realizar un diagnóstico del estado de conservación de sus poblaciones y (iii) analizar los factores determinantes de su persistencia. Además (iv), se ha puesto de manifiesto la existencia de una población de *Sonchus fragilis* con un sistema sexual probablemente mixto. Esta especie supone un material excepcional para estudios futuros sobre la evolución de los sistemas de reproducción, facilitado por la presumiblemente alta esperanza de vida de estas plantas y a la estabilidad demográfica de sus poblaciones.

1. BIOGEOGRAFÍA DE LOS TAXONES DE *SONCHUS* SECCIÓN *PUSTULATI*

1.1 Origen y antigüedad de los taxones de Sonchus sección Pustulati

Una vez confirmada el carácter monofilético de la sección *Pustulati* del género *Sonchus* (Kim *et al.*, 2007 y 2008; Capítulo 4), se han podido datar fiablemente los períodos de origen y diversificación de sus taxones mediante un reloj molecular, y así inferir algunos aspectos sobre su biogeografía (Capítulo 4). Los resultados de la Tesis apuntan que el origen y diversificación de las especies de la sección *Pustulati* tuvieron lugar en el arco de Gibraltar durante la crisis del Messiniense (5.96–5.33 Ma) y la subsecuente reinundación Zancliense del mar Mediterráneo (5.33–3.60 Ma), en un período previo al establecimiento del clima Mediterráneo (3.2–2.8 Ma; Suc, 1984). Por lo tanto, la sección *Pustulati* es un grupo antiguo (aproximadamente 5.5 Ma), y sus componentes podrían representar paleoendemismos (Thompson, 2005). Dentro del género *Sonchus* en sentido amplio (Kim *et al.*, 2007), la sección *Pustulati* parece ser relativamente ancestral, superando la antigüedad de la alianza de los *Sonchus* leñosos macaronésicos (4.23 Ma; Capítulo 4) o a la radiación del antiguo género *Dendroseris* (actualmente *Sonchus* subgénero *Dendroseris* (D. Don) S.-C. Kim & Mejías; Mejías & Kim, 2012) de las islas de Juan Fernández y Desventuradas en el Pacífico suroccidental (3.3 Ma; Sang *et al.*, 1994).

La distribución geográfica actual de los taxones de *Sonchus* sección *Pustulati*, restringida a la Zona Interna Bético-Rifeña (Capítulo 1), su origen antiguo (Capítulo 4), su alta especificidad ecológica (Capítulo 3) y la presumible baja capacidad de dispersión sugieren que su historia evolutiva podría haber tenido lugar por completo en la región Bético-Rifeña. Con la información disponible, no es posible determinar si los antecesores de estas especies se encontraban en la microplaca Bético-Rifeña o en el norte de África de manera previa a su unión con el Rif (Capítulo 1). No obstante, la clara predominancia de las especies en el rango Rifeño (Capítulo 2) y la restringida distribución de la especie más antigua, *S. masguindalii* (Capítulo 4), en el Rif central, parecen sugerir que la diversificación de las especies de la sección *Pustulati* se dio en las áreas más meridionales (Rifeñas) del arco de Gibraltar durante las edades Messiniense y Zancliense (Capítulo 1; Figura 4c-f del Capítulo 4).

1.2 Condición relicta y refugio microclimático

Durante los períodos Messiniense y Zancliense (7.6 -3.6 Ma; Mioceno tardío-Plioceno temprano), intervalo de tiempo geológico en que se sitúa el origen de la sección *Pustulati*, el clima en la región Mediterránea fue generalmente más cálido y húmedo que el régimen mediterráneo típico actual (Mai, 1989; Thompson, 2005) el cual se estableció hace 3.4–2.8 Ma (Suc, 1984). Por tanto, las especies de la sección *Pustulati* pueden considerarse parte de la flora pre-Mediterránea (Herrera, 1992; Capítulo 4). La distribución geográfica tan restringida de estas especies, su presumible estabilidad demográfica (al menos en el rango Rifeño; Capítulo 2 y 5) y restricción ecológica actual (Capítulo 3) sugieren que estas han persistido hasta hoy en un ambiente de régimen climático mediterráneo gracias a una alta resiliencia mediado por el hábitat que colonizan.

Además de proporcionar protección contra perturbaciones de carácter antropogénico (Davis, 1951; Polunin, 1980; Albert *et al.*, 2001; Lavergne *et al.*, 2004; Larson *et al.*, 2005; Thompson, 2005; aunque ver Capítulo 2), los roquedos en los que se asientan las poblaciones del grupo *Pustulati* suponen refugios contra la fuerte estacionalidad del régimen Mediterráneo actual. La orientación norte, el sustrato rocoso, la proximidad al mar y la baja altitud de estos roquedos facilitan el mantenimiento de la humedad ambiental, tamponando así las altas temperaturas y minimizando el estrés hídrico, especialmente en primavera y otoño (Médail & Quézel, 1999). Durante los meses secos y calurosos del verano, las plantas de la sección *Pustulati* entran en letargo vegetativo mediante la lignificación de las ramas y la pérdida casi completa de las hojas (J.L. Silva, observaciones personales). La cercanía al mar y la baja altitud también ayudaron seguramente a amortiguar temperaturas frías durante las glaciaciones del Cuaternario (Thompson, 2005).

Es de suponer que los cañones profundos formados por la reincisión de los ríos tras la bajada del nivel del mar Mediterráneo a lo largo de toda su cuenca (Loget & Van Den Driessche, 2006) proporcionaron abundante disponibilidad de paredones rocosos durante el período Messiniense. Esto, junto con un clima presumiblemente más benigno para estas plantas, seguramente determinó que las especies de la sección *Pustulati* ocuparan áreas de distribución mucho más extensas dentro de la región Bético-Rifeña que en la actualidad. En tal caso, la reinundación progresiva del mar Mediterráneo en el período Zancliense, la cual fue cubriendo la zona que hoy día conocemos como mar de Alborán, habría dejado sumergida gran parte de las poblaciones de estas de especies. Además, el subsiguiente establecimiento del clima Mediterráneo, con su marcada estación cálida y seca, debió producir también una fuerte constricción en la distribución geográfica de estas especies. Estos dos últimos aspectos dan lugar a su condición de relictos.

En síntesis, el número reducido de poblaciones actuales de la sección *Pustulati* podría ser el resultado de una restricción geográfica y demográfica de un grupo de plantas que una vez prosperaron posiblemente en áreas más extensas y más conectadas entre sí que las actuales en los tiempos del Mioceno tardío-Plioceno temprano. Esta idea está apoyada por la actual baja capacidad de dispersión de sus especies y una tasa de reclutamiento muy reducida en sus poblaciones, así como por la alta especificidad de hábitat y poca abundancia de sitios con condiciones ambientales apropiadas para el establecimiento de nuevos individuos.

1.2.1 Origen de la distribución disyunta de Sonchus pustulatus

Uno de los aspectos más interesantes de la biogeografía de las especies estudiadas es la distribución disyunta, a ambos lados del mar de Alborán, de *Sonchus pustulatus*. En el Capítulo 4 de esta Tesis se propusieron como hipótesis alternativas para esta distribución un evento de dispersión a larga distancia frente a un proceso de vicarianza. Integrando todos los resultados obtenidos en la presente Tesis, se considera más congruente la hipótesis de un proceso vicariante.

El análisis filogenético basado en marcadores moleculares y el patrón biogeográfico actual, claramente asimétrico, de la sección *Pustulati* sugieren que *S. pustulatus* se originó en la zona Rifeña del arco de Gibraltar, y que desde aquí se expandió a la zona Bética (Capítulo 4). Esta expansión podría haberse producido antes (hipótesis de la vicarianza) o después (hipótesis de la dispersión a larga distancia) de la reinundación del mar Mediterráneo. La marcada diferenciación genética detectada en esta especie entre los rangos Bético y Rifeño (Capítulo 4) y su presumible reducida capacidad dispersiva se corresponde mejor con un proceso de vicarianza entre ambos rangos que con la ocurrencia de eventos de dispersión a larga distancia entre ellos a través de un mar de Alborán ya formado, durante el Cuaternario. Esta hipótesis de un proceso vicariante implica una expansión antigua del rango desde el suroeste (Rifeño) hacia el noreste (Bético) siguiendo un proceso de stepping-stone (Kimura, 1953) durante los tiempos del Messiniense tardío o Zancliense temprano. Hacia el final del periodo Zancliense, el rango de distribución de la especie debió restringirse por la reinundación del mar Mediterráneo la cual fue dejando los rangos Bético y Rifeño cada vez más alejados, hasta constituir una barrera efectiva para estas especies, resultando una distribución claramente disyunta.

Las diferencias morfológicas y fisiológicas observadas entre las plantas de *S. pustulatus* de ambos lados y la similitud ecológica (menos el régimen de precipitaciones) detectada entre las dos áreas apoyan la hipótesis de la vicarianza (Capítulo 3). Las plantas rifeñas tienen hojas menos suculentas y pinnas mayores y más agudas que las béticas, que presentan hojas más redondeadas (Apéndice: Figura 1a-b). A su vez, las plantas rifeñas muestran también mayores tasas de supervivencia y resistencia a infecciones fúngicas y raíces más desarrolladas, bajo las mismas condiciones de invernadero (J.L. Silva, observaciones personales). Igualmente, la alta especificidad de hábitat, la baja tasa de reclutamiento y un ciclo de vida considerablemente largo, características típicas de las especies rupícolas (Capítulos 2 y 3; Larson *et al.*, 2005), disminuyen las posibilidades de eventos exitosos de colonización. Además, la posibilidad de una diferenciación mediada por efecto fundador, a partir de unos pocos individuos que cruzasen el mar de Alborán hasta alcanzar el sureste de la Península Ibérica en tiempos geológicos más recientes, durante el Pleistoceno o el Holoceno, no resulta muy plausible teniendo en cuenta la presumible baja capacidad de dispersión de sus semillas. Dicha baja capacidad se basa en sus características morfológicas (Capítulo 1) y en la estructuración genética relativamente alta detectada entre las poblaciones (Capítulo 4).

Las diferencias en el régimen climático entre áreas tras el establecimiento del clima mediterráneo, con una mayor sequía en el rango Bético (Capítulo 3; Hijmans *et al.*, 2005), y la presumible mayor repercusión de las glaciaciones del Cuaternario en esta vertiente pudieron favorecer un proceso de diferenciación ecológica y posteriormente de diferenciación genética (Capítulo 4). Según esta hipótesis, la estimación de 1.2 Ma de edad estimada para la divergencia genética entre ambos rangos, ya en el Pleistoceno, respondería a la diferenciación genética acumulada desde la disyunción hacia el final del periodo Zancliense y la posterior diferenciación climática. Hasta la fecha no existen prácticamente estudios que sugieran un fenómeno de vicarianza en la región Bético-Rifeña en especies que combinen baja capacidad de dispersión con una distribución disyunta pero alejada del estrecho de Gibraltar en alguno de los rangos Bético o Rifeño*,* como es el caso de *S. pustulatus* (pero ver Caujapé-Castells & Jansen, 2003). Es en estos casos donde se puede poner claramente de manifiesto la efectividad de las barreras marinas para la expansión de las plantas.

Independientemente del mecanismo responsable de la distribución disyunta de esta especie, la tendencia climática del sureste de la Península Ibérica (Ruiz-Sinoga *et al.*, 2012) puede ser uno de los factores responsables de la fuerte restricción actual de la distribución de las poblaciones Béticas de *S. pustulatus*, promoviendo el declive demográfico y, a su vez, la baja diversidad genética neutral y en el locus *S* (Capítulos 2 y 5).

2. ESTADO DE CONSERVACIÓN DE LOS TAXONES DE *SONCHUS* SECCIÓN *PUSTULATI*

Los resultados de esta Tesis Doctoral indican que los taxones de *Sonchus* sección *Pustulati* constituyen rarezas históricas que encontraron un refugio microclimático en determinados roquedos de la Zona Interna de la región Bético-Rifeña tras la total reinundación del mar Mediterráneo y el posterior establecimiento del clima mediterráneo. Según se ha visto, tales refugios permiten una alta resiliencia demográfica. Las especies no parecen estar especialmente amenazadas en el rango Rifeño, más allá de la amenaza que en sí misma supone su rareza. Por el contrario, la situación en el rango Bético resulta ser más preocupante, ya que dos de sus tres únicas poblaciones, de *S. pustulatus*, parecen estar en declive (Capítulo 2).

2.1 Rango Rifeño

Los resultados obtenidos a lo largo de los capítulos anteriores sugieren una alta persistencia de las poblaciones estudiadas en el rango Rifeño (Tabla 1). Este rango es el más poblado, pues cuenta con 16 de las 19 poblaciones detectadas, y sólo tres poblaciones de *S. pustulatus* están presentes en el rango Bético. Además, en el rango Rifeño se detectaron extensiones de la ocurrencia y áreas de ocupación mayores para las tres especies, siendo las poblaciones más grandes y densas (Capítulo 2), y la especie correspondiente a la sección *Pustulati* siempre el taxón dominante dentro de sus conjuntos de plantas (Capítulo 3). Por otra parte, todas las poblaciones Rifeñas presentaron valores de fructificación en condiciones naturales relativamente altos (Capítulo 2), además de valores de diversidad y estructuración genética moderados-altos entre ellas (Capítulo 4). Por otro lado, la diversidad de alelos *S*, responsables de la auto-incompatibilidad, parece no ser limitante para el desarrollo de cruzamientos compatibles dentro de las poblaciones (Capítulo 5) los cuales aseguran un nivel elevado y suficiente de fructificación (Bush *et al.*, 2014). A pesar de ello, en todas las poblaciones se detectó la presencia en mayor o menor medida de individuos parcial o totalmente auto-compatibles, lo cual favorece la resiliencia en caso de limitación de cruzamientos.

No obstante, se debe tener en cuenta la fragilidad intrínseca de estos taxones restringidos demográficamente, pues determinadas contingencias pueden promover fácilmente su extinción, especialmente las perturbaciones de origen antropogénigo que actúen directamente sobre el hábitat. En este aspecto, las poblaciones rifeñas más vulnerables parecen ser las más orientales de *S. masguindalii*, aisladas y diferenciadas genéticamente dentro de la especie (poblaciones QUE y SFI; Capítulo 4), junto con las poblaciones de *S. fragilis*, las cuales en conjunto presentan una extensión de la ocurrencia muy limitada (Tabla 1; Capítulo 2). Ninguno de estos dos grupos de poblaciones está incluido en espacios protegidos, y en casi en todas estas poblaciones se detectaron ciertas perturbaciones debidas a factores de carácter antropogénico (Capítulo 2).

2.2 Rango Bético

A lo largo de esta Tesis se ha puesto de manifiesto un conjunto de síntomas de depresión por endogamia en las poblaciones béticas de *S. pustulatus* (Tabla 1). La posición periférica de estas poblaciones dentro de la distribución global de la sección *Pustulati*, obviando la posible presencia de *S. pustulatus* en el área argelina (Capítulo 1 y 2), podría estar acentuando su vulnerabilidad desde los puntos de vista demográfico, ecológico, genético y reproductivo (Capítulos 2–5). Esta mayor vulnerabilidad o disminución de su resiliencia podría deberse al cambio climático global, a variaciones en el régimen de precipitaciones en este rango (Ruiz-Sinoga *et al.*, 2012), y/o a algunas perturbaciones de carácter antropogénico en su hábitat (Capítulo 2).

Tabla 1 Resumen de los resultados obtenidos en la presente Tesis sobre el estado de conservación de los taxones de *Sonchus* sección *Pustulati*. E.O., extensión de la ocurrencia; A.O., área de ocupación; FR, nivel de fructificación.

	Rango Bético	Rango Rifeño		
	S. pustulatus	*S. pustulatus*	*S. fragilis*	*S. masguindalii*
DEMOGRAFÍA				
E.O. (Km^2)	7	≈240	16	≈100
A.O. (m^2)	≈3500	≈15000	≈40000	≈40000
Nº poblaciones	3	5	4	7
Nº individuos	≈1000	≈10000	≈34000	≈40000
Densidad (individuos/100m^2)	≈30	≈50	≈50	≈100
Éxito reproductivo				
- Pre-emergente:				
FR	0.56	0.74	0.75	0.68
Densidad semillas/100m^2	78.6	114.6	215.3	1864.6
- Post-emergente:				
Densidad plántulas/100m^2	0.8	5.6	5.7	5.4
Poblaciones en declive	Sí (2)	-	-	-
ECOLOGÍA				
Especie dominante	Otras	*S. pustulatus*	*S. fragilis*	*S. masguindalii*
GENÉTICA				
Diversidad genética (AFLP)	Baja	Moderada	Moderada	Moderada-alta
SISTEMAS DE REPRODUCCIÓN				
Diversidad de alelos *S*	Baja	Moderada-alta	Moderada-alta	-

2.3 Medidas de conservación

Dada la naturaleza dispersa de las poblaciones de *Sonchus* sección *Pustulati* y que muchas de ellas se encuentran en espacios no protegidos (Tabla 1 del Capítulo 2), el principal esfuerzo de conservación de estas poblaciones debería implicar la protección localizada de sus hábitats con el fin de evitar perturbaciones de carácter antropogénico. Para esta finalidad, el uso de microreservas ha constituido una herramienta esencial para la protección efectiva de la flora en algunas regiones de la región Mediterránea occidental (p. ej., Laguna *et al.*, 2004). Su ventaja es evidente en áreas de relieve complejo y paisaje muy diversificado, como la región Mediterránea, donde la sucesión de microclimas diferentes permite la coincidencia de comunidades vegetales independientes, con rasgos ecológicos bien contrastados, en espacios muy reducidos (Thomspon, 2005). El uso de microreservas resultaría especialmente interesante en las poblaciones Béticas de *S. pustulatus*, que son sin duda las más amenazadas, y en la población GHO1 de *S. fragilis*, en la cual la protección se extendería al número altísimo de taxones de plantas acompañantes (Capítulo 3). Por otro lado, esta labor se debería completar con la incorporación de semillas de cuantas poblaciones fuese posible a bancos de germoplasma.

La alta vulnerabilidad detectada de *S. pustulatus* en el rango Bético requiere que se tengan en cuenta más medidas de conservación sobre sus poblaciones. De hecho, la extinción parece inevitable en las poblaciones en declives demográficos del Barranco del Caballar (CAB) y el Faro de San Telmo (TEL) en caso de no actuar especialmente sobre ellas (Capítulo 2). Independintemente de su integración en microreservas, una buena parte de los esfuerzos de conservación debería dirigirse a la eliminación de los factores negativos de carácter antropogénico (Capítulo 2). Por un lado, sería deseable el vallado de la zona usada como vertedero de la población CAB, y por otro lado, deberían restringirse las zonas de acceso libre para personas y ganado en el entorno de la población TEL, ya que ésta se localiza en un espacio de fácil acceso y gran concurrencia. También, dado que en 2013 se detectó una gran concentración de vías de escaladas en el extremo sur de la población del Barranco de San Antonio (ANT), la única estable demográficamente del taxón en el rango Bético, habría que limitar el número y/o la localización de éstas, puesto que suponen una amenaza potencial a tener en cuenta para la futura conservación de la población (p. ej., Vogler & Reisch, 2011).

En el rango Bético, además de las medidas de conservación anteriores, deberían aplicarse algunas complementarias. Se propone el desarrollo de un rescate genético, especialmente para las poblaciones CAB y TEL (Capítulo 2 y 4). Este rescate podría llevarse a cabo mediante siembras controladas de semillas procedentes de la misma población durante el invierno o principios de primavera. Sería conveniente la selección de parentales maternos vigorosos con localizaciones diversas. Debido a las dificultades que tienen estas plantas para establecer nuevos individuos (Capítulo 2), se debería llevar a cabo un seguimiento y protección de la siembra hasta que los individuos alcanzaran la edad adulta, por ejemplo mediante una malla fijada al sustrato. Adicionalmente, el uso de esquejes podría ser utilizado para perpetuar individuos, introduciendo los plantones en enclaves con una disponibilidad de sustrato suficiente para su establecimiento.

En tales actuaciones (siembra de semillas o esquejes) sería recomendable la selección de individuos de la población con capacidad de autofecundación, aunque son poco frecuentes en *S. pustulatus*, con el fin de promover un nivel mínimo de fructificación que favorezca la resiliencia de las poblaciones (Capítulo 5). Las plantas auto-incompatibles son menos adecuadas que las auto-compatibles totales o parciales para la recuperación de poblaciones pequeñas o altamente fragmentadas dada su mayor susceptibilidad a una posible limitación de cruzamientos y al consecuente efecto Allee *S* (Capítulo 5; Wagenius *et al.*, 2007).

Como medida de refuerzo, el rescate genético podría también llevarse a cabo mediante un programa polinizaciones masivas manuales entre individuos de diferentes poblaciones (e.g. Paschke *et al.*, 2002; Willi & Fischer, 2005; Pickup & Young, 2008). Dado que las poblaciones parecen haber perdido diversidad de alelos *S*, responsables de la auto-incompatibilidad (Capítulo 5), los cruces entre individuos de poblaciones distintas deberían aliviar los problemas posibles de limitación de cruzamientos compatibles (Busch & Schoen, 2008).

3. PERSPECTIVAS TAXONÓMICAS

Los resultados ecológicos, filogenéticos y reproductivos (Capítulo 3–5) junto con las observaciones morfológicas y fisiológicas realizadas en el desarrollo de la presente Tesis ponen en evidencia algunas cuestiones taxonómicas de interés que, aunque colaterales al enfoque propio del trabajo, conviene reseñar.

Por un lado, *S. fragilis* y *S. pustulatus* están claramente segregados bajo un concepto genealógico de especie, a pesar de que *S. pustulatus* ha sido originado a partir de *S. fragilis* mediante transferencia horizontal de ADN cloroplástico (Kim *et al.*, 2008). Además, ambas especies presentan características ecológicas muy similares, particularmente en el rango Rifeño (Capítulo 3), donde la distancia geográfica más cercana entre las poblaciones de cada especie es únicamente de unos 20 Km. Por tanto, resultaría de interés analizar si tal segregación está mediada por factores de esterilidad entre clados (concepto biológico de especie) relacionados con la transferencia indicada, o por un proceso de especiación alopátrica como sugieren los bajos niveles de flujo génico estimados entre ambas áreas (Capítulo 4). El desarrollo de este objetivo podría poner de manifiesto el papel que la distribución espacial puede desarrollar en la diferenciación de los taxones rupícolas.

Por otro lado, la diferenciación genética, ecológica y fisiológica detectada entre los individuos de *S. pustulatus* procedentes de los rangos Bético y Rifeño (Capítulos 3–4) sugiere que sería interesante desarrollar un análisis taxonómico morfológico con el fin de promover su segregación en dos taxones diferentes, por ejemplo con categoría infraespecífica. Esta posibilidad también puede conllevar intereses conservacionistas, facilitando el reconocimiento administrativo de la conservación de la especie en ambos rangos.

Por último, dada la diferenciación genética detectada entre las poblaciones más orientales de *S. masguindalii* y el resto de ellas dentro de su área de distribución sería conveniente analizar su coincidencia con la diferenciación morfotípica propia del extremo occidental de la distribución de la especie, basada en la presencia de indumento lanoso y ausencia relativa de los pelos glandulares, propios de la forma típica (Apéndice: Figura 1c y f). Esta diferenciación pudo haber dado lugar al nombre *S. masguindalii* f. bocoianus Pau & Font Quer in Font Quer Iter maroccanum, 1929: 474, aunque esta designación constituye un *nomen nudum*, es decir, sin estatus científico. Además, en el presente trabajo, se han observado diferencias en la morfología de las pinnas, siendo más pedunculadas en la forma típica (Apéndice: Figura 1d, e y g).

4. CONSIDERACIÓN FINAL

Las especies de la sección *Pustulati* Boulos del género *Sonchus* L. constituyen muy probablemente un grupo de paleoendemismos relictos de origen pre-Mediterráneo restringidos a la región Bético-Rifeña, uno de los puntos calientes de biodiversidad en la región Mediterránea. Su presente distribución parece reflejar uno de los episodios geológicos y climáticos más llamativos de la región Mediterránea: la consecución de la virtual desecación del mar durante el Messiniense, su reinundación durante el Zancliense y el posterior establecimiento del clima mediterráneo. La persistencia de estas especies hasta nuestros días pone de manifiesto la importancia que tienen los hábitats rupícolas como refugio microclimático y antropogénico de no pocas especies antiguas, las cuales se caracterizan principalmente por su alta longevidad y estabilidad demográfica. Por otro lado, el conocimiento de su biología nos brinda nuevas e interesantes perspectivas para la investigación, en especial las relacionadas con la naturaleza de los sistemas sexuales mixtos y la evolución sexual en plantas. Es nuestras manos está el aprovechar su legado y conservar las piezas que quedan de un conjunto de plantas que encierran historias fascinantes.

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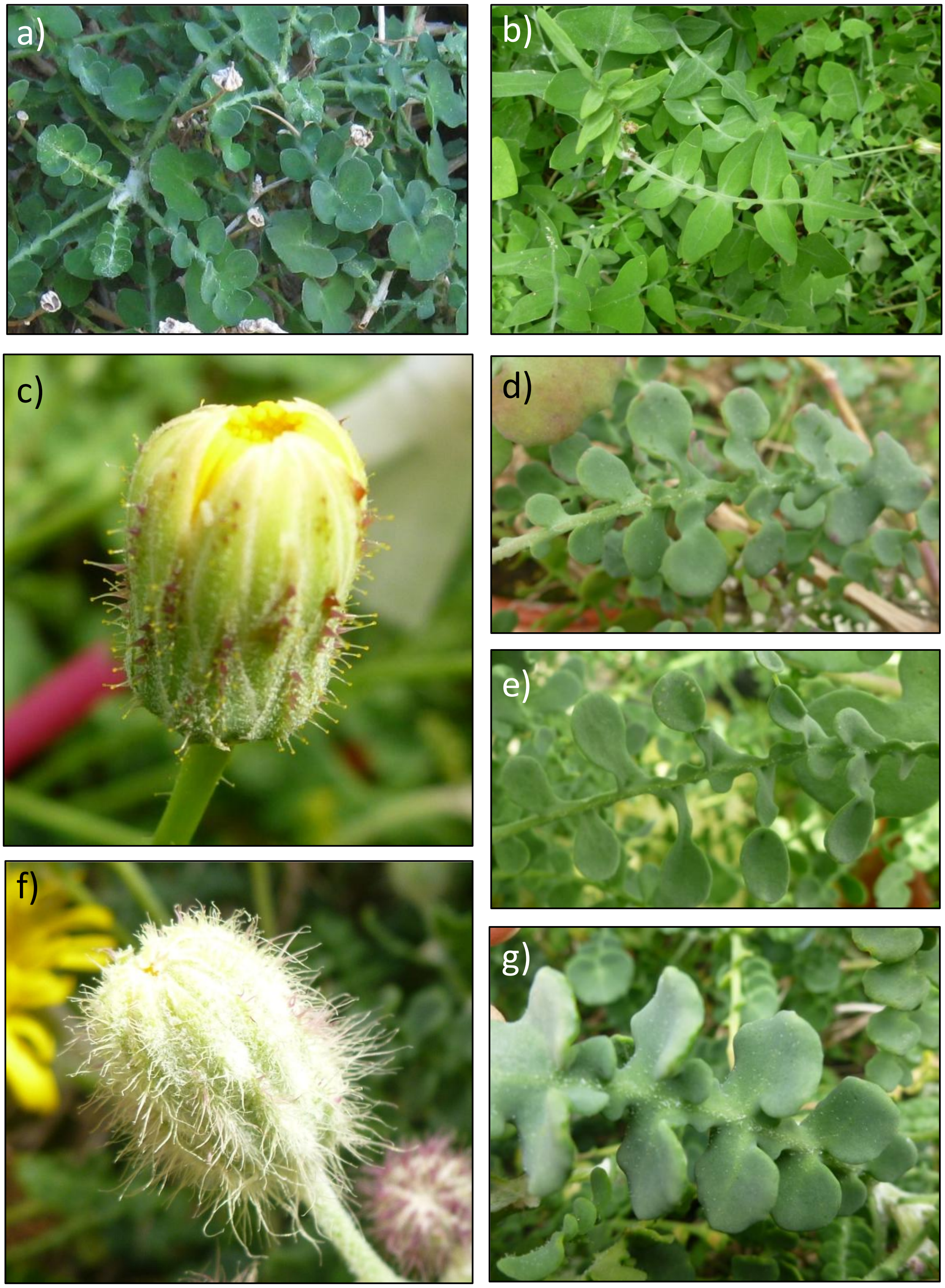
APÉNDICE



Apéndice: Figura 1 Detelles morfológicos foliares y de los capítulos de ejemplares de *S. pustulatus* de los rangos Bético (a) y Rifeño (b) y de *S. masguindalii* (c-e) y de la f. boccoianus de esta última (f-g), crecidos bajo las mismas condiciones de invernadero.

CONCLUSIONES GENERALES

GENERAL CONCLUSIONS

1. Las especies endémicas del género *Sonchus* L. sección *Pustulati* Boulos (*S. masguindalii* Pau & Font Quer, *S. fragilis* Ball y *S. pustulatus* Willk.) se encuentran restringidas a pequeñas áreas no superpuestas dentro la región Bético-Rifeña (sureste de la Península Ibérica y noroeste de África), siendo el rango Bético, ocupado por *S. pustulatus*, el de menores área de ocupación, tamaño y número de poblaciones.

2. Todas las poblaciones de la sección *Pustulati* muestran una estructura demográfica similar, con proporciones de plántulas y juveniles muy bajas, y predominancia de adultos bien establecidos con una esperanza de vida moderadamente alta. El reclutamiento es limitado debido a la estenoicidad de sus hábitats.

3. Las poblaciones béticas de *S. pustulatus* parecen sufrir cierto grado de depresión por endogamia, según indican el declive demográfico de dos de sus tres poblaciones y las densidades de individuos, valores de fructificación en campo y diversidades genéticas neutras y de alelos *S* (responsables de la auto-incompatibilidad) menores que las detectadas en las poblaciones rifeñas. A pesar de que *S. pustulatus* en el lado rifeño es el taxón con mayor amplitud ecológica dentro de la sección *Pustulati*, parece estar en el límite de su rango ecológico en el lado bético. El cambio climático global y el régimen de precipitaciones pronosticados en esta área pondrán presumiblemente en peligro la persistencia de *S. pustulatus* en el futuro cercano.

4. Los conjuntos de plantas asociadas a las especies de la sección *Pustulati* están formados por unas pocas especies específicamente rupícolas pero abundantes, y muchísimas especies propias de otros hábitats muy poco frecuentes, típicas de la vegetación circundante. Estos conjuntos mostraron proporciones similares en riqueza de especies por tipo corológico, siendo las mediterráneas las más representadas, seguidas de las ibéricas y/o norteafricanas. Por el contrario, las cosmopolitas e invasoras fueron casi inexistentes.

5. En cada una de las áreas de distribución de los taxones de *Sonchus* sección *Pustulati*, especialmente en el caso de *S. masguindalii*, se detectó una estructuración genética relativamente alta, lo que sugiere un flujo génico entre poblaciones muy restringido. La baja tasa de reclutamiento, la presumible estabilidad demográfica y alta resiliencia de estas plantas sugieren que los eventos de colonización y/o extinción poblacionales no deben ser recurrentes, y por lo tanto, no se considera que las poblaciones formen un sistema real de metapoblaciones en ninguna de las áreas.

6. El origen y diversificación de las especies de *Sonchus* sección *Pustulati* (5.5–3.89 Ma) parecen haber tenido lugar en el arco de Gibraltar durante la crisis del Messiniense (5.96–5.33 Ma) y la subsecuente reinundación progresiva del mar Mediterráneo de la edad Zancliense (5.33–3.60 Ma), de manera previa al establecimiento del clima mediterráneo (3.2–2.8 Ma). Por lo tanto, estas especies se consideran parte de la flora pre-Mediterránea y, dentro del género *Sonchus* en sentido amplio, la sección *Pustulati* parece tener una posición relativamente ancestral.

7. Las especies de la sección *Pustulati* se consideran relictas de finales del Terciario. Las similitudes ecológicas detectadas entre los hábitats de las poblaciones actuales apoyan la hipótesis de que estas especies debieron ocupar áreas más extensas y conectadas entre sí en el arco de Gibraltar durante los tiempos del Mioceno tardío-Plioceno temprano, cuando el clima era suave y más húmedo en la región. La reinundación Zancliense habría ido sumergiendo gran parte de las poblaciones de estas especies, especialmente de *S. pustulatus*. El subsiguiente establecimiento del clima mediterráneo debió suponer también una fuerte restricción en la distribución geográfica de las especies.

8. Estas especies han sido lo suficientemente resilientes frente al clima mediterráneo desde su establecimiento y a las glaciaciones del Cuaternario persistiendo refugiadas en roquedos de orientación predominantemente norte, próximos al mar y de baja altitud. Estos sitios mantienen niveles suficientes de humedad ambiental, lo cual ayuda a tamponar las temperaturas extremas altas y bajas propias del clima mediterráneo y de las glaciaciones del Cuaternario, respectivamente.

9. Entre las características de estas especies que les hacen resilientes en su hábitat destacan la esperanza de vida relativamente alta, la presumiblemente alta estabilidad demográfica, la polinización entomófila no especialista y la plasticidad en la respuesta de auto-incompatibilidad. Esta plasticidad permite a las poblaciones ser menos susceptible al efecto Allee cuando éste es provocado, por ejemplo, por una baja diversidad de alelos *S*.

10. Los cruces dialélicos realizados confirman el control genético esporofítico de la auto-incompatibilidad en el grupo, ya que la gran mayoría de los resultados pudieron ser explicados mediante relaciones de dominancia o, en menor medida, de codominancia entre alelos *S*, de acuerdo a un modelo esporofítico. La diversidad de alelos *S* a nivel poblacional es bastante pobre en el rango Bético, mientras que alcanza un número elevado en el rango Rifeño.

11. La posible presencia de poblaciones, particularmente de *S. fragilis*, con sistemas sexuales mixtos se pone de manifiesto mediante i) la diferenciación detectada en la capacidad de autofertilizarse entre individuos dentro de las poblacionales y ii) la dependencia general de polinizadores que tienen estas plantas para ser autofertilizadas. Una población de *S. fragilis* extensamente muestreada consituyó el caso más evidente, ya que la predominancia detectada tanto de individuos altamente auto-incompatibles como auto-compatibles se correspondió con una agregación espacial significativa y diferenciaciones en la morfología floral y *fitness*.

1. The endemic species of the genus *Sonchus* L. section *Pustulati* Boulos (*S. masguindalii*, *S. fragilis* and *S. pustulatus*) are restricted to small non-overlapped areas within the Baetic-Rifan region (southeastern of the Iberian Peninsula and northwestern Africa), the Baetic range, occupied by *S. pustulatus*, showing the smallest areas of occupancy, size and number of populations.

2. All populations of the section *Pustulati* show a similar demographical structure, with very low proportions of seedlings and juveniles, and predominance of well established adults which have a moderately high lifespan. The recruitment is limited due to the stenoicity of their habitats.

3. The Baetic populations of *S. pustulatus* seem to suffer certain degree of inbreeding depression, as indicated by the demographical decline of two of its three populations and the individual density, field fructification values and neutral and *S* allele (responsible for the self-incompatibility) genetic diversities, which are lower than the detected in the Rifan populations. Despite of *S. pustulatus* in the Rifan side shows the greater ecological amplitude within the *Pustulati* section, this seems to be on the limit of its ecological range in the Baetic side. The climate warming and precipitation regime forecasted in this area will presumably jeopardize the persistence of *S. pustulatus* in the near future.

4. The plant assemblages associated to the species of the section *Pustulati* are constituted by a few specifically cliff-dwelling species but with high frequencies and many non-cliff-dwelling species but with very low frequencies, typical from the surrounding vegetation. These plant assemblages show similar proportions of species richness by chorological type, being the Mediterranean plants the most represented, followed by the Iberian and/or North African ones. Conversely, there are hardly cosmopolitan and invasive plants.

5. In each one of the distribution areas of the taxa of *Sonchus* section *Pustulati*, especially in the case of *S. masguindalii*, it was detected a relatively high genetic structure that suggests a very restricted gene flow among populations. The low recruitment rate, the presumably demographical stability and the high resilience of these plants suggest that neither population extinctions nor colonization events should be recurrent, and it is not therefore considered that populations constitute an actual system of metapopulations in any of the areas.

6. The origin and diversification of the species of *Sonchus* section *Pustulati* (5.5–3.89 Ma) appear to have occurred in the Gibraltar arc during the Messinian Salinity Crisis (5.96–5.33 Ma) and the subsequent progressive reflooding of the Mediterranean sea of the Zanclean age (5.33–3.60 Ma), previously to the establishment of the Mediterranean climate (3.2–2.8 Ma). These species are therefore considered part of the pre-Mediterranean flora and, within the genus *Sonchus s.l.*, the section *Pustulati* appears to have a quite ancestral position.

7. The species of the section *Pustulati* are considered relict of the late Tertiary. The ecological affinities detected among the habitats of the current populations support the hypothesis that these species may have occupied greater areas or more connected among them in the Gibraltar arc in the late Miocene-early Pliocene times, when the climate was mild and wetter. The Zanclean reflooding would have been leaving submerged much of the populations of these species, especially of *S. pustulatus*. The subsequent establishment of the Mediterranean climate should have also supposed a strong restriction in the geographical distribution of the species.

8. These species have been sufficiently resilient to the Mediterranean climate from its establishment and to the Quaternary glaciations, taking refuge in cliffs with predominantly north orientation, low altitude and close to the sea. These sites maintain enough level of air humidity which helps to buffer the extreme high and low temperatures, characteristic of the Mediterranean climate and the Quaternary glaciations, respectively.

9. Among the characteristics of these species that make them resilient in its habitat highlight the relatively high lifespan, the presumably high demographic stability, the non-specialist entomophilous pollination and the plasticity in the self-incompatibility response. Such plasticity allows populations to be less susceptible to the Allee effect when this is caused, for instance, by a low diversity of *S* alleles.

10. The results of the diallel crosses confirmed the sporophytic genetic control of the self-incompatibility in the group, since most of them could be explained by dominance or, to a lesser extent, codominance interactions between *S* alleles, according to a sporophytic model. The population diversity of *S* alleles is quite poor in the Baetic range while it reaches a high number in the Rifan range.

11. The possible presence of populations, particularly of *S. fragilis*, with mixed mating systems has been highligthed by i) the differentiation in the selfing capacity detected among individuals within populations and ii) the general dependence on pollinators that these plants have to be self-fertilized. An extensively sampled population of *S. fragilis* constituted the most obvious case of mixed mating systems because the predominance detected both of highly self-incompatible and self-compatible individuals was corresponded to a significant spatial aggregation and to differentiations in the floral morphology and *fitness*.

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