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Evolution and conservation genetics of an insular hemiparasitic plant lineage at the limit of survival: the case of *Thesium* sect. *Kunkeliella* in the Canary Islands

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Abstract

Premise: The diversification of island flora has been widely studied. However, the role of environmental niches in insular radiation processes has been less investigated. We combined population genetic analyses with species distribution modelling to clarify the genetic relationships, diversification patterns, species niche requirements, and conservation of *Thesium* sect. *Kunkeliella*, a clade of rare hemiparasitic plants endemic to the Canaries.

Methods: We studied the three extant *Thesium* species and a new taxon from La Palma Island. We developed 12 microsatellites and performed population genetic analysis and studied the demographic history of the group. To evaluate the role of niche conservatism in the diversification of the group, we performed species distribution modelling (ESM) with four algorithms.

Results: All species presented moderate genetic diversity values for rare endemics. *Thesium canariense* (Gran Canaria) showed high differentiation, whereas *T. subsucculentum*, *T. retamoides* (Tenerife), and La Palma populations are closely related. The lineage may have undergone a recent diversification with colonization proceeding east to west, with *T. canariense* as sister to the others. We detected a climatic niche shift, as taxa showed different distributions across the temperature gradient. There is enough evidence to describe La Palma populations as a new species.

Conclusions: We characterized the evolutionary history of *Thesium* sect. *Kunkeliella* by integrating genetic and ecological assessments. Our results indicate that this clade has undergone a recent radiation process with niche differentiation among species. The results increase our knowledge about insular radiations and will inform the conservation management of the study species.

KEYWORDS

endangered species, endemic, insular radiation, microsatellites, niche conservatism, Santalaceae, species distribution modeling

Island biogeography has become a stand-alone scientific discipline, in part because isolation provides a useful context for studying speciation and radiation (Warren et al., 2015). Range disjunction for species distributed across islands has facilitated the emergence and diversification of distinct lineages; this allopatric speciation has led to high shares of endemic biota, even for islands of small area (Veron et al., 2019). In these cases, lineages find opportunities to diversify not only across territories within islands, but also across them, as oceanic boundaries act as barriers for gene flow. Thus, allopatry usually leads to non-adaptive radiations with little ecological differentiation due to the absence of competition (Rundell and Price, 2009). On the other hand, adaptive radiation and ecological specialization in sympatry has also played an important role in insular diversification, especially in young lineages (Whittaker and Fernández-Palacios, 2007). In this sense, it has been recently argued that genome-wide adaptive evolution with positive selection is much more common in rapid evolutionary radiations than in more slowly diversifying lineages (Nevado et al., 2019).

Many studies have documented these radiations within archipelagos (Stuessy et al., 2006; García-Verdugo et al., 2014; Simões et al., 2016), but the underlying drivers of species diversification within and across islands remain less well-studied. Niche conservatism in oceanic islands for narrow-ranged species, and even newly discovered species, is even less well understood (García-Verdugo et al., 2014; Steinbauer et al., 2016). The study of ecological niches is especially relevant for oceanic archipelagos because several of their characteristics make niche conservatism crucial to explaining many radiations, while at the same time presenting a challenging scientific question. First, oceanic islands tend to be recent, and so will be the speciation events (Caujapé-Castells et al., 2017). Second, relatively small island sizes imply narrower species distributions and thus higher probabilities of overlapping ranges. Finally, habitat heterogeneity can offer opportunities for niche diversification between taxa. Given this context, competitive displacement can be a driver of sympatric speciation. However, in contrast to the competitive displacement hypothesis and niche conservatism for closely related species, Steinbauer et al. (2016) and Albaladejo et al. (2020) found high niche differentiation and lability to be greater for allopatric sister species than sympatric species for some radiating plant groups in the Canary Islands.

Island organisms present inherent characteristic, like small population sizes or the inability to shift environments, that make them particularly vulnerable to anthropogenic threats (Pouteau and Birnbaum, 2016). Therefore, work to understand habitat suitability and genetically characterize these species is crucial to the efficacy of conservation plans (Rodríguez-Rodríguez et al., 2018a; Albaladejo et al., 2020). Moreover, such work ties into the broader idea of protecting island biodiversity because knowledge about ecological or genetic differentiation, potentially quite subtle, can be the basis for recognizing new endemic species in need of protection through conservation (Crawford and Stuessy, 2016).

The Canarian Archipelago, with eight main islands, is located in the Macaronesian Biogeographic region, off the northwestern coast of Africa. The archipelago is part of the Mediterranean biodiversity hotspot (Medail and Quezel, 1997), with many endemic species. In fact, 26% of the Canarian flora is threatened, with a high density of threatened species per unit area (Moreno-Saiz et al., 2015). The Canarian archipelago has been a frequent subject of studies concerning insular radiation and speciation in vascular plants (Sosa et al., 2013; Jones et al., 2014; Vitales et al., 2014). The Canary Islands harbor a very rich flora, with over 680 endemic taxa (Santos-Guerra, 2001), including species and subspecies, with many single island endemics and 22 endemic genera (Whittaker and Fernández-Palacios, 2007). This remarkable diversity likely stems in part from the high degree of isolation between islands, close proximity to the mainland, strong altitudinal gradients, and topographic complexity (Francisco-Ortega et al., 2000; Caujapé-Castells et al., 2017).

Thesium sect. *Kunkeliella* is endemic to the Canary Islands. Belonging to the Santalaceae family, it comprises four described species: *T. canariense* on Gran Canaria Island; *T. subsucculentum*, *T. retamoides*, and *T. psilotocladum* on Tenerife Island (Bañares-Baudet et al., 2004). *Thesium psilotocladum,* which has only been found in the Teno massif on Western Tenerife, is extinct (Martín-Cáceres et al., 2004, 2011) and was not included in this study. All the species are root hemiparasitic, like many other species in Santalaceae (Ascaso and Pedrol, 2002), but the hosts plants of the Canarian *Thesium* have not been well identified yet. However, field observations and greenhouse experiments suggest that they are very generalist and can parasitize a wide range of companion species (P. L. Pérez de Paz, La Laguna University, personal communication). This broad host range is expected as there is evidence that other *Thesium* species parasitize most of the companion species in their habitat (Dostálek and Münzbergová, 2010).

Unexpectedly, some populations were discovered in June 2011 in La Palma Island, in la Caldera de Taburiente National Park, at higher altitudes than the other species. Its distinctive morphological features indicated that it could be treated as a new taxon. Moreover, its small population sizes with the fact that there are no other *Thesium* species in La Palma are enough reasons to determine the genetic relationships of those populations with the other Canarian *Thesium* species. Only *T. subsucculentum* has been studied in more detail from a conservation genetics point of view (González-Pérez et al., 2013).

In this study, we used molecular markers and coarse-resolution ecological data to analyze the population genetics and demographic history of the extant species and the newly discovered populations, characterize ecological niche differentiation, and assess the implications of genetic diversity for conservation measures in the clade. Our aims in this study were to (1) characterize the genetic diversity and structure of the *Thesium* sect. *Kunkeliella* populations and their genetic relationships, (2) determine the taxonomic status of the newly discovered populations in La Palma, (3) to ascertain the ecological niche differences among the species to test the niche conservatism of the lineage, and (4) to assess the conservation and restoration needs of the clade.

MATERIALS AND METHODS

Study species and populations

Thesium canariense (W. T. Stearn) F. Forest & J.C. Manning, *Bhotalia* 43 (2): 216 (2013). (=*Kunkeliella canariensis* W. T. Stearn in Cuaderno Botanica Canariensis 16: 18 [1972]).

Thesium canariense is a small broomy shrub, highly branched, with glabrous branches, the lower ones woody, and tiny leaves, reduced to triangular scaly bracts, 2 mm long. Very small flowers with two opposing bracteoles at the base, are greenish-cream in color with five acute triangular lobes. Globose drupeous fruit, crowned by persistent perigonium (Table 1). *Thesium canariense* differs from *T. retamoides* and *T. psilotocladum* by the small size of its flowers and by its glabrous branches and fruits, and from *T. subsucculentum* by the succulent branches of the latter and its larger flowers. It is an endemic species of Gran Canaria with one known population in the Barranco de Guayadeque between 500 and 700 m a.s.l.; it has not been found in any other locality to date (Stearn, 1972; Martín-Cáceres et al., 2004). It is gynodioecious; it has two types of flowers, hermaphroditic flowers that mainly produce pollen and female flowers that produce fruits but not pollen (Pérez de Paz et al., 2015). Seeds are possibly dispersed by birds or by barochory (Martín-Cáceres et al., 2004). It is found in rocky soils in arid areas of the thermophilus woodland forests. It is considered as "Critically Endangered" (CR B2ab) in the *Red List of Spanish Vascular Flora* (Moreno-Saiz, 2008).

Thesium psilotocladum Svent. in Additamentum ad Floram Canariensem 1: 5 (1960). (=*Kunkeliella psilotoclada* (Svent.) W. T. Stearn: 20 [1972]).

Thesium psilotocladum was the first species discovered in the Canary Islands, described as *Thesium psilotocladum* (Sventenius, 1960) and transferred to the genus *Kunkeliella* by Stearn (Stearn, 1972). Very branched, small shrub, erect, with woody stems at the base and setulosus branches, olive green and squamiform leaves. Inflorescence with generally 1–3 flowers, subsessile, tribracteate; bracts triangular subulate, ciliated. Pentameric flowers, the lateral ones sterile, the median fertile, shortly pedicellate; stamens half the size of the tepal; filament dilated at the base, stigma truncated. Fertile sessile flower, bibracteate; fairly prominent disc; ovary ovoid-cylindrical, style thickened, as long as stamens; discoid stigma, diffusely trilaciniate. Fruit and seed unknown to Sventenius. Oviform to elliptical fruit, light green, shiny (Ludwig, 1984) (Table 1). Collected for the first time by Sventenius on 1 March 1948 in Masca at 800 m a.s.l.; it was not found again until March 1983, when two specimens were located at the locus classicus: "*K. psilotoclada* was found again by me in March 83, at the locus classicus. This species had disappeared years ago, and after intensive searches only two specimens were found" (Ludwig, 1984). Later in an expedition organized by the Department of Botany of the University of La Laguna in the company of Ludwig and Metlesic, they did not find any specimen, but herbarium testimony was collected, currently deposited in the TFC Herbarium of the University of La Laguna. "Masca, at the foot of the last cliff, 975 m s.m.", EBT, WW, ORD, SS, Metlesic & Ludwig, 5.XII.83. (TFC 13,172). "Altos de Masca, at 950 m s.m. and in SW orientation. Sventenius cites it at an elevation of 800 m, which suggests that it may be present along that altitude difference. However, the inaccessibility of the land has prevented us from testing such a hypothesis" (E. Beltrán in Gómez Campo et al., 1996). Holotype deposited in the ORT Herbarium of the Botanical Garden of La Orotava. Tenerife, Masca, 800 m, 1 March 1948 (ORT 2,949). Other herbarium testimonies deposited in ORT are: Masca *loc. class.*

TABLE 1 Morphological characteristics of the *Thesium* sect. *Kunkeliella* taxa

	T. canariense	*T. psilotocladum*	*T. retamoides*	*T. subsucculentum*	*T. palmense*
Size/Habit	Small shrub up to 80 cm	Small shrub of 60–100 cm	Large shrub up to 200 cm	Small shrub up to 80 cm	Large shrub up to 150 cm
Stems	Very branched, glabrous	Very branched, setulous	Branching abundant with pruinous and tomentose stems	Dense and divergent branching, succulent, setulous branches	Dense branching, divaricate, subsucculent, striated and puberulent
Leaves	Squamous triangular, 2 mm long	Squamiform of 1–1.5 mm. linear-subulate with ciliated margin; adaxial side puberulous	Squamiform. triangular-subulate, apex more or less acute	Fallen, 1.5 mm, subtriangular, with an acute apex, squamiform	Linear-subulate, 1–1.5 mm
Flowers	Pedicellate with two opposite bracteoles at the base, hermaphrodite, 2–3 mm diameter	Sessile, the lateral ones sterile and the median fertile, 3–4 mm, prominent disc	Shortly pedicellate, the lateral ones sterile, the middle fertile, 3–4 mm, disc not prominent	Sessile, 2–2.5 mm diameter, hermaphrodite; disc not prominent	Subsessile, 3.5–4 mm diameter, tiny subacute bracteoles, hermaphrodite, disc not prominent
Perigonium lobes (tepals)	Triangular, acute	Ovate-deltoid with obtuse apex	Acute	Triangular, setulous on the outside	Deltoid, subacute, greenish and puberulent on the outside, cream internal face
Fruit	Globose, 3–6 mm, whitish at maturity, topped by persistent perigonium	Oviform to elliptical, light green, setulous, 3–5 × 2.5–4 mm	Setulous, 6–7 × 5–6 mm	Globose, setulous, about 6 mm, Surmounted by remains of the persistent perigonium	Globose about 6 mm diameter, pearly, subpuberulent, crowned by persistent ocher stigmata

January 15, 1963, TFC s.n.; Masca November 1976. TFC 24,846. The species is currently catalogued as "Ex" (extinct) (Moreno-Saiz, 2008). "Species last seen in 1983; since then repeated searches of classical localities have been unsuccessful. The uncontrolled cattle seems to be the cause of its extinction" (Martín Cáceres et al., 2004).

Thesium retamoides (A. Santos) F. Forest & J. C. Manning, *Bhotalia* 43 (2): 216 (2013). (=*Kunkeliella retamoides* A. Santos, Anales del Jardín Botánico de Madrid 51: 145 [1993]).

Thesium retamoides is a relatively large shrub, with abundant ramifications, old branches and the entire plant pruinous; acute perigonial lobes, disc not prominent. It differs from *K. canariense* by its larger size, tomentosity, acute bracts, shape of the perigonial lobes, longer stigma subtrilobed and not capitate, and by its somewhat larger fruits (Table 1). Located for the first time in 1969, on the "Ladera de Güímar" (Santos, 1993). Later it was located in Las Hiedras, in the upper part of the Tamadaya ravine (Arico): Barranco de las Hiedras. Arico, Tenerife, 16.V.1992. V. Voggenreiter. (TFMC 3.427) and in the Barranco del Río (Arico): Barranco del Río. Lomo largo, 1400 m s.m., Tenerife. 1.IX.1994. R. Mesa, C. Samarín & A. Vera. (TFMC 3.997 + duplicate). In 1999, it was located above Vilaflor, in the Cuervo ravine: Barranco del Cuervo Vilaflor. 1600 m s.m., 4.IV.1999. R. Mesa & J.P. Oval (TFMC 4,484). Finally, a new population nucleus was located in the escarpment of Salto Tonásaro, in the Barranco de Erques: Barranco de Herques Sic.! Guia de Isora. 7.III.1999 R. Mesa & J.P. Oval (TFMC 4,482). Another herbarium testimony was collected at the same site: Barranco de Erques. Guia de Isora. 10.III.1999 R. Mesa & C. Massanet (TFMC 4,483). These two new populations are unpublished and extend the distribution of the species to the southwest slope of the island of Tenerife. The population of Ladera de Güímar is the largest and best preserved. In Tamadaya, it has recently suffered a drastic decline as a result of predation by mouflon. The other three populations have fewer individuals. It is also hermaphroditic, with myrmecophilous pollination, and the seeds are dispersed by barochory (Mesa-Coello et al., 2004). It is included in the category "Endangered (EN B2)" in *Red List of Spanish Vascular Flora* (Moreno-Saiz, 2008).

Thesium subsucculentum (Kämmer) F. Forest & J. C. Manning, *Bhotalia* 43 (2): 216 (2013). (=*Kunkeliella subsucculenta Kämmer*. Cuadernos de Botánica Canaria 23–24: 72 [1975]).

Thesium subsucculentum is a small succulent shrub, with a broom-like appearance, with stems and branches without apparent leaves. Woody stems at the base, with cracked bark, grayish in color. Succulent, setulosus branches, the juveniles glaucous green, the old branches thickened, greenish yellow. Very small leaves, reduced to drooping scales, squamiform, setulous on the margin. Small flowers, stellate, yellowish-greenish-white, with very short pedicels, with five small, triangular floral parts, setulous on the outside, hermaphroditic. Globose fruit, setulous, small, fleshy (drupaceous), crowned by remains of a persistent perigonium, with a single seed (Table 1). It presents myrmecophilous pollination and zoochorus dispersal (Valido, 1990). It is distributed along the northern coastal area of Tenerife between Icod and La Guancha where the two main population centers are located: Costa from Santo Domingo and El Frontón. The population numbers have been in a continuous decline from the approximately 10,000 specimens estimated by Kämmer and Vogenreiter in 1971 to 500 specimens in 2016. The species was mainly affected by an extraction of aggregates that destroyed part of the habitat and fragmented the population in the two nuclei that are currently known. The ongoing decline in numbers today is attributed to low recruitment as a result of droughts and predation by herbivores, mainly rabbits (Mesa-Coello, 2016). Due to its restricted area, it has been catalogued as "Critically Endangered" by the IUCN (Barrera-Acosta et al., 2011).

The populations from La Palma are located in the Caldera de Taburiente National Park on La Palma Island. The first locality to be discovered was Los Calzones Rotos (LP-CR), and later, two more localities were found, Hoyo Verde (LP-HV) and Los Breñuscos (LP-BR), with a total of 60–65 individuals. The individuals are perennial shrubs up to 1.5 m, with lignified stems and abundant branching, of ascending growth with hermaphroditic flowers. Material from the natural populations, herbarium specimens at TFC, and field observations were used to assess the morphological distinctiveness of the *Thesium* populations in La Palma (from now on, *Thesium palmense*) (Table 1).

Species sampling and DNA extraction

Two-hundred thirty-six leaf samples were collected and analyzed across all *Thesium* populations in Gran Canaria, Tenerife, and La Palma Islands (Figure 1C). This study set included 22 samples from *T. canariense*, 92 from *T. retamoides*, and 43 from *T. subsucculentum*, and 79 samples from *T. palmense* (Table 2). Legal permission for the collections was granted by the local administration of each island and the Caldera de Taburiente National Park (Resolution 369/109, Canary Islands Government). The samples were stored in silica gel immediately after collection and herbarium voucher specimens were brought to the TFC Herbarium at the University of La Laguna. Potential host species found during fieldwork are described in Appendix S1 for the four taxa. Genomic DNA for the development of markers and genotyping was extracted from leaf tissue using the protocol of Dellaporta et al. (1983) modified by Corniquel and Mercier (1994). DNA quality was visually assessed by means of electrophoresis. The DNA was purified with a commercial kit (MO BIO Laboratories, Carlsbad, CA, USA) when necessary.

Microsatellite isolation and amplification

Four samples of each taxon were sent to the company Allgenetics & Biology S.L. (A Coruña, Spain) for primer

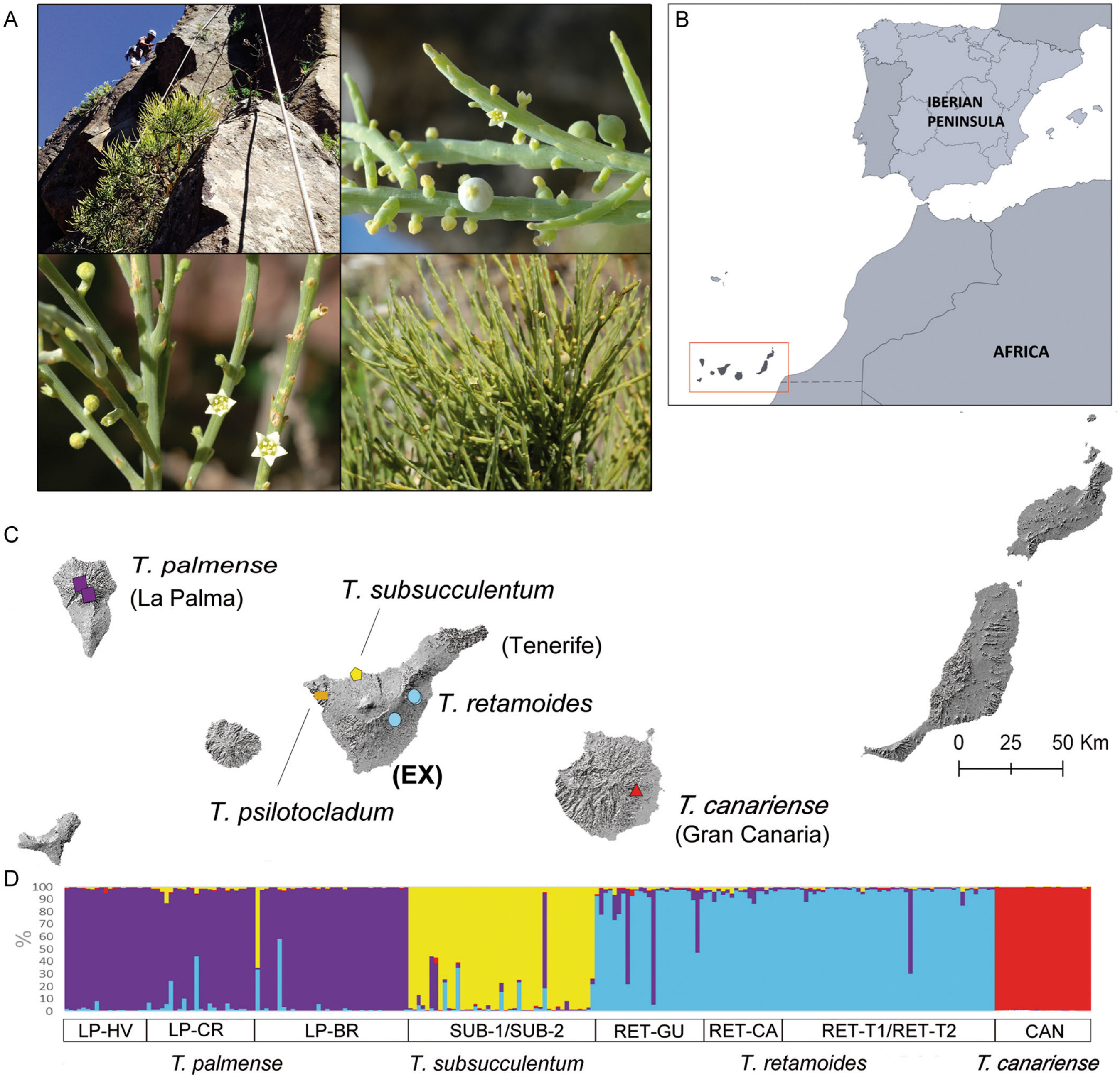


FIGURE 1 (A) Photographs of *Thesium* species included in the study. From left to right, *Thesium palmense*, *T. subsucculentum*, *T. retamoides*, *T. canariense*. *Image credits:* Ángel Palomares (*T. palmense*) and Pedro Luis Pérez Paz (*T. subsucculentum*, *T. retamoides*, *T. canariense*). (B) Geographic context of the Canary Islands (in red box). (C) Localities sampled for every studied taxon, and the former distribution of the extinct *T. psilotocladum*. (D) Bar plot of co-ancestry inferred from Bayesian cluster analysis implemented on STRUCTURE and CLUMPP ($K = 4$)

development. They extracted the DNA and selected the primer pairs employed in this study. From the initial 60 selected primers, 47 yielded some product during the amplification process, and 12 yielded enough polymorphism to be considered for genotyping the whole data set (Appendix S2). Any of the potential microsatellites that did not amplify a fragment or yield any variation were excluded from subsequent PCR procedures. These selected microsatellites were marked with fluorescent probes FAM and HEX and amplified with a PCR protocol performed in 12.5-μL reaction volumes: 10 ng of DNA, 6.25 μL of Multiplex PCR Master Mix (Qiagen, Hilden, Germany), 4 μL of dH_2O, and 1.25 μL of primer mix at 0.2 μM. The PCR consisted of a touchdown protocol with 5 min at 95°C; 30 cycles of 30 s at 95°C, annealing for 90 s at 56°C, and 30 s at 72°C; 8 cycles of 30 s at 95°C, 90 s at 52°C, and 30 s at 72°C; with a final extension of 30 min at 68°C.

TABLE 2 *Thesium* species and populations from the Canary Islands included in this study. Voucher = TFC herbarium in La Laguna University; Lat. = latitude; Long. = longitude; N = sample size

Species	Island	Location	Acronym	Voucher	Lat. N	Long. W	N
T. canariense	Gran Canaria	Guayadeque	CAN	TFC-51.517	27.936972	15.480692	22
	TOTAL (*T. canariense*)						**22**
T. palmense	La Palma	Andén de Los Calzones Rotos	LP-CR	TFC-50.728	28.702602	17.850942	27
		Pared de Los Breñuscos	LP-BR	-	28.703468	17.849646	35
		Cabecera del Barranco de Hoyo Verde	LP-HV	TFC-50.729	28.747302	17.886393	17
	TOTAL (*T. palmense*)						**79**
T. retamoides	Tenerife	Barranco de Tamadaya-1	RET-T1	TFC-51.512	28.211852	16.526310	26
		Barranco de Tamadaya-2	RET-T2	TFC-51.512	28.211852	16.526310	26
		La Hidro sobre San Juan. Güimar	RET-GU	TFC-51.514	28.297876	16.436029	24
		Barranco Badajoz, Los Marreros, El Cabuco.	RET-CA	TFC-51.515	28.304604	16.442472	16
	TOTAL (*T. retamoides*)						**92**
T. subsucculentum	Tenerife	Punta de Juan Centellas. Icod-1	SUB-1	TFC-51.518	28.392289	16.692310	21
		Punta de Juan Centellas. Icod-2	SUB-2	TFC-51.518	28.392289	16.692310	22
	TOTAL (*T. subsucculentum*)						**43**
	TOTAL (*Thesium* sect. *Kunkeliella*)						**236**

PCR products were then sequenced on an ABI 3730 Genetic Analyzer and fragments were sized against the LIZ (500-250) size standard (Applied Biosystems, Waltham, MA, USA) and visualized using Genemapper 4.0 (Applied Biosystems). We identified allelic peak profiles at each locus and assigned a genotype to each individual.

Genetic analysis

From the obtained data set of allelic markers, linkage disequilibrium and deviation from Hardy–Weinberg equilibrium (HWE) were calculated using GENEPOP version 4.2 (Rousset, 2008). Estimation of null alleles for each population was carried out with MICRO-CHECKER 2.2.3 (Van Oosterhout et al., 2006). Additionally, the neutrality of all microsatellites used in this study was tested with BayeScan 2.1 (Foll and Gaggiotti, 2008) considering the data sets per species and setting prior odds at 100. Subsequently, genetic diversity indices were obtained with GenAlex 6.5 software (Peakall and Smouse, 2012): average number of alleles per locus (Na), number of private alleles (PA), observed heterozygosity (H_o), and unbiased expected heterozygosity (H_e). Estimates of selfing were calculated for each species and populations of La Palma with the method implemented in SPAGeDi (Hardy and Vekemans, 2002; David et al., 2007).

To identify any recent genetic drift events for all the sampling sites, BOTTLENECK 1.2.02 software was used (Cornuet and Luikart, 1996). The two-phase mutation model (TPM), which is a modification of the stepwise mutation model (SMM) and has been shown to be a better fit for most microsatellite data sets, was implemented (Piry et al., 1999). In the TPM model, a proportion of SMM in the TPM = 0.000 and a variance of geometric distribution for TPM = 0.36 were chosen.

Moreover, differentiation indices were calculated to account for pairwise relationships between populations: Wright's F_{ST} (1948) was calculated with software GENEPOP version 4.2 (Rousset, 2008). To compare the effect of null alleles on the genetic distances, F_{ST} values per locus were calculated using the program FreeNA (Chapuis and Estoup, 2009), performing a two-sided *t*-test to evaluate significant differences with or without correction for null alleles. A neighbor joining (NJ) dendrogram was constructed with Nei's DA genetic distance (Nei et al., 1983) using the software Populations (Langella, 2002), with 1000 bootstraps with each locus. The tree was visualized and edited on FigTree (Rambaut, 2009). In addition, a principal coordinate analysis (PCoA), using the covariance standardized method of pairwise Euclidian distances between individuals, was implemented in R 3.6 software (R Core Team, 2019) with the package ade4 (Dray and Dufour, 2007).

To check whether the taxonomic status of the study species is in concordance with the genetic structure, we screened all the genotypes using a Bayesian admixture procedure with the software STRUCTURE (Pritchard et al., 2000). The model was assumed to be of population admixture and correlated allele frequencies. Ten independent runs were conducted for each value of *K* (from 1 to 10), and the analysis consisted of 10^5 burn-in period and a run length of 10^6 replicates. The optimal number of clusters was found by the Δ*K* method (Evanno et al., 2005) visualized with STRUCTURE HARVESTER (Earl and vonHoldt, 2012). Results of 10 replicates of the best fit *K* were processed using CLUMPP 1.1.2 (Jakobsson and Rosenberg, 2007) to estimate the optimal clustering.

A molecular analysis of the variance (AMOVA) was conducted with GENODIVE (Meirmans, 2020) to quantify gene flow between *T. subsucculentum*, *T. retamoides*, and populations of La Palma, excluding *T. canariense*, to estimate the genetic differentiation among the closest related taxa. Therefore, three different AMOVA were conducted for each of the pairwise combinations of taxa.

Finally, the approximate Bayesian computation (ABC) method (Beaumont, 2010) implemented in DIYABC v2.1 (Cornuet et al., 2014) was used to explore the demographic history that may have generated the current genetic structure and speciation in the living *Thesium* populations. Therefore, the individuals were grouped by species. Five demographic scenarios characterized by divergence times in generations (t1, t2, t3), effective population size of every species (N1, N2, N3, and N4 for populations of *T. palmense*, *T. canariense*, *T. retamoides*, and *T. subsucculentum*, respectively) and three putative ancestral populations (Na, Na2, Na3) were compared (Appendix S3). The scenarios and priors were chosen for computation after initial trials. In the simplest scenario (Scenario 1), all four species diverged simultaneously at time *t* from a common ancestor. Scenarios 2 and 3 considered an east to west island colonization, from Gran Canaria, Tenerife, and La Palma consecutively. In these two scenarios, La Palma populations could have shared ancestors either with *T. retamoides* or *T. subsucculentum*, both from Tenerife. On the contrary, scenarios 4 and 5 hypothesized that Tenerife could have been the center of radiation, colonizing the islands of Gran Canaria and La Palma afterward. A detailed methodology of the ABC analysis, such as the priors and settings for choosing the most probable scenario, the estimation of the demographic parameters and the confidence in the parameters and the scenario choice can be found in Appendix S4.

Ecological niche modeling

We assessed the extent of bioclimatic niche differences among the three species and La Palma populations in the present localities using available bioclimatic data. We retrieved occurrence data from field sampling and assigned the occurrences to the corresponding cell of the stack of grids of environmental predictors. We incorporated additional sources of information, herbarium at the Botanical Garden in Madrid (MA-RJB) and recorded GBIF occurrences. For both sources of information, we checked for duplicities, and filtered occurrences with accuracy over 0.648 arc seconds and validated identification.

Bioclimatic data were generated in R following procedures described by Rodríguez-Rodríguez et al. (2018a, 2019) and Villa-Machío et al. (2020), in which monthly climatic layers were modeled from raw data from meteorological stations and digital elevation models. Monthly layers were used to derive the presumably meaningful 19 bioclimatic layers for explaining the distribution of organisms, plus evapotranspiration. Topographic layers (slope and topographic position) were also derived from digital elevation models at a resolution of 20 m/pixel.

Because of the small number of samples, a traditional ecological niche modeling approach was substituted with ensemble of small model techniques (Breiner et al., 2015, 2018), which avoids overfitting due to an excess of variables considered in the models. We followed the initial approach of Breiner et al. (2015), based on the assemblage of combinations of bivariate models, but we modified the evaluation procedure. Instead of Sommers' *D* coefficient to weight the contribution of each individual model, we used the true skill statistic (TSS) that ranges from −1 (worst score) to 1 (perfect score). Before the ESM procedure, we selected the five predictors with the package Ecospat (Di Cola et al., 2017), using a correlation threshold between variables of 0.75. We obtained 10 combinations of bivariate models. We then selected those predictors with the highest score in the first three axes of a principal component analysis (PCA). Models were built for each *Thesium* taxa from the known localities and random pseudoabsences generation. We developed five sets of pseudoabsences to run five iterations of the models, selecting randomly for each iteration 80% of data for calibration and 20% for evaluation with the TSS score. We kept only models with TSS over 0.8. We used GLM, random forest, and GBM algorithms to build sets of models. We conducted thus 150 ESM models to build an ensemble model for each species.

Given the hemiparasitic life form of the group, we took an additional approach to the BAM framework (Soberón and Peterson, 2005) to account for the distribution of host plants. Due to our uncertainty of the host plants, we made a list of possible hosts cited in the literature and companion species identified during fieldwork. Given the high number of possible host plants identified, we mapped occurrences of every host species (Appendix S1) to build an envelope encompassing all the potential areas available for the ensemble. Occurrences of the potential host species were obtained from the same data sources as the Canarian *Thesium* species.

Assessment of niche differentiation

We computed the niche differentiation between species and La Palma populations with the R package hypervolume using Gaussian kernel density estimation (Blonder et al., 2018) to compute species niche hypervolumes and then the R package ecodist (Goslee and Urban, 2007) to calculate niche ecological differences among taxa.

RESULTS

The 12 microsatellite markers chosen for the final analysis amplified consistently and showed polymorphism for the entire pool of samples. The percentage of polymorphic loci was 91.67% (populations from La Palma, KP), 83.33% (*T. canariense*), 100% (*T. retamoides*), and 91.67% (*T. subsucculentum*). KU01 and KU29 was monomorphic in *T. canariense*, while KU34 was monomorphic in *T. subsucculentum* and La Palma populations. There was no linkage disequilibrium for any pairwise locus comparisons. Nonetheless, we detected the presence of null alleles for locus Ku-13 in *T. canariense*, *T. subsucculentum* (Icod1 and Icod2), and La Palma populations (Breñuscos, Calzones Rotos, and Hoyo Verde). Ku-32 also showed the presence of null alleles in all populations from La Palma. The neutrality test carried out with BAYESCAN did not show signs of selection for any of the loci.

Genetic diversity

Genetic diversity indices were similar among the four taxa (Table 3). In general, we found heterozygote excess for all localities, with significant deviations from HWE. Observed heterozygosity values were the most divergent, ranging from 0.512 (LP-HV) to 0.795 (RET-CA). The H_o values were also different across species, with the lowest being *Thesium palmense* ($H_o = 0.570$) and the highest *T. retamoides* at 0.722. However, expected heterozygosity values were similar among populations and species, ranging from 0.398 (LP-HV) to 0.515 (RET-CA). The presence of private alleles also differed among species, with *T. canariense* showing the highest number of private alleles (17).

The estimates of selfing rate per taxa (from 0 to 1) based on the distribution of multilocus heterozygosity, showed low values; *T. canariense* (0.000 ± 0.015), *T. palmense* (0.061 ± 0.049), *T. retamoides* (0.062 ± 0.031) and *T. subsucculentum* (0.003 ± 0.046), with few signs of selfing for all taxa.

Results from BOTTLENECK presented significant values ($P < 0.05$) of heterozygosity excess under TPM for the Wilcoxon test in *T. canariense* and *T. retamoides*. *Thesium palmense* and *T. subsucculentum* did not show signs of recent genetic drift events.

Genetic structure

Pairwise F_{ST} indices showed a high genetic differentiation of *T. canariense* (Appendix S5) from the other three taxa, with F_{ST} levels ranging from 0.381 to 0.446. Other pairwise F_{ST} values were lower, indicating closer genetic relationships between species. Intraspecific F_{ST} values were markedly lower. The Tamadaya population of *K. retamoides* (RET-T1 and RET-T2), which was artificially split for the analysis, showed no differentiation (F_{ST}). The F_{ST} values per locus calculated with FreeNa did not show significant differences between the corrected and uncorrected F_{ST} estimates for null alleles (Appendix S6), so we assumed that the null

TABLE 3 Genetic diversity indices per sampled population of *Thesium* species in the Canary Islands

								Bottleneck		
Species	**Site**	***N***	**Na**	**PA**	**H_o**	**H_e**	**F_{IS} (HW)**	**H_d/H_e**	***P***	**Shifted mode**
T. canariense	**CAN**	20.75	3.167	17	0.637	0.488	-0.335^{ns}	1/9	0.001^{**}	No
T. palmense	**LP-CR**	26.42	2.750	1	0.621	0.431	-0.476^{***}	3/8	0.074^{ns}	No
	LP-BR	32.75	2.750	0	0.555	0.422	-0.308^{*}	3/8	0.120^{ns}	No
	LP-HV	15.42	2.500	1	0.512	0.398	-0.367^{*}	3/8	0.139^{ns}	No
	Total	74.58	3.333	3	0.570	0.428				
T. retamoides	**RET-T1**	24.33	2.667	1	0.673	0.445	-0.508^{***}	2/10	0.065^{ns}	No
	RET-T2	23.83	2.500	1	0.695	0.455	-0.582^{***}	2/10	0.021^{*}	Yes
	RET-GU	23.25	2.750	1	0.760	0.505	-0.517^{***}	1/11	0.003^{**}	No
	RET-CA	15.33	2.583	1	0.795	0.515	-0.580^{***}	2/10	0.001^{**}	Yes
	Total	86.75	3.333	5	0.722	0.500				
T. subsucculentum	**SUB-1**	18.50	2.583	0	0.604	0.419	-0.431^{**}	4/7	0.073^{ns}	No
	SUB-2	19.00	2.583	0	0.655	0.453	-0.447^{***}	4/7	0.087^{ns}	Yes
	Total	37.50	2.833	1	0.630	0.434				

Note: N = average number of individuals; Na = number of different alleles; PA = number of private alleles; H_o = observed heterozygosity; H_e = unbiased expected heterozygosity; F_{IS} = inbreeding coefficient with Hardy–Weinberg (HW) equilibrium tested for heterozygosity excess; H_d/H_e = number of loci with heterozygote deficiency (H_d) and heterozygote excess (H_e) according to the TPM model in the bottleneck test; P = probability of the Wilcoxon test for heterozygote excess in the bottleneck test; shifted mode of the L-shape distribution of alleles. Not significant (ns); $^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$.

alleles detected did not overestimate the genetic distances. Therefore, we used the original data set to calculate distances and build the NJ dendrogram. Hence, the NJ dendrogram (Figure 2A) showed a clear separation of *T. canariense* from the rest of the taxa, which proved to be a monophyletic cluster. The PCoA (Figure 2B) yielded consistent results, separating *T. canariense* from the other species across the first axis, which alone captured a large share of the variance (47.98%). The second axis, which also captured a high amount of variance (almost 30%), allowed the segregation of the three remaining taxa.

Consistent with the other results for population differentiation, the Bayesian structure analysis identified two genetic clusters based on the highest Δ*K* (*K* = 2), with a high genetic differentiation of *T. canariense* (Appendix S7). On the other hand, following the second highest Δ*K* (*K* = 4), there was a clear aggregation of the individuals according to their taxonomic origin (Figure 1D), with a strong assignment also for *T. palmense*. However, there were few individuals from *T. palmense*, *T. retamoides*, and *T. subsucculentum* that showed admixture in their percentage of coancestry with other clusters. Due to biases of the Evanno method over Δ*K* that give a higher importance to *K* = 2 (Janes et al., 2017), we represented the *K* = 4 clustering in Figure 1D, in concordance with the *L*(*K*) graphic (Appendix S7).

The AMOVA analysis to detect gene flow among the different pairs of species (Table 4), excluding *T. canariense*,

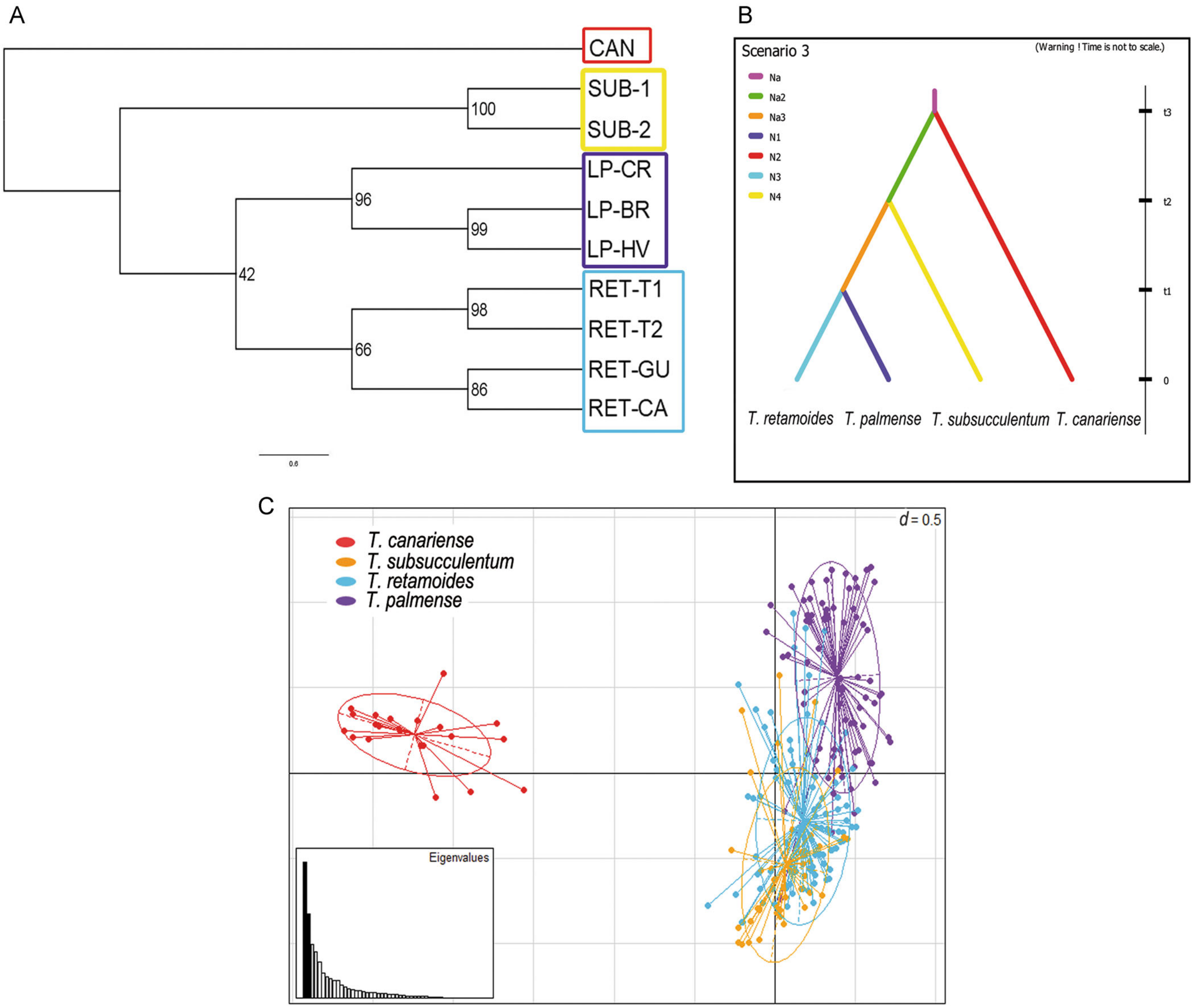


FIGURE 2 (A) Neighbor joining dendrogram based on Nei's (1983) genetic distance among the 10 localitites of *Thesium*. (B) Representation of the most probable demographic scenario (Scenario 3) with the ABC method implemented in DIYABC (Cornuet et al., 2014). t1, t2, t2a: time scale of divergence times measured in generations since the present ($t = 0$). N1, N2, N3, and Na refer to effective population sizes, respectively, of *T. palmense* (LP), *T. canariense*, *T. retamoides*, *T. subsucculentum*, and from a nonsampled ancestral population. (C) Principal coordinate analysis based on the genetic distance among individuals

did not show high levels of variation between taxa. However, the variation between species was similar among the comparisons (between 7.8% and 14.6%) and higher than the variation between localities.

Demographic history

The DYABC results showed that, among the five tested scenarios, Scenario 3 gave the highest posterior probability (PP = 0.695 [0.686, 0.704]) using a logistic approach with 10% closest points (Appendix S8). Therefore, demographic parameters were calculated under Scenario 3 (Table 5). The oldest splitting event occurred between *T. canariense* and *T. subsucculentum*, which derived from an unknown ancestral population at the event t3 (9210 [3010, 19200] generations; mode and 2.5–97.5% quantiles of the posterior distribution, respectively). *Thesium retamoides* and *T. subsucculentum* diverged at t2 (769 [348, 6800] generations). The most recent colonization event was t1 (274 [60.4, 1770] generations), during which *T. palmense* likely derived from Tenerife, sharing ancestors with *T. retamoides*. In general, mean bias estimates for the divergence times were low, with high factor 2 values (Appendix S9). Following field and greenhouse observations, it is estimated that the Canarian *Thesium* individuals can live for around 20 years (unpublished data). Given that generation time, the divergence between *T. subsucculentum* and *T. canariense* occurred 184,200 [60,200, 384,000] years ago, between *T. retamoides* and *T. subsucculentum* 15,380 [6960, 136,000] years ago, and between *T. retamoides* and La Palma populations 5480 [1208, 35,400] years ago. Nonetheless, these estimations should be taken with caution, as we do not have enough demographic data for *Thesium* in the Canary Islands to provide exact measures of generation time.

Niche modelling and niche differentiation

The seven predictors retained for modeling were slope, bio01 (average annual temperature), bio10 (temperature of the driest quarter), bio12 (annual precipitation), bio04 (temperature seasonality), bio15 (precipitation seasonality), and bio16 (precipitation of the driest quarter). Of the ESMs scored, 72% showed a TSS value above 0.8 and were therefore retained for the ensemble modeling. ESM did not indicate any suitable areas for the eastern islands of Lanzarote and Fuerteventura under the suitability thresholds that maximized the TSS score. In general terms, there were no overlaps between potential ranges of the species. *Thesium retamoides* was the only species with suitable geographic areas in only one island (Tenerife, Figure 3A). On the other hand, *T. subsucculentum* showed the most differentiated range in ecological space, obviously constrained to coastal desert areas in La Gomera, La Palma, and Tenerife, but not in Gran Canaria and El Hierro. The only niche overlap occurred in Tenerife between *T. retamoides* and *T. canariense*. The latter species had a remarkably extended potential geographic distribution despite being restricted to Gran Canaria. Regarding islands, Tenerife showed the highest potential to host *Thesium* populations.

The ensemble of occurrences of potential host plants of each taxon was remarkably large and indicated complete overlap with suitable geographic areas of all taxa and thus the absence of limitation to the distribution due to potential host species (Appendix S10).

TABLE 4 AMOVA analysis performed for *Thesium* sect. *Kunkeliella*

Source of variation	df	Sum of squares	Variance components	Variance	Percentage of variation
T. retamoides–T. subsucculentum					
Among taxa	1	45,137	45,137	0.301	8.4
Among populations	4	39,738	9935	0.152	4.2
Within populations	264	825,880	3128	3,128	87.4
T. subsucculentum–T. palmense					
Among taxa	1	63,940	63,940	0.516	14.6
Among populations	4	25,113	6278	0.084	2.4
Within populations	238	696,304	2926	2926	83.0
T. retamoides–T. palmense					
Among taxa	1	54,846	54,846	0.262	7.8
Among populations	6	59,566	9928	0.165	4.9
Within populations	334	987,167	2956	2956	87.4

TABLE 5 ABC demographic parameters estimates for Scenario 3

Parameter	Mean	Median	Mode	Quantile 2.5%	Quantile 97.5%
Effective population size					
N1 (*T. palmense*)	3610	3350	3140	852	8340
N2 (*T. canariense*)	4340	4180	4040	1490	8380
N3 (*T. retamoides*)	3530	3210	2600	737	8410
N4 (*T. subsucculentum*)	4400	4270	4480	1420	8630
Na	26,300	27,000	31,200	3090	47,100
Na2	6430	3020	334	137	34,200
Na3	27,600	28,100	40,020	3260	49,000
Time scale in generations					
t1	524	388	274	60.4	1770
t2	2030	1530	769	348	6800
t3	10,600	10,200	9210	3010	19,200

Note: The mean, median and mode for each parameter are given, along with 95% credibility intervals. N1, N2, N3, N4, Na, Na2, and Na3 refer to effective population sizes of every taxon and from three nonsampled ancestral populations from which they have diverged as modelled in the most likely of the five tested scenarios (Appendix S3). t1, t2, t3: divergence times of the standing populations from the populations they have derived.

The PCA to select predictor variables (Figure 3B) served to assess the extent of niche differentiation. The first PCA axis encompassed a large part of the variance (72.6%) and was associated with temperature gradients, therefore explaining the display of the coordinates across the first component. Only 11% of the variance attributed to the second axis and was related to slope. The four species were distributed and well differentiated across the first axis, indicating differences in temperature variables. *Thesium retamoides* and *T. canariense* showed a certain overlap in the PCA sharing similar temperature values and differing in topographic conditions and was consistent with the overlap detected in potential ranges.

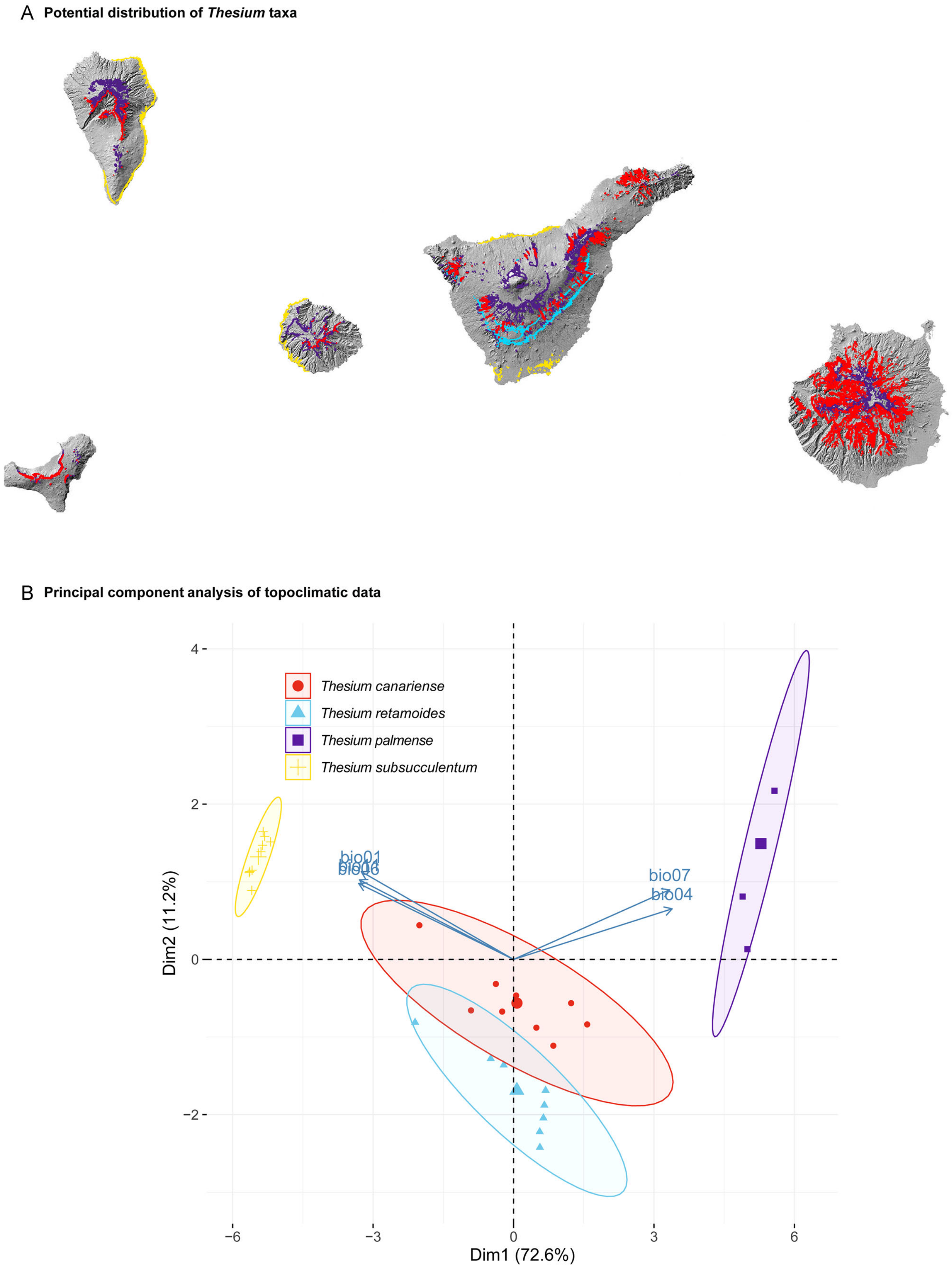
DISCUSSION

In this study, we assessed both genetic and niche evolution of four plant taxa with limited distributions from *Thesium* section *Kunkeliella*. Population genetic indices showed slightly higher diversity than expected, considering their endangered status and rarity. Our results showed that *T. canariense* is genetically differentiated, situating Gran Canaria as the source of radiation toward other niches across islands and revealed the niche differentiation across all available temperature ranges. Instead, we could identify only a partial evolution of some of the climate parameters. The combined assessment of actual distribution, potential niche, and overlap between species, together with the genetic relationships, allows a first hypothesis about the plausible scenarios of niche evolution. However, there are still uncertainties of the dispersal limitations and interspecific interactions in *Thesium* sect. *Kunkeliella* and how they impact the distribution of the species.

Genetic diversity and species differentiation

All *Thesium* sect. *Kunkeliella* taxa showed moderate levels of genetic diversity, which in comparison to other Canarian taxa would be considered intermediate values for studies with microsatellite markers (Sosa et al., 2013; Rodríguez-Rodríguez et al., 2018a, 2018b, 2019). The lowest values were found for *T. palmense*. This low genetic diversity in the youngest island (2 my) is a common pattern and has been detected previously in *Phoenix canariensis* (Saro et al., 2015) and *Lavatera acerifolia* (Villa-Machío et al., 2020). However, it is remarkable that we found heterozygosity excess in all populations because it is uncommon in restricted and endangered taxa. The amplification of different genomic regions in the same locus could lead to heterozygosity excess, but it is unlikely that there were amplification errors for 10 of 12 loci in all the taxa. Biological explanations for these results include the small reproductive population sizes, associative overdominance, negative assortative mating (migration or admixture of different populations), or asexual reproduction (Stoeckel et al., 2006; Waples and Allendorf, 2015). Because we did not detect clear signs of migration and admixture of individuals across the different taxa and the lack of repeated genotypes indicated a lack of asexual reproduction, the bottleneck signs detected in some populations together with small population sizes likely explain the heterozygosity excess in *Thesium* sect. *Kunkeliella*. Heterozygosity excess can occur when allelic frequencies differ among sexes, even in hermaphrodites without self-fertilization, enhanced by small population sizes (Waples and Allendorf, 2015). Moreover, the bottleneck tests might be underestimated in our results, due to the limitations of the heterozygosity excess test to detect population declines (Peery et al., 2012).

One of the main objectives of this study was to determine whether the genetic differences of *Thesium* of La Palma from other species in the section *Kunkeliella* were similar to the differences among the other described taxa. Despite the high genetic differentiation found for *T. canariense*, the other three taxa in the archipelago are closely related. In *T. palmense*, most of the private alleles are relatively rare and in low frequencies, so results indicate a recent differentiation in concordance with the DYABC results. On the contrary, *T. canariense* had a high frequency of private alleles. Although the STRUCTURE analysis ($K = 4$) revealed a clear aggregation for each taxon, some individuals were assigned to other clusters, which could reveal recent gene flow between the species in Tenerife and La Palma.



A Potential distribution of *Thesium* taxa

B Principal component analysis of topoclimatic data

FIGURE 3 (A) Contours of topoclimatic suitability defined by Ensemble of Small Models (ESM), defined as the threshold value of maximum True Skill Statistics (TSS) evaluation score. (B) Principal component analysis of topoclimatic niche hypervolume

All the analyses, including the NJ dendrogram, the PCoA, and the AMOVA, detected a close relationship between *T. palmense* and *T. retamoides*, with localities located in the southeastern part of Tenerife, but not with *T. subsucculentum*, with localities geographically closer to La Palma Island. These results agree with the demographic history results because the highest probability scenario indicated that *T. palmense* shares a common ancestor with *T. retamoides*. Indeed, the individuals from La Palma populations are morphologically more similar to *T. retamoides* than *T. subsucculentum*, supporting the genetic results (Table 1).

Thesium retamoides and *T. subsucculentum* are different species, well characterized morphologically (Table 1), and have 8.4% genetic variation between them. Therefore, the same treatment should be given for the new taxon found in La Palma because the percentages of genetic variation are similar when compared to *T. retamoides* (7.8%) and to *T. subsucculentum* (14.6%). Indeed, similar variation values were detected among *Pericallis* species in the Canaries (Jones et al., 2016). It is possible that the genetic differentiation detected could be due to geographic isolation or an incipient speciation. There are many other examples of monophyletic young radiations in the Canary Islands with closely related species found in different islands, such as *Echium wildpretii* and *E. perezii* (Graham et al., 2021) or the highly diversified macaronesian *Cheirolophus* (Vitales et al., 2014). Given the circumstances and the morphological distinctiveness, the *Thesium* from La Palma could be treated as a new species. The description of this species, named *Thesium palmense*, is detailed in the section Taxonomic treatment.

Climate niche evolution: general overview

The assessment of ecological niche relationships through modeling provided evidence against the hypothesis of niche conservatism in *Thesium*. We did not detect significant overlap between the potential geographical distributions of the species, and we established two sources of evidence that the segregation of species in the niche hyperspace is related only to temperature. First, at the spatial level, the only potential overlap we detected between species (*T. canariense* and *T. retamoides*) was in Tenerife, where the first species is not actually present according to the available data. Species' distributions are clearly segregated in altitudinal ranges, which we can interpret in terms of temperature variation, consistent with the separation of the four species across the first principal component (Figure 3B), where the overlap between *T. retamoides* and *T. canariense* occurs in the ecological space. A second source of evidence comes from an incomplete climate niche filling in the archipelago. While the range of temperatures is fully covered by the potential distribution of the four taxa, from the coastland deserts to the subalpine zone, there is a clear gap in respect to moister habitats in the northern slopes of the island (laurel forests and thermophilous shrublands). The extinct *T. psilotocladum* provides similar evidence of the niche filling because the localities where it was recorded in the Teno mountain range in Tenerife had similar semi-arid precipitation regimes to those of the extant *Thesium* species. On the contrary, European species of *Thesium* have a more widespread niche across species with a much broader spectrum of moisture conditions (Dostálek and Münzbergová, 2010). Two complementary hypotheses can explain the lack of niche filling. First, the hemiparasitic biology can contribute to a physiological balance because the water supply depends on the host plants rather than natural conditions (López-Sáez et al., 2002). This characteristic of hemiparasitism would remove direct selective pressures on precipitation but does not explain why niche filling does not take place. In addition, root hemiparasites tend to prefer environments with a high availability of light (Těšitel et al., 2015), which is not the case in laurel forests, where *Thesium* is not found. The second hypothesis is that the radiation of the *Thesium* sect. *Kunkeliella* is too recent to promote the occupation of a different dimension of the niche space with higher moisture conditions. Our results were consistent in part with the findings of niche lability from Steinbauer et al. (2016) for highly diversified and flagship lineages (*Aeonium*, *Argyranthemum*, *Descurainia*, *Echium*, *Lotus*, and *Sonchus*) and Albaladejo et al. (2020) for *Helianthemum* taxa. In both studies, niche lability was also the dominant trend but was related more to climatic traits including precipitation.

Considering the results obtained for the studied taxa, dispersal and colonization to other islands where radiation occurred alongside thermal niche shifts works out as the simplest and most plausible scenario. *Thesium canariense*, with the largest potential area and widest niche, is the sister species to all the others, suggesting a colonization from Gran Canaria to the rest of the islands, with classical east–west colonization pattern of the Canary Islands biota. The extreme rarity of the entire clade in comparison with the much larger potential distribution can leave other scenarios open. Taxa may have been more widespread in the past with the occurrence of competitive displacement leading to niche shifts. The overlaps of potential distribution shown by the ESM allow such possibility. However, in spite of the detected bottleneck events, especially in *T. canariense* and *T. retamoides,* it is possible that rarity is inherent to *Thesium* sect. *Kunkeliella*, enhancing the vulnerability of the populations to external factors and reducing population sizes. Nevertheless, this study provides new insights for population management from the perspective of species delimitation and areas for reintroduction and conservation genetics.

Taxonomic treatment

Thesium palmense P. Pérez, et P. Sosa sp. nov.

Affinis ad *T. psilotocladum* (Svent.) Stearn et *T. retamoides* A. Santos ex Tenerife (Nivaria), differunt:

Ramis densior, divaricati, subsucculentus; ramis vetustis brunneas, juvenalibus glaucis, leviter striato, puberulis.

Foliis linearibus subulato, mínima ca. 1–1.5 mm long, applicatis, vel flabidis in senectute demun fuscus. Floribus subsessilibus, ad minima bracteis; perigonii lobulis discoloribus, internè lutescenti, externè viridi, breviter puberulis; laciniis deltoides, ápice subacutis, médium inter *T. psilotocladum* (obtusis) et *T. retamoides* (acutis). Staminubus subinclusis, perigonium longitudine tubi similiter. Estigma trilobato, aliquantum diutius quam stamina. Fructus globosis, usque 6 mm diametri, margarita lucis color, stigmatibus fusci persistentibus coronatus.

Collecta cum flore et fructus die 14 matii 2012; Ángel Palomares et al.

Related to *T. psilotocladum* (Svent.) Stearn and *T. retamoides* A. Santos de Tenerife, it differs in being a perennial shrub up to 1.5 m, with lignified stems and abundant branching, of ascending growth. Branches thicker, divaricate, subsucculent; the old ones dark beige, the young ones, glaucous subtly striated and pubescent. Linear-subulate leaves, 1–1.5 mm, attached, yellowish, which turn brown with age. Inflorescence racemiform in appearance, with very short twigs. Flowers subsessile, with tiny bracts; with cream-colored tepals on the inside, green and slightly pubescent on the outside; subacute, intermediate between *T. psilotocladum* (obtuse) and *T. retamoides* (acute). Subinclusive stamens matching the perigonial tube. Trifid stigma, slightly longer than stamens. Globose fruits approximately 6 mm in diameter, pearly in color, crowned by persistent ocher stigmas.

Holotype: Andén de Calzones Rotos, 1.650 m. Caldera de Taburiente National Park, La Palma (Canary Islands). NW. UTM 221413-3178337. 14 March 2012. Legit: A. Palomares, R. Arocha, A. Toledo, A. Rodríguez, G. Balsera, J.E. Lorenzo y A. Acevedo. Deposited in University of La Laguna herbarium: TFC 50.728 + 7 isotype (Figures 4 and 5).

CONCLUSIONS AND IMPLICATIONS FOR CONSERVATION

Our results provide a foundation for the conservation of all *Thesium* taxa in the Canary Islands, using a multidisciplinary approach that considered not only the genetic

FIGURE 4 Photographs of *Thesium palmense* sp. nov. (Location: Andén de Los Calzones Rotos). (A) Shrub, (B) flower, (C) fruit. *Image credit:* Ángel Palomares

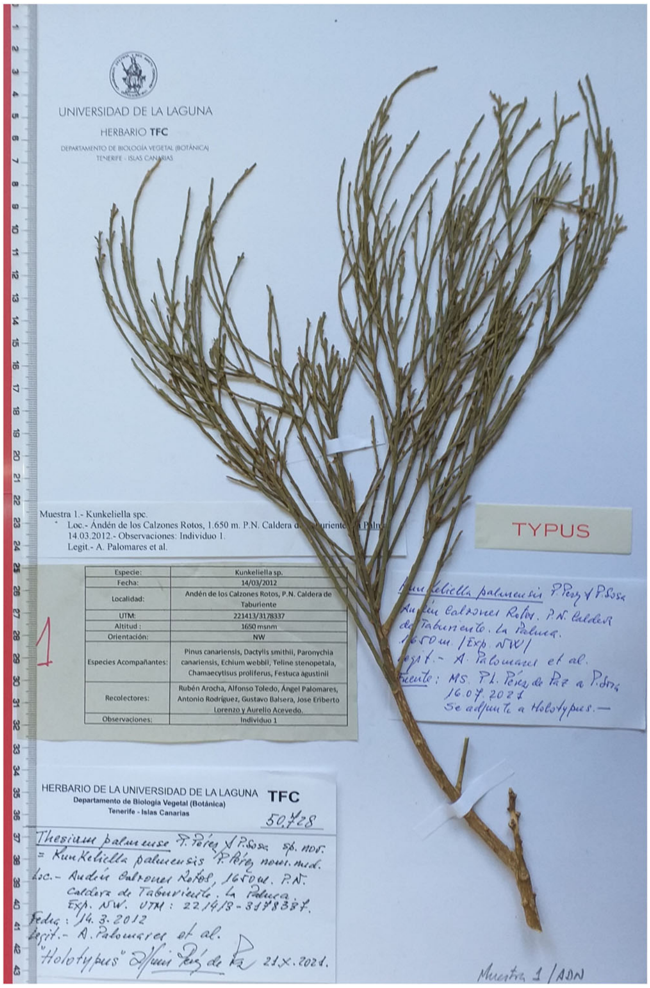


FIGURE 5 Holotype of *Thesium palmense* sp. nov., deposited in the TFC Herbarium, University of La Laguna

status of each species, but also their differences in ecology, in light of the available habitat. Moreover, the discovery of the new localities in La Palma where the presence of *Thesium* was previously unknown is highly relevant and deserves conservation attention, regardless of the taxonomic treatment that it will receive, as a new species. *Thesium palmense* presents a restricted distribution with few individuals and putative self-incompatibility. Self-incompatible species with isolated populations are more vulnerable to mate limitation, especially animal-pollinated species (Aguilar et al., 2008). Although *T. palmense* is distributed in a protected area (Caldera de Taburiente National Park), threats such as climate change and herbivory pressure should be considered (Irl et al., 2014; Harter et al., 2015). Therefore, a conservation plan is urgently needed for *T. palmense*, taking into consideration the results of this study, demographic characteristics, and threats to the populations. The genetic differentiation among *T. palmense* localities is quite low, which is not surprising as Calzones and Breñuscos are geographically close, and these could be managed as a single population. Therefore, to simplify conservation measures, managers should consider one or two genetic pools for the germplasm or a reintroduction plan if necessary. Habitat conservation, like the protection of areas against herbivores will help the conservation, not only of *Thesium*, but many other endemic species in the National Park. In this sense, it will be necessary to include the new species and the habitat in the guiding plan for use and management ("Plan Rector de Uso y Gestión") of La Caldera de Taburiente National Park and to study the distribution, reproductive biology and possible threats. Moreover, it should be included in the regional and national checklists and *Red List of Spanish Vascular Flora* (Moreno-Saiz, 2008). The combination of niche modeling and population genetics can serve as a guideline for the conservation genetics of the *Thesium* sect. *Kunkeliella* in the Canary Islands and the population reinforcement or the introduction of individuals to other areas.

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AUTHOR CONTRIBUTIONS

P.R.R., A.G.F.C., and P.A.S. performed the analysis and wrote the manuscript. P.L.P.P., A.P., and P.A.S. designed the project and the fieldwork. P.L.P.P described the new *Thesium* species. A.A. and R.M. contributed to the collection of samples and the description of *Thesium* species. L.C. did the laboratory work. and data compilation. All authors contributed to the revision of the manuscript before submission.

DATA AVAILABILITY STATEMENT

The raw microsatellite data set is available in the FigShare repository: https://figshare.com/articles/dataset/kunkeliella_species_xlsx/17081720.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

Appendix S1. Potential host species of *Thesium* sect. *Kunkeliella* inventoried during fieldwork.

Appendix S2. Characteristics of 12 microsatellite loci developed for *Thesium* sect. *Kunkeliella*.

Appendix S3. Demographic scenarios characterized by divergent times in generations (t1, t2, t3, t3a), effective population size of every species (N1, N2, N3, and N4 for *Thesium* species and three putative ancestral populations (Na, Na2, Na3).

Appendix S4. Methodology of the ABC analysis, such as the priors and settings for choosing the most probable scenario, the estimation of the demographic parameters and the confidence in the parameters and the scenario choice.

Appendix S5. Pairwise F_{ST} distances between sampled populations.

Appendix S6. F_{ST} values per locus with corrected and uncorrected matrices for null alleles with the program FreeNa.

Appendix S7. Bar plots of co-ancestry inferred from Bayesian cluster analysis ($K = 2$ and $K = 4$) and outputs from STRUCTURE HARVESTER.

Appendix S8. Posterior probabilities of the five tested scenarios in the DIYABC using a logistic approach with 10% of the closest simulated points.

Appendix S9. Description of demographic parameters and tested population history scenarios.

Appendix S10. Maps of the ensemble of the potential hosts occurrences for every *Thesium* taxon included in this study.

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