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Tracking the desert's edge with a Pleistocene relict

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ABSTRACT

In addition to the Sky Islands of the southwestern U.S. and northwestern Mexico, a series of 900–1200 m desert peaks surrounded by arid lowlands support temperate affiliated species at their summits. The presence of disjunct long-lived plant taxa on under-explored desert mountains, especially Isla Tiburón at 29° latitude in the Gulf of California, suggests a more southerly extent of Ice Age woodlands than previously understood. The phylogeography of the desert edge species *Canotia holacantha* (Celastraceae) was investigated to test the hypothesis that insular desert peak populations represent remnants of Pleistocene woodlands rather than recent dispersal events. Sequences of four chloroplast DNA regions totaling 2032 bp were amplified from 74 individuals of 14 populations across the entire range of *C. holacantha* as well as nine individuals that represented the other two species in its clade (*C. wendtii* and *Acanthothamnus aphyllus*) and two outgroups. Results suggest that a *Canotia* common ancestor occurred on the landscape, which underwent a population contraction ca. 15 kya. The Isla Tiburón *C. holacantha* population and the Chihuahuan Desert microendemic *C. wendtii* have the greatest genetic differentiation, are sister to one another, and basal to all other *Canotia* populations. Three haplotypes within *C. holacantha* were recovered, which correspond to regional geography and thus identified as the Arizona, Sonora, and Tiburón haplotypes, within which *Acanthothamnus aphyllus* is nested rather than as a sister genus. These results indicate a once broad distribution of *Canotia*/*Acanthothamnus* when the current peripheral desert ecotone habitat was more widespread during the Pleistocene, now present in relict populations on the fringes of the southern desert, in the Chihuahuan Desert, with scattered populations on desert peaks, and a common or abundant distribution at the northern boundary of the Sonoran Desert. These results suggest *Canotia* has tracked the shift of the desert's edge both in latitude and elevation since the end of the last Ice Age.

1. Introduction

The story of the Pleistocene is written on the high elevations of mountains across North America. When the cooler and wetter environments of the last glacial maximum (LGM; ca. 21 kya) began to transition to warmer and drier conditions, species once widespread in the lowlands followed favorable habitat and moved up in elevation and latitude (Butterfield et al., 2019). Novel communities replaced them in lower elevation habitats (e.g., Van Devender, 1977). A considerable amount of research has revealed profound changes to the landscapes of the world from the LGM to present. These include a fundamental shift from a mesic to more arid climate (e.g., Metcalfe et al., 1997), a ca. 120 m rise in global sea level (Clark and Mix, 2002), and a full-scale reorganization of species and communities (e.g., Van Devender, 1977; Graham and Grimm, 1990). However, the history and origin of a substantial portion of modern biodiversity remains unknown, especially in arid regions. By looking up in elevation and into the genetic and biogeographic patterns of disjunct taxa, we can further understand and appreciate the imprint of past ecosystems on modern biodiversity.

Analysis of fossil packrat middens has reconstructed the vegetation of the northern portion of the current Sonoran Desert in North America (e.g., Betancourt et al., 1990) with recent promising and refined insights from aDNA metagenomic analysis of these ancient rodent middens (Moore et al., 2020). Macrofossils from across arid North America dating to over 50,000 ya provide data on species composition and confirm the presence of relatively mesic woodland vegetation and flora in lowland

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regions (<300 m) prior to 11,000 ya (e.g., Van Devender, 1990). Pleistocene woodlands were variously characterized by presence to dominance of single-leaf pinyon (*Pinus monophylla*), Utah juniper (*Juniperus osteosperma*), shrub live oak (*Quercus turbinella*), and Joshua tree (*Yucca brevifolia*) across the broader region now inhabited by desertscrub in the greater southwest U.S. The early Holocene from 11,000 to about 9000 ya was a transitional period and modern communities were established ca. 6000 ya (Van Devender, 1977; Butterfield et al., 2019) at the same time modern sea levels were attained. However, the majority of fossil packrat midden studies have focused on the northern portion of the Sonoran Desert with few in-depth paleo records in the vicinity of the Gulf of California. Comprehensive fossil packrat midden series in the rain shadow of the eastern escarpments of the Sierra San Pedro Mártir and Sierra Juarez in northern Baja California reveal that woodland elements dominated and co-occurred at low elevations with Sonoran Desert taxa during the Pleistocene (Holmgren et al., 2011, 2014). This finding argues against a desert refugium in one of the most stable arid regions of North America. Where was desert habitat during the Ice Age? The occurrence of Ice Age woodlands into southern latitudes, areas along the Gulf of California, and into the core of the current Sonoran Desert remain unknown.

Beyond the iconic and well-studied sky island mountains of the southwestern U.S. and northwestern Mexico many lower but perhaps equally informative desert peaks that reach ca. 900–1200 m in elevation occur across the region. While lacking the full suite of community transitions from desert valleys to oak woodlands and pine forests at the summits, the desert peaks are mountains in a sea of arid lowlands. As esteemed desert ecologist Forrest Shreve stated, "Among the mountains which lie wholly within the Sonoran Desert, the vast majority are of such low elevation that their summits are clothed solely by desert plants. There are, however, a number of small ranges which rise 700 to 1200 m above the surrounding plains and furnish congenial habitats for plants that are common in the larger mountains just outside the desert, but are never or rarely found on the desert floor" (Shreve, 1951:16). These elevations often harbor species characteristic of Pleistocene woodlands. Floristic inventories of a select few of these mountain peaks, many of which remain unexplored, have identified mixed grasslands, chaparral, and long-lived mesic-adapted perennial species on peaks throughout Arizona (Brown, 1978; Felger et al., 2013), the length of the Baja California peninsula (Moran, 1983; Bullock et al., 2008), and parts of Sonora (Yatskievych and Fischer, 1984; Wilder et al., 2008).

Isla Tiburón, the largest island in Mexico (1218 km^2), is a land-bridge island in the Gulf of California a few kilometers off the west coast of Sonora that was connected to the mainland as recently as 6000 ya (Felger and Wilder, 2012). Botanical studies revealed the presence of five species confined to higher elevations on the island (>500 m; sotol, *Dasylirion gentryi*; canyon hackberry, *Celtis reticulata*; desert olive, *Forestiera phillyreoides*; Goodding's ash, *Fraxinus gooddingii*; and mock buckthorn, *Sageretia wrightii*) in addition to *C. holacantha* that are isolated by more than 200 km of intervening Sonoran Desert from their nearest conspecific populations (Felger and Wilder, 2012). The occurrence of relatively mesic-adapted perennial species on insular mountains can be likened to a "sky island within a desert island." These disjunct taxa have present day distributions in the Sky Island Mountains at the northern fringe of the Sierra Madre Occidental and northwards in temperate regions. The presence of these species on Tiburón may be relicts dating from a time when Ice Age woodlands were more extensive, including at southern latitudes (Turner et al., 1995; Wilder et al., 2008). However, that conjecture has remained untested. This paper is the first phylogeographic study of a disjunct plant species found on desert peaks in the Sonoran Desert.

To better understand paleovegetation patterns of the Sonoran Desert, including towards its southern edge and the shores of the Gulf of California, the phylogeography of *Canotia holacantha* was investigated. One of the unrelated taxa of thorny shrubs known as crucifixion thorns, this species is characteristic of the northern edge of the Sonoran Desert, and intriguingly occurs on the highest ridge of Isla Tiburón (Fig. 1; Wilder et al., 2008). We test the hypothesis that the insular population of *Canotia holacantha* is a relict resulting from a contraction of once widespread and near contiguous populations, rather than recent dispersal events.

Phylogeographic analyses of DNA sequences have been extremely useful to understand the shifts in the biota associated with glacial/interglacial periods and to distinguish when a disjunct population represents remnants of previous climatic and vegetation patterns or the result of a recent dispersal event (e.g., Hewitt, 2004). If the population of *C. holacantha* from Tiburón island represents a relict population then it should show signs of population contraction during the Holocene and phylogeographic structure where Tiburón sequences have diverged due to long-term isolation and small population sizes that could have reduced genetic diversity via genetic drift. In contrast, under a scenario of a recent dispersal event, the observed genetic variation in Tiburón is predicted to be a subset of the variation present in the current range of the species. Likewise, if the island population is relatively new then we expect a lack of phylogeographic structure and small or null sequence differences relative to the closest likely source populations.

2. Methods

2.1. Study species

Canotia (Celastraceae) is a genus comprising two species, *C. holacantha* Torrey and *C. wendtii* M.C. Johnston. The genus in addition to the monotypic southern Mexican endemic *Acanthothamnus aphyllus* (Schlechtendal) Standley has been shown to form a well-supported monophyletic clade via morphology (Johnston, 1975) and genetics (Simmons et al., 2012). Outside of this three-taxon clade, relationships become harder to reconcile within the family. *Canotia* and *Acanthothamnus* are supported, although with less significance, as sister to *Euonymus* (Coughenour et al., 2010; Simmons et al., 2012), a genus of evergreen shrubs and small trees mostly native to East Asia but also present in North America, Europe, Australasia, and Madagascar. In turn, that clade is sister to *Wimmeria*, the largely North American tropical genus that includes *W. mexicana*, which occurs in the tropical dry forests of southern Sonora, Mexico. *Mortonia*, another arid-adapted genus in Celastraceae occurs on the periphery of the Sonoran Desert where it is common on limestone in Chihuahua and is shown to be an early evolving member of the Celastraceae and distantly related to *Canotia* (Simmons et al., 2012).

Canotia is a hardwood shrub or small tree, essentially leafless with scale like leaves that are quickly drought deciduous. *Canotia holacantha* is one of a small group of unrelated desert shrubs with photosynthetic stems termed "crucifixion thorns". The crucifixion-thorn growth form occurs in a handful of genera in the deserts of North America including *Castela* (*C. emoryi* and *C. polyandra*; Simaroubaceae), *Koeberlinia* (Koeberliniaceae), *Thamnosma montana* (Rutaceae), and *Ziziphus obtusifolia* (Rhamnaceae) in addition to *Canotia* (Ávila-Lovera and Ezcurra, 2016). These plants share the holacanthoid (Muller, 1941; meaning [hola] wholly - [cantha] thorny) or the retamoid syndrome (Shmida, 1981; named after the legume *Retama* with spiny photosynthetic stems that is found in North Africa, the Levant, and some parts of southern Europe). These spinescent shrubs and trees are a fascinating adaptation to arid environments (Muller, 1941), a growth form and syndrome more common and perhaps first derived in the arid lands of South America (Johnston, 1940).

Canotia holacantha is a widespread and occasional dominant in central Arizona where it is characteristic of northern and upper elevations of the Sonoran Desert in desert grassland and juniper-chaparral communities. *Canotia holacantha* in general has a strong affinity for limestone soils, especially in central Arizona (Turner et al., 1995). Plants are often thick-trunked shrubs or small trees 3–6 (occasionally to 10) m tall (Turner et al., 1995). The disjunct populations in the southern

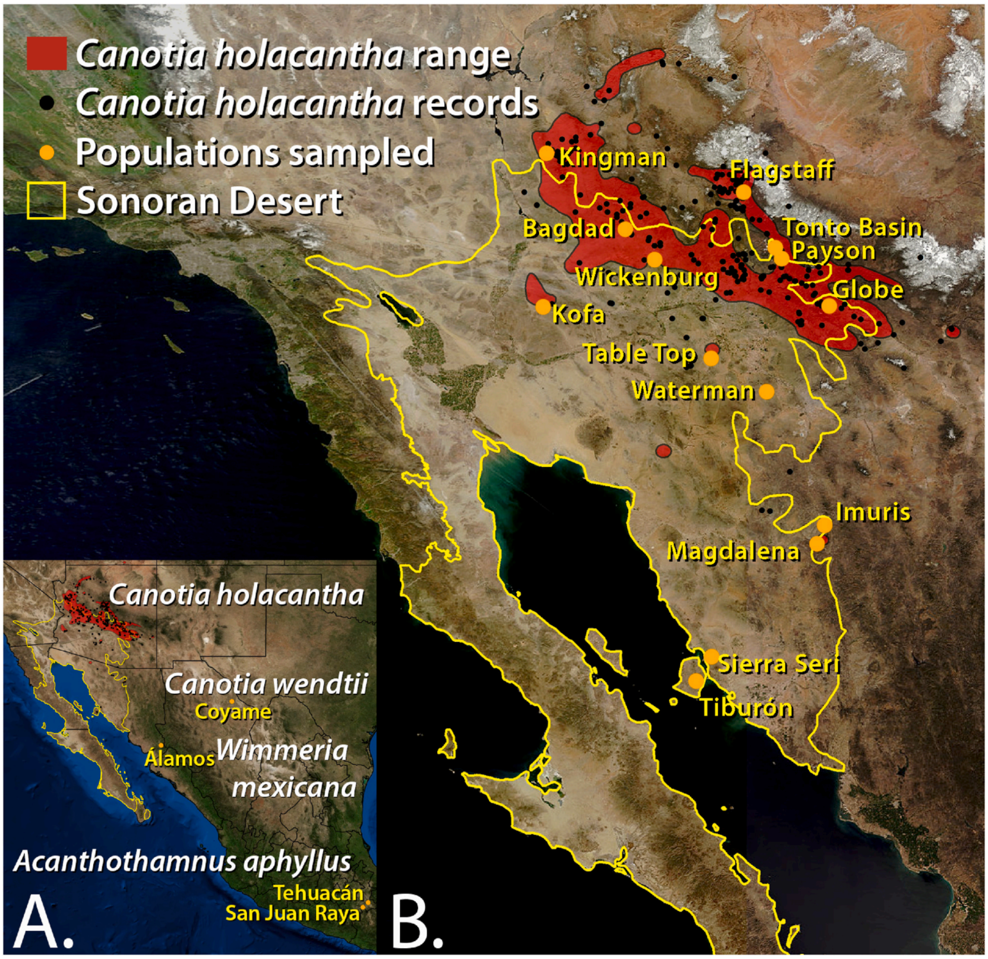

Fig. 1. A and B. Range and populations sampled of *Canotia holacantha* and outgroups. (A) Range of *Canotia holacantha* and *C. wendtii* and sampling locations of *Acanthothamnus aphyllus* and *Wimmeria mexicana*. Note, the range of the outgroup *Euonymus americanus* is not visible on this map. (B) *Canotia holacantha* distribution, the 14 sampled populations are marked by orange dots and population name (Table 1). Black dots are herbarium records of *C. holacantha* (SEINet, 2014) and the red polygon is the present range of *C. holacantha*. Golden outline is boundary of the Sonoran Desert (Shreve, 1951) courtesy of Charlotte González Abraham. Background image courtesy of NASA LANCE Rapid Response MODIS imagery. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

portion of its range in southern Arizona and northern Sonora mostly contain plants that are 0.5–1.3 m tall on exposed ridges, though favorable niches sometimes support small trees ca. 3 m tall (Wilder et al., 2008; Felger and Wilder, 2012). The five-merous white flowers are minute with no clear pollination syndrome. The fruits are persistent woody capsules 1.5–2.0 cm long, with 5 carpels that split apically into awned valves containing seeds 7–8 mm long with a thin, aril-derived basal wing (Coughenour et al., 2010). The seeds are likely gradually or tardily wind-dispersed relatively short distances. The plants are believed to be long-lived (Turner et al., 1995).

Canotia wendtii is a microendemic in the Chihuahuan Desert in the vicinity of Coyame, Chihuahua and the Chihuahua/Texas border near Big Bend National Park. It is reminiscent of a dwarf version of *C. holacantha*, 0.7–1.2 m tall, lacking a winged seed, and found on dry "badland" hillsides of lime-cemented rounded alluvial gravels in typical Chihuahuan Desert vegetation (Johnston, 1975). *Acanthothamnus aphyllus* is likewise a small spiny shrub 1.0–1.5 m tall with photosynthetic stems. This species is restricted to the southern extent of North American deserts in the Mexican states of Hidalgo, Puebla, Querétero, San Luis Potosí, and Tamaulipas (Clevinger and Clevinger, 2010). Johnston (1975:119) states, "The thorny shrubs of *Acanthothamnus* … are essentially holacanthoid, though they have a few, small, scattered leaves. In the minutest details of epidermis, bracts, calyx, petals, stamens, gynophore, ovaries, and ovules, *Acanthothamnus* is a diminutive replica of *Canotia*."

2.2. Sample collections

A total of 74 individuals of *Canotia holacantha* from 14 populations throughout the species range (Figs. 1 and 2; Table 1), with 1–10, and an average of 5 individuals per population were analyzed. Four individuals of *Canotia wendtii* from Coyame, Chihuahua and two *Acanthothamnus aphyllus* from Puebla (one herbarium specimen and one field collection) were also sampled and analyzed. *Wimmeria mexicana* (field collection from the vicinity of Álamos, Sonora) and *Euonymus americanus* (herbarium sample of a collection from Lincoln Parish, Louisiana) were included as outgroups (Table 1). Because *Canotia* usually lacks leaves, collections were made of the photosynthetic stem tissue, which was obtained by scrapping away the thick waxy cuticle with a knife, cleaned with alcohol between samples, and then making thin strips of the green stem tissue. The strips were placed in separate coin envelopes for each individual and kept in silica gel until DNA extraction was performed.

2.3. DNA extraction, sequencing, and alignment

DNA was extracted following the standard CTAB based extraction protocol of Doyle and Doyle (1987) using a bead beater to pulverize the liquid nitrogen-frozen plant tissue. Preliminary studies identified four regions of the chloroplast genome (cpDNA) to be reliably amplified, sequenced, and variable, which were used in this study: three intergenic spacers (atpB-rbcL, trnL-F, rpl20-rps12), and one intron (trnL) (Taberlet et al., 1991; Weising and Gardner, 1999). The chloroplast genome is circular, non-recombinant, and maternally inherited in angiosperms, and continues to be a widely used marker in plant phylogenetics since early successes. A number of studies have shown the chloroplast genome to evolve at a rate that retains intraspecific patterns and processes, allowing biogeographic inference within species (e.g., Fehlberg and Ranker, 2009; Duran et al., 2012).

Fig. 2. *Canotia holacantha* throughout its range. (A) Kingman, AZ; (B) Table Top, AZ, summit of mountain; (C) Silverbell, Waterman Mountains, AZ, with Janos Wilder, making tissue collection; (D) Payson, AZ, *C. holacantha* and saguaro (*Carnegiea gigantea*); (E) Sierra Seri, SON, N-slope of mountain looking N with Gulf of California on left; (F) Isla Tiburón, summit of island with Jose Ramon Torres (left) and Brad Boyle (right), note Canal del Infiernillo and Sierra Seri on mainland Sonora in background; (G) Kofa, AZ, with B. Wilder making tissue collection; (H) Bagdad, AZ with Joshua tree (*Yucca brevifolia*); and (I) Flagstaff, AZ, *C. holacantha* to left of alligator juniper (*Juniperus deppeana*). All photos by B. Wilder except Kofa (bottom right) by Rebecca Wilder.

Each of the four cpDNA fragments were amplified by polymerase chain reaction (PCR). All amplifications had a total volume of 25 μL using: 1.5 μL MgCl2, 0.2 μL deoxynucleotides (dNTPs), 2.5 μL of each primer at 10 μM concentration, 0.2 μL Taq polymerase, 2.5 μL Promega 10 reaction buffer, 1.0 μL Bovine Serum Albumin (BSA), 2.0 μL genomic DNA, and 12.6 μL of autoclaved DI water treated in a UV oven. Thermocycling conditions for the trn L, trnL-F, and atpB-rbcL markers were: 94 °C for 2min; 35 cycles of 94 °C for 15sec, 48 °C for 30sec, 72 °C for 90sec; and 72 °C for 3min. The following PCR profile was used for the rpl20-rps12 marker: 80 °C for 5min; 35 cycles of 94 °C for 30sec, 52 °C for 30sec, 72 °C for 2min; and 72 °C for 5min. Sanger sequencing was carried out on PCR amplicons at the High Throughput Genomics Unit at the University of Washington in Seattle and the University of Arizona Genetics Core. Sequences were assembled and edited in the software Sequencher (version 5.0, Gene Codes Inc., Ann Arbor, MI.). The four cpDNA data sets were combined for phylogenetic analyses in the software MEGA-X (version 10.0, Penn State University) and aligned using Clustal Omega through Geneious Prime (Biomatters, Inc., San Diego, CA.).

2.4. Genetic diversity and historical demography

Population genetic diversity parameters were estimated across study locations for *Canotia holacantha* to identify the number of polymorphic sites (S), number of haplotypes (H), haplotype diversity (Hd), nucleotide diversity (π), and average number of nucleotide differences (k) in the software DnaSP 5 (Librado and Rozas, 2009).

The demographic history of *C. holacantha* was estimated by undertaking (1) neutrality tests and (2) a Bayesian skyline plot. First, to determine whether molecular variation corresponded to signatures of recent population expansion or contraction, we estimated Tajima's *D* and Fu's F_S in DnaSP 5 (Librado and Rozas, 2009). The significance of each neutrality test was assessed with 10,000 coalescent simulations. Tajima's *D* and Fu's F_S are expected to approach zero under constant population size conditions, while significant negative values are signatures of a sudden population expansion and significant positive values indicate population contraction.

Historical population dynamics were estimated using Bayesian skyline plot approach implemented in BEAST 1.8.4 (Drummond and Rambaut, 2007). The substitution model used was HKY + G with estimated base frequencies and piecewise-constant coalescent Bayesian skyline tree prior with five starting groups. We set a strict clock with a uniform prior distribution for the clock rate with an upper and lower mutation rate ranging from 1×10^{-8} to 0.5×10^{-8} substitutions per site per year, respectively. Five independent MCMC runs of 100 million generations each were run, with trees and parameters sampled every 10, 000-iterations, with a burn-in of 20%. We combined log and tree files from each independent run using LogCombiner 1.7.4 (Drummond and Rambaut, 2007), then viewed the combined log file in Tracer 1.7 (Rambaut et al., 2018) to ensure that effective sample sizes (ESS) for all priors and the posterior distribution were >200 and parameter values were fluctuating at stable levels. The Bayesian skyline plot was generated in Tracer 1.5.0 and contraction times were identified from the skyline output as the point when the effective population size started to decrease.

2.5. Phylogenetic analysis

Phylogenetic analyses were performed using maximum parsimony and Bayesian inference. The phylogenetic analyses using the maximum parsimony inference were performed in Mega5 (Tamura et al., 2011) and branch support of the trees was evaluated by bootstrapping with 1000 replicates. The model HKY + G was used to infer phylogenetic relationships using Bayesian inference and was performed using MrBayes on XSEDE v3.2.6 in CIPRES Science Gateway. Markov Chain Monte Carlo (MCMC) simulations were run with two sets of eight chains for 20,000,000 generations, discarding the first 4,000,000 as burn-in, and sampling every 2000 generations to infer the posterior probability distribution. The run parameter convergence was assessed by average standard deviation of split frequencies (<0.005) and ESS >200 examined using the software Tracer 1.5 (Drummond et al., 2012). The trees were visualized with FigTree 1.4.2, with the posterior probabilities used to assess confidence of support for each branch. Trees were rooted using the distantly related Celstraceae taxa *Wimmeria mexicana* and *Euonymus americanus* as outgroups.

To examine the genealogical relationships among the haplotypes of *C. holacantha*, a phylogenetic network was built using Haploviewer program. The construction of the network was done using the genealogy resulting from the Bayesian inference. These networks have the advantage of showing the frequency of analyzed haplotypes and their phylogenetic relationships. In contrast to Neighbor-joining networks which use genetic distances, Haploviewer uses the consensus tree obtained by the phylogenetic inference that is based on evolutionary models of nucleotide substitution.

3. Results

The concatenated data set of four cpDNA fragments for 74 individuals across 14 populations of *Canotia holacantha*, the singular population of *C. wendtii*, two *Acanthothamnus aphyllus* populations, and the outgroups *Wimmeria mexicana* and *Euonymus americanus* consisted of a total aligned length of 2032 bp. Among all sequences there were 15 variable sites including indels.

Three unique haplotypes were identified within *Canotia holacantha* (Figs. 3 and 4). The three haplotypes have strong geographic concordance and were labeled as the Tiburón haplotype, Sonora haplotype that consists of the Sierra Seri, Magdalena, and Imuris populations as well as the Silverbell plants from southern Arizona, and then an Arizona haplotype made up of all remaining populations.

The Bayesian phylogenetic reconstruction (Fig. 4) provides support for the previously identified clade that contains *Acanthothamnus* and *Canotia* (Johnston, 1975, Simmons et al., 2012). A common ancestor for *Canotia* is identified with strong support, as is a well supported and deeply divergent relationship between *Canotia wendtii* and the Isla Tiburón population of *C. holacantha* that predates the relationships of all other *C. holacantha* populations. The rest of *C. holacantha* are more recently diverged with far less genetic differentiation among populations. Two clades are recovered though, one composed of the populations from Sonora, the "Sonora haplotype", which also contains *Acanthothamnus aphyllus*, and is sister to all other *C. holacantha* populations throughout Arizona, the "Arizona haplotype".

There was relatively little genetic diversity among populations of *C. holacantha* (Table 2). Tajima's D was found to not significantly differ from 0, though the stricter Fu's Fs was calculated to be significantly positive, indicating a population contraction. Additional evidence for a population decrease was seen in the Bayesian skyline plot (Fig. 5) with a decline in effective population size starting about 15 kya.

4. Discussion

Our analyses rejected the hypothesis of the C. *holacantha* population on Tiburón island being the result of a recent dispersal event and supported the scenario of the island population as a relict now found in an interglacial microrefugia based on the strong phylogeographic structure observed, low genetic diversity, and the large sequence divergence present in the Tiburón haplotype compared to other nearby *C. holacantha* populations. The genetic characteristics of long-term isolation and divergence of *C. holacantha* from Tiburón is mirrored in the related *C. wendtii*, which is a recognized narrow endemic. Overall, the historical demography of *C. holacantha* differs from the better known glacial refugium phenomenon where molecular data from xerophytic taxa show demographic signs of population contraction 100–15 kya into southern glacial refugia followed by postglacial range expansion during aridification in the Holocene in the Sonoran Desert (e.g., Fehlberg and Ranker, 2009).

The disjunct occurrences of *Canotia*, a desert edge specialist, suggest previously unidentified vegetation patterns and connections in what is currently the lower half of the Sonoran Desert. The current range of *C. holacantha*– a wide distribution in central Arizona and isolated small populations in northern Sonora, including the peaks of Isla Tiburón – along with the enigmatic Chihuahuan microendemic *C. wendtii* and the central Mexican desert *Acanthothamnus aphyllus* hint at a once more widespread occurrence. The results of these chloroplast genetic analyses indeed support a *Canotia* common ancestor in the Sonora-Chihuahua region, indicated by the relict Tiburón and Coyame populations that maintain a signal of past connectivity. The rest of *C. holacantha* populations seem to be the remnants of a general contraction to the northern and upper desert edge ecotone near the start of the Holocene as shown by the positive Fs statistic and the Bayesian skyline plot, that still maintain a relationship to the more southerly aridland *Acanthothamnus aphyllus*. It is possible that we are observing the remaining "tips of the branches" of a *Canotia* common ancestor, the "trunk" of which has been eroded by time through contraction and extirpation.

On the present-day landscape, *Canotia holacantha* occupies ecotones at the northern and eastern upper elevation reaches of the Sonoran Desert where plants often occur on limestone substrates. It is broadly distributed in central Arizona, where the northward gradation upslope between the Sonoran Desert and semidesert grassland, chaparral, and coniferous woodlands is extensive (Brown, 1994). Near Magdalena, Sonora the upper elevational edge of the desert grades into semi-desert

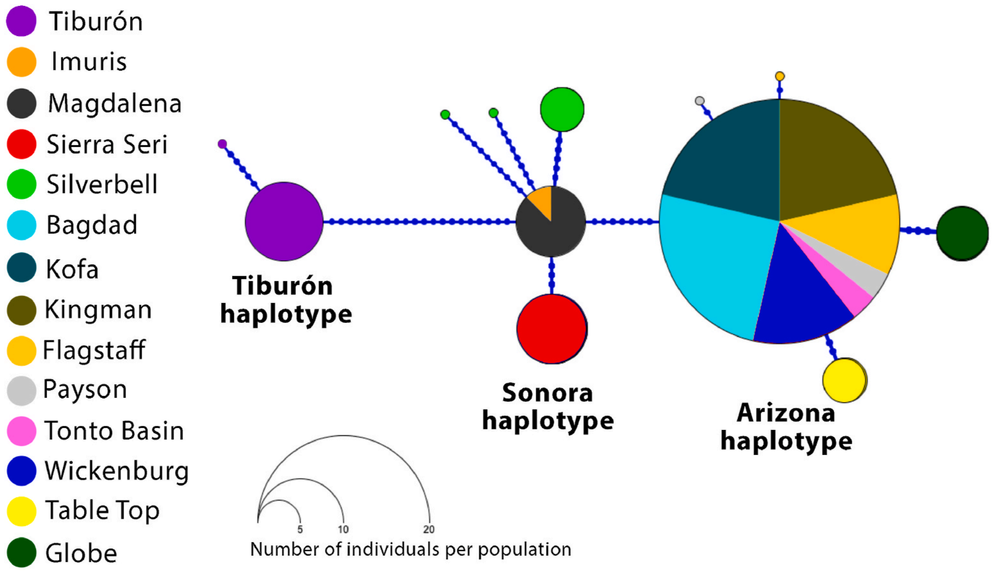

Fig. 3. Haplotype network and associated populations of *Canotia holacantha*. Tick marks between population clusters represent number of substitutions/indels between haplotypes.

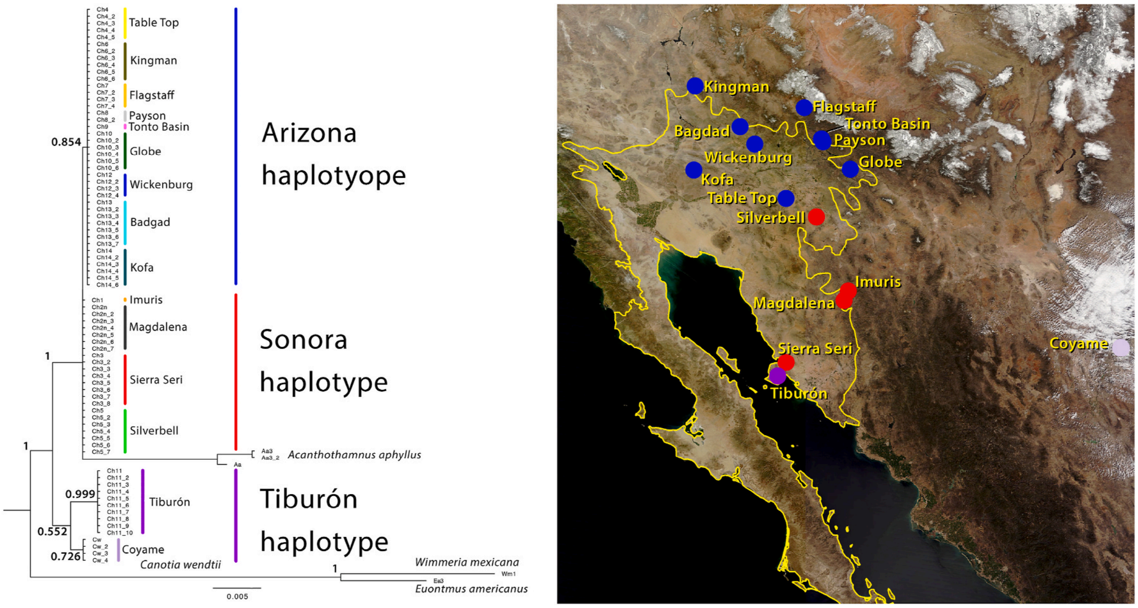

Fig. 4. Phylogenetic structure based on Bayesian inference and geographical pattern of cpDNA results. Golden outline is boundary of the Sonoran Desert as in Fig. 1. Numbers above each node represent Bayesian posterior probabilities. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

grassland to the east. *Canotia holacantha* occurs along this transition just below 1200 m where oaks and junipers become dominant. Populations in southern and western Arizona, northern Sonora, and the coast of the Gulf of California on the Sierra Seri and Isla Tiburón are restricted to isolated desert peaks.

The Isla Tiburón population of *Canotia holacantha* and the Chihuahuan Desert microendemic *C. wendtii* are supported as sister taxa, significantly divergent from all other *C. holacantha* populations (Fig. 4). *Canotia wenditii* is only known from one small region within the Chihuahuan Desert, over 720 km nearly due east of Isla Tiburón, and on the opposite side of one of the greatest vicariant barriers in North America, the Sierra Madre Occidental. The uplift of the Sierra Madre Occidental and Rocky Mountains, and the uplift of the Continental Divide on the Deming Plain in southern New Mexico date to the Miocene (23.0–5.3 Ma; Ferrari et al., 1999). The great geographic distance and intervening Sierra Madre Occidental, and the large genetic divergence observed suggest that the presence of *C. wendtii* in E. Chihuahua is not likely due to a recent long-distance dispersal. A recent dispersal origin is further questioned by the apparent absence of a long-distance dispersal syndrome for *Canotia* and subtle morphological differences between the two species, primarily the lack of a winged seed on *C. wendtii* (Johnston, 1975), that were observed to be maintained in the field.

Results of recent genetic studies of mule deer (*Odocoileus hemionus*) across North America surprisingly reveal a similar disjunct Chihuahua-Tiburón connection as seen in *Canotia* (Latch et al., 2009; Alminas et al., 2021). An endemic subspecies of mule deer, *O. hemionus sheldonii* is present on Isla Tiburón and, like *Canotia* on the island, was shown to genetically vary from mule deer on the adjacent mainland (Alminas

Table 1
Locality information for population samples.

Species	Locality Name	State	Population Code	Number of Individuals	Latitude	Longitude	Elevation (m)	Herbarium Voucher[a]
Canotia holacantha	Imuris	Sonora, MX	Ch1	1	30.76241	−110.78475	1040	Wilder 10-541
Canotia holacantha	Magdalena	Sonora, MX	Ch2n	7	30.56362	−110.87667	829	Wilder s.n.
Canotia holacantha	Sierra Seri	Sonora, MX	Ch3	8	29.21498	−112.1274	610	Wilder 11-238
Canotia holacantha	Table Top	Arizona, USA	Ch4	5	32.74898	−112.1285	1332	Wilder 11-260
Canotia holacantha	Silverbell	Arizona, USA	Ch5	7	32.35134	−111.46812	983	Wilder 11-268
Canotia holacantha	Kingman	Arizona, USA	Ch6	6	35.17215	−114.07588	1025	Wilder 12-240
Canotia holacantha	Flagstaff	Arizona, USA	Ch7	4	34.70864	−111.74116	1263	Wilder 12-247
Canotia holacantha	Payson	Arizona, USA	Ch8	2	34.03617	−111.36568	992	Wilder s.n.
Canotia holacantha	Tonto Basin	Arizona, USA	Ch9	1	33.96975	−111.32192	779	Wilder s.n.
Canotia holacantha	Globe	Arizona, USA	Ch10	6	33.37453	−110.74232	1110	Wilder 12-253
Canotia holacantha	Tiburón	Sonora, MX	Ch11	10	28.92912	−112.31796	600	Wilder 12-107
Canotia holacantha	Wickenburg	Arizona, USA	Ch12	4	33.92119	−112.79398	768	Wilder s.n.
Canotia holacantha	Bagdad	Arizona, USA	Ch13	7	34.29412	−113.11359	802	Wilder s.n.
Canotia holacantha	Kofa	Arizona, USA	Ch14	6	33.36066	−114.10135	680	Wilder s.n.
Canotia wendtii	Coyame	Chihuahua, MX	Cw1	4	29.54956	−104.92725	910	Wilder 11-193
Acanthothamnus aphylllus	Tehuácan	Puebla, MX	Aa1	1	18.472222	−97.436389	1730	Chiang F2271
Acanthothamnus aphylllus	San Juan Raya	Puebla, MX	Aa3	1	18.308306	−97.634325	1720	Valiente s.n.
Wimmeria mexicana	Álamos	Sonora, MX	Wm1	1	27.06233	−108.75198	568	Wilder s.n.
Euonymus americanus	NA	Louisiana, USA	Ea1	1	32.59868	−92.69847	77	Rhodes 20571

[a] Herbarium vouchers deposited at the University of Arizona Herbarium (ARIZ).

Table 2
General genetic diversity results for *Canotia holacantha*.

Genetic diversity parameter	*Canotia holacantha*
Number of polymorphic sites (S)	15
Number of haplotypes (H)	3
Haplotype Diversity (Hd) ± SD	0.586 ± 0.00140
Nucleotide Diversity (π) ± SD	0.00201 ± 0.0000002
Average number of nucleotide differences (k)	3.818
Tajima's D (P value)	0.69488 (>0.10)
Fu's Fs (P value)	1.58357 (<0.05)

et al., 2021). Twenty-eight samples of mule deer from Isla Tiburón form a monophyletic clade that nests within a distinct haplogroup of mule deer restricted to the Guadalupe Mountains of western Texas in the Chihuahuan Desert (Alminas et al., 2021).

The Guadalupe Mountains are the exposed portion of a Permian aged limestone reef, ca. 775 km to the northeast of Tiburón and ca. 260 km due N of *C. wendtii*. Latch et al. (2009) attribute the distinctiveness of the Guadalupe Mountains mule deer haplogroup to its likely persistence through time. Their molecular data suggests this population to be older than those in the surrounding areas (15,900 ybp; 95% CI: 25,100–5300; Alminas et al., 2021). Similarly, molecular and fossil midden results support the Guadalupe Mountains as a glacial refugia for piñon pine (*Pinus edulis*; Duran et al., 2012). Fossil packrat midden studies indicate that plant communities in the Guadalupe Mountains changed from a relatively mesic woodland at the late Wisconsin (ca. 25,000 to 11,000 ya) to present day xeric Chihuahuan Desert vegetation (Van Devender, 1977). Midden records from this time period, although not a continuous record, indicate wetter conditions than today in the early Holocene followed by increasing aridity and an abrupt expansion of desert shrubs elsewhere in the northern Chihuahuan Desert not until ca. 5000–4000 yr BP, due to either a gap in midden coverage and or delayed onset of arid conditions (Holmgren et al., 2007).

North of the Sierra Madre Occidental, the Chihuahuan and Sonoran Deserts are separated by a low-elevation gap in the Continental Divide in

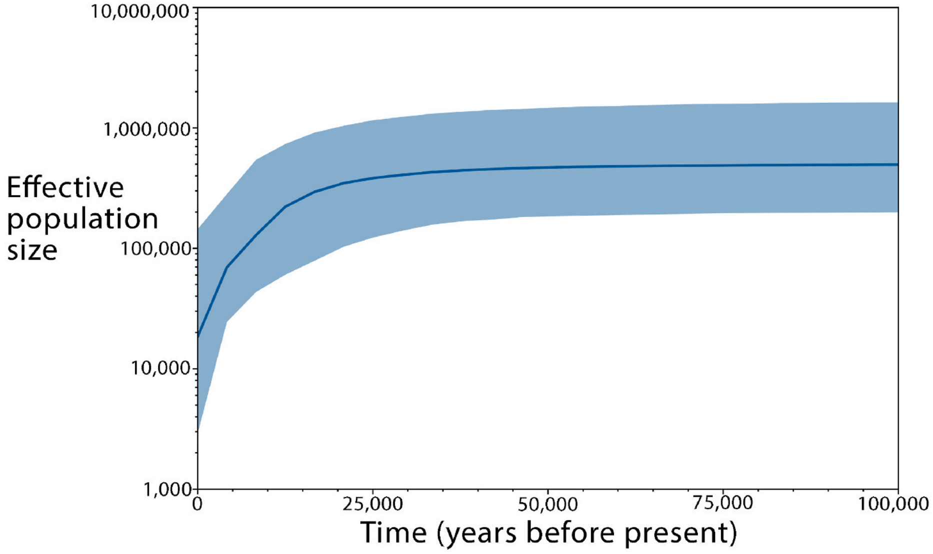

Fig. 5. Skyline plot showing effective population through time of *Canotia holacantha*.

the vicinity of the Deming Plains of southern New Mexico, termed the Cochise filter-barrier (Hafner and Riddle, 2011; Provost et al., 2021). During glacial periods the closed basins of this area filled with pluvial lakes (e.g., Smith and Street-Perrott, 1983) and C_4-dominated grasslands were present due to the likely persistence of the North American monsoon throughout the last glacial-interglacial cycle (Holmgren et al., 2007). Relatively mesic vegetation predominated, effectively closing the pass to arid elements (Hafner and Riddle, 2011). During interglacials, the lakes became playas and the filter barrier was open to arid-adapted taxa. At such times throughout the Pleistocene the Cochise filter-barrier has likely permitted the repeated dispersal of Sonoran elements east and Chihuahuan elements north and west (Hafner and Riddle, 2011; Provost et al., 2021), though no *Canotia* remains have been found in middens from this region.

The only fossil record for *Canotia holacantha* are 11,100 yr B.P. twig fragments in a fossil packrat midden from Picacho Peak, Arizona, which anchors its occurrence in Arizona by at least this time (Van Devender et al., 1991). Aside from Tiburón, all other *C. holacantha* populations as well as the two *Acanthothamnus aphyllus* populations analyzed here form a strongly supported clade with two distinct genetic groups, the Sonoran clade/haplotype that includes *A. aphyllus* and the Arizona clade/haplotype (Fig. 4).

The Sonoran clade is found in the isolated southern populations, including the Sierra Seri opposite Isla Tiburón as well as the Silverbell population in the Waterman Mountains just outside Tucson, Arizona. The Sierra Seri population, despite being found on an isolated desert peak of similar composition and geology as Isla Tiburón, is shown to not be sister to the island population as geography would lead one to believe. These two populations are separated by the narrow and shallow Canal del Infiernillo, a land-bridge connecting Tiburón to the mainland up until as recently as ca. 6 kya with the flooding of the canal (Felger and Wilder, 2012). The well-developed differentiation of *C. holacantha* on Tiburón is likely due to repeated insularity and other factors that contradict the concept of Isla Tiburón being hardly distinguishable from a chunk of mainland.

The paleovegetation of the Silverbell mountains (also known as the Watermans), an isolated block of uplifted and fractured limestone just to the northwest of Tucson, is fairly well known (Anderson and Van Devender, 1991) and provides some context for the occurrence of *C. holacantha* in these desert peaks. As in much of the northern Sonoran Desert, most woodland plants (*Juniperus*, *Berberis*, *Quercus turbinella*) disappeared by 8910 ya, and a Sonoran desertscrub of cat-claw acacia (*Senegalia* [*Acacia*] *greggii*) and brittle bush (*Encelia farinosa*) in association with saguaro (*Carnegiea gigantea*) became prevalent; blue paloverde (*Parkinsonia florida*) and creosote bush (*Larrea divaricata*) arrived by 6200 ya (Anderson and Van Devender, 1991). The preference of *Canotia* for limestone likely explains its continued presence in the Waterman Mountains despite its disappearance throughout nearly all of the adjacent low-lying areas.

The present-day center of distribution for *C. holacantha* in central and northern Arizona is genetically uniform, the populations of which form the Arizona clade. This clade encompasses widely spaced populations from the northeast to the southwest (e.g., Kingman and Globe) and those that are topographically isolated (Kofa and Table Top mountains). This range coincides with the broad ecotone where the Arizona Uplands subdivision of the northern edge of the Sonoran Desert merges with the colder and wetter interior chaparral or semidesert grassland (Brown, 1994). It is clear this is the preferred habitat of *C. holacantha* and that it can be extrapolated that this preference has been stable through time, though the location of these conditions has shifted. It should be noted that an increased fire regime in the northern Sonoran Desert largely driven by invasive grass species, as in many arid regions of the west (Fusco et al., 2019; Wilder et al., 2021), could shape the future trajectory of this and other desert edge species in ways that are not yet clear.

The taxonomic implications of the relationship of *C. wendtii* and the Tiburón *C. holacantha* population and *Acanthothamnus aphyllus* within the Sonoran clade would suggest the elevation of the Tiburón population to specific status and subsuming *Acanthothamnus* within *Canotia*. In both cases additional taxonomic and genetic work is recommended before such a revision is made, especially in regard to *Acanthothamnus*, though as noted by Johnston (1975) this relationship has been suspected.

5. Conclusion

The genetic signatures of diverse taxa provide an indication of how desert environments and arid-adapted taxa have shifted in response to post-glacial climatic change throughout the Pleistocene. Analysis of the chloroplasts of *Canotia holacantha* and related taxa suggests a long and likely much more widespread presence across the region. The disjunct connection between the northern portion of the Chihuahuan Desert, Isla Tiburón, and the southern Mexican deserts home to *Acanthothamnus aphyllus* hints at a fascinating story of past distributions and refugia. It is possible their common ancestor was once widespread across the landscape, and as climates changed the range retracted, leaving populations confined to the insular refugia of Tiburón Island, thousands of kilometers away in the limestone hills of the Chihuahuan Desert, and more amply in the arid valleys of central and southern Mexico. The timing of this is not clear and could date to the current or previous interglacials. It seems that starting about 15 kya the mainland populations of this arid-adapted hardwood perennial contracted and tracked the northern or temperate boundary of the Sonoran Desert as it concentrated between 900 and 1200 m in recent millennia. *Canotia* is an exemplar taxon to understand how more mesic elements in the Sonoran Desert and neighboring floras responded to climatic changes of the Pleistocene and the shifting boundaries of the desert today, in the past, as well as in the future.

Credit authorship contribution statement

Benjamin T. Wilder: conceived of the study and conducted the field work, undertook analyses, data interpretation, and manuscript preparation. **Amanda T. Becker:** undertook analyses, data interpretation, and manuscript preparation. **Adrian Munguia-Vega:** undertook analyses, data interpretation, and manuscript preparation. **Melanie Culver:** undertook analyses, data interpretation, and manuscript preparation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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