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Population genetics and phylogenetic relationships of beetles (Coleoptera: Histeridae and Staphylinidae) from the Sonoran Desert associated with rotting columnar cacti

Edward Pfeiler [a,*], Sarah Johnson [b], Maxi Polihronakis Richmond [b], Therese A. Markow [b,c]

[a] Centro de Investigación en Alimentación y Desarrollo, A.C., Unidad Guaymas, Apartado Postal 284, Guaymas, Sonora 85480, Mexico
[b] Division of Biological Sciences, University of California, San Diego, La Jolla, CA 92093, USA
[c] Laboratorio Nacional de Genómica de Biodiversidad, CINVESTAV, Irapuato, Guanajuato CP 36821, Mexico

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ABSTRACT

Dozens of arthropod species are known to feed and breed in the necrotic tissues (rots) of columnar cacti in the Sonoran Desert. Because the necrotic patches are ephemeral, the associated arthropods must continually disperse to new cacti and therefore the populations of any given species are expected to show very little local genetic differentiation. While this has been found to be true for the cactophilic *Drosophila*, the evolutionary histories and characteristics of other arthropods inhabiting the same necrotic patches, especially the beetles, have yet to be examined. Here we used nucleotide sequence data from segments of the mitochondrial 16S rRNA and cytochrome *c* oxidase subunit I (COI) genes to examine population structure and demographic history of three sympatric beetle species (Coleoptera: Histeridae and Staphylinidae) collected on senita cactus (*Lophocereus schottii*) from six widely-separated localities on the Baja California peninsula of northwestern Mexico. Two histerids, *Iliotona beyeri* and *Carcinops gilensis*, and an unidentified staphylinid, *Belonuchus* sp., showed little or no population structure over a broad geographic area on the peninsula, consistent with the prediction that these beetles should show high dispersal ability. Demographic tests revealed varying levels of historical population expansion among the beetle species analyzed, which are discussed in light of their ecologies and concurrent biogeographic events. Additionally, phylogenetic analyses of COI sequences in *Carcinops* collected on a variety of columnar cacti from both peninsular and mainland Mexico localities revealed several species-level partitions, including a putative undescribed peninsular species that occurred sympatrically with *C. gilensis* on senita.

1. Introduction

Desert regions worldwide provide an ideal setting for investigating the evolution, speciation and interaction of organisms adapted to extreme environmental conditions. Specifically, elucidating the historical and contemporary factors that have influenced genetic divergence among populations can provide insight into desert community ecology. The Sonoran Desert of North America is particularly rich in both plant and animal diversity compared with most desert regions of the world (Phillips and Comus, 2000; Brusca and Moore, 2013), making it especially attractive for in-depth ecological studies. In addition, the Gulf of California (also known as the Sea of Cortez) divides the Sonoran Desert into two major geographic regions, the Baja California peninsula and mainland Mexico, providing an important landscape feature for evaluating the role of vicariance in shaping genetic differentiation among populations of organisms inhabiting both regions. Because the chronology of the geological events resulting in the separation of the peninsula from the mainland is reasonably well known (Ledesma-Vázquez and Carreño, 2010), it is possible to calibrate DNA sequence evolution in sister species separated by the formation of this potential biogeographic barrier.

In the Sonoran Desert, an interesting ecological interaction has evolved involving necrotic pockets (rots) of injured tissue of various species of columnar cacti and a diverse community of desert arthropods dependent on the rots (Breitmeyer and Markow, 1998; Pfeiler and Markow, 2011). While the different arthropod species vary in features such as their trophic levels, life history characters, dispersal mechanism, and size, all are linked together in their dependence on necrotic cacti to complete their life cycle. Developing cactus necroses provide an abundant source of moisture and food, and thus represent an important microhabitat for these arthropods which are attracted to, and feed upon, the decomposing plant material. Mating often takes place within the necrotic

* Corresponding author. Fax: +52 622 225 2828.
E-mail address: pfeiler@ciad.mx (E. Pfeiler).

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pocket, and the immature stages (eggs, larvae and pupae) and adults, predominately dipterans, provide a rich food source for predatory species, including beetles and pseudoscorpions (Castrezana and Markow, 2001; Pfeiler et al., 2009, 2010). The specialized necrotic cactus microhabitat is inhabited by approximately 40 arthropod species, mostly insects, comprising 23 families and 10 orders (Castrezana and Markow, 2001). Cactus necroses, however, are patchily distributed and ephemeral, lasting from less than three months in senita (*Lophocereus schottii*) to almost a year in the more massive cardón (*Pachycereus pringlei*) and saguaro (*Carnegiea gigantea*) cacti (Breitmeyer and Markow, 1998). Although senita rots are small and relatively short-lived, distances between rots are generally less, and rot frequency is generally greater, than in the larger columnar cacti (Breitmeyer and Markow, 1998). The discrete nature of the necrotic cactus niche, and the spatial and temporal patterns of resource availability, lead to the prediction that the community of desert arthropods dependent on cactus rots for feeding and reproduction would have strong dispersal capability and high genetic connectivity among populations, thus increasing their chances of successful colonization of fresh rots. This prediction is borne out by results from population genetic studies conducted on a variety of cactophilic dipterans and one species of pseudoscorpion (reviewed in Pfeiler and Markow, 2011), but it has never been tested on the cactophilic coleopterans. Because our ability to infer evolutionary processes from population genetic data is greatly enhanced when we understand a species' ecology, the current study focuses on analyzing and comparing the population structure of several beetle species inhabiting the necrotic niche. This provides an opportunity to disentangle the effect of ecological variables on the evolutionary trajectory of desert species, using molecular approaches.

Specifically, the goals of this study were to utilize mitochondrial DNA (mtDNA) sequence data (1) to test the prediction that three cactophilic beetle species from the Baja California peninsula region of the Sonoran Desert that occur sympatrically on senita necroses (*Iliotona beyeri*, *Carcinops gilensis* and *Belonuchus* sp.) will show similar patterns of population structure as a result of utilization of the same ecological niche, and (2) to obtain estimates of the demographic history of each species. Additionally, because our survey revealed several morphospecies of cactophilic *Carcinops* collected on a variety of columnar cacti from both peninsular and mainland Mexico localities in the Sonoran Desert which were distinct from *C. gilensis*, the final aim was to elucidate the phylogenetic relationships among these specimens, and test whether they represent distinct genetic lineages.

2. Material and methods

2.1. Samples

Beetles were collected during our 2002–2003 survey of the arthropod fauna associated with cactus necroses in the Sonoran Desert of northwestern Mexico, including the peninsular states of Baja California and Baja California Sur, and the Guaymas region of mainland Sonora (Fig. 1). Here we refer to the northern peninsular state of Baja California as Baja California "Norte" (BCN) to avoid confusion with the "Baja California peninsula". All adult beetles found in a thorough search of 3–5 cactus rots per locality were removed, placed in 95% ethanol, and separated into morphologically similar groups in the field. Histerid beetles were identified in the laboratory using both morphological and molecular (mtDNA) characters (Swanson, 2008; Pfeiler et al., 2010). Ethanol-preserved staphylinids were examined independently by three beetle specialists who confirmed that they belonged to the genus *Belonuchus*, but of unknown species status. Because we were conducting a large-scale arthropod survey and not focusing on any particular species, sample sizes and species composition varied with collection locality. The entire data set comprised 108 beetles (Table 1).

On the Baja California peninsula, most of the *I. beyeri* were collected from senita; one individual, however, was found on pitahaya agria (*Stenocereus gummosus*) at San Quintín, BCN (Pfeiler et al., 2010). All peninsular samples of *C. gilensis* and *Belonuchus* sp. were collected on senita. The mainland sample of *C. gilensis* was collected at Guaymas, Sonora on cardón. The other mainland lineages of *Carcinops* were collected on cardón (*C. consors*), organ pipe, *Stenocereus thurberi*, (*Carcinops* sp. 3), and senita or organ pipe (*Carcinops* sp. 2); the peninsular *Carcinops* sp. 1 was found on senita together with *C. gilensis*. Of the beetle species treated here, only *C. gilensis* was found on both the Baja California peninsula and the mainland (Table 1).

Although several varieties of senita have been recognized (Lindsay, 1963), and allozyme studies suggest distinct mainland and peninsular phylogroups (Nason et al., 2002), we do not distinguish among these varieties as they have no apparent effect on population genetic structure of the beetles reported on here, consistent with findings on the senita-dependent *D. pachea* from the Baja California peninsula (Hurtado et al., 2004; Pfeiler et al., 2007). We also use the name *Lophocereus* for senita throughout, but recognize that there is uncertainty surrounding the correct genus assignment (Hartmann et al., 2002; Arias et al., 2003).

Iliotona beyeri (Schaeffer) (Histeridae; Histerinae; Hololeptini) is a medium-size histerid beetle (ca. 10–15 mm in length) originally described as *Hololepta* (*Lioderma*) *beyeri* (Schaeffer, 1907) based on samples collected by Gustav Beyer in 1901 from Santa Rosa, Lower California [Baja California Sur] (Michelbacher and Ross, 1942). Santa Rosa is a Cape Region locality ("rancho") about 40 km SW of our sampling site at Ensenada de los Muertos (Fig. 1). Although peninsular *I. beyeri* was later placed into synonymy with mainland *I. dorcoides* (Mazur, 1984), both morphological and genetic differences between the two taxa support the species status of *I. beyeri* (Schaeffer, 1907; Pfeiler et al., 2010). Available distributional and ecological data also suggest that *I. beyeri* may be restricted to the peninsula, and that it is closely associated with senita (Pfeiler et al., 2010).

Carcinops gilensis (LeConte) (Histeridae: Dendrophilinae; Paromalini) is a much smaller histerid beetle (ca. 2–3 mm in length) and apparently more widely distributed than *I. beyeri*, occurring from southern California and southern Arizona in the USA to the Baja California peninsula and Sonora in Mexico (Moore, 1937; Swanson, 2008). Field data suggest that *C. gilensis* is less hostplant specific than *I. beyeri*, having been found in necrotic tissue of cardón in Sonora, saguaro in southern Arizona, prickly-pear cactus (*Ferocactus* sp.) in desert regions of southern California (Swanson, 2008; M.S. Caterino, personal communication), and senita on the Baja California peninsula (present study).

The identity of the staphylinid *Belonuchus* sp. (Staphylinidae; Staphylininae; Staphylinini) (ca. 16–20 mm in length) remains uncertain. *Belonuchus ephippiatus* Say is associated with rotting cacti in San Diego County, California (Moore, 1937; M.S. Caterino, personal communication), and is also recorded from Baja California Sur (Márquez, 2006), but our specimens do not correspond to that species (J. Márquez, personal communication). Thus, this is a putative new species of *Belonuchus* which is found throughout the Baja California peninsula (Table 1). Further investigation and field collections are necessary to determine whether this is a specialist species restricted to senita, and/or to the peninsula.

2.2. Molecular protocol and data analysis

Total genomic DNA was extracted from thoracic muscle or legs of each beetle using the DNeasy™ (QIAGEN Inc., Valencia, CA) pro-

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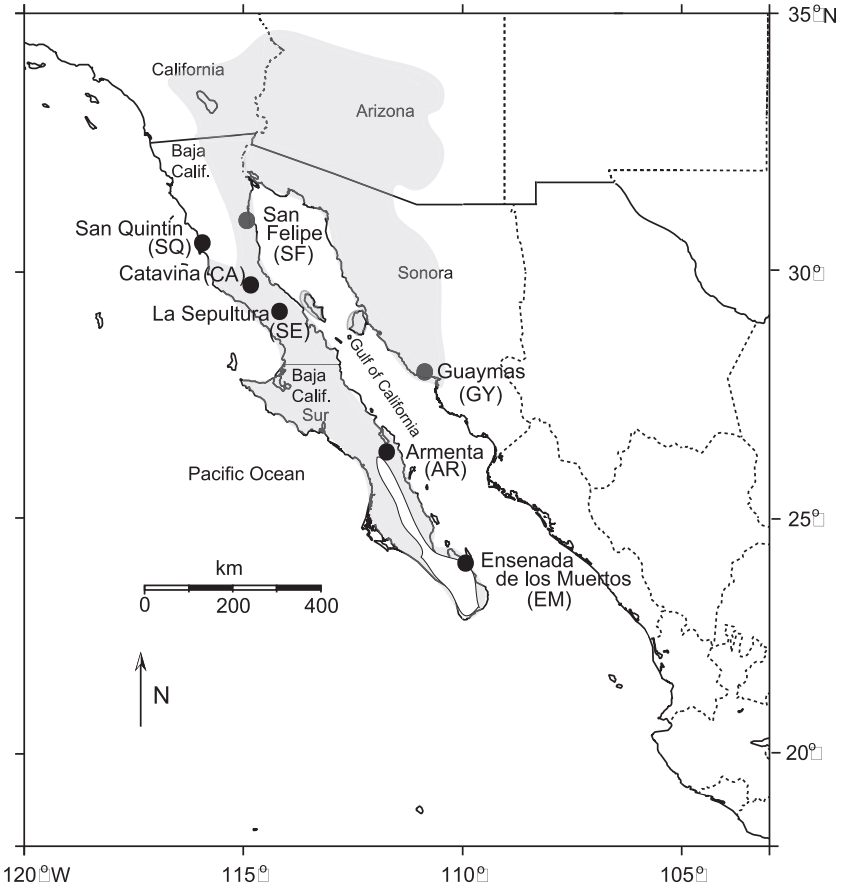

Fig. 1. Map showing localities in northwestern Mexico where necroses of columnar cacti were sampled for *Iliotona beyeri*, *Carcinops* spp. and *Belonuchus* sp. The number of each species analyzed per locality is given in Table 1. The shaded area shows the approximate boundary of the Sonoran Desert. Locality abbreviations are given in parentheses.

Table 1
Summary of numbers of cactophilic beetle species from each Sonoran Desert locality.

Species	Locality							Total
	SF	SQ	CA	SE	AR	EM	GY	
Iliotona beyeri	18	1	4	4	3	5	–	35
Carcinops gilensis	8	–	5	4	1	9	8	35
Carcinops consors	–	–	–	–	–	–	4	4
Carcinops sp. 1	4	–	–	–	4	1	–	9
Carcinops sp. 2	–	–	–	–	–	–	5	5
Carcinops sp. 3	–	–	–	–	–	–	2	2
Belonuchus sp.	5	–	1	3	4	5	–	18

Locality abbreviations are given in Fig. 1. All localities, except GY, are on the Baja California peninsula. Data for *I. beyeri* are from Pfeiler et al. (2010).

tocol. The polymerase chain reaction (PCR) was used to amplify two mitochondrial gene segments using the primer pairs 16Sar/16Sbr for 16S rRNA (Palumbi, 1996) and LCO1490f /HCO2198r for cytochrome *c* oxidase subunit I (COI) (Folmer et al., 1994). Details of PCR and sequencing reactions are found in Pfeiler et al. (2010). Sequences were proofread and aligned in ClustalX 1.81 (Thompson et al., 1997) followed by manual editing. COI sequences for all species were translated in MEGA version 5.0.5 (Tamura et al., 2011). No frameshifts or stop codons were found in any of the sequences. CG content ranged from 37% to 39%. Together these results suggest that our sequences represent mtDNA, and are not nuclear mitochondrial pseudogenes (numts) which have been reported for the COI gene in insects (Song et al., 2008). GenBank accessions numbers for 16S rRNA and COI from the beetle species treated here are given in Table S1 of Supplementary material.

Calculations of Kimura (1980) 2-parameter (K2P) genetic distances (*d*) were carried out in MEGA. Calculations of genetic diversity indices were performed in DnaSP version 5.10.01 (Librado and Rozas, 2009). Neutrality tests [Tajima's (1989) *D* and Fu's (1997) F_S] were carried out in ARLEQUIN version 3.5.1.3 (Excoffier and Lischer, 2010). Fu's Fs test is also useful for detecting signatures of population expansions signified by large negative values in the test statistic (Fu, 1997; Ramos-Onsins and Rozas, 2002). The significance of F_S at the 0.05 level is indicated when *P* values are <0.02 (Excoffier and Lischer, 2010). Haplotype networks were constructed using statistical parsimony implemented in TCS version 1.21 (Clement et al., 2000). The connection limit among haplotypes was set to the default value of 95%.

2.3. Phylogenetic analyses

Phylogenetic relationships among species and putative species of *Carcinops* based on COI sequences were assessed with Bayesian inference and maximum parsimony (MP). *Iliotona beyeri* (GenBank GU982700) was used as the outgroup. Bayesian analyses were implemented in MrBayes version 3.1 (Huelsenbeck and Ronquist, 2001). The model of nucleotide substitution that best fit the data set, determined with jModelTest 0.1.1 (Posada, 2008) using the Akaike Information Criterion, was GTR + I. Analyses were run for 1,000,000 generations, sampled every 250th generation (4000 trees sampled), using the default random tree option to begin the analysis. Clade support, expressed as posterior probabilities, was estimated utilizing a Markov chain Monte Carlo (MCMC) algo-

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rithm. The MP analyses were carried out in MEGA using the CNI heuristic search option and 100 random additions of sequences. Relative support for tree topology was obtained by bootstrapping (Felsenstein, 1985) using 1000 pseudoreplicates. Phylogenetic relationships among several species of the cactophilic Hololeptini from the Sonoran Desert, including the monophyletic *I. beyeri*, have been reported previously (Pfeiler et al., 2010).

2.4. Population structure

Hierarchical analysis of molecular variance (AMOVA, Excoffier et al., 1992) of 16S rRNA sequences, performed in ARLEQUIN, was used to test for population structure in the peninsular *I. beyeri* for the five populations where $N \geqslant 3$ (total $N = 34$). Populations were divided into two regional groups: Baja California "Norte" (SF, CA and SE) and Baja California Sur (AR and EM) (Fig. 1). The hierarchical AMOVA partitioned genetic variation among localities relative to the total sample (Φ_{ST}), among localities within regions (Φ_{SC}), and among regions (Φ_{CT}). The calculation of significance of the fixation indices Φ_{ST}, Φ_{SC}, and Φ_{CT} ($\alpha = 0.05$) was based on 10,000 permutations of the data matrix. The significance of population pairwise comparisons of Φ_{ST} was assessed using a sequential Bonferroni correction for multiple comparisons (Rice, 1989). Estimates of the number of migrants per generation (N_m) among populations were also calculated in ARLEQUIN. Hierarchical AMOVA of the COI data sets for *C. gilensis* ($N = 34$) and *Belonuchus* sp. ($N = 17$) were conducted as described above, except that the mainland population of *C. gilensis* from Guaymas (GY) was added as a third regional group.

2.5. Demographic history

Changes in effective female population size (N_{ef}) over time in *I. beyeri*, *C. gilensis* and *Belonuchus* sp. were estimated using (a) the mismatch distribution (Rogers and Harpending, 1992; Harpending, 1994) performed in ARLEQUIN, (b) the program FLUCTUATE version 1.4 (Kuhner et al., 1998) and (c) Bayesian skyline analysis implemented in BEAST version 1.3 (Drummond et al., 2005).

For the mismatch distribution, the significance of the estimated parameters of the sudden expansion model was obtained from the sum of square deviations (*SSD*) statistic and the raggedness statistic (*rg*), and their corresponding *P* values. The sudden expansion model is rejected when $P < 0.05$. FLUCTUATE provides simultaneous maximum-likelihood estimates of the mutation parameter θ (where $\theta = 2N_{ef}\mu$) and the exponential population growth parameter (*g*). After preliminary test runs, we selected a final extended run of ten short chains of 100,000 steps each and two long chains of 200,000 steps each, sampling every 20th step. Initial estimates of θ were based on number of segregating sites (Watterson, 1975), with the random tree default setting selected for the starting genealogy. Bayesian skyline analysis utilizes Markov chain Monte Carlo (MCMC) sampling of sequence data to estimate a posterior distribution of effective population size through time (Drummond et al., 2005). Bayesian skyline analyses were run using either the HKY + I + Γ (*I. beyeri* and *Belonuchus* sp.) or GTR + I (*C. gilensis*) substitution models. Five million iterations of the MCMC chains were run and sampled every 1000 iterations. The Bayesian skyline plots were generated with TRACER version 1.5 (Drummond et al., 2005).

2.6. Molecular clock calibration

Genetic divergences (K2P distances) in sister species of *Iliotona* separated by the Gulf of California, *I. beyeri* (peninsula) and *I. dorcoides* (mainland) (Pfeiler et al., 2010), were used to estimate nucleotide substitution rates in both the 16S rRNA and COI gene segments. Although a single "standardized" rate of 2.3% pairwise sequence divergence per million years (My) for mitochondrial DNA (Brower, 1994) is often used in insects, rates in separate mitochondrial genes often differ from this value (Papadopoulou et al., 2010; Pfeiler et al., 2010; Pons et al., 2010).

The complex geological history of the tectonic separation of the peninsula from the mainland, resulting in the formation of the Gulf of California, has been dated to ca. 5–8 million years ago (Mya) (Holt et al., 2000; Riddle et al., 2000; Oskin and Stock, 2003). Mean K2P distances found between *I. beyeri* and *I. dorcoides* were 7.9% and 14.6% for 16S rRNA and COI, respectively (Pfeiler et al., 2010). Using 5–8 Mya as a calibration time frame, pairwise divergences of 1.0–1.6% My^{-1} (mean 1.3% My^{-1}) for 16S rRNA and 1.8–2.9% (mean 2.4% My^{-1}) for COI were obtained. Our calibration for the 16S rRNA segment matches closely the value of 1.06% My^{-1} obtained by Papadopoulou et al. (2010) for six genera of tenebrionid beetles from the Aegean archipelago, although different methodologies were used in the calibrations. Our calibration for the COI segment, although almost identical to that of Brower's (1994) "standard" rate, is lower than the pairwise value of 3.54% My^{-1} obtained by Papadopoulou et al. (2010), and substantially lower than the single lineage value of 8.61% My^{-1} obtained by Pons et al. (2010) based on analysis of deeply divergent lineages of Coleoptera. Separate calibrations were not made for *Belonuchus*, owing to a lack of an appropriate sister species from the mainland, or for *Carcinops* because of evidence (presented herein) that some members of this genus apparently can disperse across the Gulf. Thus, for all demographic tests on the three species we used a neutral mutation rate per site per generation (μ) of 6.5×10^{-9} for 16S rRNA and 1.2×10^{-8} for COI, based on the assumption of a single generation per year in each species.

3. Results

3.1. Sequence analysis

Genetic diversity indices and results of neutrality tests (Tajima's *D* and Fu's F_S) for 16S rRNA and COI in *I. beyeri*, *C. gilensis*, and *Belonuchus* sp. (COI only; 16S rRNA sequences were ambiguous and were deleted) are shown in Table 2. Diversity indices for COI are also shown for the unidentified peninsular *Carcinops* sp. 1 collected on senita (see Table 1 and Section 3.2). Genetic diversity in the COI segment was much higher in *Carcinops* spp. and *Belonuchus* sp. compared with *I. beyeri*. The same trend, although less pronounced, was also seen in the 16S rRNA data set of *C. gilensis* (comprised of only peninsular samples) compared with *I. beyeri*. With the exception of the COI segment of *I. beyeri* and *C. gilensis*, Tajima's *D* was not significant for either gene. A significant value for Fu's F_S was seen only for COI in *C. gilensis*.

Haplotype networks for the 16S rRNA and COI genes in *I. beyeri*, *C. gilensis* and *Belonuchus* sp. are shown in Fig. 2. All haplotypes obtained for each species were connected at the 95% connection limit within their respective network, indicating that they were closely related. Several different patterns, however, can be seen among networks. *Iliotona beyeri* is characterized by low COI haplotype diversity (Table 2) and the presence of a single dominant haplotype (Fig. 2). In contrast, a predominance of singleton COI haplotypes was found for *C. gilensis* and *Belonuchus* sp. suggesting that both species may have undergone historical population expansions (see Section 3.4). None of the COI haplotypes of *C. gilensis* from Guaymas on the mainland were shared with individuals from peninsular localities, although they were usually separated by only a single mutational step. The comparison of COI haplotypes between *I. beyeri* and *Belonuchus* sp. is especially noteworthy given that sample sizes were similar in the two species. Haplotype networks

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Table 2
Summary of genetic diversity indices and results of neutrality tests (Tajima's *D* and Fu's F_S) in the 16S rRNA and COI gene segments from *Iliotona beyeri*, *Carcinops gilensis*, *Carcinops* sp. 1 and *Belonuchus* sp.

Species/gene	*N*	*L*	*k*	*K*	*h* (±SD)	π (±SD)	Tajima's *D*	Fu's F_S
I. beyeri								
16S rRNA	35	427	4	5	0.620 ± 0.047	0.00173 ± 0.00023	−0.59	−1.27
COI	16	625	4	4	0.442 ± 0.145	0.00097 ± 0.00040	−1.55*	−1.35
C. gilensis								
16S rRNA	14	453	4	5	0.758 ± 0.084	0.00230 ± 0.00046	−0.56	−1.52
COI	35	658	22	21	0.933 ± 0.027	0.00447 ± 0.00040	−1.55*	−15.87*
Carcinops sp. 1								
COI	9	658	30	9	1.000 ± 0.052	0.01516 ± 0.00341	−0.57	−2.87
Belonuchus sp.								
COI	18	614	30	14	0.954 ± 0.039	0.01346 ± 0.00161	−0.48	−3.65

Abbreviations: *N*, number of sequences; *L*, sequence length (number of bases); *k*, number of variable sites; *K*, number of haplotypes; *h*, haplotype diversity; π, nucleotide diversity.
* Significant at the 0.05 level.

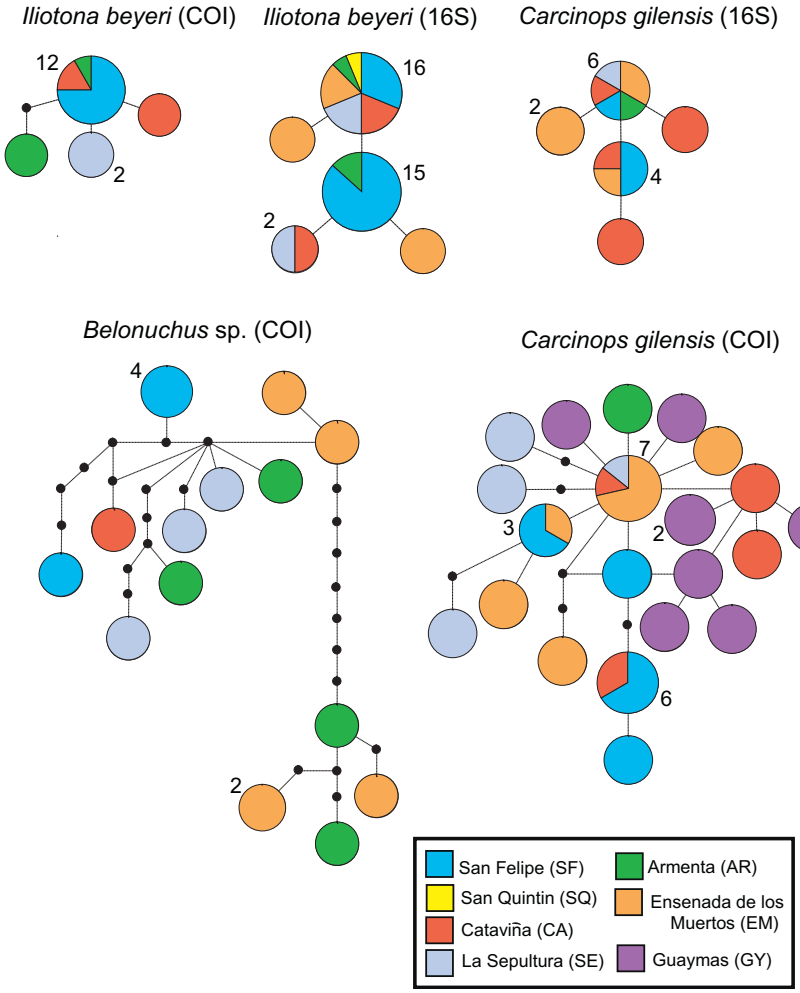

Fig. 2. TCS haplotype networks for the 16S rRNA and COI genes in *Iliotona beyeri*, *Carcinops gilensis* and *Belonuchus* sp. (COI only). Geographic distributions of haplotypes are color coded. Each line segment represents a single mutation. Inferred intermediate haplotypes that were not sampled are shown as black dots. Size of the circles is proportional to haplotype frequency. Numbers next to the circles represent number of individuals with that haplotype, if greater than one.

for the 16S rRNA gene were similar in *I. beyeri* and *C. gilensis*, with no unsampled haplotypes found in either species.

3.2. Phylogenetic analyses

Phylogenetic analyses of *Carcinops* COI sequences using both Bayesian inference and maximum parsimony yielded trees with the same topology and similar, or identical, clade support values (Fig. 3). Five distinct lineages (clades) of *Carcinops* (Table 1) were resolved. These included *C. gilensis*, *C. consors* and *Carcinops* sp. 1, 2 and 3. *Carcinops gilensis* was the only species found both on the mainland and peninsula. *Carcinops* sp. 2 and sp. 3 were both found on the mainland and both have been characterized and provisionally named (Swanson, 2008). Because these names have not been

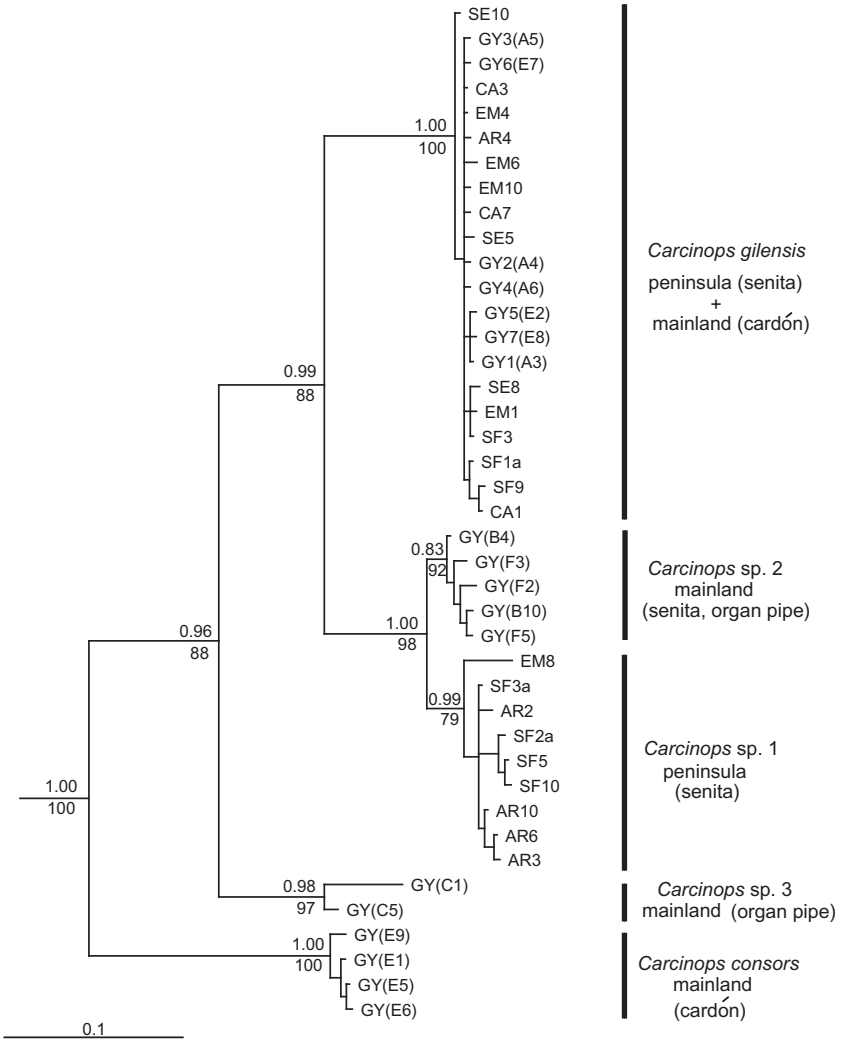

Fig. 3. Bayesian 50% majority rule consensus tree showing relationships among 41 COI haplotypes identified in 55 specimens of *Carcinops* collected from cactus necroses on the Baja California peninsula and mainland regions of the Sonoran Desert in northwestern Mexico. Clade support expressed as posterior probabilities is shown above the branches. Bootstrap support values for the MP tree (length = 332; CI = 0.641; RI = 0.873; 171 variable sites; 151 parsimony informative sites) are shown below the branches. The outgroup (not shown) was *Iliotona beyeri*. Scale shows substitutions per site. Branch terminals are labeled with locality abbreviation and sample identification number (see Fig. 1). Columnar cacti from which specimens were collected are shown in parentheses for each taxon.

formally published, we will refer to them here as undescribed species. *Carcinops* sp. 1, found only on the peninsula, was not included in the study of Swanson (2008) and appears to represent a previously unrecognized species. The peninsular *Carcinops* sp. 1 and the mainland *Carcinops* sp. 2 resolved as sister lineages separated by a mean K2P genetic distance (*d*) of 0.036, a value consistent with the view that they represent separate species-level taxa. Within group *d* values were 0.014 (*Carcinops* sp. 1) and 0.006 (*Carcinops* sp.2). In addition, TCS analysis showed that COI haplotypes of *Carcinops* sp. 1 and sp. 2 resolved in separate networks (not shown), as expected for distinct species. *Carcinops* sp. 1 and *C. gilensis* showed a large genetic divergence (*d* = 10.6%) and were found together in senita at most localities on the peninsula.

3.3. Population structure

The hierarchical AMOVA conducted on populations of *I. beyeri* from five peninsular localities using the 16S rRNA data set revealed no significant structure (overall $\Phi_{ST} = 0.119$; $P = 0.067$), with 88.10% of the genetic variation distributed within populations. None of the variation was attributed to differences between Baja California "Norte" and Baja California Sur ($\Phi_{CT} = -0.068$; $P = 0.799$). The remaining genetic variation was distributed among localities within regions ($\Phi_{SC} = 0.175$; $P = 0.076$). None of the pairwise comparisons of Φ_{ST} between populations were significant using a sequential Bonferroni correction (Table 3). Values for all pairwise comparisons of the estimated number of migrants per generation (N_m) between populations were $\geqslant 1.0$. Also, no evidence of structure between the northern peninsular populations of *I. beyeri* from San Felipe ($N = 9$) and Cataviña ($N = 3$) was seen using the smaller COI data set ($\Phi_{ST} = 0.379$; $P = 0.246$), consistent with results obtained with 16S rRNA.

The AMOVA conducted on populations of *C. gilensis* from four peninsular localities and the single mainland locality using the COI data set revealed significant population structure (overall $\Phi_{ST} = 0.241$; $P < 0.001$). However, 75.96% of the genetic variation was distributed within populations, with only 4.03% of the variation found among the three geographic areas (Baja California "Norte", Baja California Sur and mainland Sonora) ($\Phi_{CT} = 0.040$; $P = 0.603$). The remainder of the genetic variation (20.01%) was found among localities within regions ($\Phi_{SC} = 0.209$; $P = 0.066$). Only three of the ten pairwise comparisons of Φ_{ST} between populations were significant using a sequential Bonferroni correction (Table 3). The significant pairwise comparisons included the two

Table 3
Pairwise comparisons of Φ_{ST} (below the diagonal) and number of migrants per generation (N_m; above the diagonal) for populations of *Iliotona beyeri*, *Carcinops gilensis* and *Belonuchus* sp. from the Sonoran Desert of northwestern Mexico. Individual values for each locality are arranged as follows: *I. beyeri* (top), *C. gilensis* (middle) and *Belonuchus* sp. (bottom).

	Baja California "Norte"			Baja Calif. Sur		Sonora
	SF	CA	SE	AR	EM	GY
SF		1.23	1.23	Inf	1.02	ND
	–	10.82	1.08	ND	0.81	0.86
		ND	0.96	0.82	0.46	ND
CA	0.29		Inf	Inf	Inf	ND
	0.04	–	3.69	ND	2.17	7.30
	ND		ND	ND	ND	ND
SE	0.29	−0.33		Inf	Inf	ND
	0.32	0.12	–	ND	15.00	2.15
	0.34	ND		8.48	1.32	ND
AR	−0.23	−0.02	−0.02		20.29	ND
	ND	ND	ND	–	ND	ND
	0.38	ND	0.06		Inf	ND
EM	0.33	−0.10	−0.10	0.02		ND
	0.38*	0.19	0.03	ND	–	1.51
	0.52*	ND	0.27	−0.13		ND
GY	ND	ND	ND	ND	ND	
	0.37*	0.06	0.19	ND	0.25*	–
	ND	ND	ND	ND	ND	

Significant pairwise Φ_{ST} values after a sequential Bonferroni correction are indicated with asterisks. The number of individuals from each locality for each species is shown in Table 1. Localities with only a single sample were omitted. Locality abbreviations are given in Fig. 1; inf, N_m infinite and undefined. ND, no data. Values for *I. beyeri* were based on 16S rRNA sequences; values for *C. gilensis* and *Belonuchus* sp. were based on COI sequences.

Table 4
Effective female population sizes (N_{ef}) and exponential growth rates (g) in *Iliotona beyeri*, *Carcinops gilensis* and *Belonuchus* sp. calculated with FLUCTUATE.

Species	Gene	N	θ	N_{ef}	g ($1/\mu$ generations)
I. beyeri	16S	35	0.007364 (±0.00562)	5.66×10^5	3713 (±4594)
C. gilensis	COI	35	0.164762 (±0.09121)	6.87×10^6	2436 (±545)
Belonuchus	COI	18	0.066586 (±0.03135)	2.77×10^6	327 (±149)

Values for maximum-likelihood estimates of θ and g (±95% confidence intervals, CI) are shown. Neutral mutation rates per site per generation (μ) of 6.5×10^{-9} (16S rRNA) and 1.2×10^{-8} (COI) were assumed.

most geographically distant peninsular localities (SF and EM) and the mainland (GY) (Fig. 1). All values for pairwise comparisons of the estimated number of migrants per generation (N_m) between populations, with the exception of the three comparisons mentioned above, were $\geqslant$1.0. There was no evidence of structure between populations of *C. gilensis* from SF (N = 3), CA (N = 4) and EM (N = 5) using the smaller 16S data set (overall Φ_{ST} = 0.022; P = 0.379), and none of the pairwise comparisons of Φ_{ST} were significant (not shown).

The hierarchical AMOVA conducted on populations of *Belonuchus* sp. from four peninsular localities revealed significant population structure (overall Φ_{ST} = 0.354; P = 0.017), but as with *C. gilensis*, most of the genetic variation in the COI gene (64.59%) was distributed within populations. Although 32.29% of the genetic variation was found among Baja California "Norte" and Baja California Sur, the value was not significant (Φ_{CT} = 0.322; P = 0.339). The remainder of the genetic variation (3.11%) was found among localities within regions (Φ_{SC} = 0.046; P = 0.243). Only one of the six pairwise comparisons of Φ_{ST} between populations was significant using a sequential Bonferroni correction (Table 3), a comparison comprised of the geographically distant SF and EM populations. All values for pairwise comparisons of the estimated number of migrants per generation (N_m) between populations, with the exception of SF vs. EM, were either near 1.0, or >1.0.

3.4. Demographic history

Based on evidence that peninsular *I. beyeri* represents a single panmictic population (Table 3), data from all localities were combined for the demographic tests described below. As most of the pairwise comparisons of Φ_{ST} in *C. gilensis* and *Belonuchus* sp. (Table 3) were not significant, data from all localities for each species also were combined. For graphical analyses of the mismatch distribution and Bayesian skyline analyses, and for the program FLUCTUATE, only results for genes with the largest sample sizes are presented (i.e. 16S rRNA for *I. beyeri* and COI for *C. gilensis* and *Belonuchus* sp; Table 2).

Results from FLUCTUATE (Table 4) showed that the population growth parameter (g) was positive in *I. beyeri*, indicating population growth, but the value was not significantly different from zero, or no growth. In contrast, values for g in both *C. gilensis* and *Belonuchus* sp. were positive and significantly different from zero. Table 4 also shows that the estimate of N_{ef} is smaller in *I. beyeri* than in *C. gilensis* and *Belonuchus* sp., with *C. gilensis* showing the largest N_{ef} value.

Summary statistics from the mismatch distribution analysis (Table 5) showed a significant value for the raggedness statistic (rg) in *I. beyeri* 16S rRNA which rejected the null hypothesis of a population expansion. The value for the sum a square deviations (SSD) statistic, however, was not significant. For COI, both SSD and rg were not significant in *I. beyeri*, a result also seen in *C. gilensis* (both COI and 16S) and *Belonuchus* sp., consistent with populations that have experienced expansions. Graphical representation of the distribution of pairwise differences among mtDNA haplotypes in the three species is shown in Fig. 4. Populations that have undergone an historical expansion are expected to show a unimodal distribution whereas populations in equilibrium are gener-

Table 5
Summary statistics from mismatch distribution analysis for *Iliotona beyeri*, *Carcinops gilensis* and *Belonuchus* sp.

Species	Gene	N	τ (95% CI)	θ_0	θ_1	SSD	rg
I. beyeri	16S	35	0.90 (0.08, 1.55)	0.002	>1000	0.025 (P = 0.09)	0.184 (P = 0.03)
I. beyeri	COI	16	0.80 (0.00, 2.64)	0.063	1.33	<0.001 (P = 0.92)	0.109 (P = 0.85)
C. gilensis	COI	34[a]	3.25 (1.73, 4.16)	0.000	460.63	0.005 (P = 0.25)	0.032 (P = 0.48)
C. gilensis	16S	14	1.21 (0.00, 2.32)	0.004	>1000	0.027 (P = 0.20)	0.187 (P = 0.12)
Belonuchus	COI	17[a]	2.82 (0.55, 16.81)	7.752	78.67	0.025 (P = 0.24)	0.051 (P = 0.16)

Abbreviations: τ, moment estimator of mutational time, where $\tau = 2ut$, and u is the mutation rate for the entire gene segment (see Table 2 for segment size) and t is the number of generations since the expansion; θ_0 and θ_1, mutation parameters where $\theta_0 = 2uN_0$, and $\theta_1 = 2uN_1$ (N_0 and N_1 are the population sizes before and after the expansion, respectively) with 95% confidence intervals (CI); SSD, sum of square deviations; rg, raggedness statistic (Rogers and Harpending, 1992; Harpending, 1994).

[a] One sequence with missing bases was deleted for the analyses.

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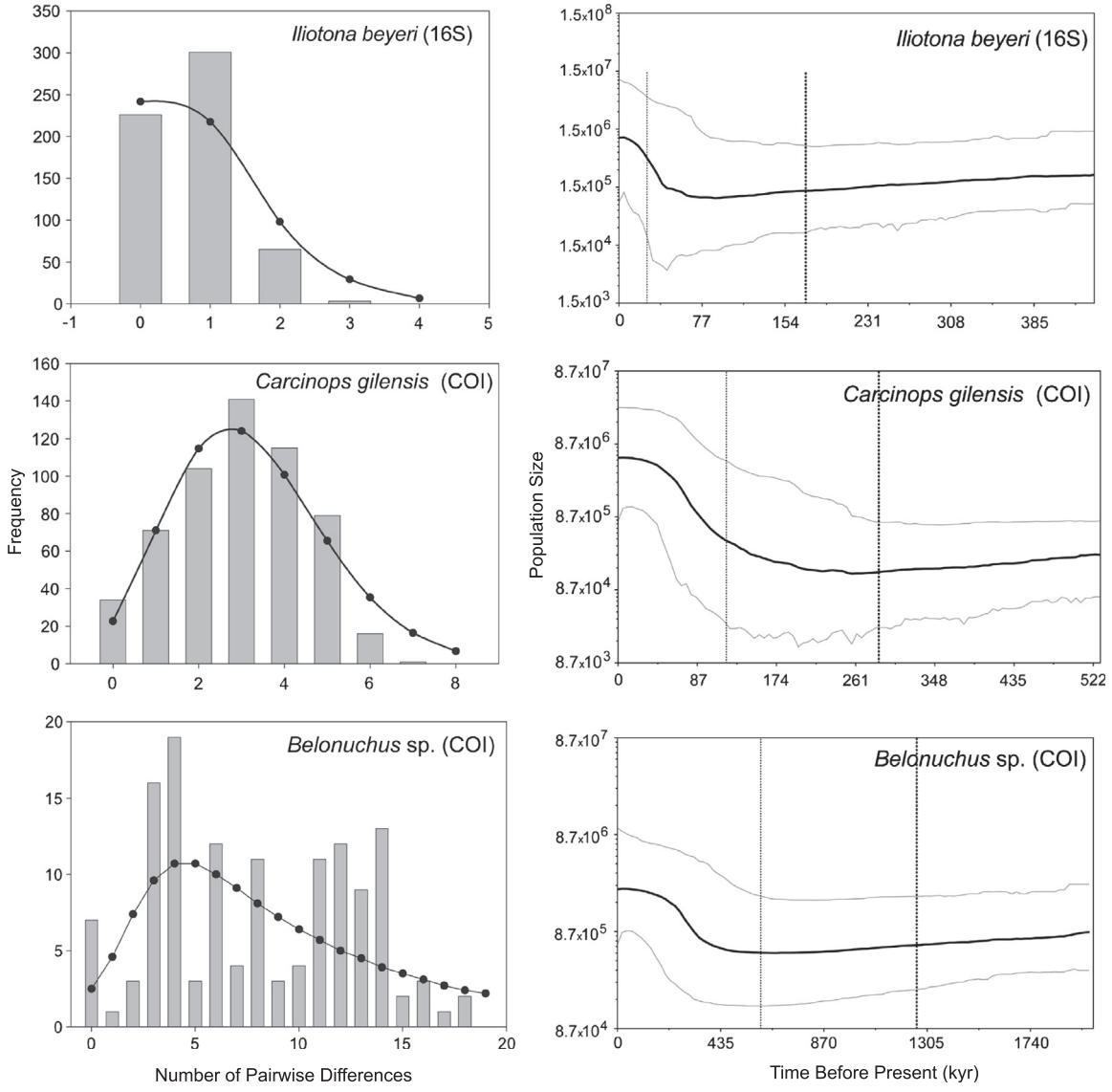

Fig. 4. Demographic history of *Iliotona beyeri*, *Carcinops gilensis* and *Belonuchus* sp. estimated from the mismatch distribution (left) and Bayesian skyline analysis (right). Vertical bars of the mismatch distribution show the observed distribution of pairwise differences among haplotypes, with the solid line representing the expected distribution under the sudden expansion model. Bayesian skyline plots show the estimated changes in effective female population size (N_{ef}) over time given on a logarithmic scale. The thick solid lines represent the median estimates of population size; the thin solid lines show the 95% highest posterior density (HPD) intervals. The vertical lines represent the median estimate (thick dotted line) and lower 95% HPD (thin dotted line) of time to the most recent common ancestor. Data for both analyses were obtained from either 16S rRNA (*I. beyeri*) or COI (*C. gilensis* and *Belonuchus* sp.) sequences.

ally characterized by a multimodal distribution (Harpending, 1994). *Belonuchus* sp. showed a poor fit and a multimodal distribution suggesting a stable population size, although the *SSD* and *rg* statistics did not reject the sudden expansion model.

The strongest graphical evidence for a population expansion from mismatch distribution analyses is seen in *C. gilensis* (Fig. 4). Assuming 2.4% and 1.3% pairwise sequence divergence per million years in the COI and 16S rRNA genes (see Materials and methods), the mean mutation rate per site per generation (u) is 7.9×10^{-6} for COI (658 bp) and 2.9×10^{-6} for 16S rRNA (453 bp). Using these estimates of u, and values for τ shown in Table 5, the estimated time to the population expansion (t) in *C. gilensis* was about 205,000 generations ago. Each of the two gene segments yielded essentially identical estimates. This value compares well with the t value of 355,000 generations ago for the beginning of the expansion in *Drosophila pachea* (Pfeiler et al., 2009), a potential prey of *C. gilensis*.

Results from Bayesian skyline analyses (Fig. 4) showed that all three beetle species showed signatures of historical population expansions, although the patterns and timing of the expansions varied. In these analyses we assumed a single generation per year. Because the actual number of generations per year is not known for these beetles, the timing of the expansions and population sizes shown in Fig. 4 are only rough estimates and are intended to show comparative trends only. These trends, however, are consistent with results from the other demographic tests and place the expansions within the Pleistocene. The relative difference in present-day N_{ef} among species seen in Fig. 4 is consistent with values shown in Table 4, with the largest N_{ef} and highest support for a population expansion seen in *C. gilensis*. The smallest present-day N_{ef} was seen in *I. beyeri*.

We also conducted a Bayesian skyline analysis on the smaller COI data set ($N = 16$) in *I. beyeri* for comparisons with the COI plots for *C. gilensis* and *Belonuchus* sp. shown in Fig. 4 and found a population size change similar to that seen for the 16S data set, except

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that the population size increase was less abrupt (not shown). Thus, both gene segments yielded similar Bayesian skyline results in *I. beyeri*, although sample sizes differed substantially.

4. Discussion

4.1. Population structure

The population genetics of three species of cactophilic coleopterans collected on senita from several localities throughout the Baja California peninsula have been compared to determine whether common patterns of dispersal and demographic history could be detected in ecologically similar organisms utilizing the same resource. Previous studies have shown that other arthropods, principally dipterans, dependent upon the necrotic cactus microhabitat in the Sonoran Desert generally show high dispersal capability within mainland and peninsular regions (Pfeiler and Markow, 2011), as would be predicted in organisms utilizing an ephemeral and patchily distributed resource for feeding and reproduction. Here we assume that dispersal of the three beetle species is by flight, but the possible effects of differences in body size and morphology on dispersal ability are not known. We also recognize that the low samples sizes from most localities, especially for *Belonuchus* sp. (Table 1), will result in reduced statistical power in the AMOVA tests used to infer population structure. Thus the conclusions discussed below should be considered preliminary and in need of confirmation using larger samples sizes and additional molecular markers.

Results from AMOVA indicated a lack of structure among populations of *I. beyeri* from the Baja California peninsula, suggesting high gene flow and dispersal capability between San Felipe (SF) in the north and Ensenada de los Muertos (EM) in the Cape Region, a distance of about 900 km (Fig. 1; Table 3). This result is consistent with the high genetic connectivity found throughout the peninsula from DNA studies on the senita-dependent *Drosophila pachea* (Hurtado et al., 2004; Pfeiler et al., 2007), a potential prey species for *I. beyeri* (see below). Peninsular populations of other cactophilic arthropods not restricted to senita, including *Drosophila mojavensis baja* and the chernetid pseudoscorpion *Dinocheirus arizonensis*, also show high genetic connectivity (Ross and Markow, 2006; Reed et al., 2007; Pfeiler et al., 2009).

In contrast to results seen in *I. beyeri*, overall Φ_{ST} values revealed significant structure among peninsular populations of both *C. gilensis* and *Belonuchus* sp. For both species, however, most of the pairwise comparisons of Φ_{ST} among peninsular localities were not significant (Table 3); significant values were seen only in the geographically distant SF and EM populations. The lack of structure between populations of *C. gilensis* from Guaymas (GY) on the mainland and the mid-peninsula region (CA and SE) (Table 3), together with the pattern on geographic distribution of COI haplotypes in *C. gilensis* (Fig. 2), suggests that these beetles may be using the Midriff Islands in the upper Gulf of California as dispersal "stepping stones" between the peninsula and mainland (Pfeiler and Markow, 2011). The strong northwesterly winds occurring during the winter in the northern Gulf of California region (Badan-Dangon et al., 1991), and the presence of host cacti (cardón, saguaro and senita) on these islands (Turner et al., 1995), could potentially aid peninsular to mainland dispersal of these very small beetles. Overall, our results suggest a general lack of population genetic structure and good dispersal capability in peninsular *I. beyeri*, *C. gilensis* and *Belonuchus* sp, consistent with results on other cactophilic arthropods utilizing the same resource and suggesting that differences in body size of these beetles do not limit dispersal capability and successful colonization of new rots.

4.2. Demographic history

Genetic evidence for historical population expansions dating to the Pleistocene and late Pliocene has been found for several taxa of Sonoran Desert arthropods associated with columnar cactus rots, including the peninsular *Drosophila* (*D. pachea*, *D. nigrospiracula*, *D. mojavensis baja*, *D. arizonae*, *D. mettleri*) and the pseudoscorpion *Dinocheirus arizonensis* (Hurtado et al., 2004; Reed et al., 2007; Machado et al., 2007; Pfeiler et al., 2007, 2009; Pfeiler and Markow, 2011). In the cactophilic beetles, evidence of an historical population expansion was strongest for *C. gilensis*. The large negative F_S value (Table 2), results from FLUCTUATE (Table 4), mismatch distribution and Bayesian skyline analyses (Table 5; Fig. 4) all support the conclusion that a population expansion dating to the Pleistocene occurred in *C. gilensis*, consistent with results from the previous studies cited above. As found for the senita-dependent *D. pachea* (Pfeiler et al., 2007), the Pleistocene population expansion seen in *C. gilensis* predates the proposed postglacial (Holocene) northern range expansion of senita on the Baja California peninsula (Nason et al., 2002).

When results from all demographic tests were taken together, evidence for population expansions in *I. beyeri* and *Belonuchus* sp. was weaker than in *C. gilensis*. The F_S values were not significant for either species (Table 2), and for *I. beyeri*, FLUCTUATE showed the growth parameter (g) was positive, but not significantly different from zero. For *Belonuchus* sp., g was statistically significant, but was about 8-fold less than the value obtained for *C. gilensis* (Table 4). Results for the mismatch distribution were equivocal in *I. beyeri* (Table 5; Fig. 4), although Bayesian skyline analysis (Fig. 4) supported a population expansion. Given the low haplotype diversity in 16S rRNA gene in *I. beyeri* (Table 2), this gene segment may not be ideal for assessing demographic history in this species. Although statistical analysis of the mismatch distribution in *Belonuchus* sp. did not reject the sudden expansion model (Table 5), graphical representation of the distribution of pairwise differences suggested a stable population (Fig. 4). Bayesian skyline analysis, however, indicated a population expansion (Fig. 4). In summary, although there is some evidence for population expansions in *I. beyeri* and *Belonuchus* sp., more data with larger sample sizes will be required to obtain a better understanding of the demographic history in both species.

Results from FLUCTUATE (Table 4) indicated that the largest effective female population size (N_{ef}) was found in *C. gilensis*. Although the 95% confidence intervals for θ (and thus N_{ef}) were large, and effective population sizes are typically smaller than census population sizes (Barker, 2011), the relative differences in N_{ef} values among the three beetle species are consistent with our field observations showing that *Carcinops* is usually the most abundant of the beetle species found in cactus rots. A higher present-day N_{ef} in *C. gilensis* is also apparent in Bayesian skyline plots (Fig. 4).

4.3. Ecology and life history

Although the feeding ecology of *I. beyeri*, *C. gilensis* and *Belonuchus* sp. has not been studied, we have assumed that all three species are predatory and are feeding at the same trophic level within senita necroses. The large differences in the size of adults, however, suggest that some resource partitioning may occur, with the larger species, *I. beyeri* and *Belonuchus* sp., most likely exploiting the largest food resources. Potential prey for the three beetle species include the different life history stages of *Drosophila pachea* (Drosophilidae), *Odontoloxozus longicornis* (Neriidae), and species from other families of Diptera, principally the Cecidomyiidae, Chironomidae and Muscidae (Castrezana and Markow, 2001). The assumption that these beetles are predatory is supported by abundant observations on staphylinids and histerids, including the

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well-studied *C. pumilio* (Erichson), an important predator of the house fly (*Musca domestica* Linnaeus). Larval and adult stages of members of both Histeridae and Staphylinidae typically (but not exclusively) feed on eggs and larvae of Diptera and other soft-bodied organisms found in many different habitats, including poultry manure and decaying vegetation (Geden and Axtell, 1988; Achiano and Giliomee, 2005). Some staphylinids, however, are parasitic with development occurring in puparia of syrphid flies found in decaying cacti (Coquillett, 1891).

It is also possible that beetles found in senita rots, which we assume compete for the same dipteran food resources, may be preying upon each other, a behavior referred to as intraguild predation, or IGP (Polis and Holt, 1992; Holt and Polis, 1997). In Brazil, evidence for IGP has been found in *Carcinops troglodytes* (Paykull) which preys upon larvae of the tenebrionid beetle *Alphitobius diaperinus* (Panzer) in poultry manure (Santoro et al., 2010). Both species are known predators of *M. domestica*. It is also possible that adult *Carcinops* found in senita may be cannibalistic, a behavior seen in *C. pumilio* which preys upon its own eggs (Kaufman et al., 2001). Both cannibalism and IGP would have important implications for community structure and population dynamics within the senita necrosis microhabitat. For example, Swanson (2008) was unable to find any larval stages of cactophilic *Carcinops*, even after exhaustive searches, an observation consistent with cannibalism. During our survey we noted a few beetle larvae in the rots, but because larval stages were not our main focus, they were not collected or identified. Although we assume below that the beetles are breeding in the necroses, and not just tracking their prey, this aspect of their reproductive biology also needs to be confirmed.

Based on the short period of time that a particular senita necrosis is available for the community of arthropods inhabiting this microhabitat, a relatively short development time would provide an adaptive advantage to both predator and prey. In the laboratory, an assumed prey, *D. pachea*, completes its development from egg to adult in about 18 days (T.A.M., personal observation). The development time of the three beetle species studied here has not been determined, but one would predict that development in these species would be relatively fast as well. Short development times (<3 weeks from egg to adult) have been documented in non-cactophilic staphylinids (Echegaray, 2012) and histerids, including *C. pumilio* (Fletcher et al., 1991; Achiano and Giliomee, 2005), thus the prediction of short development times in cactophilic beetles is not unreasonable, but also needs to be determined.

4.4. Biodiversity of beetles in senita necroses

Overall, the order Coleoptera is well-represented in senita necroses in the Sonoran Desert, including both peninsular and mainland regions. In the family Histeridae alone, two species from the genus *Iliotona*, *I. beyeri* and *I. cacti*, and four from the genus *Carcinops*, *C. gilensis*, *C. opuntiae*, and the unidentified species 1 and 2, have been recorded (Swanson, 2008; Pfeiler et al., 2010; present study), and it is probable that other histerids, as well as representatives from other families, especially Staphylinidae, remain to be discovered. As mentioned earlier, *Carcinops* sp. 2 has been diagnosed and provisionally named (Swanson, 2008). Swanson's (2008) study did not include peninsular samples, thus *Carcinops* sp. 1, the sister species to *Carcinops* sp. 2 (Fig. 3), also awaits morphological analysis and formal description. *Carcinops* sp. 3 also has been provisionally named, but to date has only been found on organ pipe cactus (Swanson, 2008; present study). The staphylinid *Belonuchus* sp., a putative new species, is widely-distributed on the peninsula, being collected on senita at all localities where *C. gilensis* was found (Table 1). Although sample size for the peninsular *Carcinops* sp. 1 was small ($N = 9$) our survey suggests that it too is widely distributed, being found from San Felipe in the north to Ensenada de los Muertos in the Cape Region. The lack of samples of *Carcinops* sp. 1 from Cataviña (CA) and La Sepultura (SE) (Table 1) may reflect the generally low sample sizes. *Carcinops* sp. 1 and *C. gilensis* are broadly sympatric and genetically distinct (mean $d = 10.6\%$). Determining whether *Carcinops* sp. 1 is restricted to senita will require additional sampling.

In conclusion, mtDNA data have shown a common pattern of little or no population structure in beetles and other arthropods associated with the ephemeral senita necroses on the Baja California peninsula for feeding and development, consistent with the prediction that these taxa should show good dispersal capability and high genetic connectivity. In addition, mtDNA data are beginning to reveal a general pattern of population expansions dating to the Pleistocene in a variety of arthropods from the Sonoran Desert dependent upon the necrotic tissue of a several species of columnar cacti (Pfeiler and Markow, 2011). This pattern complements a growing literature showing that Pleistocene population expansions in insects from a variety of taxonomic groups and habitats occurred well before the end of glacial/interglacial cycling about 15,000 years ago (Smith and Farrell, 2005; Huang and Lin, 2010; Smith et al., 2011). The fact that this pattern has been demonstrated in insects with different life history traits, dispersal mechanisms and body sizes, suggests that insect population dynamics during the Pleistocene was probably much more complex than originally thought, a suggestion also proposed for other animals, including mammals (Hofreiter and Stewart, 2009).

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2013.07.030.

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