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Short Communication

Phylogenetic relationships of Sonoran Desert cactus beetles in the tribe Hololeptini (Coleoptera: Histeridae: Histerinae), with comments on the taxonomic status of *Iliotona beyeri*

Edward Pfeiler [a,*], Joel E. Vergara-Quintanar [b], Sergio Castrezana [c], Michael S. Caterino [d], Therese A. Markow [c]

[a] Centro de Investigación en Alimentación y Desarrollo, A.C., Unidad Guaymas, Apartado Postal 284, Guaymas, Sonora C.P 85480, Mexico
[b] Escuela de Ciencias, Universidad de las Américas Puebla, Cholula, Puebla C.P 72820, Mexico
[c] Division of Biological Sciences, University of California, San Diego, La Jolla, CA 92093, USA
[d] Department of Invertebrate Zoology, Santa Barbara Museum of Natural History, Santa Barbara, CA 93105, USA

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ABSTRACT

Nucleotide sequences from 16S rRNA and cytochrome *c* oxidase subunit I (COI) were used to examine phylogenetic relationships and evolution of beetles from the tribe Hololeptini (Coleoptera: Histeridae: Histerinae) that inhabit necrotic tissue of columnar cacti in the Sonoran Desert. Phylogenetic and morphological analyses revealed the presence of seven separate lineages, three representing species in the genus *Iliotona*, including *I. beyeri* **stat. nov.**, and four species belonging to the genus *Hololepta* (sensu lato). The possible roles of historical vicariance and host plant associations on the evolution of the Hololeptini from the Sonoran Desert are discussed.

1. Introduction

The coleopteran family Histeridae, hister beetles or clown beetles, comprises about 330 genera and 3900 described species worldwide (Mazur, 1997). This diverse group of beetles is commonly found in rotting and decaying vegetation where both larvae and adults prey upon soft bodied insects, especially the eggs and larvae of dipterans. In the Sonoran Desert, necroses (rots) of columnar cacti [saguaro (*Carnegiea gigantea*), cardón (*Pachycereus pringlei*), etcho (*P. pecten-aboriginum*), organ pipe (pitahaya dulce; *Stenocereus thurberi*), pitahaya agria (*S. gummosus*), cina (*S. alamosensis*) and senita (*Lophocereus schottii*)], provide an important microhabitat for the breeding, feeding and development of a variety of insects and other arthropods, including histerid beetles (Castrezana and Markow, 2001). The family Histeridae has been subdivided into eleven currently recognized subfamilies and a number of different tribes (Caterino and Vogler, 2002). Several hypotheses on phylogenetic relationships among these groups based on morphological and molecular analyses have been presented (Ohara, 1994; Ślipiński and Mazur, 1999; Caterino and Vogler, 2002), but many details remain to be resolved. Importantly, only a single molecular phylogenetic analysis of the family has appeared (Caterino and Vogler, 2002).

The tribe Hololeptini of the subfamily Histerinae is comprised of six genera *Dimalus*, *Petalosoma*, *Platyeutidium*, *Hololepta* (sensu lato), *Iliotona* and *Oxysternus*. Two subgenera within the genus *Hololepta* (s.l.) are also currently recognized, *Leionota* and *Hololepta* (sensu stricto). Many of these taxa are extremely dorsoventrally flattened, and are mainly found beneath the bark of dead trees. A distinct subset, however, shows obligate associations with rotting succulents, especially Cactaceae, although *Yucca* is also used. The present study focuses on *Iliotona* and *Hololepta* (s.l.), the latter of which contains most of the described species (106 in total; Mazur, 1997) belonging to the Hololeptini. Several species of *Iliotona* and *Hololepta* (s.l.) have been reported to occur in rotting cactus from the Sonoran Desert region of northwestern Mexico [including the Baja California peninsula] and southwestern USA, but little is known of their phylogenetic relationships and ecology. This fauna is known to include *H. pervalida* Blaisdell, *H. yucateca* (Marseul), *H. vicina* LeConte, *H. vernicis* Casey, *H. quadridentata* (Olivier), *H. excisa* Marseul, and *H. princeps* LeConte, as well as *Iliotona cacti* (LeConte) and *I. dorcoides* (Lewis) (Lewis, 1888; Carnochan, 1917; Moore, 1937; Navarrete-Heredia and González Estrada, 2003). In addition, Schaeffer (1907) described *I. beyeri* [as *Hololepta* (*Lioderma*) *beyeri*] from Santa Rosa, Lower California [Baja California Sur] and listed morphological characters that distinguish it from

* Corresponding author. Fax: +52 622 221 6533.
E-mail address: pfeiler@ciad.mx (E. Pfeiler).

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I. dorcoides. Mazur (1984), however, placed *I. beyeri* into the synonymy of *I. dorcoides* without explanation.

The aim of the present study was to utilize mitochondrial gene sequences [16S rRNA and cytochrome *c* oxidase subunit I (COI)] from individuals of Hololeptini collected over a wide geographic area of the Sonoran Desert, including mainland and peninsular localities, in an attempt to understand phylogenetic relationships and help clarify the taxonomic status of species within this group. We were particularly interested in determining if evidence of molecular diversification could be detected that might be related to restriction of gene flow by the Gulf of California ("Gulf" and "Gulf of California" are used interchangeably here; also known as the Sea of Cortez).

2. Materials and methods

2.1. Samples

Beetles were collected from necrotic tissue of columnar cacti from nine localities on the Baja California peninsula ($N = 43$) and eight localities on the mainland of northwestern Mexico and Arizona, USA ($N = 18$) during 2002 and 2003 (Fig. 1). Four individuals from Agiabampo, Sonora (thornscrub habitat) and two individuals from Rancho San Isidro, Sinaloa (tropical deciduous forest) are outside of the Sonoran Desert proper, but these samples were collected from necroses of columnar cacti and were included with the Sonoran Desert samples. Beetles were either removed directly from rots in the field, or a section of the rot was taken and transported to the laboratory where beetles were collected using the procedure described in Castrezana and Markow (2001). Live beetles were immediately placed in 95% ethanol.

DNA samples were also obtained from six Hololeptini deposited in the M.S. Caterino collection (MSCC) and Santa Barbara Museum of Natural History (SBMNH) that were not collected on columnar cacti. These included: *Iliotona cacti*, Anza-Borrego Desert State Park, San Diego Co., CA, USA, collected on barrel cactus (*Ferocactus* sp.) (located in the extreme northwestern portion of the Sonoran Desert, a region where columnar cacti are absent), 1 April 2005 (DNA No. MSC1447; Voucher No. CBP0067817); *Hololepta populnea*, Upper Sespe Creek, Ventura Co., CA, 28 September 2002 (MSC1859, CBP0036500); *Hololepta* (*Leionota*) sp. 3., Las Cuevas Field Station, Cayo, Belize, 27 May 2000 (MSC1860); two individuals of *H. vicina*, (a) Anza-Borrego Desert State Park, San Diego Co., CA, USA, 1 April 2005, on *Ferocactus* (MSC1861, CBP0080913) and (b) Whitewater Canyon, Riverside Co., CA, USA, 15 May 2006 (MSC1862, CBP0046548); *Hololepta* (*Hololepta*) sp. 4, Barro Colorado Island, Panama, 10 August 2005 (MSC1863).

2.2. Molecular analyses

Total genomic DNA was extracted from thoracic muscle, or one or two legs of each beetle, using the DNeasy™ (QIAGEN Inc., Valencia, CA) protocol. The beetles were then pinned, or placed in ethanol, to serve as vouchers. The polymerase chain reaction (PCR) was used to amplify the two mitochondrial gene segments using the primer pairs 16Sar/16Sbr for 16S rRNA (Palumbi, 1996) and LCO1490f/HCO2198r for COI (Folmer et al., 1994). All PCR cycling conditions included an initial denaturation at 94 °C for 3 min, followed by 35 cycles of 94 °C for 30 s of denaturation, 45 °C for 1 min of annealing, and 72 °C for 1 min of extension, with a final extension of 7 min at 72 °C. Verification of successful amplification was assessed by agarose gel electrophoresis.

Sequencing reactions were performed on an Applied Biosystems (Foster City, CA) ABI 3730XL DNA sequencer at the DNA Sequencing Facility, University of Arizona, Tucson, using the amplifying primers. Alignments were performed in ClustalX 1.81 (Thompson et al., 1997) followed by manual editing. Calculations of genetic distances among sequences [*p*-distances and Kimura's (1980) 2-parameter (K2P) distances] were carried out in MEGA version 4.0 (Tamura et al., 2007). Relative rate tests (Tajima, 1993) of sequence evolution in *I. dorcoides* from the Baja California peninsula and the mainland were carried out in MEGA using *I. cacti* (16S rRNA data set) and *Hololepta* sp. 1 (COI data set) as outgroups. *H. populnea* was the outgroup for relative rate tests of the 16S rRNA data set in the sister taxa *Hololepta* sp. 1 and sp. 2.

All new sequences for both genes are deposited in GenBank (Accession Nos. GU982678–GU982708).

2.3. Phylogenetic analyses

Relationships among 16S rRNA haplotypes of the 63 sequences obtained for the ingroup tribe Hololeptini [including an available GenBank sequence for *Hololepta plana* (AM287075)] were initially assessed with the neighbor-joining (NJ) algorithm of Saitou and Nei (1987) carried out in MEGA using a matrix of K2P distances. Relative support for tree topology was obtained by bootstrapping (Felsenstein, 1985) using 1000 pseudoreplicates. The unique haplotypes identified in the NJ tree were then used to further assess relationships using maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference. The outgroup used for 16S rRNA trees was the histerid *Margarinotus brunneus* from the tribe Histerini (GenBank Accession No. AM287074).

The MP analyses were carried out in MEGA using the CNI heuristic search option and 100 random additions of sequences. Gaps were coded as missing data. The ML analyses were performed in PHYLIP version 3.68 (Felsenstein, 2004) using randomized input order and the nucleotide substitution model described below for the Bayesian analyses. Relative support for MP and ML tree topology was obtained by bootstrapping using either 1000 (MP) or 100 (ML) pseudoreplicates. Bayesian methods were implemented in MrBayes version 3.1 (Huelsenbeck and Ronquist, 2001). The model of nucleotide substitution that best fit the data set, determined with Modeltest 3.7 (Posada and Crandall, 1998) using the Akaike Information Criterion, was HKY + I + Γ. Bayesian analyses were run under the parameters of the HKY nucleotide substitution model (nst = "2"; rates = "invgamma") for 5,000,000 generations, sampled every 250th generation (20,000 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) algorithm. Log-likelihood values from four simultaneous MCMC chains (three hot and one cold) stabilized at about 6000 generations. The first 24 trees, therefore, were discarded from the analysis (burnin = 24).

3. Results

3.1. Sequence data

Sequencing of the mitochondrial 16S rRNA gene yielded a segment of 426–430 bp. Alignments were relatively straightforward, with a final segment size of 431 bp (including gaps) used for the phylogenetic analyses. These sequences represented 17 distinct haplotypes. Relationships among these are not fully resolved, but we recognize seven clades (referred to below and in Fig. 2 as clades A–G) found on columnar cacti from the Sonoran Desert. Their relationships are discussed further below. A limited data set ($N = 22$) obtained for the protein coding mitochondrial gene, COI, consisted of 625 bp. No insertions or deletions were found, as expected. The COI data set included three of the seven Sonoran Desert clades (A, B and D).

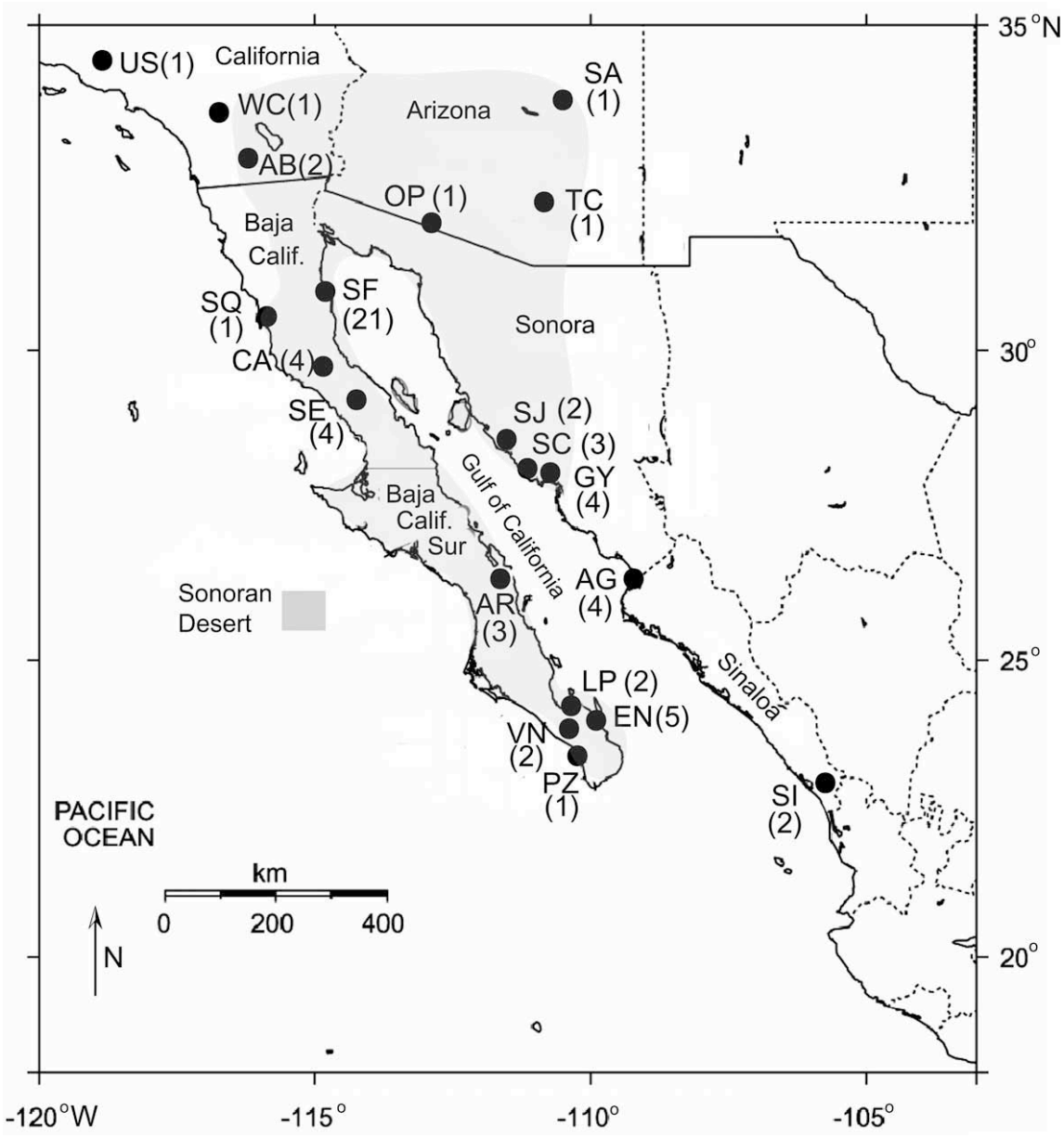

Fig. 1. Map showing collecting localities in northwestern Mexico and southwestern USA. Number of beetles collected at each locality is shown in parentheses. Abbreviations (Arizona): SA, Sierra Ancha; TC, Tucson; OP, Organ Pipe Cactus National Monument; (California): US, Upper Sespe Creek, Ventura Co.; WC, Whitewater Canyon, Riverside Co.; AB, Anza-Borrego Desert State Park, San Diego Co.; (Sonora): SJ, San Juanico; SC, San Carlos; GY, Guaymas; AG, Agiabampo; (Sinaloa): SI, San Isidro Ranch; (Baja California Sur): LP, La Paz; EN, Ensenada de los Muertos; PZ, Pozo 100; VN, Vinateria; AR, Armenta; (Baja California): SQ, San Quintin, SF, San Felipe; SE, Sepultura; CA, Cataviña.

3.2. Phylogenetic analyses

The seven main clades of the Sonoran Desert Hololeptini resolved in phylogenetic reconstruction of 16S rRNA sequences are shown in Fig. 2. The MP, ML and Bayesian analyses yielded identical trees. Two clades (A and D) were found exclusively on the Baja California peninsula. Clade A, which comprised most of the specimens (Table 1), was found throughout the peninsula associated with senita (a single individual was collected on pitahaya agria at San Quintin in the northern state of Baja California), but was absent from all mainland localities, including those where senita was sampled. Individuals from clade D ($N = 4$) were found only in the Cape Region of Baja California Sur associated with cardón rots (Table 1). A single individual from Clade F was found in the Cape Region on cardón, but was also taken in southern California. Four clades (B, C, E and G) were found only at mainland localities. Individuals assigned to clade B, sister to clade A, were all taken on organ pipe cactus in Sonora, Mexico. Individuals comprising clade C ($N = 4$) were collected on senita, cardón and barrel cactus (Anza-Borrego). Of the four individuals assigned to clade E, sister to the peninsular clade D, two were collected on saguaro in Arizona and two were found on cardón at Guaymas, Sonora. Finally, of the four individuals assigned to clade G, two were collected on etcho cactus in Sinaloa, Mexico, one was found on organ pipe cactus in southern Arizona, and one was taken in southern California. A sister relationship was seen between clades F and G, with the unidentified *Hololepta* sp. 4 from Panama resolving basal to this pair.

Morphological characters (Lewis, 1888; Schaeffer; 1907; Carnochan, 1917), together with results from molecular phylogenetic analyses (Fig. 2), support assigning five of the Sonoran Desert clades of Hololeptini to the following species: clade A, *Iliotona*

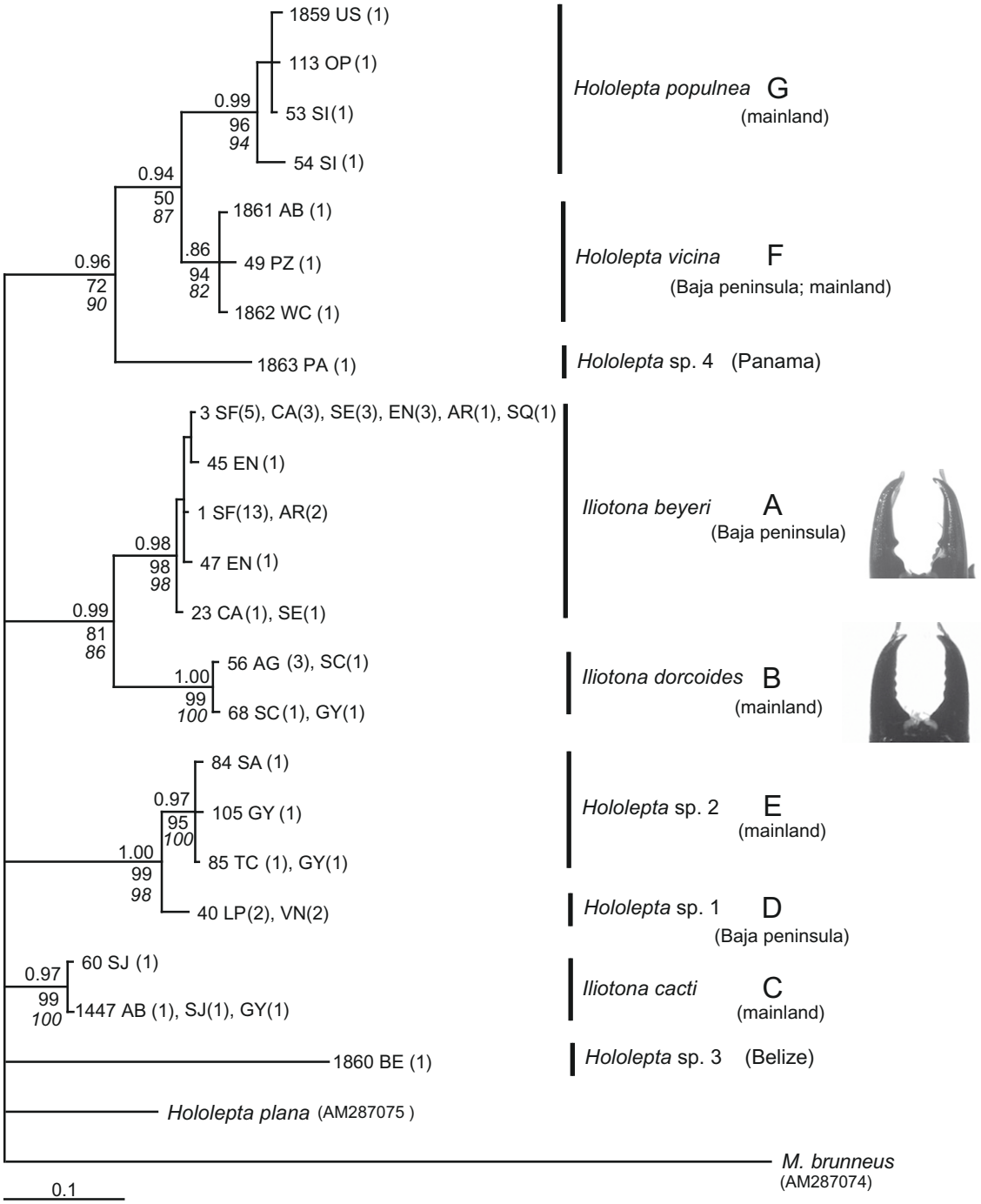

Fig. 2. Bayesian 50% majority rule consensus tree showing relationships among haplotypes in Hololeptini collected from northwestern Mexico and southwestern USA based on analysis of a 431 bp segment of the 16S rRNA gene. Unidentified specimens of *Hololepta* from Belize (sp. 3) and Panama (sp. 4), and a GenBank sequence for *H. plana* (AM287075), were also included. The tree was rooted with the histerid *Margarinotus brunneus* (Histerinae: Histerini). Clade support expressed as posterior probabilities is shown above branches. Bootstrap support values for the MP tree (length = 256; CI = 0.641; RI = 0.778; 142 variable sites; 102 parsimony informative sites) and ML tree (log-likelihood score = −2011.0) are shown below the branches (ML bootstrap values are in italics). Scale shows substitutions per site. Branch terminals are labeled with sample identification number and locality abbreviation (see Fig. 1). The number of individuals with the same haplotype at each locality is given in parentheses. Capital letters (A–G) represent the seven different clades identified for Hololeptini from the Sonoran Desert. Insets are dorsal views of the mandibles of *Iliotona beyeri* and *I. dorcoides* showing the diagnostic characters originally described by Lewis (1888) and Schaeffer (1907) that separate the two species. In *I. beyeri*, the mandible is broadly dilated at the base with one obtuse tooth above the dilation, with the apical part of the inner edge above the tooth being smooth. In *I. dorcoides*, the inner edge of the mandible is serrulate, with a blunt tooth at the base.

beyeri **stat. nov.** (=*I. dorcoides* peninsular form); clade B, *I. dorcoides* (mainland form); clade C, *I. cacti*; clade F, *Hololepta vicina*; clade G, *H. populnea*. A fairly deep split was seen between the sister taxa *I. beyeri* from the Baja California peninsula and *I. dorcoides* from the mainland. The mean 16S rRNA genetic distances between the two species were 7.5% (uncorrected *p*-distance) and 7.9% (K2P distance). The mean genetic distances between *I. beyeri* and *I. dorcoides* based on the relatively fast evolving mitochondrial COI gene were about twofold higher (13.1% and 14.6%, respectively). Although *I. beyeri* and *I. dorcoides* are superficially similar, differences in the shape of the mandibles (insets on Fig. 2), originally noted by Schaeffer (1907), are characteristic and can easily

Table 1
Summary of species of cactus beetles (tribe Hololeptini) from the present study, with columnar cactus species from which the beetles were taken, geographic region and number of sequences of mitochondrial 16S rRNA and COI gene segments. Clade designations (A–G) are from Fig. 2 and represent the seven 16S rRNA genetic lineages identified in beetles from the Sonoran Desert, including adjacent areas in southern California, USA. Two specimens from Central America (Belize and Panama) were also included in the analyses. State abbreviations: (Mexico) BC, Baja California; BCS, Baja California Sur; Son., Sonora; Sin., Sinaloa; (USA) AZ, Arizona; CA, California. *N*, total number of beetles of each species collected.

Species	Clade	*N*	Host cactus	Geographic region						No. of sequences	
				BC	BCS	Son.	Sin.	AZ	CA	16S	COI
Iliotona beyeri	A	38	Senita; agria	X	X					35	16
Iliotona dorcoides	B	8	Organ pipe			X				6	5
Iliotona cacti	C	4	Senita; cardón			X			X[a]	4	
Hololepta sp. 1	D	4	Cardón		X					4	1
Hololepta sp. 2	E	4	Saguaro; cardón			X		X		4	
Hololepta vicina	F	3	Cardón		X				X[a]	3	
Hololepta populnea	G	4	Organ pipe; etcho				X	X	X[b]	4	
Hololepta sp. 3		1	(Belize)[c]							1	
Hololepta sp. 4		1	(Panama)[c]							1	
Total		67								62	22

[a] *Iliotona cacti* and *H. vicina* from southern California were collected on barrel cactus (*Ferocactus* sp.).
[b] *Hololepta populnea* from southern California was collected on *Populus* sp.
[c] *Hololepta* sp. 3 and sp. 4 from Central America were not collected on host plants.

separate the two geographically isolated species. Thus, both on morphological and molecular grounds we argue that *I. beyeri* should be recognized as a valid taxon and removed from the synonymy of *I. dorcoides*. We were unable to make species assignments for two of the Sonoran Desert clades (clade D, *Hololepta* sp. 1; clade E, *Hololepta* sp. 2), as well as for two species from Central America (*Hololepta* sp. 3 and sp. 4). The sister taxa *Hololepta* sp. 1 and sp. 2, which apparently are geographically isolated, were separated by mean 16S rRNA genetic distances of 3.1% (*p*-distance) and 3.2% (K2P distance), less than half that seen between *I. beyeri* and *I. dorcoides*. The final sister lineages identified in the 16S rRNA tree, *H. vicina* and *H. populnea*, were separated by mean genetic distances of 5.5% (*p*-distance) and 5.7% (K2P distance), values that were also lower than those seen between *I. beyeri* and *I. dorcoides*.

3.3. Vicariance and speciation in the Sonoran Desert Hololeptini

Molecular divergences (see Section 3.2) between the sister species *I. beyeri* from the Baja California peninsula and *I. dorcoides* from the mainland, and between *Hololepta* sp. 1 (peninsular) and *Hololepta* sp. 2 (mainland), together with geological estimates for the age of the Gulf, were used to address the question of whether the process of speciation in each pair of sister species is consistent with a scenario of population disjunction resulting from the separation of the peninsula from the mainland. Relative rates tests (Tajima, 1993) of sequence evolution in both species' pairs were not significant, indicating that a molecular clock could not be rejected for either COI or 16S rRNA. Independent calibrations of a COI molecular clock in insects, including Coleoptera, typically range from 1.5% to 2.3% pairwise sequence divergence per million years (Brower, 1994; Emerson et al., 1999; Farrell, 2001; Quek et al., 2004; Sota and Hayashi, 2007). With the exception of the peninsular Cape Region, the complex geological events that resulted in the separation of the Baja California peninsula from mainland Mexico have been dated to about 5–8 million years ago (Ma) (Holt et al., 2000; Riddle et al., 2000; Oskin and Stock, 2003). Both fossil and geological evidence, summarized by Carreño and Helenes (2002), suggest a more recent age (3–4 Ma) for the separation of the Cape Region from the mainland. These considerations lead to the prediction that COI divergences in geographically isolated sister species that evolved in allopatry following the formation of a presumed Gulf dispersal barrier should range from about 7.5–18.4%, or 4.5–9.2% for a species inhabiting the Cape Region. The mean K2P distance between *I. beyeri* and *I. dorcoides* for the COI gene segment was 14.6%, consistent with the prediction. Although COI sequences were not available for *Hololepta* sp. 1 and sp. 2, we have estimated a value of 6% for the COI divergence between this species pair by assuming a value of about twice that of the measured 16S rRNA divergence of 3.2%. In this estimation we have assumed that the relative difference in K2P distances found between *I. beyeri* and *I. dorcoides* for 16S rRNA (7.9%) and COI (14.6%) (i.e. the estimated COI divergence is about twice the 16S rRNA divergence) would be similar in *Hololepta* sp. 1 and sp. 2. Our samples of *Hololepta* sp. 1 were obtained entirely from the Cape Region localities of La Paz ($N = 2$) and Vinateria ($N = 2$) (Fig. 1). Thus, the estimated COI divergence between *Hololepta* sp. 1 and sp. 2 falls within the predicted range of 4.5–9.2%.

4. Discussion

Although the deeper nodes in 16S rRNA phylogenetic tree (Fig. 2) remained unresolved, the tree does provide important new information on sister group relationships that can be used to infer evolutionary histories of the Hololeptini associated with columnar cacti in the Sonoran Desert. In addition to providing support for the recognition of *I. beyeri* as a valid species, and confirming that both *H. populnea* and *H. vicina* are faunal components of the necrotic cactus microhabitat, our results suggest that vicariance has played a role in shaping genetic diversification and evolution of the Hololeptini in the Sonoran Desert. It seems probable that evolution of the sister species *I. beyeri* and *I. dorcoides*, and the putative sister species *Hololepta* sp. 1 and *Hololepta* sp. 2, resulted largely from disruption of dispersal and gene flow owing to the formation of the Gulf of California. Our data also suggest that *Hololepta* sp. 1 and sp. 2 diverged more recently than *I. beyeri* and *I. dorcoides*, as would be predicted from the more recent age estimate for the separation of the Cape Region from the mainland (Carreño and Helenes, 2002). Disjunct populations (currently classified as different subspecies) of the kissing bug *Triatoma rubida* (Hemiptera: Reduviidae) from the Cape Region locality of La Paz, Baja California Sur (*T. r. cochimiensis*) and Guaymas, Sonora on the mainland (*T. r. sonoriana*) show a mean K2P divergence of 5% for COI (Pfeiler et al., 2006), similar to that estimated for *Hololepta* sp. 1 and sp. 2 (6%), which is consistent with a scenario in which both sister lineages each began to diverge from its common ancestor under similar circumstances.

Although we have focused on vicariance to explain speciation among peninsular and mainland populations of the Hololeptini, other factors also may have been involved. For example, we cannot

rule out the possibility that host plant specialization prior to habitat fragmentation contributed to genetic differentiation and speciation of *I. beyeri* and *I. dorcoides*. Marko (2002) has discussed this topic in detail in relation to the calibration of molecular clocks. We found that almost all individuals of *I. beyeri* (37 of 38 individuals) collected on the Baja California peninsula were taken on senita (a single individual was taken on pitahaya agria). Senita is distributed throughout the peninsula as well as throughout the mainland desert region of Sonora and a small area of southern Arizona (Turner et al., 1995). In contrast, the eight specimens of *I. dorcoides* from the mainland were all found on organ pipe cactus. The few senita sampled on the mainland contained only *I. cacti*. Although host plant specialization remains a possibility, increased sampling, especially on the mainland, will be required to assess its possible role on the evolution of the Hololeptini from the Sonoran Desert.

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