USCANA ESPINAE (HYMENOPTERA: TRICHOGRAMMATIDAE) IN CENTRAL MEXICO: NEW HOSTS, HOST PLANTS, DISTRIBUTION RECORDS, AND CHARACTERIZATION

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Abstract

In the framework of a biological control program with hymenopteran parasitoids to reduce the population densities of the bean weevil, *Acanthoscelides obtectus* (Say), and the Mexican bean weevil, *Zabrotes subfasciatus* (Boheman), that attack bean seeds in storage facilities in central Mexico, the parasitoid, *Uscana espinae* Girault (Hymenoptera: Trichogrammatidae), was collected. This program developed new records on the distribution, hosts, and host-plants associated with *U. espinae*. No evidence was found of cryptic species among 6 *U. espinae* populations from central Mexico by use of morphological characters, mitochondrial gene analysis (cytochrome oxidase I), and intra- and inter-population reproductive crosses. The original geographic distribution of *U. espinae* in Chile and Uruguay has been expanded to include the states of Morelos, Puebla, and Veracruz in Mexico.

Key Words: Bruchinae, egg parasitism, biological control, morphology, reproductive compatibility

Resumen

Dentro del marco de un programa de control biológico con parasitoides himenópteros para reducir las densidades de los gorgojos común y mexicano [*Acanthoscelides obtectus* (Say) y *Zabrotes subfasciatus* (Boheman)] que atacan semillas de frijol almacenado en el centro de México, fue colectado el parasitoide *Uscana espinae* Girault (Hymenoptera: Trichogrammatidae). La colecta de este parasitoide provee de nuevos registros sobre su distribución, hospederos y plantas hospederas. No se detectó evidencia de especies crípticas en seis poblaciones del centro de México mediante la utilización de caracteres morfológicos, un análisis del citocromo oxidasa I (COI) y cruzas reproductivas dentro y entre poblaciones. Se amplía la distribución geográfica de *U. espinae* de Chile y Uruguay para ahora incluir a los estados de Morelos, Puebla y Veracruz en México.

The genus *Uscana* Girault (Hymenoptera: Trichogrammatidae) is comprised of solitary and idiobiont endoparasitoids, with 90% of its species using eggs of the coleopteran subfamily, Bruchinae, as hosts. It is a little studied cosmopolitan genus, and is among the most derived in accordance with the latest molecular phylogenetic classification of Trichogrammatidae (Owen et al. 2007). Some species parasitize the eggs of cosmopolitan insect pests such as the bean weevil *Acanthoscelides obtectus* (Say), and the cowpea weevils *Callosobruchus maculatus* F. and *Bruchidius atrolineatus* Pic (Coleoptera, Chrysomelidae, Bruchinae), which feed on legume seeds, including beans of the genera *Phaseolus* and *Vigna*. *Uscana* spp. are specialists, achieving a high level of parasitism, making them possible biological control agents of bruchid beetles that feed

on stored beans (van Huis et al. 1990; van Huis 1991; Fursov 1995; Bonet et al. 2002). Worldwide, 28 species have been identified (Pinto 2006); of these, 3 have been recorded as endemic to the New World: *Uscana semifumipennis* Girault (also known in Japan and Hawaii), *U. espinae* Pintureau & Gerding, and *U. chiliensis* Pintureau & Gerding (Fursov 1995, Pintureau et al. 1999, Pinto 2006). The type species *U. semifumipennis* has been recorded in the U.S., Mexico, and Guatemala (Pinto 2006). In Chile, there are records of *U. espinae* and *U. chiliensis* (Pintureau et al. 1999), and specimens of *Uscana* spp. have been recorded in Brazil and Argentina (Pinto 2006). In Mexico, the only representative of the genus that has been recorded is *U. semifumipennis*, a natural enemy of Mexican bruchids (Pinto 2006).

To date, research has been conducted on the reproductive and behavioral aspects of some parasitoid species of the genus *Uscana*, with data on their hosts in order to determine whether they would make good biological control agents of pests of stored seeds. Studies have focused on African species such as *U. caryedoni* Viggiani (Delobel 1989), and *U. lariophaga* Steffan (van Alebeek & van Huis 1997), on the European species *U. olgae* Fursov and *U. senex* (Grese) (Fursov 1995), and on the Indian species *U. mukerjii* (Mani) (Sood & Pajni 2006). Regarding *U. lariophaga*, research has addressed several aspects of its behavior in order to control the bruchid *C. maculatus*, which feeds on stored *Vigna* seeds (van Huis et al. 1998, 2002, van Alebeek et al. 2007). Nevertheless, the taxonomy and other aspects of the biology of New World *Uscana* species are still poorly known.

The importance of studying *Uscana* wasps in Mexico relates to the possibility of using the genus in biological control of the bean and Mexican bean weevils (*Acanthoscelides obtectus* Say and *Zabrotes subfasciatus* Boheman), which cause significant losses to farmers who produce *Phaseolus* beans (Leroi et al. 1991; Bonet et al. 2000; Alvarez et al. 2005). Of particular concern is finding native biological control agents capable of mitigating the damage done by weevils to Mexico's bean cultivars, especially in the rustic storage facilities of self-sufficient farmers in central Mexico, where weevils damage 20% of all stored dried beans (Bonet et al. 2000). Research has shown that in field conditions, wasps of the genus *Uscana* parasitize up to 85% of the eggs of bruchids that attack wild bean seed populations (*Phaseolus vulgaris* var. *aborigeneus* L.) in Mexico (Pérez & Bonet 1984; Delgado et al. 1988; Leroi et al. 1990).

The purposes of this study were to taxonomically identify *Uscana* species collected from 6 populations in Central Mexico, and provide records of their hosts, host associations with plants, and geographic distribution. The presence of cryptic *Uscana* species among populations was also investigated using morphological characters, genotype analysis (COI), and intra- and inter-population reproductive crosses.

Materials and Methods

Collection of Insects

The analyzed individuals came from insects that were bred in the laboratory on the bean weevil, *Acanthoscelides obtectus*. The original stock of weevils had been collected from 5 locations in Central Mexico at altitudes of 697 to 1900 masl.: Pantitlán and Tepoztlán, in the State of Morelos; Río Ahuehueyo in Puebla; and Estanzuela and El Campanario I and II in Veracruz (Table 1). The initial *Uscana* individuals used for breeding were collected from 4 bruchid hosts and 3 host plants species (Table 1). During breeding, fecundity remained constant.

Morphological Description of Adults

Male and female *Uscana* adults were slide-mounted in Canada balsam (Platner et al. 1998). Measurements of the main taxonomic characters used to classify Trichogrammatidae species, especially *Uscana,* were taken; these include the antennal club and venation of the forewing in males and females, as well as the male genitalia (Table 2). The length of each morphological structure refers to the maximum length in micrometers.

Specimen identification was done by B. Pintureau and A. Bonet using the descriptions and key of Pintureau et al. (1999). Voucher samples on microscope slides were deposited in the IEXA insect collection at Instituto de Ecología A. C.

Cryptic Species Analysis through Morphological Characters

To determine if the wasp samples collected in the different populations corresponded to one or several species, comparisons of their morphological characters were performed (Pintureau et al. 1999; Pinto 2006). In both males and females, 2 antennal and one forewing measurements were compared, and for males, the aedeagus lengths were also compared (Table 2).

Cryptic Species Analysis through COI Barcode Analysis

The COI barcode technique was used to detect the presence of cryptic *Uscana* species as well as possible haplotypes (Herbert et al. 2003a, 2003b, 2004; Smith et al. 2005; Ratnasinham & Herbert 2007; Waugh 2007). Barcoding molecular gene analysis was carried out in 4 adult individuals from each of the 6 populations (Tables 1 and 3). DNA was extracted from each ethanol-preserved adult using the DNeasy Tissue Kit (Quiagen, Hilden, Germany). The whole adult body was

TABLE 1. RECORDS OF GENE BANK ACCESSION NUMBERS, LOCALITIES, HOSTS, HOST-PLANTS, YEAR OF COLLECTION, GEOGRAPHICAL LOCATION, AND ALTITUDE OF *USCANA* POPULATIONS ANALYZED.

GenBank accession No.	Host (Coleoptera: Bruchidae)	Host plant (Leguminosae)	Locality	Year of collection	Geographic location	Altitude (m asl)
AB600898 – AB600901	*Acanthoscelides obtectus* (Say) and *Acanthoscelides obvelatus* Bridwell	*Phaseolus vulgaris* L.	Río Ahuehueyo, Puebla	1996	18°37'0.36"N, 98°33'55.18"W	1250
AB600902 –AB600905	*Mimosestes humeralis* (Gyllenhal)	*Acacia pennatula* (Schltdl. & Cham.) Benth.	Estanzuela, Veracruz	2003	19°27'12.8", 96°51' 47.6"W	1038
AB600906 – AB600909	*Acanthoscelides obtectus* (Say)	*Phaseolus vulgaris* L.	Pantitlán, Morelos	2000	18°55'0.6"N, 98°59'36.6"W	1285
AB600910 – AB600913	*Acanthoscelides obtectus* (Say) and *Acanthoscelides obvelatus* Bridwell	*Phaseolus vulgaris* L.	Tepoztlán, Morelos	2005	18°59'51.9"N, 9°7'30.4"W	1900
AB600914 – AB600917	*Acanthoscelides oblongoguttatus* Fåhraeus)	*Acacia sphaerocephala* Schltdl. & Cham.	El Campanario I, Veracruz	2006	19°21'49.5", 96°50'44.1"	697
AB600918 – AB600921	*Mimosestes humeralis* (Gyllenhal)	*Acacia pennatula* (Schltdl. & Cham.) Benth.	El Campanario II, Veracruz	2006	19°21'49.5", 96°50'44.1"	697

put in a 180µL ATL buffer with 20µL proteinase K and incubated at 55 °C for 36 h. After incubation, total genomic DNA was extracted following the manufacturer's instructions. A DNA fragment of the mitochondrial cytochrome C oxidase subunit I (COI) gene was amplified using the polymerase chain reaction (PCR) with the primers LCO1490 and HCO2198 (Folmer et al. 1994). The template profile was as follows: 94.0 °C for 5 min; 35 cycles at 94.0 °C for 45 s, 48.0 °C for 45 s, and 72.0 °C for 90 s; and 72.0 °C for 8 min. PCR was performed in a reaction volume of 40 µL using 10 × EX Taq Buffer (Takara Bio, Tokyo, Japan), 0.2 mM each dNTP, 0.5 µM each primer, 0.5 U/µl EX Taq DNA polymerase (Takara Bio), and 0.2 µM temperate DNA. The PCR product was purified using Montage PCR (Millipore, Billerica, Massachusetts) and served as a template for cycle sequencing reactions with CEQ quick start mix (Beckman Coulter, Fullerton, California) following the manufacturer's instructions. After ethanol precipitation, the cycle sequencing products were sequenced using the CEQ8000 Genetic Analysis System (Beckman, Coulter). DNA sequences obtained in both directions were assembled and edited using ATGC version 4.0 (Genetyx, Tokyo, Japan). The assembled sequences were aligned manually and edited using Bioedit version 5.0.9 (Hall 1999). 531 base pairs of COI gene were compared for 4 *Uscana* individuals from each population in order to discern polymorphisms within them. The DNA sequences determined were deposited in the GenBank under the accession numbers AB600848-AB600921.

Reproductive Compatibility

Reproductive compatibility was analyzed for the 2 populations that were furthest apart (230 km) (Pantitlán, Morelos and Estanzuela, Veracruz). Crossings between male and female adults were done in group and single-pairs in order to obtain a reproductive compatibility coefficient between them (Pinto et al. 1991; Pintureau 1991; Stouthamer et al. 2000). Group mating provides some choice for mates and therefore would be more likely to reveal reproductive incompatibility; however, single-pair matings provide better quantification, in a 'no-choice' situation, of the level of incompatibility (Liu et al. 2002).

The different levels of reproductive compatibility seen in crosses between different populations help to explain the intra-specific variation observed in the species' geographic distribution (Hopper et al. 1993). A reproductive compatibility under 80% permits the separation of species in *Trichogramma* (Pinto et al. 1991), and cryptic species are present when heterogamic crosses are < 75% homogamic ones (Stouthamer et al. 2000). Pintureau (1991) and Pinto et al. (1991)'s procedure for crossing males (m) and females (f) was followed, with the reproductive compatibility

TABLE 2. MORPHOLOGICAL CHARACTERS (MEAN ± SE IN MICRONS)[1] OF ADULT MALES AND FEMALES OF *USCANA ESPINAE* COLLECTED IN FIVE LOCATIONS IN CENTRAL MEXICO.

Adult characters	Pantitlán	Tepoztlán	Río Ahuehueyo	Estanzuela	El Campanario
Males (N)	14	5	5	13	10
Fimbria length/forewing width	0.21 ± 0.00 a	0.19 ± 0.00 b	0.21 ± 0.01 a	0.22 ± 0.00 a	0.23 ± 0.01a
	H = 13.58; df = 4,48; P = 0.0087				
Mean lengths of the C1/C2 antennal segments	1.01 ± 0.03	1.06 ± 0.03	1.02 ± 0.04	1.05 ± 0.02	1.04 ± 0.03
	F = 0.59; df = 4,47; P = 0.6728				
Mean lengths of the C3/C2 antennal segments	1.27 ± 0.03	1.26 ± 0.06	1.10 ± 0.03	1.29 ± 0.03	1.25 ± 0.02
	F = 0.29; df = 4,47; P = 0.8776				
aedeagus length	52.37 ± 0.70	56.61 ± 1.87	53.44 ± 0.90	53.94 ± 0.79	55.02 ± 1.04
	F = 2.28; df = 4,47; P = 0.0769				
Females (N)	10	5	5	14	8
Fimbria length/anterior wing width	0.18 ± 0.00	0.18 ± 0.00	0.18 ± 0.00	0.18 ± 0.00	0.17 ± 0.01
	H = 2.22; df = 4,40; P = 0.6962				
Mean lengths of the C1/C2 antennal segments	1.12 ± 0.03	1.10 ± 0.02	1.15 ± 0.04	1.10 ± 0.04	1.12 ± 0.04
	F = 0.22; df = 4,40; P = 0.9231				
Mean lengths of the C3/C2 antennal segments	1.34 ± 0.04	1.19 ± 0.02	1.34 ± 0.04	1.28 ± 0.03	1.33 ± 0.04
	F = 1.76; df = 4,42; P = 0.1581				

[1]F: F-statistic, H: Kruskal–Wallis test statistic. Means with different letters are significantly different ($P < 0.05$).

TABLE 3. CHARACTERIZATION OF *USCANA ESPINAE* HAPLOTYPES IN CENTRAL MEXICO. POLYMORPHISMS ARE LOCATED ON THE THIRD CODON, AND SYNONYMS.

		Segregating position on COI fragment		
Haplotype	Populations (specimen #)[1]	121	211	355
Haplotype 1	CA I	A	C	G
Haplotype 2	ES, PA (2)	A	T	A
Haplotype 3	RA, PA (1, 3, 4), TE, CA II	G	T	A

[1]The numbers appearing in brackets correspond to the individual used. Population abbreviations are given in Table 1.

coefficient of 2 populations (A × B) measured as the average number of female progeny in heterogamic combinations (A m × B f) divided by the mean in each homogamic combination (A m × A f), or (B m × B f), as well as their respective reciprocal crosses.

In the present study, heterogamic crosses were carried out with virgin individuals from each population without *a priori* knowledge of the sex of the 2 individuals or, for group mating, the sex of all individuals. When female progeny were recorded, they were *a posteriori* interpreted as resulting of crosses. As the sex of virgin adults could not be determined before each mating, it was assumed that the 35 replicates in both directions that resulted in female progeny included heterogamic crosses.

Two intra-population crosses were done (PA m × PA f) and (ES m × ES f) as homogamic controls, as well as one heterogamic inter-population cross (PA mf × ES mf). The individuals used for these crosses came from laboratory breeding on *A. obtectus* over 6 and 7 generations of wasp and host, respectively. Intra-population crosses were done under environmental conditions of 23 ± 2 °C, with 63 ± 10 % RH and at 12:12 h L:D. The embryonic development of wasp progeny occurred in a breeding chamber at 25 ± 1 °C, with a 55 ± 5 % RH.

For all crosses, both single-pairs and group, adult virgin males (m) and females (f) born on the same day were left alone with honey so that they would mate. Afterward, each female was isolated in a gelatin capsule with 50 eggs of the host *A. obtectus* (24 to 48 hours old); the eggs were changed daily, so that oviposition could occur until death of the adult female. For single-pair crosses (N = 35), mating occurred in one-half of a gelatin capsule (2.5 cm long × 0.6 cm diam). In the case of group crosses, adults (N = 80) were placed in a container (4 cm high × 7 cm base diam) for 24 h with honey in order for mating to take place. Afterward, 40 randomly chosen female individuals were isolated in gel capsules with host eggs. Parasitized host eggs were placed in a breeding chamber until progeny emerged. The sex of both adult progenitors and progeny was confirmed after they had died.

For each type of cross, the number of parasitized hosts per wasp female was recorded, as well the number and sex ratio of progeny that emerged and the percentage of survival from egg to adult stage. The reproductive compatibility coefficient was calculated following Pinto et al. (1991). It is estimated as 2 percentages that compare levels of female progeny arising from heterogamic mating versus the homogamic control (Pinto et al. 1991): 100 × mean sex ratio of progeny (= proportion of female progeny) (A m × B f) / mean sex ratio of progeny (A m × A f), with the same calculation done for reciprocal mating.

Statistical Analyses

The morphological characters of individuals from different populations and the results of reproductive crosses between populations were compared with one way ANOVA. When significant differences were found, Tukey's multiple comparisons test was used to detect differences between populations. When ANOVA assumptions were not met, a non-parametric Kruskal-Wallis analysis of variance and multiple comparisons using the "Fisher's least significant difference on the ranks" test were used (Conover 1980; Sokal & Rohlf 1995).

RESULTS

The individuals collected from the 6 central Mexican populations belong to the species *U. espinae*. This is the first Mexican record for *U. espinae*; and *Acanthoscelides obtectus*, *A. obvelatus* Bridwell, *A. oblongoguttatus* (Fåhraeus), and *Mimosestes humeralis* (Gyllenhal) are new host records; and *Phaseolus vulgaris*, *Acacia pennatula* (Schltdl. and Cham.) and *A. sphaerocephala* Schltdl. and Cham. are new host plants for the wasp (Table 1).

Morphological Analysis

Measurements of the morphological characters (antennae, forewings and aedeagus) of individuals from different populations were similar (Table 2). The only difference was found in Tepoztlán, where the ratio of male fimbria length and anterior wing width was significantly different

from the corresponding ratios of all other populations (Table 2).

Molecular Analysis of the COI Gene

In the 6 populations analyzed, 3 haplotypes were found (Table 3). Variation within and among populations was less than 1% for the 531 base pairs of the COI gene. Haplotype 1 was found only at one location, El Campanario I, Veracruz, on *Acanthoscelides oblongoguttatus* on the plant, *Acacia sphaerocephala*. Haplotype 2 was found in Pantitlán, Morelos and Estanzuela, Veracruz. Haplotype 3 was found in 4 populations in 3 states (Morelos, Puebla, and Veracruz). The population in Pantitlán was the only one with more than one haplotype (haplotypes 2 and 3) (Table 3).

Reproductive Compatibility

No reproductive isolation between the 2 populations crossed was found. Adults from these 2 populations (Pantitlán and Estanzuela) had a reproductive compatibility coefficient of 85% for single-pair crosses and 88-92% for group crosses, with no detectable element of reproductive incompatibility (Table 4).

The group cross in the Pantitlán population produced 15 % more parasitoids in its progeny (F = 5.96; df = 2,102; P = 0.0036) than that in the Estanzuela population (Table 4). The sex ratio in progeny of inter-population heterogamic crosses per group was lower (H = 8.81; df = 2,102; P = 0.0122) than those recorded from homogamic crosses in Pantitlán and Estanzuela (Table 4), but this difference did not reach the threshold of 75% used by Stouthamer et al. (2000) as an indication of species separation. Survival percentage of progeny to adulthood did not differ significantly within and among populations in the case of single-pair crosses and group crosses (Table 4).

Discussion

The search for a biological agent to control *A. obtectus* and *Z. subfasciatus* led to the discovery

Table 4. Progeny (mean ± SE)[1], sex ratio of progeny, percent survival from egg to adult, reproductive compatibility resulting from intra- and interpopulation crosses in *Uscana espinae* (pair and group matings). Males (m), females (f), and male-female mixture (mf) that originated from Pantitlán, Morelos (pa) and Estanzuela, Veracruz (es).

Biological parameters	Inter-population cross PA (MF) x ES (MF)	Intra-population cross PA x PA (M x F)	Intra-population cross ES x ES (M x F)
Single-pair matings			
Number of females	35	35	35
Proportion of females in progeny	0.52 ± 0.04	0.62 ± 0.03	0.61 ± 0.02
	H = 2.74; df = 2,105; P = 0.2541		
No. of parasitoids produced	25.4 ± 0.61	25.37 ± 1.00	25.91 ± 0.79
	F = 0.14; df = 2,105; P = 0.8689		
% of progeny surviving to adulthood	0.86 ± 0.01	0.85 ± 0.02	0.84 ± 0.01
	H = 0.52; df = 2,105; P = 0.7714		
Reproductive Compatibility		85 %	85 %
Group matings			
Number of females	35	35	35
Proportion of females in progeny	0.53 ± 0.02a	0.61 ± 0.03b	0.58 ± 0.04b
	H = 8.81; df = 2,102; P = 0.0122		
No. of parasitoids produced	28.70 ± 0.91a	28.74 ± 0.90a	24.62 ± 1.09b
	F = 5.96; df = 2,102; P = 0.0036		
% of progeny surviving to adulthood	0.81 ± 0.02	0.79 ± 0.02	0.80 ± 0.02
	F = 0.01; df = 2,105; P = 0.4242		
Reproductive Compatibility		88%	92%

[1]F: F-statistic, H: Kruskal–Wallis test statistic. Means with different letters are significantly different (P < 0.05).

of the endoparasitoid *U. espinae*, parasitizing weevil eggs in several localities of central Mexico. It was identified on the basis of its morphology, using the species' diagnostic characters, and no cryptic species could be detected. It was determined that all *Uscana* specimens from Pantitlán, Tepoztlán, Río Ahuehueyo, Estanzuela, and El Campanario I and II belong to the same species, which appears to be distributed throughout central Mexico. This is a new record for Mexico, with new hosts and host plant associations. *Uscana espinae* had been recorded only from Chile and Uruguay attacking the eggs of the bruchids *Pseudopachymerina spinipes* (Erichson 1833), *Scutobruchus ceratioborus* (Philippi) and *Stator furcatus* on *Acacia caven* (Molina) Molina and *Prosopis chilensis* (Molina) (Pintureau et al. 1999, Rojas-Rousse 2006). Thus, 4 new hosts have been identified (*Acanthoscelides obtectus*, *A. obvelatus*, *A. oblongoguttatus*, and *Mimosestes humeralis*) as well as 3 new host plants (*Phaseolus vulgaris*, *Acacia pennatula*, and *A. sphaerocephala*).

The COI barcode technique was used to test for the presence of cryptic *Uscana* species at the different locations. According to Herbert et al. (2003b) and Waugh (2007), if intra-population variation is < 2%, variation can be considered to be intra-specific. The variation recorded in the 6 populations considered was less than 1% for the 531 base pairs of the COI gene analyzed, leading to the conclusion that no cryptic species were present.

Among the 3 different haplotypes identified on the basis of the COI mitochondrial gene, two were found (2 and 3) on the same host plant, *P. vulgaris,* in Pantitlán. Haplotype 3 was found in individuals from 3 locations, Río Ahuehueyo, Tepoztlán, and El Campanario II, while haplotype 2 was also present in Estanzuela. This suggests that haplotypes 2 and 3 represent 2 morphs of the same species. Haplotype 1, more differentiated, was only present at location Campanario I where wasp individuals were collected inside *A. oblongoguttatus* eggs on the plant *Acacia sphaerocephala* pods.

Reproductive isolation was not found between individuals of Pantitlán and Estanzuela, as reproductive compatibility was 85% for single-pair crosses and 88-92% for group crosses, thus not under the 75% indicated by Stouthamer et al. (2000) to discriminate species. However, some variability was observed among populations in terms of number and sex ratio of progeny.

The use of native biological control agents is indispensable to reduce densities of bruchid beetles by environmentally friendly means. Phylogeographical research is needed throughout the geographic distribution area of *U. espinae* in order to confirm its native or non-native status in Mexico (e.g., Alvarez et al. 2005), because the species was known only from Chile and Uruguay. Its distribution has now been expanded to include the Mexican states of Morelos, Puebla, and Veracruz. *U. espinae* populations from central Mexico could be recommended in mass rearing laboratories to be used in an augmentative program to control the common and Mexican bean bruchids (van Huis 1991, Bonet et al. 2005).

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