Uscana espinae (Hymenoptera: Trichogrammatidae) in Central Mexico: New Hosts, Host Plants, Distribution Records, and Characterization

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**Uscana espinae** (Hymenoptera: Trichogrammatidae) in Central Mexico: New Hosts, Host Plants, Distribution Records, and Characterization

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

**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 cripticas en seis poblaciones del centro de México mediante la utilización de caracteres morfológicos, un análisis del citocromo oxidasa I (COI) y cruas reproductivas dentro y entre poblaciones. Se amplía la distribución geográfica de *U. espinae* en Chile y Uruguay para ahora incluir a los estados de Morelos, Puebla y Veracruz en México.

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

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. esiniae Pintureau & Gerding, and U. chilensis 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. esiniae and U. chilensis (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 Zabrotos 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; Rio Ahuehuevo 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...
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.

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

\(^1\)F: F-statistic, \(H\): Kruskal–Wallis test statistic. Means with different letters are significantly different (\(P < 0.05\)).
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 as 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 (Fähraeus), 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 those of the other populations.
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>
<thead>
<tr>
<th>Biological parameters</th>
<th>Inter-population cross</th>
<th>Intra-population cross</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PA (MF) x ES (MF)</td>
<td>PA x PA (M x F)</td>
</tr>
<tr>
<td>Single-pair matings</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of females</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Proportion of females in progeny</td>
<td>0.52 ± 0.04</td>
<td>0.62 ± 0.03</td>
</tr>
<tr>
<td>No. of parasitoids produced</td>
<td>25.4 ± 0.61</td>
<td>25.37 ± 1.00</td>
</tr>
<tr>
<td>% of progeny surviving to adulthood</td>
<td>0.86 ± 0.01</td>
<td>0.85 ± 0.02</td>
</tr>
<tr>
<td>Reproductive Compatibility</td>
<td>85 %</td>
<td>85 %</td>
</tr>
<tr>
<td>Group matings</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of females</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Proportion of females in progeny</td>
<td>0.53 ± 0.02a</td>
<td>0.61 ± 0.03b</td>
</tr>
<tr>
<td>No. of parasitoids produced</td>
<td>28.70 ± 0.91a</td>
<td>28.74 ± 0.90a</td>
</tr>
<tr>
<td>% of progeny surviving to adulthood</td>
<td>0.81 ± 0.02</td>
<td>0.79 ± 0.02</td>
</tr>
<tr>
<td>Reproductive Compatibility</td>
<td>88%</td>
<td>92%</td>
</tr>
</tbody>
</table>

$F$: F-statistic, $H$: Kruskal–Wallis test statistic. Means with different letters are significantly different ($P < 0.05$).
of the endoparasitoid U. espiniae, 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 Usca specimens from Pantitlán, Tepoztlán, Rio Ahuehuey, 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. Usca espiniae 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 (Philippi) and Scutobruchus ceratioborus. This is a new record for Mexico, cies, which appears to be distributed throughout El Campanario I and II belong to the same species. Haplotype 1, more differentiated, was only found (2 and 3) on the same host plant, A. oblongoguttatus, and A. sphaerocephala.

The COI barcode technique was used to test for the presence of cryptic Usca 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, Rio Ahuehuey, 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. espiniae 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. espiniae populations from central Mexico could be recommended in mass rearing laboratories to be used in an augmented program to control the common and Mexican bean bruchids (van Huis 1991, Bonet et al. 2005).

Acknowledgments

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