Troglobites from the Lava Tubes in the Sierra de Chichinautzin, Mexico, Challenge the Competitive Exclusion Principle

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Introduction

The Sierra de Chichinautzin is located south of Mexico City and north of Cuernavaca, in Mexico. This volcanic mountain range had, in relatively recent times, (Holocene) at least seven lava flows with the formation of lava tubes (Espinasa-Pereña, 1999). Multiple caves of great extension can be found in this mountain range, including the Cueva del Ferrocarril, the largest lava tube in the Americas, at about 6 km, and Cueva de la Iglesia, at about 5 km. A detailed description of most of the cave systems in the area can be found in Espinasa-Pereña (1999, 2006).

Some of the Sierra de Chichinautzin lava tubes are inhabited by cave adapted silverfish insects (Cubacubaninae: Nicoletiidae: Insecta). Nicoletiids are one of the most important and common representatives of cave adapted fauna in the Neotropics and southern North America. While studying the relationships within the subfamily Cubacubaninae, Espinasa et al. (in press) included three troglobitic individuals from three different lava tubes from the Sierra de Chichinautzin: Cueva de la Iglesia, Cueva del Aire, and Cueva del Naranjo Rojo. Contrary to what might be expected due to the geographical proximity of the caves, the sequence data from five loci showed that the individuals belonged to two different species. The individual from Cueva de la Iglesia actually appeared to be more closely related to a species from a near surface locality, the town of Alpuyeca, than to its neighboring troglobite (Fig. 1).

The purpose of this study is to better understand how many species of troglobitic nicoletiid insects inhabit the lava tubes of the Sierra de Chichinautzin, their distribution, and their dispersal capabilities among caves.

Material and methods

Samples were collected by hand and deposited in 95% ethanol. Dissections were made with the aid of a stereo microscope. Total DNA was extracted from one leg of each individual using Qiagen's DNEasy® Tissue Kit. Molecular data have been obtained for 13 terminals, sometimes including more than one individual per locality (Table 1)

Markers were amplified and sequenced as a single fragment using the 16Sar and 16Sb primer pair for 16S rRNA (Edgecombe et al., 2002). Amplification was carried out in a 50 μl volume

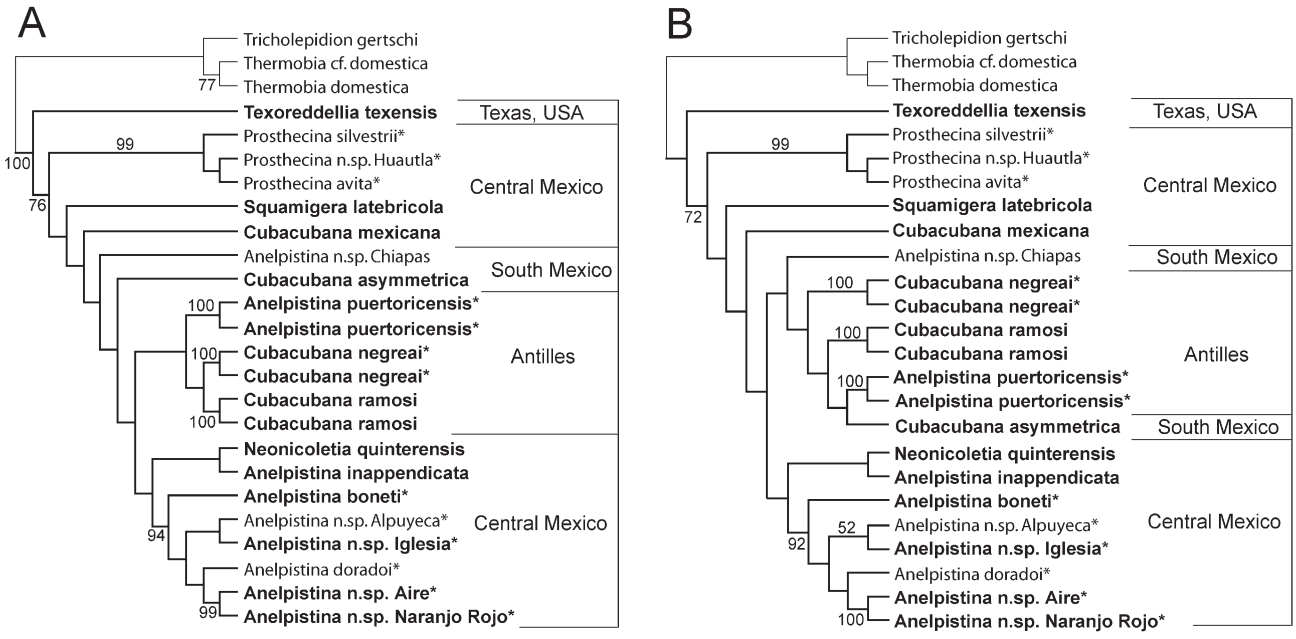

Figure 1. Figure taken from Espinasa et al. (In press). Two equally costly trees derived from the analysis of the combined analysis of sequence data from five loci and morphology. Bold species names indicate cavernicolous species. Asterisks denote species in which adult males have articulated submedian appendages on urosternite IV. Numbers on branches indicate jackknife support values. Notice that the specimen labeled *Anelpistina* n. sp. Iglesia is more closely related to *Anelpistina* n. sp. Iglesia than it is to both *Anelpistina* n. sp. Aire and *Anelpistina* n. sp. Naranjo Rojo.

Table 1. Samples studied, locality of collections, and references.

Samples	Locality of sample used	References
Anelpistina n.sp. Alpuyeca 1 individual	Alpuyeca, Morelos, Mexico (18º43' N, 99º15' W)	Google Earth
Anelpistina n.sp. Iglesia 6 individuals	Cueva de la Iglesia-Mina Superior, San Juan Tlacotenco, Morelos, Mexico (19º01' 02'' N, 99º05' 29'' W)	Espinasa, 1999; Espinasa-Pereña, 1999
Anelpistina n.sp. Aire *1 individual*	Cueva del Aire, Ajusco, DF, Mexico (19º13'30'' N, 99º10'24'' W)	Espinasa-Pereña, 1999
Anelpistina n.sp. Naranjo Rojo 4 individuals	Cueva del Naranjo Rojo, km 6.5 on the Cuernavaca-Tepoztlan highway, Morelos, Mexico (18º58' 46''N, 99º10' 54'' W)	Tapie, 1987; Google Earth
Anelpistina n.sp. Herradura 1 individual	Cueva de la Herradura, km 6.5 on the Cuernavaca-Tepoztlan highway, Morelos, Mexico (18º59' N, 99º11')	Google Earth

reaction, with 1.25 units of AmpliTaq® DNA Polymerase (Perkin Elmer, Foster City, CA, USA), 200 μM of dNTPs, and 1 μM of each primer. The PCR program consisted of an initial denaturing step at 94 °C for 60 sec, 35 amplification cycles (94 °C for 15 sec, 49 °C for 15 sec, 72 °C for 15 sec), and a final step at 72 ºC for 6 min in a GeneAmp® PCR System 9700 (Perkin Elmer).

PCR amplified samples were purified with the AGTC® Gel Filtration Cartridges (Edge BioSystems, Gaithersburg, MD, USA), and directly sequenced using an automated ABI Prism® 3700 DNA analyzer. Cycle-sequencing with AmpliTaq® DNA polymerase, FS (Perkin-Elmer) using dye-labeled terminators (ABI PRISM™ BigDye™ Terminator Cycle Sequencing Ready Reaction Kit, Foster City, CA, USA) was performed in a GeneAmp® PCR System 9700 (Perkin Elmer). The sequencing reaction was carried out in a 10 μl volume reaction: 4 μl of Terminator Ready Reaction Mix, 10-30 ng/ml of PCR product, 5 pmoles of primer and dH_20 to 10 μl. The cycle-sequencing program consisted of an initial step at 94 °C for 3 min, 25 sequencing cycles (94 °C for 10 sec, 50 ºC for 5 sec, 60 °C for 4 min), and a rapid thermal ramp to 4 °C and hold. The BigDye-labeled PCR products were cleaned with AGTC® Gel Filtration Cartridges (Edge BioSystems). Chromatograms obtained from the automated sequencer were read and contigs made using the sequence editing software Sequencher™ 3.0. Complete sequences were edited in MacGDE (Linton, 2005), where they were split according to conserved secondary structure features. All external primers were excluded from the analyses.

Individuals whose sequence was different from other members of the same locality received a second DNA extraction and sequencing to verify that no contamination or human error had occurred.

Results and Discussion

Individuals from all localities belong to genus *Anelpistina* and are similar to *Anelpistina cuaxilotla* (Espinasa, 1999). Those of the cave localities were very similar in morphology, sharing troglobitic characters such as enlarged antennae, caudal appendages and legs. On the contrary, the Alpuyeca samples were easily differentiated by their comparatively smaller appendage/body ratio, as befits surface nicoletiids.

Table 2. Partial sequence alignment of mitochondrial 16S rRNA spanning nucleotides 298-354. In bold, specimens with a distinctive sequence corresponding to a species different to the majority of individuals of that cave locality. Dots = same nucleotide; lines = insertions or deletions; letters = nucleotides.

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Iglesia          TGACTAACCCTCTTGTAGGCAAGATTGTTTTATGGGCATG--TTGTTGATCCTTT-TATTAAGATTAATA
Iglesia          ............................................................
Iglesia          ..............................A.............................
Iglesia          ............................................................
Iglesia          ............................................................
Naranjo Rojo     ............................................................
Aire             .A.C....--.......T...T.GG.TAC..T.AA.T...GT..A..........A...........T..
Heradura         .A.T....--.......T...T.GG.TAC..T.AA.T...GT..A..........A...........T..
Naranjo Rojo     .A.T....--.......T...T.GG.TAC..T.AA.T...GT..A..........A...........T..
Naranjo Rojo     .A.T....--.......T...T.GG.TAC..T.AA.T...GT..A..........A...........T..
Naranjo Rojo     .A.T....--.......T...T.GG.TAC..T.AA.T...GT..A..........A...........T..
Iglesia          .A.T....--.......T...T.GG.TAC..T.AA.T...GT..A..........A...........T..
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Sequence data from thirteen individuals were obtained. Length of fragment analyzed was of 499 nucleotides. Sequence analysis (Table 2) showed that individuals could be arranged into three distinct groups. The first group was composed of the individuals from Cueva del Aire, Cueva de la Herradura, three individuals from Cueva del Naranjo Rojo and one individual from Cueva de la Iglesia. Nucleotide differences among this group averaged 1.2 nucleotides, ranging from a minimum of zero to a maximum of four. The second group was composed of one individual from Naranjo Rojo and five individuals from Cueva de la Iglesia. Nucleotide differences among this second group averaged 2.1 nucleotides, ranging from a minimum of zero to a maximum of five. The last group was composed of the single individual from Alpuyeca. Members of group one against members of group two differed on 71 nucleotides on average, ranging from a minimum of 55 and a maximum of 78. Members of group one differed from the Alpuyeca individual by an average of also 71 nucleotides, spanning from 62-73 nucleotide differences. Members of group two differed from the Alpuyeca individual by an average of 54 nucleotides, spanning from 49-56 nucleotide differences.

Differences between individuals within a group are within the boundaries of members of a species for the Cubacubanininae, on the contrary, the differences among groups are those typically found across different species (Espinasa et al. in press). It appears that members of group one belong to an as of yet undescribed species, different from the also undescribed species of group two. This group two also appears to be more closely related to the surface species from Alpuyeca than they are to the troglobitic specimens of group one, which is in agreement with what was found by Espinasa et al. (In press) and shown in figure 1.

An interesting aspect of the two troglobitic species is that they can be found in several cave localities, regardless of the lava tube being formed from different lava flows. Members of group one species can be found along the entire Sierra de Chichinautzin in both the northern and southern lava tubes. This implies that these troglobites have the capability to disperse across lava flows, even in the absence of cave connections.

Another interesting aspect is that the two species appear to be sympatric in their geography. Both Naranjo Rojo and Iglesia cave were inhabited by members of both species. The competitive exclusion principle establishes that this is an ecological unstable situation, as two similar species can not occupy the same niche. The two species may have recently and independently colonized and adapted to the cave environment. Since the formation of these lava tubes is fairly recent (Holocene), with even Cueva de Naranjo Rojo and Cueva de la Herradura being formed less than 5,000 years B.P. (Siebe et al. 2004), it is likely that their dispersal has only recently put them in contact and we are in the remarkable position of witnessing a unique point in time and evolution where two sympatric species are in the process of a still unresolved competition for the same niche.

Conclusions

Cave Nicoletiids can disperse among lava flow systems.

The Sierra de Chichinautzin lava tube systems have independently been colonized by at least two different species of Nicoletiids.

The morphology of both species has converged as a result of troglobitic evolution.

Both species are sympatric (overlapping habitats), which represents an unstable ecological condition.

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