

Identifying the Area of Origin of the California Population of the Invasive Goldspotted Oak Borer, *Agrilus auroguttatus* Schaeffer (Coleoptera: Buprestidae)

Borer, *Agrilus auroguttatus* Schaeffer (Coleoptera: Buprestidae)

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ABSTRACT

Molecular analyses were conducted on goldspotted oak borer specimens collected in Arizona (AZ), Southern Mexico (MX), and California (CA). DNA was extracted from individuals, amplified by polymerase chain reaction (PCR), and then sequenced. Mitochondrial (COI) and nuclear (28S) DNA sequences of individuals from CA, AZ, and MX were used to estimate the relatedness of these populations. The goal of this work is to identify the potential population source that invaded California. This information will guide foreign exploration efforts for natural enemies.

INTRODUCTION

The goldspotted oak borer (GSOB), *Agrilus coxalis*, a buprestid beetle native to oak forests of Southeastern Arizona, Central Mexico, and Northern Guatemala, is causing substantial injury and mortality to three oak species in southern California. It has been estimated that more than 21,000 trees have been killed by GSOB over 4900 km² since 2002. In its native range, GSOB is not a pest. This may be due to efficient population control by natural enemies and natural levels of resistance by oak species that have co-evolved with GSOB. The integration of modern DNA-based techniques into the development of a classical biological control program for GSOB may greatly enhance the success of this effort by identifying the area of origin of CA's invasive population, thereby allowing the collection of natural enemies that have evolved to exploit the genotype of the invading pest population.



Figure 1. Oak mortality in the Cleveland National Forest.

METHODOLOGY

Collection Methods:

GSOB were collected from infested oak trees in CA, AZ, and from Chiapas and Oaxaca in southern Mexico. The CA individuals were collected from purple and green prism sticky traps which were placed throughout the Cleveland National forest (Fig. 2) in San Diego County in Southern CA. The AZ and southern MX individuals were collected by chipping into the bark and removing the larvae that were found inside these trees (Fig. 3).



Figure 2. Distribution of *Agrilus coxalis* in southern California (Coleman and Seybold 2008).

DNA Extraction, Amplification, and Sequencing:

Whole genomic DNA was extracted from individual specimens by using either a standard Chelex® extraction method or the Qiagen DNeasy Plant Tissue Kit. The polymerase chain reaction was used to amplify 658 bp of the mitochondrial gene (mtDNA) cytochrome oxidase c subunit 1 (COI) ($N=172$), and 536 bp of the D2 domain of 28S (28SD2) nuclear ribosomal DNA (rDNA) ($N=23$) with the respective primer pairs:

LCO 1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') with HCO 2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al. 1994); and, 28sF3663 (5'-TACCGTGAGGGAAAGTTGAAA-3') with 28Sr4076 (5'-AGACTCCTTGGTCCGTGTTT-3') (Choudhury, R. and J. H. Werren. 2006).

Sequence Analysis:

Sequences were aligned manually in BioEdit 7.0.5.3 (Hall 1999). COI sequences were translated using the EMBOSS-Transeq website to confirm the absence of nuclear pseudogenes (Song et al. 2008). A haplotype network was constructed using TCS version 1.21 (Clement et al. 2000). In light of major differences revealed by the COI sequences (see results), 28S sequences were used to investigate the potential existence of cryptic species and to investigate the phylogenetic relationship between the CA, AZ, and MX specimens. This was done using the 'One Click' mode on the Phylogeny.fr platform (Dereeper et al. 2008).

RESULTS & DISCUSSION

The COI gene region was sequenced from 172 individuals: 73 from AZ, 84 from CA, and 15 from MX. From these sequences, 35 haplotypes were identified (Fig. 4). The only shared haplotype was Haplotype 1, which was shared among 28 individuals from CA ($N=6$) and AZ ($N=22$) populations. Thus, the area of origin for the CA population currently remains elusive. We can rule out southern MX as the area of origin since the specimens from this area differed by nearly 11% in their COI sequences from the CA and AZ material. This level of divergence suggests that the MX specimens may in fact be a different species. To further look into this "different species" hypothesis, we sequenced the 28S gene region of a representative sample of specimens. The 28S is typically highly conserved within a species and indeed 28S sequences were identical for the CA and AZ populations. However, the 28S sequences of the MX specimens differed from the CA-AZ sequence by 8 bps. This is strong evidence that the specimens from MX represent a different species to the one from AZ and CA. The phylogenetic relationship between these specimens are shown in Fig. 5.

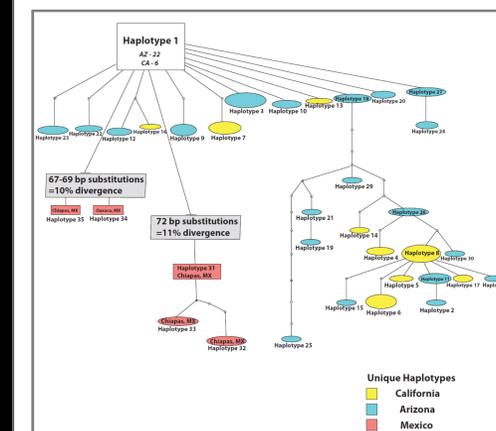


Figure 4. COI haplotype network for GSOB collected from California, Arizona, and Mexico.

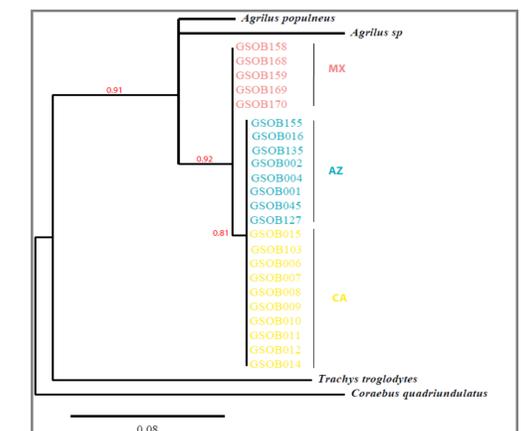


Figure 5. Phylogenetic tree that was constructed using 28S sequences. Branch support for this tree is based on an approximate likelihood ratio test (Anisimova and Gascuel 2006).

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