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Can the destruction of California's oak woodlands be prevented? Potential for biological control of the goldspotted oak borer, *Agrilus auroguttatus*

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Abstract The goldspotted oak borer (GSOB), *Agrilus auroguttatus* Schaeffer (Coleoptera: Buprestidae), is an introduced and aggressive phloem/wood borer infesting native oaks in southern California. Elevated levels of oak mortality have occurred continually for the last nine years on three oak species in San Diego Co., California, USA. Biological control is being assessed as an option for long-term and widespread management of the invasive population of GSOB. Foreign exploration in the native ranges of GSOB and a related sibling species (*Agrilus coxalis* Waterhouse) was conducted to determine life history information, to assess the natural enemy complex, and to collect specimens for molecular analyses that could help to identify the area of origin of California's introduced population. Two species of parasitoids, *Calosota elongata* Gibson (Eupelmidae) and *Atanycolus simplex* Cresson (Braconidae), were discovered with GSOB populations in Arizona and California. No insect natural enemies were found with populations of *A. coxalis* in southern Mexico. However, *Quercus conzatti* Trel. and *Quercus peduncularis* Nee in Oaxaca and Chiapas, respectively, were recorded as the first known hosts of *A. coxalis*. A comparative analysis of our understanding of the natural enemy complexes for other pestiferous *Agrilus* with that of GSOB suggests that more effort should be directed at uncovering potential egg parasitoids and microbial pathogens of GSOB. Analyses of mitochondrial and nuclear ribosomal DNA (rDNA) revealed that the California population of GSOB was more similar to the Arizona population. Specimens of *A. coxalis* from southern Mexico were confirmed as a separate species. Additional surveys and sampling are needed across the complete native range of the GSOB species complex to develop a comprehensive inventory of parasitoid species that could be considered for use in a classical biological control program in California and to delineate the area of origin of California's population.

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Introduction

The invasive goldspotted oak borer (GSOB), *Agrilus auroguttatus* Schaeffer (Coleoptera: Buprestidae), was first detected in San Diego Co., California by the California Department of Food and Agriculture in 2004 (Westcott 2005). Populations of this flatheaded borer were linked first to on-going oak mortality in 2008 after nearly six years of tree death had been misdiagnosed and attributed to prolonged drought (Coleman and Seybold 2008b). As the oak mortality continued unabated, this insect represents a significant threat to native oak woodlands in California. Analyses of tree mortality patterns suggests that since 2002, GSOB has killed more than 21,500 oaks in San Diego Co., covering an estimated 212,460 ha (Bohne and Rios 2006, 2007, 2008; Geiger and Woods 2009; Heath 2010). Tree mortality has occurred in a radially expanding pattern primarily on private, federal, and tribal lands in the mountains and foothills of eastern San Diego Co. (Heath 2010), but in 2009 an isolated population of GSOB was found killing trees in Marion Bear Memorial Park located in the suburban, coastal area of San Diego approximately 32 km from the closest known infestation.

In California, GSOB is an aggressive borer that kills coast live oak, *Quercus agrifolia* Née, California black oak, *Quercus kelloggii* Newb., and canyon live oak, *Quercus chrysolepis* Liebm. by feeding primarily at the interface of the phloem and xylem (Coleman and Seybold 2008a). In California, *Q. agrifolia* and *Q. kelloggii* are the primary hosts colonized and killed by GSOB. On rare occasions, Engelmann oak, *Quercus engelmannii*, is also colonized, but in these instances tree mortality has not been attributed solely to GSOB (Coleman and Seybold 2011).

Agrilus auroguttatus was first described in 1905 from specimens collected in the Huachuca Mountains of southeastern Arizona (Fisher 1928; Schaeffer 1905). A related species, *Agrilus coxalis* Waterhouse, had been described in 1889 from material collected in Oaxaca, Mexico (Waterhouse 1889). These species were later synonymized as *A. coxalis* because of their extensive similarity in morphology (Hespenheide 1979). Hespenheide and Bellamy (2009) assigned subspecies status (Arizona: *A. c. auroguttatus*; Mexico: *A. c. coxalis*) to the two former species based on differences in the size of the spots of setae on the elytra, in a minor morphological structure on the pronotum, and in geographic distribution and impact on host oaks. Hespenheide et al. (2011) returned the two taxa to their original species status following further assessment of the morphology of the male genitalia (Arizona: *A. auroguttatus*; Mexico: *A. coxalis*). Morphologically, the California population resembles the Arizona population (Hespenheide and Bellamy 2009). Coleman and Seybold (2008b, 2011) hypothesized that GSOB was introduced into California in firewood from southeastern Arizona or northern Mexico. Thus, this indigenous exotic species was probably moved intra-continentally from its region of origin (southeastern Arizona or northern Mexico) to a novel ecosystem via unregulated movement of firewood (Coleman and Seybold 2011; Dodds et al. 2010).

The high-levels of tree injury and mortality observed in California are hypothesized to result from the new association of GSOB with ecologically-naïve hosts and from a reduced level of impact of a co-evolved natural enemy complex on the regulation of the population of the pest (Coleman and Seybold 2011). In southeastern Arizona, GSOB's native hosts are Emory oak, *Quercus emoryi* Torrey, and silverleaf oak, *Quercus hypoleucoides* A. Camus, and oak mortality from GSOB in this area is negligible in comparison to southern California (Coleman and Seybold 2011). Extensive tree mortality in southern California is challenging management goals and altering decision making for land managers. Dead oaks can represent significant hazards around dwellings, high-use recreation areas, and wildfire corridors. Dead trees reduce aesthetic and property values and habitat quality for wildlife is degraded. These susceptible oak species hold dominant and co-dominant positions in the canopy and represent a major component in the forests of southern California, either in oak woodlands or in mixed conifer forests.

Wood boring insects in large trees are difficult to manage due to their cryptic habits, complex and lengthy life cycles, hurdles involving early detection of infested trees, limited efficacy and duration of insecticidal treatments, and cost of insecticide application. An integrated pest management program for GSOB needs to be developed and could incorporate the following: monitoring, use of trap trees, preventive insecticide applications, removal of infested trees coupled with sanitation of infested wood, and classical biological control coupled with conservation of natural enemies. In combination, these strategies will be needed to protect high-value sites and slow-the-spread of GSOB in California.

Classical biological control, the deliberate introduction of host-specific natural enemies from the pest's home range, has the potential to provide long-term, low-cost, environmentally benign, and species-specific management of GSOB across the landscape. Consequently, classical biological control is being pursued as an important tool for controlling GSOB in California. The objective of this nascent program is to use host specific natural enemies to prevent or slow tree mortality from GSOB by reducing pest population pressure, possibly to levels similar to those observed in the native range of GSOB. Initial objectives for this program were to survey GSOB's native and introduced regions to collect life history data and assess the natural enemy complex associated with larvae and pupae. Specimens collected from these regions have been used in an analysis of mitochondrial and nuclear DNA in an attempt to determine the origin of California's population.

Methods

Field surveys

Collection data from museum holdings and the literature were used to identify specific localities for surveys of GSOB and its natural enemies in its native regions of southeastern Arizona and for surveys of *A. coxalis* and its natural enemies in southern Mexico (Coleman and Seybold 2011). During 2008–2010, ground surveys were conducted in Arizona for dead and dying oaks when GSOB was dormant at the interface of the phloem and outer bark. The focus of the surveys was the collection of GSOB larvae and associated larval parasitoids and predators. Surveys were conducted in the Santa Rita, Sierra Vista, Santa Catalina, and Chiricahua Ranger Districts of the Coronado National Forest (Cochise, Pima, and Santa Cruz Cos.), which span four mountain ranges (Santa Rita, Huachuca, Santa Catalina, and Chiricahua Mountains, respectively). Aerial survey data for hardwood tree mortality is not available for this region as a result of historical low-levels of tree mortality and recreation and fuel reduction management objectives.

The four Arizona mountain ranges surveyed are represented by a diverse number of tree genera, including: *Abies* spp., *Fraxinus* spp., *Juglans* spp., *Juniperus* spp., *Picea* spp., *Pinus* spp., *Populus* sp., *Pseudotsuga* sp., and *Quercus* spp. (Elmore 1976). In these areas, Emory oak, *Q. emoryi*, Arizona white oak, *Quercus arizonica* Sarg., silverleaf oak, *Q. hypoleucoides*, and Gray oak, *Quercus grisea* Liebm., dominate lower elevation oak woodlands (1,372–1,982 m), whereas Gambel oak, *Quercus gambelii* Nutt., is more prevalent at higher elevations (1,219–2,590 m). Surveys for *A. coxalis* in southern Mexico (Chiapas and Oaxaca) were conducted between 28 March and 10 April 2010. Pine-oak and oak woodlands were surveyed around San Cristobal de las Casas, Ocosingo, Teopisca, Altamirano, and Laguna de Montebello National Park in Chiapas, and near Mitla, Oaxaca. *Fraxinus* sp., *Juniperus* spp., *Liquidambar* sp., *Pinus* spp., *Platanus* sp., *Podocarpus* sp., and *Quercus* spp. were common genera in these forest stands (Miranda 1975). Due to high oak species diversity in Mexico and lack of access to identification keys, some species of oaks were not identified from field surveys.

Recently dead and dying oaks in Arizona and Mexico were inspected for GSOB injury symptoms (Coleman et al. 2011; Hishinuma et al. 2011). If D-shaped exit holes were encountered on the lower portion of the main stem, the outer bark was removed to determine the presence/absence of larval feeding and immature life stages. If encountered in Arizona, *Agrilus* sp. larvae and the outer bark were collected from these trees and returned to California under permit for rearing of adult wood borers and natural enemies. For genetic studies, GSOB larvae were extracted from the outer phloem and bark of oaks in 2009–2010, preserved in 95% ethanol in labeled vials, and stored below 0°C until DNA extractions were made.

Concurrently, ground surveys were conducted in California for natural enemies of GSOB. In southern California, low elevation woodlands (<1,372 m) are dominated by *Q. agrifolia*. At higher elevations

(1,524–2,438 m), *Q. kelloggii* is a co-dominant species with Jeffrey pine, *Pinus jeffreyi* Balf., and ponderosa pine, *P. ponderosa* C. Lawson (Roberts 1965). *Q. chrysolepis* and interior live oak, *Quercus wislizeni* A. DC. commonly span these elevational ranges, but occur at lower densities. *Q. engelmannii* has a restricted range in southern California and is often found interspersed with *Q. agrifolia* (Griffin and Critchfield 1972). During 2008–2011, rearing of GSOB from infested oak logs and hand sampling of infested *Q. agrifolia* and *Q. kelloggii* were conducted in the Descanso Ranger District of the Cleveland National Forest. Recently killed *Q. agrifolia* were felled and logs were collected from William Heise County Park (Julian, CA, USA) and surrounding areas from January to March 2010. Lower parts of the main bole from infested trees were quartered and placed in greenhouse rearing cages (2.5 m × 2.5 m, Bioquip, Rancho Dominguez, CA, USA). Cages were monitored for insect emergence (GSOB and natural enemies) from April to September 2010.

Genetic analyses of GSOB populations

A subsample of adults and larvae collected from Arizona, California, and Mexico (larvae only) were subjected to molecular analyses in an attempt to determine the area of origin of California's GSOB population. Whole genomic DNA was extracted from individual specimens by using either a standard Chelex® 100 (Bio-Rad, Hercules, CA, USA) extraction method (Walsh et al. 1991) or a DNeasy® Plant Mini Kit (Qiagen, Valencia, CA, USA). Genetic variation was initially examined by amplifying 658 bp of the mitochondrial gene (mtDNA) cytochrome oxidase *c* subunit 1 (COI) of 172 specimens with the polymerase chain reaction (PCR). Reactions were performed in 25 μl volumes containing 2 μl of DNA template (concentration not determined), 1× ThermoPol PCR Buffer (New England BioLabs, Ipswich, MA, USA), an additional 1 mM $MgCl_2$, 200 μM of each dNTP, 4% (v/v) BSA (NEB), 1 U *Taq* polymerase (NEB), and 0.2 μM each of the primers LCO1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and HCO2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′) (Folmer et al. 1994). PCR was performed in a Mastercycler® ep gradient S thermocycler (Eppendorf North America Inc., New York, NY, USA) with the following settings: 2 min at 94°C; followed by five cycles of 30 s at 94°C, 1 min 30 s at 45°C, and 1 min at 72°C; followed by a further 35 cycles of 30 s at 94°C, 1 min 30 s at 51°C, and 1 min at 72°C; and a final extension of 5 min at 72°C. Successful amplification was confirmed by agarose gel electrophoresis and PCR products were subsequently cleaned by using the Wizard® PCR Preps DNA purification system (Promega, Madison, WI, USA) and sequenced directly in both directions at the University of California Riverside Genomics Institute, Core Instrumentation Facility.

Sequences were aligned manually in BioEdit 7.0.5.3 (Hall 1999) and primers sequences were removed from the analysis. COI sequences were translated by using the EMBOSS-Transeq website (http://www.ebi.ac.uk/Tools/emboss/transeq/index.html) to confirm the absence of nuclear pseudogenes (Song et al. 2008). A haplotype (H) network was constructed by using TCS version 1.21 with default run settings (Clement 2000). Representative sequences were deposited in GenBank (Benson et al. 2008) (accession numbers: JF719839–JF719888).

In light of large differences revealed by the COI sequences (see "Results" section), we also sequenced a section of the D2 domain of 28S (28S-D2) nuclear ribosomal DNA (rDNA) of a subsample of individuals ($N = 23$). Sections of 28S were sequenced from specimens from Arizona, California, and specimens with H-31, H-32 and H-33 haplotypes from Mexico. A 536 bp section of 28S-D2 was amplified by using the primers 28sF3663 (5′-TACCGTGAGGGAAAGTTGAA-3′) with 28sR4076 (5′-AGACTCCTTGGTCCGTGTTT-3′) and a protocol previously described in Rugman-Jones et al. (2010). 28S-D2 sequences were aligned with the sequences of four species retrieved from GenBank: two species of *Agrilus*, *A. populneus* Schaeffer (AJ810781) and *Agrilus* sp. (FJ000409); and two outgroup taxa, *Trachys troglodytes* Gyllenhal in Schoenherr (AJ810782) and *Coraebus quadriundulatus* Motschulsky (AB232645). Phylogenetic relationships based on 28S-D2, among the California, Arizona, and Mexico specimens were investigated by using the "One Click" mode on the online Phylogeny.fr platform (http://www.phylogeny.fr/version2_cgi/index.cgi) (Dereeper et al. 2008). Again, representative 28S-D2 sequences were deposited in GenBank (accession numbers: JF719839–JF719888).

Results

Field surveys

Agrilus auroguttaus

In southeastern Arizona, late instar larvae were observed in the outer phloem of *Q. emoryi* and *Q. hypoleucoides* (Coleman and Seybold 2011). The advanced state of development of these larvae was supported by their presence in pupal cells with the anterior and caudal portions of the body folded over one another into a "hairpin" configuration (Coleman and Seybold 2008b; Hishinuma et al. 2011). No *Q. arizonica* and *Q. grisea* were observed with GSOB injury. Since 2008, eleven recently dead oaks have been observed in Arizona with symptoms of GSOB damage and late instar *Agrilus* sp. larvae present under the bark. One individual of *Q. hypoleucoides* surveyed in Madera Canyon, Santa Rita Mountains was found with symptoms of GSOB injury, but larvae were not sampled from this tree.

Infested trees in Arizona had similar injury symptoms to those observed in California, including meandering larval galleries on the wood surface, D-shaped exit holes, and bark staining (Coleman et al. 2011). Larval galleries were packed with frass and dark-colored when the bark was freshly removed. D-shaped exit holes and larval feeding were more frequently observed along the main stem (<2.5 m in height). Bark staining was also observed on some of the infested trees. Excessive crown thinning/die back was not always present on GSOB-infested trees and predisposing factors were not observed except for one tree in the Chiricahua National Monument that had a minor infection from true-leaf mistletoe, *Phoradendron coryae* Trel.

In May 2009, 27 late instar *Agrilus* larvae were collected from the outer phloem of one individual of *Q. emoryi* surveyed in Box Canyon, Santa Rita Mountains, Arizona (Table 1; Coleman and Seybold 2011). A total of 27 larvae were recovered from the outer bark during sampling. All specimens were reared to adult stage and confirmed as GSOB from adult morphology. Bark samples from this site yielded an additional 104 adult GSOB (emerged between 13 May and 2 June 2009; peak emergence of 16 adults on 16 May). GSOB adults were the only wood boring insects collected or reared from this material between September and January 2010, additional late instar *Agrilus* sp. larvae were extracted from six *Q. emoryi* in the Chiricahua Mountains, from two *Q. emoryi* in the Santa Catalina Mountains, and from one *Q. emoryi*, and one *Q. hypoleucoides* in the Huachuca Mountains (Table 1). These larvae were used in molecular analyses to investigate the genetic structure of GSOB populations, and all were confirmed as *A. auroguttatus* based on their mitochondrial DNA COI sequences (see below).

Two species of parasitoids were associated with GSOB in Arizona. Larvae of *Calosota elongata* Gibson (Hymenoptera: Eupelmidae) were discovered in the outer phloem feeding gregariously on four late-instar GSOB larvae within pupal cells in Box Canyon, representing a parasitism rate of 15% for these late-instar GSOB larvae (Table 1; Gibson 2010; Coleman and Seybold 2011). When bark was collected from this tree in May 2009, additional adult specimens of *C. elongata* were reared from samples on 6 July (four specimens), 7 July (three specimens), 12 July (two specimens), 13 July (two specimens), and 14 July 2009 (one specimen). These specimens of *C. elongata* were all female except for one. Seven (six females, one male) additional specimens were collected from the rearing cages when they were dismantled in October 2009 (Coleman and Seybold 2011). While sampling GSOB-infested trees on the Coronado National Forest during January 2010, larvae and pupae of *C. elongata* were encountered again in the outer phloem of *Q. emoryi* in the Huachuca Mountains, Miller Canyon; and in the Chiricahua Mountains, Chiricahua National Monument, Pinery Canyon, and Rucker Canyon (Table 1).

Atanycolus simplex Cresson (Hymenoptera: Braconidae) was also reared from the bark collected from the GSOB-infested *Q. emoryi* from Box Canyon. Adults of this parasitoid emerged prior to the emergence of adult GSOB in the lab (6 May 2009–18 May 2009) (Table 1; Coleman and Seybold 2011). A bark gnawing beetle (Coleopetera: Trogossitidae) was also found associated with GSOB from samples collected at Box Canyon and Miller Canyon (30 October 2009). An unidentified species of click beetle (Coleoptera: Elateridae) was found in association with GSOB pupal cells in the outer bark of an *Q. emoryi* in the Chiricahua National Monument (31 October 2009) (Table 1).

Table 1 Collection data of the goldspotted oak borer, *Agrilus auroguttatus* (southeastern Arizona and California), and *Agrilus coxalis* (southern Mexico) and their arthropod natural enemies

Region	Locality	*Quercus* spp. host	*Agrilus* spp.	Natural enemies
Arizona			*A. auroguttatus*	
	Coronado National Forest			
	Santa Rita Mountains, Box Canyon (N 31.79961°, W 110.75921°)	*Q. emoryi*	104 adults	19 *Calosota elongata* 6 *Atanycolus simplex* 5 Coleoptera: Trogossitidae
	Huachuca Mountains, Miller Canyon (N 31.742687°, W 110.25411°)	*Q. emoryi* and *Q. hypolueucoides*	15 larvae	16 *Calosota elongata* 1 Coleoptera: Trogossitidae
	Chiricahua Mountains, Chiricahua National Monument (N 31.00854°, W 109.38030°), Rucker Canyon (N 31.74999°, W 109.39748°), and Pinery Canyon (N 31.97136°, W 109.34537°)	*Q. emoryi*	36 larvae	*Calosota elongata* 1 Coleoptera: Elateridae
	Santa Catalina Mountains, Chihuhua Pine Picnic Area (N 32.43441°, W 110.75285°) and Molino Basin (N 32.337619°, W 110.690072°)	*Q. emoryi*	7 larvae	–
California	William Heise County Park, Julian, CA (N 33.039219°, W 116.591958°)	*Q. agrifolia*	540 adults	7 *Atanycolus simplex* 6 *Temnochila* sp. (Trogossitidae)
Mexico			*A. coxalis*	
	Chiapas			
	4.4 km south of Teopisca (N 16.48499°, W 92.49679°)	*Q. peduncularis*[a]	1 larva	–
	12 km south of Altamirano (N 16.76813°, W 92.02338°)	*Q. peduncularis*	1 adult and 61 larvae	–
	Laguna de Montebello National Park (N 16.09562°, W 91.72961°)	*Q. peduncularis*	2 larvae	–
	Oaxaca			
	4 km east of Mitla (N 16.92929°, W 96.29912°)	*Q. conzatti*	2 adults and 3 larvae	1 Coleoptera: Trogossitidae

Larval and adult *Agrilus* sp. collected at these sites were used to investigate the population genetics of the species complex

[a] We thank Kevin C. Nixon (Liberty Hyde Bailey Hortorium Herbarium, Cornell University) for identifying these Mexican oak species

GSOB larvae and adults were also collected for genetic analysis from infested logs of *Q. agrifolia* felled in California (Table 1; Coleman and Seybold 2011). Adult emergence began on 3 May and continued until 18 June 2010 in the laboratory. Four oak cordwood borers, *Xylotrechus nauticus* (Mannerheim) (Coleoptera: Cerambycidae), a flatheaded appletree borer, *Chrysobothris femorata* (Olivier) or *Chrysobothris wintu* Wellso and Manley (Coleoptera: Buprestidae), and western oak bark beetles, *Pseudopityophthorus pubipennis* (LeConte) and ambrosia beetles, *Monarthrum* spp. (both Coleoptera: Scolytidae) emerged from the caged logs during the observation period. Seven *A. simplex* were reared from the cut logs between 24 May and 4 June. Six adult *Temnochila* sp. (Coleoptera: Trogossitidae) were also recovered (3 March 2010) (Table 1).

During ground surveys in the Descanso Ranger District of the Cleveland National Forest, a snake fly larva, *Agulla* sp. (Neuroptera: Raphidiidae), was collected from a GSOB pupal cell in *Q. agrifolia* at Pine Creek Trailhead (N 32.836697°, W 116.54258°). Additional sampling in plots in the Descanso Ranger District revealed pupae of *C. elongata* in one GSOB pupal cell in the outer phloem from a single *Q. agrifolia* on 19 January 2011 at Noble Canyon Trailhead (N 32.84889°, W 116.52235°).

Agrilus coxalis

Surveys in March–April 2010 in Chiapas and Oaxaca, Mexico revealed four recently dead oaks with evidence of colonization by *A. coxalis*. D-shaped exit holes were observed on the lower main stem of three recently dead *Quercus peduncularis* Nee. in Altamirano, Laguna de Montebello National Park, and Teopisca, Chiapas; and recently dead *Quercus conzatti* Trel. in Mitla, Oaxaca (Table 1). *Q. peduncularis* is a white oak that is widespread in southern Mexico, Guatemala, El Salvador, and Honduras (Standley and Steyermark 1952), whereas *Q. conzatti* is a red oak with a limited distribution in Oaxaca Valley (K.C. Nixon, personal communication). Crown thinning/die back and bark staining were not evident on these trees. Exit holes and larval feeding were more common on the lower stem and showed the same irregular pattern and localized high-density feeding as observed in Arizona and California.

Mature *Agrilus* sp. larvae were recovered from the outer bark of a single tree in each of the following locations: 4.4 km south of Teopisca (30 March 2010), 12 km south of Altamirano (31 March 2010), Laguna de Montebello National Park (1 April 2010), and 4 km east of Mitla (5 April 2010). Sixty-seven larvae were recovered from the four trees at heights <1.5 m along the main stem. A subsample of these larvae from each location was used in the genetic analyses, and all were confirmed as *A. coxalis* based on their mitochondrial and rDNA sequences (see below).

At the Altamirano site, two larvae were beginning to constrict for pupation and one unemerged adult was found in a pupal cell in the outer phloem. Two dead *A. coxalis* adults, which had failed to emerge from pupal cells, were collected from the single dead tree at Mitla. A bark gnawing beetle (Coleoptera: Trogossitidae) was also found associated with *A. coxalis*-infested bark.

Genetic analyses

The COI gene region was sequenced from 172 individuals: 73 from Arizona (adults and larvae), 84 from California (adults and larvae), and 15 from Mexico (larvae only). Among these sequences, 34 haplotypes were identified, which were grouped into three separate networks (Fig. 1). The geographic distribution of these haplotypes appears to be very structured. Arizona and California individuals all grouped into a single network (haplotypes: H-1 to H-30), but within this, only one haplotype occurred in both regions H-1 (Arizona, $N = 22$ and California, $N = 6$). Twenty haplotypes were identified only from Arizona and nine only from California. The COI sequences of specimens from southern Mexico (H-31 to H-34) differed from the Arizona and California material by nearly 11% confirming that the Mexican specimens were a different species (Fig. 1). Further evidence that the Arizona/California haplotypes represent a different species from the Mexican haplotypes was found by examination of 28SD2 sequences which revealed that the Arizona and California populations were identical, but that the Mexico specimens differed from the Arizona–California sequence by 8 bp (1.49%). In our phylogenetic reconstruction, the U.S. and Mexican populations investigated here formed sister taxa in a clade also containing the two outgroup *Agrilus* spp. (Fig. 2).

Discussion

Agrilus auroguttatus represents a significant threat to oak woodlands of southern California. This exotic buprestid fills a vacant niche on native California oak species (Brown and Eads 1965; Swiecki and Bernhardt 2006), which in recent history have never been exposed to this type or level of herbivory. Elevated levels of oak mortality are expected to continue as the infestation spreads within and from San Diego Co. (Coleman and Seybold 2011). Due to GSOB's neighboring historical range in neighboring Arizona and its unlikely behavior as an aggressive tree pest in California, regulation of this indigenous exotic has proven difficult and no quarantines have been imposed to limit oak firewood movement from this area into uninfested areas. Long-term and landscape-level management plans are needed to mitigate tree mortality from GSOB. Classical biological control has the potential to be an important long-term component of these management objectives.

Initial "foreign" exploration efforts in southeastern Arizona revealed a small ensemble of natural

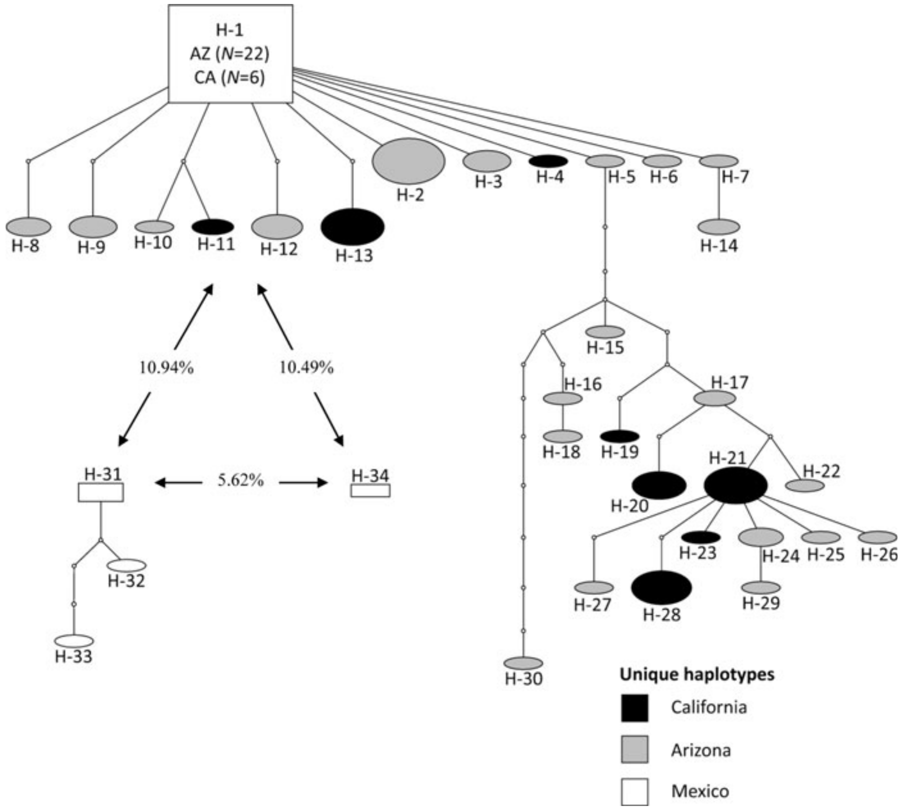
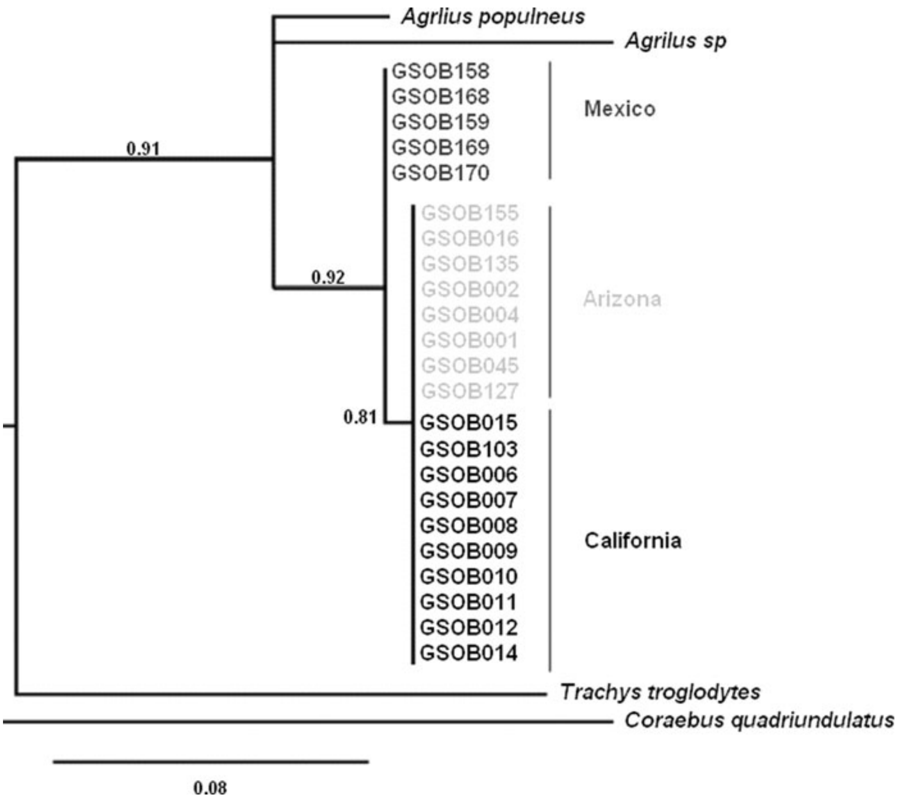


Fig. 1 Cytochrome oxidase *c* subunit 1 (COI) haplotype network for goldspotted oak borer populations collected from California and southeastern Arizona (*Agrilus auroguttatus*), and southern Mexico (*Agrilus coxalis*). Overall, there were 34 haplotypes which fell into three networks (most likely ancestral haplotypes of each appear as rectangles). Divergence between the three ancestral is given as % sequence difference. The three colors for the haplotype network correspond to the three populations

enemies associated with GSOB, but several of these species are generalist predators and not suitable as classical biological control candidates for importation and establishment in California (Table 1). *C. elongata* was the dominant parasitoid species found associated with *A. auroguttatus* pupal cells in Arizona. This gregarious larval ectoparasitoid was described as a new species by Gibson (2010), thus no life history, impact, or ecological data are available for it. Other species of *Calosota* are known parasitoids of bark beetles (Scolytidae), Cecidomyiidae flies (Diptera), Eurytomidae (Hymenoptera), and gall wasps (Gibson 2010; Mendel 1986). Initial levels of larval parasitism for GSOB from *C. elongata* (15%) were comparable to other larval parasitism rates of *Agrilus* spp. in the U.S. (Table 2; Haack and Acciavatti 1992; Katovich et al. 2000; Liu et al. 2007).

Due to its only known association with GSOB, we infer *C. elongata* may be very host specific. Thus we have considered that *C. elongata* would be a promising candidate for classical biological control in California. However, in December, 2010, *C. elongata* was collected for the first time in San Diego Co. (L.J. Haavik, unpublished data), suggesting that it has already been introduced with the invasive population of GSOB. Because of the prevalence of *C. elongata* with GSOB-infested trees and its presence in pupal cells during the dormant season in southeastern Arizona, it was not surprising that this larval parasitoid may have been introduced with its host into southern California via firewood movement. Although the parasitoid has been identified on multiple occasions, to date *C. elongata* has a limited distribution in the Pine Creek riparian area in the Descanso Ranger District of the Cleveland National Forest near the communities of Descanso, Guatay, and Pine Valley, California where tree mortality was initially mapped in 2002 (Coleman and Seybold 2008b). The distribution of *C. elongata* appears restricted when compared to the spread of GSOB in San Diego Co., and its infestation rate in California is considerably lower (<1%) than what has been observed in southeastern Arizona (~15%). These observations on the parasitoid further support the hypothesis of an introduction of GSOB into southern

Fig. 2 Phylogenetic tree constructed by using 28S sequences that indicate divergence of Mexican goldspotted oak borer populations (*Agrilus coxalis*) from California and Arizona populations (*Agrilus auroguttatus*). Four outgroups are also included in this analysis. Branch support based on an approximate likelihood test is given above the branches (Anisimova and Gascuel 2006). The different colors for the 28S sequences correspond to the three populations



California from elsewhere, and not a naturally-occurring range expansion by adults dispersing out of Arizona or northern Mexico (Coleman and Seybold 2011). Because the distributions of the parasitoid and its host do not appear completely sympatric in California, *C. elongata* may be amenable for augmentative releases into new areas of California where GSOB is present but *C. elongata* is absent.

Atanycolus simplex has a broad distribution in the U.S. and has been associated as a larval ectoparasitoid with other *Agrilus* spp. and various other buprestid and cerambycid species (Table 2; Krischik and Davidson 2007; Quicke and Sharkey 1989; USDA Forest Service 2010). *A. simplex* has also been associated with the oak cordwood borer, which is found associated with recently killed *Q. agrifolia* in California (Coleman and Seybold 2008b; Swiecki and Bernhardt 2006). Parasitism rates of GSOB from *A. simplex* in California may be lower than what we have observed in southeastern Arizona, and are not known definitely because of the presence of other potential hosts in cut logs that we have reared from. This species is likely opportunistically exploiting an abundant resource.

A diverse complex of hymenopterous parasitoids and predators is associated with all life stages of *Agrilus* wood borers in the U.S. (Table 2). This survey of the literature (Table 2) suggested that due to the frequency of egg parasitoids associated with other *Agrilus* spp., we might hypothesize that this group of natural enemies may contribute, in part, to the low GSOB populations observed in southeastern Arizona. Conversely, the potential absence or low density of egg parasitoids from the invasive population of GSOB in California may have contributed to the high population density of the herbivore in San Diego Co. (Coleman and Seybold 2011). The literature review has also revealed that our knowledge of the larval parasitoid complex is rather limited vis-à-vis the other species of *Agrilus* and that more survey and research could be done to elucidate the role if any of fungal pathogens in regulating the populations of GSOB in Arizona and *A. coxalis* in Mexico. In the northeastern U.S., native parasitoids have been reported to switch from indigenous hosts to the exotic emerald ash borer, *Agrilus planipennis* Fairmaire (Bauer et al. 2005; Cappaert and McCullough

Table 2 Comparison of natural enemies associated with several economically important *Agrilus* species and the goldspotted oak borer, *Agrilus auroguttatus*

Species	Natural enemies^{a}				
	Egg parasitoids	Larval parasitoids	Predators	Woodpeckers	Fungi and pathogens
Emerald ash borer, *Agrilus planipennis* Fairmaire					
In China	*Oobius agrili* (12–61.5%) *Avetianella xystrocerae*	*Spathius agrili* (30–90%) *Tetrastichus planipennisi* (4–40%)	–	–	–
In U.S.	*Pediobius* sp. (0.3%)	*Atanycolus cappaerti* (9–71%) *Atanycolus hicoriae* (1–20%) [*Spathius simillimus* *Atanycolus simplex* *Heterospilus* sp. *Phasgonophora sulcata* *Balcha indica* (0.05%)] [four spp. of native parasitoid species (<4%)] [*Dolichomitus vitticrus* *Orhizema* sp. *Cubocephalus* sp. *Euplemus pini* *Balcha indica* 3.6%] *Leluthia astigma*	*Enoclerus* sp. *Catogenus* sp. *Tenebroides* sp.	*Melanerpes carolinus* *Picoides pubescens* *Picoides villosus*	[*Beauveria bassiana* *Paecilomyces farinosus* *Paecilomyces fumosoroseus* *Verticillium lecanii* *Metarhizium anisopliae* (<2%)]
Twolined chestnut borer, *Agrilus bilineatus* Weber					
	Trichogramma sp.	*Phasgonophora sulcata* (10%) *Cymatodera bicolor* *Spathius simillimus* *Phasgonophora sulcata* *Wroughtonia ligator* *Doryctes anatolikus*	*Tenebroides corticalis* *Phyllobaenus verticalis* *Phyllobaenus* sp. *Adelocera oculatus* *Tenebroides bimaculatus*	[*Picoides pubescens* *Picoides villosus* (78%)]	–
Bronze birch borer, *Agrilus anxius* Gory					
	[13 species of *Ooencyrtus*, 6 species of *Avetianella*, *Ablerus* sp. 6 species in tribe Signiphorini (7%)] nr. *Coccidencyrtus* sp. *Thysanus* sp.	[*Atanycolus charus* *Spathius simillimus* *Phasgonophora sulcata* *Tetrastichus* sp. *Eurytoma* sp. (mean of 18%)] *Spathius* sp. *Doryctes fartus* *Doryctes rufipes* *Atyancolus* sp. *Wroughtonia ligator* *Dolichomitus messor perlongus* *Ichneumon* sp.	–	[*Picoides pubescens* *Picoides villosus* 60–90%]	–
Goldspotted oak borer, *Agrilus auroguttatus*					
In Arizona	–	*Calosota elongata* (15%) *Atanycolus simplex*	Elateridae Trogossitidae	–	–

Table 2 continued

Species	Natural enemies[a]				
	Egg parasitoids	Larval parasitoids	Predators	Woodpeckers	Fungi and pathogens
In California	–	*Calosota elongata* discovered with limited range *Atanycolus simplex*	*Temnochila* sp. *Agulla* sp.	*Melanerpes formicivorus* *Picoides nuttallii*	–

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[a] The parasitism rate for a species or a complex of species (where known) is listed parenthetically following each taxon

2009). In California, a complex of parasitoids associated with the native oak twig girdler, *Agrilus angelicus* Horn, has not been observed switching to GSOB populations, although the two congeners both feed on *Quercus* (Burke 1920; Krombein et al. 1979; Swiecki and Bernhardt 2006). Woodpeckers are ubiquitous predators of *Agrilus* spp. wood borers throughout the U.S. (Table 2), and also prey frequently on GSOB in California (Coleman et al. 2011), but the extent of their impact on larval and pupal populations is unknown and has so far appeared to be insufficient for controlling GSOB.

Surveys of Chiapas and Oaxaca in southern Mexico have not revealed insect natural enemies associated with quiescent *Agrilus* larvae, but they did document the first host plant records for *A. coxalis* (*Q. conzatti* and *Q. peduncularis*). The distributions of these two hosts plants may provide additional insight into the geographic and host range of *A. coxalis* and indicate additional areas to prospect for potential natural enemies. It is curious that we have evidence that *A. coxalis* colonizes *Q. peduncularis*, which is in the white oak subgroup of *Quercus* (Nixon 1993). In our more extensive surveys of Arizona and California, GSOB has only been observed on one species of white oak (*Q. engelmannii* Greene), and those observations have been relatively rare (Coleman and Seybold 2011). Injury symptoms from *A. coxalis* on *Q. conzatti* and *Q. peduncularis* were generally similar to injury symptoms described for GSOB on various oak species in Arizona and California (Coleman et al. 2011; Coleman and Seybold 2011). However, crown thinning and bark staining were not evident on infested *Q conzatti* and *Q. peduncularis*. This observation may have been more likely a result of the timing of surveys than any biology difference. The surveys in southern Mexico occurred at the end of the dry season. Presumably with the arrival of the wet season and subsequent leaf flush on these evergreen trees, symptoms related to crown decline and sap flow may be more easily observed.

Although until recently, *A. auroguttatus* and *A. coxalis* have been obscure in the economic entomology literature and the taxonomic confusion surrounding the identification of these two beetles has been problematic for over 30 years (Hespenheide 1979). The preliminary molecular analyses reported here help to resolve the species status of the U.S. and Mexican populations. Thirty-four haplotypes were identified from the three populations and the geographic distribution of these haplotypes was very structured. This is an unusually high number of COI haplotypes given the relatively small sample sizes from the populations, and this result suggests that the members of this species complex are highly variable in this gene region. The high number of haplotypes in California (nine haplotypes) may suggest a large founding population or multiple smaller introductions from different areas. However, 31 specimens collected from the bark and phloem of a single tree in Arizona yielded six haplotypes, which suggests that the California population may have possibly arisen from a single truck load of firewood. Only one haplotype was shared between the Arizona and California populations. Had we sampled the source of California's population we would expect to see a much greater overlap between haplotypes from Arizona and California. Therefore, the area of origin for the invasive California population has most likely not been sampled. However, we can rule out southern Mexico as the area of origin for California's population because the specimens from this area differed by nearly 11% in their COI sequences from the California and Arizona material.

This level of divergence suggests that the Mexico specimens used in this study represent a different species (Hebert et al. 2003), supporting the morphological distinction proposed by Hespenheide et al. (2011). Further, strong evidence that the Mexican specimens represent a different species from the Arizona and California specimens is provided by our analysis of the 28S-D2 sequences. The nuclear 28S-D2 is a highly conserved gene region that makes it useful for detecting species-level differences (e.g., Ballman et al. 2011; Rugman-Jones et al. 2007). 28S-D2 sequences of specimens from Arizona and California populations were identical, whereas the sequences from the Mexican specimens differed by 8 bp (~1.5%). However, the 28s-D2 sequences for haplotypes H-31 to H-34 were identical, which contradicts the COI sequence data comparison. The latter suggested that there may be two species among our specimens from southern Mexico. Pinned specimens of *A. coxalis* from Costa Rica and Honduras have been suspected to represent one or more separate species from *A. coxalis*, even though these specimens are overwhelming similar in morphology (Coleman and Seybold 2011; H.A. Hespenheide, personal communication). Molecular analyses may help resolve uncertainty over species status for these populations.

Determining the area of origin of the California population within the native range of *A. auroguttatus* will help us locate natural enemies most closely adapted to GSOB genotypes in California and potentially provide a suitable climatic match, which may increase the likelihood of successful biological control (Stouthamer 2008). Discovering natural enemies of *A. auroguttatus* and *A. coxalis* populations from both native regions may enable the development of a diverse natural enemy complex to enhance the ultimate efficacy of the integrated pest management program in California. Consideration of new association natural enemies from *A. coxalis* for use against *A. auroguttatus* in California may facilitate the construction of an "extraordinary" guild of GSOB parasitoids, which collectively may greatly increase levels of biotic mortality on *A. auroguttatus* that may not be achieved if just parasitoids associated with *A. auroguttatus* are used. A thorough examination of the natural enemy fauna associated with *A. auroguttatus* and *A. coxalis* populations in Arizona and Mexico will allow us to assess all available options for suppressing this invasive pest with biological control agents (i.e., classical biological control, and new association biological control).

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