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Establishment of a non-native xyleborine ambrosia beetle, *Xyleborus monographus* (Fabricius) (Coleoptera: Curculionidae: Scolytinae), new to North America in California

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Abstract

Specimens of an ambrosia beetle, *Xyleborus monographus* (Fabricius), were found infesting oak trees in California. This is the first record of this species established in North America. Based on collection information, this species most likely has been established in the Napa County area for several years. A modified key to *Xyleborus* in North America, and diagnosis of the species is provided.

Key words: Invasive ambrosia beetle, adventive species, oaks, key to species, cytochrome c oxidase subunit I, ambrosia fungi

Introduction

Bark and ambrosia beetles (Coleoptera: Curculionidae: Scolytinae) are one of the most commonly intercepted, introduced, and established taxa in many countries (Haack & Rabaglia 2013) and in North America several of these species have had significant impacts on natural and urban forests (Fraedrich *et al*. 2008; Eskalen *et al*. 2013; Smith & Hulcr 2015; Stouthamer *et al*. 2017). Due to their wide host range, cryptic nature, fungus farming, and inbreeding, ambrosia beetles in the tribe Xyleborini have been one of the most successful groups at colonizing new and non-native habitats (Atkinson *et al*. 1990; Rabaglia *et al.* 2006; Smith & Hulcr 2015; Gomez *et al.* 2018a). Nearly half of the more than 60 species of non-native scolytines established in the continental United States are xyleborine ambrosia beetles (Gomez *et al.* 2018a, 2018b). This paper reports on the first establishment of another xyleborine ambrosia beetle*, Xyleborus monographus* (Fabricius), in North America.

In September 2019, specimens of an ambrosia beetle in a valley oak, *Quercus lobata*, were collected in Calistoga, Napa County, California. These specimens were compared to beetles in the collection at the United States Museum of Natural History, Smithsonian Institution and determined to be *Xyleborus monographus*. Additional specimens were collected in Calistoga on October 16 and 17, 2019 (see below for collection details). These collections were thought to represent the first records of this species established in North America; however, previous specimens collected in September 2017 from the same area (see below for collection details) were recently identi-

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fied as *X. monographus*. By early 2020, infested trees were found throughout a 15-mile-long area in Napa County, as well as neighboring Lake and Sonoma Counties, indicating a well-established population in the area.

In 2018, one specimen of *X. monographus* was detected in a multi-funnel trap baited with an 'oak pinhole borer' lure (consisting of ethanol, leaf alcohol, sulcatol and sulcatone [Synergy Semiochemicals, Delta, B.C., Canada]) in Fairview, Multnomah County, Oregon (W. Williams, Oregon Department of Forestry, unpublished data), however, trapping in 2019 did not detect additional beetles nor were infested trees found in the Portland area.

Molecular confirmation of the species identification was also sought by sequencing a section of the cytochrome c oxidase subunit I gene (COI) commonly used in DNA barcoding studies (Hebert *et al.* 2003). Two beetles from the initial collection were subject to non-destructive DNA extraction and sequencing following Stouthamer *et al.* (2017). Subsequent BLAST searches of the GenBank database (Benson *et al*. 2013) revealed 100% similarity to COI sequences derived from known European specimens of *Xyleborus monographus* (GenBank accessions: HQ953471, KM286137, and KM285995; also see BOLD Barcode Index Number - BOLD:AAO0226 [Ratnasingham & Hebert 2013]). In addition, a section of the D2 region of 28S ribosomal RNA was also sequenced according to Stouthamer *et al*. (2017). Our 28S sequences represent the first deposition in GenBank attributed to *X. monographus*, but the most similar existing matches were 28S sequences attributed to the congeneric species *X. affinis* [GU808581] and *X. bispinatus* [HM099741]; both ~98.6% similar. Sequences generated from the specimens were deposited in GenBank (accession numbers MN974135-36 [COI] and MN970511-12 [28S]) and the coinciding morphological vouchers were deposited in the Entomology Research Museum, University of California, Riverside, CA.

Specimens are deposited in the following collections:

NMNH—US National Museum of Natural History, Washington, DC
RJRC—Robert J. Rabaglia Collection, Annapolis, MD
UCRC—Entomology Research Museum, University of California, Riverside, CA
EMEC—Essig Museum of Entomology, University of California, Berkeley, CA

Xyleborus monographus (Fabricius, 1792)

Figure 1

Bostrichus monographus Fabricius, 1792: 365.
Xyleborus monographus (Fabricius), Eichhoff 1864: 704.
Bostrichus tuberculosus Herbst, 1793: 113. Synonymy Eichhoff 1878: 397.

Diagnosis. Specimens of *Xyleborus monographus* can be distinguished from most species of *Xyleborus* in North America by the tubercles on declivital interstriae 1 that are distinctly larger than those on other interstriae. It is very similar to the eastern species *X. celsus* Eichhoff, but *X. monographus* is smaller (3.0–3.2 mm *vs* approximately 3.6–4.5 mm for *X. celsus*), and the width of interstriae 2 on the declivity is approximately half the width of interstriae 3 on *X. monographus*, *vs* twice the width of interstriae 3 on the declivity of *X. celsus*.

This is only the fourth species of *Xyleborus* reported from California; the other three species are native. It can be distinguished from *X. ferrugineus* (F.) which lacks tubercles on interstriae 1 and has tubercles on interstriae 3 larger than on others. *Xyleborus intrusus* Blandford and *X. xylographus* (Say) both have tubercles on interstriae 1 but they are not larger than those on interstriae 3. There are five other species of xyleborine ambrosia beetles in California, *Anisandrus dispar* (F.), *Cyclorhipidion bodoanum* (Reitter), *Euwallacea fornicatus* (Eichhoff), *E. kuroshio* Gomez & Hulcr, and *Xyleborinus saxesenii* (Ratzeburg), which can be distinguished from *Xyleborus* and each other by the keys and images in Gomez *et al*. (2018a) and updated in Smith *et al*. (2019).

The key to female *Xyleborus* species in America north of Mexico in Gomez *et al.* (2018a) is modified below to include *X. monographus*. Alterations are in bold.

3. Tubercles on declivital interstriae 1 distinctly larger than tubercles on other interstriae 4
\- Tubercles on declivital interstriae 1 either similar in size to tubercles on other interstriae or absent (except at base or apex). 5
4. Elytral disc and declivity setose; all declivital interstriae armed by strong tubercles at base; declivital interstriae 1 armed by two very large pointed tubercles, declivital interstriae 3 armed by several smaller tubercles . . . . 4'

- Elytral disc and declivity glabrous; all declivital interstriae armed by small granules, gradually decreasing in size toward apex; interstriae 1 near apex armed by one or two small tubercles . . . . *glabratus* Eichhoff

4'. Total body length 3.6−4.5 mm; declivital interstriae 2 1.5–2x as wide as interstriae 3, punctures deep and large (Fig. 2B); in *Carya* species. . . . . *celsus* Eichhoff

- Total body length 3.1−3.2 mm; width of declivital interstriae 2 .5–1x as wide as interstriae 3, punctures shallow and small (Fig. 2A); mostly in *Quercus* species . . . . *monographus* (Fabricius)

Description: Female- Length 3.1−3.2 mm, 3 times as long as wide; color reddish brown. Frons convex, surface reticulate, not smooth, punctures sparse, shallow; setae sparse, longer near epistomal margin. Pronotum about 1.5 times as long as wide, anterior rounded and convex, coarsely asperate on anterior half, basal half smooth with shallow, sparse punctures. Elytra approximately twice as long as wide, and slightly less than twice as long as pronotum, disc shining, striae shallowly impressed, punctures shallow, interstriae with fewer, shallow punctures; declivity steep, less than 25% of elytral length, flat, surface dull, strial punctures small and shallow, in rows curving away from suture at middle of declivity, then towards suture at apex, interstriae 1 wide, smooth with two large tubercles at middle of declivity, one smaller denticle at base of declivity, interstriae 2 smooth, about half as wide as interstriae 3, two small denticles on interstriae 3 at about the same level as those on interstriae 1. Elytral setae on striae minute, in rows, interstrial setae longer and fine.

Male. Not examined.

Distribution. The distribution records are based on Wood & Bright (1992) and supplements (Bright & Skidmore 1997, 2002; Knížek 2011; Bright 2014).

Africa: Algeria, Morocco; **Asia**: Azerbaijan, Iran, Iraq, South Korea, Turkey; **Europe**: Albania, Austria, Belgium, Bulgaria, Croatia, Czech Republic, Denmark, Estonia, France, Great Britain, Germany, Greece, Hungry, Italy, Latvia, Luxemburg, Macedonia, Montenegro, Netherlands, Norway, Poland, Portugal, Romania, Russia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Ukraine.

New records in **North America**: United States: California, Napa Co., Calistoga, 14 September, 2017, M. Garbelotto coll., ex. *Quercus lobata* (2, NMNH); as previous except:, 10 September, 2019, L. Burkhardt coll., ex. *Quercus lobata* (1, USNM; 2, UCRC [UCRC_ENT 00528716 and UCRC_ENT 00528703]); as previous except: Silverado Trail, 16 October, 2019, Sheri Smith coll., ex. *Quercus lobata* (2, RJRC); as previous except: 17 October, 2019, Sheri Smith coll., ex. *Quercus lobata* (2, RJRC); as previous except: Bothe Napa Valley State Park, 38.551877, -122.522836, 29 January, 2020, Cutis Ewing coll., ex. *Quercus kelloggii* (10, EMEC1332925-34); as previous except: 38.552298, -122.522948, 29 January, 2020, Cutis Ewing coll., ex. *Quercus douglasii* (1, EMEC1332924); as previous except:, Silverado Tr. & Brannan St., 38.5856, -122.5724, 18 November, 2019, Curtis Ewing coll., ex. *Quercus lobata* (5, EMEC 1332915-19); as previous except: vineyard between Silverado Tr. & Rt. 128, 38.57737, -122.57563, 18 November, 2019, Curtis Ewing coll., ex. *Quercus lobata* (4, EMEC 1332920-23); as previous except: Middletown Hwy., Mayacmas Mts., 38.6670, -122.5974, 10 January, 2020, Curtis Ewing coll., ex. *Quercus lobata* (4, EMEC 1332935-38); California, Lake Co., Middletown, Graham Ln., 38.7525, -122.6217, 10 January, 2020, Curtis Ewing coll., ex. *Quercus lobata* (1, EMEC 1332939); as previous except: Calistoga, Silverado Tr. & Glass Mt. Rd., 38.53529, -122.59057, 29 January, 2020, Curtis Ewing coll., ex. *Quercus lobata*, (5, EMEC 1332940-44); as previous except: Middletown Hwy., Mayacmas Mts., 38.6670, -122.5974, 02 February, 2020, Curtis Ewing coll., ex. *Quercus lobata* (4, EMEC 1332945-48).

Hosts and biology. In Europe, the most commonly reported hosts of *X. monographus* are various species of oaks (*Quercus*) and other genera of Fagaceae (*Fagus* and *Castanea*). Wood & Bright (1992) report *Quercus* spp., and state it is uncommon in *Castanea vesca* and *Fagus orientalis*. Bright and Skidmore (1997) cites Koch (1992) and lists: *Betula pendula (=B. verrucosa), Carpinus betulus, Castanea sativa, Fagus sylvatica, Fraxinus excelsior, Juglans regia, Prunus avium, Quercus canariensis, Q. castaneifolia* var. *incana, Q. ceris, Q. coccifera, Q. ilex, Q. lusitanica, Q. petraea, Q. pubescens, Q. pyrenaica, Q. robur, Q. rubra, Q. suber, Ulmus laevis*. Schedl (1964) states it is most frequently found in *Quercus*, but lists several of the non-oak species above as hosts also.

In California, the original infested trees were mostly valley oaks (*Quercus lobata*), but some blue oaks (*Q. douglasii*) also were found infested in the area. A very limited infestation was found in a single limb of California black oak (*Quercus kelloggii*) with extensive heart rot.

Schedl (1964) stated that he found most attacks by this species in trunks of downed oaks felled in winter or early spring, and in branches larger than 20 cm in diameter. He also stated that most attacks occurred on the sides or undersides of logs, and only rarely on the upper, sun-exposed surfaces. In California, we found a similar attack pattern by this beetle on valley oaks. Most of the trunks were heavily colonized by the beetle, but we also have seen attacks on mostly larger branches and in branches as small as 6.35 cm. diameter in the upper crowns of apparently healthy oaks.

Data from Schedl (1964) and his reference to Eichhoff (1881) and Escherich (1929), indicated two generations per year for this species in Germany, but he questioned if this was the case throughout Europe, and cited Palm (1959) who found only one generation per year in Sweden. More recent work in Greece (Markalas & Kalapandia 1997) and Slovakia (Galko *et al.* 2014) found one generation per year based on trapping data. These later two studies also found peak trap catch in late May and June. In Israel, adults were active for nearly the entire study period, March–September, and no activity peaks were detected, suggesting multiple overlapping generations (Buse *et al.* 2013).

There have been several studies in Europe that have tested the response of ambrosia beetles to ethanol-baited funnel traps (Markalas & Kalapandia 1997; Galko *et al.* 2014 and references therein), and they have shown positive response of *X. monographus* to ultra-high release (UHR) ethanol-baited traps.

As with all xyleborine ambrosia beetles, *X. monographus* exhibits sib-mating, with haploid and wing-less males (Kirkendall 1993). Schedl (1964) reports a sex ratio of 8.5:1 females to males. Other species of xyleborines have been reported to have ratios similar to this or more females to males (Smith & Hulcr 2015), and additional studies may show a more female biased sex ratio for this species as well.

FIGURE 1. Dorsal and lateral habitus of *Xyleborus monographus*. Scale bar represents 0.5 mm.

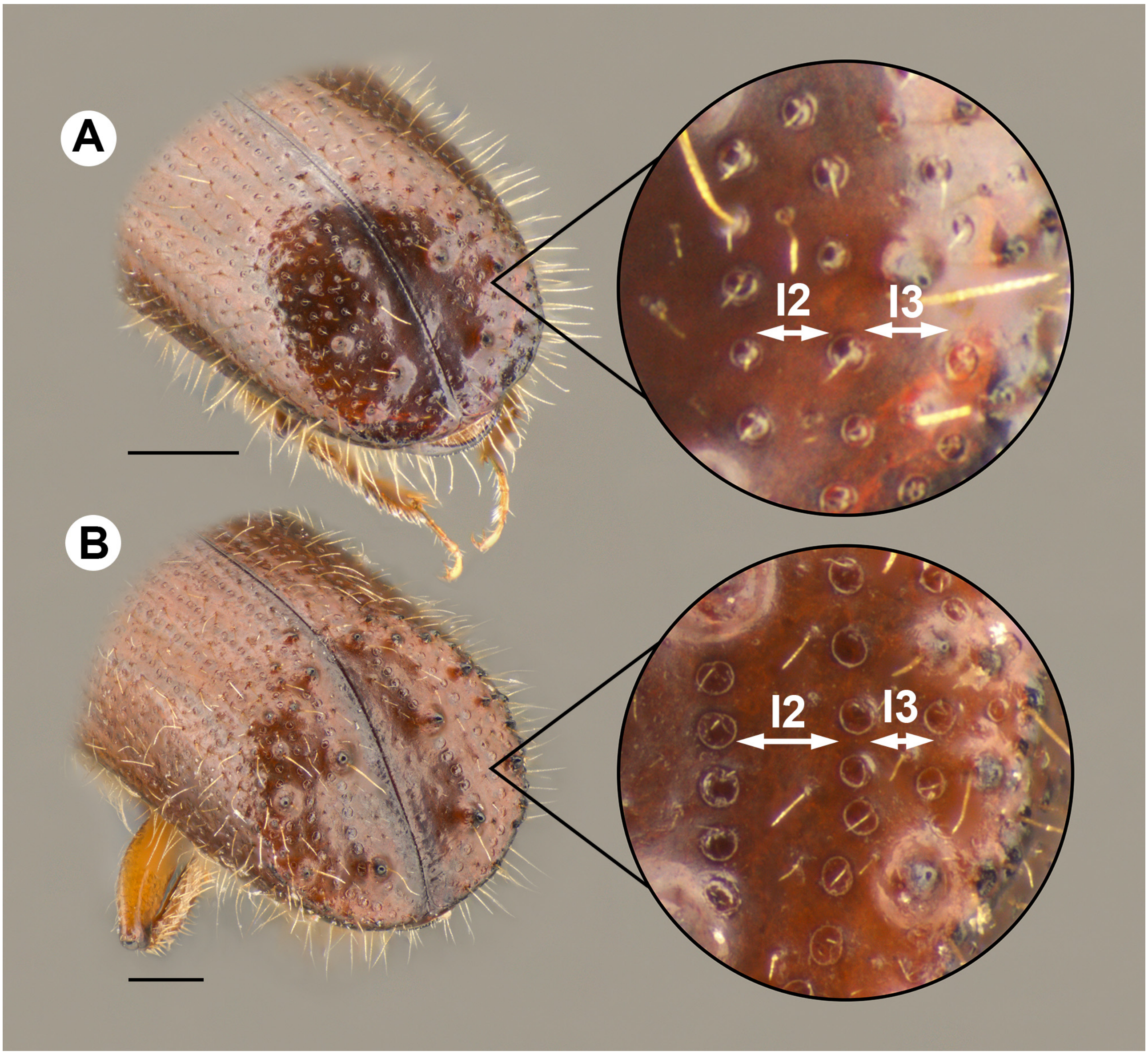

FIGURE 2. Elytral declivities of *Xyleborus monographus* (A) and *X. celsus* (B). I-2 and I-3: Interstriae 2 and 3 respectively. Scale bars represent 0.2 mm.

Fungal symbionts. Ambrosia beetles carry symbiotic fungi which they introduce into the xylem and are used as food for adults and larvae (Beaver 1989). Species in the Xyleborini, as do most other ambrosia beetles, have special structures, mycangia, in which the spores of their symbiotic fungi are carried. In *Xyleborus monographus*, as in all *Xyleborus* species, the mycangia are in the mandibles (Schedl 1964). In most cases the fungal associate is not pathogenic to the host tree, however, the fungal associate of the non-native *X. glabratus* Eichhoff (the red bay ambrosia beetle), *Raffaelea lauricola* is very pathogenic and has caused extensive mortality of several species of Lauraceae in the southeastern US (Fraedrich *et al.* 2008; Harrington *et al.* 2010). Gebhardt *et al.* (2004) found *Raffaelea montetyi* associated with *X. monographus* in Germany. They also found this fungus in *X. dryographus* (Ratzeburg) and *Platypus cylindrus* (F.). Inácio *et al.* (2012) tested the pathogenicity of *R. montetyi* strains from Portugal on cork oak (*Quercus suber*) saplings and had 100% mortality within 60 days.

Ambrosial fungal species from both beetle and plant tissues infested by *X. monographus* were collected with methods of Eskalen *et al.* (2013). Specimens were collected from infested valley oaks in Calistoga, CA. A total of 10 beetles and infested wood samples were collected from each of three infested trees. Fungal isolations from symptomatic tissues and female beetle mycangia were recovered following the methods of Lynch *et al.* (2016). Based on the morphological characterization and BLAST's query comparison of the ITS sequence data in the present study and other isolates in GenBank, the fungal species, *Raffaelea montetyi* (UCD8134), *Paecilomyces formosus* (UCD8140), *Fusarium solani* (UCD8043), undescribed species of *Fusarium* (UCD8376) and *Leptographium* sp. (UCD8382), and a yeast species, *Saccharomyces microspore* (UCD8112) were recovered.

Further identification of the fungal species using multi loci gene sequence analyses is underway. Currently, pathogenicity tests of fungal species on young valley oak trees are being conducted.

Conclusion

At this time, it is hard to predict what impacts this species may have on the oak resources of California or North America. Most of the more than 30 species of xyleborine ambrosia beetles established in North America have had little impact on the health of forests, but, as noted above, *Xyleborus glabratus* and species in the *Euwallacea fornicatus*-complex, along with their fungal associates, have had a significant impact on trees in the southeastern US and California, respectively. Sometimes the symbiotic fungi that ambrosia beetles vector from host to host can be plant pathogens and the mutualistic relationship becomes destructive when introduced to new habitats from a native habitat such as Southeast Asia (Hulcr & Stelinski 2017). Although *X. monographus* and fungal associates have not shown pathogenicity in their native range, how these species interact with new, naïve hosts in North America still needs to be investigated.

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