Distribution, Host Records, and Symbiotic Fungi of Euwallacea fornicatus (Coleoptera: Curculionidae: Scolytinae) in China

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Distribution, host records, and symbiotic fungi of *Euwallacea fornicatus* (Coleoptera: Curculionidae: Scolytinae) in China

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_Euwallacea_ (Coleoptera: Curculionidae) is a genus of mostly Asian ambrosia beetles (Storer et al. 2015). The genus includes over 50 recognized species and is increasingly important due to several globally invasive pest species (Mendel et al. 2012; Eskalen et al. 2013; Li et al. 2014). Currently, the most damaging are several populations within the species complex called _Euwallacea fornicatus_ (Eichhoff), associated with fungal mutualists in the Ambrosia _Fusarium_ Clade and the fungal genus _Raffaelea_ (Freeman et al. 2013; Kasson et al. 2013). This beetle–fungus complex is able to injure or kill trees by mass accumulation, in which each beetle inoculates the mildly pathogenic symbiont (Smith & Hulcr 2015). _Euwallacea fornicatus_ has a vast distribution throughout Asia and Oceania, and has recently been introduced and established in Mesoamerica and several locations in the United States (Rabaglia et al. 2006; Kirkendall & Ødegaard 2007). This species (or complex of species) has a broader host range than previously thought (Browne 1961; Danthanarayana 1968), and has much wider distribution (James 2007; CABl 2015).

Little is known regarding the distribution of this increasingly important pest in China (Li et al. 2014, 2015). Even the Catalog of Scolytidae and Platypodidae (Wood & Bright 1992), an essential reference for scolytine biogeography, contains few records of this species from China. Browne (1961) and Danthanarayana (1968) comprehensively recorded its host range in Sri Lanka, India, and Southeast Asia, but neither included any records from China. Consequently, any research on the biogeographic, ecological, and climate-related aspects of this beetle is currently likely to suffer a significant gap in the baseline data.

Here we present previously unpublished host records of _E. fornicatus_ deposited in the National Zoological Museum of China (NZMC), Institute of Zoology, Chinese Academy of Sciences, Beijing, and from extensive field investigation in China from 2013 to 2015. Chinese host tree names were associated with scientific names according to Iconographia Cymophytorum Sinicorum Tomus website (http://pe.ibcas.ac.cn/tujian/tjsearch.aspx). The collection at the NZMC in Beijing contains 193 specimens of _E. fornicatus_ collected from 1960 to 1999. Huifen Yin and Fusheng Huang identified the specimens.

The collection data show that this beetle is mainly distributed in the humid and subtropical southern China, but it also occurs in distinctly temperate and dry habitats (Fig. 1). Seven tree species are recorded for the first time as host plants of _E. fornicatus_ (Table 1). Three of them belong to plant families from which the beetle has not been recorded before, namely, Actinidiaceae, Oleaceae, and Pinaceae. One of the authors (Y. L.) observed a complete family (eggs, larvae, pupae, and adults) on a weakened _Pinus massoniana_ (Pinaceae) in Oct 2015. To our knowledge, this is the first record of _E. fornicatus_ from a conifer. Although a single record from a particular host plant is not necessarily indicative of a stable host association, _E. fornicatus_ is known to have broad host specificity, and it suggests that the fungal mutualist is viable in conifers.

Our data suggest that in its native habitat, _E. fornicatus_ is capable of colonizing still-living tissues of angiosperm hosts. This may help explain the beetle’s unique semiochemical ecology (Kendra et al. 2011). However, most of our data do not suggest that the beetle is an aggressive colonizer of living and healthy trees, because nearly all individuals in our collection were collected from weak, diseased, or dead host plants. We were not able to corroborate the supposed aggressive attacks on _Litchi chinensis_ (Sapindaceae) in the south of China reported previously (Wang & Yuan 2003) even after our visit to the sites from which the event was recorded. We only found this beetle mass attacking relatively healthy _Acer buergerianum_ (Sapindaceae) and _Platanus orientalis_ (Platanaceae) in an urban area of Kunming City. The NZMC collection labels do not contain information on whether the trees were killed by the beetle.

In Guiyang, Guizhou (26.3857°N, 106.6731°E) on a log of black locust _Robinia pseudoacacia_ (Fabaceae), we found more than 10 dead individuals of _E. fornicatus_ bearing distinct signs of having been parasitized by an unknown natural enemy. The parasitoid consumed the abdomen of _E. fornicatus_ and bored an exit hole through the elytral declivity (Fig. 2). Unfortunately, the parasitoid...
was not collected. This symptom is known from other Scolytinae
beetles parasitized by Hymenoptera (Nierhaus-Wunderwald 1993),
and this observation suggests that a search for natural enemies as
a part of biocontrol efforts may be fruitful.

Additionally, two fungi isolated from _E. fornicatus _and its gallery
in Guizhou were identified. The first fungus was morphologically
similar to a recently described _Paracremonium pembeum_, a known
mycangial commensal of the polyphagous shot hole borer (_E. forni-
About 15,000 colony forming units (CFUs) of it were isolated from the
oral mycangia of one individual. Cultures were slimy to moderately
floccose, and pale pink to salmonaceous in color. The conidia were
generated in simple verticillate phialides. The fungus was identified
by amplifying the ribosomal DNA (rDNA) internal transcribed spacer
(ITS) and querying the GeneBank database of the National Center for
Biotechnology Information. Three representative ITS rDNA sequences
(Hulcr12051 and LL84) were 100% identical to the hypocrealean fun-
gus _Sarocladium strictum_ and an uncultured _Acremonium_ (GenBank
accessions KM249080 and HG936339, respectively).

Acremonium sp. had been isolated from _E. fornicatus_ (Freeman et al. 2016).
The second fungus was consistent with the known nutritional
mutualist of _Euwallacea_, a representative of the Ambrosial
_Fusarium_ Clade (AFC, Kasson et al. 2013). It produced abundant aerial
mycelia and clavate macroconidia forming in sporodochia; 8,000
CFUs were isolated from the oral mycangia of one individual. Por-
tions of the translation elongation factor 1-α (_EF1-α_) and the sec-
ond largest subunit of RNA polymerase 2 (_RPB2_) were used to con-
firm placement among known AFC members (Kasson et al. 2013).
Initial GenBank BLAST searches revealed isolate 12049A, 12049B,
and LL74 _RPB2_ sequences were 99 to 100% identical to _Fusarium euwallaceae_ strains NRRL 62626 and FD31 ACVI (GenBank acces-
sions KU171702 and JX892009, respectively). A BLAST search of _EF1 _sequences revealed that strains Hulcr12049 and LL74 had 99% similarity to _Fusarium sp. AF-12_, _AF-5_, and _AF-4 _and _Fusarium am-
brosium_ (GenBank accessions KM406629, KC691542, KC691537,
and KC691528, respectively). Conclusively, our sequencing results
indicate that the _Fusarium _sp. associated with _E. fornicatus _in China
is a member of the monophyletic AFC. However, sequencing of ad-
ditional loci is needed to confirm whether or not these strains rep-
resent a novel species.

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nese Academy of Sciences) for facilitating access to the collection
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and Plant Health Inspection Service (APHIS). It may not necessarily
express APHIS views.
Euwallacea fornicatus (Eichhoff) (Coleoptera: Curculionidae) is an emerging invasive tree pest, but its native distribution remains incompletely known because minimal records have been published from China. We report the distribution of E. fornicatus in China from records in the National Zoological Museum of China and from our own samples, including the first family-level host records in the Actinidiaceae, Oleaceae, and Pinaceae. We also report a parasitoid of E. fornicatus from Guizhou, China, and two fungi associated with E. fornicatus: a putatively new Fusarium sp. belonging to the monophyletic Ambrosial Fusarium Clade and an anamorphic hypocrelean fungus, Sarocladium strictum.

Key Words: Actinidiaceae; Oleaceae; Pinaceae; natural enemy; Fusarium; Sarocladium strictum

### Summary

*Euwallacea fornicatus* (Eichhoff) (Coleoptera: Curculionidae) is an emerging invasive tree pest, but its native distribution remains incompletely known because minimal records have been published from China. We report the distribution of *E. fornicatus* in China from records in the National Zoological Museum of China and from our own samples, including the first family-level host records in the Actinidiaceae, Oleaceae, and Pinaceae. We also report a parasitoid of *E. fornicatus* from Guizhou, China, and two fungi associated with *E. fornicatus*: a putatively new *Fusarium* sp. belonging to the monophyletic Ambrosial *Fusarium* Clade and an anamorphic hypocrelean fungus, *Sarocladium strictum*.

Key Words: Actinidiaceae; Oleaceae; Pinaceae; natural enemy; *Fusarium*; *Sarocladium strictum*

### Table 1. Host trees of *Euwallacea fornicatus* specimens in the National Zoological Museum of China (NZMC) and our new field collection from 2013 to 2015.

<table>
<thead>
<tr>
<th>Province</th>
<th>Location</th>
<th>Family of host</th>
<th>Host</th>
<th>Source</th>
<th>Number of specimens</th>
</tr>
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<tr>
<td>Beijing</td>
<td>Beijing (greenhouse)</td>
<td>Malvaceae</td>
<td><em>Theobroma cacao</em></td>
<td>NZMC</td>
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<tr>
<td>Chongqing</td>
<td>Taojiaxiang</td>
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<tr>
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<td>Nanjing</td>
<td>Sapindaceae</td>
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<tr>
<td>Fujian</td>
<td>Zhangpu</td>
<td>Sapindaceae</td>
<td><em>Litchi chinensis</em></td>
<td>NZMC</td>
<td>9</td>
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<tr>
<td>Guangdong</td>
<td>Jiangmen</td>
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<td><em>Ricinus communis</em></td>
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<tr>
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<td>Xianjiang</td>
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<td><em>Hevea brasiliensis</em></td>
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<td>10</td>
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<td>Guangdong</td>
<td>Zhongshan</td>
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<td>Guyang</td>
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<td><em>Robinia pseudoacacia</em></td>
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<td>Oleaceae</td>
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<td>Guyang</td>
<td>Pinaceae</td>
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<td>Ledong</td>
<td>Sapindaceae</td>
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<td>Wuzhishan</td>
<td>Fabaceae</td>
<td><em>Acacia sp.</em></td>
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<td>1</td>
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<tr>
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<td>Chengdu</td>
<td>Fabaceae</td>
<td><em>Robinia pseudoacacia</em></td>
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<td>11</td>
</tr>
<tr>
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<td>Emei Mountain</td>
<td>Fabaceae</td>
<td><em>Robinia pseudoacacia</em></td>
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<td>4</td>
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<td>Motuo</td>
<td>Actinidiaceae</td>
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<tr>
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<tr>
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<td>Kunming</td>
<td>Sapindaceae</td>
<td><em>Acer buergerianum</em></td>
<td>field</td>
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</tr>
<tr>
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<td>Betulaceae</td>
<td><em>Betula alnoides</em></td>
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</tr>
<tr>
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<td>Euphorbiaceae</td>
<td><em>Hevea brasiliensis</em></td>
<td>field</td>
<td>5</td>
</tr>
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<td>Xishuangbanna</td>
<td>Euphorbiaceae</td>
<td><em>Ricinus communis</em></td>
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<td>Fabaceae</td>
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<td>Fabaceae</td>
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<td>Fabaceae</td>
<td><em>Cassiea sp.</em></td>
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<td>Fabaceae</td>
<td><em>Camellia sinensis</em></td>
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<td>3</td>
</tr>
</tbody>
</table>

*a* New record of host plant family.

*b* New record of host plant species.

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**Fig. 2.** The elytral declivity of *Euwallacea fornicatus* after being parasitized by an unknown natural enemy.
References Cited


