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

You Li[1], Xinyao Gu[2], Matthew T. Kasson[3], Craig C. Bateman[4], Jianjun Guo[2], YinTse Huang[1], Qiao Li[5], Robert J. Rabaglia[6], and Jiri Hulcr[1,4,]*

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; CABI 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Here we present previously unpublished host records of *E. fornicatus* deposited in the National Zoological Museum of China (NZMC), Institute of Zoology, Chinese Academy of Sciences, Beijing, and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 Cormophytorum 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 in 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 tree 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

[1]School of Forest Resources and Conservation, University of Florida, Gainesville, Florida 32611, USA; E-mail: youli88@ufl.edu (Y. L.), ythuang@ufl.edu (YT. H.), hulcr@ufl.edu (J. H.)
[2]The Provincial Key Laboratory for Agricultural Pest Management of Mountainous Region, Institute of Entomology, Guizhou University, Guiyang 550025, China; E-mail: guxinyaojy@hotmail.com X. G.), jjguo@gzu.edu.cn (J. G.)
[3]Division of Plant and Soil Sciences, West Virginia University, Morgantown, West Virginia 26505, USA; E-mail: mtkasson@mail.wvu.edu (M. T. K.)
[4]Entomology and Nematology Department, University of Florida, Gainesville, Florida 32611, USA; E-mail: batemanc@gmail.com (C. C. B.)
[5]Key Laboratory of Forest Disaster Warning and Control in Yunnan Province, Southwest Forestry University, Kunming 650224, China; E-mail: lqfcb@126.com (Q. L.)
[6]Forest Health Protection, United States Department of Agriculture Forest Service, Washington, District of Columbia 20250, USA; E-mail: brabaglia@fs.fed.us (R. J. R.)
*Corresponding author; E-mail: hulcr@ufl.edu (J. H.)

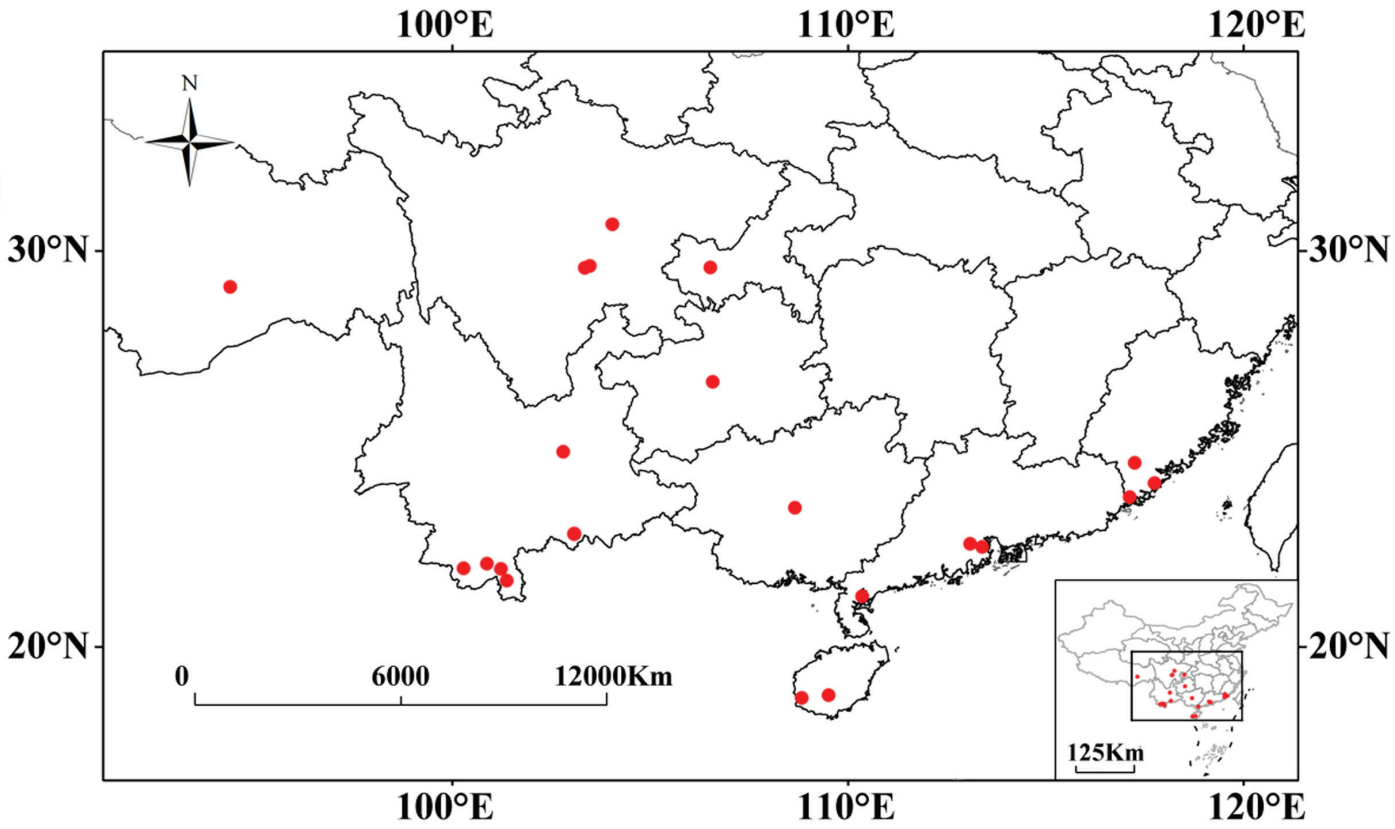

Fig. 1. The distribution of *Euwallacea fornicatus* in the south of China.

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. fornicatus* species complex) in California and Vietnam (Lynch et al. 2016). 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 fungus *Sarocladium strictum* and an uncultured *Acremonium* (GenBank accessions KM249080 and HG936339, respectively). *Sarocladium* has previously been reported to be associated with bark and ambrosia beetles throughout the northern temperate region (Hutchison 1999; Jankowiak et al. 2007; Jankowiak & Kolařík 2010), as well as from mites *Steneotarsonemus spinki* Smiley (Acari: Tarsonemidae) in Taiwan (Hsieh et al. 1980). *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. Portions of the translation elongation factor 1-α (*EF1-α*) and the second largest subunit of RNA polymerase 2 (*RPB2*) were used to confirm 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 accessions 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 ambrosium* (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 additional loci is needed to confirm whether or not these strains represent a novel species.

We thank Jian Yao and Menglei Zhang (Institute of Zoology, Chinese Academy of Sciences) for facilitating access to the collection records. Additional thanks to Yingchao Ji (Beijing Forestry University, China) for improving the distribution map, Guangyu Liu and Wei Mo (Guizhou University, China) for assisting with beetle collections, Angie Macias and Matt Berger (West Virginia University) for help with DNA sequencing and molecular identification, and Paloma C. de Grammont and Claudia Paez for translating the summary. The authors Y. L. and J. H. were supported by the USDA-FS-FHP Coop agreement 12-CA-11420004-042, USDA Farm Bill agreement 12-8130-0377-CA, and the National Science Foundation DEB 1256968. This material was made possible, in part, by a Cooperative Agreement from the United States Department of Agriculture's Animal and Plant Health Inspection Service (APHIS). It may not necessarily express APHIS views.

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.

Province	Location	Family of host	Host	Source	Number of specimens
Beijing	Beijing (greenhouse)	Malvaceae	*Theobroma cacao*	NZMC	3
Chongqing	Taojiaxiang	Rutaceae	*Citrus* sp.	NZMC	2
Fujian	Nanjing	Sapindaceae	*Litchi chinensis*	NZMC	20
Fujian	Zhangpu	Euphorbiaceae	*Ricinus communis*	NZMC	43
Fujian	Zhaoan	Sapindaceae	*Litchi chinensis*	NZMC	9
Guangdong	Jiangmen	Euphorbiaceae	*Ricinus communis*	NZMC	10
Guangdong	Zhanjiang	Euphorbiaceae	*Hevea brasiliensis*	NZMC	10
Guangdong	Zhongshan	Sapindaceae	*Litchi chinensis*	NZMC	32
Guizhou	Guiyang	Fabaceae	*Robinia pseudoacacia*	field	8
Guizhou	Guiyang	Oleaceae[a]	*Ligustrum compactum*[b]	field	16
Guizhou	Guiyang	Pinaceae[a]	*Pinus massonian*[a]b	field	11
Hainan	Ledong	Euphorbiaceae	*Hevea brasiliensis*	NZMC	1
Hainan	Wuzhishan	Fabaceae	*Acacia* sp.	NZMC	1
Sichuang	Chengdu	Fabaceae	*Robinia pseudoacacia*	NZMC	11
Sichuang	Emei Mountain	Fabaceae	*Robinia pseudoacacia*	NZMC	4
Tibet	Motuo	Actinidiaceae[a]	*Saurauia tristyla*[b]	NZMC	1
Tibet	Motuo	Euphorbiaceae	*Mallotus barbatus*[b]	NZMC	2
Tibet	Motuo	Fagaceae	*Castanopsis fargesii*[b]	NZMC	1
Yunnan	Kunming	Fabaceae	*Dalbergia odorifer*a	NZMC	2
Yunnan	Kunming	Platanaceae	*Platanus orientalis*b	field	5
Yunnan	Kunming	Sapindaceae	*Acer buergerianum*	field	3
Yunnan	Xishuangbanna	Betulaceae	*Betula alnoides*	NZMC	1
Yunnan	Xishuangbanna	Euphorbiaceae	*Hevea brasiliensis*	field	5
Yunnan	Xishuangbanna	Euphorbiaceae	*Ricinus communis*	NZMC	7
Yunnan	Xishuangbanna	Fabaceae	*Acacia mearnsi*i	NZMC	25
Yunnan	Xishuangbanna	Fabaceae	*Cassia siamea*	NZMC	3
Yunnan	Xishuangbanna	Fabaceae	*Erythrina variegata*	NZMC	1
Yunnan	Xishuangbanna	Fagaceae	*Castanea* sp.[b]	NZMC	1
Yunnan	Xishuangbanna	Theaceae	*Camellia sinensis*	NZMC	3

[a]New record of host plant family.
[b]New record of host plant species.

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*

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

Sumario

Euwallacea fornicatus (Eichhoff) (Coleoptera: Curculionidae) es una plaga invasora emergente de árboles, cuya distribución nativa no es completemente conocida todavia debido a que únicamente se ha publicado un número limitado de registros en China. Reportamos la distribución de *E. fornicatus* en China a partir de registros recolectados en el Museo Nacional de Zoología de China y de nuestras propias muestras, los cuales incluyen los primeros registros de las familias Actinidiaceae, Oleaceae y Pinaceae. Reportamos también un parasitoide de *E. fornicatus* encontrado en Guizhou, China, así como dos hongos asociados con *E. fornicatus*: una especie de *Fusarium* perteneciente al monofilético clado de *Fusarium* ambrosial y un hongo hypocrelano anamorfico, *Sarocladium strictum*.

Palabras Clave: Actinidiaceae; Oleaceae; Pinaceae; enemigo natural; *Fusarium*; *Sarocladium strictum*

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