The Coleopterists Bulletin, 72(4): 713–716. 2018.

First Record of the Invasive Neotropical Ambrosia Beetle *Euplatypus parallelus* (Fabricius, 1801) (Coleoptera: Curculionidae: Platypodinae) Infesting Arecanut in Karnataka, India

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Abstract

An infestation of the ambrosia beetle, *Euplatypus parallelus* (F.) (Coleoptera: Curculionidae: Platypodinae) on arecanut, *Areca catechu* L., is reported from the state of Karnataka in southwestern India for the first time. The visible symptoms on healthy and younger palms include oozing of a yellowish brown resinous exudation from infested stems. Small, pinhead-size holes were visible after removal of the resinous exudation. Symptoms on older, stressed, and diseased palms appear as extrusion of sawdust frass in the form of loose, cylindrical strings. Fronds of infested palms lose vigor and turn yellow. The size of the larval galleries varied 1.40–1.46 mm in diameter. Large numbers of larvae, pupae, and adults were found in the galleries of affected palms. Adult beetles are slender, 4.0–4.2 mm long, and brownish with yellowish hairs. Male and female specimens were distinguished based on the elytral declivity. The taxonomic identity of the species was confirmed by amplification of 649 base pairs of the mitochondrial cytochrome oxidase I gene. In a climate change scenario, this beetle may become a serious threat to arecanut production in India and elsewhere.

Key Words: distribution, *Areca catechu*, resinous exudation, *mtCOI*, adventive species

DOI.org/10.1649/0010-065X-72.4.713

Invasive bark and ambrosia beetles (Curculionidae: Scolytinae and Platypodinae) are a major emerging problem in many parts of the world (Haack 2001). They are often associated with fungi, which may be phytopathogenic and, in some cases, cause tree mortality (Fraedrich *et al.* 2008). The subfamily Platypodinae (pinhole borers) contains over 1,400 species (Beaver and Shih 2003), and they are abundant in the tropics where they are responsible for severe economic damage to timber and the wood industry. They generally attack weakened, dying, and recently cut or killed trees, but some species can attack living trees, especially if the tree is stressed or unhealthy (Bumrungsri *et al.* 2008; Kirkendall *et al.* 2015). Species like *Platypus quercivorus* (Murayama) and *Platypus koryoensis* (Murayama) act as carriers for wilt diseases in Fagaceae, especially oaks (Kobayashi and Ueda 2005; Hong *et al.* 2006).

One important species is *Euplatypus parallelus* (Fabricius), which is considered to be the most destructive platypodine in the world (Wood and Bright 1992). *Euplatypus parallelus* is widely

distributed in the Americas, the Caribbean, Africa, Madagascar, and throughout Southeast Asia and Indonesia to Australia (Beaver 1999, 2013). In the Indian region, it was first reported from Sri Lanka in 1975 (Krombein 1981), from Bangladesh in 2003 (Kirkendall and Islam 2003), and more recently from Goa in India (Beaver 2013; Maruthadurai *et al.* 2014). This polyphagous beetle can breed in live trees, although it prefers to breed in trees stressed by drought, disease, or recent wounds (Boa and Kirkendall 2004). Its wide host range includes both broadleaf and coniferous trees and more than 20 plant families (Schedl 1965; Zanuncio *et al.* 2005).

Arecanut (also known as betel nut; *Areca catechu* L., Arecaceae) is an important cash crop in the Western Ghats, East Coast, and North Eastern regions of India. Arecanut is also an important component of religious, social, and cultural themes as well as the economic life of people in India. India is the largest producer and consumer of arecanut in the world. There is a danger that *E. parallelus* may become a serious threat to arecanut cultivation in the future. The present investigation was carried out to expand our knowledge on the morphology, infestation symptoms, incidence, and distribution of *E. parallelus* in the arecanut ecosystem of India.

Material and Methods

Samples were collected from several sites (Table 1) by cutting open the main stem of infested arecanut trees. The beetles were identified by Dr. Roger Beaver and voucher specimens are maintained at the ICAR-Central Plantation Crops Research Institute, Regional Station, Vittal, Karnataka.

One adult specimen identified via morphological criteria was subjected to molecular characterization using the mitochondrial cytochrome *c* oxidase I (*mtCOI*) gene. DNA was extracted from the abdomen by using the QiagenDNeasy Blood and Tissue Kit® following the manufacturer's protocols. PCR reactions were performed to amplify partial *mtCOI* gene sequences with a 50-µl reaction volume containing 25.0 µl of GoTaq® Green Master Mix (Promega Corporation, USA), 2.0 µl each of forward (LCO1490 5'-GGTCAACAAATCATAAAGATATTGG-3') and reverse (HCO2198 5'-TAAACTTCAGGGTGACCAAAAAATCA-3') primers (Folmer *et al.* 1994) (10 pmol/µl), 2.0 µl of DNA, and 19.0 µl of sterile water. Thermocycling (Bio-Rad T100) consisted of an initial denaturation at 94° C for 3 min, followed by 30 cycles of denaturation at 94° C for 20 sec, annealing at 50° C for 30 sec, extension at 72° C for 30 sec, and final extension at 72° C for 5 min. The amplified product was evaluated with electrophoresis using a 1.0% agarose gel (Sambrook and Russell 2001). Purified PCR products (Bioline International, Canada) were sent for Sanger sequencing (Agrigenome Pvt. Ltd., Cochin, India). Sequence similarity was analyzed by aligning our sequence along with available sequences in the National Center for Biotechnology Information (NCBI) (www.ncbi.nlm.nih.gov) and Barcode of Life Data database (www.boldsystems.org) using BioEdit (Biological sequence alignment editor). Confirmed sequence was deposited in NCBI.

Symptoms of infestation on palms of different ages were standardized by observing and classifying the type of damage symptoms caused by *E. parallelus* on palms belonging to each age group. We surveyed arecanut gardens of different ages in major arecanut growing regions of India (Karnataka (Table 1), Kerala, and Tamil Nadu) to understand the distribution pattern of this pest. Since arecanut is a monocot and consists of single main stem, only destructive sampling (cutting of the whole tree) allowed for isolation of beetles from the trees. Cutting large numbers of trees from farmers' fields for isolation of beetles is not economical. Hence, the incidence of *E. parallelus* was recorded on the basis of symptoms of infestation. Percentage incidence of *E. parallelus* was determined by calculating (number of apparently infested palms per plot / number of all palms per plot) * 100.

Results and Discussion

Male (Fig. 1A) elytral striae are darker, deeply impressed, and subequal in width to interstriae at

Table 1. *Euplatypus parallelus* in infested gardens in Karnataka, India.

Village	GPS coordinates		Age of Palms (years)	Incidence (%)	Palm status before attack
	Latitude	Longitude			
Bantwal	12°53'13.18"	75°01'33.84"	3	34.0	Healthy
Vittal	12°46'43.60"	75°06'58.60"	6	0.2	Healthy
Seethur	13°33'21.00"	75°25'29.99"	28	2.0	Healthy
Kanur	13°32'33.00"	75°26'14.00"	13	1.0	Healthy
Melige	13°40'04.00"	75°17'14.99"	25	40.0	Healthy
Vaggade	13°31'00.00"	75°30'16.00"	28	2.0	Healthy
Honnur	14°25'32.32"	75°59'22.99"	14	0.2	Diseased (Crown rot)

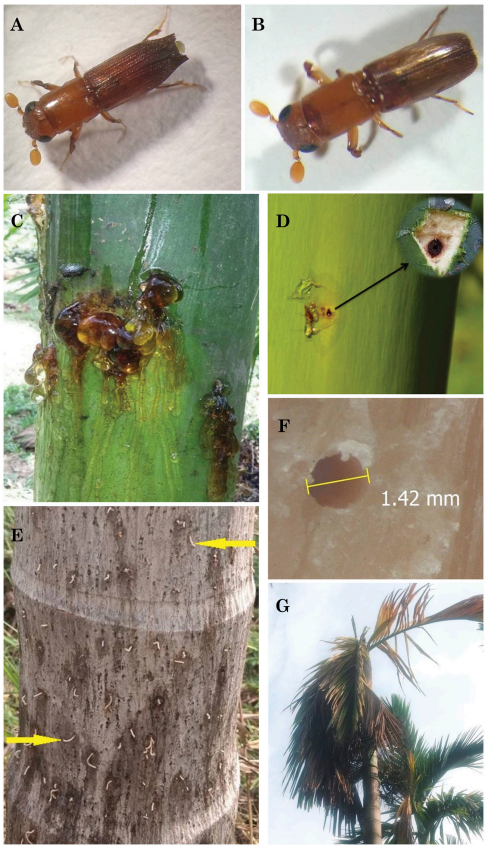

Fig. 1. *Euplatypus parallelus* infesting arecanut in Karnataka, India. A) Male, B) Female, C) Oozing of yellowish brown resinous exudation, D) Presence of small pinhead size holes under resinous exudation, E) Extrusion of sawdust frass in the form of loose, cylindrical strings (arrows), F) Round gallery entrance hole in the center of a stem, G) Yellowing of infested palm.

base of declivity. In the female (Fig. 1B), they are lighter, shallow in depth, and equal in width to interstriae at base of declivity. These are considered important characteristics for distinguishing male and female specimens.

Molecular characterization of the beetle was confirmed by amplifying 649 bp of the *mtCOI* gene. The nucleotide sequence of the *mtCOI* gene was submitted to GenBank (accession number MH628467) and showed 99% similarity with *E. parallelus* (GenBank accession No. KR261327.1) submitted from the University Museum of Bergen, University of Bergen, Norway.

Euplatypus parallelus was first found in Bantwal, Karnataka (12°53'13.18" N, 75°1'33.84" E) in a 4-year-old arecanut plantation, and subsequently in Honnur, Karnataka (14°25'32.32" N,75°59'22.99" E) on older (more than 14 years) crown rot-infested palms. Damage by *E. parallelus* to arecanut attracted attention from the farming and scientific community very rapidly due to the presence of very typical and new damage symptoms that made the palms appear unhealthy. *Euplatypus parallelus*-infested arecanut palms showed two types of symptoms under field conditions: (1) oozing of yellowish brown resinous exudation from infested stems (Fig. 1C) and the presence of small pinhead-sized holes underneath the resinous exudation on young infested palms (Fig. 1D); and (2) extrusion of sawdust frass in the form of loose, cylindrical strings from older infested palms (Fig. 1E). After dissection of affected palms, we noticed numerous bore holes and galleries ranging 1.40–1.46 mm in diameter directly through the bark into the center of the main stem (Fig. 1F). Gallery length varied 1.2–4.6 cm. A large number of eggs, larvae, pupae and adults were observed in the galleries of the infested palms. Fronds of infested palms turned yellow with reduced vigor (Fig. 1G).

Our survey conducted in the major arecanut growing regions of India confirmed that this beetle is distributed only in the southern part of Karnataka, but it has not been found in Kerala and Tamil Nadu. In Karnataka, the incidence of infestation varied from 0.2 to 40% (Table 1). Arecanut palms of ages 3–28 are susceptible to attack, and the infestation incidence is apparently not correlated with tree age (Table 1).

Most Platypodinae infest only freshly dead, dying, or stressed plants. However, *E. parallelus* is one of the few species that can infest live trees (Boa and Kirkendall 2004; Beaver 2013; Kirkendall *et al.* 2015). In our study, we noticed that apparently healthy arecanut palms were damaged by this polyphagous beetle (Table 1). *Euplatypus parallelus* has been reported to attack live rubber trees, *Hevea brasiliensis* (Willd. ex A. Juss.) Müll. Arg. (Euphorbiaceae), in Brazil (Silva *et al.* 2013), Indian rosewood, *Dalbergia sissoo* Roxb. Ex DC. (Fabaceae), in Bangladesh (Kirkendall and Islam 2003), and *Pterocarpus indicus* Willd. (Fabaceae), in Thailand (Bumrungsri *et al.* 2008). As larvae feed on ambrosia fungus, and ambrosia fungi are considered to be non-specific to plant species, polyphagy should be expected (Bumrungsri *et al.* 2008). In Asia and the Seychelles, presence of a wilt fungus, *Fusarium oxysporum* Schlecht., in several leguminous crops attacked by *E. parallelus* has been reported (Sanderson *et al.* 1997; Philip 1999).

Arecanut is an important cash crop in most South Asian countries. Changing climate may support the emergence of *E. parallelus* in the arecanut ecosystem as the stressed palms are more vulnerable to infestation. Being polyphagous in nature, this beetle may spread easily to new host trees and may also act as a vector of plant pathogens. Hence, occurrence and infestation of *E. parallelus* on the economically

important arecanut crop is of very serious concern. Detailed studies on the pest's biology, pathogenic fungi association, population dynamics, altitudinal distribution, and management aspects are in progress.

Acknowledgments

The authors thank Dr. Roger Beaver for identifying the beetle. They also thank the arecanut growers who allowed destructive sampling to isolate the beetles.

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(Received 6 August 2018; accepted 10 October 2018. Publication date 28 December 2018.)