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A NEW INVASIVE JACK BEARDSLEY MEALYBUG, *PSEUDOCOCCUS JACKBEARDSLEYI* (HEMIPTERA: PSEUDOCOCCIDAE) ON PAPAYA IN INDIA

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Mealybugs (Hemiptera: Pseudococcidae) are major pests of a wide range of agricultural, horticultural and ornamental plants worldwide (Miller et al. 2002). Certain attributes of the Pseudococcidae, viz., wide host range, short generation time, cosmopolitan nature, ability to transmit some important plant viruses, etc., have contributed to their enormous damage potential (Meyer et al. 2008). In this regard, *Pseudococcus jackbeardsleyi* Gimpel and Miller (Hemiptera: Pseudococcidae), known as the Jack Beardsley mealybug, is a polyphagous species of neotropical origin that is known to attack 93 plant species including several vegetable and fruit and ornamental crop species (CAB Intl. 2001). The earliest record of this mealybug in Asia was from Singapore in 1958, followed by Malaysia in 1969, Indonesia in 1973, Philippines in 1975, Brunei in 1979, Thailand in 1987, the Maldives, and Vietnam in 1994 (Williams 2004a,b; Muniappan 2011) and not reported yet from India. In the current report during the survey for monitoring the activity of papaya mealybug, *Paracoccus marginatus* Williams and Granara de Willink and its parasitoid *Acerophagus papayae* Noyes & Schauff in Satyamangalam (N 11.5200° E 77.2500°) Tamilnadu, India, a short tailed mealybug and *P. marginatus* were found colonizing 2 papaya plantations on Jun 2012. Mealybugs were found scattered on the leaves, fruits and trunks and were observed sucking the sap from various parts of the papaya plants including leaves, stems, flowers and fruits. No serious damage was observed on the papaya plant caused by this new mealybug. Specimens of this mealybug were collected and preserved in ethyl alcohol (100%) and were stored at 20 °C until further use. The mealybug was identified as the Jack Beardsley mealybug, *P. jackbeardsleyi*, at the National Bureau of Agriculturally Important Insects, Bangalore.

Morphological characters used for identifying *P. jackbeardsleyi* are as follows: Body of the female oval to elongate. Antenna 8 segmented. Legs well developed, translucent pores present on hind femur and hind tibia. Discoidal pores present near eye on sclerotised rim. Cerarii 17 pairs. Anal lobe cerari with 2 conical setae and many

trilocular pores on sclerotised area. All other cerarii on a membranous area. Anterior cerari with relatively smaller setae and with 2 to 3 auxiliary setae. Cerarii number C₇ and cerarii on head with 3-4 conical setae. Dorsal surface with small setae and trilocular pores scattered almost evenly. All frontal cerarii with oral rim tubular duct just behind them. Other oral rim tubular ducts present submedially and submarginally on thorax and abdomen. Anal lobe and penultimate cerari with oral collar tubular duct between them. Ventral surface with slender setae. Multilocular disc pores present on V to VII abdominal segments. Oral rim tubular duct and oral collar tubular ducts same as dorsum. (Fig. 1).

The invasive mealybug is grayish in color; thin filaments around the body, caudal pair about one half of the length of the body, and ovisac covering hind part of the body. All other morphological characters also match the description given by Williams (2004a). Quick and correct identification of an invasive pest like *P. jackbeardsleyi* is important in order to initiate suitable management measures that may include quarantine. Molecular identification, at this juncture is handy as it is not limited by life stage, polymorphism and gender. Incursion of this pest into India has been proven so far by identifying it by morphological or molecular methods. Hence, in this paper we are providing evidence that identifies *P. jackbeardsleyi* in India by both morphological and molecular methods.

Standard protocols were followed for DNA extraction, Polymerase Chain Reaction, sequencing of the mitochondrial cytochrome oxidase I (CO-I) gene fragment and sequence alignment (Hajibabaei et al. 2005). Small portion of the abdomen from individual *P. jackbeardsleyi* was used for genomic DNA and PCR was carried out in a thermal cycler (ABI-Applied Biosystems, Veriti, USA) with the following cycling parameters; 94 °C for 4 min as initial denaturation followed by 35 cycles of 94 °C for 30 s, 47 °C for 45 s, 72 °C for 45 s and 72 °C for 20 min as final extension using universal CO-I primers (LCO-1490- 5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3' and HCO-2198- 5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3',

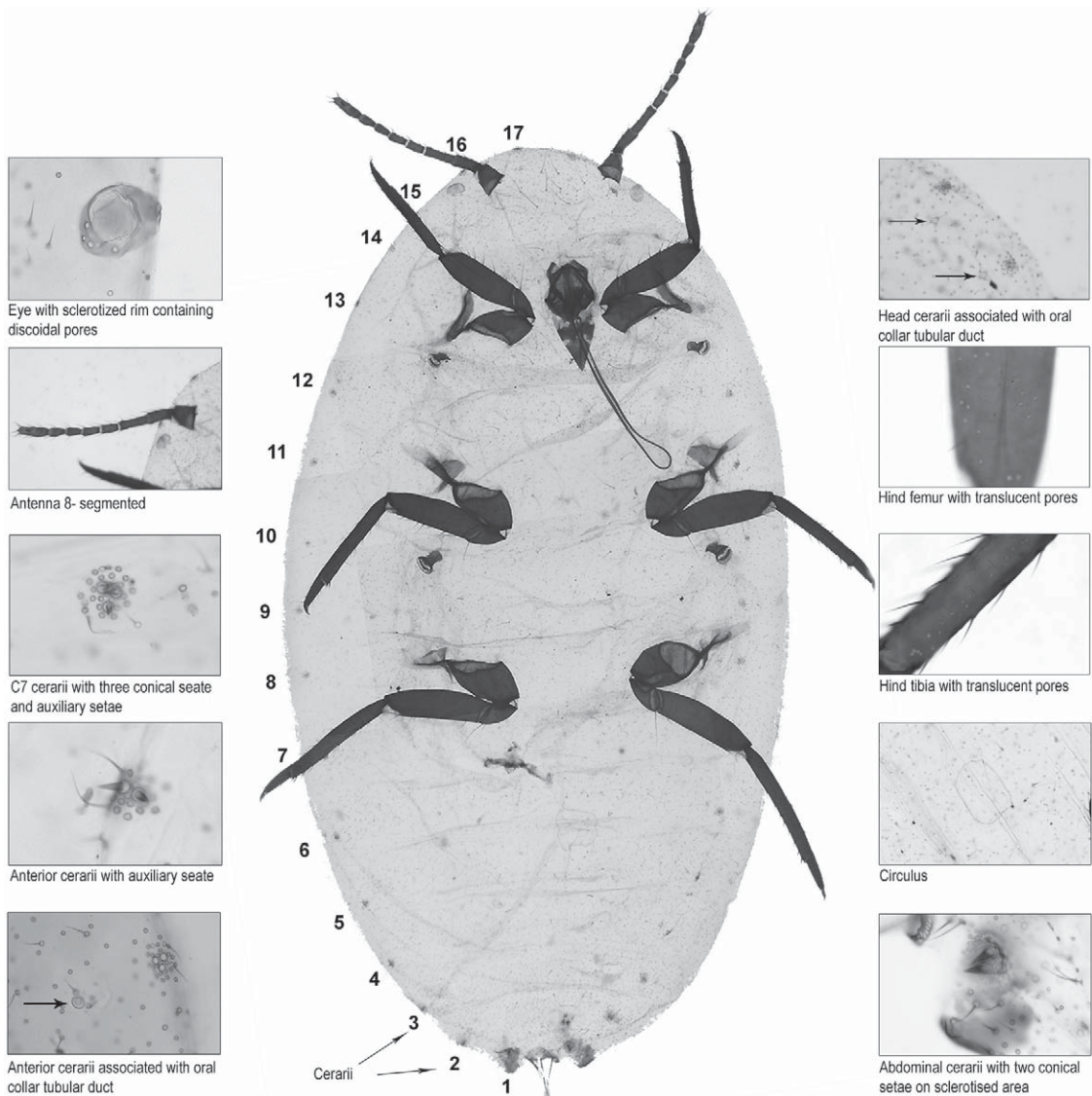


Fig 1. Morphological characters used for identification of *Pseudococcus jackbeardsleyi* Gimpel and Miller, collected on papaya from Satyamangalam, Tamilnadu.

Hebert et al. 2004). PCR was performed in 25- μ L total reaction volume containing 20 picomoles of each primer, 10 mM Tris HCl (pH-8.3), 50 mM KCl, 2.5 mM $MgCl_2$, 0.25 mM of each dNTP and 0.5 U of Taq DNA polymerase (Fermentas Life Sciences, EU). The voucher specimen deposited in the National Pusa Collection, (NPC), New Delhi. The amplified products were resolved in 1.0% agarose gel, stained with ethidium bromide (10 μ g/mL) and visualized in a gel documentation system (UVP). The PCR amplified fragments were eluted using Nucleospin® Extract II according to the manufacturer's protocol (MN, Germany) and ligated into the general purpose-

cloning vector, InsT/Aclone and transformation using *Escherichia coli* (DH5 α) cells was carried out (Fermentas GmbH, Germany) according to the manufacturer's protocol. Blue/white selection was carried out and all the white colonies (colonies harbouring the insert) were maintained on LBA containing ampicillin (100 mg/mL), incubated at 37 °C overnight and stored at 4 °C until further use. Plasmids were isolated by GeneJET™ Plasmid Miniprep Kit (Fermentas, Germany) according to manufacturer's protocol, from overnight cultures of the 5 randomly selected clones multiplied in LB broth. Sequencing was carried out in an automated sequencer (ABI Prism 310;

Applied Biosystems, USA) using M13 universal primers both in forward and reverse directions. Homology search was carried out using BLAST (<http://www.ncbi.nlm.nih.gov>) and the differences in CO-I sequences of *P. jackbeardsleyi* were determined using the sequence alignment editor 'Bioedit'. A sequence for *P. jackbeardsleyi* was deposited with the NCBI database, and the accession number is KC119455. Sequence generated in the present study along with the other *Pseudococcus* spp. viz. *Pseudococcus comstocki* (Kuwana), *Pseudococcus viburni* (Signoret) and *Ferrisia virgata* (Cockerell) (as an outgroup) (Retrieved from NCBI) were aligned using BioEdit. 7. 0 program using Clustal W.

Mitochondrial cytochrome oxidase-1 (CO-I) was successfully sequenced from an individual *P. jackbeardsleyi*. A comparison of the triplicate sequences showed no evidence of mismatch, indicated there were no sequencing errors. A total fragment size of 647 bp of the CO-I was analyzed for *P. jackbeardsleyi*. Evidence of nuclear copies was not found, which was supported by the absence of stop codons within the sequences and the base composition was similar with no indels (Rebijith et al. 2012). *Ferrisia virgata* was selected as an out group. Phylogenetic analysis of aligned sequences was done using MEGA. 5. 0 (Tamura et al. 2011). The method of neighbor-joining (NJ) with the Kimura two-parameter model (Kimura 1980) was selected to build the phylogenetic tree. To assess the robustness of the tree, 1000 bootstrap replicates were selected. *Pseudococcus jackbeardsleyi* showed 8.0% (52/649) and 8.1% (53/649) sequence variation with *Pseudococcus comstocki* and *Pseudococcus viburni* respectively. Phylogenetic tree for sequences of all *Pseudococcus* spp. used in the present study are shown in Fig. 2. This figure and the sequences suggest that *P. jackbeardsleyi* collected on papaya had 100%

similarity with the other 3 accessions found in the BLAST search and used for constructing the phylogenetic tree.

As many as 22 plant species have been reported as hosts of *P. jackbeardsleyi* in Asian countries. There is no record of this mealybug on papaya in south Asian countries (Williams 2004a,b). Thus, we report *P. jackbeardsleyi* for the first time in India and for the first time breeding on papaya.

No classical biological control attempt has been made against the Jack Beardley mealybug, and possibly it is kept under control by the local natural enemies in certain invaded countries (Muniappan et al. 2011). Also we observed that the coccinellid, *Cryptolaemus montrouzieri* Mulsant, was capable of checking Jack Beardley mealybug populations in the present study. Hence there is no need for any panic to cope with the newly invasive *P. jackbeardsleyi* in India. However, our study will aid in proper quarantine measures and emergency management plans by which further spread into other states may be hindered or delayed to avoid economic losses.

SUMMARY

Mealybugs are major factors limiting the productivity of papaya in India. In June 2012 during a survey for the papaya mealybug, *Paracoccus marginatus*, and its parasitoid *Acerophagus papayae*, in Satyamangalam, Tamilnadu, India, a short tailed mealybug was found together with *P. marginatus* colonizing papaya in 2 plantations. This mealybug was identified as the Jack Beardsley mealybug, *P. jackbeardsleyi* Gimpel and Miller (Hemiptera: Pseudococcidae), by an integrated taxonomic approach. This work appears to be the first report of *P. jackbeardsleyi* in India and of papaya as a host of this pest.

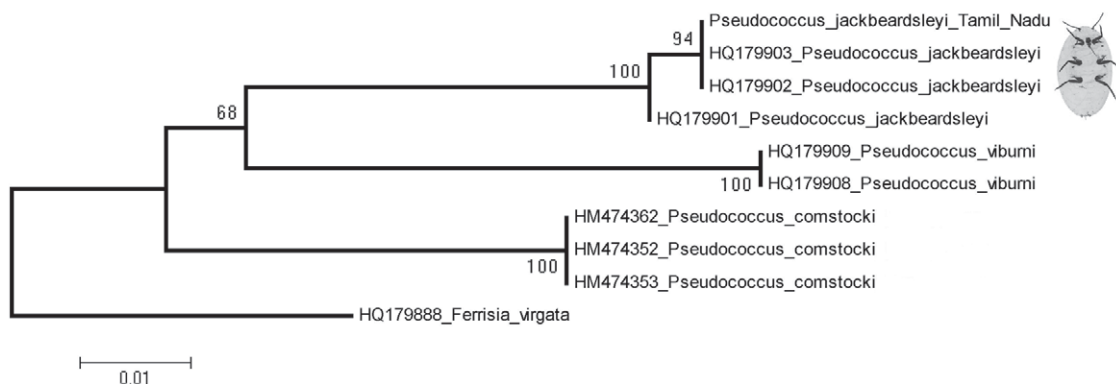


Fig 2. Neighbor joining (NJ) tree (MEGA. 5.0) with bootstrap support (1000 replicates), showing clustering of *Pseudococcus* species for CO-I sequences. All the *P. jackbeardsleyi* sequences formed a single clade with > 99% similarity in sequences. Bootstrap values greater than 65% are indicated above the branches. *Ferrisia virgata* (HQ179888) were used as an outgroup.

Key Words: CO-I, phylogenetic tree, parasitoid, *Acerophagus papayae*, integrated taxonomy

RESUMEN

Las cochinillas es factor principal que limita la productividad de papaya en la India. En este sentido, en junio del 2012, durante un estudio de la cochinilla de papaya, *Paracoccus marginatus* y su parasitoide, *Acerophagus papayae*, en Satyamangalam, Tamil Nadu, India, una cochinilla de cola corta fue encontrada junto con *P. marginatus* colonizando papaya en dos plantaciones. Esta cochinilla fue identificada como la cochinilla Jack Beardsley, *Pseudococcus jackbeardsleyi* Gimpel y Miller (Hemiptera: Pseudococcidae), por métodos morfológicos y moleculares. Este parece ser el primer informe del establecimiento de *P. jackbeardsleyi* en la India.

Palabras Clave: CO-I, árbol filogenético, parasitoide, *Acerophagus papayae*, caracteres morfológicos

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