First Report of the Papaya Mealybug, Paracoccus marginatus (Hemiptera: Pseudococcidae), in China and Genetic Record for Its Recent Invasion in Asia and Africa

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First report of the papaya mealybug, *Paracoccus marginatus* (Hemiptera: Pseudococcidae), in China and genetic record for its recent invasion in Asia and Africa

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Abstract

The papaya mealybug, *Paracoccus marginatus* Williams and Granara de Willink (Hemiptera: Pseudococcidae), is a polyphagous pest that damages many tropical crops. It is a native of Central America and spread to the Caribbean region and South America in the 1990s; since then it has accidentally been introduced to some islands in the Pacific region and some countries in Africa and Asia. We recorded its presence in China for the first time in 2013 from Guangdong Province and Yunnan Province in southern and southwestern China, respectively. Our genetic analysis revealed that only 1 haplotype of *P. marginatus* has been recorded in all of Asia, including China, reflecting the very recent invasion of *P. marginatus* in Asia. This study also includes a summary of the global geographical distribution of *P. marginatus*.

Key Words: genetic identity; haplotype; global distribution; insect pest

Resumen

La cochinilla de la papaya, *Paracoccus marginatus* Williams y Granara de Willink (Hemiptera: Pseudococcidae), es una plaga polífaga que daña muchos cultivos tropicales. Es originario de América Central y se extendió a la región del Caribe y América del Sur en la década de 1990; desde entonces ha sido introducida accidentalmente en algunas islas de la región del Pacífico y algunos países de África y Asia. Se registró su presencia en China por primera vez en el año 2013 en las provincia de Guangdong y de Yunnan en el sur y suroeste de China, respectivamente. Nuestro análisis genético reveló que sólo un haplotipo de *P. marginatus* se ha registrado en toda Asia, incluyendo China, lo que refleja la reciente invasión de *P. marginatus* en Asia. Este estudio también incluye un resumen de la distribución geográfica mundial de *P. marginatus*.

Palabras Clave: identidad genética; haplotipo; distribución mundial; plaga insecto

The papaya mealybug, *Paracoccus marginatus* Williams and Granara de Willink (Hemiptera: Pseudococcidae), is a small, yellowish, polyphagous sucking insect and is considered a significant pest of many tropical and subtropical fruits, vegetables, and ornamental plants (Miller et al. 2002). It feeds on the sap of plants by inserting its stylets into the epidermis of the leaf and into the fruit and stem. The leaves become crinkled, yellowish, and withered (Tanwar et al. 2010; Suganthy et al. 2012; Kirsur et al. 2014; Ben-Dov 2015). Sooty mold develops on honeydew excreted by this mealybug; this mold eventually covers the leaves, fruits, and stems, impeding photosynthesis and gaseous exchange. The result is chlorosis, plant stunting, leaf deformation, early dropping of the leaves and fruits, a heavy accumulation of honeydew, and death of the host plants. The mealybug also deposits a thick, white, waxy material that can make plants inedible (Muniappan 2011). Although the papaya mealybug originated in Mexico, it was not a serious pest there or in Central America as its natural enemies kept it under control (Miller et al. 1999). However, when it spread to the Caribbean and to Florida, USA, the mealybug caused significant yield losses not only in papaya but in more than 60 other crops, particularly horticultural species (Miller et al. 2002; Meyerdirk et al. 2004; Walker et al. 2006; Heu et al. 2007).

Paracoccus marginatus was first collected in Mexico in 1955 but first described by Williams and Granara de Willink in 1992 from Mexican specimens collected on cassava (*Manihot esculenta* Crantz; Malpighiales: Euphorbiaceae); it was thus thought to be native to Mexico and South/Central America (Williams & de Willink 1992; Miller et al. 1999, 2001; Miller & Miller 2002). It was first reported as a pest of papaya (*Carica papaya* L.; Capparales: Caricaceae) in the Caribbean during the 1990s, and by 2000 it had spread to many Caribbean countries as well as Florida in the United States (Pollard 1999; Muniappan 2009). Over the next decade, there were

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additional reports from many countries in Oceania, Asia, and Africa (Meyerdirk et al. 2004; Muniappan et al. 2006, 2008, 2009; Muniappan 2009).

Paracoccus marginatus was first reported in India in 2008, and in subsequent years it has invaded 9 additional Asian countries (Muniappan 2009, 2011; Chen et al. 2011; Myrick et al. 2014). In this study, we provide the first report of its presence in southern and eastern parts of China and identify its genetic haplotype in China and other Asian and African countries. These results provide the only genetic survey of *P. marginatus* from Asia and enhance our understanding of the invasion of this detrimental insect pest.

Materials and Methods

SAMPLE COLLECTION

We designated in total 12 sites in mainland China, Cambodia, Malaysia, and Mozambique (Table 1) and used 3 individuals from each. The samples from Cambodia, Malaysia, and Mozambique were intercepted at the entry port of China. However, the Chinese samples were taken randomly from those sites wherever the pest was reported in China. Specimens of *P. marginatus* were collected with forceps and preserved in 95% alcohol. Species identifications were confirmed using both morphological characters (Miller & Miller 2002) and DNA barcodes from BOLD System V3 (Ratnasingham & Hebert 2007). For the haplotype analysis, we also incorporated genetic data from specimens collected at an additional 9 sites in India, Indonesia, and Thailand; sequences were downloaded from GenBank, BOLD, and EMBL-EBI (Table 1).

DNA EXTRACTION

The total genomic DNA was extracted from individual specimens of *P. marginatus* using a Promega Wizard® Genomic DNA Purification Kit (Madison, Wisconsin, USA). We used Wizard® Genomic DNA Purification Kit animal tissue protocols as described in their technical manual, #TM050 (http://www.promega.com/ resources/ protocols/technical-manuals/0/wizard-genomic-dna-purification-kit-protocol/).

PCR AMPLIFICATION

Polymerase chain reaction (PCR) amplification was performed in 25 µL volume reactions using final concentrations of the following reagents: 2.5 µL of 1× PCR buffer, 1.5 µL of 25 mM $MgCl_2$, 2.5 µL of 2 mM dNTP mix with 0.2 mM of each nucleotide, 1.25 µL of each 0.5 µM primer, 2.0 µL of 50–125 ng DNA template, 0.5 µL of 0.5 U of DNA polymerase containing 5 U/µL (Takara), and 14.75 µL of nuclease-free water. We amplified partial sequences (~800 bp) of the cytochrome oxidase subunit I (*COI*) gene using the universal primer pairs CJ-J-2183 (alias Jerry, 5'-CAACATTTATTTTGATT TTTTGG-3') and TL2-N-3014 (alias Pat, 5'-TCCAATGCACTAATCTGCCATATTA-3') (Simon et al. 1994). The *COI* gene was amplified under the following conditions: 3 min at 95 °C, followed by 30 cycles at 94 °C for 1 min, 57 °C for 30 s, and 72 °C for 1min, and a post-cycle incubation at 72 °C for 5 min. After that, 5 µL of the PCR product underwent electrophoresis on 1.2% agarose gels. When bands of the expected size, ~800 bp, were visible in the gels, the other 20 µL of the PCR product was used for sequencing using an ABI 3730xl sequencer (Invitrogen Life Tech Co. Ltd., Shanghai, China) with the same primers for both directions.

ALIGNMENT

Sequences were aligned using multiple sequence alignment with the "GINSI" algorithm in MAFFT (Tamura et al. 2011). The ends with incomplete codons were then trimmed to produce 712 bp alignments. In order to exclude possible nuclear mitochondrial pseudogenes (numts) commonly found in invertebrates (Buhay 2009), we screened translated *COI* sequences for stop codons.

Table 1. Identification and distribution for the haplotype of *Paracoccus marginatus* samples along with its genetic identities.

Accession number	Location	Host plant	Reference	Genetic identity
KP745312	Pnom-penh, Cambodia	*Manihot esculenta*	This study	Haplotype1
KP745312	Pnom-penh, Cambodia	*Manihot esculenta*	This study	Haplotype1
KP745312	Pnom-penh, Cambodia	*Manihot esculenta*	This study	Haplotype1
KP745312	Tianhe, Guangzhou, China	*Jatropha integerrima*	This study	Haplotype1
KP745312	Baiyun, Guangzhou, China	*Carica papaya*	This study	Haplotype1
KP745312	Panyu, Guangzhou, China	*Carica papaya*	This study	Haplotype1
KP745312	Guangzhou, China	*Jatropha curcas*	This study	Haplotype1
JN797604	India	*Manihot esculenta*	Unpublished	Haplotype1
JN797603	India	*Carica papaya*	Unpublished	Haplotype1
JN797602	India	*Tagetes erecta*	Unpublished	Haplotype1
JN797601	India	*Morus nigra*	Unpublished	Haplotype1
JN797600	India	*Gossypium hirsutum*	Unpublished	Haplotype1
KF686748	India	*Carica papaya*	Unpublished	Haplotype1
JF933768	India	N/A	Unpublished	Haplotype1
HM474243	Indonesia	N/A	Park et al. 2011	Haplotype1
KP745312	Malaysia	N/A	This study	Haplotype1
KP745312	Niassa, Mozambique	*Carica papaya*	This study	Haplotype1
KP745312	Niassa, Mozambique	*Carica papaya*	This study	Haplotype1
HM474242	Thailand	N/A	Park et al. 2011	Haplotype1
KP745312	Yunnan, China	*Plumeria rubra*	This study	Haplotype1
KP745312	Yunnan, China	*Carica papaya*	This study	Haplotype1

*All samples sequenced in this study were identical and were assigned same accession number by NCBI.

GENETIC NETWORKING

Analyzing closely related populations is challenging because the small genetic distances between individuals can obscure evolutionary pathways. In these cases, tree-based phylogenetic methods are poor at representing intraspecific gene evolution (De Barro & Ahmed 2011). Network analysis is a more appropriate means of exploring relationships within species, and many different networking approaches have been developed to estimate intraspecific genealogies (Fitch 1997; Bandelt et al. 1999).

The haplotype identifier refers to haplotypes belonging to various locations; the haplotype identifiers are listed in Table 1. The sequences were analyzed using statistical parsimony (Templeton et al. 1992; Posada & Crandall 2001) with the program TCS v.1.21 (Clement et al. 2000) with a cut-off set at 95% (Hart & Sunday 2007; Chen et al. 2010). Each sequence was assigned a haplotype identifier (Table 1), and the resulting network provided both the relationship between the different haplotypes and the significant number of substitutions connecting haplotypes (Templeton et al. 1992).

Twenty-one *COI* sequences were analyzed for genetic identity, 12 of which were sequenced specifically for this study. Most sequences were from China (2 from Yunnan and 4 from Guangzhou) and India (7), and the rest from Cambodia (3), Indonesia (1), Malaysia (1), Mozambique (2), and Thailand (1).

GENETIC DISTANCES

Pair-wise genetic distance values were calculated in MEGA5 (Tamura et al. 2011). The use of the genetic species concept and these values are not meant to be a strict delimitation, but are a guide to help estimate the number of possible genetic lineages in our samples.

Results

FIRST RECORD OF *P. MARGINATUS* IN CHINA AND ITS DISTRIBUTION IN THE WORLD

Our study recorded the first presence of *P. marginatus* from 2 provinces of China: the southwestern Yunnan Province and the southern Guangzhou Province. Chinese specimens were found on 4 host plants: *C. papaya*, *Jatropha integerrima* Jacquard, *Jatropha cuarcas* L. (Malpighiales: Euphorbiaceae), and *Plumeria rubra* L. (Gentianales: Apocynaceae). Our study also provides the first Southeast African record of *P. marginatus*, in Mozambique. In addition, we surveyed the invasion history of *P. marginatus* and found that it has invaded more than 54 countries and regions in the last 22 yr (Table 2).

HAPLOTYPE DIVERSITY AND DISTRIBUTION OF *P. MARGINATUS*

We analyzed the genetic identities of Chinese populations of *P. marginatus* and compared them with genetic identities of *P. marginatus* populations from 6 other countries in Asia and Africa. The results indicated that only 1 haplotype is present across all sampled countries (Table 1).

Discussion

Shortly after *P. marginatus* was first reported to have invaded India in 2008, it was found to have invaded a number of other Asian countries. Our study adds the first Chinese records, from 2 noncontiguous provinces, and the first record from southeastern Africa. This further demonstrates the potential for *P. marginatus* to rapidly invade new regions.

The presence of the same of *P. marginatus* haplotype across all sampled Asian countries suggests that all specimens stem from the same population resource that initially invaded Asia in 2008, and perhaps from the same population that entered Mozambique. This is another typical case of rapid invasion given that the reported rate of *COI* evolution for some model insects is ~1.5 to 2.3% per million years ago (Nakamine & Takeda 2008). Similar cases have been found with other invasive Sternorrhyncha. A single haplotype of the cotton mealybug *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) has undergone a very recent rapid invasion in China (Ahmed et al. 2015), and a single haplotype of an invasive whitefly has established a global distribution (De Barro & Ahmed 2011).

The low levels of variation between geographically distant populations of Asia support the hypothesis that this was a recent invasion that likely occurred during the last 4 to 6 yr. However, the route that *P. marginatus* used as it spread across regions is still unclear. More sampling and investigating of genetic identities of *P. marginatus* from its native countries in South and Central America and from other invaded countries, especially those in the Caribbean, will help to understand the precise route of its invasion.

One interesting topic we were unable to address with our data is whether certain external factors contributed to the rapid spread of *P. marginatus* populations across Asia. One report suggests trade of ornamental flowers could have facilitated the invasion (Qian et al. 2007). However, the mealybug itself was reported to have high biological adaptability, ecological adjustability, temperature tolerance, and a wide range of host plants that may help it to establish quickly in newly invaded places (Arif et al. 2009; Hodgson 2009; Vennila et al. 2011, 2013; Xin et al. 2011). This rapid spread suggests that current quarantine measures have failed to stop the invasion of *P. marginatus* into China and other neighboring countries. Therefore, officially listing *P. marginatus* as an invasive species, along with revised quarantine regulations and protocols, may help stop further invasion by this devastating pest.

The recent establishment of *P. marginatus* is of serious concern because it is a major pest of many species of tropical fruits, vegetable crops, and ornamental plants. Multiple control strategies should be developed such as early detection and eradication in the areas with new records, and biological control for areas where *P. marginatus* has been established. Within 3 mo of the first discovery of *P. marginatus* in Indonesia, the magnitude of the mealybug problem caused the government to consider development of a classical biological control program against it (Muniappan et al. 2008). Successful results with classical biological control were achieved in India, Guam, Palau, Florida, and Hawaii in recent years (Meyerdirk et al. 2004; Muniappan et al. 2006; Walker et al. 2006; Heu et al. 2007). In India, an encyrtid parasitoid of papaya mealybug, namely, *Acerophagus papayae* Noyes & Schauff (Hymenoptera: Encyrtidae), was imported from Puerto Rico and released after multiplication. The introduction of *A. papayae* provided excellent control of the papaya mealybug within 5 mo along with reduced pesticide use, increased production, and increased income (Myrick et al. 2014). This success suggests that classical biological control may also be a viable option against *P. marginatus* in China.

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Table 2. Survey of the global invasion of *Paracoccus marginatus* since the first reports of its invasiveness.

First reported	Location	Region	Origin	Reference
1992	Belize	South/Central America	Native	Williams & de Willink 1992; Miller et al. 1999; Pollard 1999; Miller & Miller 2002
1992	Costa Rica	South/Central America	Native	Williams & de Willink 1992; Miller et al. 1999; Pollard 1999; Miller & Miller 2002;
1992	Guatemala	South/Central America	Native	Williams & de Willink 1992; Miller et al. 1999; Pollard 1999; Miller & Miller 2002
1992	Mexico	South/Central America	Native	Williams & de Willink 1992; Miller et al. 1999; Pollard 1999; Miller & Miller 2002
1994	Antigua	Caribbean	Introduced	Muniappan 2009
1994	Dominican Republic	Caribbean	Introduced	Pollard 1999; Muniappan 2009
1994	Grenada	Caribbean	Introduced	Muniappan 2008
1994	U.S. Virgin Islands	Caribbean	Introduced	Pollard 1999; Muniappan 2009
1996	British Virgin Islands	Caribbean	Introduced	Muniappan 2008
1996	Saint Martin	Caribbean	Introduced	Pollard 1999; Muniappan 2009; Muniappan et al. 2009
1998	Barbuda	Caribbean	Introduced	Pollard 1999
1998	Guadeloupe	Caribbean	Introduced	Matile-Ferrero & Etienne 1998, Muniappan 2009
1998	Haiti	Caribbean	Introduced	Pollard 1999; Muniappan 2009
1998	Manatee, Palm Beach, and Broward counties, Florida, USA	North America	Introduced	Miller et al. 1999; Pollard 1999; Miller & Miller 2002; Muniappan 2009
1998	Saint-Barthelemy	Caribbean	Introduced	Muniappan 2009
1998	St. Kitts and Nevis,	Caribbean	Introduced	Pollard 1999; Muniappan 2009
1999	Cuba	Caribbean	Introduced	Muniappan 2008
1999	French Guyana	Caribbean	Introduced	Matile-Ferrero et al. 2000; Muniappan 2008
1999	Netherlands Antilles	Caribbean	Introduced	Pollard 1999
1999	Puerto Rico	Caribbean	Introduced	Pollard 1999; Muniappan 2009
2000	Barbados	Caribbean	Introduced	Muniappan 2009
2000	Cayman Islands	Caribbean	Introduced	Muniappan 2009
2000	Montserrat	Caribbean	Introduced	Muniappan 2009
2002	Bahamas	Caribbean	Introduced	Muniappan 2009
2002	Guam	Oceania	Introduced	Meyerdirk et al. 2004; Muniappan 2009
2003	Palau	Oceania	Introduced	Muniappan et al. 2006; Muniappan 2009
2004	Hawaii, USA	North America	Introduced	Heu et al. 2007; Muniappan 2009
2005	northern Mariana Islands	Oceania	Introduced	Muniappan 2009; Muniappan et al. 2009
2008	Java, Bali, and Sulawesi Islands, Indonesia	Asia	Introduced	Muniappan 2009; Muniappan et al. 2009
2008	Luzon, Philippines	Asia	Introduced	Muniappan 2009; Muniappan et al. 2009
2008	Saint Lucia	Caribbean	Introduced	Jn Pierre 2008
2008	Sri Lanka	Asia	Introduced	Muniappan 2009; Muniappan et al. 2009; Galanihe et al. 2010
2008	Tamil Nadu, India	Asia	Introduced	Muniappan 2009; Muniappan et al. 2009; Ayyasamy & Regupathy 2010; Suresh et al. 2010
2009	Bangkok, Thailand	Asia	Introduced	Muniappan 2009; Muniappan et al. 2009
2008	Joydebpur, Bangladesh	Asia	Introduced	Muniappan et al. 2008
2009	Jammu, India	Asia	Introduced	Sharma et al. 2013
2009	Joydebpur, Bangladesh	Asia	Introduced	Muniappan 2009; Muniappan et al. 2009
2009	Kerala, India	Asia	Introduced	Krishnakumar & Rajan 2009; Lyla & Philip 2010
2009	Malaysia	Asia	Introduced	Mastoi et al. 2011
2009	Maldives	Asia	Introduced	Muniappan 2009; Muniappan et al. 2009
2009	Nsawam Kede, Ghana	West Africa	Introduced	Muniappan 2009; Muniappan et al. 2009
2009	Hilacondji, Benin	West Africa	Introduced	Muniappan 2009; Muniappan et al. 2009
2009	Lomé, Togo	West Africa	Introduced	Muniappan 2009; Muniappan et al. 2009
2009	Siem Reap, Cambodia	Asia	Introduced	Muniappan 2009; Muniappan et al. 2009

Table 2. (Continued) Survey of the global invasion of *Paracoccus marginatus* since the first reports of its invasiveness.

First reported	Location	Region	Origin	Reference
2009	Thailand	Asia	Introduced	Muniappan 2009; Muniappan et al. 2009
2010	Réunion	Africa	Introduced	Germain et al. 2010
2011	Karnataka, India	Asia	Introduced	Shekhar et al. 2011
2011	Oman	Arabian Peninsula	Introduced	CABI unpublished data; Muniappan 2011
2011	Taiwan	Asia	Introduced	Chen et al. 2011
2012	Assam, India	Asia	Introduced	Sarma 2013
2012	Rajasthan, India	Asia	Introduced	Mani et al. 2012
2014	Guangzhou, China	Asia	Introduced	This study
2014	Mauritius	Africa	Introduced	Germain et al. 2014
2014	Yunnan, China	Asia	Introduced	This study

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