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Research Article

Characterization and identification of *Acerophagus papayae* Noyes and Schauff (Hymenoptera: Encyrtidae), an introduced parasitoid of papaya mealybug, *Paracoccus marginatus* Williams and Granara de Willink through DNA barcode

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ABSTRACT: The papaya mealybug, *Paracoccus marginatus* Williams and Granara de Willink, is a serious invasive pest in India and causes severe yield loss. *Acerophagus papayae* Noyes and Schauff (Encyrtidae) is one of the efficient parasitoids for the suppression of papaya mealybug in its native range. This parasitoid was introduced from Puerto Rico in 2010 through USDA–APHIS for use against the papaya mealybug. Subsequently, natural occurrence of the parasitoid was observed in mealybug infested papaya fields at Pune and the parasitoid was identified as *A. papayae* based on morphology based taxonomy at NBAII. The study was undertaken for the DNA barcoding of *A. papayae*, using CO1 region in order to supplement and confirm that the introduced and Pune populations belonged to the same species and the study revealed that the *A. papayae* populations from Pune and USA are one and the same having fragment size of ~673bp.

KEY WORDS: Papaya mealybug, Paracoccus marginatus, Acerophagus papayae, cytochrome c oxidase-I (CO1), DNA barcode

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INTRODUCTION

India is the leader in papaya (*Carica papaya* L.) production contributing to approximately 50% of the world production of 6 million tonnes of fruits cultivated in 8000 ha in various states of the country (Shylesha *et al*., 2010). The invasive papaya mealybug (PMB), *Paracoccus marginatus* Williams and Granara de Willink has caused havoc in agricultural and horticultural crops ever since its first report from Coimbatore during 2008. The insect has assumed the status of a major pest and caused severe damage to economically important crops and huge losses in Tamil Nadu, Karnataka, certain parts of Andhra Pradesh, Kerala and Maharashtra. It is polyphagous infesting more than 60 species of economically important host plants including papaya, hibiscus, cotton, tomato, brinjal, tapioca, silk cotton, mulberry, jatropha, pigeon pea, teak, *Parthenium hysterophorus* and several other plants (Tanwar *et al.,* 2010). *Acerophagus papayae* Noyes and Schauff (Encyrtidae) (Plate 1) is one of the efficient parasitoids for the suppression of papaya mealybug in its native range (Muniappan, 2006; Amarasekare *et al*., 2009). It was imported from Puerto Rico in 2010 through the USDA–APHIS and cultures are maintained on *P. marginatus* infested papaya seedlings in the quarantine laboratory of NBAII, Bangalore. Subsequently, natural occurrence of the parasitoid was observed in significant numbers during surveys for natural enemies of papaya mealybug in papaya fields at Pune (Pokharkar *et al.,* 2010). These parasitoids were identified as *A. papayae* using morphology based taxonomy at NBAII. However, a study was undertaken for DNA barcoding of *A. papayae* in order to supplement and confirm that the introduced and Pune populations belonged to the same species.

The tool used for this study is a universally acceptable marker gene known as cytochrome c oxidase subunit I (CO1). This marker gene is used universally as its mutation rate is fast enough to distinguish between closely related species.

MATERIALS AND METHODS

The live adult parasitoid of *A. papayae* was freeze-killed at –80°C and transferred to an Eppendoff tube and homogenization was done by crushing the adult in 20µl of 5% Chelex 100 MB (BIO–RAD) (Walsh *et al*., 1991). This was followed by incubation for 3 h at 56°C and then

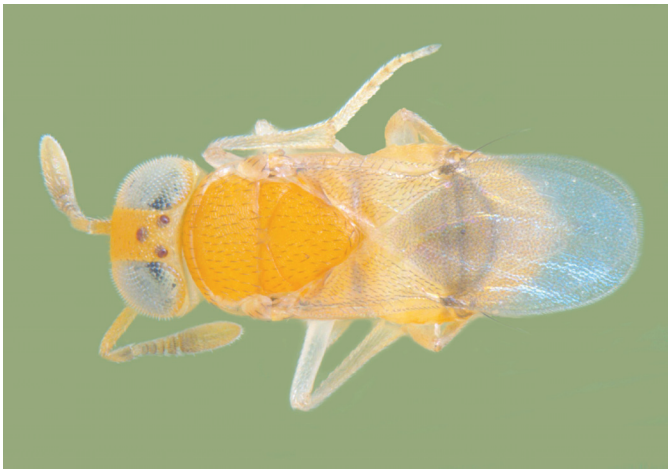

Plate 1. *Acerophagus papayae,* **an important parasitoid of papaya mealybug**

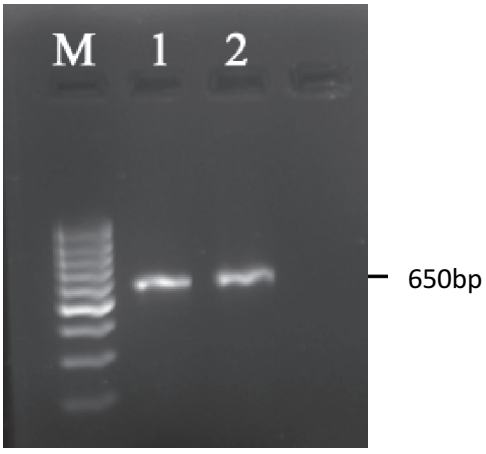

Fig. 1. PCR amplification of CO1 region of *A. papayae* (M: 50bp Ladder, Lane 1: *A. papayae* (673bp) USA, Lane 2: *A. papayae* Pune (~670bp)

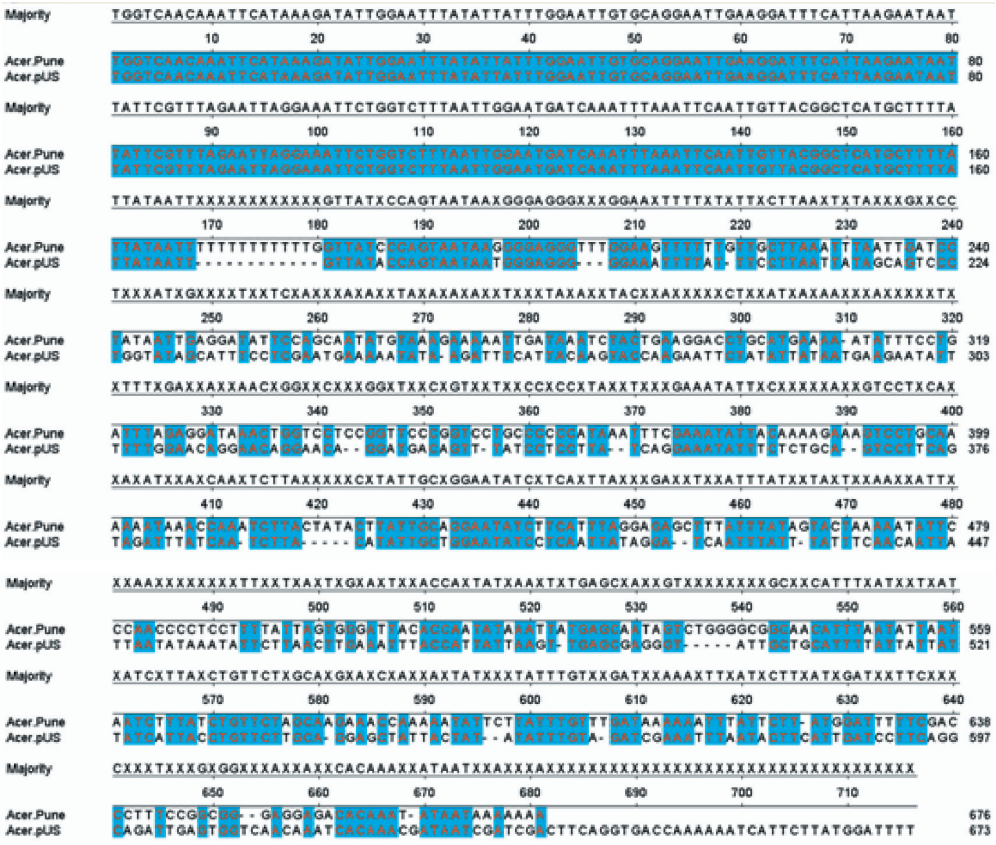

Fig. 2. Pair-wise alignment of *A. papayae* (US & Pune) using DNASTAR

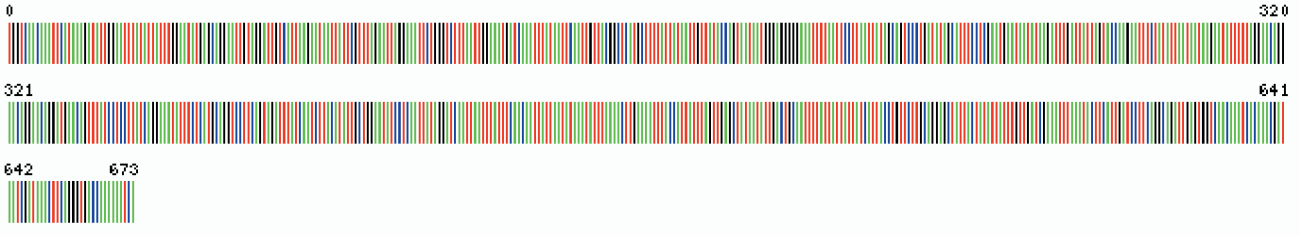

Fig. 3. DNA Barcode ID of *A. papayae* (USA): ACERO001-10

at 100ºC for 10 min. PCR was performed with a total reaction mixture of 50 µl consisting of 10x Taq buffer (complete), 10mM dNTP mix, universal primer HCO1–2198 (20pm/µl), LCO1–1490(20pm/ µl) (Folmer *et al*., 1994), template DNA(50ng), Taq polymerase(1U/ µl) and sterile water. The DNA extracted was amplified under the following conditions: Initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation (94° C for 1 min), annealing (54°C for 1 min), extension (72°C for 1 min) and a final extension step at 72°C for 10 min. The PCR amplification was performed in a thermal cycler (Bio-Rad).

RESULTS AND DISCUSSION

CO1 (674bp) region of *A. papayae* from USA and Pune were visualized on 1.8% gel (Fig. 1) with a low range ladder (Fermentas Mass Ruler 1000bp). The PCR products were purified with MinElute PCR purification kit (Qiagen). The PCR product was sequenced using an ABI prism 310 DNA sequencer by Big Dye Terminator reaction. The sequence was edited by BioEdit software and aligned using BLAST2 and verified. The DNA sequence of *A. papayae* (USA) was submitted to GenBank (Acc. no–HQ231257). The BLAST2 (www.ncbi.nlm.nih.gov) and BioEdit tools were used to find the similarity between the two populations for the conserved CO1 region.

The sequence length of 673 bp along with specimen information, taxonomical and collection details were submitted to BOLD, Canada (Barcode of life Data Systems) and DNA barcode was generated (www.boldsystems.org) (Fig. 2) with the published sequence in NCBI of *A. papayae* (USA) by BLAST analysis tool and the similarity was found to be 98%. The study revealed that the *A. papayae* populations from Pune and USA are one and the same. The barcode developed for *A. papayae* is a diagnostic tool for the identification of the parasitoid.The CO1 gene has proved to be suitable for species identification in a large range of animal taxa, including butterflies and moths (Hebert *et al*., 2004; Burns *et al*., 2008), spiders (Greenstone *et al*., 2005), mosquitoes (Kumar *et al*., 2007) and wasps (Smith *et al*., 2008). It appears that *A. papayae* might have got introduced fortuitously into India along with papaya mealybug from Sri Lanka.

REFERENCES

Amarasekare, K. G., Mannion, C. M. and Epsky, N. D. 2009. Efficiency and establishment of three introduced parasitoids of the mealybug *Paracoccus marginatus* (Hemiptera: Pseudococcidae). *Biological Control*, **51**: 91–95.

Avise, J.C. 2000. *Phylogeography. The history and formation of species.* Cambridge, MA; Harvard University Press.

Folmer, O., Black, M., Howh, W., Lutz, R. and Vrijenhoek, R. 1994. DNA primer for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, **3**: 294–299.

Greenstone, M. H., Rowley, D. L., Heimbach, U., Lundgren, J. G., Pfannenstiel, R. S. and Rehner, S. A. 2005. Barcoding generalist predators by polymerase chain reaction: Carabids and spiders. *Molecular Ecology,* **14**: 3247–3266.

Hebert, P. D. N., Penton, E. H., Burns, J. M., Janzen, D. H. and Hallwachs, W. 2004. Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly, *Astraptes fulgerator*. *Proceedings of the National Academy of Sciences, USA,* **101**: 14812–14817.

Kim, J.M., Yoon, J.H., Im, H.H., Jeong, U.H., Kim, I.M., Kim, R.S. and Kim, I. 2009. Mitochondrial sequence variation of the bumblebee, *Bombus ardens* (Hymenoptera: Apidae). *Journal of Asia-Pacific Entomology*, **12**: 133–139.

Kumar, N. P., Rajavel, A. R., Natarajan, R. and Jambulingam, P. 2007. DNA barcodes can distinguish species of Indian mosquitoes (Diptera: Culicidae). *Journal of Medical Entomology*, **44**: 1–7.

Miller, J. S., Brower, A. V. Z. and Desalle, R. 1997. Phylogeny of the neotropical moth tribe Josini (Notodontidae: Dioptinae): Comparing and combining evidence from DNA sequences and morphology. *Biological Journal of the Linnean Society*, **60**: 297–316.

Muniappan, R., Meyerdirk, D. E., Sengebau, F. M., Berringer, D. D. and Reddy, G. V. P. 2006. Classical biological control of papaya mealybug (Hemiptera: Pseudococcidae) in the Republic of Palau. *Florida Entomologist*, **89**: 212–217.

Pokharkar, D. S., Nakat, R. V., Tamboli, N. D., Dhane, A. S., Jadhav, S. S., Chandele, A. G. and Rabindra, R. J. 2010. A new record of *Acerophagus papayae* Noyes and Schauff on papaya mealybug, *Paracoccus marginatus* Williams and Granara de Willink, in Pune region of Maharashtra state. *Journal of Biological Control*, **24**: 291.

Ratnasingham, S. and Hebert, P. D. N. 2007. BOLD: The Barcode of Life Data System (www. Barcodinglife.org). *Molecular Ecology Notes*, **7**: 355–364.

Shylesha, A. N., Joshi, S., Rabindra, R. J. and Bhumannavar, B. S. 2010. Classical biological control of the papaya mealybug. Newsletter, NBAII, Bangalore, India.

Smith, M. A., Rodriguez, J., Whitefield, J., Deans, A., Janzen, D. H., Hallwachs, W. and Hebert, P. D. N. 2008. Extraordinary diversity of parasitoid wasps exposed by iterative integration of natural history, DNA barcoding, morphology and collections. *Proceedings of the National Academy of Sciences USA*, **105**: 12359–12364.

Tanwar, R. K., Jeyakumar, P. and Vennila, S. 2010. *Papaya mealybug and its management strategies*. Technical Bulletin 22, NCIPM, New Delhi, India, 22 pp.

Walsh, P. S., Metzger, D. A. and Higuchi, R.1991. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *Biotechniques*, **10**: 506–513.