



Research Article

Record of coccinellid predator, *Hyperaspis maindroni* Sicard (Coleoptera: Coccinellidae) on invasive cassava mealybug, *Phenacoccus manihoti* Matile-Ferrero (Hemiptera: Pseudococcidae)

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ABSTRACT: The cassava mealybug, *Phenacoccus manihoti* Matile-Ferrero is one of the most serious pests of cassava worldwide, which got recently introduced into India (traced in Kerala) as a potential invasive pest. Studies were carried out to record the incidence of mealybug, *P. manihoti* and its natural enemies on different varieties of cassava in Namakkal dt. of Tamil Nadu during June 2020. The observations were taken in the field *in situ* as well in the collected mealybug infested twigs in the laboratory. Results revealed that all the three surveyed cassava varieties *viz.*, Mulluvadi, Thailand white and Shree Atulya were found severely infested with cassava mealybug. A huge number of coccinellid beetles were collected from the *P. manihoti* colonies, which were identified as *Hyperaspis maindroni* Sicard, 1929 (Coccinellidae: Coccinellinae: Hyperaspidini). Molecular characterization of both grub and adult beetle of *H. maindroni* was carried out to confirm its identity.

KEY WORDS: Cassava mealybug, Coccinellidae, *Hyperaspis maindroni*, *Phenacoccus manihoti*, predator

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INTRODUCTION

Cassava mealybug, *Phenacoccus manihoti* Matile-Ferrero (Hemiptera: Pseudococcidae) is known to infest mainly cassava and its wild relative, *Manihot glaziovii* Mull. Arg. The crawlers and the adult mealybugs colonise on the leaves and suck sap causing distortion of the leaves. In severe cases of infestation, the plants show wilting symptoms and exhibit die-back. The mealybug infestation also leads to the development of sooty mould that affects photosynthesis and thereby affecting the yield.

A number of ladybird beetles belonging to Coccinellidae of Coleoptera function as important biocontrol agents of several sucking pests especially mealybugs. Around 90 % of coccinellid species are predacious in nature serving as biocontrol agents (Hodek, 2012). Of several genera of predatory ladybird beetles, *Hyperaspis* is promising in predated on a wide range of sucking insects. The genus *Hyperaspis* Chevrolat, 1837 comprises of nearly 400 species worldwide (Kovář 2007; Bogaert *et al.*, 2012) that are mainly predators on scale insects, mealybugs and aphids

belonging to Hemiptera (Gordon and Canepari, 2008). Several species of *Hyperaspis* have been successfully introduced into newer areas to attain biological control.

On cassava mealybug, five *Hyperaspis* spp. were found to predate besides *Cyra* spp. in Africa. The *Hyperaspis* spp. got introduced in Africa from South America to control cassava mealybug (Neuenschwander 2001), of which *H. notata* Mulsant got established in Zaire, Burundi and Mozambique and recovered too from the fields. Similarly, *Hyperaspis senegalensis hottentotta* Mulsant was found to be the most endemic species on cassava mealybug in the Congo, Central Africa on cassava (Fabres and Kiyindou, 1985).

In South Asia, *Hyperaspis maindroni* Sicard, 1929 is known to occur widely and is an important species of Hyperaspines. The species predated on wide range of mealybug species *viz.*, *Maconellicoccus hirsutus* (Green) *Phenacoccus solenopsis* Tinsley and *Saccharicoccus sacchari* (Cockerell) (CABI, 2017). *Hyperaspis maindroni*

got established in India from New Guinea to bring control of hibiscus mealybug (Rao *et al.*, 1971) and found to be widely distributed in South India (Poorani, 2002).

The recent report on the inadvertent entry of cassava mealybug, *P. manihoti* into India during April 2020 (Joshi *et al.*, 2020) has led to a search for its further spread. So, surveys were carried out in neighboring state of Tamil Nadu to record the occurrence of this mealybug on cassava crop and its natural enemies especially coccinellid predator, *Hyperaspis* sp.

MATERIALS AND METHODS

The surveys were carried out for cassava mealybug infestation and occurrence of *Hyperaspis* sp. on cassava plants in Tamil Nadu. The cassava plants belonging to three varieties *viz.*, Mulluvadi, Thailand White and Shree Atulya were observed for mealybug infestation and the associated coccinellid predator (Table 1) in the Namakkal district of Tamil Nadu during June 2020. Data on the incidence of mealybug infestation and the number of coccinellid predator were recorded in the field *in situ*. Later, the collections of cassava mealybug colonies along with the infested twigs were brought to the laboratory and observed for the occurrence of *H. maindroni* grubs carefully, which camouflages with the mealybug. The number of grubs and adults of *Hyperaspis* sp. from each infested twig (of 30 cm length approximately) were recorded. Likewise 30 infested twigs were observed for the presence of *Hyperaspis* sp. grubs and adults.

The mealybugs and coccinellid predator were then separated from the cassava twigs and identified. The detailed examination of both grubs and adults of *Hyperaspis* sp. was carried out and the morphological characters were observed through Stereozoom microscope, Leica M60. Morphometric studies of the coccinellid adults were taken *viz.*, Total length (TL) - from the apex of the clypeus to apical margin of elytra; Total width (TW)-at the widest part of both elytra; Pronotal Length (PL)- from the middle of anterior margin to the pronotal base; Pronotal Width (PW)- at the widest part of the pronotum; Elytral Length (EL)- along the suture from apex to base.

Images of the adult and grub specimens were taken using Leica M 205A having automontage software. Photographs were edited and plates were prepared using Adobe Photoshop 7.0. The identified specimens are deposited in the National Insect Museum, ICAR-National Bureau of Agricultural Insect Resources, Bengaluru, Karnataka, India.

Molecular characterization

The molecular characterization of both grub and adult of *Hyperaspis* sp. was carried out through the mitochondrial Cox 1 gene sequencing.

Insect sample collection and preservation

The adult beetles and grubs of *H. maindroni* collected from Namakkal district of Tamil Nadu were identified taxonomically and used for molecular characterization. Five beetles and grubs were transferred to 100% ethanol for further processing of DNA extraction and the remaining specimens were kept as voucher specimens as dry collections and alcohol preserved specimens and stored at 20°C (Robert, 2002), at the Indian Council of Agricultural Research-National Bureau of Agricultural Insect Resources (ICAR-NBAIR) Bengaluru, India.

DNA extraction

DNA was extracted from the adult and grub of *H. maindroni* using QiagenDNeasy® kit, following the manufacturer's protocols.

PCR amplification of mitochondrial cytochrome oxidase subunit 1 gene

The extracts were subjected to PCR amplification of a 658 bp region near the 5' terminus of the *cox1* gene following standard protocol (Hebert *et al.*, 2003). Primers used were: forward primer (LCO 1490: 5'-GGTCAACAAATCATAAAGATATTGG-3'), and reverse primer (HCO 2198: 5'-TAAACTTCAGGGTGACCAAAAAATCA-3'). PCR reactions were carried out in 96-well plates, 50 µL reaction volume containing: 5 µL GeNeiTMTaq buffer, 3 µL GeNeiTM 10mM dNTP mix, 1 µL (20 pmol/µL) forward primer, 1 µL (20 pmol/µL) reverse primer, 1 µL GeNeiTMTaq DNA polymerase (1 U/µL), 8 µL DNA (50 ng/µL), and 31 µL sterile water. Thermo cycling consisted of an initial denaturation of 94°C for 5 min, followed by 34 cycles of denaturation at 94°C for 1 min, annealing at 45°C for 30 Sec and extension at 72°C for 1 min. PCR was performed using a BioRad C1000™ Thermal Cycler. The amplified products were analyzed on a 1.5% agarose gel electrophoresis as described by Sambrook and Russell (2001). The amplified products were sequenced by M/s Barcode Bioscience, Bengaluru. The COI generated sequences have been deposited in NCBI Genbank database.

RESULTS AND DISCUSSION

All three varieties of cassava *viz.*, Mulluvadi, Thailand white and Shree Atulya were found to be severely infested with cassava mealybug in Namakkal dt. of Tamil Nadu. Surveys revealed heavy infestation of mealybugs on cassava plants and associated population of coccinellid predator adults and grubs feeding on the mealybugs (Table 1). The collected mealybug was identified and confirmed as *P. manihoti* and the coccinellid beetles as *H. maindroni*.

Abundance of *H. maindroni* in cassava mealybug colonies

Around 20-25 cassava mealybug infested twigs from the infested cassava fields were assessed for the cassava mealybug population density (number of adults and active crawlers) and its coccinellid predator, *H. maindroni*. Around 14 to 35 adult beetles of *H. maindroni* were found per infested twig in all the surveyed areas of Namagiripettai and Sendamangalam blocks of Namakkal district of Tamil Nadu. The cassava fields in Pottanam region of Sendamangalam block exhibited highest *H. maindroni* population (34.7±3.5) (Table 1).

The lab studies also revealed the occurrence of *H. maindroni* grubs and adults in the cassava mealybug colonies. The adults of *H. maindroni* ranged from 4 to 24 per mealybug infested twig while grubs ranged from 11 to 39 grubs per infested twig (Table 2).

Morphological characters

The adult beetle of *H. maindroni* is oval bodied, head and pronotum yellow, elytra pale yellow with three longitudinal black stripes: two black stripes in a mid-dorsal position on each elytron, not reaching both the basal or apical margins and a sutural stripe reaching apex (Fig. 1A). All legs yellow and ventral body yellow to orange coloured (Fig. 1B). The morphometrics of the adult beetles of *H. maindroni* presented in Table 3. The total length (TL) ranged from 2.8 to 3.1 mm with a mean of 2.95, total width (TW) ranged from 2.12 to 2.16 mm with a mean of 2.14 mm, pronotal length (PL) ranged from 0.68 to 0.75 mm with a mean of 0.71 mm, pronotal width (PW) ranged from 1.68 to 1.72 mm with a mean of 1.69 mm, elytral length (EL) ranged from 2.09 to 2.15 mm with a mean of 2.11 mm (Table 3).

The grub of *H. maindroni* is stout, light yellowish brown, segmented body (Fig. 1C, D) absolutely camouflaging with the mealybug with its mealy coat on dorsum.

Table 1. Population density of cassava mealybug, *P. manihoti* and its coccinellid predator, *H. maindroni* in the field

Sample No	Details of collection site	Mealybug/twig Mean±SE	<i>Hyperaspis maindroni</i> /twig (Mean±SE) (n=30)
1.	Pachudayampalayam, Namagiripettai Block, Namakkal Dt., Tamil Nadu	373.5±7.2	33.6±2.1
2.	Perumagoundampalayam, Namagiripettai Block, Namakkal Dt., Tamil Nadu	459.8±13.5	18.6±1.3
3.	Oduvankurichi, Namagiripettai Block, Namakkal Dt., Tamil Nadu	389.6±3.3	21.6±0.9
4.	Thoppapatti, Namagiripettai Block, Namakkal Dt., Tamil Nadu	223.3±9.6	13.4±1.4
5.	T. Jedarpalayam, Namagiripettai Block, Namakkal Dt., Tamil Nadu	259±14.5	26.9±2.3
6.	Kalkurichi, Sendamangalam Block, Namakkal Dt., Tamil Nadu	412.8±11.3	17.5±1.0
7.	Pottanam, Sendamangalam Block, Namakkal Dt., Tamil Nadu	601±23.8	34.7±3.5
8.	Belukurichi, Sendamangalam Block, Namakkal Dt., Tamil Nadu	442±17.4	29.4±2.9

Table 2. Occurrence of *H. maindroni* in the cassava mealybug colonies collected from Namakkal district of Tamil Nadu (in the laboratory)

S. No.	Mealybug infestation	No. of samples examined	No. of <i>H. maindroni</i> beetles	No. of <i>H. maindroni</i> grubs
1.	I	30	4	11
2.	II	30	7	16
3.	III	30	12	17
4.	III	30	24	39
5.	III	30	16	22
6.	III	30	18	31
7.	II	30	8	14

Grade I – Sparse incidence on cassava twig (of 30 cm length); Grade II – Medium incidence on the twig; Grade III – Severe incidence on the twig

Molecular characterization

The mitochondrial gene sequencing of adult and grubs confirmed the identity of coccinellid predator, *H. maindroni* and the accession numbers obtained (MT679210 for grub and MT679211 for adult) were submitted to NCBI database and BOLD. The barcodes were generated for the same.

In natural unsprayed cassava fields, *H. maindroni* was found associated with the mealybug, *P. manihoti* colonies effecting the biological control. The field surveys revealed that the species *H. maindroni* was the abundant coccinellid species found in the mealybug colonies. It is reported to be distributed widely in India and Pakistan (Poorani, 2002). Sankar *et al.* (2011) recorded the natural incidence of *H. maindroni* on solenopsis mealybug, *P. solenopsis* in Permabulur district of Tamil Nadu but was recorded low as compared to the other coccinellid predator, *Scymnus*

nubilus (Mulsant). In the present study, the cassava mealybug samples collected from Namakkal district of Tamil Nadu showed higher percent incidence of *H. maindroni*. Mukherjee and Suman (2017) reported the occurrence of *H. maindroni* (1.25%) in Bhubhaneswar on cowpea and ridge gourd while Poorani and Lalitha (2018) reported its occurrence on *M.hirsutus* in mulberry.

CONCLUSION

High incidence of cassava mealy bug, *P. manihoti* was observed on three varieties, Mulluvadi, Thailand white and Shree Atulya of cassava in the Namakkal district of Tamil Nadu. Occurrence of high number of coccinellid predator, *H. maindroni* in *P. manihoti* colonies was recorded, which can naturally bring down the pest population. The natural occurrence of this predator in mealybug colonies implies the need for conservation of the same, the important component

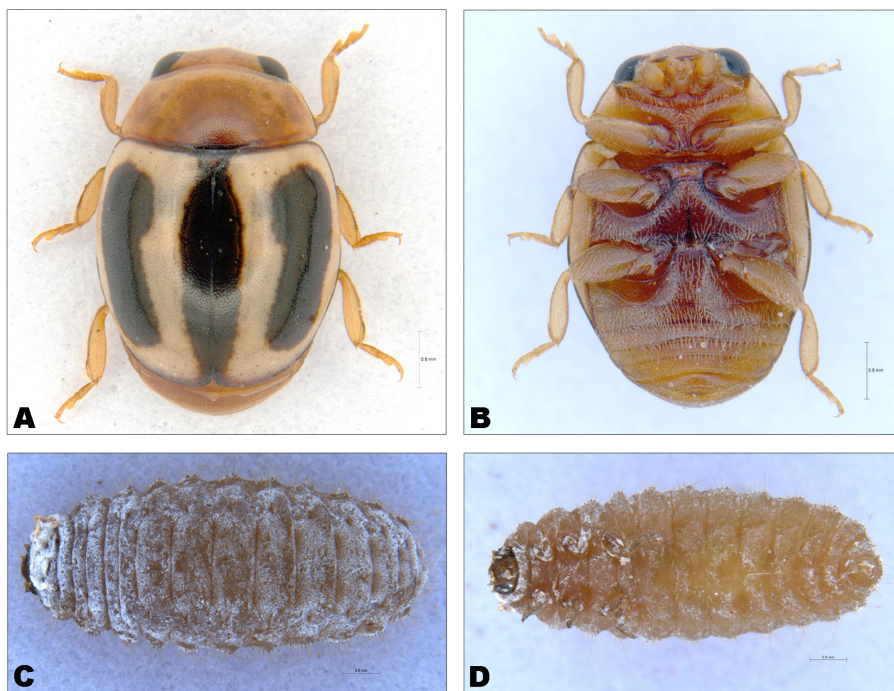


Figure 1. *Hyperaspis maindroni* A. Adult dorsal view B. ventral view C. Grub dorsal view D. ventral view

Table 3. Measurements of adult beetle of *H. maindroni*

S. No.	Parameter	Mean ± SE
1.	Total length (TL)	2.95 ± 0.06
2.	Total width (TW)	2.14 ± 0.01
3.	Pronotal length (PL)	0.71 ± 0.01
4.	Pronotal width (PW)	1.69 ± 0.02
5.	Elytral length (EL)	2.11 ± 0.01

of the biological control. The farmers should look for this natural predator, *H. maindroni* carefully while taking up the management practices for cassava mealybug as *H. maindroni* grubs camouflages with the mealybug and becomes difficult to differentiate or identify. The farmers need to be educated to observe for the presence of this coccinellid predator and accordingly plan management strategies.

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