

## Screening alcohol-preserved samples for cassava mealybug, *Phenacoccus manihoti* Matile-Ferrero (Hemiptera: Pseudococcidae), a recent invasive introduction to India

J. Poorani<sup>1,\*</sup>, Sunil Joshi<sup>2</sup>, R. Thanigairaj<sup>1</sup> and J. Diraviam<sup>3</sup>

<sup>1</sup>ICAR-National Research Centre for Banana, Thogamalai Road, Thayanur Post, Tiruchirappalli 620 102, India

<sup>2</sup>ICAR-National Bureau of Agricultural Insect Resources, PB No 2491, HA Farm Post, Bellary Road, Hebbal, Bengaluru 560 024, India

<sup>3</sup>Krishni Vigyan Kendra, Pulutheri, R.T. Malai Post, Kulithalai, Karur district 621 313, India

***Phenacoccus manihoti* Matile-Ferrero (Hemiptera: Coccothraupidae: Pseudococcidae), one of the most serious pests of cassava worldwide, was recently introduced to India. Surveillance, early detection and mapping of its distribution in cassava-growing areas are essential to prevent it from spreading further. Several species of mealybug are known to infest cassava in India; their authoritative identification normally requires preparation of slide mounts, which is a highly skilled and time-consuming process. A simple method is described here for screening alcohol-preserved samples of mixed-species infestations for cassava mealybug.**

**Keywords:** Alcohol-preserved samples, cassava mealybug, invasive pests, Sternorrhyncha.

MEALYBUGS (Hemiptera; Coccothraupidae: Pseudococcidae) are among the most destructive crop pests, sucking sap from all parts of the host plant<sup>1</sup>. In the last two decades, many exotic mealybug species such as *Paracoccus marginatus* Williams & Granara de Willink, *Phenacoccus solenopsis* Tinsley, *Phenacoccus madeirensis* Green and *Pseudococcus jackbeardsleyi* Gimpel & Miller have been recorded from India for the first time. All have established and cause significant economic damage to several agricultural and horticultural crops.

The cassava mealybug, *Phenacoccus manihoti* Matile-Ferrero, is the most recent invasive mealybug to be reported from India<sup>2</sup>. It is established in parts of Kerala and Tamil Nadu; some cassava-growing regions of Tamil Nadu witnessed serious outbreaks in 2020–21 (ref. 3). Several mealybug species occur on cassava and mixed infestations of multiple species are common. Joshi *et al.*<sup>2</sup> listed the other species on cassava in different parts of India as *Ferrisia virgata* (Cockerell), *Ps. jackbeardsleyi*, *Pa. marginatus* and *Ph. madeirensis*, and reported mixed infestations of *Ps. jackbeardsleyi* and *Phenacoccus manihoti* in Kerala. Parsa *et al.*<sup>4</sup> included *Ph. solenopsis* in

their key to mealybugs recorded on *Manihot* spp. worldwide. The species is also found on cassava in India (unpublished data).

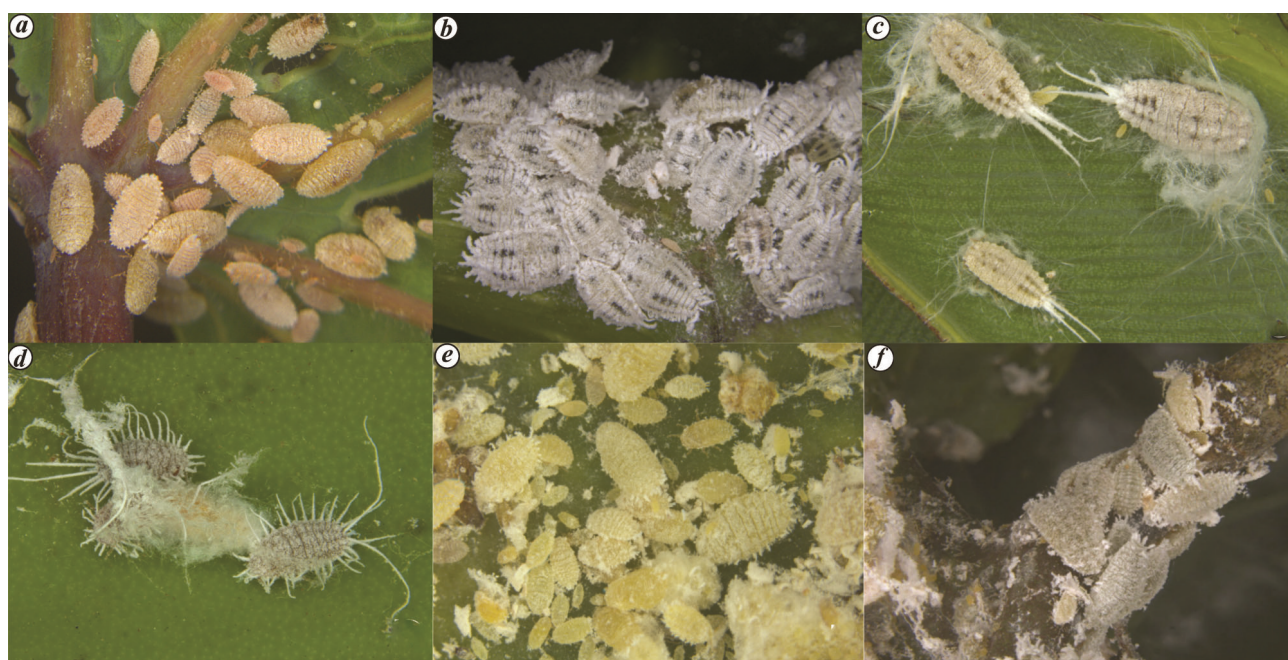
For effective management of mealybugs on cassava, it is important to ascertain whether the damage is due to a single or multiple mealybug species. If multiple species are involved, the extent of damage and the relative proportions of their populations must be assessed in order to determine the most appropriate management strategies. In India, effective biological control agents are only available for use against papaya mealybug. Cassava mealybug has no major natural enemies in India at present and efforts are in progress to introduce an exotic parasitoid, *Anagrus lopezi* (De Santis) (Hymenoptera: Encyrtidae) for its management<sup>3</sup>.

In August 2021, samples of mealybug-infested cassava from Karur district, Tamil Nadu were submitted for identification. Mixed colonies of two species were found, *Pa. marginatus* and *Ph. manihoti*, with the latter being predominant. In life, it is not easy to differentiate these two species by their body colour because although the early instars of the cassava mealybug are predominantly pink to rose pink, the adult female is pale yellowish (Figure 1 a) and the papaya mealybug is also yellowish (Figure 1 e). Besides, it is difficult to recognize different mealybug species on severely infested plant parts, which often have a tangled mass of mealybugs and their wax secretions. While assessing the relative proportions of mealybug species in the samples, we found a simple way to distinguish immature and adult female *Ph. manihoti* from *Pa. marginatus* and other mealybugs known to infest cassava in India.

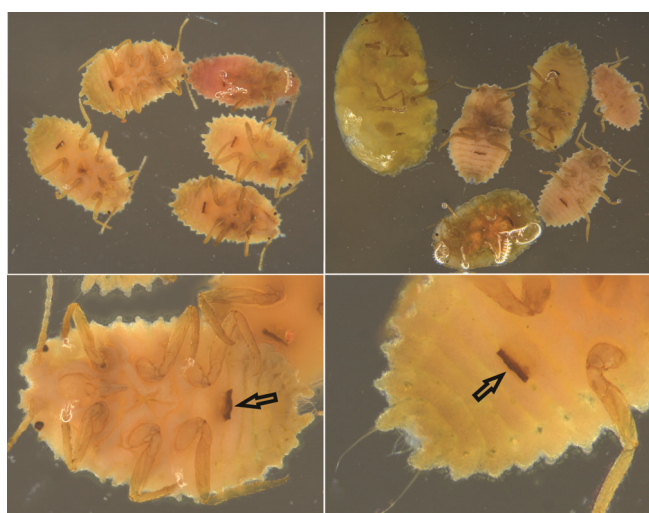
Cassava twigs infested by mixed colonies of *Ph. manihoti* and *Pa. marginatus* were collected from the fields in Kulithalai and nearby areas in Tamil Nadu. Samples of other mealybugs known to feed on cassava in India (*Ferrisia virgata*, *Ps. jackbeardsleyi*, *Ph. madeirensis* and *Ph. solenopsis*) were collected from other host plants. The morphological terminology used in this study follows Williams<sup>1</sup>. Images of mealybugs were taken using a Leica M205A stereo microscope fitted with a DMC 4500 digital camera and composite images were generated using CombineZP<sup>®</sup>. Slide-mounted specimens were examined under a Leica DM500 microscope and photomicrographs were captured with a Leica EC4 digital camera attachment. Permanent slide mounts and alcohol-preserved specimens of the mealybugs studied have been deposited in the collections at ICAR-National Research Centre for Banana, Tiruchirappalli, and the ICAR-National Bureau of Agricultural Insect Resources, Bengaluru.

Images of live mealybugs known to infest cassava in India [*Ph. manihoti* (Figure 1 a), *Ph. solenopsis* (Figure 1 b), *Pa. marginatus* (Figure 1 e), *Ph. madeirensis* (Figure 1 f), *F. virgata* (Figure 1 c), and *Ps. jackbeardsleyi* (Figure 1 d)] are provided for reference. Of these, only two species, *Ph. manihoti* and *Pa. marginatus*, were found on the cassava twigs examined in this study.

\*For correspondence. (e-mail: pooranij@outlook.com)

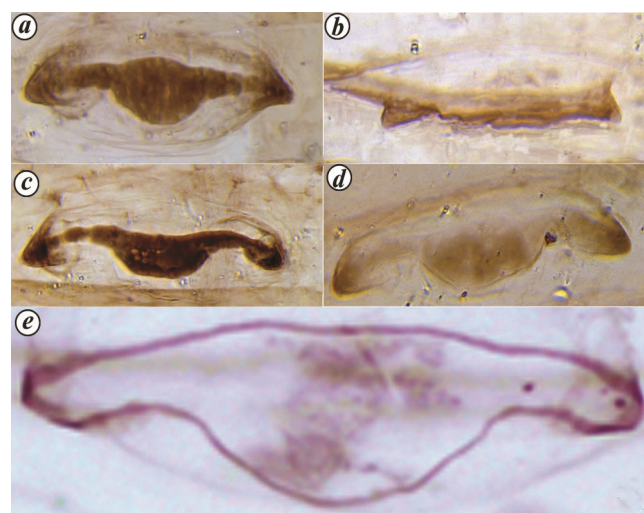


**Figure 1.** Mealybugs recorded as cassava pests from India: *a*, *Phenacoccus manihoti*; *b*, *Phenacoccus solenopsis*; *c*, *Ferrisia virgata*; *d*, *Pseudococcus jackbeardsleyi*; *e*, *Paracoccus marginatus*; *f*, *Phenacoccus madeirensis*.



**Figure 2.** Cassava mealybug specimens with circuli clearly visible in glycerine (circulus indicated by an arrow).

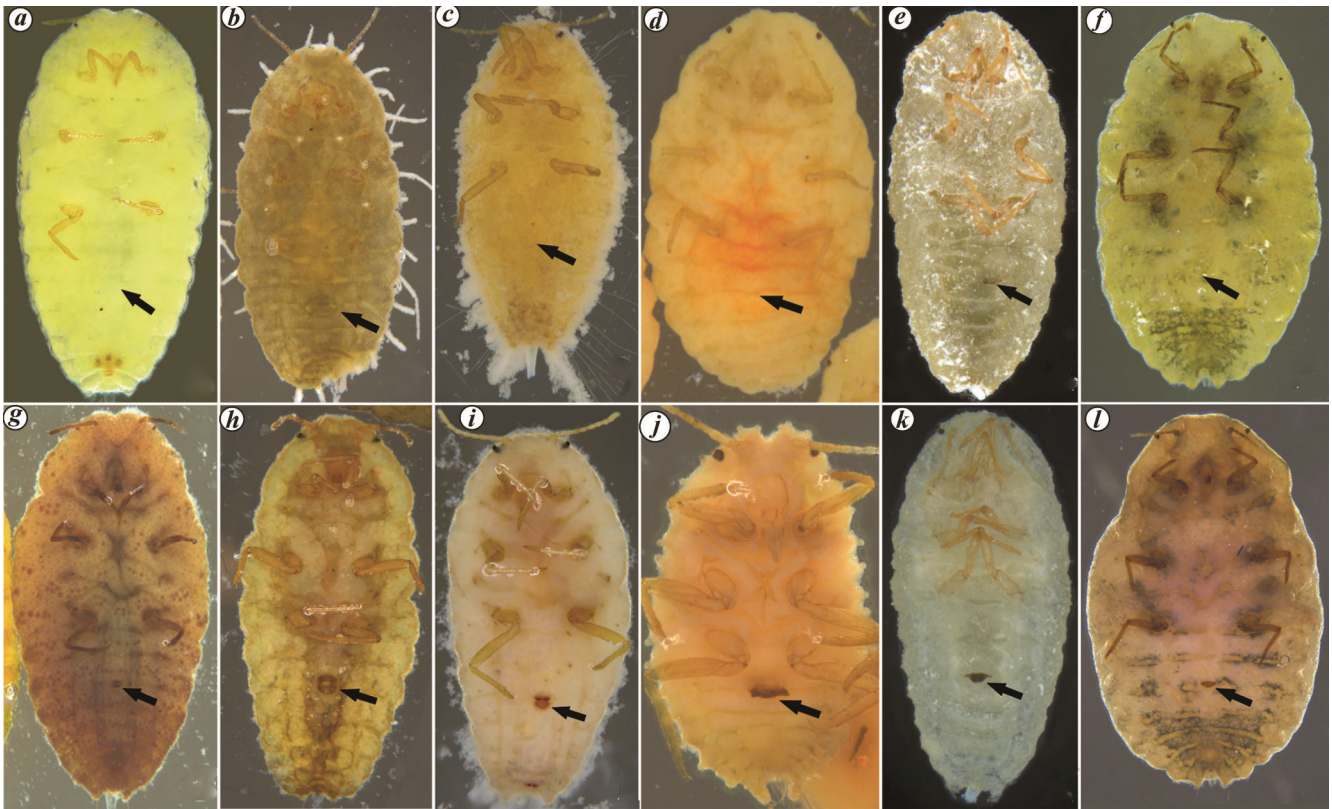
In all immature and adult female *Ph. manihoti* placed in 70% ethanol, the circulus on the ventral surface between abdominal segments III and IV gradually became visible to the naked eye after 30 min and was obvious after 2 h of preservation (Figure 2). In the adult female the circulus is well developed, being narrow, transversely elongate and laterally tapered and curved, giving it the characteristic shape of a double yoke (clearly seen in slide-mounted specimens (Figure 3 *a-d*)). In *P. marginatus*, specimens placed in alcohol remained bright yellow for many hours before turning dark brown to black after



**Figure 3.** Circuli as seen in permanent slide mounts: (*a-d*) in *Ph. manihoti*; (*e*) in *Ph. madeirensis*.

one day (Figure 4 *a*). In darker specimens, the circulus could be seen (Figure 4 *g*). In *Ps. jackbeardsleyi*, *F. virgata*, *Ph. madeirensis* and *Ph. solenopsis*, the circulus also became clearly visible after 2–3 h of preservation in ethanol. In *Ps. jackbeardsleyi* the circulus is large, with two lip-like halves located on either side of the intersegmental crease (Figure 4 *b* and *h*). In *F. virgata*, it is shaped like a small pot (Figure 4 *c* and *i*).

*Ph. madeirensis* has a circulus similar in shape to that in *Ph. manihoti* (Figures 3 *e* and 4 *e* and *k*). However, in *Ph. madeirensis*, the circulus is clearly visible and its



**Figure 4.** Circuli in mealybug species found on cassava in India: (a–d, f) freshly killed in alcohol, (g–l) after 3 h in alcohol, and (e) after prolonged storage in alcohol. Each circulus is indicated by an arrow. (a and g) *Pa. marginatus*; (b and h) *Ps. jackbeardsleyi*; (c and i) *F. virgata*; (d and j) *Ph. manihoti*; (e and k) *Ph. madeirensis*; (f and l) *Ph. solenopsis*.

shape is well defined only in the immature instars (Figure 4 k). In mature females it loses its dark brown colour and the shape is distorted (Figure 4 e). In addition, when seen in profile the circulus in *Ph. madeirensis* is only slightly elevated from the derm, whereas in *Ph. manihoti* it is much more protruberant.

In *Ph. solenopsis* in alcohol, the circulus is transverse with a median bulge (Figure 4 l). Hodgson *et al.*<sup>5</sup> reported that the circulus in *Ph. solenopsis* is dark in live specimens, but in the present study shape is not clear in freshly killed specimens (Figure 4 f). In permanent slide mounts of adult female *Ph. manihoti* the circulus becomes much more transparent (Figure 3 a–d) but the typical ox-yoke shape is retained (sometimes referred to as mushroom-shaped or ‘transversely oval with narrow lateral extensions’<sup>1</sup>). In the earlier instars it is transverse-elongate with lateral extensions (Figure 3 b; also illustrated by Matile-Ferrero<sup>6</sup> and Joshi *et al.*<sup>2</sup>). To the best of our knowledge, the circulus has been mentioned previously in the literature only as a diagnostic character for identifying slide-mounted specimens.

Our observations indicate that in large alcohol-preserved samples of cassava bearing infestations of mixed mealybug species, the appearance of the circulus can be used to recognize *Phenacoccus* specimens, and probably other mealybug species as well. In alcohol the circulus could be

clearly seen because it becomes dark brown; the characteristic yoke shape in *Ph. manihoti* is evident and, when seen in lateral view, it clearly protrudes from the surface of the derm. In the second- and third-instar nymphs also, the circulus turns dark brown and hence is useful for separating the species. When we substituted isopropyl alcohol, which is often used as a cheaper alternative for ethanol, we obtained similar results.

Matile-Ferrero<sup>6</sup> mentioned that the circulus of *Ph. manihoti* was well developed in the first instar and illustrated the same. Williams<sup>1</sup> in his diagnosis of the genus *Phenacoccus* also mentioned that the circulus in some species has lateral extensions and is elevated from the surface of the derm. He covered 14 species of *Phenacoccus*, among which only *Ph. madeirensis* and *Ph. takagii* Williams (described from Haryana, North India) had a circulus similar in shape to that of *Ph. manihoti*. *Ph. madeirensis* is greenish-grey in contrast to the pink/yellow *Ph. manihoti*. In the other species of *Phenacoccus* known from this region, the circulus is either absent or differently shaped from that in *Ph. manihoti*<sup>1</sup>.

*Ph. solenopsis*, introduced in India in 2005, was not included by Williams<sup>1</sup>. It has a characteristic appearance in life, being white with dark dorsomedial spots on the submedian intersegmental areas of the thorax and abdomen. In alcohol-preserved material also, dark dorsomedial and

lateral markings can be seen clearly. Hodgson *et al.*<sup>5</sup> mentioned that the circulus in *Ph. solenopsis* is usually large, flaccid and of variable shape in slide-mounted material.

The significance of the circulus as a diagnostic character in Pseudococcidae appears to be poorly explored, possibly because it is a soft, membranous structure that can be distorted by slide preparation. In stained permanent mounts of mealybugs, when a single circulus is present it can be seen as a faint, weakly sclerotized ring, usually lying across the ventral intersegmental fold between abdominal segments III and IV, with the enclosed area being free of pores and setae<sup>1</sup>. A few species have more than one circulus, whereas in other species in the same genus it may be absent. When present it occurs in all the developmental stages of the female. In living specimens, the circulus can appear as a rather prominent tumid area without wax, but it is often retracted and invisible<sup>7</sup>. Ferris and Murdock<sup>8</sup> suggested that the structure is secretory, with the products discharged internally. It is known to be an adhesive organ, used to enable movement over or attachment to smooth surfaces<sup>9</sup>. Lloyd and Martini<sup>7</sup> studied six mealybug species and concluded that 'the circulus when present may function as a rudimentary adhesive organ in most Pseudococcidae but this is not necessarily its sole function'. They suggested that it might be used for adhesion to the substrate only during the latter half of development of the mealybug. Our observations indicate that in alcohol-preserved cassava mealybugs, the circulus is clearly visible from the second instar onwards.

Slide mounting of mealybugs is needed to identify the species authoritatively, but this is a laborious and time-consuming process even for experts. Examination of mealybug circuli under a dissection microscope could be used by economic entomologists in India as a rapid, simple laboratory method for screening a large number of mealybug-infested cassava samples preserved in alcohol to detect those that contain *Ph. manihoti*. However, it is empha-

sized that confirmation of species identity by an expert taxonomist should be done before embarking on large-scale, area-wide biological control initiatives. Mealybug taxonomists usually provide the details of colour change of mealybug species in alcohol and it may be useful to add details on the shape of the circulus as well.

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