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Taxonomy of Cassava (*Manihot esculenta* Crantz) Mealybugs (Hemiptera: Pseudococcidae) - 1: Identification and Local Distribution of the Predominant Species on Witches' Broom-Diseased Plants in the Philippines

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Samples of cassava (*Manihot esculenta* Crantz) plants infected with witches' broom disease were gathered from 13 provinces in the Philippines. Mealybugs were observed to occur in nine out of the 13 cumulative samples. The most predominant among several mealybug species observed was identified as *Pseudococcus jackbeardsleyi* Gimpel and Miller. *P. jackbeardsleyi* is not yet known to transmit phytoplasma, especially the one causing cassava witches' broom (CWB). However, its occurrence in almost 70% of the CWB-infected samples provides circumstantial evidence that it may be related to the spread of the pathogen. This formal report of identification is in support of the studies on the possible role of mealybug species on CWB phytoplasma transmission.

Keywords: cassava phytoplasma disease, cassava witches' broom, phytoplasma, *Pseudococcus jackbeardsleyi*, transmission, vector

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is an important root crop, being a source of human food and animal feed and raw material for ethanol production in the Philippines, as in many parts of the world. Several species of scale insects, including mealybugs, are among the known insect pests that attack cassava plants at different stages. For scale insects, Lit et al. (1990) reported as new faunal and host records two species for cassava from Leyte and enumerated three other species that occur on crop in other areas in the Philippines. More recently, however, the cassava witches' broom (CWB) also known as Cassava Phytoplasma Disease (CPD) (Fig. 1), was observed to be a greater production constraint in major cassava-growing areas in the country. The pathogen is known to be transmitted by insects and the possible vector(s) in the Philippines have not yet been identified.

CWB is caused by phytoplasma, pleomorphic, wall-less, obligate, phloem-limited phytopathogenic bacteria, and spread by insects that feed on the phloem of an infected plant (Bertacinni and Duduk 2009). Dolores, Langres, Pinili et al. (2023) reported the incidence, distribution, and genetic diversity of a '*Candidatus Phytoplasma luffae*'-related strain (16SrVIII) associated with the CWB disease in the Philippines. A related strain belonging to the same species has also been recently reported to cause the Bamboo Witches' Broom (Dolores, Langres, Cortaga et al. 2023). Phytoplasma transmission, in general, is reported to be in a persistent propagative manner by more than 90 phloem-feeding insect species belonging to the order Hemiptera (Bosco and Tedeschi 2013). The reported vectors so far belong to Cicadellidae (leafhoppers), Fulgoromorpha (planthoppers), and Psyllidae (psyllids) (Weintraub and Beanland 2006; Weintraub and Gross 2013). The nature and epidemiology of phytoplasma diseases in plants, particularly in cassava, will be understood if vector-phytoplasma interactions are known (Weintraub and Beanland 2006).

As an initial step, a nationwide survey of insects on cassava plants that show symptoms of CWB was conducted. This paper specifically reports the identification of the mealybugs that were infesting CWB-infected plants. This hopes to provide initial clues to possible vectors and push for studies on cassava-cassava mealybug-CPD interaction or at least, their interrelationship(s).

MATERIALS AND METHODS

Survey and Mapping

In the conduct of survey and collection of specimens, coordination with and assistance was requested from the Regional Crop Protection Center, Bureau of Plant Industry, Department of Agriculture, and the respective Local Government Units. Based on their advice and the information they provided, provinces and sites were selected for the visits. The respective local agencies also provided guides during visits to farms within their respective areas of jurisdiction. The presence of cassava witches' broom disease was determined and the details of symptoms as well as insects present among the diseased plants were recorded. Collection sites (i.e., provinces and localities) were tabulated to reflect the distribution of the cassava witches' broom and cassava mealybugs.

Collection of Mealybugs from Cassava Farms Around the Philippines

Insects and other arthropods that were present on the CWB-infected plants were observed, recorded, and collected from October 2017 to July 2020. All samples were properly labeled, clearly indicating locality data, dates of collection, and the health status of the host plant. Flying or more active insects were collected with a sweep net but crawling, less active, or stationary insects were collected manually using a pair of fine-pointed forceps or a small watercolor brush. Based on published literature, especially those mentioning insect groups that are possible candidates for vector identification, special attention was given to mealybugs and other sucking insects. For mealybugs, portions of the affected plants were cut and placed together with the mealybug colonies in clear plastic bags and sealed. Photographs were taken whenever possible, and all insects were preserved in 80% – 95% ethyl alcohol.

Processing and Identification of Collected Mealybugs

The mealybugs preserved in ethyl alcohol were brought to the laboratory for processing as slide-mounted specimens are needed for identification. Adult females were macerated by soaking overnight in a 10% aqueous solution of potassium hydroxide under ambient room temperature. Dissolved as well as partially-digested body contents were teased out of the mealybug integument through a slit made earlier before maceration using mounted blunt insect pins. Cleared specimens were then washed with distilled water and two to three changes of 95% ethyl alcohol consecutively. Specimens that contained stubborn fat globules were immersed for at least 30 min in a 1:1 mixture of phenol and xylene and again in two to three changes of 95% ethanol. Samples were then stained by soaking for at least 1 h or longer in small dishes fashioned out of cut-glass vials, half-filled with alcoholic acid fuchsin overnight. Destaining was done by running them through an alcohol series (50%, 75%, 85%) and then 95% ethyl alcohol for 15 min twice, and finally, two changes of absolute (100%) ethyl alcohol for 10 min. During periods of very high relative humidity, the second alcohol was changed to 100% propanol as it is less hygroscopic. Samples were then treated in two successive changes of xylene, each for 15 min. Specimens were finally mounted on microscope slides using Canada Balsam. Voucher specimens were labeled and deposited in the UPLB-Museum of Natural History Entomological Collection.

Mounted specimens were examined under a compound microscope (Carl Zeiss PrimoStar 2016) connected to a computer monitor. Images were captured to serve as photomicrographs, with scale bars. Details of cerarii, multilocular pores, tubular ducts, and translucent pores were also taken. Published identification keys, illustrations, and descriptions were consulted. Specimens were also compared with museum collections, i.e., types when possible and vouchers determined by specialists.

RESULTS AND DISCUSSION

Distribution of Cassava Witches' Broom and Mealybugs

The survey was conducted to 19 provinces with representative municipalities (Table 1). In Luzon Island, among the 10 provinces visited, CWB was present in six, based on symptomatology and molecular detection (Dolores, Langres, Pinili et al. 2023a). Six provinces—Ifugao, Isabela, Laguna, Batangas, Occidental Mindoro, and Camarines Sur—were positive for CWB, while Ilocos Norte, Ilocos Sur, Pampanga, and Pangasinan were not.

Table 1. Provinces and localities surveyed for the presence (+) or absence (-) of cassava witches' broom and cassava mealybugs.

Province	Locality	Date	CWB Disease	Cassava Mealybugs
Luzon				
1. Ifugao	Alfonso Lista	20-Feb-18	+	-
2. Ilocos Norte	Dingras	07-Feb-18	-	-
	Laoag City	07-Feb-18	-	-
3. Ilocos Sur	Vigan City	08-Feb-18	-	-
4. Pangasinan	Mangaldan	01-Mar-18	-	-
5. Isabela	Ilagan City	18-Feb-20	+	+
	Naguilian	24-Jun-18	+	+
6. Pampanga	Porac	08-Mar-19	-	-
	Guagua	08-Mar-19	-	+
	Floridablanca	08-Mar-19	-	-
7. Laguna	UPLB Campus	20-25 November 2018	+	+
	San Pablo City	31-Jan-18	-	-
8. Batangas	Santo Tomas City	04-May-20	+	-
9. Occidental Mindoro	Rizal; San Jose	26-Oct-17	+	+
	Mamburao	18-Jan-18	+	+
10. Camarines Sur	Naga City	13-Aug-18	+	-
	Baao	13-Aug-18	+	-
	Tigaon	13-Aug-18	-	-
	Goa	13-Aug-18	-	-
	Pili	13-Aug-18	-	-
Visayas				
11. Capiz	Jamindan	30-May-18	+	+
12. Antique	Hamtic	31-May-18	+	+
13. Iloilo	Santa Barbara	31-May-18	+	-
14. Cebu	Daan Bantayan	14-Mar-18	+	+
	Camotes Island	14-Mar-18	+	+
15. Bohol	Trinidad	15-Mar-18	+	+
	Ubay	16-Mar-18	+	+
	San Miguel	16-Mar-18	+	+
16. Leyte	Baybay City	22-Nov-17	+	+
	Matalom	22-Nov-17	+	+
	Villaba	22-Nov-17	+	+
Mindanao				
17. Bukidnon	Baungon	09-Jan-18	+	+
18. Davao de Oro	New Bataan	17-Mar-18	-	-
19. Davao del Sur	Digos City	04-Dec-19	-	-
	Davao City	05-Dec-19	-	-

In Visayas, all six provinces surveyed—Capiz, Antique, Iloilo, Cebu, Bohol, and Leyte—had CWB infestation, while in Mindanao, only the province of Bukidnon was positive for CWB; Davao de Oro (formerly Compostela Valley) and Davao del Sur were negative during the visit.

Regardless of CWB incidence, the initial results on the diversity and abundance of arthropods observed during visits to cassava farms showed that the orders Hemiptera, Diptera, and Hymenoptera (mainly the ants, Formicidae) were the most commonly encountered, with a cumulative total of 65 insect species. Among the provinces visited, mealybugs (Hemiptera: Pseudococcidae) were observed infesting cassava plants which were also infected with the CWB in the provinces of Isabela, Laguna, Occidental Mindoro, Capiz, Antique, Cebu, Leyte, Bohol, and Bukidnon. The presence of this pest draws attention to its possible association with CWB. However, mealybugs were not included in the previous reports on vectors of phytoplasma (Weintraub and Beanland 2006; Weintraub and Gross 2013), which listed only sucking insects such as leafhoppers, planthoppers, and psyllids.

Taxonomy/Identification

Order Hemiptera

Suborder Coccomorpha

Superfamily Coccoidea

Family Pseudococcidae

Genus *Pseudococcus* Westwood

Pseudococcus Westwood 1840: 447. Type species: *Dactylopius longispinus* Targioni Tozzetti by subsequent designation.

Diagnosis. Adult female body normally broadly oval but may be more gibbose when gravid, around 1 – 4 mm long, 0.5 – 2.5 mm wide; antennae 8-segmented; legs well -developed, claw without a denticle; translucent pores usually on coxae, and/or other larger leg segments; tarsal and claw digitules both capitate, claw digitules thicker than tarsal digitules. Circulus usually present, well-developed, and divided by an intersegmental line; rarely small and not divided, usually wider than long. Quinquelocular pores always absent (Gimpel and Miller 1996).

Remarks. *Pseudococcus* is among the genera of mealybugs commonly encountered among cassava plants in the Philippines, the others being *Ferrisia*, *Paracoccus*, and *Phenacoccus*. However, *Ferrisia* species are easily distinguished, having very large dorsal tubular ducts with sclerotized orifices. On the other hand, both *Paracoccus* and *Pseudococcus* have oral rim tubular ducts and 8-segmented antennae, *Paracoccus* has 18 pairs of cerarii and a well-defined ventral sclerotized bar on the anal lobe, while *Pseudococcus* has 12 – 17 pairs of cerarii and occasionally only a faint or not well-defined ventral anal lobe bar sclerotization. Lastly, species of *Phenacoccus* always have short lanceolate setae and further, except for a few species, 9-segmented antennae and 18 pairs of cerarii. In areas where pineapples or bananas are present, the genus *Dysmicoccus* may also be encountered. However, *Dysmicoccus* as represented by *D. brevipes* (Cockerell) and *D. neobrevipes* Beardsley do not have oral rim tubular ducts and tend to be more rotund when the females are gravid. For the identification of other *Pseudococcus* species, some of which may also occur in cassava, Williams' (2004) key to Southern Asian species may be used. For other remarks, see those under *Ps. jackbeardsleyi*.

Species *Pseudococcus jackbeardsleyi* Gimpel and Miller (Fig. 1)

Pseudococcus jackbeardsleyi Gimpel and Miller 1996: 66. Type data: Mexico: Chiapas, on fruit of *Musa* sp. Holotype, female. Type depository: Washington: United States National Entomological Collection, U.S. National Museum of Natural History.

Pseudococcus elisae Borchsenius: Lit, Calilung, and Villacarlos 1990: 707; Lit and Calilung 1994: 258.

Material Examined. Two paratypes on one slide, *Pseudococcus jackbeardsleyi* Gimpel and Miller: Honduras: ex *Yucca* sp., 19-vi-1974, A. Boston (on loan from the USNM). Mexico: Tabasco, Rancherias Barrancar y Amate, Mpio Villahermosa Centro, ex *Euphorbia* sp., 04-vi-1999, H. Gonzalez and D.R. Miller (on loan from the USNM). Numerous adult females, Philippines, all ex CWB-infected *Manihot esculenta*: Luzon Island – Ilocos Norte Province: Lumban, Dingras (10); Isabela Province: CVRC, San Felipe, Ilagan (19); Laguna Province: NSF, NPGRL and Entomology Greenhouses and Screenhouses, UPLB Campus. IPB Experimental Site, Tranca, Bay. Paciano Rizal, Bay (37); Mindoro Island – Occidental Mindoro Province: Sitio Lagdaan, Kayamaan, Mamburao (10); Leyte Island – Leyte Province: Sitio Cogon, Zaragoza, Matalog (10); Bohol Island – Province: Bohol Experiment Station, Gabi, Ubay (19); Mindanao Island – Bukidnon Province: Lingating, Baungon, Bukidnon (28) – leg R.A.P. Laude / M.C. Lit. x.2017-vii.2020 (UPLB MNH).

Diagnosis. Adult female, as mounted, mostly oval, at times elongate oval, about 2.5 mm long, 2.25 mm wide. Morphologically quite close to *Ps. elisae* Borchsenius

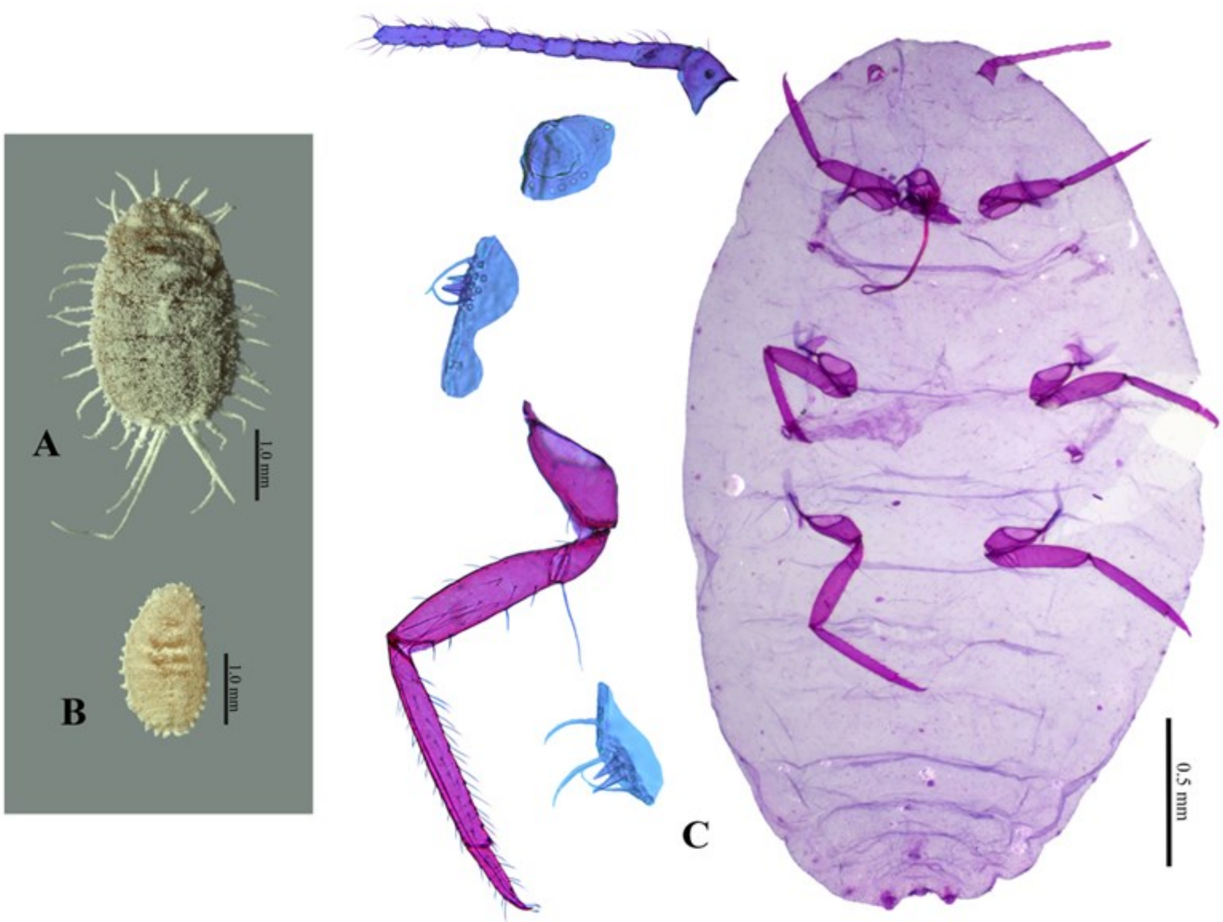

Fig. 1. Jack Beardsley's mealybug, *Pseudococcus jackbeardsleyi* Gimpel and Miller. Adult females. a-b. habitus. a. gravid individual. b. young adult. c. as mounted on slide, with the antenna, eye, cerarii (thoracic), and hind leg highlighted.

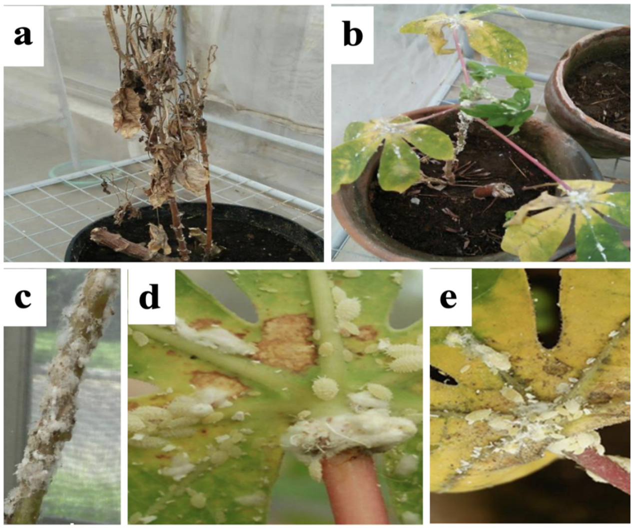

Fig. 2. Colonies of Jack Beardsley's mealybug, *Pseudococcus jackbeardsleyi*, infesting Witches' Broom-infected cassava. a-b: Whole plants with high mealybug infestation. c: numerous mealybugs infest the stem. d-e: Leaves: d: abaxial (ventral or underside). e: adaxial (dorsal or above).

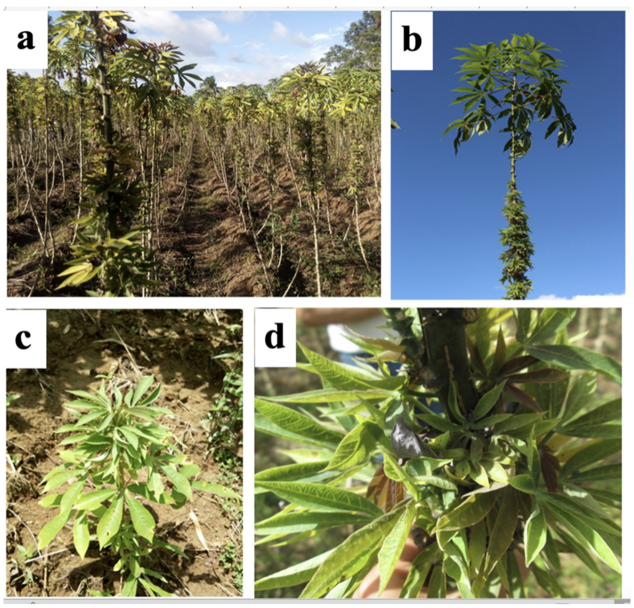

Fig. 3. Cassava Witches' Broom disease symptoms as potential and actual hosts of mealybugs especially *Pseudococcus jackbeardsleyi*. a: tall but thin-stemmed cassava plant during high incidence of CWB disease in the field. b: excessively proliferated leaves and side shoots at the middle part of the stem. c: excessive side shoots. d: close-up of small, proliferated leaves on shortened internodes.

known from Central America and northern South America but differs by having: (1) more oral-rim tubular ducts (13 – 27) on the dorsum, lateral oral-rim ducts on abdominal segment VII; (2) fewer (14 or less) or no multilocular disc pores on abdominal segment III; and (3) having translucent pores on the hind femur and tibia.

Remarks. The specimens at hand agree quite well with the characters as enumerated in the original description of *Ps. jackbeardsleyi* (Gimpel and Miller 1996: 67 – 69) as well as with those of the two paratypes examined (Fig. 1) and the non-paratypic material from Mexico determined by D.R. Miller.

Among the mealybugs found in cassava farms, *Ps. jackbeardsleyi* may occur in mixed colonies with *Ferrisia virgata* (Cockerell), *Paracoccus marginatus* Williams and Granara de Willink, and/or *Phenacoccus manihoti* Matile-Ferrero. However, the available data showed that Jack Beardsley's mealybug is the most common in cassava.

Its previous local record as *Ps. elisae* Borchsenius (Lit et al. 1990; Lit and Calilung 1994) is based on a comparison with a reference slide (Honduras: La Lima, on *Rivina humilis* L., ix-x.1967, leg C. Evers, det. J.W. Beardsley, Jr.) generously shared with the first author by the late Dr. John "Jack" W. Beardsley, Jr., of the University of Hawaii. A subsequent comprehensive study of species in the *Pseudococcus maritimus*-complex by Gimpel and Miller (1996) concluded that those from the Philippines, often confused with *Ps. elisae*, are different. What was then known as populations of *Ps. elisae* from the Philippines and elsewhere have been named and described as *Ps. jackbeardsleyi*.

CONCLUSION

Contrary to expectations, considering that the recently introduced *Ph. manihoti* is included in the enumeration of Kondo et al. (2022), *Ps. jackbeardsleyi* was identified in nine out of the 13 cumulative samples of cassava witches' broom (CWB)-infected cassava plants. Colonization, settlement, and proliferation of colonies of mealybugs (Fig. 2) were apparently encouraged by excessively proliferated and, therefore, denser—albeit smaller—branches and leaves of CWB-diseased plants (Fig. 3), where the dense foliage protected them better from environmental factors like rainfall, larger predators, etc.

The relationship of Jack Beardsley's mealybug with microorganisms, especially bacteria and allied groups, is not well established. Choi and Lee (2022) identified a species of Betaproteobacteria, specifically *Candidatus Tremblaya princeps* as associated with this mealybug, as an endosymbiont, just as other mealybugs are. *Ps. jackbeardsleyi* is not yet known to transmit phytoplasma, especially the one causing CWB. However, its occurrence in nine, i.e., almost 70%, out of the 13 cumulative CWB-infected samples provides circumstantial evidence that it may be related to the spread of the pathogen. This formal report of identification is in support of studies on the possible role of this mealybug species in CWB phytoplasma transmission. The molecular detection of the CWB-causing phytoplasma among local populations of *Ps. jackbeardsleyi* and the experimental transmission of the pathogen using laboratory-reared individuals of this mealybug species are recommended.

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