



First report of the enset root mealybug *Paraputo ensete* (Williams & Matile-Ferrero) (Hem.: Pseudococcidae) on banana in Ethiopia

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Abstract

Enset root mealybugs, a major pest affecting the cultivation of the enset crop in the Ethiopian highlands, have for the first time been observed on banana mats indicating the potential host status of *Musa* spp. These observations were made under natural conditions in backyard gardens in the Gedeo zone, southern Ethiopia, on the root system of banana mats of the ‘Pisang Awak’ (local name ‘Feranji Muz’, ABB genome group) landrace. Here, we confirm the identification of the collected enset root mealybug specimens on banana mat root systems as *Paraputo ensete* (Williams & Matile-Ferrero) (Hemiptera: Pseudococcidae) through DNA analysis.

Keywords DNA analysis · *Ensete ventricosum* · Host plant · *Musa* spp

Introduction

Enset (*Ensete ventricosum* (Welw.) Cheesman) is a major food crop in the south and south-western highlands of Ethiopia, where it is a staple starch food for ~20 million people (Brandt et al. 1997; Borrell et al. 2019). While enset cultivation on small-holder subsistence farms is a critical factor for food security, livelihood and sustainability in this region, its production is affected by multiple pests and pathogens of varying severity (Yemataw et al. 2020). Its most important biotic constraints include enset Xanthomonas wilt (a bacterial disease) and enset root mealybugs (*Paraputo ensete* (Williams and Matile-Ferrero)) (Hemiptera: Pseudococcidae) (Addis et al. 2008a; Blomme et al. 2017). The enset root mealybug

is an insect pest infesting the roots and corm of enset plants (Addis et al. 2008a), causing a reduced number of roots, dried outer leaves, reduced pseudostem circumference (Addis et al. 2008a; Azerefegne et al. 2009), retarded plant growth and occasional death, especially under drought stress.

Recently, Erenso et al. (2022) encountered enset root mealybugs on the root system of banana mats of the ‘Pisang Awak’ (local name ‘Feranji Muz’, ABB genome group) landrace under natural conditions in three backyard gardens of the Dilla Zuria district in the Gedeo zone, southern Ethiopia. *Ensete* and *Musa* genus are closely related, both being monocarpic perennial herbaceous plants of the Musaceae family, similar in morphology and physiology (Borrell et al. 2019). The root mealybugs observed and collected on the roots of the Pisang Awak mats were morphologically identified as *Paraputo ensete* (Williams and Matile-Ferrero) (Hemiptera: Pseudococcidae), providing a first record of the enset root mealybug on banana in Ethiopia.

In this short communication, we combine an in-depth morphological assessment with DNA analysis on the enset root mealybug specimens collected by Erenso et al. (2022) to verify these findings.

Materials and methods

Additional DNA analysis was performed on the enset mealybug specimens collected by Erenso et al. (2022). In short, Erenso et al. (2022) surveyed 60 backyard gardens of

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small-holder farmers cultivating bananas within or adjacent to enset crops in the Gedeo zone in the Southern Nations, Nationalities, and Peoples' Region (SNNPR) of Ethiopia. The surveys took place in the woredas (districts) of Dilla Zuria and Wonago (two bordering woredas) and Yirgachefe. Root systems and corms of five enset plants and five banana mats were investigated at each farm, and root mealybug specimens were collected when present. Specimens were stored in vials with 70% ethanol and sent to the IITA research station in Benin Republic for identification. Mealybugs from both banana and enset collections were individually slide mounted and examined under a transmitted-light microscope (Leitz LaborLux S) at a magnification range of 40X to 1000X using the keys by Williams and Matile-Ferrero (1999) and Watson and Kubiriba (2005). Permanent mounts of voucher specimens were deposited in the reference collection at the IITA Biodiversity Center.

To cross check the identification results obtained using morphological characters, mealybug samples collected from enset and banana were sent at the Virology and Molecular Diagnostics Unit at the IITA Headquarters in Ibadan, Nigeria. DNA barcodes based on the mitochondrial cytochrome oxidase I (COI) gene were generated using standard protocols. PCR amplification of the barcode fragment was performed with the primers C1-1554F (5'-CAGGAATAATAGGAACATCAATAAG-3') and C1-2342R (5'-ATCAATGTCTAATCCGATAG TAAATA-3') (Deng et al. 2012). Amplified products were purified and sequenced in both orientations, and

the sequences (~736 bp) were deposited in the NCBI GenBank. Extractions of following material were submitted to a homology test using sequences available from the Barcoding of Life Data system (BOLD) (<http://www.boldsystems.org/>) and additional sequences from the GenBank® database (<http://www.ncbi.nlm.nih.gov/genbank/>): Ethiopia, Dilla Zuria, SNNP, 3. ii. 2020, on cord root of *Ensete ventricosum*, Coll. G. Blomme; Ethiopia, Dilla Zuria, SNNP, 3. ii. 2020, on cord root of banana, cultivar 'Feranji Muz' [*Musa* ABB genome], Coll. G. Blomme. The CO-I sequences presently generated were deposited in GenBank under accessions AB49136067 and AB49136038, respectively. Ranking of the best species matches on GenBank was performed based on the Max Score values of the BLAST results.

Results

Root mealybug specimens collected on enset plants at Dilla Zuria and Yirgachefe and on the *Musa* cultivar Pisang Awak in 3 backyard gardens at the Dilla Zuria district in the Gedeo Zone (Fig. 1) were morphologically identified as *Paraputo ensete* (Williams and Matile-Ferrero) (Hemiptera: Pseudococcidae) (Erenso et al. 2022).

Slide-mounted adult female specimens from both enset and banana (Fig. 2) keyed out as *Paraputo ensete* in the key published by Watson and Kubiriba (2005) and fully

Fig. 1 Location of the first observation of the enset root mealybug *Paraputo ensete* (Williams and Matile-Ferrero) (Hem.: Pseudococcidae) on banana by Erenso et al. (2022) indicated by a red star. The observation was made in the Dilla Zuria district (woreda) in the Gedeo Zone of the Southern Nations, Nationalities, and Peoples' (SNNP) Region of Ethiopia

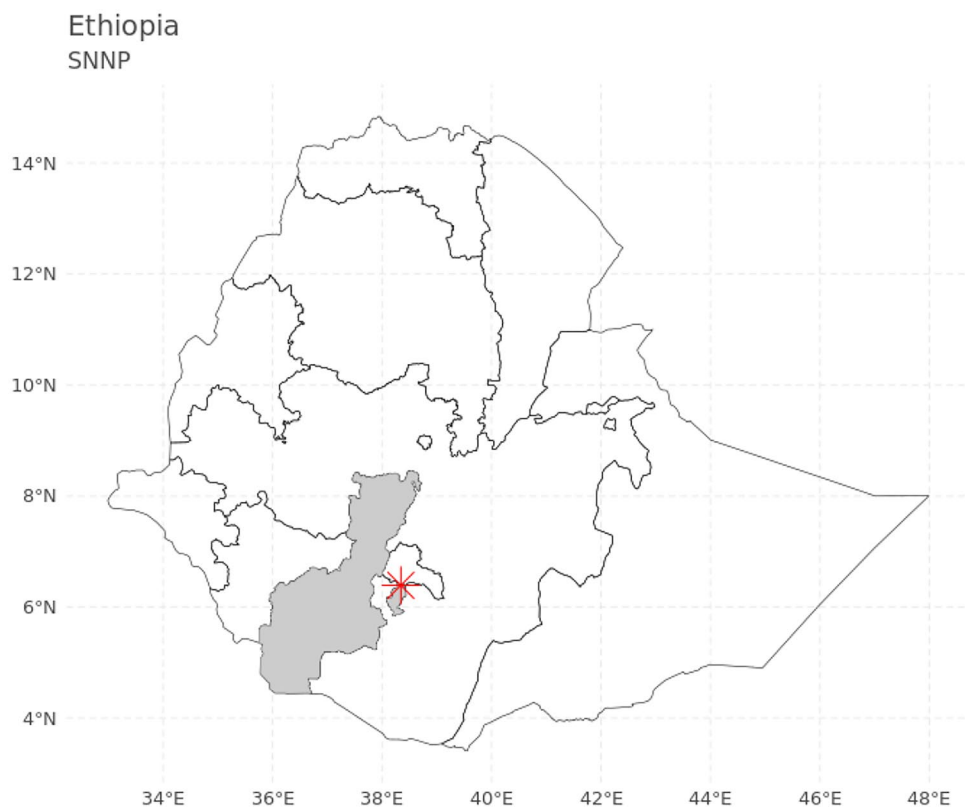
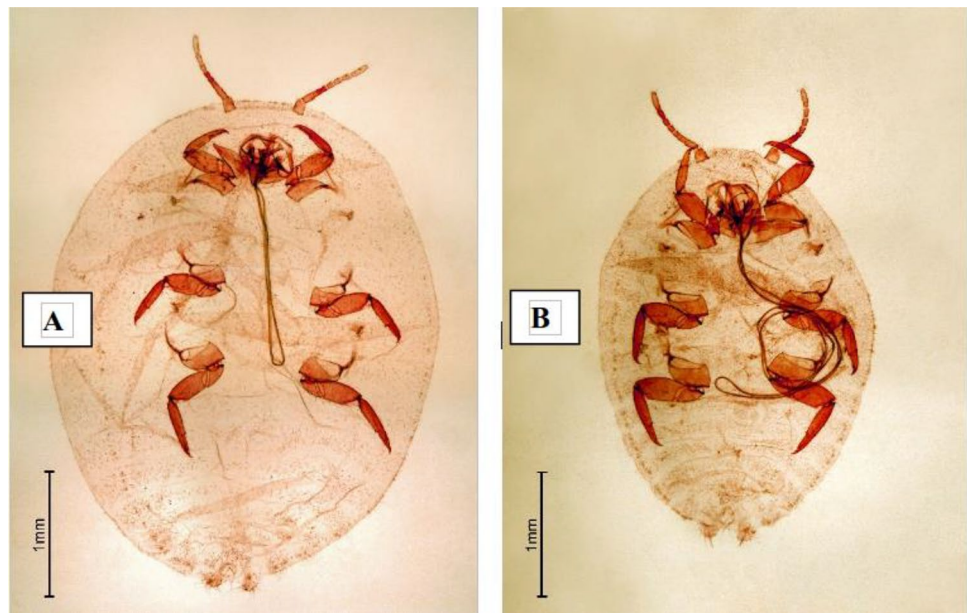


Fig. 2 Slide mount picture of the enset root mealybug that was sampled on enset (**A**) and on the banana cultivar Pisang Awak (ABB genome) (**B**) in mixed enset-banana backyard gardens at Dilla Zuria, Southern Ethiopia



matched the species original description by Williams and Matile-Ferrero (1999). Important distinguishing features observed in the material studied, and which confirmed its identity, were the presence of antennae with eight segments, only moderately developed anal lobes each containing 2 to 29 cerarian setae, and venter of anal lobe widely sclerotized. Two pairs of ostioles are well visible on the dorsum. The venter has a single circulus notched at each side and divided by an intersegmental line. Legs are well developed and contain numerous translucent pores on anterior and posterior surfaces of each hind coxa, moderate numbers on hind trochanter and a few present distally on each hind tibia. The number of cerarii is difficult to determine as they create a nearly unbroken marginal band, due to presence of numerous intermediate cerarii.

The recognition of the genus *Cataenococcus* as a junior synonym of *Paraputo* led to numerous nomenclatural changes including the assignment of the enset root mealybug into the genus *Paraputo* (Tang 1992; Ben-Dov and Williams 2006). Currently, this pantropical genus encompasses 88 species of which more than half occur in the Oriental region (García Morales et al. 2016). Among the 13 species recorded in the Afrotropical Region, *P. ensete* is morphologically closest to *Paraputo hypogeus* (De Lotto 1961) (Williams and Matile-Ferrero 1999).

Morphologically, the root mealybug specimens collected on Pisang Awak at Dilla Zuria were notably smaller in size compared to the specimens found on enset (Fig. 2; Erenso et al. 2022). The smaller size range of the specimens originating on the banana host plants does however fit within the size variability recorded by the original description running under *Cataenococcus ensete* namely

2.80–4.00 mm long, and 2.85–3.70 mm wide (Williams and Matile-Ferrero 1999).

The analysis of the DNA barcode sequences revealed a 100% similarity between the root mealybugs collected on enset and banana (Fig. 3) confirming thereby the identification based on morphological characters. As publicly accessible DNA barcode data for *P. ensete* are yet unavailable, a BLAST against reference databases gave no clear matching with the nucleotid sequences presently gained. The nearest species match was found for *Paracoccus marginatus* Williams and Granara de Willink with a relatively low similarity score of the top 100 matches (GenBank, identity 92.26%, coverage 100%; Bold, similarity 91.75%).

Discussion

DNA analysis confirms that the root mealybugs observed on the root system of the *Musa* cultivar Pisang Awak are in fact *Paraputo ensete* (Williams and Matile-Ferrero) (Hemiptera: Pseudococcidae). *P. ensete* had until now exclusively been recorded on enset in Ethiopia. Conversely, literature sources indicate that enset is a poor host plant for any other mealybug species which makes an identification based on morphological and biological features relatively easy among possible candidates. So far, only *Paracoccus burnerae* (Brain 1915) and *Planococcus ficus* (Signoret, 1875) were observed to attack enset on rare occasions, however these species are not feeding on roots (De Lotto 1964; Williams and Matile-Ferrero 1999).

Molecular identification of *P. ensete* based on the COI gene region inferred the enset root mealybug with the papaya

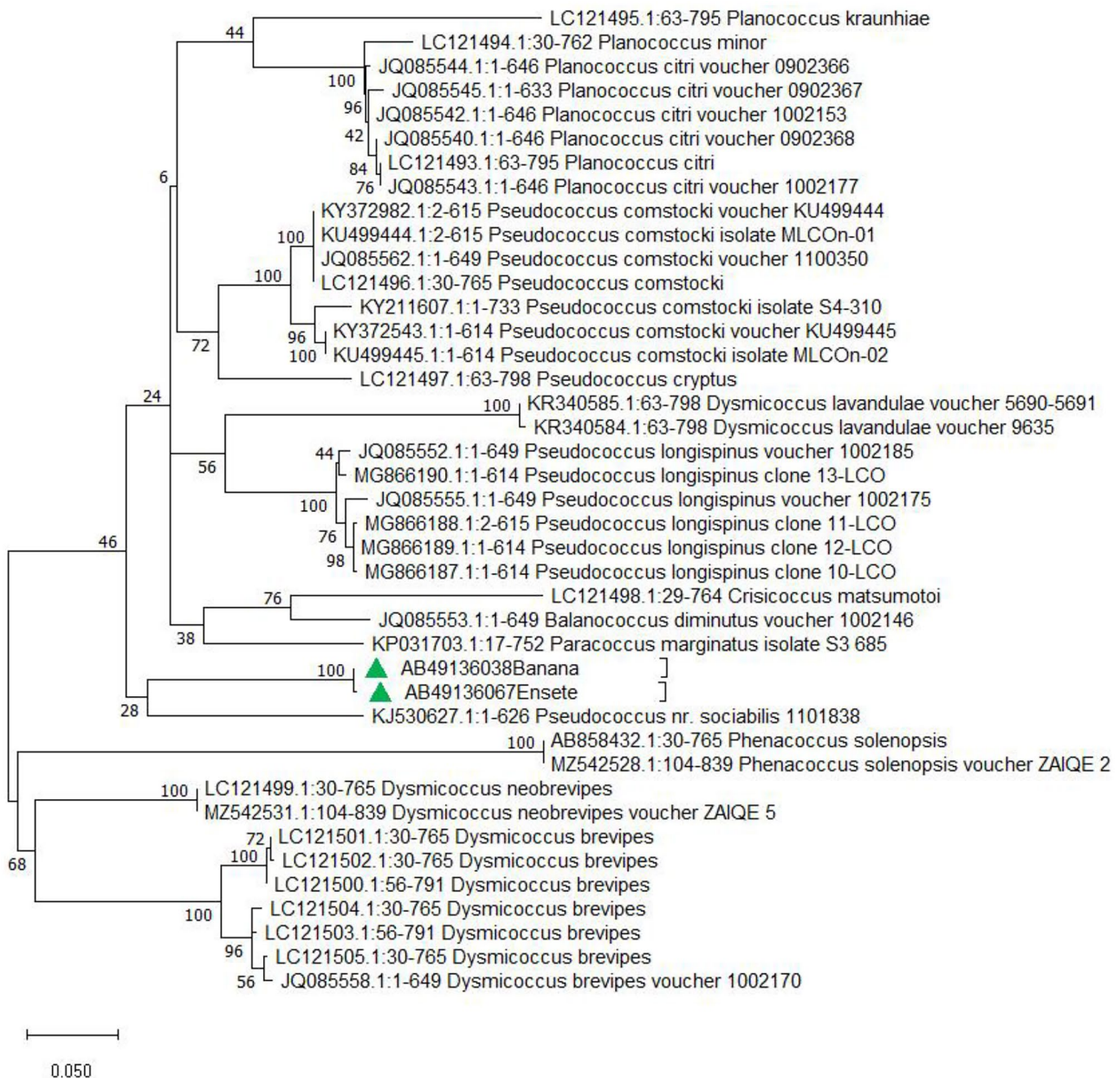


Fig. 3 Neighbor-Joining tree from mealybug samples collected on cord roots of *Ensete ventricosum* and banana, cultivar 'Feranji Muz' [*Musa* ABB genome] at Dilla Zuria, Ethiopia, inferred from the 736 bp mitochondrial cytochrome oxidase I (COI) sequences using the

Maximum Likelihood method (ML) and General Time Reversible model (Nei and Kumar 2000). Evolutionary analyses were conducted in MEGA X (Kumar et al. 2018). Numbers above branches indicate ML bootstrap values

mealybug *Paracoccus marginatus*. However, this grouping is not well-supported by the present genetic analysis, as indicated by a low bootstrap value. The absence of further *Paraputo* species in the present phylogenetic tree suggests a wide genetic distance between various member of this biodiverse genus. Yet, the consultation of BOLD and GenBank databases indicate that nucleotides of *Paraputo* species are available for only three species of which two are unnamed. Thus,

additional specimens and species from various geographic origin are needed to clarify the phylogenetic position of *P. ensete* among congeners. Furthermore, such analyses may include information from 28S, TEF1a and COII gene regions to investigate the degree of relatedness among pseudococcid mealybugs with more confidence and accuracy.

As discussed by Erenso et al. (2022), the host suitability of *Musa* spp. for enset root mealybugs remains relatively

low. *P. ensete* was only observed on mats of the *Musa* cultivar Pisang Awak at backyard gardens where very high infestations of *P. ensete* on enset plants were present, and in which the rooting zones of the two crops were located closely together. Additionally, the mealybug specimens collected on Pisang Awak plants were smaller in size, potentially indicating a moderate host suitability. For now, we therefore do not expect that the enset root mealybugs pose a large threat to *Musa* cultivation. Nevertheless, further research is needed on the impact of enset root mealybugs on banana yield and health.

The presence and survival of enset root mealybugs on *Musa* cultivars does pose an additional risk for the spread of the pest. Enset root mealybugs are naturally dispersed through active movement of *P. ensete* during the nymphal stages or when adult mealybugs are disturbed (Addis et al. 2008b), through association with ants (Malsch et al. 2001) or displacement by water. Farmers can further contribute to local spread by uprooting and replanting infested enset suckers (Addis et al. 2008b) and the use of contaminated tools. The exchange and trade of enset planting material could contribute to wide-spread dispersal, but in practice enset plants are most often traded within the local community. The presence of enset root mealybugs on banana suckers/ planting materials could further introduce and establish the pest to new farms and regions, if the mealybugs have the opportunity to transfer to enset hosts at planting location. Banana suckers used as new planting material, through local and regional trade / exchange, should therefore be visually inspected for the presence of mealybugs, and should not be planted in the vicinity of enset plants to minimize mealybug survival. As such, integrated pest management systems developed for enset (Megersa and Lemma 2022) should account for the host suitability of banana cultivars to the enset root mealybug.

To assess the risk of spread of the enset root mealybug, updated and improved geographical distributions of the pest across the enset-growing belt of Ethiopia should be developed. The most recent survey (to our knowledge) by Yemataw et al. (2020) observed that low lying areas along the Great Rift Valley are most severely affected by the enset root mealybug, in line with previous survey findings that mealybugs are most severe at an elevation between 1,600-1,800 masl in the Gedeo, Sidama and Amaro Zones (Azerefegne et al. 2009; Addis et al. 2008b, 2010). While known as a locally important pest, field surveys generally find a relatively large percentage of sampled farms to be infested with enset root mealybug, respectively 30-33% (Azerefegne et al. 2009; Addis et al. 2008b, 2010; Yemataw et al. 2020). Risk assessments of the spread of pests and diseases affecting enset cultivation highly rely on updated

geographical distribution maps, combined with the assessment of dispersal pathways. The dispersal risk for enset root mealybugs by accounting for farms cultivating enset and bananas in close vicinity, combined with the host status of bananas and pathways of planting material trade, should in the future be introduced into overall enset pest and disease management systems.

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Authors' contributions Fikadu Erenso conceived and developed the research concept, contributed to methodology development, data collection, analysis, result interpretation, manuscript writing and editing. Guy Blomme mobilized the resources for the study, conceived and developed the research concept, contributed to methodology development, data analysis, result interpretation, manuscript writing and editing. Georg Goergen contributed to mealybug sample identification and manuscript editing. Mitiku Muanenda contributed to data collection. Elizabeth Kearsley contributed to data analysis, result interpretation, data visualization, manuscript writing and editing. Temesgen Addis contributed to methodology development, result interpretation and manuscript editing.

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Data availability The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

Code availability Not applicable.

Declarations

Ethics approval Not applicable.

Conflicts of interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as or result in a potential conflict of interest.

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