Research Note


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The lanzones scale insect, *Unaspis mabilis* Lit & Barbecho (Hemiptera: Diaspididae), is reported to infest cacao trees (*Theobroma cacao*) in Brgy. Nalook, Kalibo, Aklan. Despite minor morphological differences, analysis of DNA barcode region of the *cytochrome c oxidase subunit I* (COI) generated from the cacao-infesting scale insects (GenBank Accession Number MN114100.1) matched the DNA barcodes obtained from a complementary study (GenBank Accession Number MN114099), and hence, supported the species identity based on morphological characters. Notes on the distribution, host plant association and insights on possible control measures were provided.

Key words: *cytochrome c oxidase subunit I*; DNA barcode; Hemiptera; new host record

Abbreviations: COI—*cytochrome c oxidase subunit I*, DNA—Deoxyribonucleic Acid, PCR – polymerase chain reaction, UPLB—University of the Philippines Los Baños

INTRODUCTION

The lanzones scale, *Unaspis mabilis* Lit & Barbecho (Hemiptera: Diaspididae), has been known since 2008 to exclusively infest lanzones leaves (*Lansium domesticum*). Despite that, the species was first named and described by Lit and Barbecho (2014), who also enumerated areas of occurrence within the Philippines. The same species was named *U. lansivora* by Watson (2015a), which is now considered a synonym (Watson, 2015b). This armored scale insect has demonstrated aggressive spread throughout almost all lanzones plantations from North and South Cotabato, Davao and Sarangani in Mindanao to various provinces in Luzon and, more recently, in Mindoro, Negros and Panay Islands (Lit and Barbecho 2014).

In 2017, what initially appeared to be “mussel scales” were collected from cacao trees (*Theobroma cacao*) in Brgy. Nalook, Kalibo, Aklan. The samples showed field characteristics that were quite similar to lanzones scales, albeit somewhat smaller. Upon detailed morphological examination of the samples of the scale insects collected from cacao complemented with molecular analysis, its identification has been proven to be *Unaspis mabilis*. This paper reports that cacao is a new host record for the lanzones scale, *Unaspis mabilis* and provides insights on host plant associations and pest management.
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**MATERIALS AND METHODS**

**Sample Collection**

During the nationwide survey of armored scale insects and other coccoids found in coconuts and associated crops in 2017, populations of the scale insect *U. mabilis* were found infesting cacao leaves in Brgy. Nalook, Kalibo, Aklan. Samples were collected, and temporarily preserved in 95% ethyl alcohol for transport.

**Morphological Identification**

Scale insects, initially preserved in 95% ethyl alcohol, were macerated in 10% ethyl alcohol overnight. They were then washed with distilled water and 95% ethyl alcohol, consecutively. Samples were then stained with alcoholic acid fuchsin, also overnight. Destaining was done by an alcohol series: 95% ethyl alcohol for 15 minutes twice, absolute ethyl alcohol for 10 minutes. Samples were dehydrated in xylene twice for 15 minutes each and mounted in microscope slides using Canada Balsam (Schaff 2001). Voucher specimens were labeled and deposited in the UPLB-Museum of Natural History Entomological Collections. Specimens were identified using keys to genera of Williams and Watson (1988) and key to species by Watson (2015a). Based on the data of identified specimens, the distribution map of the scale insect species was updated.

**Molecular Identification**

The total genomic DNA of each scale insect infesting cacao leaves was extracted following the protocol described in Alvarez et al. (2020). The DNA barcode region of the COI gene was then amplified using the PcoF1 and LepR1 primer pair. Polymerase chain reactions were carried out using 25 μL PCR reaction mixture containing 12.5 μL of 2X Taq Master mix (Vivantis, Malaysia), 10 pmol PcoF1, 10 pmol LepR1, 4 mM MgCl2, and 50 ng genomic DNA. PCR thermocycling was done under the following conditions: 2 min at 95°C; 5 cycles of 40 s at 94°C, 40 s at 45°C, 70 s at 72°C; 40 cycles of 40 s at 94°C, 40 s at 51°C, 70 s at 72°C; 5 min at 72°C; held at 4°C (Park et al. 2010). The amplicons were resolved in 1% agarose gel in a 0.5x TBE buffer stained with GelRed® (Biotium, Inc., Fremont, CA, USA). The molecular size of the PCR products was estimated using VC100 bp Plus DNA Ladder (Vivantis, Malaysia). The gels were viewed and photographed using Alpha Imager Mini (Protein Simple, San Jose, CA, USA) at 302 nm. Samples that matched with the expected molecular weight of the PCR products was estimated using VC100 bp Plus DNA Ladder (Vivantis, Malaysia). The gels were viewed and photographed using Alpha Imager Mini (Protein Simple, San Jose, CA, USA) at 302 nm. Samples that matched with the expected molecular weight of the PCR product were then trimmed using BioEdit software (Hall 1999) and then subjected to BLASTn (https://blast.ncbi.nlm.nih.gov) for data mining. A dendrogram was generated by neighbor-joining using K2P distance model and 1000 bootstraps using MEGA X (Kumar et al. 2018).

**RESULTS AND DISCUSSION**

**Identification**

Samples of scale insects collected from cacao were slightly smaller (average length 1.8 mm) than typical lanzones scales (average length 2.0 mm) but of the same general color and appearance (Figure 1A). Those of females are dark gray with whitish border, elongate, somewhat mussel-shaped, with a median longitudinal ridge. The exuviae of earlier instars were light to dark brown, and all placed at the anterior tip of the scale cover. The scales of males are cottony white, at most a fifth the size of adult female scale.

Examination of slide-mounted specimens from cacao showed that they were indeed conspecific with those infesting lanzones, the species identified earlier as *Unaspis mabilis* Lit & Barbecho. The occurrence of this species is hereby reported as a new host record in the following taxonomic account:

Species *Unaspis mabilis* Lit & Barbecho (Figure 1A-G)

*Unaspis mabilis* Lit & Barbecho 2014: 1-4. Type data:

**Philippines:** Luzon, Mount Makiling, Los Baños, Laguna, on *Lansium domesticum*, 2/14/2014, by N.M. Barbecho. Holotype, female, Type depository: Los Baños: Museum of Natural History, University of the Philippines Los Baños.

*Unaspis lansivora* Watson 2015a: 432. Type data:


**Host Plants:** *Lansium parasiticum* (Osbeck) Sahni and Bennet 1974; *Theobroma cacao* L., 1753 (new host record)

Remarks: *U. mabilis* from cacao vary slightly from those in lanzones in terms of the membranous lateral lobes of free abdominal segments (sclerotized in those from lanzones). Despite that, other distinguishing characters fit the description of *U. mabilis*: head and thorax membranous; perivulvar pores numerous, in five
groups; posterior spiracle with near equal number of pores to anterior spiracle; interlobular space with one simple gland spine; distance between median lobes less than half the width of a median lobe; median lobe weakly serrated; and, abdominal sternite 1 with glands spine and duct tubercles on the margin and submarginal area.

The DNA barcode region of the COI gene extracted from the cacao-infesting scale insects matched the DNA barcode generated from a complementary study (GenBank Acc. Nos. MN114099, MN14101, and MN114102), and hence, supported the species identity based on morphological characters.

The aligned nucleotide sequence of the scale insect collected from cacao (MN114100) (Voucher Accession no. UPLBMNH HEM-02576) has 100% similarity with confirmed U. mabilis collected from Bagong Silang, Los Baños, Laguna (GenBank Acc. No. MN114099) and other sequences from Alvarez et al. (2020) (Figure 2). Despite the minor morphological differences in the sclerotization of populations of scale insects collected from cacao and lanzones, results of molecular analysis revealed that they belong to the same genus and species, U. mabilis. This has been shown also by the monophyly of the scale insect samples from cacao with those of other lanzones scale samples/accessions (Figure 3).

Distribution

Lit and Barbecho (2014) enumerated the following islands among the known distribution records of U. mabilis, based on their examined material as well as field observations: Luzon, Mindoro, Panay, Mindanao. Watson (2015a) also included Negros Island (Negros Occidental Province) and also confirmed its occurrence in Camiguin Island. The species was not present among the lanzones trees we observed during our trip in 2019 to Tawi-tawi, the southernmost province of the Philippines.

This species is currently known only from the Philippines but both Lit and Barbecho (2014) and Watson (2015a) doubted whether it is native or endemic. Lit and Barbecho (2014) mentioned that in 2009, the infestation was first noticed in outbreak levels in areas around Makilala, North Cotabato in Mindanao. Almost a year since then, the infestation has spread to other areas in Mindanao and the Philippines. The species has also been reported from Negros Island and Camiguin Island.

Fig. 1. Unaspis mabilis Lit & Barbecho, 2014 from Theobroma cacao: (A) male (white) and females scales; (B) habitus, ventral; (C) antenna; (D) anterior spiracle; (E) posterior spiracle; (F) lateral margin of first and second abdomen, ventral; (G) pygidium, dorsal (left) and ventral (right) view.

Fig. 2. Nucleotide sequence alignment cytochrome c oxidase I gene of Unaspis mabilis Lit & Barbecho infesting lanzones, Lansium parasiticum, collected in Bagong Silang (MN114099.1), Los Baños, Laguna and cacao, Theobroma cacao L. (MN114100.1) from Kalibo, Aklan, Panay.
later, reports of similar infestations and specimens were received from Nagcarlan, Laguna in Luzon and other places including Los Baños and Bay, also in Laguna Province. The occurrence in Aklan Province on Panay Island was reported in 2014. Its aggressive spread and behavior is characteristic of invasive alien species. Generally, introduced species exhibit exponential population growth, having escaped from the regulation by natural enemies in their native ranges. To date, there are no known naturally occurring natural enemies in the Philippines and we have not observed any mark of hymenopteran parasitization among *U. mabilis* individuals we observed both on lanzones and on cacao.

Host Plant Associations and Pest Management Insights

The type host, lanzones or *Lansium parasiticum* (Osbeck) Sahni & Bennet (*L. domesticum* Correa) belongs to the family Meliaceae, the same family that includes the invasive mahogany (*Swietenia macrophylla* King) and the medicinal/insecticidal neem tree (*Azadirachta indica* A. Juss). On the other hand, the new host plant record here reported, *Theobroma cacao* L., was formerly included in the Sterculiaceae but is now part of the broad family Malvaceae. These families are remotely related to each other and thus, the new host record makes *U. mabilis* polyphagous. In terms of distribution, lanzones is native to the tropical areas of the Oriental Region, specifically native to the Malesian biogeographic region of Southeast Asia, from Peninsular Thailand and Peninsular Malaysia, to Java, Sumatra, Borneo, the Philippines (Luzon, Camiguin, Basilan, and Mindanao), Sulawesi, the Moluccas, and Western New Guinea (Pelser et al. 2020; Hassler 2020).

In contrast, cacao is native to the neotropics, from the southern part of Mexico to the Amazon basin. The genus *Unaspis* is largely Oriental although one or two species have been introduced, most probably with their *Citrus* hosts to many parts of the world. This increases the possibility that the native host is, indeed, lanzones. However, the newly observed occurrence on cacao presents evidence of its capability to switch hosts or expand its host range and hence, also its potential to become a polyphagous pest.

This trend make it possible for this pest to have the ability to spread further in the future. Therefore, an aggressive effort should be made to monitor especially in areas where cacao is now largely planted. The need for pest management research and development for *U. mabilis* is emphasized considering that cacao is a high value crop. To date, there are only few studies conducted like the mass rearing of the black lady beetle *Chilocorus nigrita* (Fabricius) (Pableo and Simpao 2013) and the systemic resistance to the lanzones scale accorded by *Trichoderma* microbial inoculant (Silva et al. 2019). Additionally, monitoring other plants belonging to the families Meliaceae and the subfamily Byttnerioideae of the Malvaceae must be done.

Fig. 3. Dendrogram showing relationship of *Unaspis mabilis* associated with cacao (n = 4) and selected species (n = 28) of Diaspididae based on partial nucleotide sequences of *cytochrome C oxidase I* analyzed by neighbor-joining using K2P distance model and 1000 bootstraps, with Pseudococcidae species, namely: *Pseudococcus jackbeardsleyi* (KY373149.1) and *Planococcus citri* (EU250560.1) as outgroups. Note monophyly with 100% identity of all *U. mabilis* samples whether from cacao (CAO) or lanzones (BS).

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Lit and Barbecho (2014) noted the same.
SUMMARY AND CONCLUSION

The lanzones scale, *Unaspis mabilis* Lit & Barbecho (Hemiptera: Diaspididae), has been known since 2008 to exclusively infest lanzones leaves. It has demonstrated aggressive spread throughout almost all lanzones plantations from North Cotabato and Davao in Mindanao to various provinces in Luzon and, more recently, in Mindoro and Panay Islands. In 2017, what initially appeared to be “mussel scales” collected from cacao trees in Kalibo, Aklan, showed field characteristics that were quite similar to lanzones scales. Examination of slide-mounted specimens showed that they were indeed conspecific with those infesting lanzones. The DNA barcode region of the *cytochrome c oxidase subunit I* (COI) generated from the cacao-infesting scale insects (GenBank Accession Number MN114100.1) matched the DNA barcodes obtained from a complementary study (GenBank Accession Number MN114099), and hence, supported the species identity based on morphological characters. *Unaspis mabilis* which was historically known to feed solely on lanzones (Meliaceae) is now recorded to be feeding on cacao (Malvaceae) too. The newly observed occurrence on cacao presents evidence of its capability to switch hosts or expand its host range and hence, also its potential to become a polyphagous pest.

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