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A new species of planthopper belonging to the genus *Oecleus* Stål, 1862 (Hemiptera: Fulgoroidea: Cixiidae) from coconut palm (*Cocos nucifera* L) in Jamaica

WAYNE MYRIE[1], ERICKA E. HELMICK[2], CHARLES R. BARTLETT[3],
ASSUNTA BERTACCINI[4] & BRIAN W. BAHDER[5]
[1]*Coconut Industry Board, 18 Waterloo Road, Kingston 10, Jamaica W. I. E-mail: cocomax@cwjamaica.com*
[2]*University of Florida, Department of Entomology and Nematology—Fort Lauderdale Research and Education Center; 3205 College Ave., Davie, FL 33314-7719, USA. E-mail: ehelmick@ufl.edu*
[3]*University of Delaware, Department of Entomology and Wildlife Ecology; 250 Townsend Hall, Newark, DE 19716, USA E-mail: Bartlett@udel.edu*
[4]*University of Bologna, Department of Plant Pathology; Viale Fanin 42, 40127, Bologna, Italy. E-mail: assunta.bertaccini@unibo.it*
[5]*University of Florida, Department of Entomology and Nematology—Fort Lauderdale Research and Education Center; 3205 College Ave., Davie, FL 33314-7719, USA. E-mail: bbahder@ufl.edu*

Abstract

A new species of cixiid planthopper (Hemiptera: Fulgoroidea) in the genus *Oecleus* Stål, *Oecleus mackaspringi* **sp. n.**, is described from Spring Garden, Jamaica. This new taxon is associated with coconut palm (*Cocos nucifera* L.) and was found while surveying palm plots with active cases of lethal yellowing (LY). This is the first report of the genus *Oecleus* from Jamaica. Sequence data for the COI gene and 18S gene are also provided.

Key words: Cixiidae, coconut, lethal yellowing, palm, survey, Caribbean

Resumen

Una nueva especie de chicharrita de la familia *Cixiidae* (Hemiptera: Fulgoroidea) del género *Oecleus* Stål, *Oecleus mackaspringi* **sp. n.**, se describe para Spring Garden, Jamaica. Este nuevo taxón está asociado con la palma de coco (*Cocos nucifera* L.) y se encontró durante el muestreo de parcelas de palma con casos activos de amarillamiento letal (LY). Este es el primer informe del género *Oecleus* para Jamaica. También se proporcionan datos de secuencia para el gen COI y el gen 18S.

Introduction

The genus *Oecleus* Stål, 1862 is a large taxon in the tribe Oecleini and is widespread throughout the new world (Bartlett *et al*. 2014, 2018). The Oecleini are recognized by lacking spines on the hind tibiae. In the United States and Canada, there are 45 species in the genus *Oecleus* and in the Neotropics, there are an additional 20 species, a total of 65 species (Bourgoin 2019). The diversity of *Oecleus* in the Neotropics is likely much higher than is currently known. The genus is easily recognized as possessing a greatly compressed vertex and five carinae (some appearing to have three) on the mesonotum. The genus as currently comprised has a wide range of host plants (Wilson *et al*. 1994). Other new-world genera in tribe Oecleini include *Antillixius* Myers, 1928, *Haplaxius* Fowler, 1904, *Notolathrus* Remes Lenicov, 1992, *Nymphocixia* Van Duzee, 1923, *Nymphomyndus* Emeljanov, 2007, *Proclytus* Emeljanov, 2007, and *Rhamphixius* Fowler, 1904. The close relationship of *Oecleus* to *Haplaxius* is important due to the role that *Haplaxius* plays in the epidemiology of palm lethal decline phytoplasmas and the unknown range of taxa within the cixiids that serve as competent vectors of the 16SrIV phytoplasmas.

Lethal yellowing (LY) is a lethal decline of various palm species (primarily coconut) and was first reported

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from Jamaica (Fawcett 1891) where it has been causing significant economic losses in coconut production since its discovery. While the vector of LY was determined to be *Haplaxius crudus* (Van Duzee) in Florida, USA (Howard *et al*. 1983), the transmission of the 16SrIV-A phytoplasma by this insect species has not been replicated elsewhere in the region, especially Jamaica. *Haplaxius crudus* is well documented from Jamaica, however, a wide variety of planthoppers are known from coconut palms (Wilson *et al*. 1994) with new taxa being discovered as a result of survey efforts in the Caribbean basin (Bahder *et al*. 2019). Any phloem-feeding auchenorrhynchan on coconut should be carefully investigated for their role in phytoplasma transmission, especially cixiids. Additionally, the cixiid *Hyalesthes obsoletus* Signoret is a vector of the 16SrXII-A phytoplasma, the causal agent of Bois Noir in grapevine (Boudon-Padieu 2003). The evolutionary relationship between cixiids and phytoplasmas in terms of vector competency is poorly studied, specifically with regard to the palm lethal decline group (16SrIV), due to the difficulty of working with palms and the inability to culture 16SrIV phytoplasmas in media or in alternate host plants. Regardless, due to the high diversity and abundance of both palms and cixiids throughout the Caribbean and the presence of various subgroups throughout the region (Myrie *et al*. 2019), the role that other cixiids aside from *H. crudus* play in the epidemiology of palm lethal decline should be investigated.

Herein, a new species of the genus *Oecleus* is described from coconut palm in Jamaica in an area with active spread of LY. Additionally, sequence data for the COI and 18S genes is provided.

Materials and methods

*Locality and specimen collection***.** Specimens were collected on May 27th, 2019 in Spring Garden, Portland Parrish, Jamaica by sweep net. Individuals were stored in 95% ethanol and shipped to BWB for identification and molecular classification. All specimens were collected from coconut palm (*Cocos nucifera* (L.)).

Morphological terminology and identification. Morphological terminology generally follows that of Bartlett *et al*. (2014) with wing venation following Bourgoin *et al*. (2015). Voucher specimens, including primary types, are stored at the University of Florida – Fort Lauderdale Research and Education Center (FLREC) in Davie, FL, U.S.A and the Florida State Collection of Arthropods (FSCA) in Gainesville, FL, U.S.A. Label information of type is quoted, with '/' indicating a new line. All specimens were measured and photographed using a Leica M205 C stereoscope. Images of specimens and all features photographed were generated using the LAS Core Software v4.12.

*Dissections and DNA extraction***.** The genitalia that were dissected also served as the source of tissue for DNA extraction. The terminal end of the abdomens with genitalia were removed and placed directly into a solution of tissue lysis buffer (buffer ATL) and proteinase K (180 µl ATL and 20 µl proteinase K) from the DNeasy® Blood and Tissue Kit (Qiagen). The genitalia was left to lyse for 24 hours at 56°C. Following lysis, eluate was transferred to a new 1.5 ml microcentrifuge tube and DNA extraction proceeded as per the manufacturer's instructions. The genitalia were then immersed in 200 µl of buffer ATL and 200 µl of buffer AL from the same kit and placed at 95°C for 24 hours to remove fat, wax, and residual tissue. The cleared genitalia was then used for morphological characterization and photography.

*PCR parameters, sequence data, and analysis***.** To obtain COI sequence data, DNA template from specimens was amplified using the primers LCO1490 (5'-GGTCAACAAATCATAAAGATATTG-3') and HCO2198 (5'-TCAGGGTGACCAAAAAATCA-3') (Folmer *et al*. 1994). To obtain 18S sequence data, the primers designed by Bahder *et al*. (2019) were used, 18S/Forward (5'-ACTGTCGATGGTAGGTTCTG-3') and 18S/Reverse (5'-GTCCGAAGACCTCACTAAA-3'). PCR reactions contained 5x GoTaq Flexi Buffer, 25 mM $MgCl_2$, 10 mM dNTP's, 10 mM of each primer (for both COI and 18S reactions), 10% PVP-40, and 2.5U GoTaq Flexi DNA Polymerase, 2 µl DNA template, and sterile dH_20 to a final volume of 25 µL. Thermal cycling conditions for COI were as follows: 5 min initial denaturation at 95°C, followed by 40 cycles of 1 min denaturation at 95°C, 30 sec annealing at 55°C, 1 min extension at 72°C, followed by a 5 min extension at 72°C. Thermal cycling conditions for 18S were as follows: 5 min initial denaturation at 95°C, followed by 40 cycles of 1 min denaturation at 95°C, 30 sec annealing at 59°C, 2 min extension at 72°C, followed by a 5 min extension at 72°C. All products were run on a 1.5% agarose gel stained with 1% GelRed (Biotium, Fremont, California, USA). PCR products of the appropriate size were purified using the Exo-SAP-IT™ PCR Product Cleanup Reagent (ThermoFisher Scientific, Waltham, Massachusetts, USA). Purified PCR product was quantified using a NanoDropLite spectrophotometer (ThermoFisher Scientific, Waltham, Massachusetts, USA) and sent for sequencing at Eurofins Scientific (Louisville, KY, USA). Contiguous files were assembled using DNA Baser (Version 4.36) (Heracle BioSoft SRL, Pitesti, Romania), aligned using ClustalW as part of the package MEGA7 (Kumar *et al.* 2016). A matrix of pairwise differences using number of differences among COI and 18S haplotypes were calculated with MEGA7 (Kumar *et al.* 2016).

Systematics

Order Hemiptera Linnaeus, 1758

Family Cixiidae Spinola, 1839

Subfamily Cixiinae Spinola, 1839

Tribe Oecleini Muir, 1922

Genus *Oecleus* Stål, 1862

Type species: *Oecleus seminiger* Stål, 1862: 306; by subsequent designation by Oshanin 1912: 117.

Modified diagnosis. Small to midsize (3.3–8.5 mm); large eyes and head narrower than pronotum in dorsal view. Vertex narrow, trough-like and parallel-sided, lateral and anterior margin carinate. Slightly raised; proximally narrowed and distally produced beyond eyes for a variable distance. In lateral view, apex of head acutely or obtusely angled, eyes emarginate, ocellus present under each eye and near midline above frontoclypeal suture. In frontal view, frons elongate and narrowing towards vertex, carina on midline of frons present (sometimes obsolete). Clypeus triangular to subtriangular. Antennae originating from a large socket, scape reduced to collar-like form, pedicel rounded with sensoria, flagellum beadlike basally and filamentous distally. Pronotum with irregular ridges, narrowest on midline, indented on posterior margin, carinate on posterior and lateral margins. Mesonotum longer at midline than vertex and pronotum combined, flattened with five longitudinal carinae. Carinae flanking midline sometimes reduced to pigmented lines. Hind tibiae lacking lateral spines. Forewings transparent, rarely with patterns, veins usually dotted with pustules, often bearing setae.

***Oecleus mackaspringi* sp. n.**
(Figures 2–8)

Type locality. Spring Garden, Portland Parrish, Jamaica

Diagnosis. A moderate sized species with five carinae on mesonotum and a projected head with yellow/orange color scheme in males and fuscous-yellow color scheme in females. Male terminalia with a broad, rounded ventral lobe of the pygofer, two processes on the ventral surface of the aedeagus pointed distally and two processes on the right lateral side of aedeagus (four total). Two processes on the flagellum.

Description. *Color*. Ground color of body uniformly stramineous with a triangular orange patch on the abdominal tergites with dark bands running laterally within the orange patch in dorsal view (males) (Fig. 2) while females are a lighter shade of yellow as the ground color with the dorsum of the abdomen slightly darkened and three longitudinal darkened bands running from the terminus of the abdomen to the metathorax with the lateral two bands lighter than the band running along the midline (Fig.2). *Structure.* Body length males: 7.96–7.99 mm (*n* =15) with wings; 5.42–5.45 mm without wings; females (*n*=16): 8.33–8.36 mm with wings; 7.01–7.04 mm without wings. <u>Head</u>. Anterior margin of head in lateral view pointed and slightly curved upward (Fig.3). Vertex extremely narrowed in dorsal view so that posterior margin is hidden by eyes, eyes meeting at posterior margin, then expanding slightly at the midline of eyes with the widest point beginning at anterior margin of eyes (Fig. 3).Vertex length males: 1.10–1.15 mm; females: 1.30–1.34 mm. Vertex width at hind margin males: 0.044–0.046 mm; females: 0.044–0.045 mm. Vertex width at distal margin males: 0.125–0.128 mm; females: 0.183–0.186 mm. Frons with lateral carinae strongly keeled and infuscate, median ocellus present above frontoclypeal suture, conspicuous in frontal view (Fig. 3). Lateral margins of frons sinuate and widest at the postclypeal suture, constricting at the midline then expanding briefly before slightly constricting again at the dorsal margin (Fig. 3). Transverse carina at juncture with vertex evident in frontal view (Fig. 3). Frons length males: 1.03–1.05 mm; females: 1.08–1.10 mm. Frons dorsal width males: 0.123–0.126 mm; females: 0.126–0.127 mm. Frons frontoclypeal margin width males: 0.418–0.420 mm; females: 0.421–0.422 mm. Clypeus length males: 0.387–0.390 mm; females: 0.412–0.415 mm.

FIGURE 1. Habitat in Spring Garden, Jamaica where *Oecleus mackaspringi* **sp. n.** was collected.

<u>Thorax</u>. Anterior margin of pronotum following posterior margin of head and posterior margin strongly concave in dorsal view (Fig. 3). In lateral view, posterior margin of pronotum sinuate. Pronotum length at midline males: 0.157–0.160 mm; females: 0.205–0.207 mm. Mesonotum with five carinae – lateral carinae closer to each other that to the midline carina. Indentations present on the inner lateral carinae near the posterior margin in dorsal view (Fig. 3). Mesonotum length at midline males: 1.50–1.52 mm; females: 1.68–1.70 mm. Mesonotum width males: 1.23–1.26 mm; females: 1.55–1.59 mm.

Wings transparent with conspicuous pustules along veins (Fig. 4). The radial vein is 3-branched and median vein 4-branched. The CuA is 2-branched and PCu joining with A1 at basal third of clavus. Forewing length males: 5.98–5.99 mm; females: 6.51–6.52 mm.

<u>Terminalia</u>. Pygofer in later view wide, widest on dorsum and narrowing dorsally (Fig. 5), distal margin convex and basal margin concave (Fig. 5). In ventral view, opening of pygofer bearing a subtriangular lobe, widest at the base an attenuating distally to a rounded apex (Fig. 5). Parameres in lateral view with three lobes. Dorsal lobe appearing tooth-like and sclerotized while distal lobe and ventral lobe more rounded (Fig. 6). In ventral view, parameres with subparallel margins basally and distally rounded with a lateral tooth on the inner margin (Fig. 6). Anal segment in lateral view with parallel dorsal and ventral margins. Apex angled downwards with constriction prior to expanding into a truncate terminus (Fig. 5). Aedeagus with two large lateral, anterior pointed spines on the right side with the larger spine arching ventrally then angled dorsally at the terminus (Fig. 7, 8) and the shorter spine arching dorsally then angled ventrally at the terminus (Fig. 6, 7). Pair of spines on the ventral side of aedeagus pointing posterior (Fig. 7, 8). Flagellum scaly in appearance with two large spines pointed towards the anterior and angled upward (Fig. 7, 8). Spines on flagellum slightly curved away from each other (Fig. 7, 8).

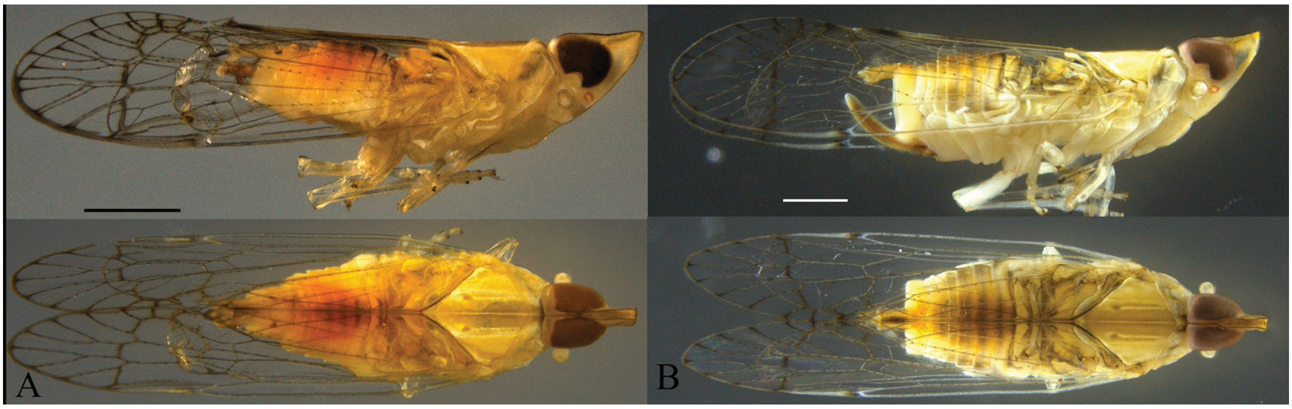

FIGURE 2. Adult habitus *Oecleus mackaspringi* **sp. n.**; A. male and B. female, scale=1mm.

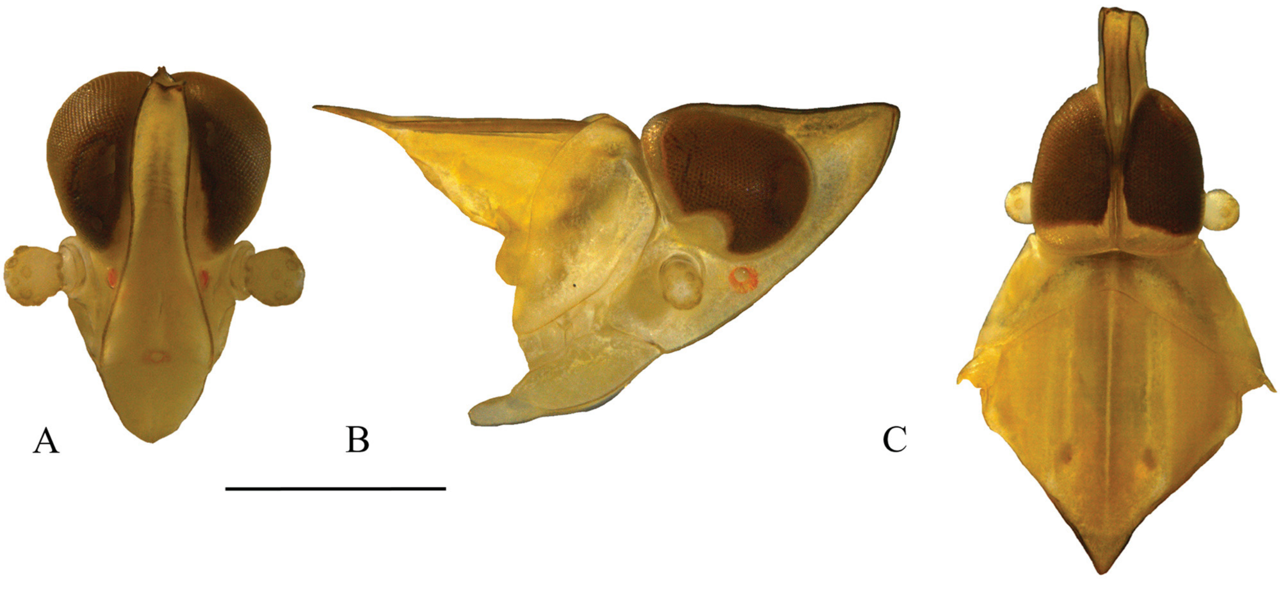

FIGURE 3. Adult male *Oecleus mackaspringi* **sp. n.**; A. head frontal view, B. head, pronotum, and mesonotum lateral view, C. head, pronotum, and mesonotum dorsal view, scale=1mm.

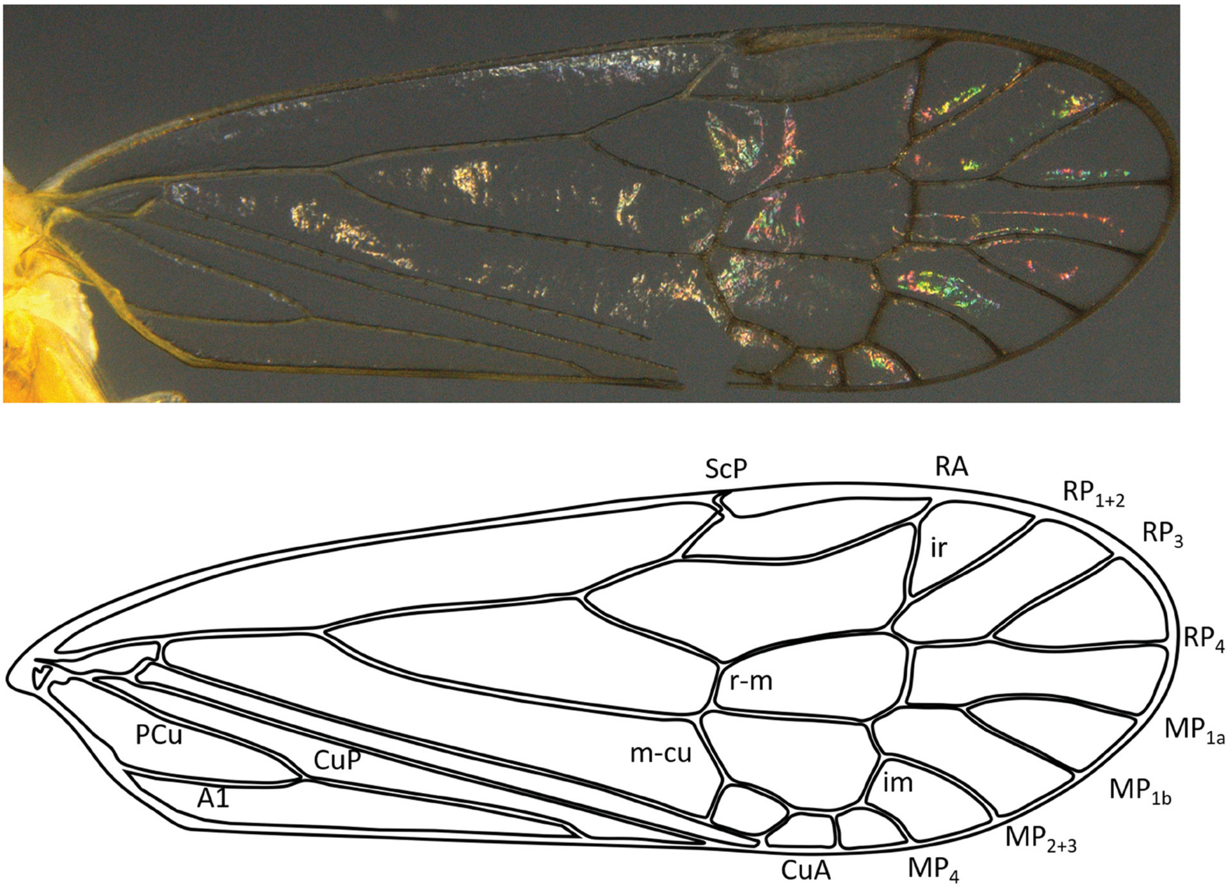

FIGURE 4. Forewing venation of *Oecleus mackaspringi* **sp. n.**

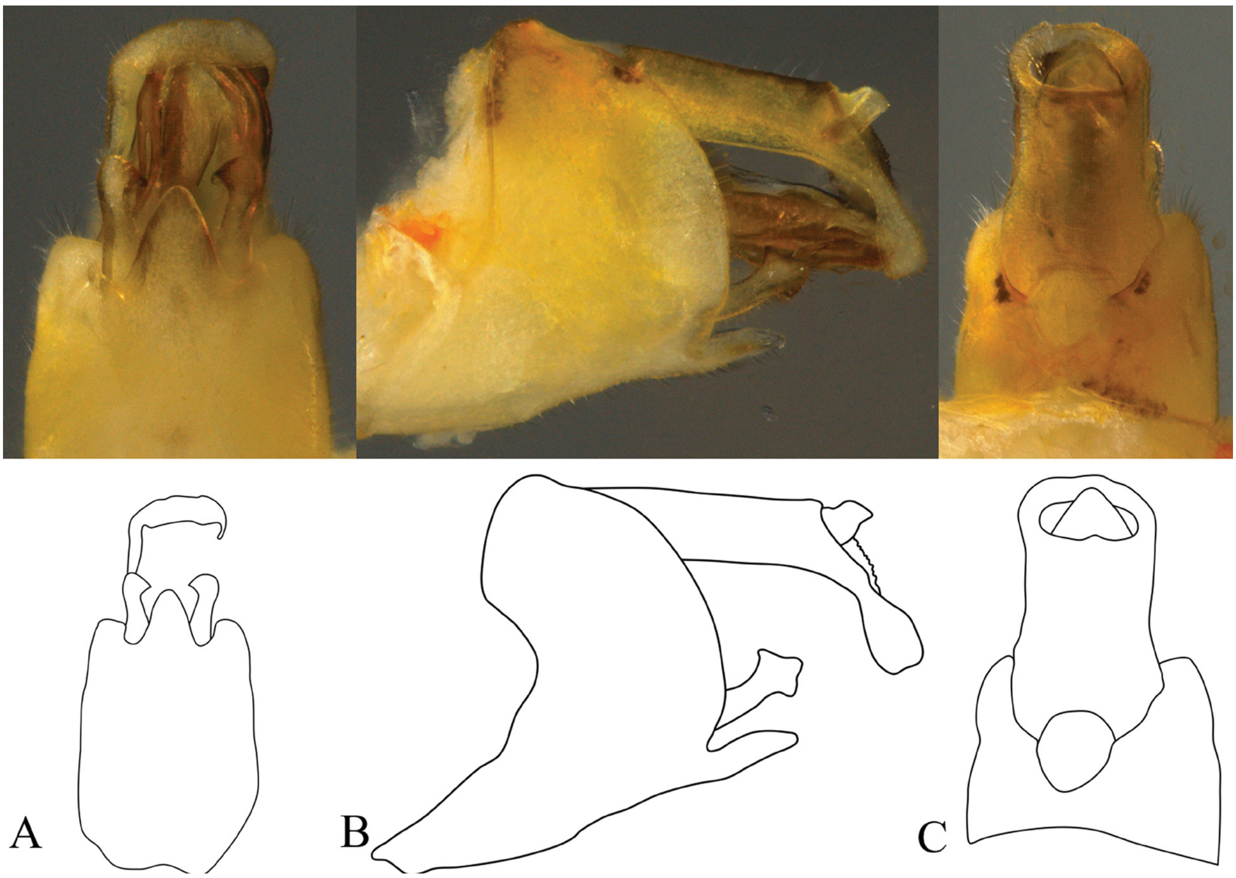

FIGURE 5. Male terminalia of *Oecleus mackaspringi* **sp. n.**; A. lateral view, B. ventral view, and C. dorsal view.

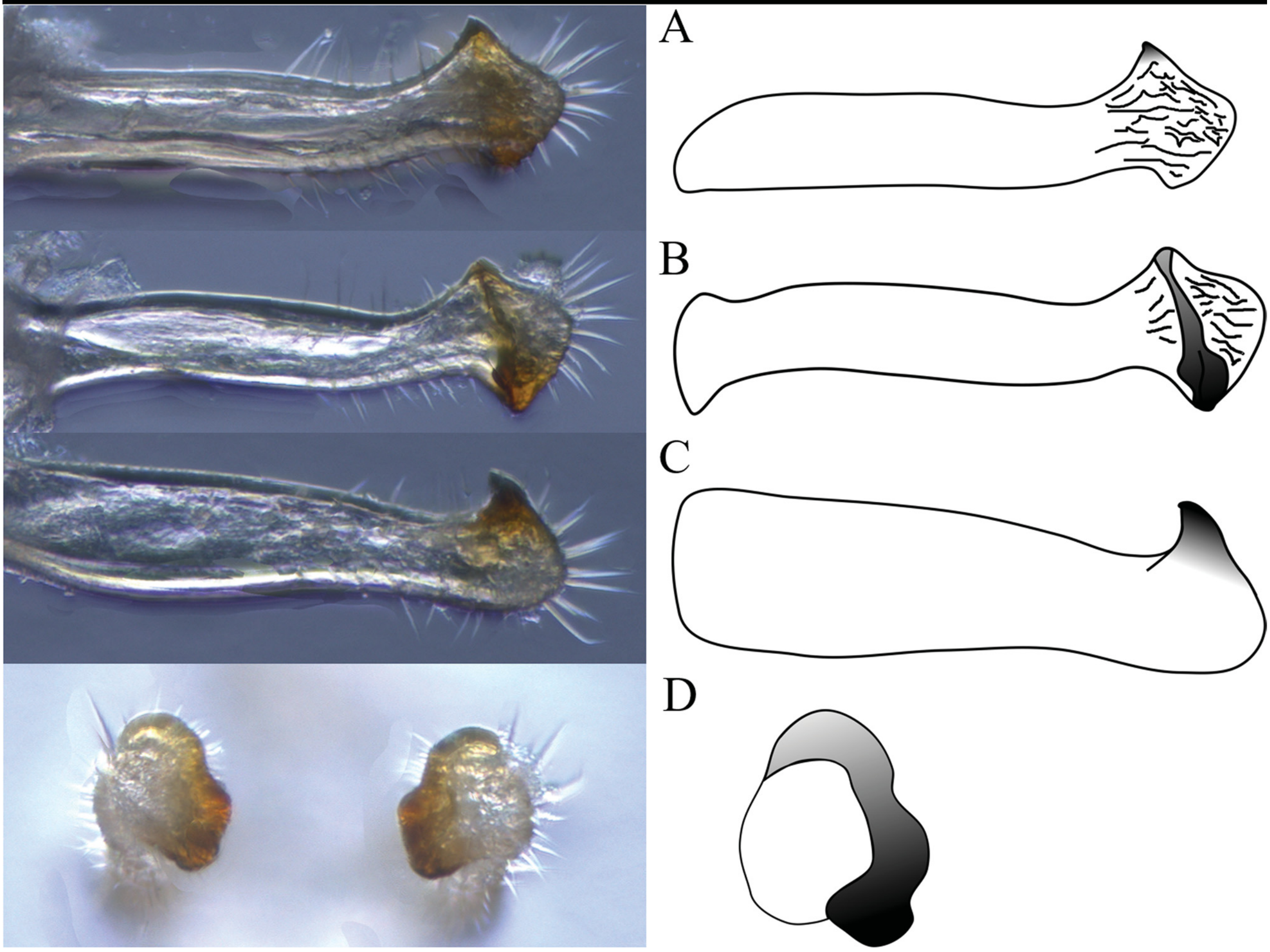

FIGURE 6. Paramere; A. exterior lateral view, B. interior lateral view, C. ventral view, D. caudal view.

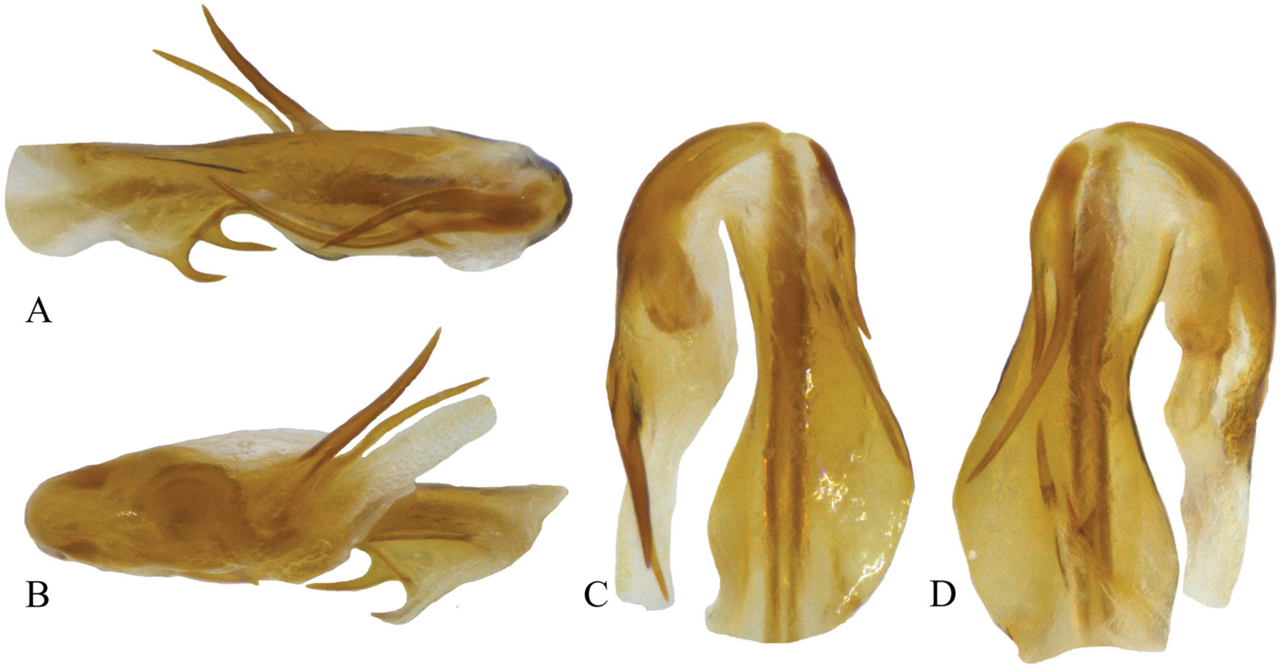

FIGURE 7. Aedeagus of adult male *Oecleus mackaspringi* **sp. n.**; A. right lateral view, B. left lateral view, C. dorsal view, D. ventral view.

Plant associations. Coconut palm, *Cocos nucifera*, Arecaceae

Distribution. Jamaica (Portland Parrish, Spring Garden)

Etymology. The specific name given is an amalgamation of the Jamaican slang “macka” which denotes something spiny and references the unique pair of spines on the ventral surface of the aedeagus as well as the locality where the species was discovered, Spring Garden.

Material examined. Holotype male “Jamaica, Portland Parrish / Spring Garden / 24-V-2019 / Host: *Cocos nucifera* / Coll.: W. Myrie/ Holotype/ /” (FLREC). Paratype males (n=14) and females (n=16) “Jamaica, Portland Parrish / Spring Garden / 24-V-2019 / Host: *Cocos nucifera* / Coll.: W. Myrie/”.

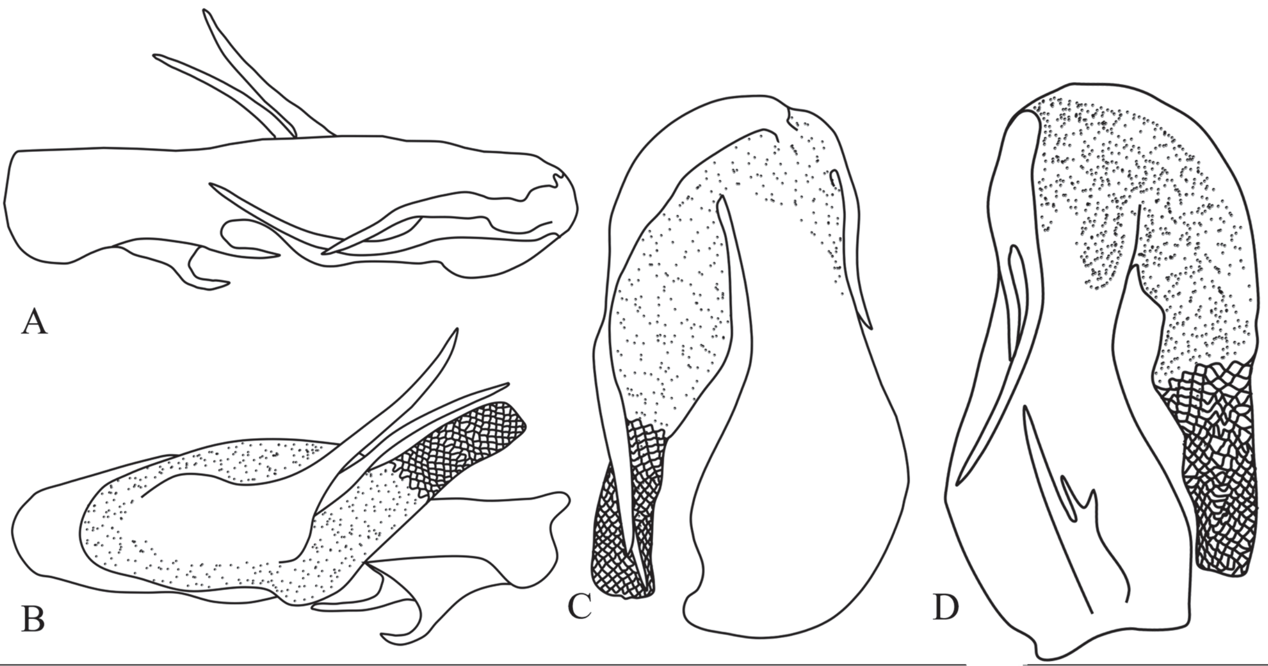

FIGURE 8. Aedeagus of adult male *Oecleus mackaspringi* **sp. n.**; A. right lateral view, B. left lateral view, C. dorsal view, D. ventral view.

Sequence data. For the COI gene, a 698 bp sequence was generated for *Oecleus mackspringii* **sp. n.** For the 5’ region of the COI gene that was amplified in this study, no data was available for any other taxa within the Oecleini for the region amplified. The closest taxa available at 100% query coverage was *Melanoliarus humilis* (Say) (as *Oliarus humilis*) (GenBank Accession No. KR562306.1) and was 83.3% similar (16.7% variance). For the 18S gene, a 1,354 bp product was generated for *Oecleus mackaspringi* **sp. n.** (GenBank Accession No. MN422261). Pairwise distances showed 0.9% difference from *Oecleus perpictus* Van Duzee (GenBank Accession No. JQ982515.1), about 2.2% different from the genus *Haplaxius*, 2.5% different from *Nymphomyndus* and 3.9% different from *Myndus taffini* Bonfils (Table 1). The Maximum Likelihood tree generated using the same 18S sequences showed both *Oecleus mackaspringi* **sp. n.** and *Oecleus perpictus* grouping together relative to the other Oecleini (Fig. 9).

TABLE 1. Pairwise comparison using Maximum Composite Likelihood method based on the 18S gene for various cixiid species within the Oecleini (bottom) and standard error (top)

	Species	1	2	3	4	5	6
1	*Oecleus mackaspringii* **sp. n.**		0.003	0.004	0.004	0.005	0.006
2	*Oecleus perpictus*	0.009		0.004	0.004	0.005	0.006
3	*Haplaxius crudus*	0.022	0.023		0.002	0.004	0.006
4	*Haplaxius pictifrons*	0.022	0.023	0.005		0.004	0.006
5	*Nymphomyndus caribbea*	0.025	0.027	0.015	0.016		0.006
6	*Myndus taffini*	0.039	0.038	0.045	0.043	0.045	

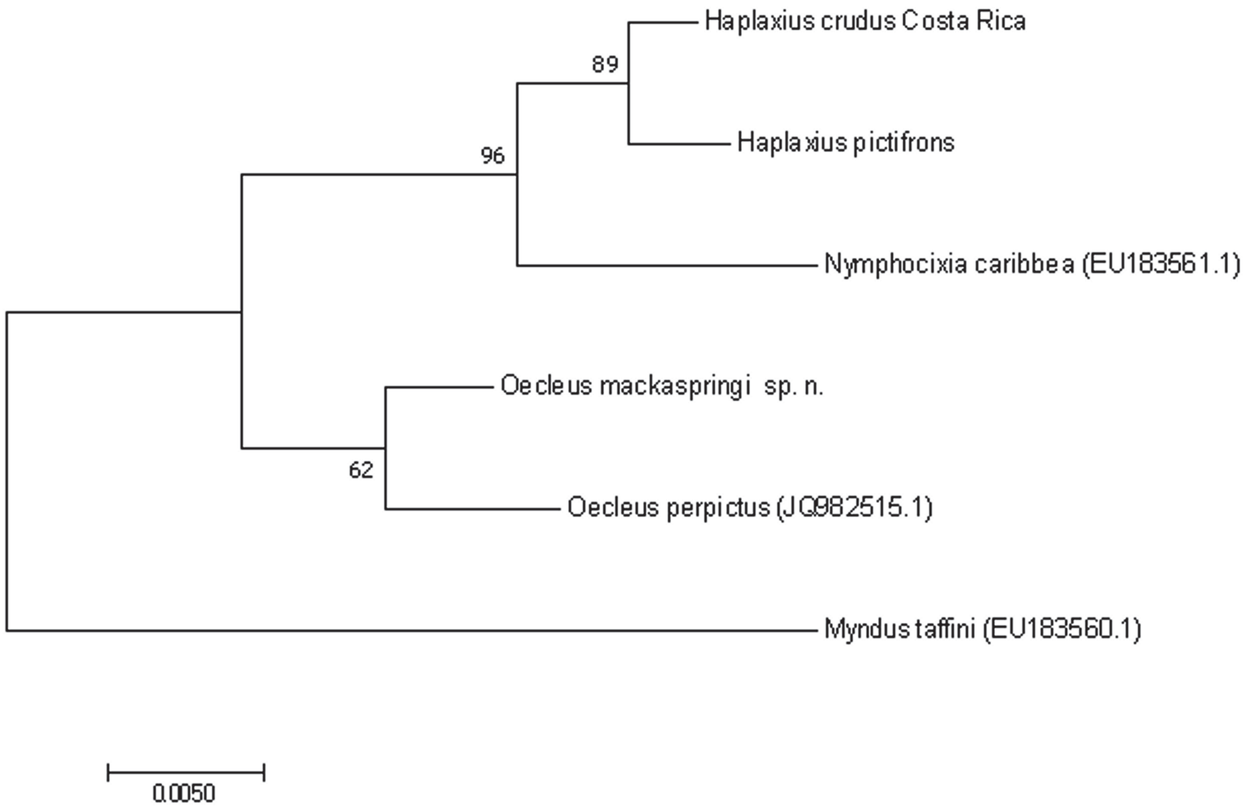

FIGURE 9. Maximum Likelihood phylogenetic tree based on 18S sequence data. Branch support was assessed using 1,000 bootstrap replicates with *Haplaxius*, *Myndus*, and *Nymphomyndus* representing outgroups within the Oecleini.

Remarks. The general form of the aedeagus in *Oecleus mackaspringi* **sp. n.** is similar to many of the species illustrated by Kramer (1977), however, the arrangement and positioning of the spines in *Oecleus mackaspringi* **sp. n.** appear unique. The closest in terms of aedeagus structure appears to be *Oecleus lyra* Kramer, but this species is described as darker with some markings on the wing which the new taxon lacks. The parameres and process on the ventral margin of the pygofer of *Oecleus mackaspringi* **sp. n.** are very similar to many other species of *Oecleus* and agree with the assertion by Ball & Klingenberg (1923) that these features are not reliable for species identification in *Oecleus*. What does appear unique to *Oecleus mackaspringi* **sp. n.** and distinguishes it as a new species in the ventral pair of spines present on the aedeagus.

Discussion

Oecleus as currently defined is rather broad and because of this, the wide geographic range of the genus as currently defined and the high number of species, it is possible that *Oecleus* is not monophyletic and an in-depth analysis of the taxa assigned to the genus is needed. However, it is acknowledged by Ball & Klingenberg (1923) that the form of the parameres, which is highly informative in other taxa, is not useful in species identification within *Oecleus*. The parameres in *Oecleus* as reported by Ball & Klingenberg (1923) and Kramer (1977) exhibited a club-like appearance with a sclerotized spine, which the novel taxon clearly exhibits. Due to the structure of the parameres observed in the novel taxon in combination with the highly constricted vertex and the presence of five carinae, the novel taxon clearly fits within *Oecleus* as currently defined. The molecular analyses were very preliminary and based on very few taxa and not meant as definitive for placement of the novel taxon in *Oecleus*. The molecular data was provided so that in future works, the data presented herein can be built upon as more taxa are sequenced and analyzed more thoroughly. Despite the lack of sequence data for many closely related taxa, the data that is available seems to support the novel taxon being placed in the genus *Oecleus*. The description of *Oecleus mackaspringi* **sp. n.** brings the species number to 66 for the genus *Oecleus* and represents the first documented species of this genus from Jamaica.

Due to the diversity of this genus in the southwestern United States and Mexico (Kramer 1977, Caldwell 1944) and the expected undiscovered diversity throughout the Neotropics, the documentation of a novel taxon from Jamaica is not unexpected.

The novel taxon was encountered while searching for *H. crudus* in plots of coconut that were affected by LY. Specimens were collected from coconut palms and interestingly, somewhat resemble *H. crudus* in color scheme with males being smaller, yellow/orange and the females larger, yellow and with a higher degree of sclerotization on the body resulting in an overall darker coloration. Due to *Oecleus mackaspringi* **sp. n.** occupying a seemingly similar niche, at least for adults, as *H. crudus* and the fact that *Oecleus* is within the same tribe as *Haplxaius*, future efforts should determine if *Oecleus mackaspringi* **sp. n.** can carry palm infecting phytoplasmas and assess the role it plays in the epidemiology of LY in Jamaica. In similar survey work conducted in Brazil, *Oecleus sergipensis* Bartlett, Dos Passos, Gonçalves da Silva, Diniz & Dollet was a newly discovered species from coconut palm (Bartlett *et al*. 2018). While LY is not known from Brazil and no specimens of *O. sergipensis* were tested for phytoplasma, Brazil is at risk for the introduction and spread of LY due to large scale coconut production in Northern Brazil as well as the presence of *H. crudus* in the region (Silva *et al*. 2019). Because of this, documenting cixiid diversity on coconut palm is essential and the discovery of two new species of *Oecleus* from Brazil and Jamaica is interesting and potentially important from an epidemiological standpoint.

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