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A new species of planthopper in the genus *Bothriocera* (Hemiptera: Auchenorrhyncha: Cixiidae) from coconut palm (*Cocos nucifera*) in Jamaica

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Abstract

Recent survey work in Jamaica on palm-associated planthoppers seeks to identify putative vectors of the lethal yellowing phytoplasma. Herein, a new species of planthopper, *Bothriocera harthi* **sp. n.**, is described from coconut palm. Molecular data for the cytochrome *c* oxidase subunit I (COI), 18S rRNA gene, histone 3 (H3) gene, and 28S rRNA gene is provided to support placement of the novel taxon in *Bothriocera*. These findings are important because it provides novel data to help better understand the diversity and evolution of this unique group of planthoppers.

Key words: biodiversity, Caribbean, barcoding, taxonomy, phylogeny

Resumen

Durante un reciente trabajo de investigación en Jamaica sobre chicharritas asociados a las palmeras busca identificar posibles vectores del fitoplasma amarillento letal. En este documento, se describe el nuevo taxón, *Bothriocera har*thi **sp. n.**, de la palmera de coco. Se proporcionan datos moleculares para la subunidad I del citocromo c oxidasa (COI), el gen 18S rARN, el gen de la histona 3 (H3) y el gen 28S rARN para corroborar la ubicación del nuevo taxón en en Bothriocera. Estos hallazgos son importantes porque proporcionan datos novedosos para ayudar a comprender mejor la diversidad y evolución de este grupo único de chicharritas.

Introduction

The genus *Bothriocera* Burmeister currently comprises 45 species (Bourgoin 2023). *Bothriocera* is one of six genera (four extinct, two extant) in the tribe Bothriocerini, which until recently was placed in the subfamily Bothriocerinae but has recently been subsumed into Cixiinae (Luo et al 2021), a placement in line with the recent suggestion that Bothriocerini may be allied with the Oecleini (Le Cesne *et al.* 2022).

The extant genera of Bothriocerini consist of the New World *Bothriocera* and *Bothrioceretta* Caldwell. The Bothriocerini are readily diagnosed by the location of the antennae, which are directly in front of the eyes (with the lateral ocelli above the antennae at the anterodorsal edge of the eyes), and the head anterior to the eye forming a reversed "C"-shaped cavity within which the antennae rest (e.g., Fig 3C). *Bothrioceretta* is a poorly known genus comprising four Mesoamerican species putatively differing from *Bothriocera* by the presence of a 'prominent transverse carina' at midlength of the vertex (in addition to an apical carina), the head not as forward-produced as *Bothriocera*, and by the wing orientation (forewings distinctly overlapping apically in *Bothrioceretta* and slightly

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—or not—overlapping in *Bothriocera*), and unspecified features (in Caldwell 1950) of the male terminalia, which we interpret to include the form of the medioventral process of the pygofer (elongate in *Bothrioceretta* and short in *Bothriocera*) and the aedeagus with the flagellum bearing slender, internal processes in *Bothriocera* that are lacking in *Bothrioceretta*).

The highest diversity of *Bothriocera* is in Mesoamerica and the Caribbean, with ten species known from the continental United States (Kramer 1983, Bartlett *et al*. 2014), and (by our count, see also O'Brien 2006) about 15 species in Mesoamerica, 25 species in the Caribbean region and five species from South America (including some doubtful records; Bourgoin 2023). The genus as a whole has never been revised and some species are incompletely described, circumstances that can lead to taxonomic challenges. The relatively high diversity of *Bothriocera* species in the Caribbean may be partially an artifact of greater taxonomic effort in that region (e.g., by Fennah 1943, 1971).

Many *Bothriocera* have patterning on the wings that may be distinctive (as asserted by Caldwell 1943) but possess considerable variability, e.g., see notes in Kramer (1983: 27) concerning *B. drakei* where specimens from Florida "are unaccountably lighter in color than those from other parts of its range". The best features for species diagnosis appear to be those of the male terminalia.

Recent survey efforts in Jamaica focusing on putative vectors of lethal yellowing (LY) (a fatal phytoplasma infection of palms have resulted in the discovery of multiple new species of planthoppers in the families Cixiidae (Myrie *et al*. 2019, Bahder *et al*. 2023) and Derbidae (Bahder *et al*. 2020). The cixiid species *Haplaxius crudus* Van Duzee (Cixiinae: Oecleini) is a known vector of LY (Howard & Thomas 1980) and was originally described from Jamaica (Van Duzee 1907). While the ability of other species of *Haplaxius* and closely related genera remains unverified, understanding what species of Cixiidae are associated with palms in areas where LY is considered endemic is an important aspect of elucidating the evolutionary relationships among cixiids and their ability to transmit LY and closely related strains. Herein, a novel species of *Bothriocera* is described from palm survey efforts seeking potential LY vectors, with accompanying molecular data to test genus-level placement.

Materials and Methods

Locality and Specimen Collection. Individuals of the novel taxon were collected by sweeping coconut palms, aspirated, and immediately transferred to 95% ethanol. Specimens were collected at a site on the northeastern coast of Jamaica labeled Hart Hill (Fig. 1) on 16-II-2021, Saint Mary Parish, Jamaica (18.222908, -76.601503), and imported to the U.S.A. under permit number P526-170201-001. All specimens collected were measured, photographed, and dissected using a Leica M205 C stereoscope and Leica DFC25 camera. Images of specimens and all features photographed were generated using the LAS Core Software v4.12. 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.

Morphological terminology and identification. Morphological terminology generally follows Kramer (1983) except with male terminalia nomenclature updated after Bourgoin (1988) and Bourgoin & Huang (1990) and forewing venation following Bourgoin *et al*. (2015). New taxa are to be attributed to Bahder and Bartlett. Kramer (1983) uses the term 'transverse carina' to refer to the carinae of the vertex extending near the anterior margin of the eyes that meet at the vertex midline, but this structure might be homologous to the carinae we had previously called sublateral carinae (e.g., Myrie *et al.* 2023), and that Locker *et al.* (2006, fig. 1E) termed subapical carina. Here we are adopting 'subapical transverse carina' for this structure, and intend this label to be understood as topological rather than as a hypothesis of homology until the nature of this structure is clarified.

Dissections and DNA Extraction. The terminalia that were dissected also served as the source of tissue for DNA extraction. The terminal end of the abdomen was 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 abdomen was left to lyse for 24 hours at 56°C. Following lysis, the eluate was transferred to a new 1.5 ml microcentrifuge tube and DNA extraction proceeded as per the manufacturer's instructions. The terminalia 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 terminalia were then used for morphological characterization and photography.

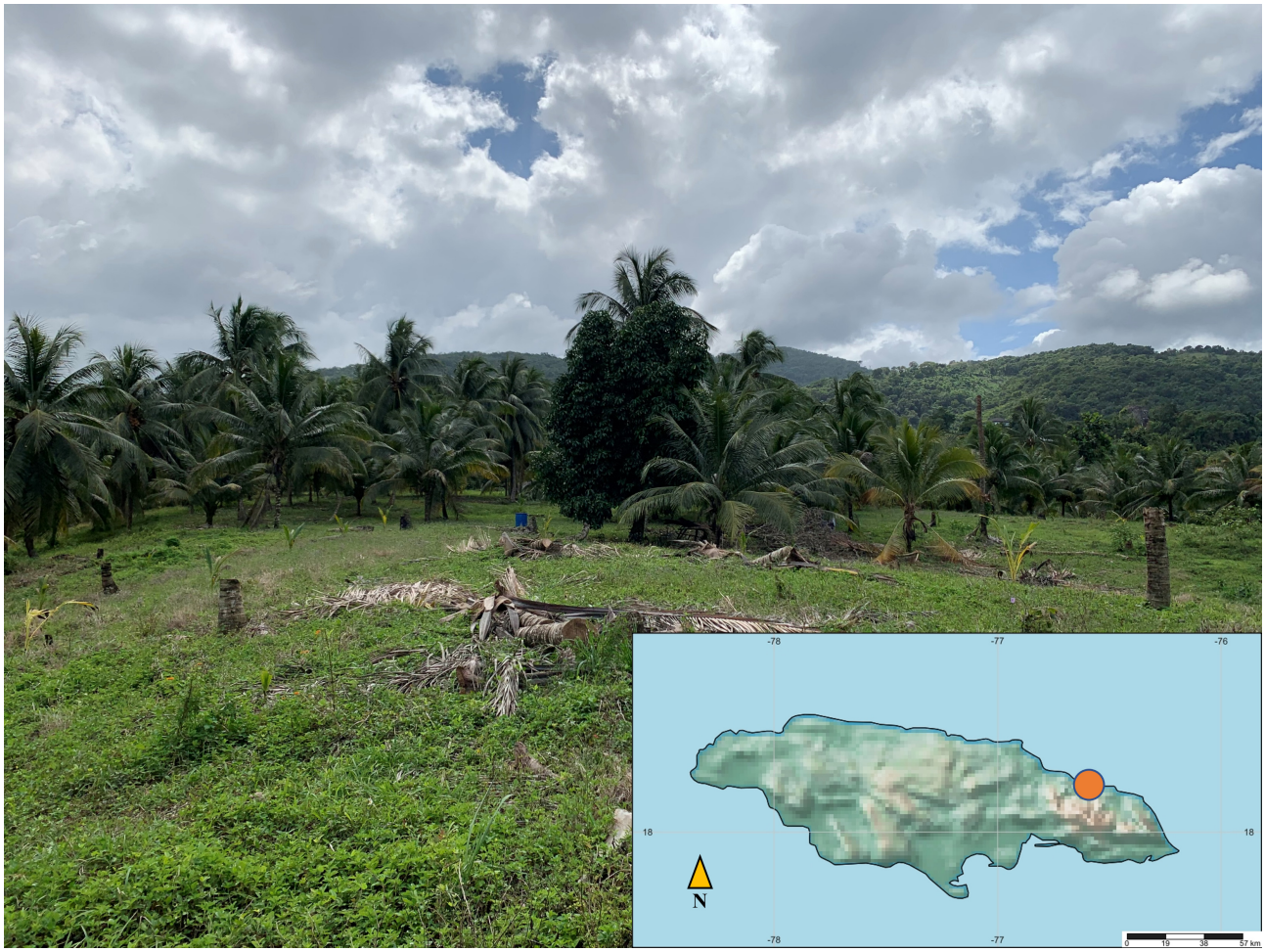

FIGURE 1. Habitat and locality of *Bothriocera harthi* **sp. n.**

PCR Parameters, Sequence Data, and Analysis. To obtain COI, 18S, H3, and 28S sequence data, previously published primers were used in all PCR reactions (Table 1). PCR reactions contained 5x GoTaq Flexi Buffer, 25 mM $MgCl_2$, 10 mM dNTP's, 10 mM of each primer, 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 all loci involved were as follows: 2 min initial denaturation at 95°C, followed by 35 cycles of 30-sec denaturations at 95°C, 30-sec annealings, and extension at 72°C. Specific annealing temperatures and extension times for respective loci are presented in Table 1. Products were visualized on a 1.5% agarose gel stained with GelRed (Biotium). PCR products of the appropriate size were purified using the ExoSAP-IT™ Express PCR Product Cleanup Reagent per the manufacturer's protocol (ThermoFisher Scientific, Waltham, Massachusetts, USA). The purified PCR product was quantified using a NanoDrop Lite Spectrophotometer (ThermoFisher Scientific, Waltham, Massachusetts, USA) and sequenced using the SeqStudio Genetic Analyzer (Applied Biosystems). Contiguous files were assembled using DNA Baser (Version 4.36) (Heracle BioSoft SRL, Pitesti, Romania), and aligned using ClustalW as part of the package MEGA7 (Kumar *et al.* 2016). Maximum Likelihood trees were generated using the Bootstrap method at 1,000 replicates based on the Tamura-Nei model for both the COI, 18S, and H3 loci as well as the consensus tree with concatenated data for COI, 18S, 28S and H3 data. A matrix of pairwise differences using the number of differences among 18S for a subset of taxa within each genus was calculated with MEGA7 (Kumar *et al.* 2016). The bootstrap method was used for variance estimation at 1,000 replicates and using the p-distance model.

Taxon Sampling. For morphological comparisons and molecular analyses, five species of *Bothriocera* (*B. basalis* Metcalf, *B. datuna* Kramer, *B. drakei* Metcalf, *B. maculata* Caldwell, and *B. transversa* Caldwell) were included as in-group taxa. For out-group comparisons, four species of Oecleini (*Myxia belinda* Bahder & Bartlett, *Oecleus mackaspringi* Bahder & Bartlett, *Nymphocixia unipunctata* Van Duzee, and *Haplaxius crudus* (Van Duzee)),

and one Pentastirini (*Melanoliarus maidis* (Fennah)) were used. GenBank accession numbers for all in-group and out-group taxa are presented in Table 2.

TABLE 1. Primers used to amplify corresponding gene regions used to assess the placement of novel taxon and PCR parameters for each locus.

Locus	Primer	Direction	Sequence (5' 3')	Annealing	Extension	Reference
COI	COI_D1_F C1-J-2195RC	Forward Reverse	GGAACWATAAGAAGWATAATYATYCG ACTTCTGGATGACCAAAAAATCAA	40˚C	1 min. 30 sec.	Humphries *et al.* 2021
18S	18Sfullforward 18SR	Forward Reverse	GGATAACTGTGGTAATTCTAG GTCCGAAGACCTCACTAAA	50˚C	2 min.	Urban & Cryan 2012 Bahder *et al.* 2019
H3	H3F2 H3R	Forward Reverse	GKAARTCSACCGGHGGHAARGC GTKACHCKCTTRGCGTGRAT	55˚C	30 sec.	This study Echavarria *et al.* 2021
28S	V X	Forward Reverse	GTAGCCAAATGCCTCGTCA CACAATGATAGGAAGAGCC	55 ˚C	1 min. 30 sec.	Urban & Cryan 2012

TABLE 2. Molecular taxon sampling and GenBank accession numbers.

Species	Locus			
	COI	18S	H3	28S
Bothriocera harthi **sp. n.**	OR115602	OR120252	OR133714	OR116974
Bothriocera basalis	OR115604	OR120254	OR133717	OR116975
Bothriocera datuna	OR115603	OR120253	OR133715	OR116979
Bothriocera drakei	OR115605	OR120256	OR133716	OR116976
Bothriocera maculata	OR115606	OR120255	OR133718	OR116978
Bothriocera transversa	OR115607	OR120257	OR133719	OR116977
Haplaxius crudus	MT080285	MT002393	MZ274037	OR116983
Myxia belinda	MT900605	MN200096	MZ274041	OR116981
Nymphocixia unipunctata	OM264284	OM258690	OM262389	OR116982
Oecleus mackaspringi	MN488999	MN422261	MZ274045	OR116980
Melanoliarus maidis	OP871036	OP889235	OP896208	OR116984

Systematics

Family Cixiidae Spinola, 1839

Subfamily Cixiinae Spinola, 1839 (following Luo *et al.* 2021)

Tribe Bothriocerini Muir, 1923

Genus *Bothriocera* Burmeister, 1835

Type species: *Bothriocera tinealis* Burmeister, 1835.

Diagnosis. (modified from Kramer 1983) Small to medium-sized (3.9–5.8 mm); head in dorsal view narrower than pronotum, broad and subrectangular, strongly produced beyond anterior margin of eyes, its anterior margin concave and as broad or broader than its interocular width at base, lateral and posterior margins of vertex carinate, subapical transverse carina between anterior margins of eyes medially joining median carina extending anteriorly onto frons (median carina of vertex absent posterior to subapical transverse carina), posterior margin of vertex

nearly transverse between eyes. In lateral view, head declinate to level of antennae, antennae arising from a deep elongated depression in front of eye formed by lateral carinae of the frons defining a foliate, inverted C-shaped concavity, ocellus prominent above antennae in front of anterodorsal margin of eye. Head from frontal view with sides of frons and clypeus carinate, dorsolateral portions of frons expanded and auriculate and level of antennae, longitudinal midline of frons carinate only on upper half, frontal ocellus prominent (ventral margin of frons may bear a variably expressed broadly U-shaped ridge above frontoclypeal suture). Frontoclypeal suture approximately transverse. Longitudinal midline of clypeus incompletely carinate. Pronotum extremely narrowed medially (may be entirely concealed medially by hind margin of head). Mesonotum elongated, tricarinate. Hind tibiae without spines before apex. Forewings broadly spatulate, held broadly tectiform only slightly (or not) overlapping on inner margin (versus more broadly overlapped in *Bothrioceretta*), usually boldly patterned, veins without prominent setae-bearing pustules. Male pygofer bearing short medioventral lobe (approximately as tall as wide, not elongated as in *Bothrioceretta*). Phallus bearing varied but few processes or projections from shaft, endosoma (flagellum of Kramer 1983) elongate, sinistrally curving into a partial helix, bearing varied processes.

Remarks. The position of the antennae (in front of the eye in a distinct cup-like concavity) readily separates Bothriocerini from all other New World cixiids. The only extant genera of Bothriocerini are *Bothriocera* and *Bothrioceretta*. *Bothrioceretta* is putatively distinguished from *Bothriocera* by the presence of a 'prominent transverse carina' at midlength of the vertex (in addition to the subapical transverse carina), head not as forward-produced as in *Bothriocera*, and by wing orientation (forewings distinctly overlapping apically in *Bothrioceretta* and slightly (or not) overlapping in *Bothriocera*), and the male terminalia (the medioventral process of the pygofer elongate in *Bothrioceretta* and short in *Bothriocera,* and the aedeagus with flagellum bearing processes in *Bothriocera* that are lacking in *Bothrioceretta*). It also appears that *Bothrioceretta* includes species that tend to be dark in color (both wings and body).

Bothriocera harthi Bahder & Bartlett sp. n.

(Figures 2–6)

Type Locality. Black Hill, Portland Parish, Jamaica.

Diagnosis. Medium-sized (for the genus) with strongly patterned forewings bearing a fuscous transverse band from stigma to apex of clavus and second highly irregular band along nodal line. Medioventral lobe of pygofer slightly wider than tall, rounded with slight point at apex. Phallus without processes along shaft, endosoma elongate, helically curved in sinistral direction bearing elongated basal projection (with 2 elongate subapical teeth) on left side and broad 'fin-like' projection on right side. Anal tube broadened subapically in left lateral view.

Description. *Color*. General body color brown (Fig. 2), anterior portion of head darker, posterior region and genae paler, intercarinal regions of mesonotum slightly paler than lateral region, ventrally more pallid to testaceous on legs; forewings (Fig. 4) with veins dark, cells translucent, bearing strong fuscous pattern: small dark patch around apex of Sc, broad, irregular transverse fuscous band from stigma to claval apex, very irregular transverse band at nodal level including apices of RA, RP and CA veins, small patch in cell C3aa.

Structure. Body length male (n = 2): 5.21 mm with wings; 3.28 mm without wings (Table 3), female 5.25 mm with wings, 3.31 mm without.

Head. In dorsal view (Fig. 3A), vertex broadly pentagonal, approximately 2X wide as long at midpoint, subapical transverse carinaat fastigium distinct, jointing medially in broadly obtuse angle, connecting to median carina of frons, anterior margin concave (except projected at median carina), posterior margin of head broadly concave (nearly truncate medially). In frontal view (Fig. 3B) frons broadly and irregularly quadrate, laterally expanded at antennae (widest at midpoint of eye), median carina becoming obsolete ventrad; ventral margin of frons with broadly U-shaped ventral ridge. Median ocellus prominent. Frontoclypeal suture nearly transverse (slightly convex). In lateral view (Fig. 3C), head projected in front of eyes, margins irregularly sinuate, vertex declinate to level of antennae, lateral margins of frons forming reverse C-shaped concavity ahead of antennae, antennae subtended by genal carina from ventroanterior margin of eye to ventral margin of cup formed by lateral margins of frons. Clypeus, from anterior view triangular with incomplete median carina. Antennae short, scape very short and obscure, pedicle emarginate, appearing horseshoe-shaped in lateral view (bearing many sensory plaques) with central bristle-like flagellum with a bulbous base. Lateral ocelli prominent above antennae and anterior to dorsal margin of compound eyes. Compound eyes dorsoventrally elongate, anterior margin concave, posterior margin truncate.

FIGURE 2. Adult male habitus of *Bothriocera harthi* **sp. n.**: A) dorsal view and B) lateral view; scale bar = 1 mm.

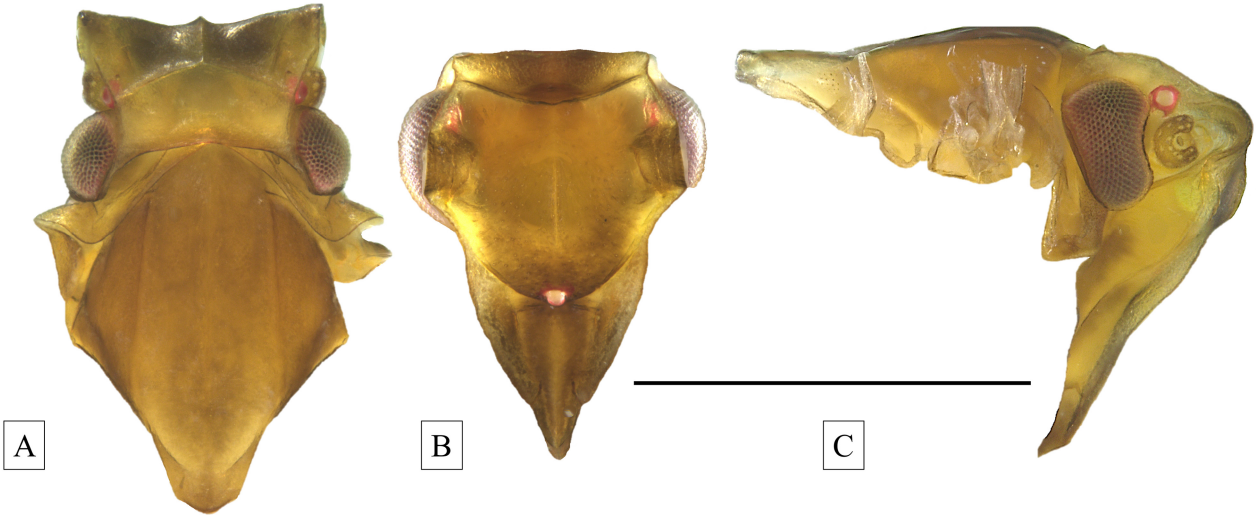

FIGURE 3. Adult male *Bothriocera harthi* **sp. n.**: A) head, pronotum and mesonotum in dorsal view, B) head in frontal view and C) head, pronotum and mesonotum in lateral view; scale bar = 1 mm.

TABLE 3. Biometric data for *Bothriocera harthi* **sp. n.**

Character	Male (*n*=2)		Female (*n*=2)	
	Range	Average ± SE	Range	Average ± SE
Body length, with wings	5.21–5.21	5.21±0.0	5.25–5.25	5.25±0.0
Body length, no wings	3.28–3.28	3.28±0.0	3.31–3.32	3.31±0.1
Forewing length	4.30–4.30	4.30±0.0	4.61–4.61	4.61±0.0
Vertex length	0.23–0.23	0.23±0.0	0.25–0.25	0.25±0.0
Vertex width, basal margin	0.66–0.66	0.66±0.0	0.67–0.67	0.67±0.0
Vertex width, distal margin	0.52–0.52	0.52±0.0	0.52–0.52	0.52±0.0
Pronotum length, midline	0.05–0.05	0.05±0.0	0.05–0.05	0.05±0.0
Mesonotum length, midline	1.09–1.09	1.09±0.0	1.09–1.09	1.09±0.0
Mesonotum width	1.06–1.06	1.06±0.0	1.07–1.07	1.07±0.0
Frons width, dorsal margin	0.56–0.56	0.56±0.0	0.56–0.56	0.56±0.0
Frons width, clypeal suture	0.49–0.49	0.49±0.0	0.50–0.50	0.50±0.0
Frons width, widest	0.72–0.72	0.72±0.0	0.72–0.72	0.72±0.0
Frons width, narrowest	0.49–0.49	0.49±0.0	0.50–0.50	0.50±0.0
Frons length, midline	0.61–0.61	0.61±0.0	0.61–0.61	0.61±0.0
Clypeus length	0.43–0.43	0.43±0.0	0.43–0.43	0.43±0.0

Thorax. Pronotum very short medially, mostly hidden beneath posterior margin of head (Fig. 3A), deeply concave on posterior margin; paradiscal region foliate in front of tegulae, lateral margin, in lateral view broad, apex squared off well below lower level of compound eyes. Mesonotum elongated, much longer than wide (about 2.5x length of head at midline; Fig. 3A), width approximately 2/3 length at midpoint, tricarinate with lateral carinae evident (subparallel, appearing to reach hind margin), median carina weak, in lateral view mesoscutum convex, inclected slightly upward at scutellum. Forewing relatively short and broadly spatulate (Fig. 4), stigma distinct, claval apex before wing midlength, apex rounded, weakly projected near apex of RP_4; crossveins r-m and m-cu in proximal half; branching pattern; RA 2-branched, RP 3-branched, MP 5-branched, CuA 2-branched.

Terminalia. Pygofer in lateral view (Fig. 5A) irregularly triangular, shortest dorsally, expanded to midlength, narrowed to ventral margin (caudal margin appearing broadly rounded), dorsal margin rounded, posterior margin broadly rounded, anterior margin irregularly sinuate. In ventral view (Fig. 5B) medioventral process present, rounded, just wider than long bearing slight point at apex. Gonostyli in lateral view (Fig. 5A) narrowest basally, angled dorsad near midlength, then expanding to rounded apex, strongly concave along ventral margin in distal half; in ventral view (Fig. 5B), narrowest proximally, expanding distad, medial concavity near midlength point on inner lateral margin. Aedeagal shaft simple (without lateral or apical processes), cylindrical (Figs. 6 & 7), approximately straight; endosoma elongate (longer than aedeagal shaft) and helically curved in sinistral direction distally narrowed to blunt, membranous apex; two primary processes arising subapically, the first (F1) arising on left lateral margin near endosoma base, comprised primarily of a process sclerotized along leading margin (otherwise membranous), curving ventrad on right lateral side with two sclerotized elongate teeth (F1a and F1b) arising subapically, F1a slightly longer and more slender than F1b; second process (F2) broadly falcate ("fin-like"), weakly sclerotized, with irregular serrulations along trailing margin; distal region of endosome bearing slender, elongated sclerotized internal rod (F3) extending nearly to apex. Anal segment in lateral view (Fig. 5A) elongated, distally downcurved; narrow basally, dorsal, and ventral margins subparallel, curved ventrad at 1/3 length, constricting slightly, then expanding near apex, generally rounded with slight point at apex; in dorsal view (Fig. 5C), broad, lateral margins sinuate, apex irregularly sinuate and slightly asymmetrical; paraproct conical, short and stout.

Plant associations. Coconut palm (*Cocos nucifera* L.).

Distribution. Black Hill, Saint Mary Parish, Jamaica.

Etymology. The specific name is given in reference to the field site where the holotype was collected (Hart Hill) that was amalgamated as "harthi". The specific name is intended to be indeclinable.

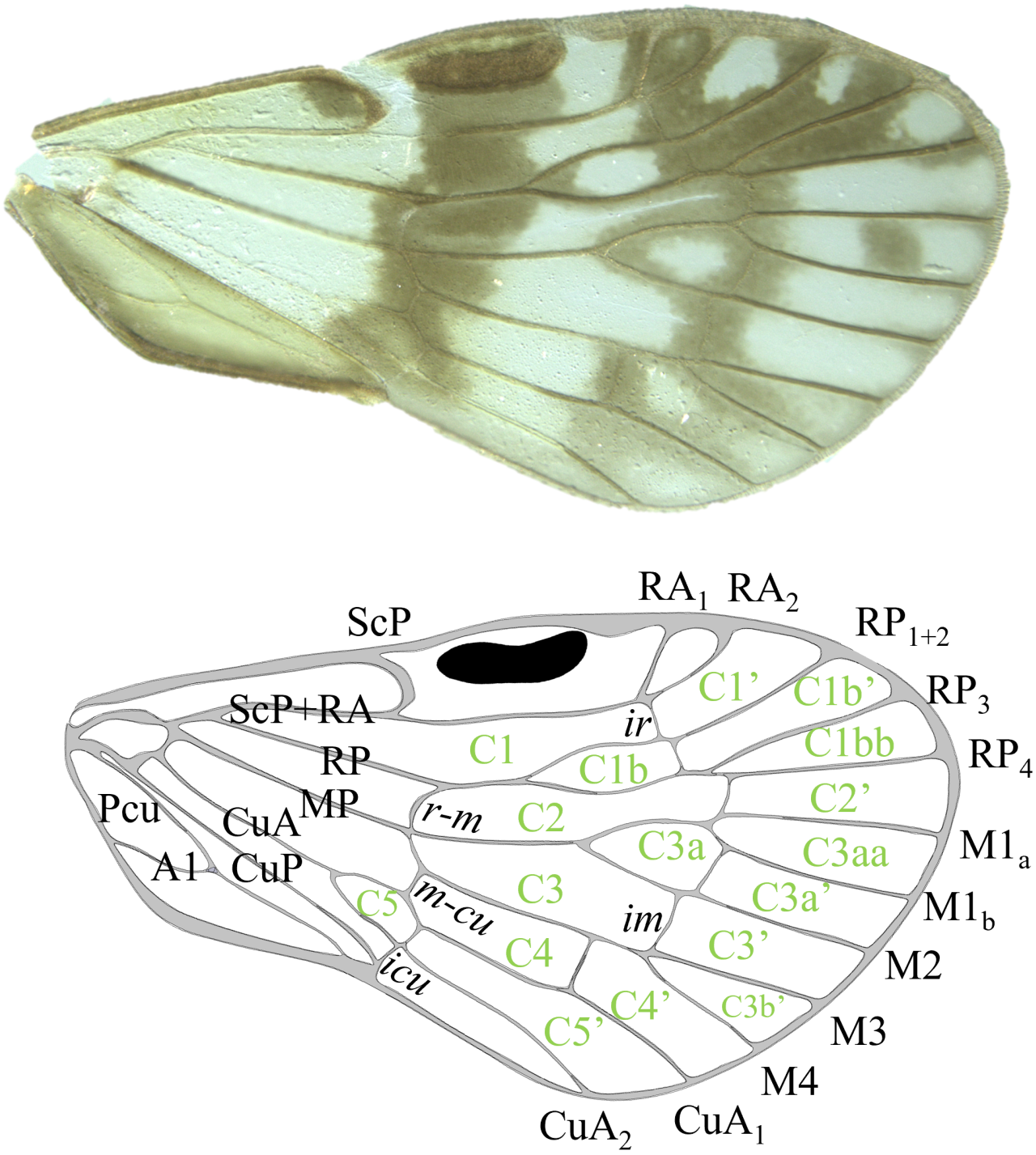

FIGURE 4. Figure 4. Adult male *Bothriocera harthi* **sp. n.** forewing; black text = vein, italic text = crossvein, green text = cell.

Material examined. Holotype male "♂" (FLREC); Paratypes 1 male, 2 females, same data as holotype (FSCA).

Sequence Data. For *Bothriocera harthi* **sp. n.**, a 642 bp product for COI, a 1,298 bp product for 18S, a 344 bp product for H3, and a 753 bp product for 28S were generated. In addition, products for COI, 18S, H3, and 28S were also generated for *B. drakei* (Fig. 8), *B. maculata* (Fig. 9), *B. transversa* (Fig. 10), *B. datuna* (Fig. 11), and *B. basalis* (Fig. 12) with corresponding GenBank accession numbers presented in Table 2. Based on the phylogenies, there is strong bootstrap support for all loci (89, 100, 95, and 100 for COI, 18S, H3, and 28S respectively) (Fig. 13) and strong bootstrap support in the consensus tree (100) for *Bothriocera* being monophyletic (Fig. 14). Furthermore, *Bothriocera harthi* **sp. n.** resolves within *Bothriocera* with strong bootstrap for COI (100, adjacent to *B. drakei*),18S

(86, adjacent to *B. datuna*, being 100% identical), H3 ((85, adjacent to *B. datuna*). Finally, while the consensus tree generated shows strong bootstrap support (100) for the monophyly of *Bothriocera* (with *Bothriocera harthi* **sp. n.** resolving within the genus), the bootstrap support for relationshipsamong species available and loci analyzed was low (<52) (Fig. 14).

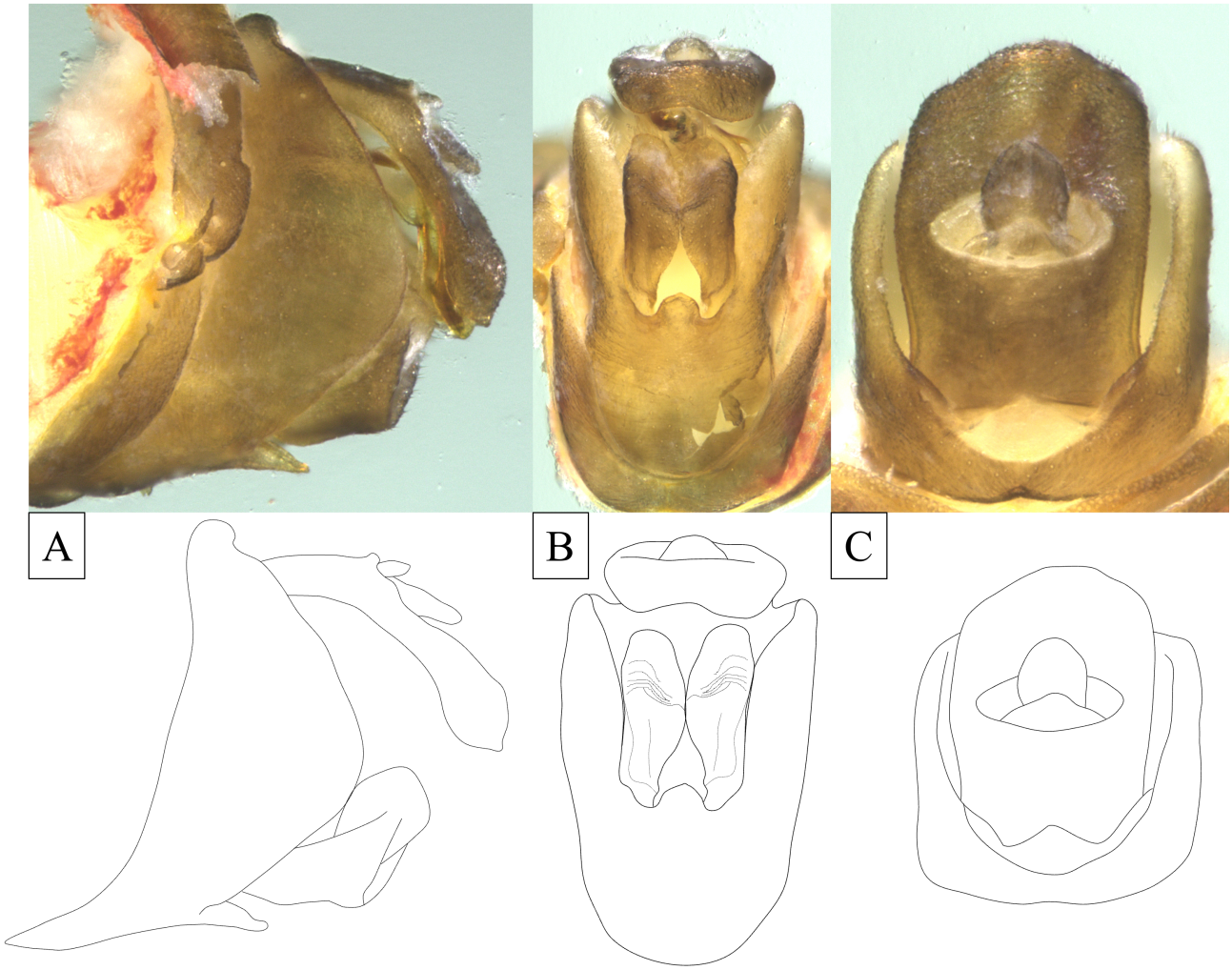

FIGURE 5. Adult male *Bothriocera harthi* **sp. n.** terminalia; (A) lateral view, (B) ventral view and (C) dorsal view.

TABLE 4. Pairwise comparison showing estimates of evolutionary divergence between sequences based on the COI gene for *Bothriocera harthi* **sp. n.** demonstrating intrageneric (orange) and intergeneric (blue) variability; the number of base differences per site from between sequences are shown. Standard error estimate(s) are shown above the diagonal and were obtained by a bootstrap procedure (1000 replicates).

		1	2	3	4	5	6	7	8	9	10	11
1	*Bothriocera harthi* **sp. n.**		0.020	0.022	0.010	0.020	0.020	0.025	0.027	0.024	0.025	0.028
2	*Bothriocera datuna*	0.207		0.021	0.017	0.019	0.021	0.026	0.025	0.024	0.023	0.028
3	*Bothriocera basalis*	0.212	0.189		0.019	0.019	0.020	0.025	0.023	0.025	0.020	0.027
4	*Bothriocera drakei*	0.061	0.156	0.176		0.016	0.017	0.023	0.024	0.023	0.023	0.027
5	*Bothriocera maculata*	0.186	0.166	0.179	0.136		0.016	0.021	0.025	0.024	0.023	0.027
6	*Bothriocera transversa*	0.191	0.198	0.190	0.150	0.140		0.024	0.025	0.024	0.022	0.025
7	*Haplaxius crudus*	0.244	0.256	0.254	0.223	0.195	0.232		0.023	0.022	0.022	0.019
8	*Myxia belinda*	0.298	0.274	0.245	0.265	0.243	0.257	0.251		0.026	0.023	0.023
9	*Nymphocixia unipunctata*	0.252	0.247	0.266	0.246	0.235	0.250	0.202	0.279		0.023	0.023
10	*Oecleus mackaspringi*	0.234	0.250	0.188	0.209	0.216	0.211	0.212	0.233	0.226		0.024
11	*Melanoliarus maidis*	0.295	0.293	0.278	0.283	0.266	0.243	0.158	0.232	0.224	0.243	

TABLE 5. Pairwise comparison showing estimates of evolutionary divergence between sequences based on the 18S gene for *Bothriocera harthi* **sp. n.** demonstrating intrageneric (orange) and intergeneric (blue) variability; the number of base differences per site from between sequences are shown. Standard error estimate(s) are shown above the diagonal and were obtained by a bootstrap procedure (1000 replicates).

		1	2	3	4	5	6	7	8	9	10	11
1	*Bothriocera_harthi_***sp._n.**		0.000	0.003	0.003	0.001	0.001	0.010	0.009	0.010	0.010	0.010
2	*Bothriocera datuna*	0.000		0.003	0.003	0.001	0.001	0.010	0.009	0.010	0.010	0.010
3	*Bothriocera basalis*	0.008	0.008		0.004	0.003	0.003	0.010	0.009	0.010	0.010	0.009
4	*Bothriocera maculata*	0.006	0.006	0.012		0.003	0.002	0.010	0.009	0.010	0.011	0.010
5	*Bothriocera drakei*	0.002	0.002	0.008	0.006		0.001	0.010	0.009	0.010	0.011	0.010
6	*Bothriocera transversa*	0.002	0.002	0.008	0.005	0.002		0.010	0.009	0.010	0.010	0.010
7	*Melanoliarus maidis*	0.035	0.035	0.035	0.034	0.036	0.036		0.005	0.007	0.006	0.006
8	*Oecleus mackaspringi*	0.031	0.031	0.031	0.031	0.032	0.032	0.015		0.006	0.005	0.005
9	*Nymphocixia unipunctata*	0.036	0.036	0.037	0.037	0.037	0.037	0.022	0.019		0.006	0.005
10	*Myxia belinda*	0.037	0.037	0.037	0.039	0.038	0.038	0.020	0.016	0.021		0.005
11	*Haplaxius crudus*	0.034	0.034	0.032	0.036	0.035	0.035	0.020	0.014	0.016	0.017	

TABLE 6. Pairwise comparison showing estimates of evolutionary divergence between sequences based on the H3 gene for *Bothriocera harthi* **sp. n.** demonstrating intrageneric (orange) and intergeneric (blue) variability; the number of base differences per site from between sequences are shown. Standard error estimate(s) are shown above the diagonal and were obtained by a bootstrap procedure (1000 replicates).

		1	2	3	4	5	6	7	8	9	10	11
1	*Borthriocera harthi* **sp. n.**		0.009	0.013	0.018	0.012	0.012	0.023	0.030	0.018	0.023	0.022
2	*Bothriocera datuna*	0.025		0.011	0.017	0.013	0.012	0.023	0.030	0.019	0.024	0.022
3	*Bothriocera drakei*	0.051	0.044		0.017	0.012	0.013	0.022	0.029	0.018	0.024	0.022
4	*Bothriocera basalis*	0.099	0.092	0.096		0.019	0.015	0.024	0.033	0.019	0.027	0.022
5	*Bothriocera maculata*	0.052	0.062	0.048	0.118		0.013	0.024	0.030	0.018	0.023	0.023
6	*Bothriocera transvera*	0.045	0.048	0.054	0.074	0.055		0.022	0.029	0.018	0.024	0.022
7	*Haplaxius crudus*	0.155	0.156	0.152	0.166	0.168	0.144		0.028	0.022	0.025	0.025
8	*Melanoliarus maidis*	0.235	0.228	0.231	0.256	0.235	0.213	0.202		0.027	0.032	0.031
9	*Myxia belinda*	0.106	0.111	0.107	0.127	0.110	0.099	0.139	0.190		0.022	0.022
10	*Nymphocixia unipunctata*	0.162	0.160	0.172	0.204	0.160	0.157	0.178	0.249	0.149		0.023
11	*Oelceus mackaspringi*	0.152	0.156	0.148	0.167	0.160	0.156	0.186	0.262	0.144	0.155	

Based on the pairwise comparison for COI nucleotide sequences, species in *Bothriocera* differed from each other by an average of 16.9% (SE±0.9) whereas variability among genera, on average, was 23.9% (SE±0.9). *Bothriocera harthi* **sp. n.** differed on average by 17.2% (SE±2.8) from other species within *Bothriocera,* and differed from genera in other higher taxa on average by 26.5% (SE±1.3) (Table 4).

Based on the multiple pairwise comparisons for the loci analyzed, the highest levels of variance were observed for COI, followed by H3 with variances observed among 28S and 18S being significantly lower (with 28S being being slightly more variable than 18S). Regardless, *Bothriocera harthi* **sp. n.** varied from other congeners by levels that were within the observed range of intrageneric variability for all loci analyzed (Table 7).

Remarks. *Bothriocera harthi* **sp. n.** is easily placed in the genus *Bothriocera* by head morphology (antennae in a cavity in front of compound eyes), the weakly overlapped wings (relative to *Bothrioceretta*), and the short medioventral lobe of the pygofer. This placement is also supported by molecular data (COI, 18S, H3, and 28S genes). In general, *Bothriocera harthi* **sp. n.** superficially resembles the other *Bothriocera* in the pattern of the forewings. The forewing pattern may (as Caldwell 1943 asserted) be useful for species diagnosis, but better documentation of variation in color pattern of the known species is needed before relying on this feature.

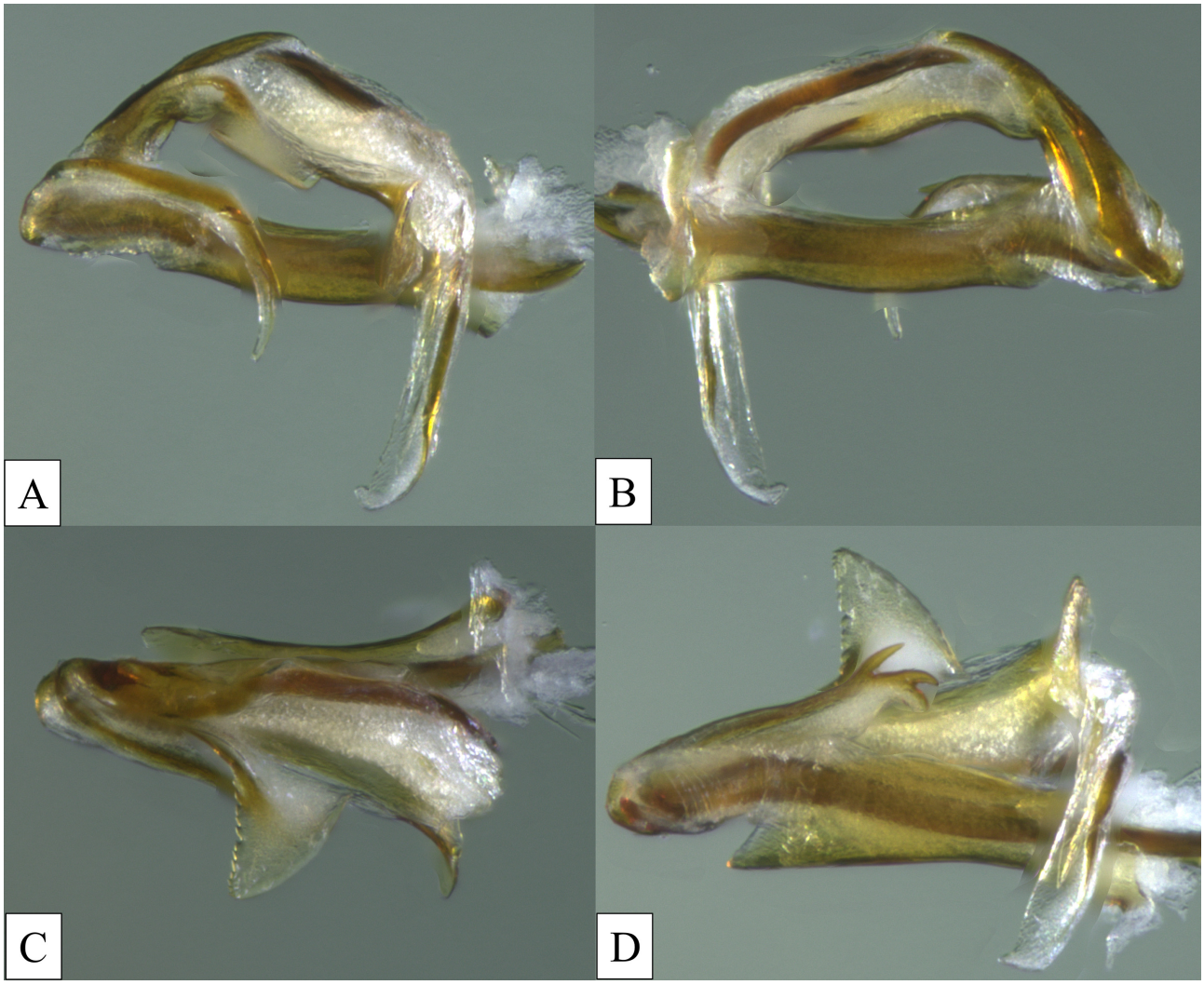

FIGURE 6. *Bothriocera harthi* **sp. n.** aedeagus; (A) right lateral view, (B) left lateral view, (C) dorsal view and (D) ventral view.

TABLE 7. Pairwise comparison showing estimates of evolutionary divergence between sequences based on the 28S gene for *Bothriocera harthi* **sp. n.** demonstrating intrageneric (orange) and intergeneric (blue) variability; the number of base differences per site from between sequences are shown. Standard error estimate(s) are shown above the diagonal and were obtained by a bootstrap procedure (1000 replicates).

		1	2	3	4	5	6	7	8	9	10	11
1	*Bothriocera harthi* **sp. n.**		0.003	0.002	0.002	0.013	0.002	0.026	0.025	0.031	0.029	0.029
2	*Bothriocera basalis*	0.005		0.003	0.002	0.013	0.003	0.025	0.026	0.030	0.029	0.028
3	*Bothriocera drakei*	0.001	0.004		0.001	0.013	0.002	0.026	0.025	0.030	0.029	0.029
4	*Bothriocera transversa*	0.002	0.003	0.001		0.013	0.002	0.026	0.025	0.030	0.029	0.028
5	*Bothriocera maculata*	0.028	0.030	0.029	0.030		0.013	0.037	0.037	0.042	0.040	0.040
6	*Bothriocera datuna*	0.001	0.005	0.001	0.002	0.029		0.026	0.026	0.030	0.029	0.029
7	*Oecleus mackaspringi*	0.059	0.058	0.059	0.058	0.079	0.059		0.017	0.016	0.014	0.018
8	*Myxia belinda*	0.058	0.058	0.057	0.057	0.079	0.058	0.040		0.023	0.019	0.020
9	*Nymphocixia unipunctata*	0.068	0.068	0.068	0.068	0.089	0.067	0.036	0.050		0.015	0.024
10	*Haplaxius crudus*	0.065	0.065	0.065	0.064	0.084	0.064	0.032	0.043	0.034		0.021
11	*Melanoliarus maidis*	0.065	0.063	0.064	0.063	0.085	0.065	0.041	0.046	0.056	0.048	

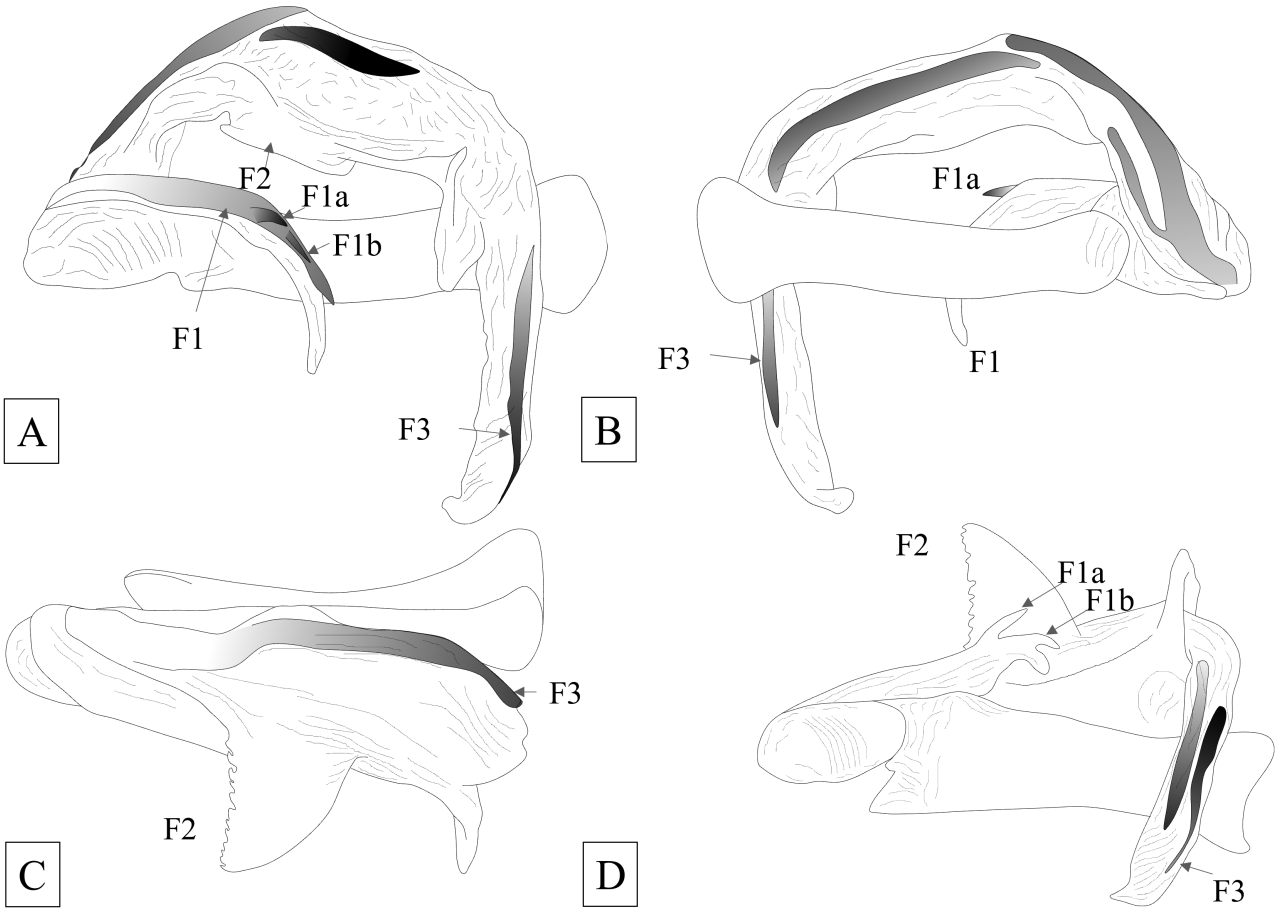

FIGURE 7. *Bothriocera harthi* **sp. n.** aedeagus line art; (A) right lateral view, (B) left lateral view, (C) dorsal view and (D) ventral view.

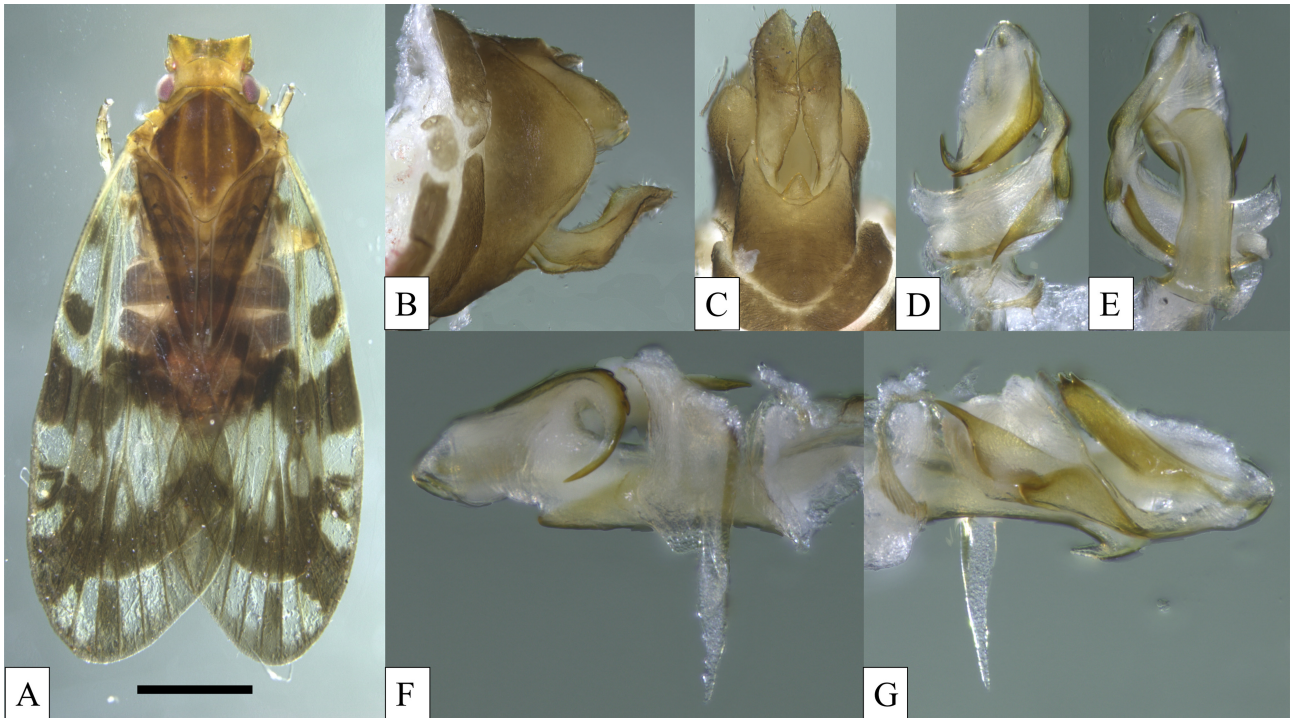

FIGURE 8. Adult male *Bothriocera drakei*; A) dorsal habitus, B) terminalia left lateral view, C) terminalia ventral view, D) aedeagus dorsal view, E) aedeagus ventral view, F) aedeagus right lateral view and G) aedeagus left lateral view, scale = 1 mm.

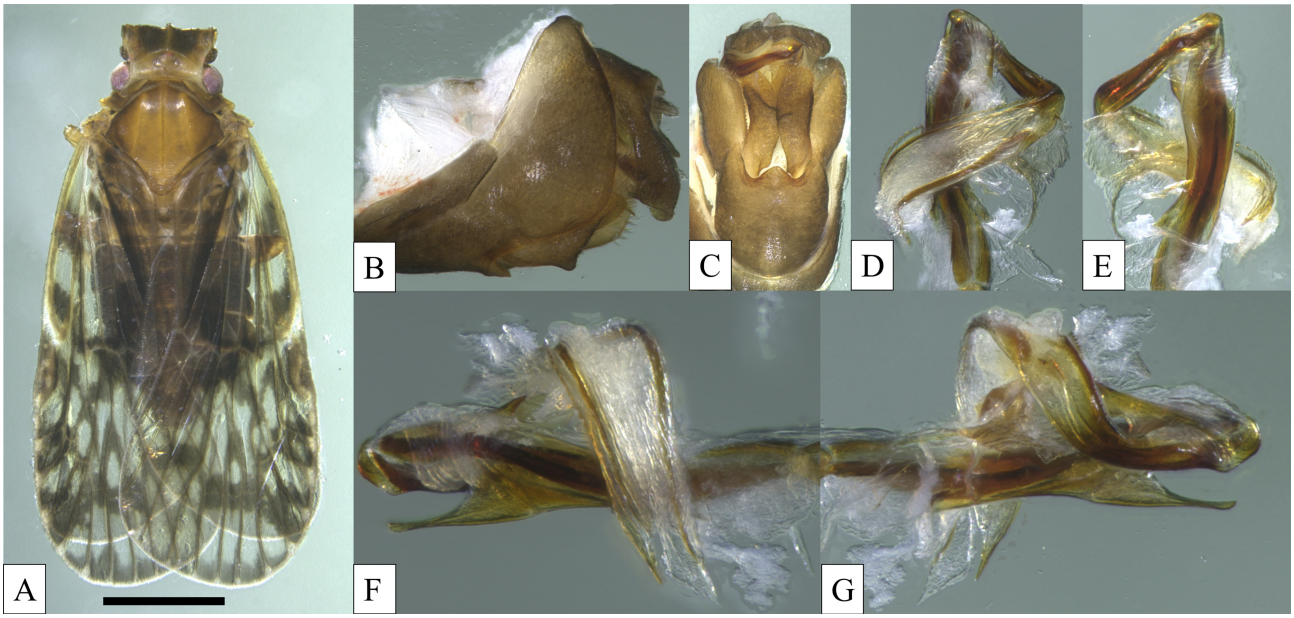

FIGURE 9. Adult male *Bothriocera maculata*; A) dorsal habitus, B) terminalia left lateral view, C) terminalia ventral view, D) aedeagus dorsal view, E) aedeagus ventral view, F) aedeagus right lateral view and G) aedeagus left lateral view, scale = 1 mm.

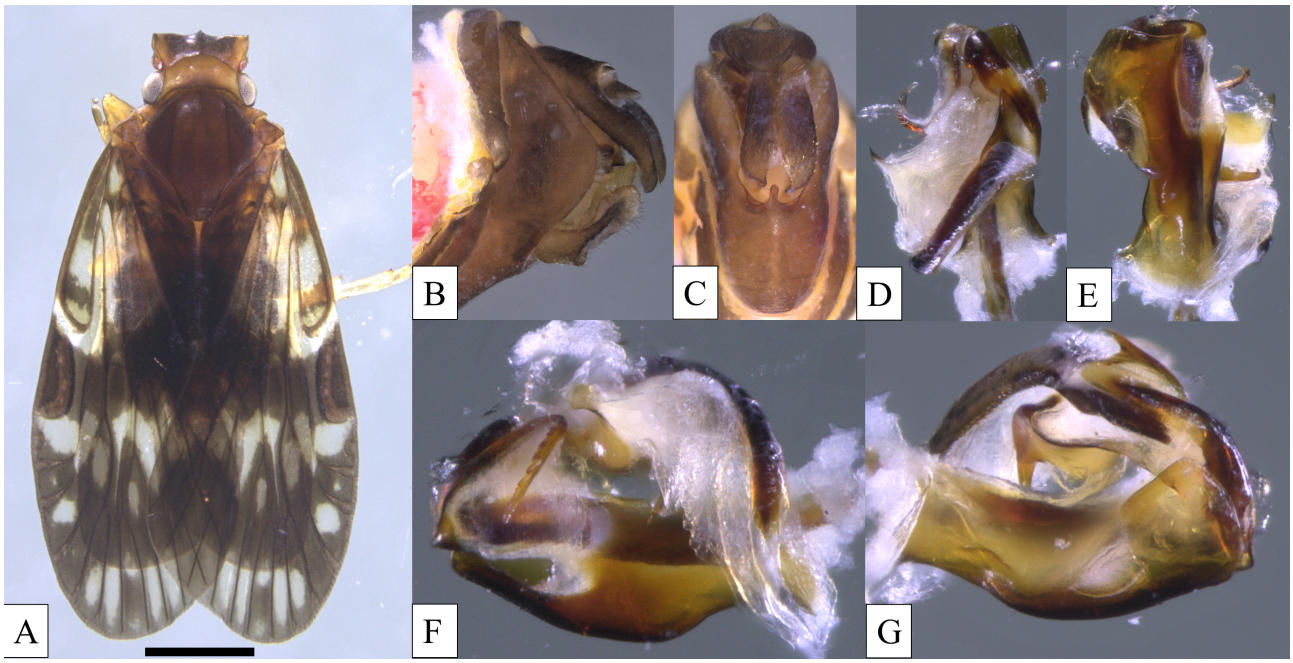

FIGURE 10. Adult male *Bothriocera transversa*; A) dorsal habitus, B) terminalia left lateral view, C) terminalia ventral view, D) aedeagus dorsal view, E) aedeagus ventral view, F) aedeagus right lateral view and G) aedeagus left lateral view, scale = 1 mm.

Bothriocera harthi **sp. n.** appears most like *Bothriocera datuna* Kramer (see Fig. 11 and Kramer 1983, figs. 39–41). Aside from geography, *Bothriocera harthi* **sp. n.** differs most readily from *B. datuna* in that the latter species has less extensive wing markings the medioventral lobe of the pygofer bears an apical projection; also, and differences in the shape of the gonostyli.

Other material examined.

Bothriocera basalis. Costa Rica, La Tarde Ecolodge, Osa Peninsula, 16.VI.2021 (3 males, 2 females).

Bothiocera datuna. Coral Springs, FL, U.S.A., Tall Cypress Natural Area, 3.IV.2022 (1 male).

Bothriocera drakei. Milford, DE, U.S.A., Woods Haven, 6.VII.2021 (1 male).

FIGURE 11. Adult male *Bothriocera datuna*; A) dorsal habitus, B) terminalia left lateral view, C) terminalia ventral view, D) aedeagus ventral view, E) aedeagus left lateral view, F) aedeagus dorsal view and G) aedeagus right lateral view, scale = 1 mm.

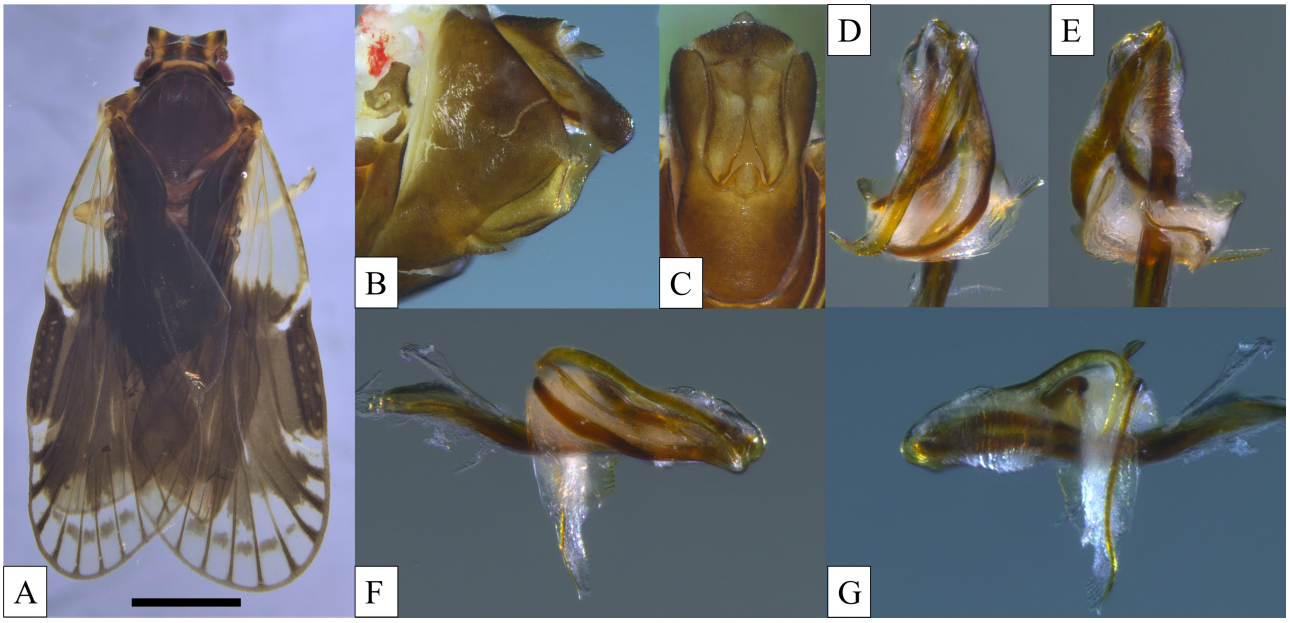

FIGURE 12. Adult male *Bothriocera basalis*; A) dorsal habitus, B) terminalia left lateral view, C) terminalia ventral view, D) aedeagus dorsal view, E) aedeagus ventral view, F) aedeagus left lateral view and G) aedeagus right lateral view, scale = 1 mm.

Bothriocera maculata. Coral Gables, FL, U.S.A., Montgomery Botanical Center, 16.V.2022 (2 males).

Bothriocera transversa. Coral Gables, FL, U.S.A., Montgomery Botanical Center, 16.V.2022 (5 males, 2 females).

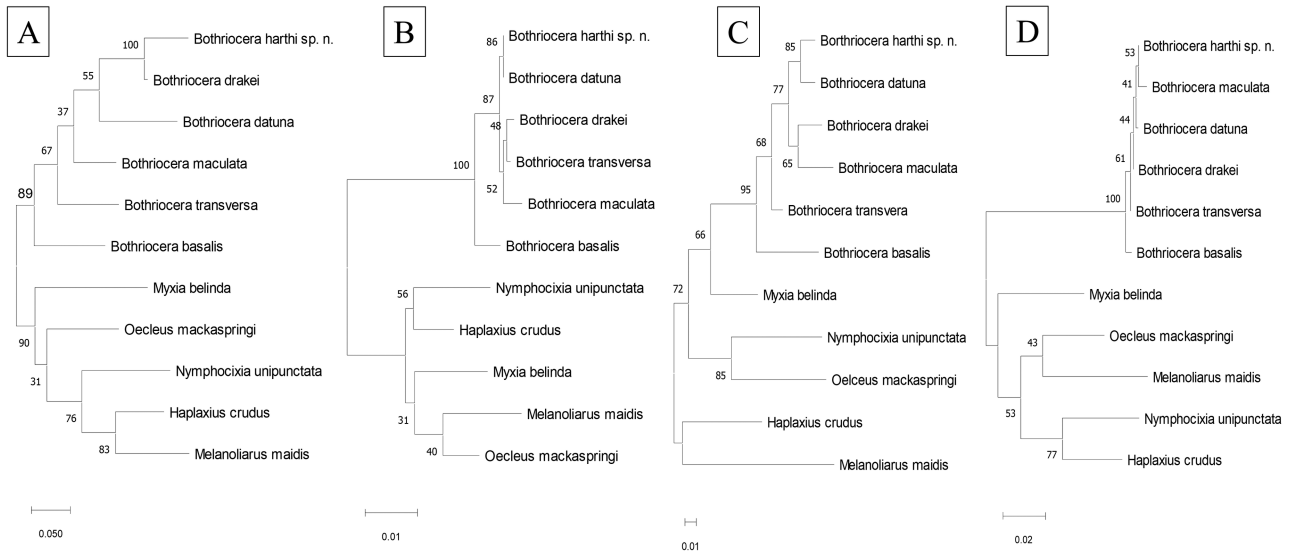

FIGURE 13. Maximum likelihood phylogenetic tree based on 1,000 replicates; (A) COI gene, (B) 18S rRNA gene, (C) H3 gene, and (D) 28S gene; scale bar = percent nucleotide difference.

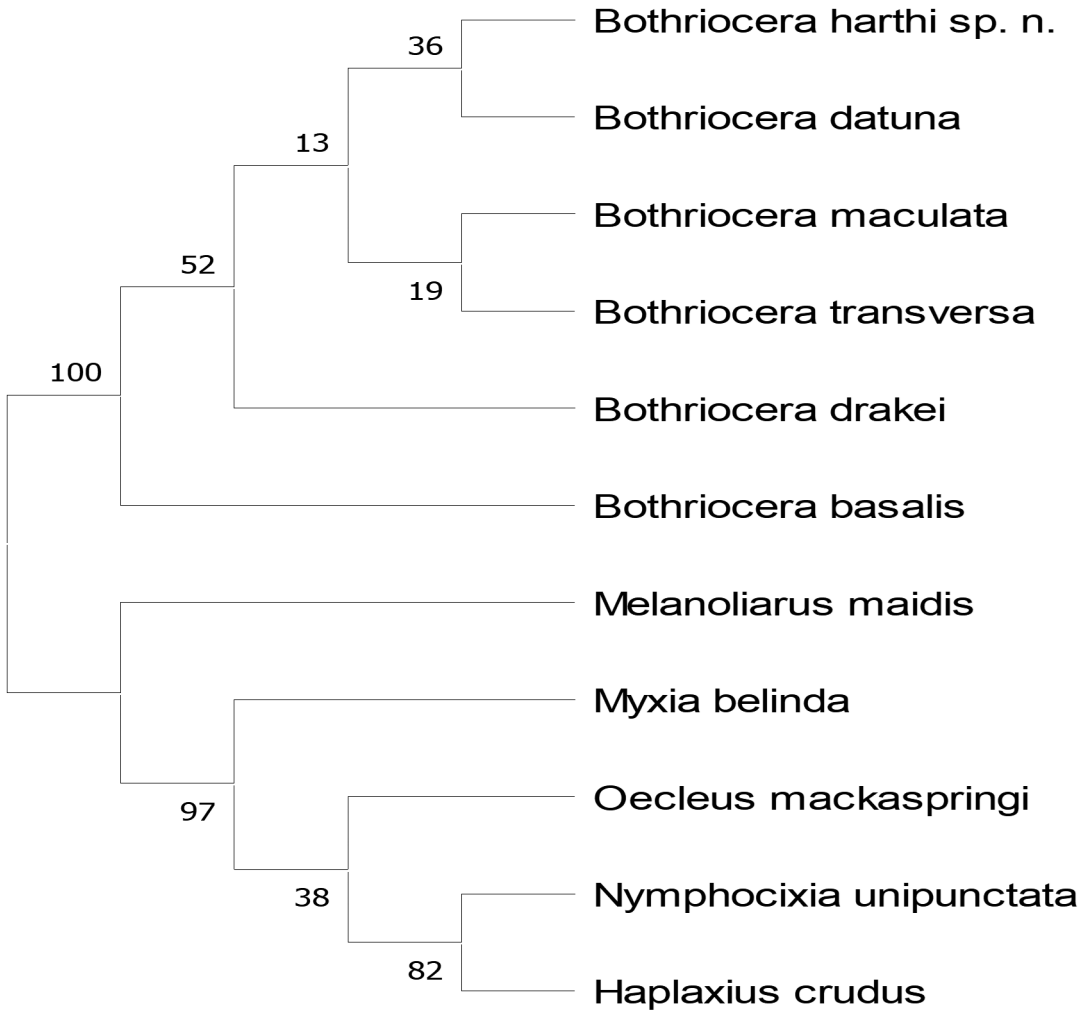

FIGURE 14. Maximum likelihood phylogenetic consensus tree based on 1,000 replicates for concatenated COI, 18S, 28S, and H3 sequence data.

Discussion

The Bothriocerini are a distinctive New World taxon whose placement relative to other cixiid taxa remains uncertain but may be related to the Oecleini (Luo *et al.* 2021, Le Cesne *et al.* 2022). A comprehensive review of *Bothriocera* is necessary for evaluating the monophyly in its current status and based on current species composition because certain taxa (such as *B. holzingeri* O'Brien and *B. emeljanovi* O'Brien; O'Brien 2006) appear to display characteristics that may serve as useful diagnostic traits to merit segratating new genera from within *Bothriocera*. However, among the species that we have molecular data from so far, the genus is monophyletic, although the relationships among species remain far from clear.

Ongoing survey work in Jamaica for putative vectors of LY has resulted in the discovery of multiple new taxa in the families Derbidae (Bahder *et al.* 2020) and Cixiidae (Myrie *et al.* 2019, Bahder *et al*. 2023) from coconut palms. The discovery of *Bothriocera harthi* **sp. n.** represents the first novel taxon in Bothriocerini documented as part of this survey. Due to the diversity and widespread distribution of the genus, the potential for the discovery of other new species is high. While there is currently no evidence *Bothriocera* is implicated in the spread of LY, it is a unique group of planthoppers and more thorough knowledge of bothriocerine diversity will be critical to better understanding the phylogeny and evolution of the Cixiidae.

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