



## A new species of planthopper in the genus *Haplaxius* (Hemiptera: Auchenorrhyncha: Fulgoroidea: Cixiidae) on palms in Costa Rica and a new country record for *Haplaxius skarphion*

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### Abstract

The genus *Haplaxius* is a large taxon of cixiid planthoppers that is of economic importance due to the ability of *Haplaxius crudus* to transmit lethal yellowing in coconut palms. *Haplaxius dougwalshi* **sp. n.** is established as a new taxon of Cixiidae in the tribe Oecleini collected from native palms in lowland tropical rainforest in Costa Rica. Placement in the genus *Haplaxius* is supported both by molecular evidence based on the COI and 18S genes as well as by morphological characters. This novel taxon was discovered during survey work in Costa Rica to look for phytoplasmas and document planthopper diversity on palms. Furthermore, *Haplaxius skarphion* was also collected from coconut palms during survey work and is reported for the first time in Costa Rica.

**Key words:** Cixiidae, taxonomy, DNA barcoding, planthopper, palm, Costa Rica

### Resumen

*Haplaxius dougwalshi* **sp. n.** fue recolectada en palmeras nativas en el bosque lluvioso tropical en las tierras bajas de Costa Rica y se establece como un nuevo taxón de la familia Cixiidae de la tribu Oecleini. La clasificación de esta especie dentro del género *Haplaxius* se basa tanto en la evidencia molecular aportada por los genes COI y 18S; así como en los caracteres morfológicos de la misma. Este nuevo taxón fue descubierto durante un trabajo de investigación que se está llevando a cabo en Costa Rica, el cual tiene como objetivo principal la evaluación de la ocurrencia de fitoplasmas y la documentación de la diversidad de chicharritas en las palmeras.

**Palabras clave:** Cixiidae, taxonomía, código de barras de AND, chicharrita, palmera, Costa Rica

## Introduction

The planthopper genus *Haplaxius* Fowler is a large taxon that is restricted to the New World with 34 species represented in North America and 30 species known from the Neotropics (Bartlett *et al.* 2014, Bourgoïn 2020). Both Bahder *et al.* (2019a) and Kramer (1979) acknowledged that *Haplaxius* as currently defined is broad and possibly paraphyletic, with significant variation in habitus and genitalia. As currently circumscribed, *Haplaxius* is recognized by having evident tegulae, frons with median carina, vertex lacking carina at midline and between eyes, mesonotum about 2X long as vertex or less, lacks denticle on forecoxae, carinae on pronotum terminate at ventro-lateral apex, lacking spines on hind tibiae (tribal feature) (Bahder *et al.* 2019a). It is expected that as new taxa are discovered and molecular data becomes available for *Haplaxius*, significant revisions may result including establishment new genus-groups. Recently a new oecleine species from Costa Rica similar to *Haplaxius* possessed sufficient morphological and molecular differences to place it as a new genus and species, *Myxia belinda* Bahder & Bartlett (Bahder *et al.* 2019a).

*Haplaxius* is of economic interest because of the putative ability of *H. crudus* (Van Duzee) to transmit the lethal yellowing (LY) phytoplasma (16SrIV-A) in Florida (Howard & Thomas 1980). However, these experiments have failed to replicate both in Florida and the Caribbean where active LY research and spread continues. Furthermore, *Myndus taffini* Bonfils has been shown to transmit the coconut foliar decay virus (CFDV) (Julia *et al.* 1985, Wefels *et al.* 2015) and *Hyalesthes obsoletus* Signoret is a vector of the 16SrXII-A phytoplasma, causal agent of Bois Noir in grapevines (Boudon-Padiou 2003). These studies highlight that there is complex and interesting relationship among cixiids and plant pathogens and that a closer look at described taxa and new taxa to assess their role in transmitting microorganisms is merited.

Because of the relationship of cixiids to plant pathogens and recent survey efforts documenting cixiid taxa on palms in the Caribbean basin (Bahder *et al.* 2019a, Myrie *et al.* 2019), there is renewed interest in the taxonomy and biodiversity of *Haplaxius* and related Oecleini. A recent expedition in Costa Rica revealed a small, bright orange planthopper on both native palms and at light traps and was tentatively identified as a cixiid belonging to the genus *Haplaxius*. Herein we describe a novel taxon in the genus *Haplaxius* with molecular data from both the 18S and COI loci. In addition, *H. skarphion* Kramer is given as a new country record for Costa Rica.

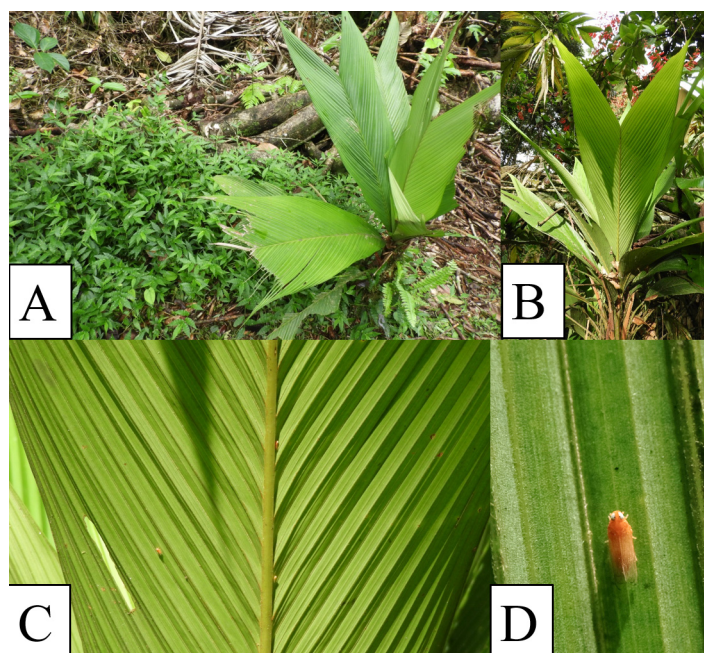
## Materials and methods

**Locality and specimen collection.** Individuals of the novel taxon were aspirated from healthy appearing specimens of the palm *Asterogyne martiana* Wendl in addition to being collected at a mercury vapor light trap and were immediately transferred to 95% ethanol. Specimens were collected (permit no. SINAC-ACC-PI-LC-072-2019) at La Selva Biological Station (Fig. 1A–B), Heredia province, Costa Rica (10.431269, -84.005961). Specimens taken from palms were observed along the trails where there were openings and noticeable levels of disturbance (recent clearing for trail maintenance, storm damage). A specimen was also collected on coconut palm in Gandoca, Costa Rica, near the Panamanian border and was identified in the field as belonging to *Haplaxius* based on resemblance to *Haplaxius crudus*. Specimens were exported under permit number DGVS-434-2019 and imported into the U.S.A. under permit number P526-170201-001. All specimens collected were measured, photographed and dissected using a Leica M205 C stereoscope. 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 that of Bartlett *et al.* (2014), except forewing venation following Bourgoïn *et al.* (2015) and with male terminalia nomenclature modified after Bourgoïn & Huang (1990). New taxa are intended to be attributed to Bahder and Bartlett.

**Dissections and DNA extraction.** The terminalia 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 abdomen 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

abdomen was then immersed in 200  $\mu$ l of buffer ATL and 200  $\mu$ l 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 were then used for morphological characterization and photography.



**FIGURE 1.** *Haplaxius dougwalshi* sp. n. habitat/host (A–B) and *in vivo* (C–D).

**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 the reverse complement of C1-J-2915 from Simon *et al.* (1994) (5'-ACTTCTGGATGACCAAAAAATCAA-3'). To obtain 18S sequence data, the primers designed by Bahder *et al.* (2019b) were used, 18S/Forward (5'-ACTGTCGATG-GTAGGTTCTG-3') and 18S/Reverse (5'-GTCCGAAGACCTCACTAAA-3'). PCR reactions contained 5x GoTaq Flexi Buffer, 25 mM MgCl<sub>2</sub>, 10 mM dNTP's, 10 mM of each primer, 10% PVP-40, and 2.5U GoTaq Flexi DNA Polymerase, 2  $\mu$ l DNA template, and sterile dH<sub>2</sub>O to a final volume of 25  $\mu$ L. Thermal cycling conditions for COI were as follows: 2 min initial denaturation at 95°C, followed by 35 cycles of 30 sec denaturation at 95°C, 30 sec annealing at 40°C, 1 min 30 sec extension at 72°C, followed by a 5 min extension at 72°C. Thermal cycling conditions for 18S were as follows: 2 min initial denaturation at 95°C, followed by 35 cycles of 30 sec denaturation at 95°C, 30 sec annealing at 50°C, 2 min 30 sec extension at 72°C, followed by a 5 min extension at 72°C. 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 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. Maximum Likelihood trees were generated using the Bootstrap method at 1,000 replicates based on the Tamura-Nei model.

**Taxon sampling.** COI sequence data was used for *Haplaxius crudus* specimens collected in Costa Rica (GenBank Accession No. MT080284) as an in-group comparison and *Oecleus mackaspringi* Bahder & Bartlett (GenBank Accession No. MN488999) and *Nymphomyndus caribbea* (Fennah) (GenBank Accession No. MT080286) as outgroups. These are currently the only oecleines that have publicly available COI sequence data for the five-prime region of the COI gene. For the 18S locus, sequence data was used for *Haplaxius crudus* specimens collected in Costa Rica (GenBank Accession No. MT002393) was used as an in-group and *Nymphomyndus caribbea* (GenBank Accession No. MT002394), *Myxia belinda* (GenBank Accession No. MN200095), *Oecleus mackaspringi* (GenBank Accession No. MN422261), and *Myndus taffini* (EU183560.1) were used as outgroups.

## Systematics

### Family Cixiidae Spinola 1839

### Subfamily Cixiinae Spinola 1839

### Tribe Oecleini Muir 1922

### Type genus: *Oecleus* Stål 1862

### Genus *Haplaxius* Fowler 1904

### Type species: *Haplaxius laevis* Fowler 1904

**Amended diagnosis.** (Modified from Kramer 1979) Small to average size cixiids (3.2–6.4 mm); head in dorsal view narrower than pronotum, eyes large; vertex elongate, moderately broad (among Oecleini), disc slightly concave, sides and apex carinate, lacking carina at midline and between eyes, apex variably produced beyond eyes. In lateral view, apex of head bluntly angled, ocellus beneath eye (anterior to antenna). In facial view, sides of frons concave (“flared”) and carinate, midline of frons carinate, interrupted near frontoclypeal suture by ocellus, clypeus subtriangular with lateral margins and midline carinate. Antennal scape reduced, pedicel robust, flagellum beadlike basally and filamentous distally.

Pronotum narrow, with irregular ridges and distinct paranotal region, length shortest on midline, posterior margin indented. Tegulae evident. Mesonotum tricarinate; longitudinal midlength of mesonotum about 2x or less that of vertex. Hind tibiae without lateral spines. Forewings tectiform, usually hyaline or transparent, but sometimes infuscated or patterned, veins usually with small setae-bearing pustules.

Male pygofer usually longest on ventral margin, hind margin variably produced. Aedeagus asymmetrical and elaborated with projections and processes, vertical connective articulating base of aedeagus with gonostyli. Gonostyli symmetrical and usually simple. Anal tube symmetrical or asymmetrical, with processes from one or both ventral margins and sometimes with projections originating between ventral margins.

### *Haplaxius dougwalshi* sp. n.

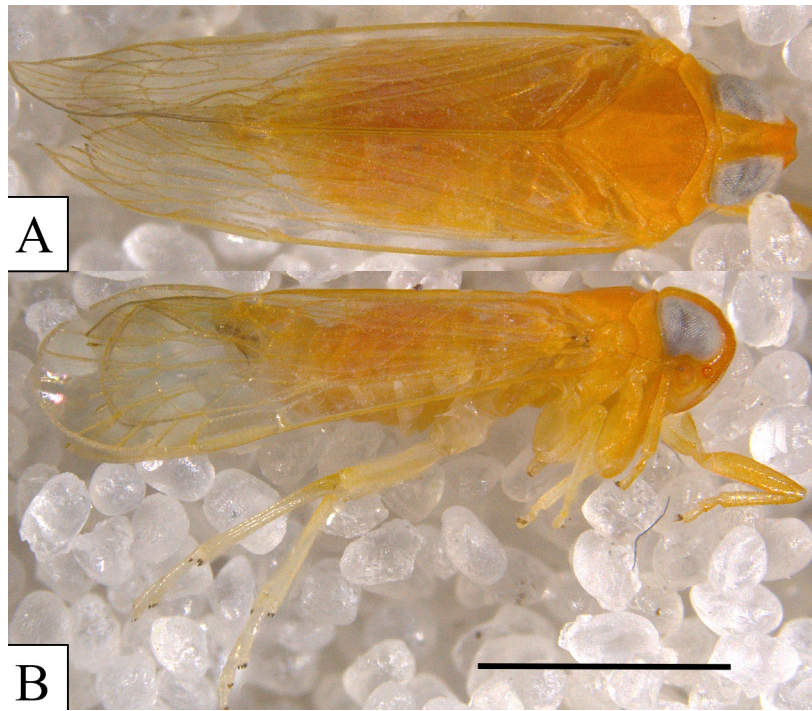
(Figures 2–7)

**Type locality.** La Selva Biological Station, Heredia, Costa Rica

**Diagnosis.** This species is distinguished by a combination of bright orange coloration, facial color pattern of an orange frons with white arch traversing width of frons resulting in a trapezoidal shape dorsad of frontoclypeal suture, large, incurved processes on lateral margins of the pygofer and aedeagus with curved spine near midlength of shaft.

**Description.** *Color.* General body color is uniformly orange with abdominal sternites, middle and hind legs paler orange (Fig. 2). Frons with median white patch merging with lateral white patches, reaching frontoclypeal suture, orange patch dorsad of frontoclypeal suture trapezoidal in shape (Fig. 3A). Forewing veins pale orange (Fig. 2). *Structure.* Body length males ( $n=14$ ): 3.16–3.19 mm with wings; 2.53–2.56 mm without wings; females ( $n=9$ ): 3.19–3.20 mm with wings; 2.56–2.58 mm without wings.

*Head.* In frontal view, frons narrowed between eyes, expanding at ventral margin, transverse carina present separating frons and vertex (Fig. 3). Fastigium rounded in lateral view (Fig. 3). In dorsal view, frons concave, vertex widest posteriorly, narrowing to almost half width at base (Fig. 3). Vertex length males: 0.33–0.34 mm; females 0.35 mm. Vertex width at hind margin males: 0.21–0.22 mm; females: 0.22–0.23 mm. Vertex width at distal margin males: 0.11–0.12 mm; females: 0.12–0.13 mm. Frons length males: 0.50–0.51 mm; females: 0.51–0.52 mm. Frons dorsal width males: 0.10–0.11 mm; females: 0.11–0.12 mm. Frons width at widest portion males: 0.36–0.37 mm; females: 0.37–0.38 mm. Frons frontoclypeal margin width males: 0.29–0.30 mm; females: 0.31–0.32 mm. Clypeus length males: 0.39–0.40 mm; females: 0.40–0.41 mm.



**FIGURE 2.** Adult habitus *Haplaxius dougwalshi* sp. n.; (A) body dorsal view male (B) body lateral male, scale = 1 mm.

Thorax. Carinae extending to lateral margin in frontal view (Fig. 3). Mesonotum tricarinate, lateral margins subparallel (Fig. 3). Pronotum length at midline males: 0.05–0.06 mm; females: 0.06–0.07 mm. Mesonotum length at midline males: 0.47–0.48 mm; females: 0.48–0.49 mm. Mesonotum width males: 0.65–0.66 mm; females: 0.66–0.67 mm. Fore wings transparent, clouded posterior to pseudostigma (Fig. 4), with conspicuous tubercles along main veins (Fig. 4). Apex of clavus just beyond midlength of wing, PCu+A1 reaching wing margin before claval apex. Fork of CuA just basad of RA(+ScP)+RP fork, both just basad of wing midlength; MP forked from ScP+R in basal fourth of wing; forewing branching pattern: RA 1-branched, RP 3, MP 5, CuA 4 (or 3 with icu crossvein); icu joining apex of clavus. PCu joining with A1 at midpoint of clavus. Forewing length males: 2.58–2.60 mm; females: 2.60–2.62 mm.



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

Terminalia. Pygofer in lateral view widest ventrad, narrowing dorsally, posterior margin with large process on median posterior margin (Fig. 5A). In ventral view, pygofer opening bearing an elongate rounded lobe, approximately 2x long as wide (Fig. 5B); in caudal view, pygofer with lateral processes, medially angled (Fig. 6). Gonostyli in lateral view angled upwards with acute apex, strongly sinuate on dorsal margin with basal curve strong, resulting

in constriction of gonostyli, appearing spoon-like (Fig. 5A). In ventral view, appearing clubbed with inner and outer margins of clubbed apex irregularly sinuate (Fig. 5B), inner margin strongly sinuate, outer margin weakly sinuate (Fig. 5B). Aedeagus asymmetrical and complex (Fig. 7A–C); aedeagal shaft with small spine at midlength on right lateral margin, strongly curved (almost 90° angle) (Fig. 7B) and larger spine at apex, angled dorsad and slightly distad on left lateral margin, nearly extending to base of aedeagus, approximately 2x length and thickness of small spine. Flagellum (endosoma) with one dorsal spine angled distad toward right lateral margin, terminating approximately at base of small aedeagal spine (Fig. 7C) and one larger, ventral spine, arching ventrad, approximately twice length of dorsal flagellar spine, terminating approximately at same point as smaller aedeagal spine (Fig. 7B). Anal segment in lateral view with parallel dorsal and ventral margins, approximately 5X long at apex as wide (Fig. 5B), apex angled slightly downward, median process angled ventrad approximately 2/3 length distad of pygofer, median process of anal segment about equal size to lateral process of pygofer (Fig. 5).

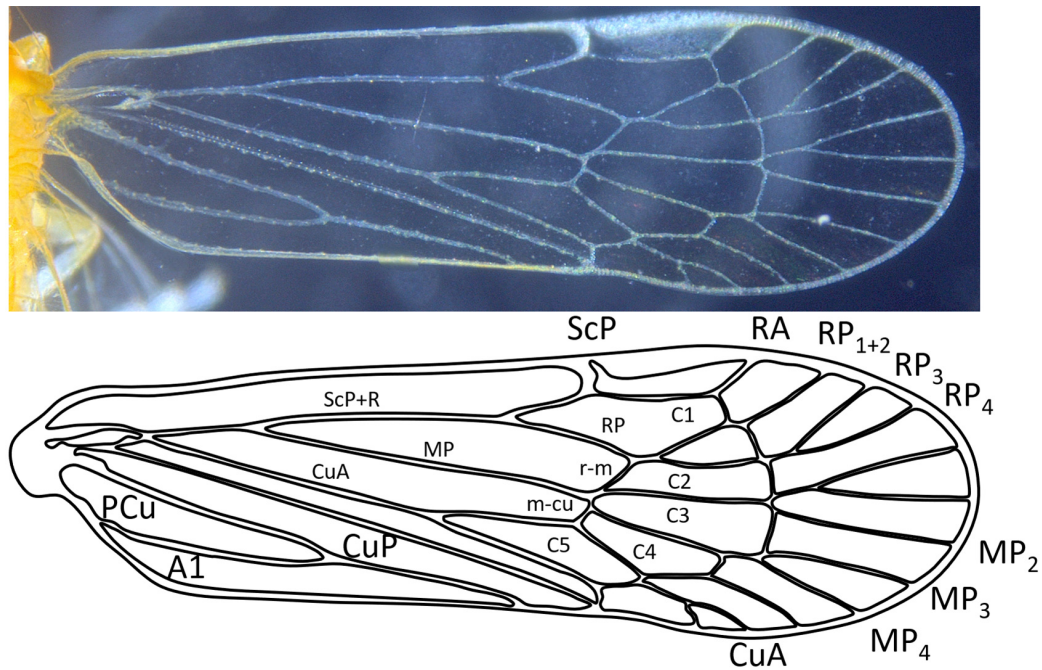


FIGURE 4. Forewing venation of *Haplaxius dougwalshi* sp. n.

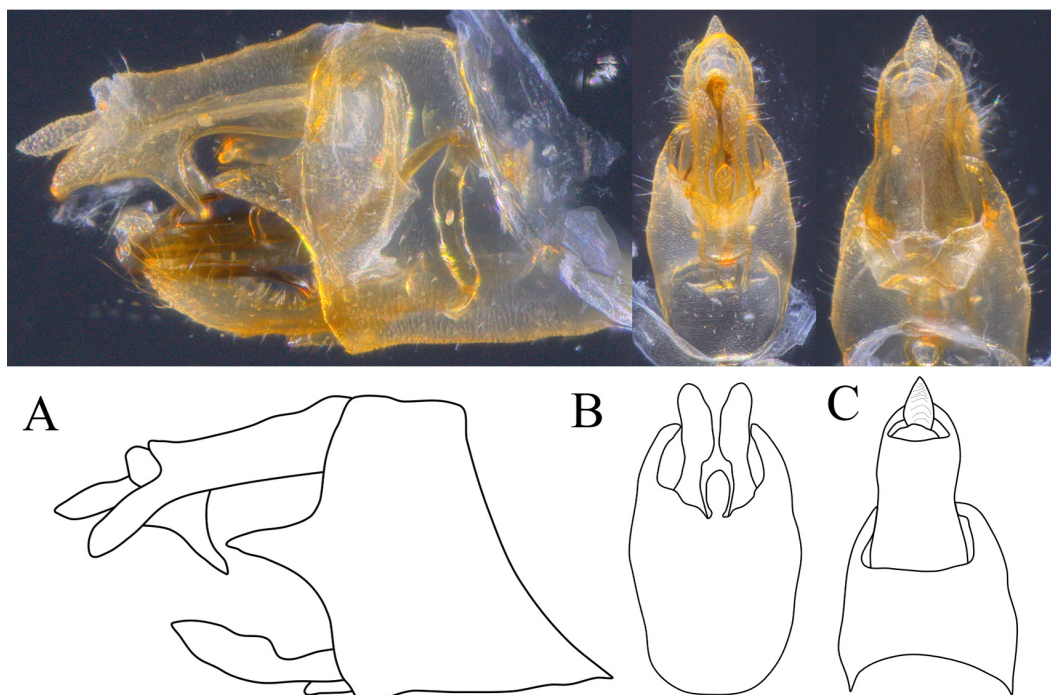


FIGURE 5. Male terminalia of *Haplaxius dougwalshi* sp. n.; (A) lateral view, (B) ventral view, and (C) dorsal view.



FIGURE 6. Caudal view of pygofer of adult male *Haplaxius dougwalshi* sp. n.

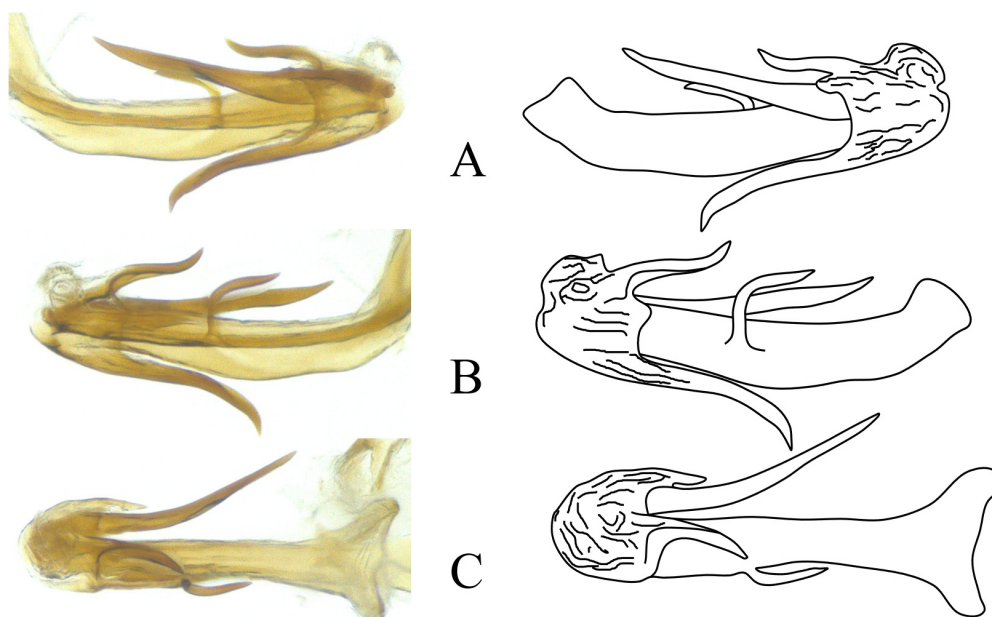


FIGURE 7. Aedeagus of adult male *Haplaxius dougwalshi* sp. n.; (A) left lateral view, (B) right lateral view, and (C) dorsal view.

**Plant associations.** Pata de gallo palm (*Asterogyne martiana*), Arecaceae.

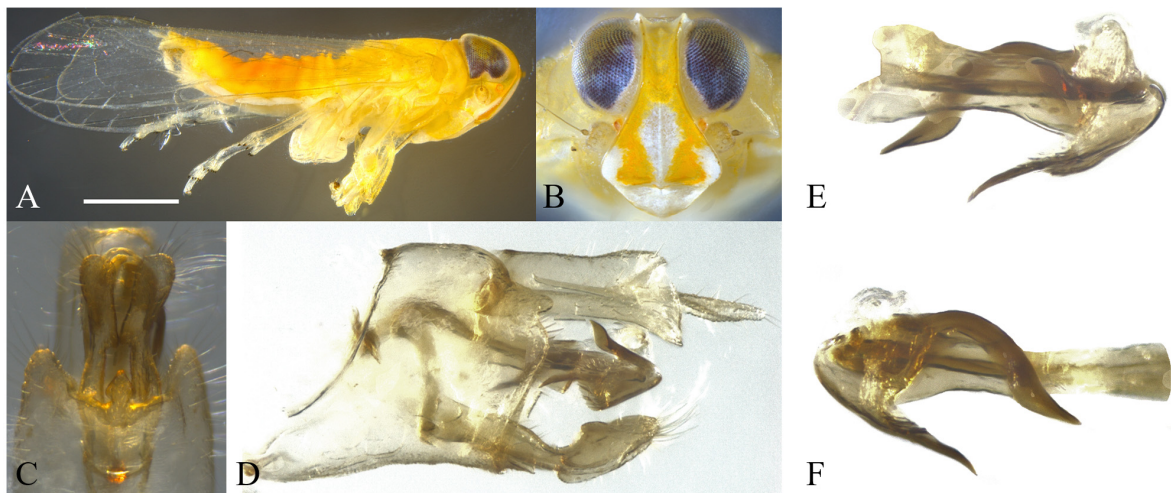
**Distribution.** Costa Rica (Heredia, Puerto Viejo de Sarapiquí)

**Etymology.** The specific name given is an honorarium in reference to the lead authors professor and chair during graduate school (Ph.D.), Dr. Doug Walsh at Washington State University, who's signature mustache resembles the color pattern observed on the frons of the novel taxon.

**Material examined.** Holotype male "Costa Rica, Heredia / La Selva Biological Station / 26.V.2019 / Sweeping *Astogyne martiana* / Coll.: B.W.Bahder // Holotype / *Haplaxius dougwalshi*" (FLREC); Paratypes, La Selva Biological Research Station [26.V.2019] (13 males, 9 females, FLREC and FSCA).

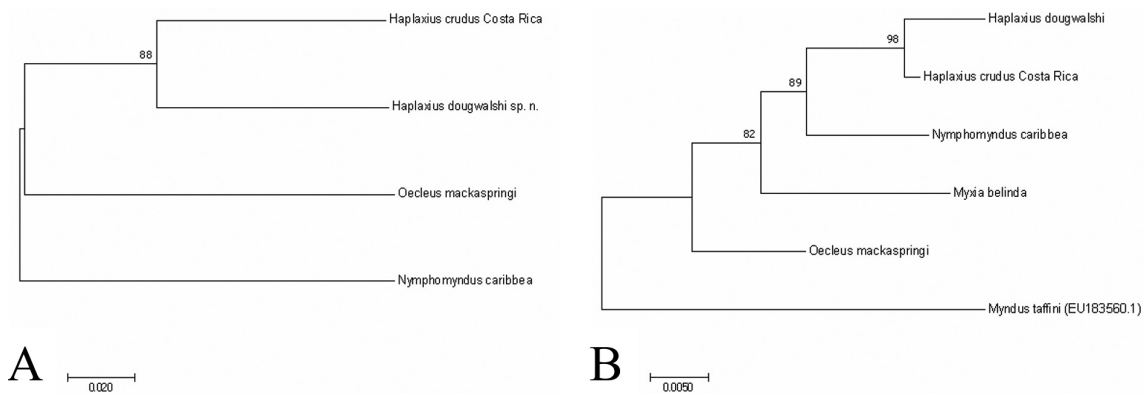
**Other *Haplaxius* species collected.**

*Haplaxius skarphion* – "Costa Rica, Limón / Gandoca, nr. Panama / B.W.Bahder; 14.V.2018 / aspirated from coconut palm" (2 males) (Fig. 8)



**FIGURE 8.** Adult male of *Haplaxius skarphion*; (A) body lateral view, (B) frontal view of head and prothorax, (C) ventral view of terminalia, (D) lateral view of terminalia, (E) left lateral view of aedeagus, and (F) right lateral view of aedeagus, scale bar = 1 mm.

**Sequence data.** For the COI locus, 683 bp of the 5-prime region of the gene were obtained for *Haplaxius dougwalshi* **sp. n.** (GenBank Accession No. MT080284) and was compared to the corresponding region for *Haplaxius crudus*, *Nymphomyndus caribbea* and *Oecleus mackaspringi*. There was strong bootstrap support (88) for *H. dougwalshi* **sp. n.** resolving next to *H. crudus* relative to the other two genera of oecleines included (Fig. 9A) and based on the pairwise comparison, was 12% different than *H. crudus*, 16.4% different than *N. caribbea*, and 17% different from *O. mackaspringi* (Table 1). For the 18S locus, 1,358 bp were obtained for *H. dougwalshi* **sp. n.** (GenBank Accession No. MT002395) and was compared to the corresponding region for *H. crudus*, *N. caribbea*, *Myxia belinda*, *Myndus taffini*, and *O. mackaspringi*. There was strong bootstrap support for *H. dougwalshi* resolving in the genus *Haplaxius* (98) relative to other genera of oecleines (Fig. 9B). Additionally, *H. dougwalshi* **sp. n.** was 0.6% different from *H. crudus* for the 18S gene whereas it was on average, 3.0% different from the other oecleine genera included, ranging from 1.8% to 5.1% different (Table 2).



**FIGURE 9.** Maximum Likelihood phylogenetic tree based on COI sequence data (A) and 18S sequence data (B). Branch support was assessed using 1,000 bootstrap replicates.

**Remarks.** The general form of the aedeagus of *H. dougwalshi* **sp. n.** is similar to many species illustrated by Kramer (1979). While the presence of a spine on the aedeagus before the apex is not unique to *H. dougwalshi* **sp. n.** (shared with *H. fulvus* (Osborn), *H. pusillus* (Van Duzee), *H. viridis* (Ball), *H. jamaicae* (Kramer), *H. simplicatus* Caldwell, and *H. vilbastei* (Kramer)), the spine on the shaft in *H. dougwalshi* **sp. n.** is distinct in being positioned further basad than the other species and strongly curved, whereas the other taxa possess a spine that is either straight or only slightly curved. The lateral processes of the pygofer of *H. dougwalshi* **sp. n.** is unique and easily diagnoses it, being much longer and narrower than any other species of *Haplaxius*. Other taxa that also possess a similar process include *H. glyphis* (Kramer), *H. gnophos* (Kramer), *H. neopusillus* (Kramer), and *H. pusillus* (Van Duzee);



however, in these taxa the base of the process is broad whereas in *H. dougwalshi* **sp. n.**, the base is strongly narrowed, producing a very well-defined process.

While the features of the genitalia are the most reliable to distinguish *H. dougwalshi* **sp. n.**, the white patterns observed on the frons also appear distinct, along with the uniform orange coloration. In addition, there is molecular support for *H. dougwalshi* **sp. n.** belonging to *Haplaxius* based on both the COI and 18S loci.

**TABLE 1.** Pairwise comparison for the COI gene based on 1,000 bootstrap replications using the p-distance method; numbers on bottom left=percent difference, numbers in upper right=standard error.

		1	2	3	4
1	<i>Haplaxius dougwalshi</i> <b>sp. n.</b>		0.013	0.014	0.013
2	<i>Haplaxius crudus</i>	0.120		0.014	0.014
3	<i>Nymphomyndus caribbea</i>	0.169	0.164		0.015
4	<i>Oecleus mackaspringi</i>	0.166	0.170	0.185	

**TABLE 2.** Pairwise comparison for the 18S gene based on 1,000 bootstrap replications using the p-distance method; numbers on bottom left=percent difference, numbers in upper right=standard error.

		1	2	3	4	5	6
1	<i>Haplaxius dougwalshi</i> <b>sp. n.</b>		0.002	0.006	0.004	0.004	0.005
2	<i>Haplaxius crudus</i>	0.006		0.006	0.004	0.003	0.005
3	<i>Myndus taffini</i>	0.051	0.048		0.006	0.006	0.006
4	<i>Myxia belinda</i>	0.026	0.022	0.046		0.004	0.005
5	<i>Nymphomyndus caribbea</i>	0.018	0.014	0.048	0.025		0.004
6	<i>Oecleus mackaspringi</i>	0.025	0.022	0.043	0.027	0.024	

## Discussion

The genus *Haplaxius* is currently broadly defined, resulting in a speciose genus that could reasonably be split as data becomes available. Using molecular data from museum specimens (when possible) and fresh material will significantly advance this effort. Recently, the genus *Myxia* Bahder & Bartlett was described after it was found that it differed from *Haplaxius* by about 16% for COI and 3% for 18S. After assessment of the male genitalia, it was evident the specimen merited genus level placement to accommodate distinct difference observed. The novel taxon, *H. dougwalshi* **sp. n.** appears to conform to the standard form of *Haplaxius* both in overall body form as well as the genitalia. It was noted by Bahder *et al.* (2019a), that *H. crudus*, a common and widely distributed species, is useful for comparison to assess the general form of *Haplaxius*. The clubbed form of the gonostyli, rounded/ovate form of the medioventral process, and form of the aedeagus between *H. crudus* and *H. laevis* Fowler are very similar and these similarities are observed in *H. dougwalshi* **sp. n.** While not all genera within the Oecleini were available for molecular study, for the COI and 18S genes, a combination of molecular data and morphological characters support the placement of the novel taxon in *Haplaxius* as it is currently defined. Very little sequence data is available for the five-prime region of COI with regard to other oecleines, making a robust analysis impossible at the moment. As more taxa are described and previously described taxa are sequenced, more thorough analyses can be performed to further validate the novel taxon's placement but also elucidate the phylogeny of the group (Oecleini) as a whole. While the morphological data and characters are the primary evidence for the placement of the novel taxon in *Haplaxius*, the molecular data will aid in future studies for establishing species and genus level relationships in the Oecleini. The discovery of a new species in the genus *Haplaxius* along with novel molecular data for this species advances the effort in understanding the taxonomy and phylogeny of *Haplaxius* as currently defined.

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