



A new species of planthopper in the genus *Anotia* Kirby (Hemiptera: Auchenorrhyncha: Derbidae) from coconut palm in Costa Rica

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Abstract

An ongoing survey for planthoppers associated with palms in the Caribbean basin is being conducted with current efforts on the Caribbean lowlands of Costa Rica. In an expedition in June of 2019, a derbid was found on coconut palms in Alajuela province and was determined to be a new species belonging to the genus *Anotia*. The novel taxon is described and named *Anotia firebugia* Bahder & Bartlett sp. n. Additionally, molecular barcode data (COI) is provided for the newly described taxon.

Key words: Derbidae, Fulgoroidea, palms, coconut, Costa Rica, planthopper

Resumen

Se está llevando a cabo una investigación con el fin de determinar las especies de chicharritas asociadas a palmeras en la vertiente Caribeña, enfocándose actualmente en las tierras bajas del Caribe de Costa Rica. En un muestreo realizado en Junio del 2019 se encontró una nueva especie de la familia derbidae en cocoteros en la provincia de Alajuela y se clasificó dentro del género *Anotia*. El nuevo taxón se denomina *Anotia firebugia* Bahder & Bartlett sp. n. Además, se proporcionan datos de códigos de barras moleculares (COI) para el taxón recientemente descrito.

Palabras clave: Derbidae, Fulgoroidea, especie nuevo, palmera, cocotero, Costa Rica, chicharrita

Introduction

The genus *Anotia* Kirby 1821 (Derbidae: Otiocerinae: Otiocerini) is currently comprised of 19 New World species (Bartlett *et al.* 2014, Bourgoin 2019) widely distributed through eastern North America, Mesoamerica and parts of the Caribbean (excluding *Interamma septentrionalis* Anufriev, 1968—from Yakovlevka, Primorsky Krai, Russia—transferred to *Anotia* without explanation by Emeljanov 1992). While 10 of the 19 New World species are known from North America and the remaining nine from throughout the Caribbean and Central America, it is likely that the highest diversity is in the Neotropics and that many species remain to be discovered (Bartlett *et al.* 2014).

The genus name *Anotia* means ‘no ear’, a reference to the lack of the subantennal processes that are usually found in Otiocerini and some other fulgoroid groups (see Liang 2005). Host associations and general biology are poorly understood, but it appears that *Anotia* prefer moist habitats. Nymphs are presumed to feed on fungal hyphae, like many other derbids (Wilson *et al.* 1994, Nickel 2003, Bartlett *et al.* 2014, 2017), while adults are associated with a variety of plants (Fitch 1851, Spooner 1937, Wilson *et al.* 1994). *Anotia* is not documented as an economically important taxon (e.g., Wilson & O’Brien 1987).

Here we describe a new species of *Anotia* found during a survey of the auchenorrhynchan fauna associated with palms in Costa Rica (Bahder *et al.* 2019a,b; 2020) as well as provide DNA sequence data to supplement the description.

Materials and methods

Locality and Specimen Collection. Coconut palms were surveyed in Costa Rica from June 12th, 2019 to June 30th, 2019 in the north-central region ranging from San Ramón to the Nicaraguan border and west to Caño Negro. Specimens were swept from the underside of a coconut frond (Fig. 1), aspirated and transferred immediately to 95% ethanol for transportation to laboratory. Samples were collected (permit no. SINAC-ACC-PI-LC-072-2019) near Santa Rosa de Pocosal, Alajuela province, Costa Rica on private property. Permission was obtained by the owner prior to collecting. Specimens were exported under permit number DGVS-434-2019 and imported in the U.S.A. under permit P526-170202-001. 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. The voucher specimen (primary type) is stored at the University of Florida—Fort Lauderdale Research and Education Center (FLREC) in Davie, FL, U.S.A. Labels for type material are quoted with ‘/’ indicating a new line and ‘//’ a new label. DNA sequence data is deposited in GenBank.



FIGURE 1. Habitat and collection site of *Anotia firebugia* Bahder & Bartlett *sp. n.*

Morphological terminology. Morphological terminology generally follows that of Bartlett *et al.* (2014), except forewing venation which follows Bourgoïn *et al.* (2015) and with male terminalia nomenclature modified after

Bourgoin (1988) and Bourgoin & Huang (1990). All measurements from the type specimen (i.e., n=1, male). The new taxon is to be attributed to Bahder and Bartlett. Label information of type is quoted, with ‘/’ indicating new line. Type material is deposited at the University of Florida’s Fort Lauderdale Research and Education Center (FLREC) in Davie, FL. New taxon 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 terminalia were 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 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.

PCR Parameters and Sequence Data Analysis. To obtain COI sequence data, DNA template was amplified using the primers LCO1490 (5’-GGTCAACAAATCATAAAGATATTG-3) (Folmer *et al.* 1994) the reverse compliment of the C1-J-2195 primer by Simon *et al.* (1994), resulting in the sequence (5’-ACTTCTGGATGAC-CAAAAAATCAA-3’) for the reverse primer. PCR reactions contained 5x GoTaq Flexi Buffer, 25 mM MgCl₂, 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 dH₂O to a final volume of 25 µL. 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. 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 haplotypes were calculated with MEGA7 (Kumar *et al.* 2016). Outgroup taxa available in the barcode of life database (BOLD) that were used in the analysis are presented in Table 1.

TABLE 1. Outgroup taxa used for COI pairwise comparison and the associated number assigned to the barcode data in the barcode of life database.

| Species | BOLD Reference No. |
|-------------------------------|--------------------|
| <i>Apache degeerii</i> | BBPEC057-09 |
| <i>Anotia bonnetii</i> | CNCHG1201-12 |
| <i>Anotia uhleri</i> | GMGSI335-12 |
| <i>Lydda elongata</i> | HEQT322-09 |
| <i>Otiocerus stollii</i> | CNCHG1206-12 |
| <i>Otiocerus wolfii</i> | CNCHG1204-12 |
| <i>Patara vanduzeei</i> | ASAHE162-12 |
| <i>Saccharodite chrysonoe</i> | HEWT663-10 |

Systematics

Family Derbidae Spinola 1839

Subfamily Otiocerinae Muir 1917

Tribe Otiocerini Muir 1913

Genus *Anotia* Kirby 1821

Amended diagnosis. Members of this genus are pale, fragile taxa, usually ~5–7 mm in length, with wings greatly exceeding body (~2x body length). The head is strongly dorsoventrally compressed, such that lateral keels of frons are nearly in contact, and projected beyond eyes for a distance about equal to eye width and angled upwards at ~45 degree angle, subtriangular at apex (lateral view). Vertex usually triangular in dorsal view, disc deeply concave, lateral carinae keeled, pustulate, strongly converging to meet prior to fastigium. First antennal segment very small, second antennal segment greatly elongated (reaching or exceeding apex of head), subantennal process absent. Forewings bearing pustules along basal portion of costal vein, humeral region of costa produced into weak lobe, or not produced; media fused with radius + subcosta near wing base, forked in proximal quarter, RP forked from RA + Sc in proximal half of wing. Combined Pcu+A1 extending to reach CuP (i.e., clavus open). Second tarsomere of hind tarsus with row of 4–5 spinules.

Remarks. Most species in this genus are incompletely described. Species diagnostics for *Anotia* are based primarily on color and patterns, especially of forewings, and few species have the terminalia characterized, leading to uncertainties in species diagnostics. While certain species appear to have distinct color patterns, others are ambiguous. Differences among specimens could be due to age of the adult when collected (coloration becoming more intense with time, teneral adults poorly marked), age of the pinned specimen (coloration fades with time), or natural variation within the species. Proper analysis of male terminalia is needed for all described species to establish the validity of taxonomic concepts within *Anotia*, ideally verified by molecular studies. While this is beyond the scope of the current, we have initiated studies on *Anotia* diversity which we hope to publish in the future.

Additionally, some putative *Anotia* possess an expanded lobe in the humeral region of the costa of the forewing, a key diagnostic feature between *Anotia* (not developed) and *Sayiana* (well-developed) (e.g., Bartlett *et al.* 2014, fig. 64J). The development of the costal projection appears to be either misleading or inconsistently evaluated among species, and the genus-level placement of *Sayiana maracasa* Fennah, 1952, *S. puertoricensis* Caldwell, 1951, and *S. viequensis* Caldwell, 1951 should be re-examined. Banaszkiwicz & Szwedó (2005) note that *Sayiana* has reduced apical spinulation of the second tarsomere of the hind leg, a feature that might be helpful in genus diagnostics.

Anotia firebugia Bahder & Bartlett sp. n.

(Figures 2–6)

Type locality. Santa Rosa de Pocosol, Alajuela, Costa Rica.

Diagnosis. In general, a small (~3 mm, excluding wings) pale species with three carinae on mesonotum and a projected head. Wings almost entirely dark, with bright red veins. Facial markings and second antennal segment bright red. Parameres with a trifurcated lobe at midline on dorsal surface with median section sclerotized. Endosoma with three anterior facing sclerotized spines.

Description. *Color.* Base color of body yellow. Head with broad bright red line extending diagonally across lateral portion of head beginning near frontoclypeal suture extending across antennae and compound eyes, continuing onto dorsolateral portions of pronotum and mesonotum. Antennae bright red. Forewings mostly dark with bright red veins (except at wing base, costa and portions of apex and along trailing margin), cells darkly embrowned (except at wing base and most of costal cell). Hindwings embrowned with red veins.

Structure. Body length: 5.19 mm with wings; 3.03 mm without wings. Head. In dorsal view, vertex narrow, approximately as wide as eye, disc deeply depressed, lateral carinae foliate, converging distally (converging on fastigium); distal margin concave (transverse carinae near vertex apex absent); hind margin strongly concave (Fig. 3A). Second antennal segment laterally compressed; narrowed basally and expanding distally; greatly exceeding head anteriorly (approximately as long as combined head, pronotum and mesonotum at midline; Fig. 3A). Head in lateral view extending beyond eyes for a distance about equal to eye length; frontal margin rounded; dorsal margin only slightly angled upward (Fig. 3B). Head in frontal view greatly compressed; lateral carinae in contact from fastigium nearly to frontoclypeal suture; frons partially concealing frontoclypeal suture along midline in frontal view (Fig. 3C). Vertex length: 0.310 mm, width at hind margin: 0.173 mm, width at distal margin: 0.783 mm. Frons length: 0.766 mm, dorsal width: 0.068 mm, frontoclypeal margin width: 0.117 mm.

Thorax. Pronotum short, shaped like inverted, obtuse “V”; anterior margin convex, following posterior margin of head; posterior margin deeply concave, acute (Fig. 3A); carina present at midline resulting in raised keel in lat-

eral view (Fig. 3B), and lateral carinae present at eye level, strongly serpentine, within red band; paradiscal fields of pronotum narrowing below eyes to rounded apex (Fig. 3A). Mesonotum with two lateral carinae, median carina obsolete (Fig. 3A). Pronotum length at midline 0.754 mm. Mesonotum length at midline: 0.837 mm, width: 0.811 mm.

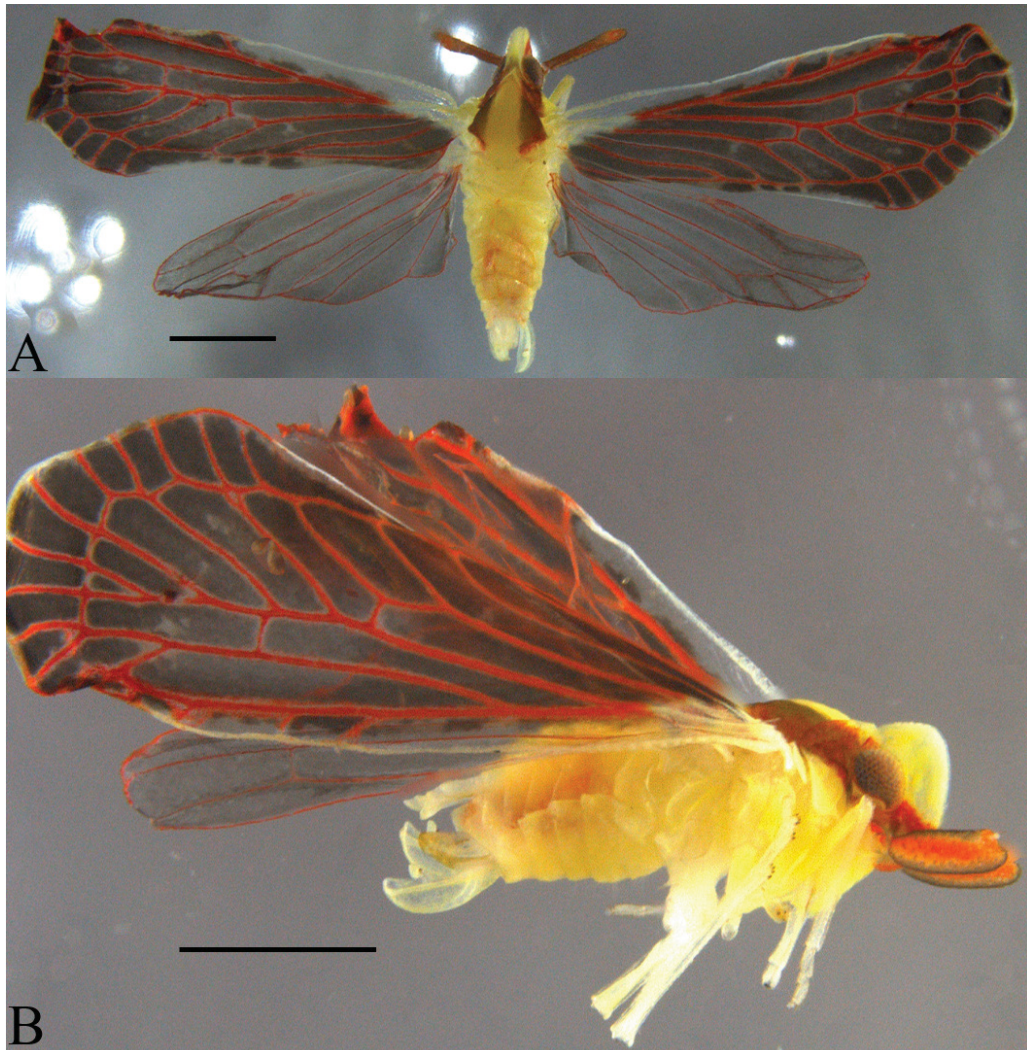


FIGURE 2. Adult male habitus *Anotia firebugia* Bahder & Bartlett **sp. n.** A. body lateral view and B. body dorsal view, scale = 1mm.



FIGURE 3. Adult male *Anotia firebugia* Bahder & Bartlett **sp. n.** A. head, pronotum and mesonotum, dorsal view; B. head, pronotum and mesonotum, lateral view; C. head, frontal view. scale=1mm.

Wings extend considerably beyond abdomen. Forewing (Fig. 4) length (male): 4.29 mm, spatulate, narrowing towards base; apex rounded near leading margin, trailing margin diagonally truncate. Forewings bearing pustules on costal vein in humeral region, humeral region of costa not produced into lobe; media branching from radius + subcosta in basal quarter of wing, RP forked from RA + Sc just before wing midlength. Combined Pcu+A1 extending to reach CuP (i.e., clavus open). General wing ground plan includes one subcostal posterior branch, six radial branches (RA 3 branched, RP 3 branched), seven branches in medial field, cubitus anterior with three branches (CuA₁, CuA₂), seven branches in posterior medial field, cubitus anterior with two branches (evidently fused apically), cubitus posterior unbranched, combined postcubitus and first anal vein unbranched. Postcubitus and first anal vein merged about level with fork of ScP+RA from RP. Fork of CuA about level with clavus apex (distad of Pcu+A1 merge).

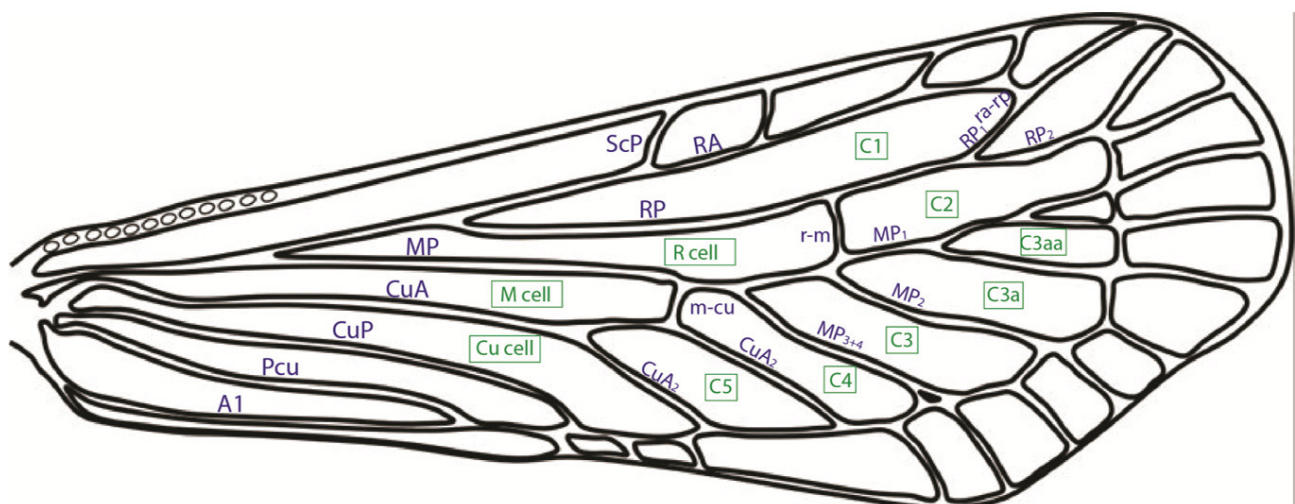
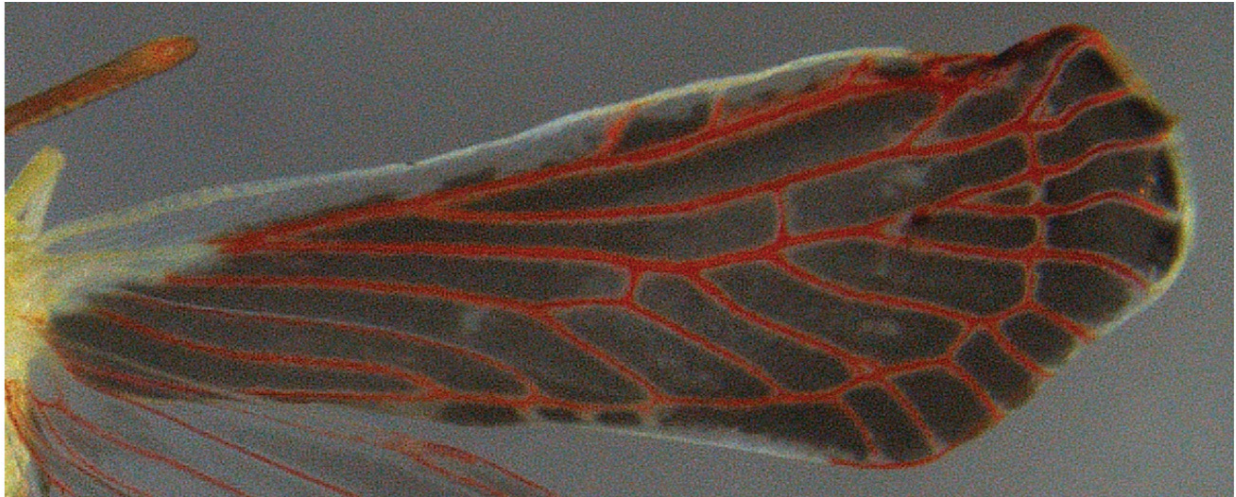


FIGURE 4. *Anotia firebugia* Bahder & Bartlett **sp. n.**, forewing venation; ScP=posterior subcostal, RA=anterior radius, RP=posterior radius, MP=posterior media, CuA=anterior cubitus, CuP=posterior cubitus, Pcu=postcubitus, A1=anal vein, lowercase=crossveins, green letters in box=corresponding cell.

Terminalia. Pygofer in lateral view very narrow with distinct, subtriangular projection near middle of caudal margin (Fig. 5A), anterior margin concave; lacking medioventral process in ventral view (Fig. 5B). Gonostyli in ventral view rounded on outer margins and irregularly sinuate on inner margin (Fig. 5B); narrowest basally, expanding distally (Fig. 5B); in lateral view (Fig. 5A), ventral margin rounded, irregular dorsad with irregularly sinuate lobe basally and trifurcated lobe distal with middle process sclerotized; all processes of distal lobe rounded at apex. Aedeagus simple (shaft without processes), weakly upcurved, with single large spine on right side at apex pointing anteriorly (Fig. 6). Flagellum (endosoma) comprised of two large, lightly sclerotized lobes both with sclerotized spines pointing anteriorly (Fig. 6). Segment 10 (anal tube) in lateral view short, rather quadrate; dorsal margin slightly sinuate; ventral margin straight with acute apex; anal column projected beyond apex of anal tube (Figs 6A, C).

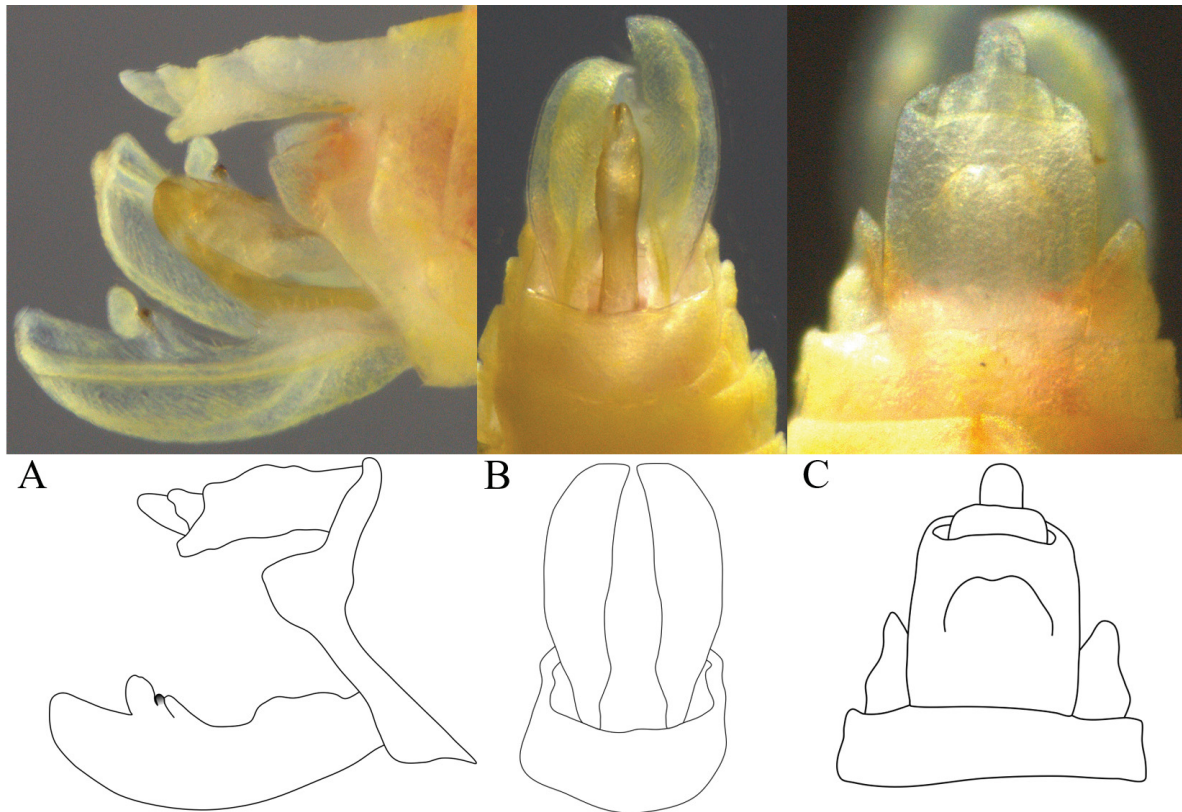


FIGURE 5. Male terminalia of *Anotia firebugia* Bahder & Bartlett **sp. n.** A. lateral view of paramere, pygofer, and anal segment; B. ventral view of pygofer and parameres; C. dorsal view of anal segment.



FIGURE 6. Aedeagus of *Anotia firebugia* Bahder & Bartlett **sp. n.** A. left lateral view; B. right lateral view; C. dorsal view; illustrations correspond directly to images on left.

Plant associations. Coconut (*Cocos nucifera* L.), Arecaceae.

Distribution. Costa Rica (Alajuela).

Etymology. The specific name given by the first author's sons observation that the novel taxon looked like a fire bug, inquired if that could be the insect's name.

Material examined. Holotype male, "Costa Rica, Alajuela Pr./Santa Rosa de Pocosal/15-VI-2019/Coll.: B.W. Bahder/Host: *Cocos nucifera*/*Anotia firebugia*, ♂" (FLREC).

Sequence data. Sequence data for the COI gene for *Anotia firebugia* Bahder & Bartlett **sp. n.** was generated (698 bp, 5') (GenBank Accession No. MT084365). Relative to the other *Anotia* spp. available in BOLD, *A. firebugia* Bahder & Bartlett **sp. n.** differed by about 16.7% (Table 2). When compared to other genera within the Otiocerini, *A. firebugia* Bahder & Bartlett **sp. n.** differed from between 18% to 20%. *Anotia firebugia* **sp. n.** also resolved with other *Anotia* spp. on the maximum likelihood tree generated using COI data (Fig. 7).

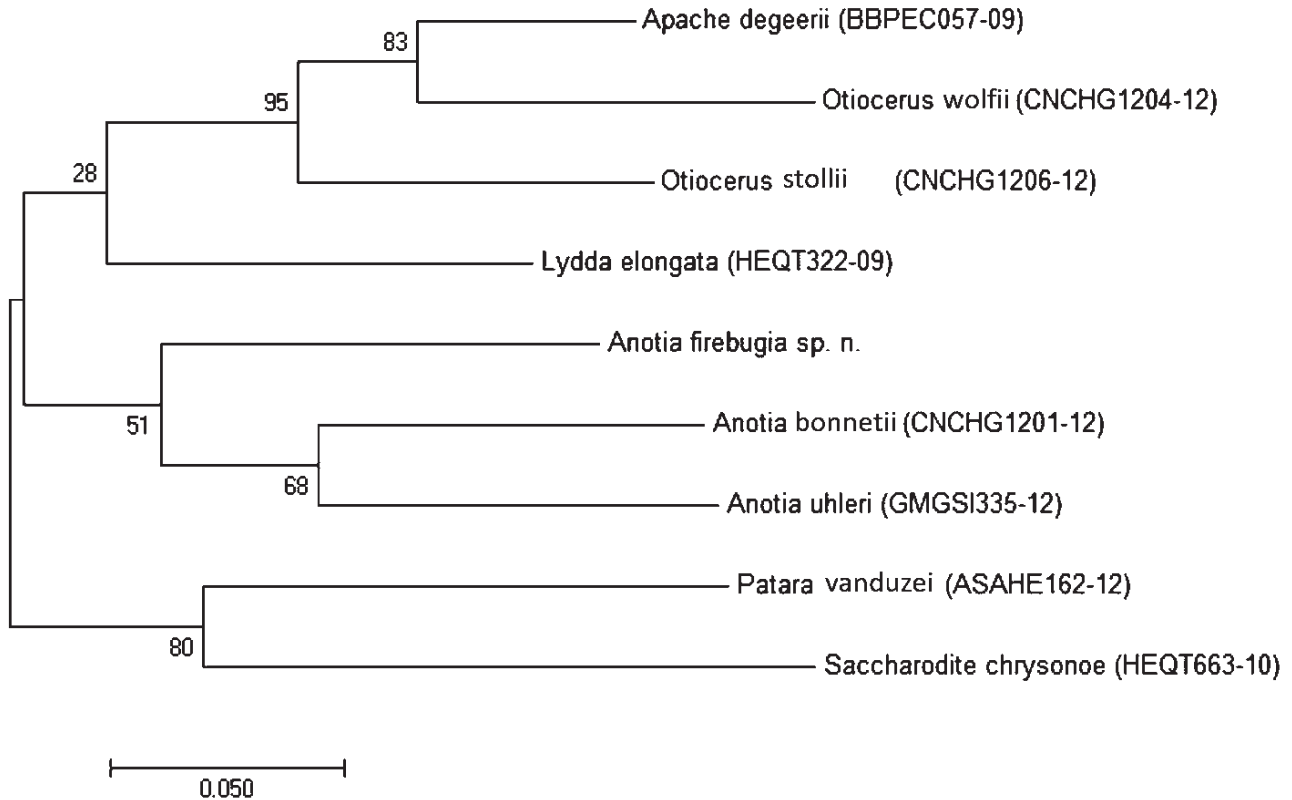


FIGURE 7. Maximum likelihood phylogenetic trees (1,000 replicates) based on the COI gene demonstrating the relationship of the novel taxon relative to other Otiocerinae.

TABLE 2. Pairwise comparison for the COI gene based on 1,000 bootstrap replications using the p-distance method; values in lower left cells=percent difference among taxa, values in upper right cells=standard error.

| No. | Species | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|-----|---------------------------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| 1 | <i>Anotia firebugia</i> sp. n. | | 0.015 | 0.015 | 0.015 | 0.016 | 0.015 | 0.015 | 0.014 | 0.016 |
| 2 | <i>Anotia bonnetii</i> | 0.169 | | 0.014 | 0.014 | 0.015 | 0.015 | 0.016 | 0.014 | 0.016 |
| 3 | <i>Anotia uhleri</i> | 0.166 | 0.149 | | 0.015 | 0.015 | 0.015 | 0.016 | 0.015 | 0.016 |
| 4 | <i>Apache degeerii</i> | 0.180 | 0.161 | 0.177 | | 0.013 | 0.013 | 0.015 | 0.014 | 0.016 |
| 5 | <i>Otiocerus wolfii</i> | 0.198 | 0.167 | 0.181 | 0.117 | | 0.014 | 0.016 | 0.015 | 0.016 |
| 6 | <i>Otiocerus stollii</i> | 0.190 | 0.177 | 0.184 | 0.123 | 0.149 | | 0.015 | 0.015 | 0.016 |
| 7 | <i>Patara vanduzei</i> | 0.195 | 0.205 | 0.202 | 0.183 | 0.201 | 0.195 | | 0.015 | 0.016 |
| 8 | <i>Lydda elongata</i> | 0.161 | 0.164 | 0.180 | 0.142 | 0.172 | 0.166 | 0.186 | | 0.016 |
| 9 | <i>Saccharodite chrysonoe</i> | 0.209 | 0.201 | 0.209 | 0.204 | 0.205 | 0.204 | 0.204 | 0.199 | |

Remarks. The extensive red markings of *A. firebugia* Bahder & Bartlett **sp. n.** are distinct within *Anotia*. Other species with extensive red markings includes *Anotia sanguinea* Fennah (from Trinidad), *A. fitchi* (Van Duzee) and *A. uhleri* (Van Duzee), all of which have less extensive (or more patchy) red markings. Interestingly, *A. fitchi* and *A. lineata* Ball both share with *A. firebugia* Bahder & Bartlett **sp. n.** the paired broad vitta beginning near the fronto-clypeal suture, extending across the antennal base and eyes and continuing along the lateral portions of the pro- and mesonota.

The form of the terminalia are generally similar to other *Anotia* that have been illustrated and those we have examined. Differences among species appear to consist of differences in the shape of the lateral margin of the pygofer (lateral view), elements of the flagellum and aedeagal apex, and differences in the shape of the anal tube, especially in lateral view. The terminalia of too few species of *Anotia* have been described for useful comparison of these features among species.

Discussion

While the bootstrap support is weak for *Anotia firebugia* Bahder & Bartlett **sp. n.** the percent similarity for the COI sequence data indicates that it is closest to *Anotia* based on available data, although *A. bonnetii* and *A. uhleri* differ by about 15% from each other, and by 17% from *A. firebugia* Bahder & Bartlett **sp. n.**, which appear to be relatively large differences. The higher percentage difference of *Anotia* spp. to other genera within the tribe would support the trend observed between the novel taxon and other *Anotia*. *Sayiana* may be phylogenetically close to *Anotia*, but data from this genus was not available. Further efforts and additional data are needed to test the relationships among species of *Anotia* and among Otiocerini that appear similar using various loci and a larger sampling of the genus. One challenge for this group is obtaining molecular data from museum specimens. Generally, obtaining COI data for fresh specimens (less than two years in ethanol) has thus far been successful for *Anotia*, but older specimens have not yielded successful amplification. In other groups, museum specimens can yield adequate sequence data if they are less than 20 years old (B.W. Bahder, *unpublished data*).

The discovery of a new species of *Anotia* on coconut palm is an important addition to the larger survey effort that seeks to understand the diversity of palm-associated planthoppers and assess the potential risk they may pose. Future efforts should seek to collect more individuals of this novel taxon and to assess microbial communities associated with this species to determine if any plant pathogens, specifically phytoplasmas are present in the population. Interestingly this is the first known association of *Anotia* with palms. Most host records are from broadleaf, hardwoods with *A. fitchi* being the exception where it has been documented from sugarcane and prairie cordgrass (Wilson *et al.* 1994).

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