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A new species of planthopper in the genus *Anotia* (Hemiptera: Auchenorrhyncha: Derbidae) from the Los Angeles cloud forest in Costa Rica

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Abstract

Recent survey work in Costa Rica has resulted in the discovery of new species of derbid and cixiid planthoppers associated with palms. During this survey, one species belonging to the genus *Anotia* has been discovered and described, *A. firebugia*. A second species was collected while sweeping trailside vegetation in the Los Angeles cloud forest in Costa Rica and determined to represent a new species of *Anotia*. Herein, *Anotia cerebro* **sp. n.** is described and supplemental molecular data for the cytochrome *c* oxidase subunit I (COI), 18S rRNA gene and D9-D10 expansion region of the 28S rRNA gene are provided.

Key words: taxonomy, phylogenetics, survey, biodiversity

Resumen

Durante un reciente trabajo de investigación llevado a cabo en palmeras en Costa Rica se encontró una gran diversidad de chicharritas pertenecientes a la familia Derbidae. Durante este estudio, se describió una especie perteneciente al género Anotia, denominada A. firebugia. Durante la investigación, se recolectó una segunda especie mientras se muestreaba la vegetación a lo largo de los senderos en el bosque nuboso de Los Ángeles en San Ramón y se determinó que representa una nueva especie de Anotia. En este documento, se describe el nuevo taxón, Anotia cerebro sp. n. y se proporcionan datos moleculares complementarios para la región del código de barras de la subunidad I (COI) del gen del citocromo c oxidasa, el gen 18S ARNr y la región de expansión D9-D10 del gen 28S ARNr.

Palabras clave: taxonomía, filogeneticas, encuesta, biodiversidad

Introduction

Anotia Kirby 1821 (Otiocerinae: Otiocerini) is a genus of derbids with 21 species, mainly from Mesoamerica, south and eastern United States (Barrantes *et al.* 2020, Bartlett & Wilson 2023, Bourgoin 2023). Of the currently

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recognized species, 11 are reported from the US, 7 from Mesoamerica, 3 from Trinidad, and 1 from Cuba (some species are reported from more than one region) with one species doubtfully placed in *Anotia* from Western Russia (Primorsky) (Emeljanov 1992, 1999).

The Otiocerini are delicate forms with laterally compressed elongated heads (narrowly triangular from dorsal view, vertex deeply concave), and the forewings extend well beyond the abdomen. The combined Pcu+A1 veins in the clavus reach the CuP before the margin (i.e., the clavus is "open"). *Anotia* are small and usually pale in color with distinctively marked wings and bodies, on which species diagnoses are often made. The second antennal segment is elongated with subantennal appendages absent. The head tends to be subconical in shape and upwardly angled. The forewings bear pustules on the costal vein, and a small, low humeral lobe is present (despite statements to the contrary, e.g., Szwedo 2005). In life, *Anotia* often holds its wings angled upward and away from the body. *Anotia* is most readily separated from *Sayiana* Ball (the genus superficially most like *Anotia*) by the humeral lobe, which is low and rounded in *Anotia*, but elongated and triangular in *Sayiana*.

Recent survey work in Costa Rica has documented a variety of novel planthopper species associated with palms (Arecaceae). Of these new species, one species of *Anotia* (*A. firebugia* Bahder & Bartlett) has been described thus far. During an expedition in the Los Angeles cloud forest in Costa Rica, a specimen of *Anotia* was collected and subsequently determined to represent a new species. The novel taxon is described herein, and supplemental molecular data for the cytochrome *c* oxidase subunit I (COI), D9-D10 expansion region of 28S rRNA, and 18S rRNA genes are provided.

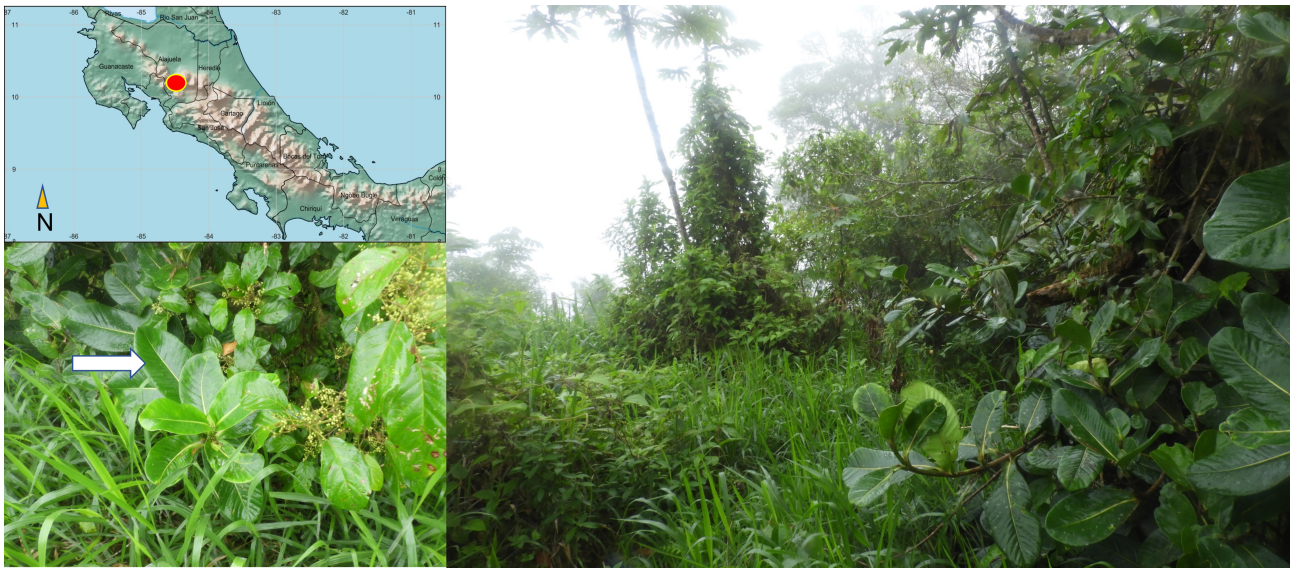

FIGURE 1. Locality and habitat with plant association (white arrow) where *Anotia cerebro* **sp. n.** was collected.

Materials and Methods

Locality and Specimen Collection. The specimen of the novel taxon was collected by sweeping vegetation along trails at Hotel Villa Blanca, Alajuela Province, Costa Rica (10.203231, -84.485094). The specimen was aspirated and transferred to 95% ethanol in the field while still alive. The specimen was collected under permit no. SINAC-ACTo-GASPPNI-016-2018, exported under permit number DGVS-256-2018 to the U.S.A. and imported under permit number P526-170201-001. The specimen was 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 holotype is stored at the University of Florida—Fort Lauderdale Research and Education Center (FLREC) in Davie, FL, U.S.A.

Morphological terminology and identification. Morphological terminology follows that of Bartlett *et al*. (2014), except for the forewing venation following Bourgoin *et al*. (2015) and with male terminalia nomenclature modified after Bourgoin (1988) and Bourgoin & Huang (1990). New taxa are intended to be attributed to Bahder & Bartlett.

Dissections and DNA Extraction. The terminalia that was dissected also served as the source of tissue for DNA extraction. The terminal end of the abdomen was removed and placed 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). Lysis of tissue of the abdomen was 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. The cleared genitalia were then used for morphological characterization and photography.

PCR Parameters, Sequence Data, and Analysis. Primers to amplify COI,18S, and 28S loci are presented in 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 were as follows: 2 min initial denaturation at 95°C, followed by 35 cycles of sec denaturation at 95°C, 30 sec annealing, extension at 72°C, followed by a 5 min extension at 72°C. Annealing temperatures and extension times for corresponding primers/loci are presented in Table 1. PCR product was run on a 2% agarose gel stained with GelRed (Biotium) amplicons of the appropriate size were purified using the Exo-SAP-IT™ PCR Product Cleanup Reagent (ThermoFisher Scientific, Waltham, Massachusetts, USA). The 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), and aligned using Clustal*W* 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 and 18S loci separately as well as one based on concatenated data forming a consensus tree between COI and 18S with MEGA7 (Kumar *et al.* 2016).

TABLE 1. Primers used to amplify loci used for assessment of *Anotia cerebro* **sp. n.** and corresponding annealing temperatures and extension times.

Gene	Name/Direction	Sequence (5'- 3')	Annealing	Extension	Reference
COI	LCO1490/Forward C1J2195RC/Reverse	GGTCAACAAATCATAAAGATATTG ACTTCTGGATGACCAAAAAATCAA	40˚C	1 min. 30 sec.	Folmer *et al.* 1994 Humphries *et al.* 2021
18S	18SFI/Forward 18SRI/Reverse	ACTGTCGATGGTAGGTTCTG GTCCGAAGACCTCACTAAA	50˚C	2 min.	Bahder *et al.* 2019
28S	V/Forward X/Reverse	GTAGCCAAATGCCTCGTCA CACAATGATAGGAAGAGCC	55˚C	1 min. 30 sec.	Cryan *et al.* 2000

Taxon Sampling. For in-group comparison, molecular data for *A. firebugia* was used for COI, 18S and 28S. Out-groups in the Otiocerini included three species of *Shellenius* Ball, 1928, *Cobacella palmensis* Bahder & Bartlett (in Echavarria *et al.* 2023), and *Sayiana sayi* (Ball, 1902). Other Otiocerinae included are 2 species of *Patara* Westwood, 1841 (Patarini), *Mula resonans* Ball, 1928 and *Sikaiana harti* (Metcalf, 1923) (Sikaianini). A non-otiocerine out-group was used to help polarize the tree—*Agoo xavieri* Bahder & Bartlett 2019 (Derbinae: Cenchreini, in Bahder *et al.* 2019). All GenBank accession numbers for in-group and out-group taxa are presented in Table 2.

TABLE 2. Representative Otiocerinae used for morphological and molecular comparisons with *Agoo xavieri* as subfamily outgroup (Derbinae).

Species	COI	18S	28S
Agoo xavieri	MK443068	MK443973	ORO50638
Anotia cerebro **sp. n.**	OR418164	OR419691	OR050637
Anotia firebugia	MT084365	MT945942	ORO50636
Cobacella palmensis	ORO44883	ORO41765	ORO50628
Mula resonans	OQ473376	OQ519977	ORO50635

.....Continued on the next page

TABLE 2. (Continued)

Species	COI	18S	28S
Patara cooki	MW332651	MW333024	ORO50634
Patara vanduzei	OQ473377	OQ519977	ORO50633
Shellenius ballii	OQ473378	OQ519976	ORO50631
Shellenius schellenbergi	OQ473379	OQ519975	ORO50630
Shellenius serratus	OQ473380	OQ519974	ORO50629
Sikaiana harti	OR418165	OR419691	OR419689

Systematics

Family Derbidae Spinola 1839

Subfamily Otiocerinae Muir 1917

Tribe Otiocerini Muir 1917

Genus *Anotia* Kirby 1821

= *Amalopota* Van Duzee, 1889, synonym by Fennah (1952: 152).

Type species: *Anotia bonnettii* Kirby 1821

Diagnosis. (Modified from Barrantes *et al.* 2020.) Pale, fragile taxa, usually 5–7 mm in length, with wings greatly exceeding body (~2x body length), often held somewhat upward and away from the body in life. Head strongly laterally compressed, with lateral keels on frons nearly in contact; 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 before fastigium. Antennae with first segment very small, second 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 + subcostal near wing base, forked in proximal quarter, RP forked from RA + ScP 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.

Anotia cerebro Bahder & Bartlett sp. n.

(Figures 2–7)

Type Locality. Costa Rica, Alajuela Province, Hotel Villa Blanca.

Diagnosis. Large, body yellowish species with orange markings (7.2 mm with wings). Head projected, inclined upwards, with orange stripe between eye and head apex. Forewings yellowish with three fuscous bands, veins along costal margin with reddish wash, cell C5 small with arched trailing margin. Aedeagal endosoma membranous and globose, lacking elongate processes. Anal tube in lateral view irregularly quadrate, relatively short, weakly bilaterally asymmetrical at apex, ventral margin of apex downturned with rounded apices.

Description. *Color.* Base color of body pale yellow, head with orange margins, genae, and orange stripe extending from eyes to head apex just below dorsal margin. Orange wash on abdominal tergites and sternites. Forewings translucent, faintly yellow, with three fuscous bands, first in basal 1/3, extending from costal margin, forked at CuA (anterior fork reaching wing margin near claval apex, posterior band continued to clavus proximad of fusion of Pcu and A1); second band near midlength, of irregular width, extending from leading to trailing margin (along MP_{3+4}); third band subapical, extending from RP_3 branch to $MP_{2.1}$ branch; veins pale yellow, reddish wash on veins along costal margin.

FIGURE 2. *Anotia cerebro* **sp. n.** *in vivo* (iNaturalist observation 42334011, San José Province, Costa Rica, Gernot Kunz).

TABLE 3. Biometric data for *Anotia cerebro* **sp. n.** (in mm).

Character	Male, *n*=3	Female, *n*=1
Body length, with wings	7.16	7.91
Body length, no wings	3.38	4.12
Forewing length	6.16	6.60
Vertex length	0.13	0.14
Vertex width, basal margin	0.82	0.83
Vertex width, distal margin	0.15	0.16
Pronotum length, midline	0.20	0.20
Mesonotum length, midline	0.73	0.74
Mesonotum width	1.09	1.10
Frons width, dorsal margin	0.04	0.04
Frons width, clypeal suture	0.08	0.08
Frons width, widest	0.08	0.08
Frons width, narrowest	0.04	0.04
Frons length, midline	1.37	1.38
Clypeus length	0.54	0.55

Structure. Body length: male (*n*=3), with wings 7.16 mm, without wings 3.38 mm, female (*n*=1) with wings 7.9 mm, without wings 4.1 mm (Table 3). Head. In dorsal view narrower than pronotum (Fig. 3A), vertex strongly depressed, lateral margins postulate, distinctly projected beyond eye, posterior margin roundly concave, anterior margin rounded. In frontal view (Fig. 3B), vertex elevated above eyes, frons greatly compressed, lateral margins broadly in contact. In lateral view (Fig. 3C), head projecting beyond eyes for distance nearly equal to eye width. Vertex inclined giving head distinctly upturned appearance, in lateral viw head conical with rounded apex. Antennal

scape very short, pedicle elongated (ovoid in cross-section), apices exceeding distal margin of head, apex dorsally emarginated. Lateral ocelli obsolete. Rostrum just reaching hind coxae, apical segment short.

FIGURE 3. Adult male habitus *Anotia cerebro* **sp. n.**; (A) dorsal view and (B) lateral view; scale bar = 1 mm.

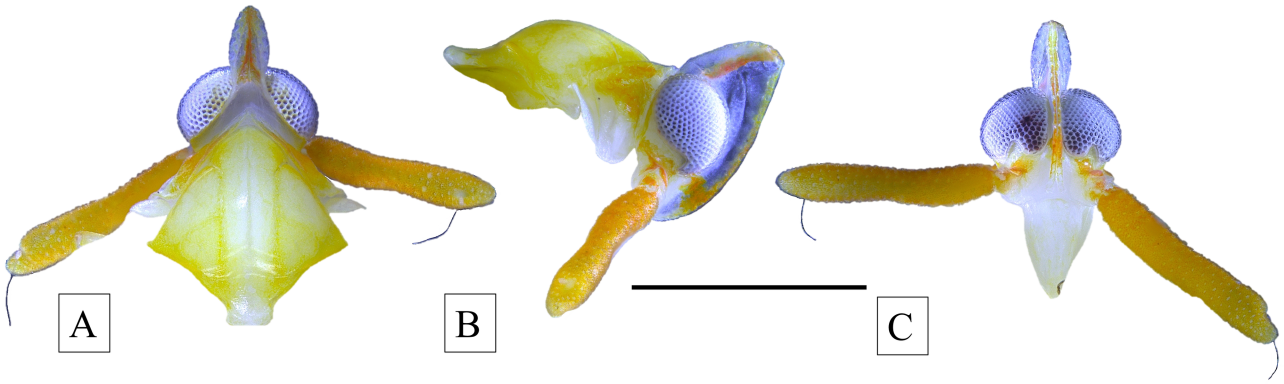

FIGURE 4. Adult male *Anotia cerebro* **sp. n.**; (A) dorsal view of head, pronotum and mesonotum, (B) lateral view of head, pronotum and mesonotum and (C) frontal view of head, pronotum and mesonotum; scale bar = 1 mm.

Thorax. Pronotum short, length along midline about equal to vertex length, anterior margin moderately convex, posterior margin concave, median carina present. Mesonotum weakly tricarinate, approximately 1.25X wider than long, strongly arched in lateral view. Spinulation of hind leg 5-5-4.

Forewing (Fig. 5) with rounded projection in humeral region of costal margin; MP fused with ScP+R for short distance from basal cell, RP forked from ScP+RA in proximal 1/3 of wing; cell C5 small with arched trailing margin, cells along apical margin of wing irregular in size (although mostly rectangular and taller than wide); branching

pattern RA 2-branched, RP 3-branched, MP 8-branched, CuA 2-branched; composite vein Pcu+A1 joining with CuA (closing cell C5) before attaining wing margin beyond wing midlength (clavus open)

Terminalia. Pygofer in lateral view narrow, approximately equal width at dorsal and ventral margins, anterior margin linear, posterior margin with lateral margins of opening bearing large triangular projection (Fig. 6A); in ventral view, medioventral process absent (Fig. 6B). Gonostyli in lateral view with ventral margin rounded, dorsal margin with three processes: basal process broad at base, apex gently rounded and curved caudad, middle process small, sclerotized, curved caudad with acute apex; distal process larger, leaning caudad, with lateral margins subparallel, subtruncate at apex, leaning caudad (Fig. 6A) in ventral view, medially scoop-like, narrowed basally, expanding at midpoint, rounded on outer margins, inner margin angulate then linear to apex (Fig. 6B). Aedeagus simple, relatively short, somewhat upcurved, tubular, broadest near base, distally narrowed, shaft simple (without processes), endosoma large, bulbous, mostly membranous semi-spherical in form, lacking elongate processes, bearing two small, slightly sclerotized patches, first patch (A1) on right, dorsal surface, second patch (A2) on ventral surface near anterior margin with small ventrad pointing spine (Fig. 7). Anal tube in lateral view irregularly quadrate, with irregularly sinuate dorsal and ventral margins, relatively short, ventral margin of apex downturned with rounded apices (Fig. 6A), in dorsal view, broad, roughly quadrate, asymmetrical, right lateral apex extending further than left lateral apex (Fig. 6C); paraproct short and conical.

Plant Associations. Unknown; sweeping damp vegetation comprised of various monocot and dicots with large tree branch overhanging sweeping site.

Distribution. Costa Rica (Alajuela).

Etymology. The specific name is given as a reference to the Spanish word for brain, “cerebro”, a reference to the resemblance of the aedeagus resembling a brain. The name is to be treated as a noun in apposition and indeclinable.

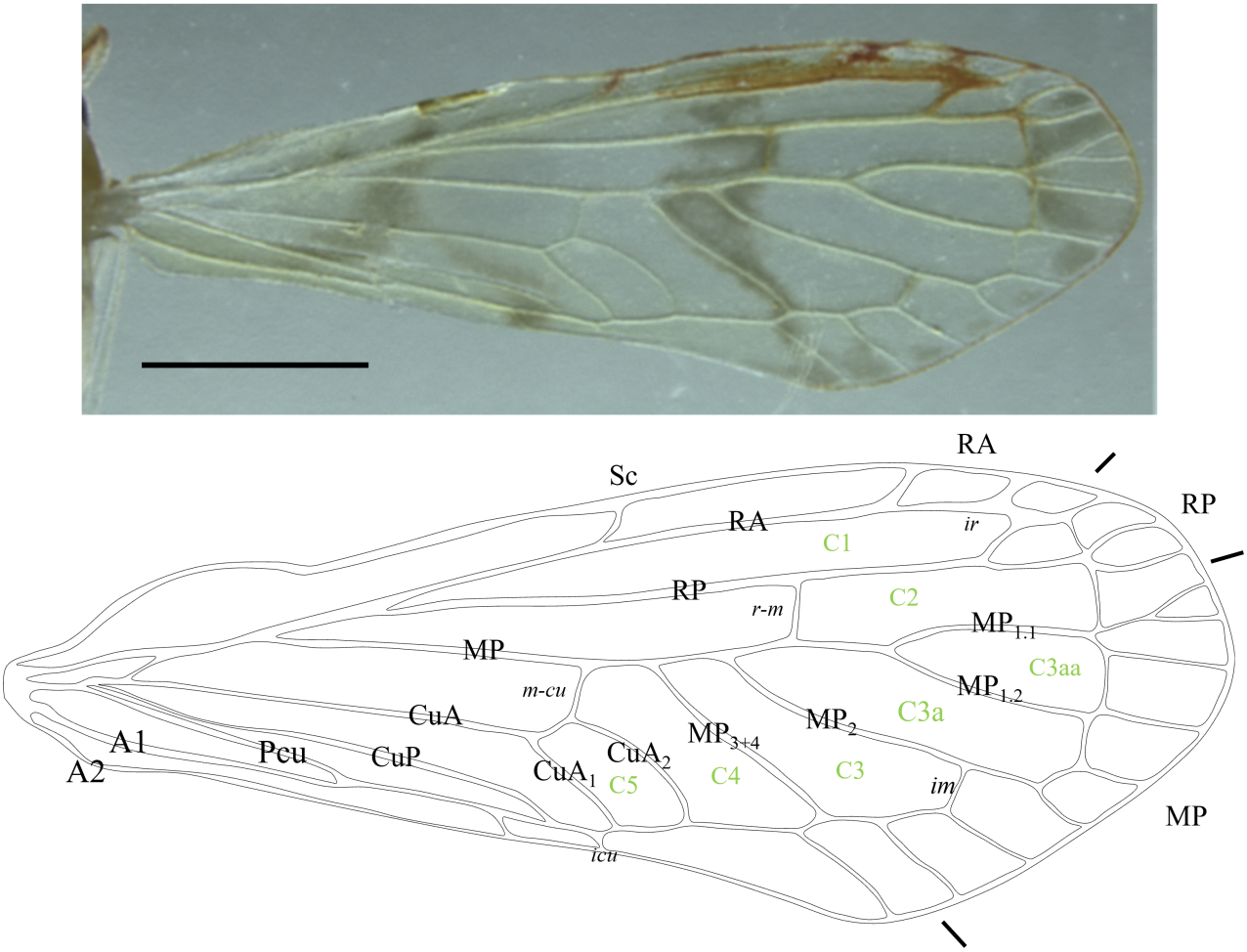

FIGURE 5. Adult male *Anotia cerebro* **sp. n.** forewing venation (veins black, crossveins italics, cells green, abbreviations following Bourgoin *et al.* 2015); scale = 1 mm.

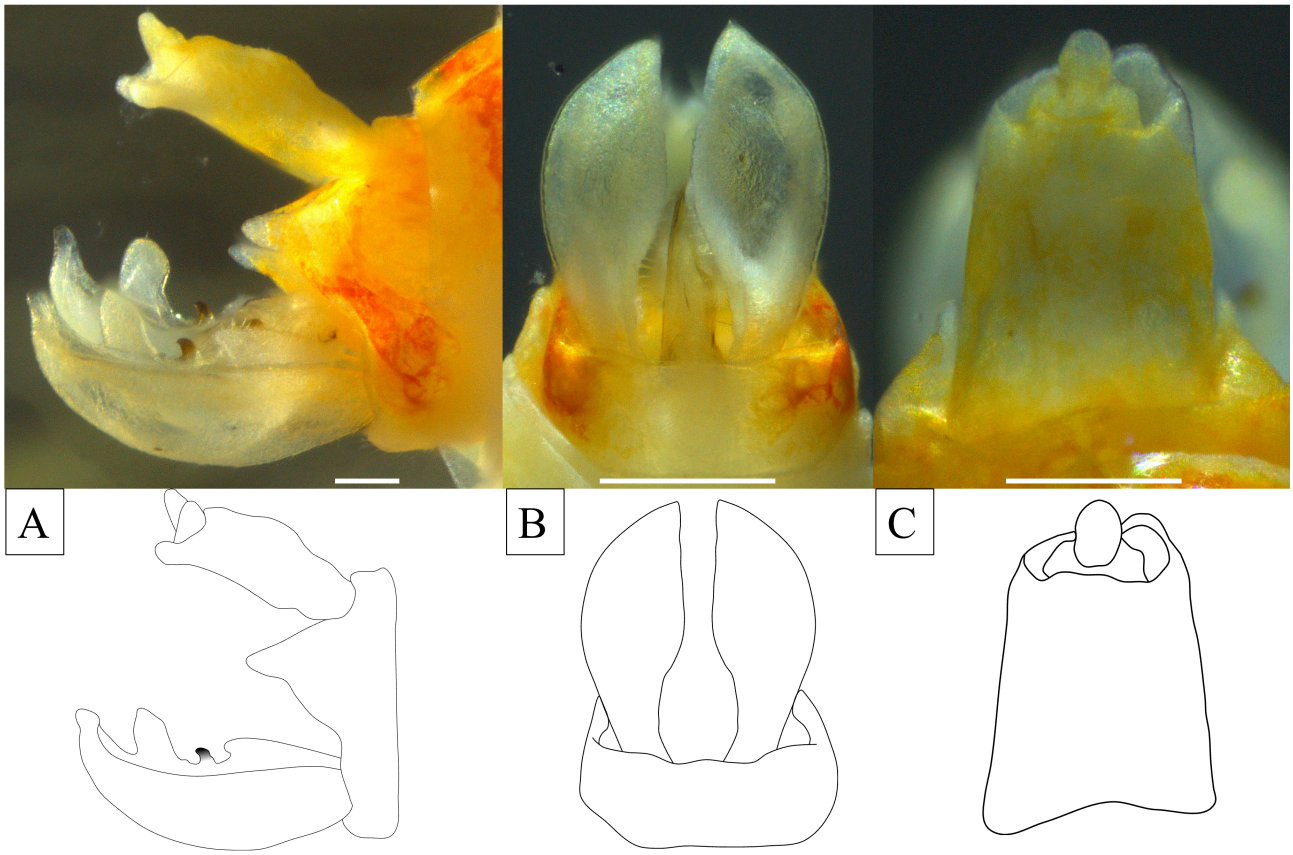

FIGURE 6. Adult male *Anotia cerebro* **sp. n.** terminalia; (A) lateral view, (B) ventral view and (C) dorsal view; scale = 0.25 mm.

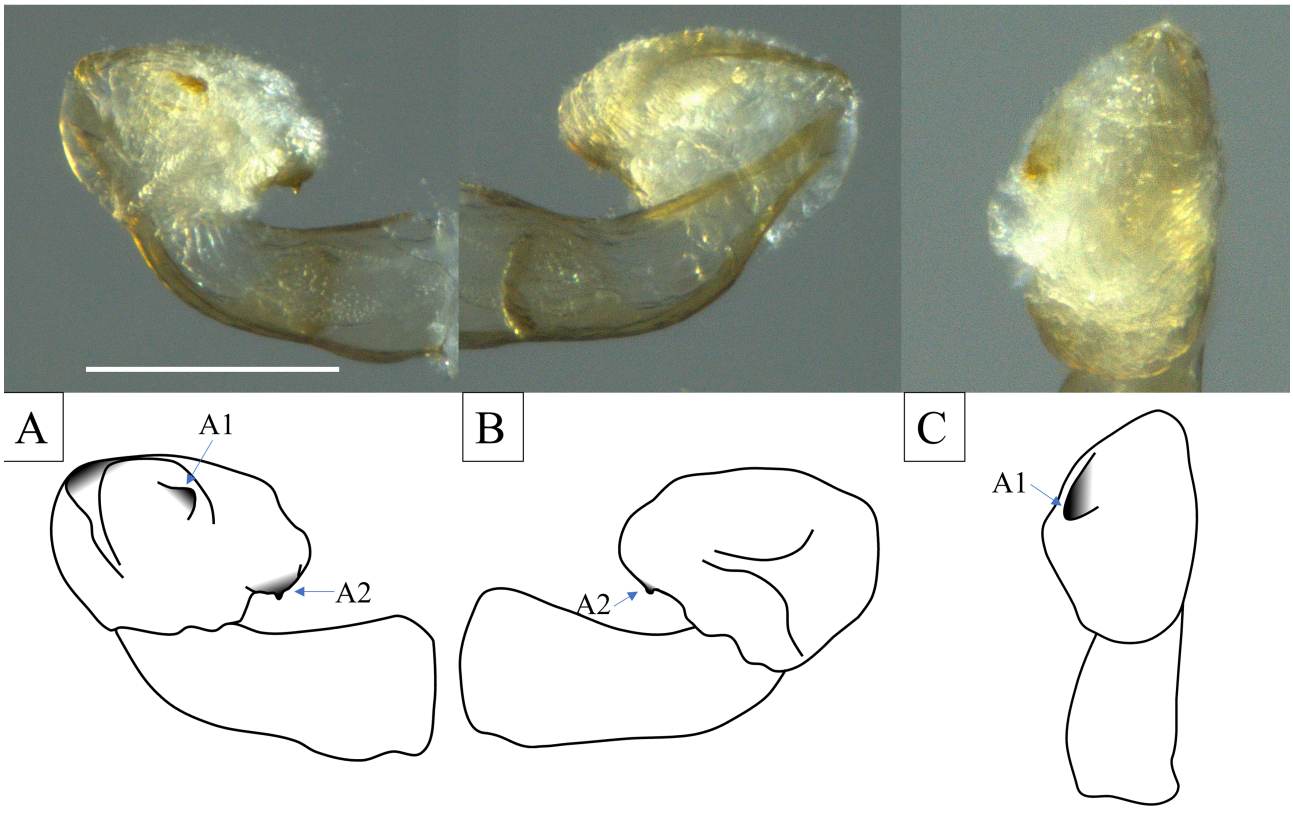

FIGURE 7. *Anotia cerebro* **sp. n.** aedeagus; (A) right lateral view, (B) left lateral view and (C) dorsal view; scale = 0.33 mm.

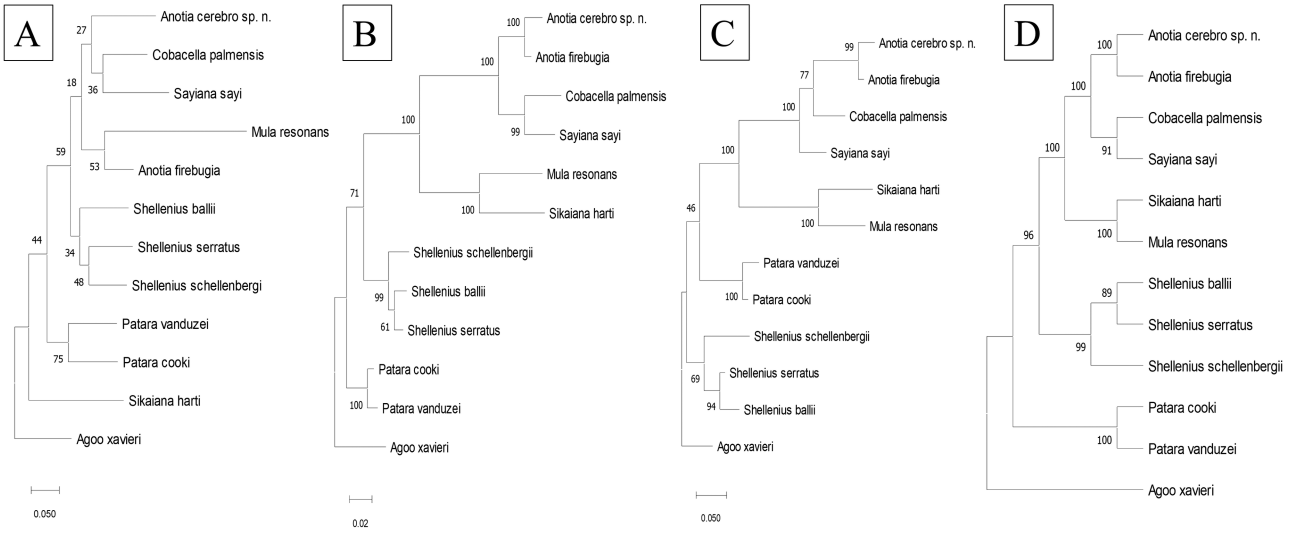

FIGURE 8. Maximum likelihood phylogenetic tree based on 1,000 replicates; (A) COI gene, (B) 18S rRNA, (C) 28S gene and (D) consensus tree of concatenated COI, 18S, and 28S data; scale bar = percent nucleotide difference.

Material Examined. Holotype male, "Costa Rica, Alajuela Pr. / Hotel Villa Blanca, Henri Pittier Trail / 24-VI-2019 / Coll.: B.W. Bahder / Sweeping near beginning of trail / *Anotia cerebro* ♂" (FLREC), paratypes same as holotype but collection date 18-VII-2023 (2 males, 1 female).

Sequence Data. For *Anotia cerebro* **sp. n.** a 684 bp product was generated for the barcoding region of COI, a 1,660 bp product was generated for the 18S gene, and a 749 bp product for the D9-D10 expansion region of the 28S gene.

The phylogenetic analysis based on the barcoding region of COI demonstrated weak (≤75) bootstrap support for all nodes (Fig. 8A). Phylogenetic analysis of the of 18S gene showed strong bootstrap support (100) for placement of *Anotia cerebro* **sp. n.** adjacent to *A. firebugia* and strong bootstrap support (100) for placement of *Anotia* adjacent to a clade comprised of *Sayiana sayi* and *Cobacella palmensis* (Fig. 8B). Finally, the phylogeny based on 28S also showed strong bootstrap support (99) for the placement of *A. cerebro* **sp. n.** adjacent to *A. firebugia* (Fig. 8C). The consensus tree based on concatenated COI,18S, and 28S data demonstrated strong bootstrap support (100) for *Anotia cerebro* **sp. n.** resolving adjacent to *A. firebugia* (Fig. 8D).

Remarks. The structure of the head, the general form of the genitalia, and the projection on the costal margin of the forewing for *Anotia cerebro* **sp. n.** support its placement in the genus *Anotia*. The molecular analyses performed provided strong supplemental support for the placement of the novel taxon in *Anotia*. While there is limited molecular data for the Otiocerinae, the resolution of *A. cerebro* **sp. n.** adjacent to *A. firebugia* based on 18S and consistent morphological characters with currently described *Anotia* is important for beginning to shed light on the phylogeny of this interesting group of insects.

Anotia cerebro **sp. n.** is not obviously similar to any of the described species from that region. The species is more closely allied to *Anotia* s.s. then the species formerly placed in *Amalapota* (synonymized with *Anotia* by Fennah, 1952). The species keys poorly to *A. marginicornis* Fowler, 1904 in Metcalf (1938; the most recent key to the non-US taxa) and *A. cerebro* **sp. n.** does not match either this illustration (Fowler 1904, table 9, fig. 4 shows a species with unmarked wings and differing venation, perhaps incorrectly assigned to *Anotia*) or the description (e.g., whitish with unmarked wings). Based on the descriptions, only *A. invalida* Fowler, 1904, is not described as white (light testaceous with a reddish tinge), but the illustrated markings (Fowler 1904, tab. 9, fig. 8) are a poor match for the new species. After Fowler (1904), neotropical species were described by Metcalf (1938, from Panama) and Fennah (1952, from Trinidad), none of which are a plausible match for *A. cerebro* **sp. n.** (*A. punctata* Metcalf 1938 had a black abdominal dorsum; the four species described by Fennah, 1952, include male genital figures—figs. 30–33—that differ markedly from the new species).

Discussion

Many species belonging to *Anotia* were not fully described (based on color, without a description of male genitalia), making a comprehensive understanding of the current species difficult and uncertain. A review of the type material of *Anotia* species from south of the US border is needed to more fully characterize described species, particularly those described by Fowler (1904; a review of the US species is in preparation). Observations of *Anotia* on iNaturalist are abundant (currently 1,569 observations attributed to the genus), and suggest that while there appear to be several (or many) undescribed species, the described species are often not sufficiently characterized to diagnose based on iNaturalist observations. They also suggest that *Anotia* occurs in South America, where it has not been reported in technical literature, although some of these observations may represent allied, but undescribed genera. Aside from taxonomic issues, a better understanding of the life history of *Anotia*, and Otiocerini in general, is needed to better understand their biology concerning plant associations in general, and palms in particular, with particular regard to whether they may be plant disease vectors.

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