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A new species of planthopper in the genus *Agoo* (Hemiptera: Fulgoroidea: Derbidae) from coquito palms (*Astrocaryum alatum*) in Costa Rica

BRIAN W. BAHDER[1], MARCO A. ZUMBADO ECHAVARRIA[2], EDWIN A. BARRANTES BARRANTES[3], GERNOT KUNZ[4], ERICKA E. HELMICK[5] & CHARLES R. BARTLETT[6]

[1]*University of Florida, Department of Entomology and Nematology - Fort Lauderdale Research and Education Center; 3205 College Ave., Davie, FL 33314-7719, USA. E-mail: bbahder@ufl.edu*
bbahder@ufl.edu; https://orcid.org/0000-0002-1118-4832
[2]*Universidad de Costa Rica—Sede San Ramón, Departamento de Ciencias Naturales, de la Iglesia el Tremedal 400 mts al Oeste carretera hacia San Pedro, San Ramón, Alajuela, Costa Rica. E-mail: marco.zumbado@ucr.ac.cr*
nassua75@gmail.com; https://orcid.org/0000-0002-2591-7662
[3]*Universidad de Costa Rica—Sede San Ramón, Departamento de Ciencias Naturales, de la Iglesia el Tremedal 400 mts al Oeste carretera hacia San Pedro, San Ramón, Alajuela, Costa Rica. E-mail: edwin.barrantes@ucr.ac.cr*
edwbarrantes@gmail.com; https://orcid.org/0000-0001-9565-2105
[4]*Karl-Franzens-Universität, Universitätsplatz 2, Zoologie, 8010 Graz, Austria. E-mail: gernot.kunz@gmail.com*
gernot.kunz@gmail.com; https://orcid.org/0000-0001-7858-0402
[5]*University of Florida, Department of Entomology and Nematology - Fort Lauderdale Research and Education Center; 3205 College Ave., Davie, FL 33314-7719, USA. E-mail: ehelmick@ufl.edu*
ehelmick@ufl.edu; https://orcid.org/0000-0002-5153-0891
[6]*University of Delaware, Department of Entomology and Wildlife Ecology, 250 Townsend Hall, Newark, DE 19716-2160, USA. E-mail: Bartlett@udel.edu*
bartlett@udel.edu; https://orcid.org/0000-0001-9428-7337

Abstract

An ongoing survey to document planthopper diversity on palms (Arecaceae) is being conducted in Costa Rica. During these efforts a new species of derbid planthopper belonging to the genus *Agoo* was found on *Astrocaryum alatum* Loomis in the Heredia province at La Selva Biological Station and is described here as *Agoo luzdenia* Bahder & Bartlett **sp. n.**, bringing the genus to four described taxa—*A. dahliana*, *A. luzdenia* Bahder & Bartlett **sp. n.**, *A rubrimarginata*, and *A. xavieri*. Sequence data for the cytochrome c oxidase subunit I (COI) and 18S genes was generated for the novel taxon and strongly supports its placement in the genus *Agoo*.

Key words: Cenchreini, new species, Derbidae

Resumen

Se está llevando a cabo una investigación con el fin de documentar la diversidad de chicharritas en palmeras (Arecaceae) en Costa Rica. Durante los muestreos, se encontró una nueva especie de chicharrita derbida perteneciente al género Agoo en *Astrocaryum alatum* Loomis en la Estación Biológica La Selva, provincia de Heredia y se describe aquí como *Agoo luzdenia* Bahder & Bartlett **sp.n**. llevando el género a cuatro taxones descritos: *A. dahliana, A. luzdenia* Bahder & Bartlett **sp. n**., *A rubrimarginata* y *A. xavieri*. Los datos de secuencia para los genes de la subunidad I del citocromo c oxidasa (COI) y 18S se generaron para el nuevo taxón y refuerzan su colocación en el género Agoo.

Palabras clave: Cenchreini, especie nuevo, Derbidae

Introduction

The planthopper genus *Agoo* Bahder & Bartlett is a recently described genus of Derbidae (Derbinae: Cenchreini), initially established as a subgenus of *Omolicna* Fennah to accommodate *Omolicna* (*Agoo*) *xavieri* Bahder & Bartlett

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found on coconut palms in Costa Rica (Bahder *et al*. 2019a). Subsequently, *Agoo* was raised to genus status, the species *Agoo dahliana* Bahder & Bartlett was described (associated with the native palm, *Astrocaryum alatum* H.F. Loomis), and *Omolicna rubrimarginata* Fennah was transferred into *Agoo* (Bahder *et al*. 2020). *Agoo* was elevated to full genus due significant difference in the terminalia relative to *Omolicna* that were consistent among the novel taxa, including a symmetrical aedeagus, subtriangular medioventral process lacking lateral teeth, as well as salient features of the head and wings. In addition to substantial morphological differences, there were significant differences for both the 18S and cytochrome *c* oxidase subunit I (COI) genes that resulted in two distinct clades based on differences among taxa relative to *Omolicna*.

The monotypic genus *Cenanges* Fennah, 1952 (including only the type species *C. spectralis* Fennah) from Dominica is superficially like *Agoo*. These taxa differ most obviously in the general form of the aedeagus: *Agoo* has a complex endosoma, with contorted processes on both the aedeagus and endosoma that are angled in different directions, whereas *C. spectralis* has a simple aedeagus and endosoma, with all processes angled dorsad.

Recent survey efforts in Costa Rica assessing diversity of planthoppers on palms have resulted in the discovery of three species: *Agoo dahliana* Bahder & Bartlett (Bahder *et al*. 2020), *Agoo xavieri* Bahder & Bartlett (Bahder *et al*. 2019a), and *Myxia belinda* Bahder & Bartlett (Cixiidae, Oecleini; Bahder *et al*. 2019b). During this survey, a derbid collected from the palm *Astrocaryum alatum* at La Selva Biological Station in Costa Rica (Fig. 1) was subsequently identified as a new species of *Agoo* based on overall morphological characters and the form of the aedeagus and is herein described as a novel taxon with supporting DNA sequence data for the cytochrome *c* oxidase subunit I (COI) and 18S ribosomal RNA (18S) genes. In addition, a revised key to species of the genus *Agoo* is provided.

FIGURE 1. Specimen of *Astrocaryum alatum* where specimens of *Agoo luzdenia* **sp. n.** were collected (A) and the understory showing suspected habitat of planthopper nymphs (B).

Materials and Methods

Locality and specimen collection. Individuals of the novel taxon were aspirated from healthy appearing examples of *Astrocaryum alatum* and were immediately transferred to 95% ethanol. Specimens were collected (permit no. SINAC-ACTo-GASPPNI-016-2018) at La Selva Biological Station (Fig. 1), Heredia province, Costa Rica (10.431269, -84.005961), and exported under permit number DGVS-256-2018 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. 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. Morphological terminology generally follows that of Bartlett *et al*. (2014), except forewing venation following Bourgoin *et al*. (2015) and with male terminalia nomenclature modified after Bourgoin (1988) and Bourgoin & Huang (1990).

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 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, 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 and sequence data analysis. To obtain COI sequence data, DNA template from specimens was amplified using the primers LCO1490 (5'-GGTCAACAAATCATAAAGATATTG-3') and HCO2198 (5'-TCAGGGTGACCAAAAAATCA-3') (Folmer *et al*. 1994). To obtain 18S sequence data, the primers developed by Bahder *et al.* (2019a) were used and are as follows; forward primer 18SF (5'-ACTGTCGATGGTAGGTTCTG-3'), reverse primer 18SR (5'-GTCCGAAGACCTCACTAAA-3'). 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 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 55°C, 2 min 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 and 18S 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 for both the COI and 18S loci.

FIGURE 2. Adult *Agoo luzdenia* **sp. n.** *in vivo*.

Taxon sampling. COI sequence data was used from three species of *Omolicna* (*O. brunnea* (McAtee)—GenBank Accession No. MK443070, *O. puertana* Caldwell—GenBank Accession No. MN496468, and *O. triata* Caldwell—GenBank Accession No. MK443069), *Anchimothon dubia* (Caldwell) (GenBank Accession No. MN496470), *Neocenchrea heidemanni* (Ball) (GenBank Accession No. MN496473), an undetermined species of *Cenchrea* Westwood (GenBank Accession No. MT085816), and an undetermined *Herpis* Stål (GenBank Accession No. MT085817) (collected in this study) as out-groups in the phylogenetic analyses. COI data for *A. dahliana* (GenBank Accession No. MN496467) and *A. xavieri* (GenBank Accession No. MK443068) was used as in-group data. 18S sequence data was used for *Anchimothon dubia* (GenBank Accession No. MN474755), *Omolicna puertana* (GenBank Accession No. MN472751), and *Cenchrea dorsalis* Westwood (GenBank Accession No. MN472756) as out-groups and *Agoo dahliana* (GenBank Accession No. MH472754) and *Agoo xavieri* (GenBank Accession No. MK443073) as in-groups. Amplification of 18S for *Herpis* sp. (Fowler) failed using current primers.

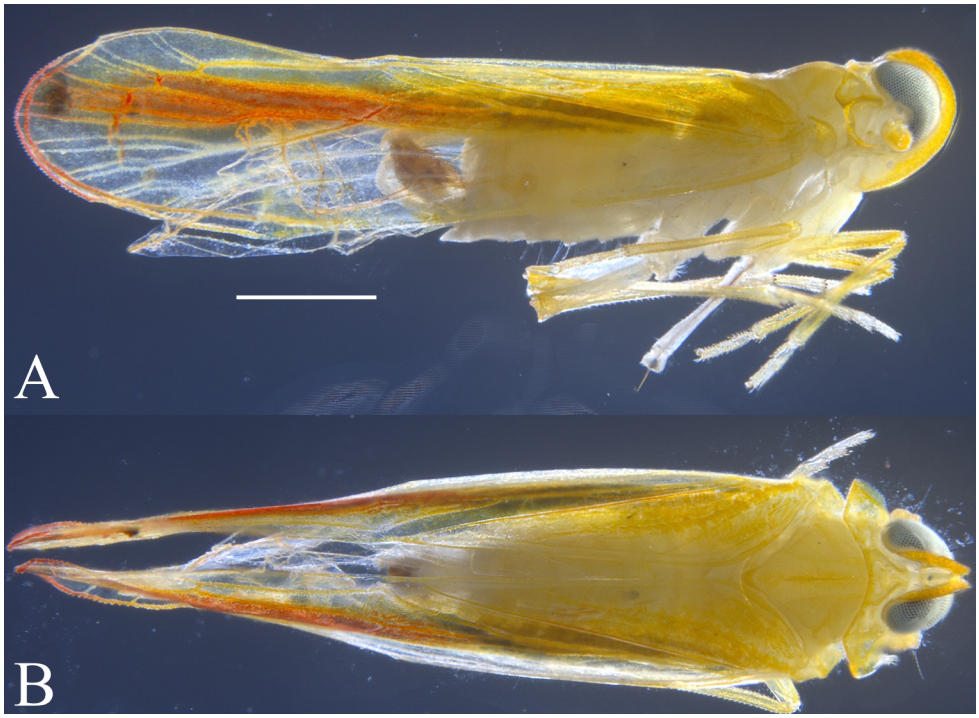

FIGURE 3. Adult male habitus *Agoo luzdenia* **sp. n.** A. body lateral view and B. body dorsal view, scale = 1 mm.

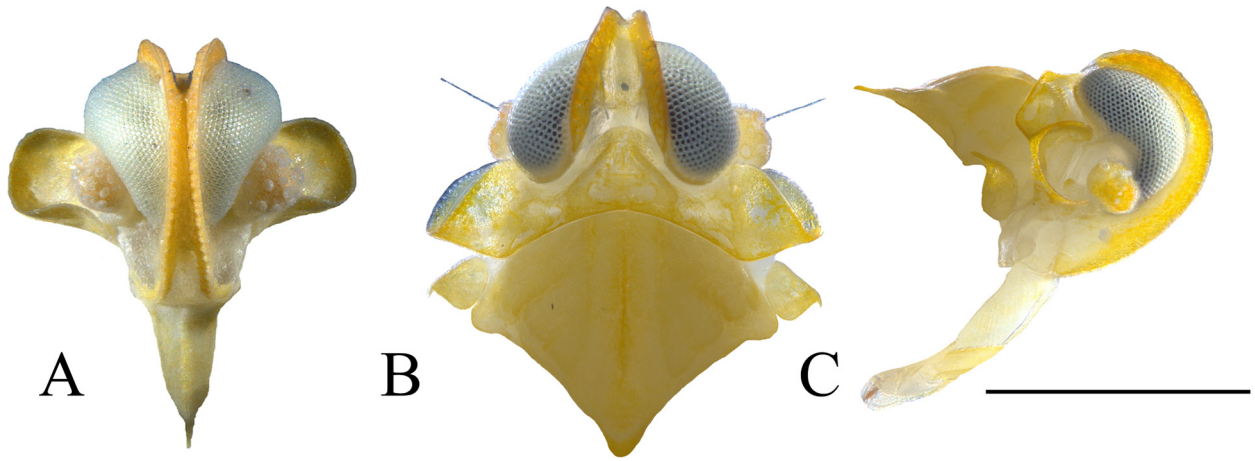

FIGURE 4. Adult male *Agoo luzdenia* **sp. n.** A. head frontal view, B. head, pronotum and mesonotum dorsal view, C. head, pronotum and mesonotum lateral view, scale=1mm.

Systematics

Family Derbidae Spinola 1839

Subfamily Derbinae Spinola 1839

Tribe Cenchreini Muir 1913

Genus *Agoo* Bahder & Bartlett 2019a

Type species: *Agoo xavieri* Bahder & Bartlett, 2019a

Amended diagnosis. Frons narrow; carinae of frons, vertex and paranota strongly foliate. Transverse carina at fastigium lacking. Head in lateral view with vertex + frons profile smoothly rounded. Paranotal folia quadrate to semiquadrate in frontal view. Ventral lobe of pygofer (ventral view) broad, distally attenuating to rounded apex (simple/subtriangular). Aedeagus and endosoma nearly bilaterally symmetrical, aedeagus with at least one pair of elongate processes at apex, extremely complex endosoma with at least two pairs of large sclerotized processes.

Key to the species of *Agoo*

1. Process present on dorsal surface of paramere; pair of processes on aedeagus situated posterior, wings with distinct black spots . . . . . *dahliana*
1'. Process absent on dorsal surface of paramere, sclerotization on parts of parameres variable 2
2. Body yellow, forewings with longitudinal stripe 3
2'. Body fuscous, wings hyaline . . . . . *rubrimarginata*
3. Stripe on forewing terminating in red with distal black spot; parameres with sclerotized lateral ridge . . . . . *luzdenia* Bahder & Bartlett **sp. n.**
3'. Stripe on forewing fuscous, no sclerotization on parameres . . . . . *xavieri*

Agoo luzdenia Bahder & Bartlett sp. n.

(Figures 2–7)

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

Diagnosis. Distinguished from congeners by the stripe on the forewing (proximally greyish, distally reddish), with a distal, black spot. Aedeagus with a greatly enlarged lateral region of flagellum.

Description. *Color*. In life, orangish-yellow with slight wax coating on wings, tufts of wax on sensory pits of vertex and wax spindle present on venter of abdomen (Fig. 2). Vertex and frons orangish-yellow (Fig. 4B), genae and ventral 1/3 of frons white, lateral carinae of head yellow, clypeus white. Thorax and abdomen slightly lighter yellow than head on dorsal surface and white on ventral surface. Terminalia white. Legs orangish-yellow with tarsi lighter in color to white. Forewings with anterior half transparent, longitudinal stripe running basad to wing apex, basal 1/4 yellow, dark yellow distad, transitioning to reddish at wing midlength, nearly reaching wing apex, terminating in black spot (Figs. 2, 5). Posterior cells tinted yellow.

Structure. Body length males (n =3): 5.24–5.26 mm with wings; 2.74–2.76 mm without wings; females (n=2): 5.26–5.28 mm with wings; 5.26–5.27 mm without. Head. In lateral view, anterior margin of head smoothly rounded (Figs. 3, 4C), labial sheath slightly longer than head. In frontal view (Fig. 4A), lateral carinae of head strongly keeled and extended above eyes, single row of sensory pits along carinae margin for entire length of vertex and frons. Vertex deeply concave posteriorly, notched distally (Fig. 4B), broadest near base, tapering distally. Vertex length males: 0.27–0.28 mm; females: 0.29–0.31 mm. Vertex width at hind margin males: 0.20–0.21 mm; females: 0.20–0.22 mm. Vertex width at distal margin males: 0.03–0.04 mm; females: 0.04–0.05 mm. Frons with lateral carinae strongly keeled, narrowest between compound eyes, diverging slightly ventrad until reaching frontoclypeal suture (Fig. 4A). Frons length males: 0.55–0.56 mm; females: 0.56–0.57 mm. Frons dorsal width males: 0.10–0.11 mm; females: 0.11–0.12 mm. Frons frontoclypeal margin width, males: 0.18–0.19 mm; females: 0.19–0.20 mm. Clypeus with lateral carinae keeled, sensorial pits absent, converging near midlength to labrum (Fig. 4A). Clypeus length males: 0.84–0.86 mm; females: 0.88–0.90 mm.

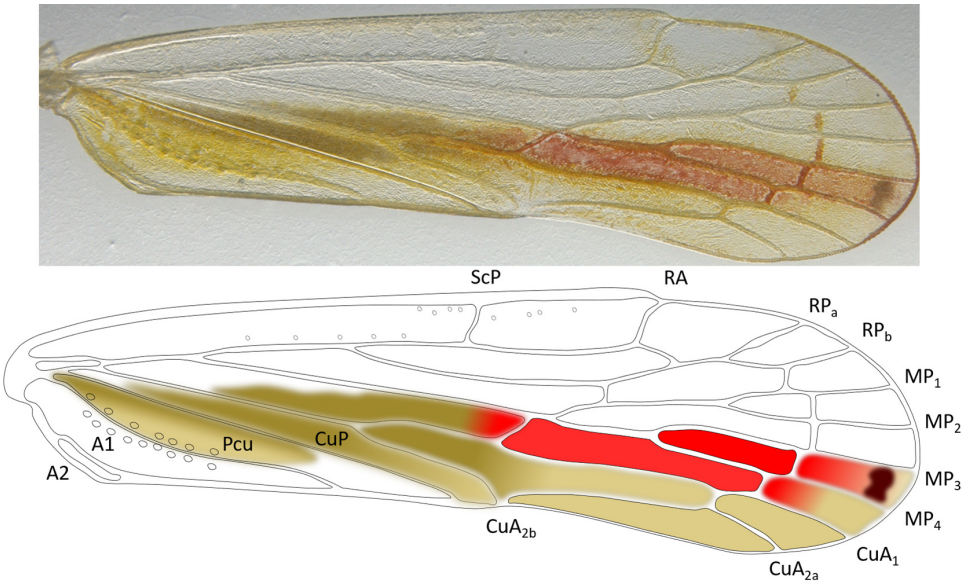

FIGURE 5. *Agoo luzdenia* **sp. n.**, forewing venation.

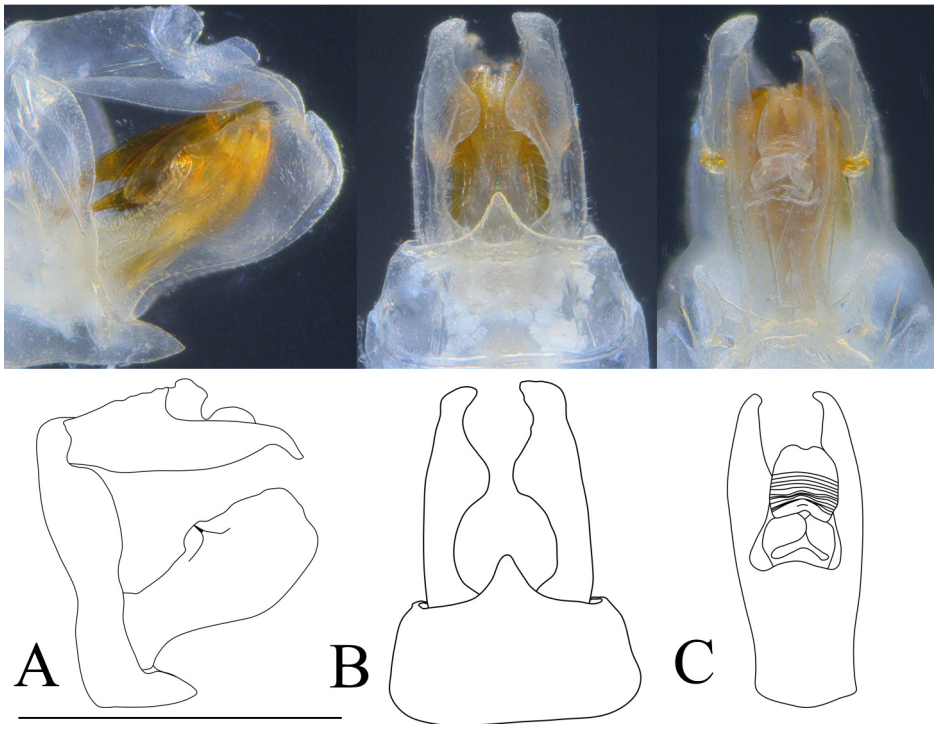

FIGURE 6. Male terminalia of *Agoo luzdenia* **sp. n.** A. lateral view, B. ventral view, and C. dorsal view.

Thorax. Pronotum relatively wide at midline, convex on anterior margin, extending about 1/3 length of eyes, concave on posterior margin, strongly foliate and subquadrate in frontal view (Figs. 3B, 4B), weak carina at midline. Pronotum length at midline males: 0.23–0.24 mm; females: 0.23–0.24 mm. Mesonotum weakly tricarinate with lateral carinae subparallel. Mesonotum length at midline males: 0.69–0.70 mm; females: 0.71–0.72 mm. Mesonotum width males: 0.85–0.86 mm; females: 0.86–0.87 mm.

Forewing (Fig. 5) with Tubercles irregularly arranged in costal cell, two rows of sensory pits running along basal 2/3 of postcubitus. Media branching from combined ScP+R in basal fourth of wing, fork of R (i.e., of ScP+RA and RP) and CuA approximately at same level, near fusion of Pcu+A1 in clavus, in basal 1/3 of wing, both basad of claval apex near midlength of wing (Fig. 5). Wing branching pattern RA 1-branched, RP 2-branched, MP 4-branched and CuA 3-branched. Combined Pcu+A1 reaching wing margin prior to CuP (i.e., clavus closed). Forewing length males: 4.94–4.96 mm; females: 4.99–5.02 mm.

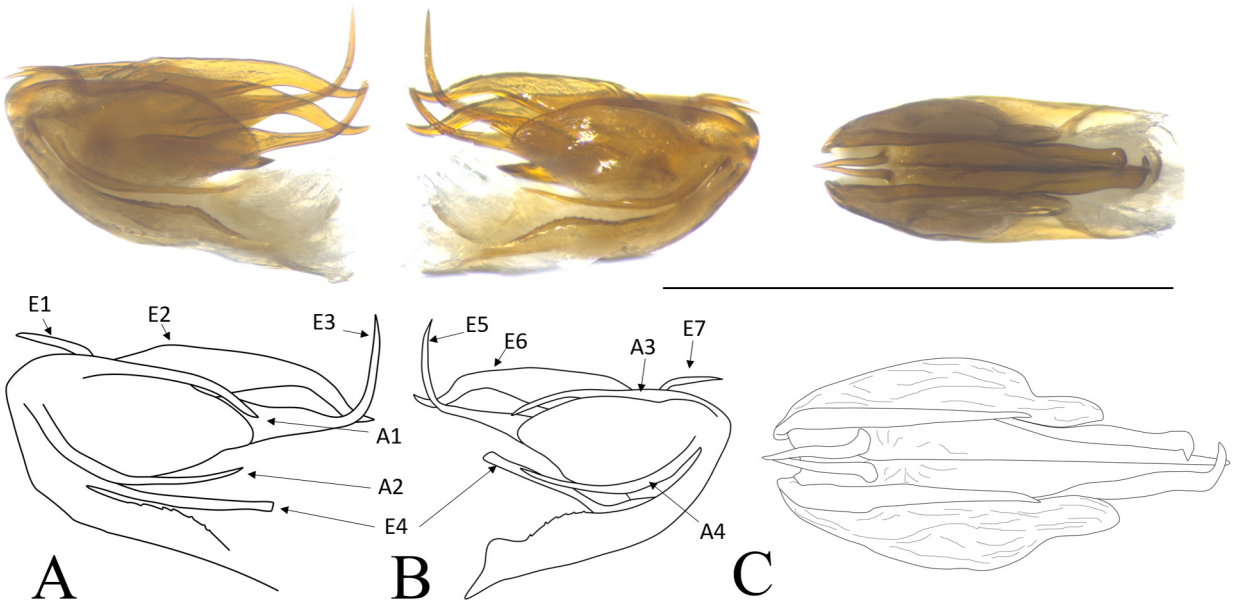

FIGURE 7. Aedeagus of adult male *Agoo luzdenia* **sp. n.** A. right lateral view, B. left lateral view, and C. dorsal view.

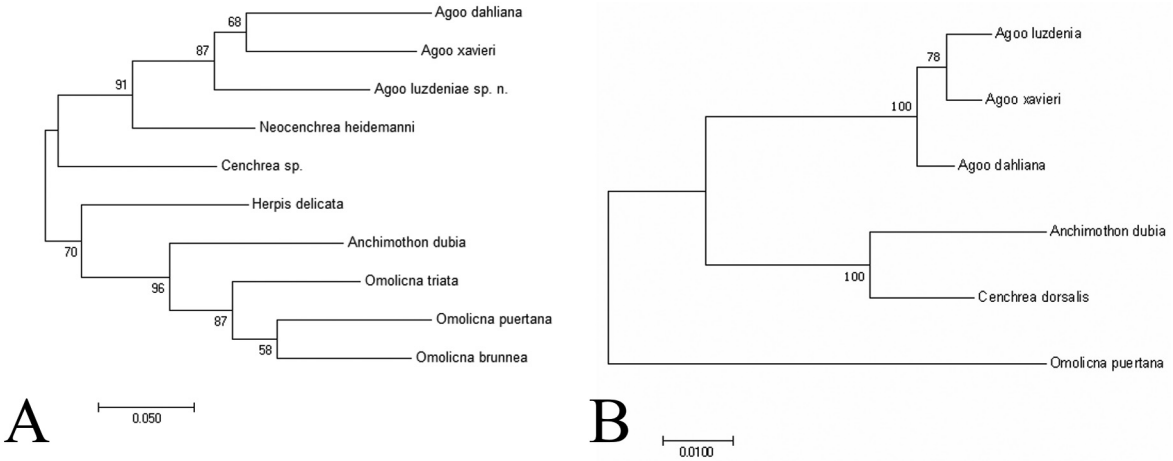

FIGURE 8. Maximum likelihood phylogenetic trees (1,000 replicates) based on the COI (A) and 18S (B) gene demonstrating the relationship of the novel taxon, *Agoo luzdenia* **sp. n.**, relative to other species in *Agoo* and other genera within the Cenchreini.

Terminalia. Pygofer in lateral view narrow, irregularly sinuate on both posterior and anterior margin, widest ventrally. In ventral view, medioventral process subtriangular (Fig. 6B), approximately wide as long. Parameres in lateral view (Fig. 6A) appearing clubbed, broad at base, distally narrowing then expanding; apex weakly narrowed and truncate, about as wide as base; ventral and dorsal margin sinuate, sclerotized ridge on outer lateral margin near midlength bearing sharp projection. Parameres in ventral view (Fig. 6B) with two medial concavities separated by rounded lobe near midlength; apex truncate. Aedeagus approximately bilaterally symmetrical, bearing two pairs of elongate processes (subequal in length, labeled A1–A4, Figs. 7A–B), one apical pair (A1 and A3), arched ventrad, angled anteriorly, arising above subapical pair (A2 and A4) that arches dorsad and angled anteriorly; processes appear to circumscribe enlarged portion of flagellum in lateral view. Dorsal surface of aedeagus bearing finely serrate convex flange in basal half (Fig. 7A–B). Endosoma complex (Figs. 7A–B), bearing three pairs of elongate processes and one medial process (labeled E1–E7) within a pair of broad, ovate lobes; dorsal spine pair near aedeagus apex (E1 and E7), distally angled to sharp, posteriorly directed apices; second pair (E2 and E6) elongate (but shorter than E2, E6), exceeding endosoma, approximated, anteriorly directed from dorsal surface of flagellum; third pair (E3 and E5) distad to second and strongly angled dorsad at apices (Fig. 7A–B). Median process present on endosoma, truncate apex (E4), approximately half length of spines on endosoma in lateral view. Anal tube in lateral view (Fig. 6A)

robust proximad with subparallel dorsal and ventral margins to anal column, tapered distally with ventrally concave margin near apex; apex down curved (Fig. 6); in dorsal view (Fig. 6C), apex deeply concave (providing appearance of paired, caudally directed projections beyond anal column).

Plant associations. Coquito (*Astrocaryum alatum* H.F. Loomis), Arecaceae.

Distribution. Costa Rica (Heredia).

Etymology. The specific name given as in honor to the lead authors wife, Luz Denia Bahder, who translated the abstract of this and other manuscripts into Spanish. The specific name is intended to be indeclinable.

Material examined. Holotype male "Costa Rica, Heredia / La Selva Biological Station / Brian W. Bahder; 22 May 2018 / aspirated from coquito // Holotype/*Agoo*/*luzdenia*" (FLREC). Paratypes, same data as holotype (3 males, 2 females, FLREC and FSCA).

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	5	6	7	8	9	10
1	*Agoo dahliana*		0.01	0.01	0.02	0.01	0.01	0.01	0.02	0.02	0.01
2	*Agoo luzdenia* sp. n.	16.4		0.01	0.02	0.01	0.01	0.01	0.01	0.02	0.02
3	*Agoo xavieri*	15.5	15.7		0.02	0.01	0.01	0.01	0.02	0.02	0.02
4	*Anchimothon dubia*	19.4	19.1	20.5		0.01	0.01	0.01	0.01	0.01	0.01
5	*Neocenchrea heidemanni*	16.6	15.5	15.2	16.9		0.01	0.01	0.01	0.01	0.02
6	*Cenchrea* sp.	18.0	17.2	17.9	17.2	15.1		0.01	0.01	0.01	0.01
7	*Herpis* sp.	18.8	18.6	19.6	17.7	16.3	14.8		0.01	0.01	0.01
8	*Omolicna triata*	20.1	19.2	20.2	14.8	17.7	17.4	16.7		0.01	0.01
9	*Omolicna puertana*	19.9	17.7	20.2	15.7	19.1	18.2	18.0	13.9		0.01
10	*Omolicna brunnea*	21.1	18.8	21.1	16.7	19.6	17.7	15.8	12.6	13.2	

Sequence Data and Analysis. A 712 bp product was generated for the COI locus (GenBank Accession No. MT085818). Based on the pairwise comparison of the COI loci for all taxa sampled, *A. luzdenia* Bahder & Bartlett **sp. n.** differs from *A. dahliana* and *A. xavieri* by 16% and 15%, respectively (Table 1). On average, *A. luzdenia* Bahder & Bartlett **sp. n.** differed by 19.6% from other genera within the Cenchreini (Table 1). However, *A. luzdenia* Bahder & Bartlett **sp. n.** differed by 16.6% from *Neocenchrea heidemanni*, a similar level seen within *Agoo*, but despite the percent similarity, the phylogenetic analysis of the COI region resolves a monophyletic *Agoo* sister to *Neocenchrea heidemanni* (Fig. 8). A 1,412 bp product was generated for the 18S locus (GenBank Accession No. MN999709). For the pairwise comparison of the 18S loci, *Agoo luzdenia* Bahder & Bartlett **sp. n.** differed from other *Agoo* by an average of 1.2% (Table 2) while differing 5.9% from *Cenchrea dorsalis*, 6.9% from *Anchimothon dubia*, and 9.6% from *Omolicna puertana* (Table 2). The maximum likelihood tree based on the 18S data shows strong bootstrap support for *A. luzdenia* Bahder & Bartlett **sp. n.** belonging in the genus *Agoo* relative to the other out-groups (Fig. 8B).

TABLE 2. Pairwise comparison for the 18 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	*Agoo luzdenia* sp. n.		0.003	0.002	0.007	0.006	0.009
2	*Agoo dahliana*	0.014		0.003	0.007	0.006	0.008
3	*Agoo xavieri*	0.010	0.012		0.007	0.006	0.008
4	*Anchimothon dubia*	0.069	0.072	0.074		0.005	0.008
5	*Cenchrea dorsalis*	0.059	0.060	0.062	0.034		0.008
6	*Omolicna puertana*	0.096	0.094	0.094	0.099	0.093	

Remarks. The overall structure of the terminalia, wing color and molecular evidence supports *Agoo luzdenia* Bahder & Bartlett **sp. n.** as distinct from *A. dahliana* and *A. xavieri*. The general features of *A. luzdenia* Bahder &

Bartlett **sp. n.** are consistent with other species in *Agoo*, such as the elongate, narrow frons, lateral carinae of vertex elevated above eyes, subquadrate projections of paranota forming fossae, spinose aedeagus and endosoma that are nearly symmetrical. Among described *Agoo* there does not appear to be sexual dimorphism, except that females are slightly larger than males, a common trend in the Auchenorrhyncha (B.W. Bahder, *pers. observ.*). One feature that appears to vary among *Agoo* species is the wing coloration patterns. The forewings of *Agoo dahliana* possessed many spots, *A. xavieri* has with a single fuscous stripe but is otherwise uniform in color, and *A. luzdenia* Bahder & Bartlett **sp. n.** with a stripe of two different colors and a spot at the apex of the wing, similar to that seen in *Cenchrea dorsalis*. Wing patterns of *A. rubrimarginata* are not detailed by Fennah (1945). These wing coloration patterns may prove useful for field identifications.

Discussion

The discovery of another species of planthopper on palms in Costa Rica further highlights both the unknown diversity of planthoppers on palms and the fascinating relationship among derbids and palms. Derbids are widely believed to be fungal feeders as nymphs (Willis 1982, Wheeler & Wilson 1996) and in tropical rainforests and cloud forest, many species likely develop in the leaf litter based on observations of adults flying in great abundance out of disturbed leaf litter (Bahder, *unpubl. data*). However, specimens of *A. luzdenia* Bahder & Bartlett **sp. n.** were collected on a palm in a large clearing with no leaf litter near it (Fig. 1). Based on observations of the trunk and canopy of the coquito palm that specimens were collected from, we suspect that the accumulation of organic material around the base of the crown (Fig. 1) created suitable habitat for nymphs develop. Future studies will seek to identify larval habitats of derbids to gain a better understanding on the life histories of the fascinating groups.

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