

Description of Four New Species for the Genus *Damasippus* Stål, 1875 (Phasmida: Prisopodidae)

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Abstract

During the review and identification of specimens of the genus *Damasippus* Stål, 1875 deposited in the National Museum of Costa Rica, some specimens were found that corresponded to four non-described species: *D. sepia* n.sp., *D. viridicorpus* n.sp., *D. duoviridis* n.sp and *D. viridilabrum* n. sp. Due to the above, a review of the literature describing the species of this group was carried out, with which an illustrated dichotomous key was developed to identify the 16 species, in addition to a partial phylogenetic analysis to corroborate that they do not correspond to the species *D. fuscipes*, due to its morphological similarity.

Keywords

Costa Rica, Stick Insects, Taxonomic Key

1. Introduction

After the closure of the National Institute of Biodiversity (INBio), the National Museum of Costa Rica assumed custody of all the specimens of the insect and plant collection, which has been subjected to multiple restoration and taxonomic revision procedures over the last five years. This has led to the discovery of new species, some of which were misidentified or unidentified at all, one such group being the genus *Damasippus* Stål.

This genus belongs to the Prisopopinae Bruner subfamily, whose distribution is reported only for Central and South America [1] [2] [3]. It differs from the other genera of the group for being small (less than 6 cm long), and for presenting the hind wings with a black distal zone and a colorless proximal ornament

[4], the latter being of vital importance, therefore, the mounting of the specimens, with at least one wing open, plays an essential role in identification.

Previously, 13 species were described for this group: *D. alejandria* Conle, Hennemann & Gutiérrez 2011 [2], *D. batesianus* Westwood 1859 [5], *D. discoidalis* Redtenbacher 1906 [6], *D. fuscipes* Redtenbacher 1906 [6], *D. hahneli* Dohrn 1910 [7], *D. piceipennis* Redtenbacher 1906 [6], *D. pulcher* Redtenbacher 1906 [6], *D. spatulatus* Piza 1937 [8], *D. staudingeri* Redtenbacher 1906 [6], *D. striatus* Redtenbacher 1906 [6], *D. unilineatus* Redtenbacher 1906 [6], *D. westwoodii* Stål 1875 [9] and *D. zymbraeus* Westwood 1859 [5]; of which *D. unilineatus* was removed from the group and transferred to the genus *Dinelytron* [10].

Due to the above, and because the descriptions refer to very similar characteristics between the species, it is necessary to deepen the study of this group, and use genetic methods to support the chromatic characteristics. The close similarity between some species limits the identification of very particular maculations, which could be thought to be the result of intraspecific chromatic variation, although in reality they are quite reliable in terms of shape and location. Even so, a genetic analysis, either complete or partial of the genus, can separate possible cryptic species [11], which are usually better differentiated in early stages of development as eggs or larvae and nymphs.

This leads us to the information gaps that, including the species described in this work, for the genus *Damasippus* would be the following: the only described egg is that of *D. zymbraeus* in the work of Bellanger *et al.* [12]. There are no works that compile the forms which the nymphs of any species present in their different stages of development, only one *D. zymbraeus* nymph is reported [3]. Nor are the females of the species *D. spatulatus* and *D. pulcher* known, nor are the males of *D. alejandria*, *D. westoodii*, *D. piceipennis*, and *D. duoviridis* n.sp.

There are currently 31 specimens of this genus in the collection, whose new records together with the previous 12 add up to a total of 16 species recorded until September 2019. The objective of this work is to describe the four new species, as well as provide a dichotomous key to the identification of the members of this group, using a partial phylogenetic analysis to rule out that the new contributions may correspond to intraspecific chromatic variations of *D. fuscipes*.

2. Materials and Methods

2.1. Specimens Review

All the specimens of the genus *Damasippus* in the collection of the National Museum of Costa Rica (MNCR), were collected in the Costa Rican territory, in an altitudinal range from 0 masl to 1600 masl, in localities located in the provinces of Puntarenas, Heredia, Guanacaste and Alajuela, strictly in protected areas, corresponding to National Parks, and private Biological Reserves.

Reviewed specimens: 2♂⁷ Station. Cacao. 1000 - 1400 m southwest side Cacao

Volcano, Guanacaste Province, Costa Rica. Nov-Dec 1989. Rec. URCG. R. Blanco & C. Chavez. 323,300 - 375,700 (Catalog No. MNCR-A 146,605 and MNCR-A 87,450); 1 ♂ Station. Cocoa. 1000 - 1400 m southwest side of Cacao Volcano, Guanacaste Province, Costa Rica. Rec. A. Guadamuz, Nov.-Dec. 1990. L N 323,300, 376,700 (No. Catalog MNCR-A 298146); 1 ♂ La Casona, 1520 m, Monteverde Biol. Res., Puntarenas Province, Costa Rica. Mar 1991. Rec. N. Obando L-N-253,250, 449,700; DNA Barconding. Rec. E. Ulate. CCDB-15935 E09. (Catalog No. MNCR-A 1193523); 1 ♂ Quebrada Bonita Station, 50 m, Carara National Park, Puntarenas Province, Costa Rica. Jan. 1993. Rec. J.C. Saborío L-N-194,500, 469,850. (No. Catalog MNCR-A 1,352,178); 1 ♂ Reserve San Lorenzo. 1050 m R.F. Cord. Guanacaste (Tenorio), Guanacaste Province, Costa Rica. Rec. C. Alvarado, Jul.-1991. L-N-287,800, 427,600. (Catalog No. MNCR-A 364,912); 1 ♂ Finca San Gabriel, 2 km southwest of Dos Ríos, Prov. Alajuela, Costa Rica, 600 m, May-1988, Rec. Janzen and Hallwachs W 85°23'50", N 10°53'19" (No. Catalog MNCR-A 1011288); 1 ♂ Estación Cacao, 1000 - 1400 m southwest side of Volcán Cacao. Porv. Guanacaste, Costa Rica. Mar.-1988. Rec. GNP Biodiversity Survey, 323,300, 375,700. (Catalog No. MNCR-A 50,489); Season. Cacao, 1000 - 1400 m southwest side Cacao Volcano, Prov. Guanacaste Costa Rica, set-1989, Rec. URCG R. Blanco, 323,300, 375,700. (No. Catalog MNCR-A 87,446); 1 ♂ Las Pailas Station, P.N. Rincón de la Vieja, Guanacaste Province, Costa Rica, 800 m 19-Jun./1-July-1993, Rec. D.G. Garcia, LN 306,300_388,600 #2189 (Catalog No. MNCR-A 1967669); 1 ♀ Las Pailas Station. 800 m. P.N. Corner of the Old Guanacaste Province. Costa Rica, July 1/22, 1992, Rec. D. García L-N 306,300, 388,600. (Catalog No. 718468); 1 ♀ Station. Maritza, 600 m, west side of the Orosí Volcano, Guanacaste Province, Costa Rica. May-1989, Rec. GNP Biodiversity Survey, 326,900 - 373,000 (No. Catalog MNCR-A 100,519); 4 ♀ Estación Cacao, 1000 - 1400 m southwest side of Volcán Cacao, Guanacaste Province, Costa Rica, Nov.-Dec. 1989, Rec. A 146,594, MNCR-A 147,695 and MNCR-A 147,720); 1 ♀ Estación Cacao, 1000 - 1400 m, Southwest side Volcán Cacao Guanacaste National Park, Guanacaste Province, Costa Rica, Rec. F.A. Quesada 21/29-May-1992, L-N-323,300, 375,700. (No. Catalog MNCR-A 378,941); 1 ♀ Sirena Station, Corcovado National Park, 0 - 100 m, Prov. Puntarenas, Costa Rica, Rec. N. Obando, Jun.-1990, L-S-270,500, 508,300 (No. Catalog MNCR-A 644,070); 1 ♀ Sect. San Ramón de Dos Ríos, Prov. Alajuela, Costa Rica, 620 m, 27-Apr./11-May-1995, Rec. C. Cano, LN 318,100 - 381,900, #5276 (No. Catalog MNCR-A 2,145,968); 1 ♀ Cacao Station, Guanacaste Province, Costa Rica, 1100 m. 7/8-Feb.-1995, Rec. M. Madrigal, LN 323,100 - 375,800, #7409. (Catalog No. MNCR-A 2,187,030); 1 ♀ Pilón Station, Guatuso, P.N. Tenorio Volcano, Prov. Alajuela, Costa Rica, 700 - 800 m, 5/20-May-2012, Rec. J.A. Azofeifa, Free Collection, LN 298,212 - 4279, #104,902 (No. Catalog MNCR-A 4,331,867); 1 ♀ Cacao Station, southwest side Vol. Cacao, Guanacaste National Park. Prov. Guanacaste. Costa Rica, 800 - 1600 m, Jul.-1993, Rec. R.M. Guzman, L. S. 323,300 - 375,700 #2215. (No. Catalog MNCR-A 1,936,199); 1 ♀ Estación Cacao, 1000 - 1400 m southwest side Volcán Cacao Guanacaste Pr. Costa Rica. Set 1989, Rec. URCG R. Blanco and C. Chávez. 323,300 - 3757. (No. Catalog

MNCR-A 87,445); 1 ♀ Cocoa Station. 1000 - 1400 m, southwest side of the Cacao Volcano Prov. Guanacaste, Costa Rica, set-1989, Rec. URCG R, Blanco & C. Chávez, 323,300, 375,700. ADN E. Ulate CCDB-15,935 F01 barcode. (No. Catalog MNCR-A 87,447); 1 ♂ Las Pailas Station, P.N. Rincón de la Vieja, Guanacaste Province, Costa Rica, 800 m, 5/24-Aug.-1994, Rec. D.G. García, LN 306,300_388,600 #3194. E. Ulate DNA barcode CCDB-15,935 E11 (No. Catalog MNCR-A 2,038,563); 1 ♀ Costa Rica. Province of Puntarenas. Corcovado National Park, La Leona Sector, Cerro Puma, 300 m, 17 set/5-oct-2003, Rec. A. Azofeifa, free collection, L-S 267,700, 518,900, #15,935, ADN E. Ulate barcode CCDB-15,935 F02 (Catalog No. MNCR-A 3,783,384); 1 ♂ (*D. sepia*) Costa Rica Prov. Puntarenas. Corcovado National Park, La Leona Sector, Cerro Puma, 302 m, 27-Jun/1-Jul.-2003, Rec. K. Caballero, light trap, L-S 267,700, 518,900, #74,482. (No. Catalog MNCR-A 3,736,798); 1 ♂ Costa Rica, Alajuela Province, Tenorio Volcano National Park, El Pilón Sector, Guatuso. Río Roble Valley 800 - 900 m, August 17, 2004, Rec. A. Azofeifa. Light Trap, L N 297,700 - 455,800, #78,106; E. Ulate DNA barcode CCDB-15,935 E08 (No. Catalog MNCR-A 3,878,725); 1 ♀ Costa Rica, Province of Guanacaste, La Cruz, A.C.G. Pque Nal. Gte., Estación Mengo, Ladera SW Volcán Cacao, 1100 m, Jul. 3, 1987, Rec. Janzen, manual collection, LN 330,200, 375,700, #52,581, ADN E. Ulate CCDB-15,935 barcode. E07 (No. Catalog MNCR-A 3,353,995); 1 ♀ June 25, 2003, La Selva Biological Station, Rec. Karin Gastreich, Prov. Heredia, Costa Rica, 50 - 150 m, 10°26"N 84°01"W, ADN E. Ulate CCDB barcode - 15,935. F03. (No. Catalog MNCR-A 3,241,544); 1 ♀ Mora Z.P., Prov. San José, Costa Rica, Finca El Rodeo, Diamond Row, 900 - 1000 m, July 21, 2005, Rec. J. Mata, A. Hoepker, J. Montero, R. Zúñiga, free collection, diurnal. L-N 209,300, 507,000 #95,199. DNA barcode E. Ulate CCDB-15,935. E15 (No. Catalog MNCR-A 4,178,893).

Although the collection does not present specimens collected in the provinces of Limón, Cartago and San José, the presence in these provinces, which may be found in future investigations, is not ruled out.

2.2. Documentation

For the identification of the specimens, a Motic SMZ-171 optical stereoscope was used, and for the photographs an OLYMPUS SZX16 stereoscope, an OLYMPUS DP74 digital camera for optical equipment and some SCHOTT KL 1600 LED accessory lights.

The descriptions of the original species were studied and a dichotomous key was developed, for which Westwood [5], Redtenbacher [6] [13], Dorn [7], and Conle *et al.* [2]. In order to facilitate the reader's use of the key, hand-drawn illustrations of the relevant structures were produced.

2.3. Phylogenetic Analysis

For the phylogenetic analysis, sequences of the genus *Damasippus* were searched in different molecular databases, they were only found in the Barcode of life data system (BOLD) [14], only 11 cytochrome oxidase I (COI) sequences were found

and used (see **Table 1**), which contains 578 base pairs, 9 of these sequences belong to the specimens guarded by the National Museum of Costa Rica (MNCR). Two sequences from the genus *Prisopus* sp. also from the IOC region. For the alignment of the samples, the Mega X program [15] was used.

To carry out the phylogeny, the best fit model was selected using JModeltest [16], which was 011020 + I + G + F and was used to run the Bayesian analysis. The selected information criterion was the Akaike criterion. In the MrBayes [17] program, the analysis was run using 10,000,000 generations, storing the most optimal tree every 1000 generations with the default parameters, running 4 markov-Montecarlo chains, obtaining a consensus tree as part of the analysis result. The tree was created using the figtree program [18].

3. Results

3.1. Species Description

3.1.1. *Damasippus viridicorpus* n.sp.

Holotype: ♀ Costa Rica, Guanacaste Province, Guanacaste National Park. 1000 - 1400 masl. September 1989. URCG collectors, R. Blanco and C. Chaves. Deposited in the Entomology Collection of the National Museum of Costa Rica. Catalog number MNCR-A 87447.

Paratype: ♂ Costa Rica, Guanacaste Province, Guanacaste National Park. 1000 - 1400 masl. September 1989. URCG collectors, R. Blanco and C. Chaves. Deposited in the National Museum of Costa Rica. Catalog number MNCR-A 87,445.

Table 1. Sequences used for phylogenetic analysis, with the suggested identification of each specimen.

Species on BOLD	BOLD Code	Correct identification
<i>Damasippus striatus</i>	ASPHA055-12	<i>Damasippus fuscipes</i>
<i>Damasippus striatus</i>	ASPHA056-12	<i>Damasippus viridilabrum</i>
<i>Damasippus striatus</i>	ASPHA057-12	<i>Damasippus viridilabrum</i>
<i>Damasippus striatus</i>	ASPHA058-12	<i>Damasippus sepia</i>
<i>Damasippus fuscipes</i>	ASPHA059-12	<i>Damasippus viridicorpus</i>
<i>Damasippus icl02</i>	ASPHA060-12	<i>Damasippus striatus</i>
<i>Damasippus fuscipes</i>	ASPHA061-12	<i>Damasippus viridicorpus</i>
<i>Damasippus batesianus</i>	ASPHA062-12	<i>Damasippus sepia</i>
<i>Damasippus icl01</i>	ASPHA063-12	<i>Damasippus duoviridis</i>
<i>Prisopus biolleyi</i>	ASPHA072-12	<i>Prisopus biolleyi</i>
<i>Prisopus cepus</i>	ASPHA073-12	<i>Prisopus cepus</i>

Diagnosis: Stigma of the membranous wing forming a single lobe. Prothorax without macules.

Coloration: Light green coloration uniformly throughout the body, with the exception of the posterior half of the forewing, it presents all the abdominal segments in the same light green hue, and the pronotum and mesonotum completely green with just a light gray central area. The species is expected to present chromatic variations all over its body between greenish yellow and light green.

Etymology: he used the compound word “viridicorpus” built from the Latin words “viridi” which means green, and “corpus” which means body, to refer to the green coloration of the entire body.

Description of the holotype: epicranial suture with two dimples on each side on the vertex. It presents a green head with the area from the vertex to the sepia-colored mouthparts (**Figure 1(C)**). The length of the antennae is two thirds the length of the body. Pronotum with a light brown central area, light green lateral margins, and a darker and thickened rear edge forming a 1 mm thick strip. Mesonotum with a sepia central line edged in gray, and light green lateral margins (**Figure 1(H)**). Tegmina 18 mm long and 5 mm wide with dark posterior half (**Figure 1(B)**). Membranous wings 30 mm long at the costal edge, and 16 mm at the anal edge. Hind wing an incomplete light green border forming a band 1 mm thick in males, and 2 mm thick located on the subapical edge, and

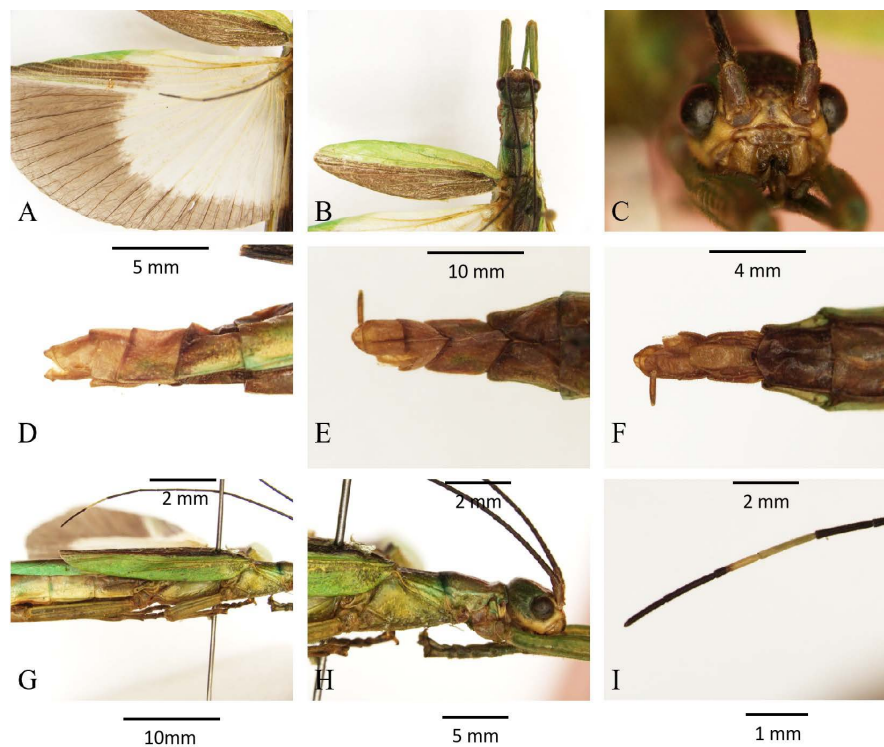


Figure 1. ♀ *Damasippus viridicorpus*. (A) Membranous wings. (B) Tegmina. (C) Head, front view. (D) Last abdominal segments, lateral view. (E) Last abdominal segments, dorsal view. (F) Last abdominal segments, ventral view. (G) Middle and hind legs, lateral view. (H) Thorax, lateral view. (I) Last segments of the antenna.

comprising one third of the length of the costal margin, followed by a nontransparent brown area reaching up to the radial vein, the rest of the wing has a transparent brown coloration, with a central colorless area that forms a single lobe with an irregular edge, which starts in the middle of the costal edge and goes down to the radial vein, forming a curve in the direction of the base of the wing, at which point the curve deviates in the direction of the apex with a forming the edge of a regular half circle until it reaches the anal vein for a distance of three quarters of its length, where it deviates again until it reaches the costal edge, forming a straight line (**Figure 1(A)**). Last three abdominal segments in sepia color (**Figures 1(D)-(F)**). Tibias and femurs light green, with sepia-colored edges of the femurs. Sepia-colored sterna and green tergi, except for the last four segments, which are sepia-colored. Rear edge of tergum thickened and with slightly darker coloration. Total body length 48 mm, with segments 22 and 23 sepia colored (**Figure 1(I)**).

Description of the male: epicranial suture with two dimples on each side on the vertex. Brown head with the area from the vertex to the sepia mouthparts (**Figure 1(C)**). Antennae completely dark. Hindwings same as female but colorless zone curved instead of straight ending at costal edge, 23 mm long at costal edge, and 11 mm at anal edge (**Figure 2(A)**). Thorax with the same characteristics as the female (**Figure 2(B)**). Tegminas with the outer half light green and the inner dark brown, 14 mm long and 4 mm. Total body length 36 mm (**Figure 2**).

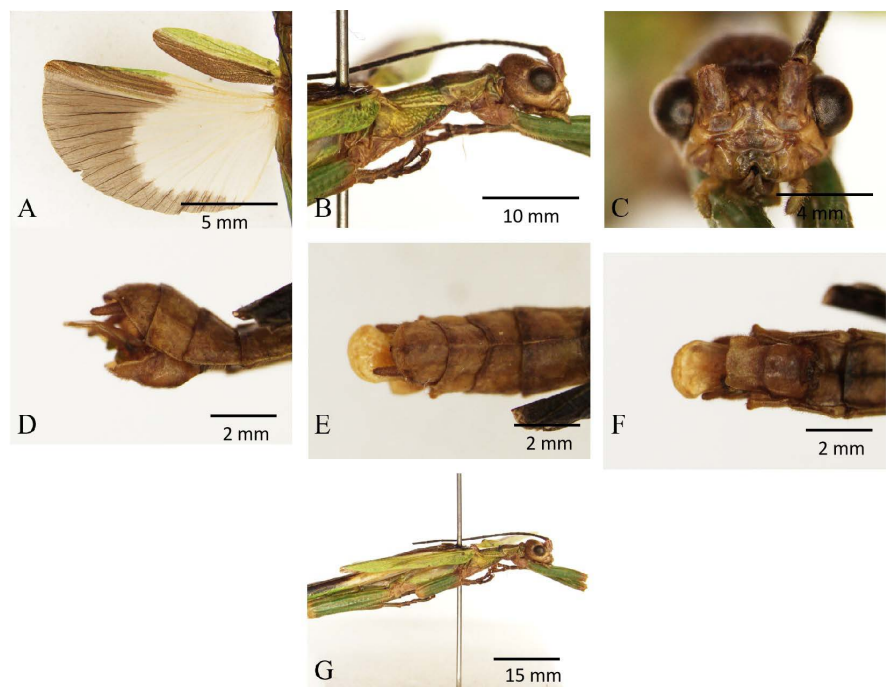


Figure 2. ♂ *Damasippus viridicarpus*. (A) wings. (B) Thorax, lateral view. (C) Head, front view. (D) Last abdominal segments, lateral view. (E) Last abdominal segments, dorsal view. (F) Last abdominal segments, ventral view. (G) Legs, side view.

3.1.2. *Damasippus duoviridis* sp.n.

Holotype: Female. Costa Rica, Heredia Province, La Selva OTS Biological Station. 50 - 150 masl. June 25, 2003. Collector Karin Gastreich. Deposited in the National Museum of Costa Rica. Catalog number 3,241,544.

Diagnosis: Presents a clear lateral maculation that forms a continuous stripe with the clear area of the tegmina. Colorless area of the wings forming a semi-rectangular curved line.

Coloration: It presents the last three dark abdominal segments, unlike *D. westwoodii* which has them yellow, and the legs are dark green instead of light green or black as in other species of the genus. The species is expected to show chromatic variations all over its body between shades of dark green and even brown.

Etymology: The name *D. douviridis* was assigned to it, constructed from the Latin words “duo” which means two and “viridi” which means green, referring to the light and dark green tones that differentiate it from the other species.

Description of the holotype: Membranous wings with a light green margin in the terminal third between the costal margin and the subcostal vein, followed by a dark brown band that covers the middle of the radial zone up to the flexion margin, the rest of the wings it is transparent dark brown, with a colorless zone that starts from the middle of the costal border and extends in a straight line to the middle of the ulnar zone, where it forms an irregular curve and deviates towards the anal border (**Figure 3(A)**). Tegmina 22 mm long and 6 mm wide, with the outer half light green and the lower half dark brown (**Figure 3(B)**). Dark green head with slight longitudinal gray bands less than 1 mm thick. Epicranial suture with two dimples on each side in the vertex area. (**Figure 3(C)**). Completely black antennae two-thirds the length of the body. Dark brown monochromatic abdomen including the last three segments (**Figures 3(D)-(F)**). Femurs and tibiae dark green, hind femurs dark brown in the first two thirds with dark gray transverse bands (**Figure 3(G)**). Total length of the body 64 mm. Dorsal part of thorax dark brown, uniform in shape. Pronotum and mesonotum with the lateral border with a light green band 1.2 mm thick, which forms a continuous maculation with the mesothorax, in which this light green coloration appears, and a dark brown band on the lateral border 1.2 mm thick (**Figure 3(H)**).

3.1.3. *Damasippus sepia* sp.n.

Holotype: Female. Costa Rica, Puntarenas Province, Corcovado National Park. 300 masl. September 17 to October 5, 2003. Collector A. Azofeifa. Deposited in the National Museum of Costa Rica. Catalog number 3,783,384.

Paratype: Male. Costa Rica, Puntarenas Province, Corcovado National Park. 300 masl. June 27 to July 1, 2003. Collector K. Caballero. Deposited in the National Museum of Costa Rica. Catalog number 3,736,798.

Diagnosis: Presents a clear lateral maculation that forms a continuous stripe with the clear area of the tegmina. Colorless area of the wings with three well-defined lobes.

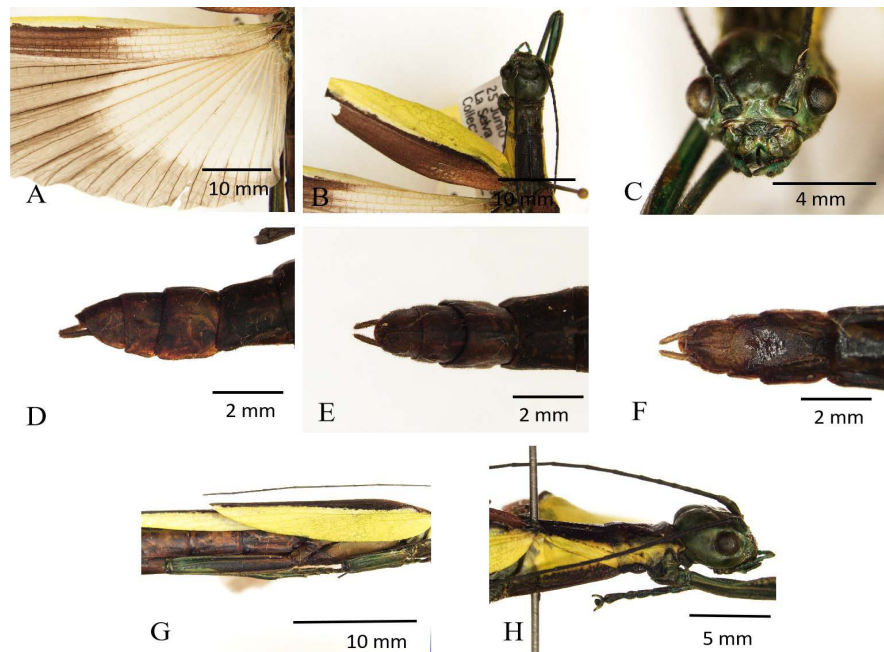


Figure 3. ♀ *Damasippus duoviridis*. (A) Membranous wings. (B) Tegminas. (C) Head, front view. (D) Last abdominal segments, lateral view. (E) Last abdominal segments, dorsal view. (F) Last abdominal segments, ventral view. (G) Middle and front legs, lateral view. (H) Thorax, lateral view.

Coloration: body completely dark brown, with light areas in a sepia tone similar to dry leaves. Head with slight linear spots typical of the genus in pinkish tones. It is expected that the specimens of this species vary their color between sepia and light green in the maculations and light and brown areas between light and dark in the rest of the body, as well as the streaks on the face could vary between shades of reddish to sepia.

Etymology: *Damasippus sepia* was named as a reference to the sepia coloration that characterizes it.

Description of the holotype: Membranous wings 30 mm long at the costal margin and 16 mm wide at the anal margin, with a non-transparent dark brown coloration from the middle of the costal edge to the terminal zone of the anal edge (a quarter of the total length). Colorless area of the trilobed membranous wings, which begins in the middle of the costal edge and descends irregularly to the radial vein, where it deviates in the direction of the apex, forming an irregular curve that descends to the cubital vein, there it deviates again in a regular straight line towards the base of the wing on the same vein to the middle of its length, and deviates again in an irregular curve to the anal margin. Tegminas 17.06 mm long and 5 mm wide, with the outer half sepia colored and the inner half dark brown (Figure 4(A)). Pronotum with a light lateral border in a reddish tone and with sepia-colored contours of 0.06 mm. Mesonotum with the lateral border with a sepia band of 0.5 mm. Sepia colored dorsal area of the mesothorax and dark brown marginal area (Figure 4(B)). Dark brown head with very fine lines in a slightly lighter tone and with a thickness close to 0.05 mm (Figure

4(C)). Epicranial suture does not present dimples on the sides in the vertex area. Sepia-colored sterna and dark brown tergi with a sepia-colored lateral margin and a pattern of lateral longitudinal lines on the last four segments (**Figure 4(D)-(F)**). Tibiae and femora dark brown with upper margins sepia 0.01 mm (**Figure 4(G)**). Total body length of 42 mm.

Description of the male: same characteristics as the female except membranous wings 20.55 mm long at the costal margin and 8.43 mm wide at the anal margin. Tegmina 11.70 mm long and 2.84 mm wide, with the outer half green in the male, and the inner half dark brown (**Figure 5(A)**). Lateral margin of the prothorax slightly darker than in the female (**Figure 5(B)**). Olivaceous maxillary palps (**Figure 5(C)**). Abdomen with uniform dark color including the last three segments (**Figure 5(D)-(F)**). Femurs and tibiae uniform dark brown (**Figure 5(G)**). Total length of the body of 33 mm.

3.1.4. *Damasippus viridilabrum* n.sp.

Holotype: Male. Costa Rica, Puntarenas Province, Monteverde Biological Reserve. 1520 masl. March 1991. Collector N. Obando. Deposited in the Entomology Collection of the National Museum of Costa Rica. Catalog number MNCR-A 1,193,523.

Paratype: 1♂ (*D. viridilabrum*) Costa Rica. Prov. Alajuela, P.N. Tenorio volcano. El Pilon Sector, Guatuso. Río Roble Valley 800 - 900 m. Aug 17, 2004, A. Azofeifa. Light Trap, L N 297,700 - 455,800 #78,106; DNA barcode. E. Ulate. CCDB-15,935 E08 (Catalog No. MNCR-A 3,878,725);

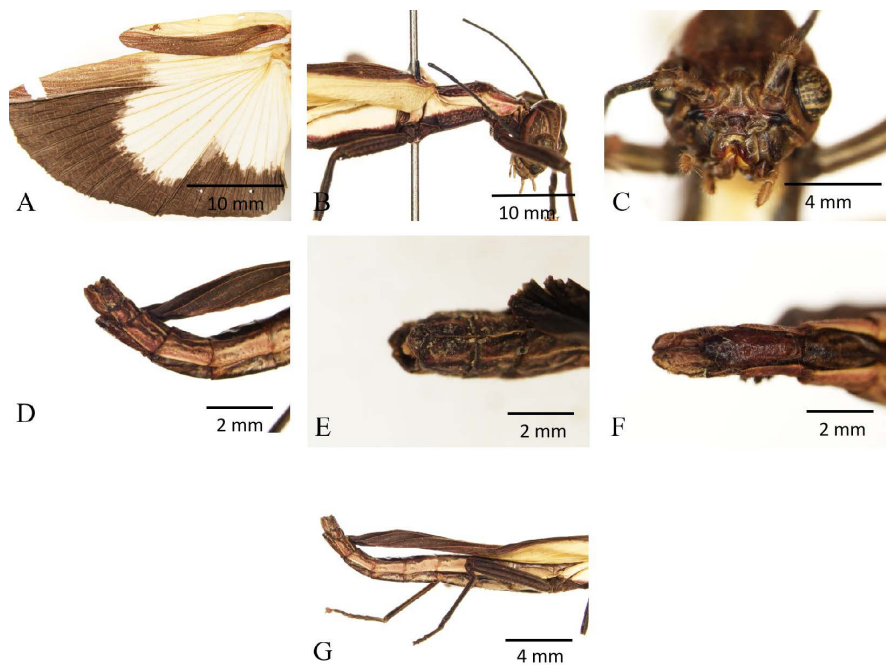


Figure 4. ♀ *Damasippus sepia*. (A) Membranous wings. (B) Thorax, lateral view. (C) Head, front view. (D) Last abdominal segments, lateral view. (E) Last abdominal segments, dorsal view. (F) Last abdominal segments, ventral view. (G) Anterior median leg, lateral view.

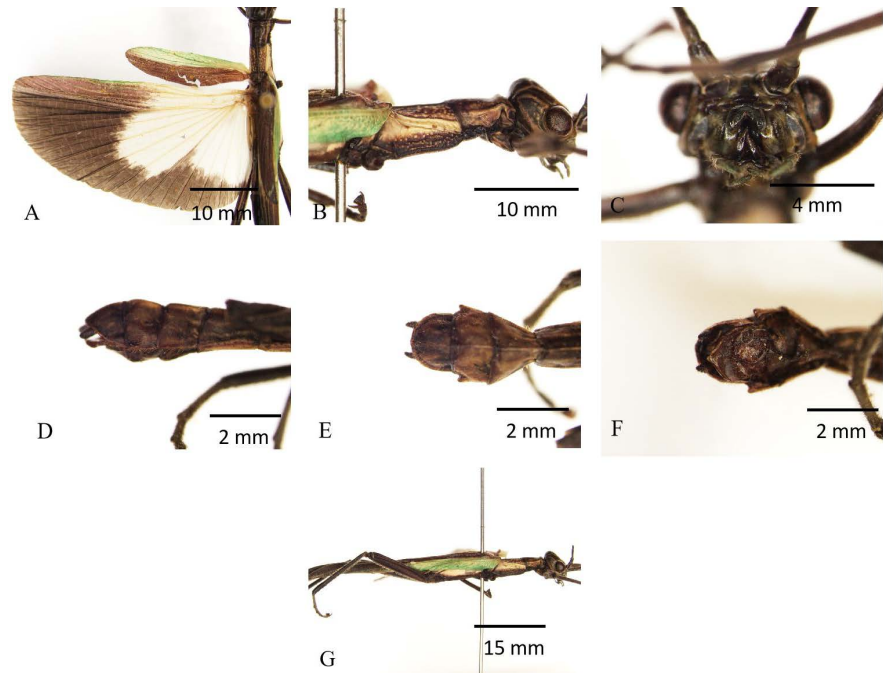


Figure 5. ♂ *Damasippus sepia*. (A) Wings. (B) Thorax, lateral view. (C) Head, front view. (D) Last abdominal segments, lateral view. (E) Last abdominal segments, dorsal view. (F) Last abdominal segments, ventral view. (G) Lateral view of the body.

Diagnosis: Labrum completely clear. Thorax of a uniform color, without macules.

Etymology: used the compound word “viridilabrum” built from the Latin words “viridi” which means green, and “labrum” which refers to the labrum, to refer to the color of the labrum that distinguishes it from *D. fuscipes*.

Holotype description: Male. Dark brown head with very fine lines in a slightly lighter tone and with various thicknesses. Epicranial suture does not present dimples on the sides in the vertex area. Pronotum and mesonotum of uniform color. Dorsal part of the mesothorax uniform dark brown color. Tibias and femurs dark brown with slightly greenish tarsi. Tegminas 12.70 mm long and 3.72 mm wide, with the outer half green and the inner half dark brown. Membranous wings 22.20 mm long at the costal margin and 10.60 mm wide at the anal margin, with a non-transparent dark brown coloration from the middle of the costal edge to the terminal zone of the anal edge (a quarter of the total length). Colorless area of the trilobed membranous wings, which begins in the middle of the costal edge and descends irregularly to the radial vein, where it deviates in the direction of the apex, forming an irregular curve that descends to the cubital vein, there it deviates again in a regular straight line towards the base of the wing on the same vein to the middle of its length, and deviates again in an irregular curve to the anal margin. Esternos and tergi brown, with a dark lateral margin. The specimen only presents the first abdominal segment, due to previous damage. Length to the apex of the plagued membranous wings 29.42 mm (**Figure 6**).

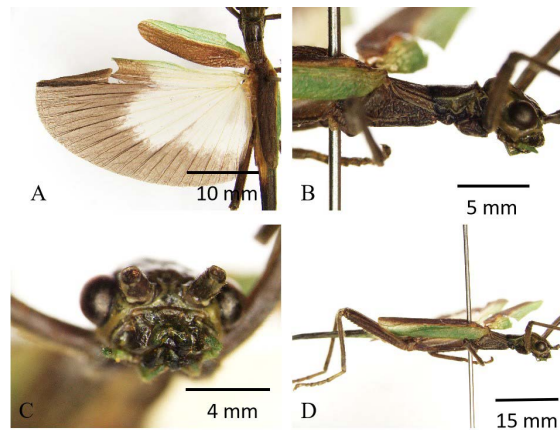


Figure 6. ♂ *Damasippus viridilabrum*. (A) Wings. (B) Thorax, lateral view. (C) Head, front view. (D) Last abdominal segments, lateral view. (E) Lateral view of the body.

Description of the female: same characteristics as the male, except that the total length of the body is 63 mm. Tegminae 24.03 mm long and 11.12 mm wide.

3.2. Identification Key for *Damasippus* Stål Species

- 1) Head with two spines on the vertex (**Figure 7(A)**).....2
- 1') Head without spines on vertex.....6
- 2) Head spines dark, hind legs completely pale.....*D. batesianus*
- 2') Head spines light, hind legs completely dark.....*D. hahneli*
- 3) Hind wings with the colorless three-lobed area (**Figure 7(B)**).....4
- 3') Hind wings do not have the colorless three-lobed zone.....6
- 4) Clear labrum.....*D. viridilabrum* n.sp.
- 4') Dark labrum.....5
- 5) Mesothorax longer than wide with entire upper margin of lateral zone clear.....*D. sepia* n.sp.
- 5') Mesothorax almost equal in length and width, with a clear, well-defined lateral maculation on the upper margin that tapers to the posterior part of the margin (**Figure 7(C)**).....*D. fuscipes*
- 6) Dark tegminas with fine light lines (**Figure 7(D), Figure 7(E)**).....7
- 6') Tegminas without clear fine lines, but with larger and more marked light areas.....10
- 7) Femurs very light, with the rest of the legs very dark.....*D. alejandria*
- 7') Femurs and tibiae light, with only dark joints.....8
- 8) light tegminas with dark longitudinal striations in the upper half (**Figure 7(G)**).....*D. striatus*
- 8') Dark tegmina, with light longitudinal lines.....9
- 9) Presence of a clear and thin line that crosses the dorsal area of the thorax longitudinally, and a wide clear stripe on the anterior edge of the median coxa.....*D. straudingeri*
- 9') Thorax of uniform color, with a very fine line on the anterior margin of the median coxa.....*D. piceipennis*

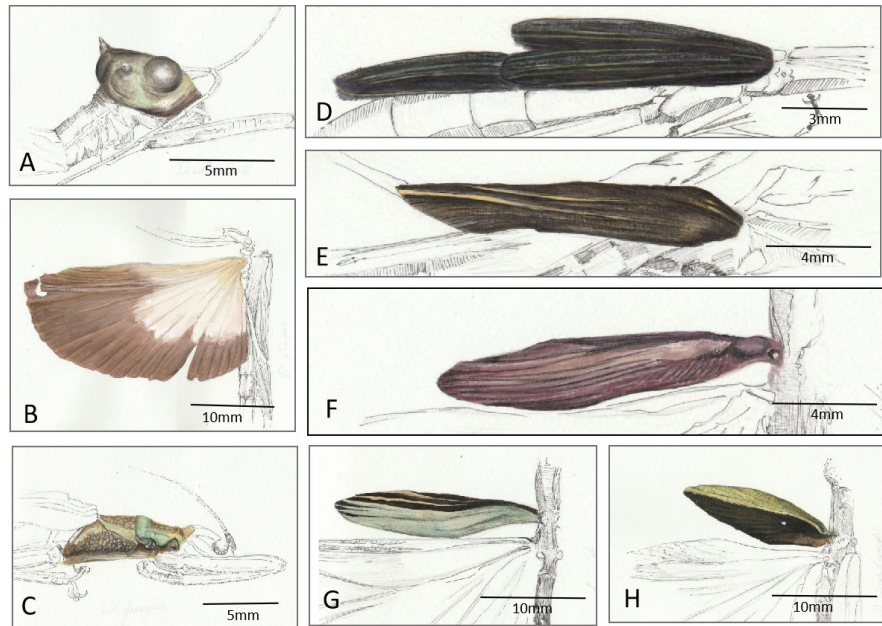


Figure 7. ♂ Structures. (A) Head of *D. hahneli*, (B) Hind wings of *D. fuscipes*, (C) Thorax of *D. fuscipes*. (D) Tegmin of *D. straudingeri*, (E) Tegmin of *D. spatulatus*, (F) Tegmin of *D. discoidalis*, (G) Tegmin of *D. striatus*, (H) Tegmin of *D. westwoodii*.

- 10) dark anterior tegmina with a single broad pale maculation never reaching the distal margin (**Figure 7(F)**).....*D. discoidalis*
- 10') Tegminas present a clear area in the middle (**Figure 7(H)**).....11
- 11) Tegminas completely light, with just a few longitudinal, slightly dark striae.....*D. zimbraeus*
- 11') Anterior tegminas with only the upper longitudinal half clear.....12
- 12) Last three abdominal segments completely dark.....13
- 12') Last three abdominal segments completely clear.....14
- 13) Legs totally dark, with some green markings.....*D. duoviridis* n.sp.
- 13') Legs very pale, with only dark knees.....*D. pulcher*
- 14) Membranous wings with the colorless area as in **Figure 1(A)** and **Figure 2(A)**, dorsal area of the thorax light, with a light gray stripe.....
.....*D. viridicorpus* n.sp.
- 14') Membranous wings with the colorless zone does not present this shape, dorsal zone of the thorax with a very dark black stripe.....*D. westwoodii*

3.3. Phylogeny

The phylogenetic analysis (using the COI gene) groups the species into two clades, in the first with *D. viridicorpus*, *D. viridilabrum* and *D. fuscipes*, and in the second with *D. sepia*, *D. duoviridis* and *D. striatus*. *D. viridilabrum* is a sister group to *D. fuscipes*, *D. striatus* and *D. duoviridis* are grouped in the same node. Bayesian analysis (**Figure 8**) indicates that both clades are monophyletic.

The divergence of the COI gene in the genus *Damasippus* ranged from 9.9% to 0.21%, the largest interspecific difference was between *D. striatus* with 8.7%

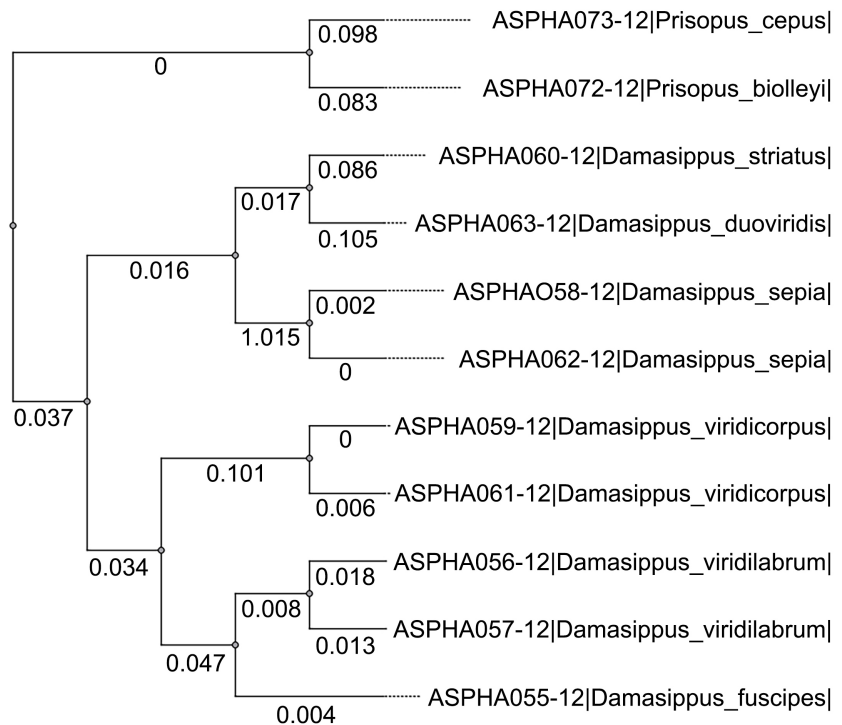


Figure 8. Bayesian analysis of the COI gene. The posterior probability support values are given at each node.

and *D. duoviridis* with 9.9% and the smallest difference was between a specimen of *D. fuscipes* 0.22% and *D. sepia* 0.20%. The results show that *D. fuscipes* individuals from different collection sites clustered together as a clade.

4. Discussion

In the collection of the National Museum, the genus *Damasippus* has very few specimens, so in order to determine the veracity of the four described species, we resorted to using the genetic sequences that were already in BOLD, extracted by the National Institute of Biodiversity. (INBio) before they handed over custody of the collections to the government. However, before using them it was necessary to correct the identification of the taxa (Table 1). Although, although the specimens were assigned a species, clear confusions were found, such as the case of *D. duoviridis*, identified as *D. batesianus*, and 24 specimens of *D. fuscipes* confused with *D. striatus*.

Therefore, it is necessary to emphasize three characteristics that are diagnostic in this group. The first, the presence of spines on the head, which is an exclusive character of *D. batesianus* and *D. hahneli*. The second is the shape and arrangement of the colorless area of the wings, used to determine the species *D. viridicarpus*, *D. sepia* and *D. duoviridis* (Figure 9) according to the presence of defined lobes, because although there is dimorphism, as in the case of *D. fuscipes* (Figure 10), this variation continues to present different patterns in each of the species, making it a diagnostic characteristic.

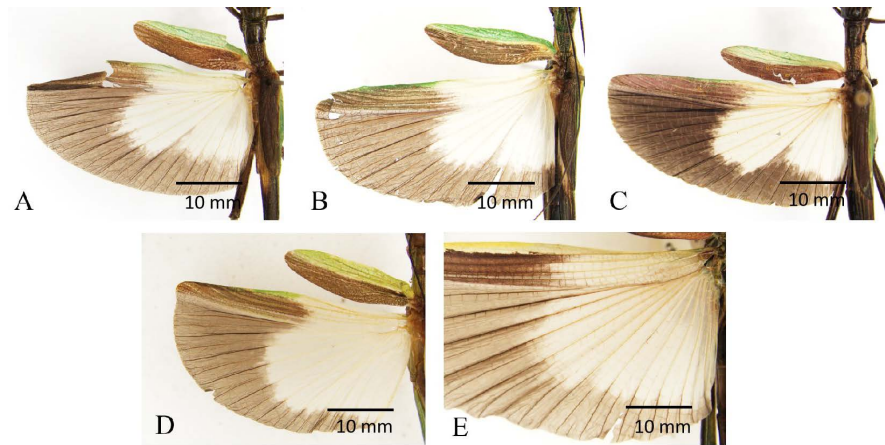


Figure 9. Wings of *Damasippus* specimens from the collection of the National Museum of Costa Rica. (A) *D. viridilabrum* ♂, (B) *D. fuscipes* ♂, (C) *D. sepia* ♂, (D) *D. viridicorpus* ♀, (E) *D. duoviridis* ♂.

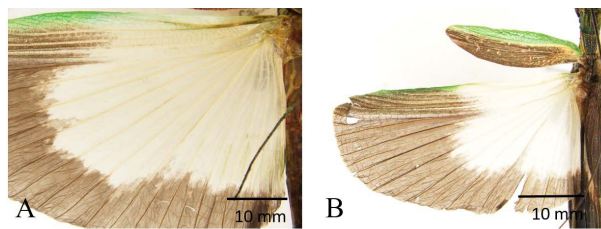


Figure 10. Membranous wings of *Damasippus fuscipes* from the collection of the National Museum of Costa Rica. (A) Female, (B) Male.

The third characteristic was the presence of very particular and well-defined maculations on the prothorax and mesothorax (**Figure 11**), which allowed us to differentiate the species *D. viridicorpus*, *D. duoviridis*, *D. viridilabrum* and *D. sepia* from *D. fuscipes*, as the green and sepia tones vary quite a bit between them, which makes the coloration as such unreliable, but the pattern of the maculations on the lateral edges of the pronotum and mesonotum are unique in each one of them. Also, the coloration of the labrum is important, because although there is intraspecific variation in the shades of brown or green (**Figure 12**), these only vary from lighter to darker, but never in base color, which is why it is a reliable diagnostic characteristic.

Considering the above, it is possible to attribute the previous erroneous identifications to the fact that this group was not properly worked on by a specialist, and not to the fact that the literature is ambiguous, or that it is in Latin. The confusion between what led to the identification of *D. duoviridis* as *D. batesianus*, and the specimens of *D. fuscipes* as *D. striatus* are the clear result of a poor or non-existent review of the original descriptions of the species in question, since that despite the fact that the texts are in Latin, the presence of cephalic horns in *D. batesianus* is clearly indicated, and the characteristic coloration of *D. striatus* forming longitudinal bands, both characteristics recognizable with the naked eye. Therefore, it is recommended that genetic sequence databases in

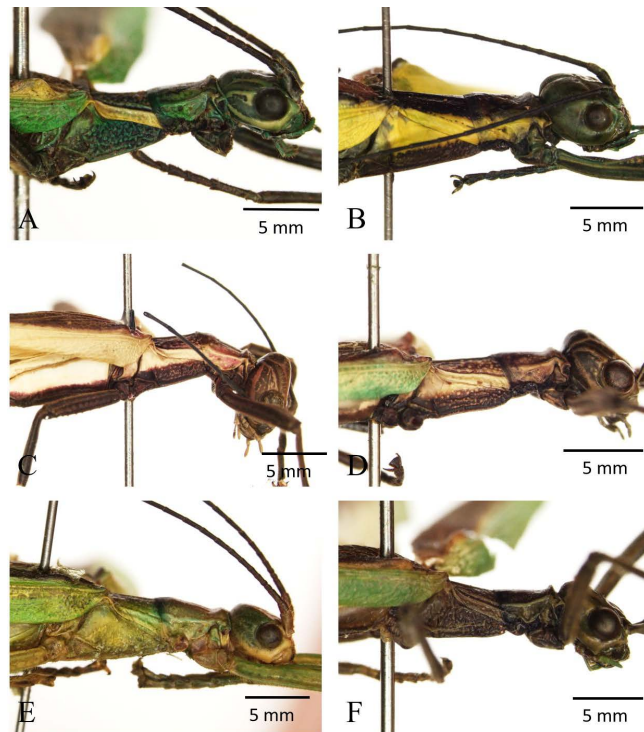


Figure 11. Profiles of *Damasippus* specimens from the collection of the National Museum of Costa Rica. (A) *D. fuscipes*, (B) *D. duoviridis*, (C) *D. sepia* ♀, (D) *D. sepia* ♂, (E) *D. viridicarpus* ♀, (F) *D. viridilabrum*.

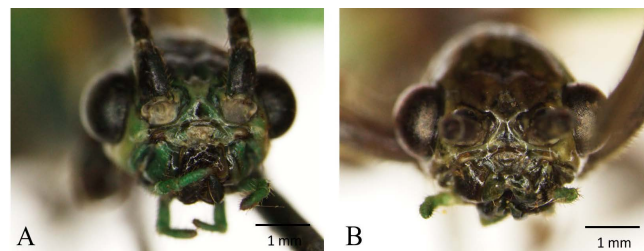


Figure 12. Head, front view. (A) *Damasippus fuscipes*, (B) *Damasippus viridilabrum*.

general verify that the deposited sequences have been previously reviewed by a specialist, and that research institutions do the same with the works that are supported.

Genetic analysis is a powerful tool that saves a lot of time in terms of efficiently separating the groups of specimens to be identified, however, it never replaces the role of the specialist, as we have seen in this genus, the specimens previously worked on had identification problems, which, if not detected, would have seriously damaged the results. This is a common mistake made by inexperienced technicians and trainees, which can easily be corrected by checking identification before uploading genetic sequences to world databases.

The results of the molecular analysis have a high affinity with the morphological comparison, being a necessary support for them when determining the separation between species. A study involving more genes and a greater number of

sequences of this genus is urgently needed to achieve a more marked genetic differentiation.

The phylogenetic analysis of the COI gene facilitates the identification of species due to the high mutability between them, its small size has the advantage that it can be preserved even in dry-preserved specimens over time without presenting degradation. In addition, said gene has enough information to assign species to a certain taxonomic group [19] [20] [21] [22]. The 578 base pairs of the COI gene allowed the *Damasippus* species guarded by the MNCR to be identified with 95% confidence, this methodology continues to give reliable results and good results when it comes to confirming the presence of cryptic or very similar species [23] [24].

It is also important to point out that although it is risky to use only the color patterns for species identification, due to intraspecific variations and possible discoloration that the specimen may suffer when it is prepared, it is quite viable to use the spots and colorless areas of wings, because each species has constant patterns, leaving chromatic variations in the background, and integrative genetic analyzes have proven to be very effective in differentiating species [25], in this case molecular analysis being the necessary support for new species, and morphological comparisons for species poorly described.

5. Conclusion

It is concluded, therefore, that in Costa Rica *D. fuscipes*, *D. striatus*, *D. sepia*, *D. viridicorpus*, *D. duoviridis* and *D. viridilabrum* are present; it is confirmed that the report of *D. batesianus* corresponds to an identification error, an additional genetic study is not necessary to confirm it, since *D. batesianus* has two easy-to-distinguish cephalic horns, which are not present in any of the national species, and that specimens collected in Costa Rican territory have not yet been contributed to the national collections; and an alert call is made to colleagues interested in using the BOLD sequences, to confirm the identifications of the specimens associated with each sequence of the genus *Damasippus*, provided in this work.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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