Ecobiology of Haemagogus Populations (Diptera: Culicidae)

Vectors of Pathogens in Brazil

Jeronimo Alencar
Cecilia Ferreira de Mello
Shayenne Olsson Freitas Silva
Jacenir Reis dos Santos Mallet
(organizers)

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PREFACE

We find ourselves amidst a global scenario of climate change, global warming, and the resurgence of arthropod-borne diseases, including arboviruses. Among these, Sylvatic Yellow Fever (SYF) has exhibited an increase in the number of cases, including epidemic outbreaks, particularly in various regions of Brazil, affecting not only humans but also native primates. Thus, in addition to implications for human health, SYF emerges as another obstacle for animals of our biodiversity deserving protection. Within this reality, a comprehensive study of SYF vectors, particularly the mosquitoes of the genus *Haemagogus* (Diptera: Culicidae), is essential. This is crucial for both enhancing existing knowledge and updating it, considering that global changes may be influencing the ecobiology of these insect populations. Such is the paradigm of the present work, whose chapters represent new studies with a modern and current approach to several aspects of the ecology, biology, and morphology of the *Haemagogus* species of greatest epidemiological importance in Brazil. Notably, this book includes a compilation of numerous studies published over the years in reputable and impactful scientific journals. This ensures that the presented texts have been refined and polished through various peer reviews conducted previously. Thus, we present a collection with content grounded in high technical and scientific rigor, which is not only useful for a better understanding of *Haemagogus* species and, consequently, of part of the Brazilian biodiversity but also contributes to a deeper understanding of these species as arbovirus vectors. By its scope, this book fills gaps in the knowledge of several aspects of the studied species. For all these reasons, the information presented may have future implications for preventive and/or interruptive actions against SYF transmission, both for humans and native primates in our forested areas. It is essential to highlight the authors' dedication to continuous improvement, as evidenced by their successive publications, resulting in a magnificent body of work for updated and in-depth knowledge of various aspects of the studied *Haemagogus* species. In conclusion, I affirm my belief that this collection is indispensable for consultation and study by all those whose field of activity or research includes SYF or *Haemagogus* species from now on.

Hélcio Reinaldo Gil-Santana

Public Health Technician

Instituto Oswaldo Cruz

ABSTRACT

The genus *Haemagogus* (Diptera: Culicidae) comprises 28 species and four formally undescribed forms, which are broadly distributed throughout the Americas. Several species are considered of medical importance as they are responsible for transmitting the yellow fever virus (YFV) and other arboviruses. In Brazil, these species primarily inhabit areas of dense gallery forests. In this book, the nine species reported for Brazil are reviewed in terms of their geographical distribution, adult and immature biology, and medical importance, emphasizing their involvement in the transmission of the YFV and other arboviruses, as well as the potential role of *Haemagogus* species in the transmission of the dengue virus (DENV). Among the species identified as potential YFV transmitters, *Hg. janthinomys* stands out as the primary vector in the Americas. This vector competence is reinforced by its geographical distribution, which coincides with the endemic areas of the disease. Vast Neotropical regions, especially the Amazon and Northeast Brazil, need further research on *Haemagogus* and other sylvatic mosquitoes. The possible role of mosquitoes from this genus in the transmission of pathogens that cause arboviral diseases, such as Dengue, Yellow Fever, and Mayaro, needs to be further explored. Therefore, studies on the ecobiology and medical importance of *Haemagogus* and other sylvatic mosquitoes are fundamental. Experimental infection research, such as that conducted with *Ae. aegypti* and YFV should also be developed with *Haemagogus* spp. and DENV. In this regard, the information obtained in this book is presented in the form of articles addressing the following aspects: ecology, biology and morphology of the genus *Haemagogus*.

KEYWORDS: Culicidae; Mosquitoes; *Haemagogus*; Bioecology; Morphology; yellow fever, dengue, arbovirus

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Rayane Dias
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Shayenne Olsson Freitas Silva
Laboratório de Diptera, Fundação Oswaldo Cruz, Rio de Janeiro, RJ, Brasil
Laboratório Interdisciplinar de Vigilância Entomológica em Diptera e Hemíptera, Fundação Oswaldo Cruz, Rio de Janeiro, RJ, Brasil

Ana Laura Carbajal de la Fuente
CONICET - Buenos Aires' University. Institute of Ecology, Genetics and Evolution (IEGEBA), Eco-Epidemiology Laboratory. Buenos Aires, Argentina

Cecilia Ferreira de Mello
Laboratório de Diptera, Fundação Oswaldo Cruz, Rio de Janeiro, RJ, Brasil
Instituto de Biologia, Pós-Graduação em Biologia Animal, Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ, Brasil

Jeronimo Alencar
Laboratório de Diptera, Fundação Oswaldo Cruz, Rio de Janeiro, RJ, Brasil

ABSTRACT: Introduction: *Haemagogus capricornii* and *Hg. janthinomys* females are considered morphologically indistinguishable. We analyzed morphometric variability between Brazilian populations of these species using wing geometric morphometry. **Methods:** Size and shape at intra- and interspecific levels were analyzed in 108 *Hg. capricornii* and *Hg. janthinomys* females. **Results:** Geometric morphometry indicated size and shape variables can differentiate these species at interspecific level. However, at intraspecific level, results show relative differentiation. Two populations of *Hg. capricornii* had a smaller centroid size with no significant differences between them, whereas all *Hg. janthinomys* populations showed significant differences. **Conclusions:** Both species were correctly identified by geometric morphometry.

KEYWORDS: *Haemagogus capricornii*. *Haemagogus janthinomys*. Culicidae. Geometric morphometry. Brazil.

One of the most important genera of mosquitoes capable of infecting and transmitting the wild yellow fever virus (WFV) is *Haemagogus* Williston, 1896, which is considered a biological vector and responsible for maintaining the natural cycle of this zoonosis in forested areas of the Americas[1]. Mosquitoes of this genus are restricted to the Americas and almost all species have a Neotropical distribution, except for *Hg. equinus* Theobald, 1903, which can even be found in some southern parts of the Nearctic region[2]. These are mainly wild, diurnal, and acrodendrophic mosquitoes inhabiting primarily dense forest and gallery areas[3].

Haemagogus is very diverse; it includes twenty-eight species of which nine are found in Brazil[1]. Some of these are epidemiologically important in the transmission of the wild-type yellow fever virus[1]. Among the nine known vector species, five stand out for the efficiency of their transmission in Brazil: *Hg. albomaculatus* Theobald, 1903, *Hg. leucocelaenus* Dyar and Shannon, 1924, *Hg. spegazzini* Bréthes, 1912, *Hg. capricornii* Lutz, 1904, and *Hg. janthinomys* Dyar, 1921. Larvae and females of *Hg. capricornii* and *Hg. janthinomys* species are currently morphologically indistinguishable, their differentiation being based primarily on characteristics of the male genitalia. Their identification is carried out based on the following: the presence (*Hg. janthinomys*) or absence (*Hg. capricornii*) of notable spiculosity on the ventral face of the aedeagus and the existence of a medial process, with a hooked shape, near the apex of the paraproct in *Hg. janthinomys*. These structures are small and only distinguishable by well-trained personnel and misidentifications can be frequent.

Although traditional morphometry contributed to the identification of these species, a more robust approach is necessary[4]. Geometric morphometry is a powerful, low-cost tool that addresses issues in taxonomy, ecology, and morphology, particularly in insects and especially in the family Culicidae, which possesses wings[5]. These bi-dimensional structures are eminently suitable for morphometrical description[6]. Geometric morphometry makes it possible to identify morphological variations and to explore their causes both within and between populations[7]. In Diptera, it has been widely used to answer questions mainly related to population studies[6]. A recent study of *Culex* mosquitoes from the state of Rio de Janeiro showed the effects of seasonal variations on phenotypic variations using this tool[3].

Considering the difficulties in the identification of *Hg. capricornii* and *Hg. janthinomys* females, the poor knowledge about them, the partial overlap of their geographical distribution, and their eco-epidemiological importance, attention must be paid to the evaluation of old reports of infection of these and similar species, especially in Brazil[1]. In this context, the aim of this study was to determine the phenotypic variability in *Hg. janthinomys* and *Hg. capricornii* females at species and population levels, using the geometric morphometry of the wings. For this purpose, we included populations of the two species that have a large proportion of their geographic distribution in Brazil.

A total of 108 right and left wings of females belonging to *Hg. capricornii* and *Hg. janthinomys* from Brazil were used in this study (**Figure 1A** and **Figure 1B**; **Table 1**). The *Haemagogus* populations came from ecological and epidemiological studies carried out by the Diptera Laboratory team and from the Entomological Collection at the Oswaldo Cruz Institute, Fiocruz, Brazil. Species were identified by direct observation of morphological characters using an optical microscope (Leica DMD108® - Morrisville, United States of

America - USA) according to Arnell (1973)[1]. Once identified, the wings were extirpated and later photographed according to Alencar et al. (2016)[3].

Fourteen type-1 landmarks were selected and included in the analyses[8]. We used coordinate data and the isometric estimator centroid size (CS) to compare overall wing sizes between species and populations. The Mann-Whitney test was applied to comparisons of CS between species and populations. The shape variables (partial warps and uniform components) were obtained using the generalized Procrustes analysis superimposition algorithm. Mahalanobis distances derived from the shape variables were used to explore shape proximity between the species and populations. Statistical significance was determined by permutation tests (1,000 runs each) and corrected by the Bonferroni method.

We represented the Mahalanobis distances between species and populations in neighbor-joining (NJ) trees. The percentage of phenotypic similarity between species and populations was calculated using the cross-check test of discriminant analysis. Shape variables were regressed onto CS by multivariate regression analysis to detect allometry. The correlation between geographic and Mahalanobis distances was determined by a Mantel test (1,000 permutations) using straight-line geographic distances between collection sites as described by Rosenberg and Anderson (2011)[9].

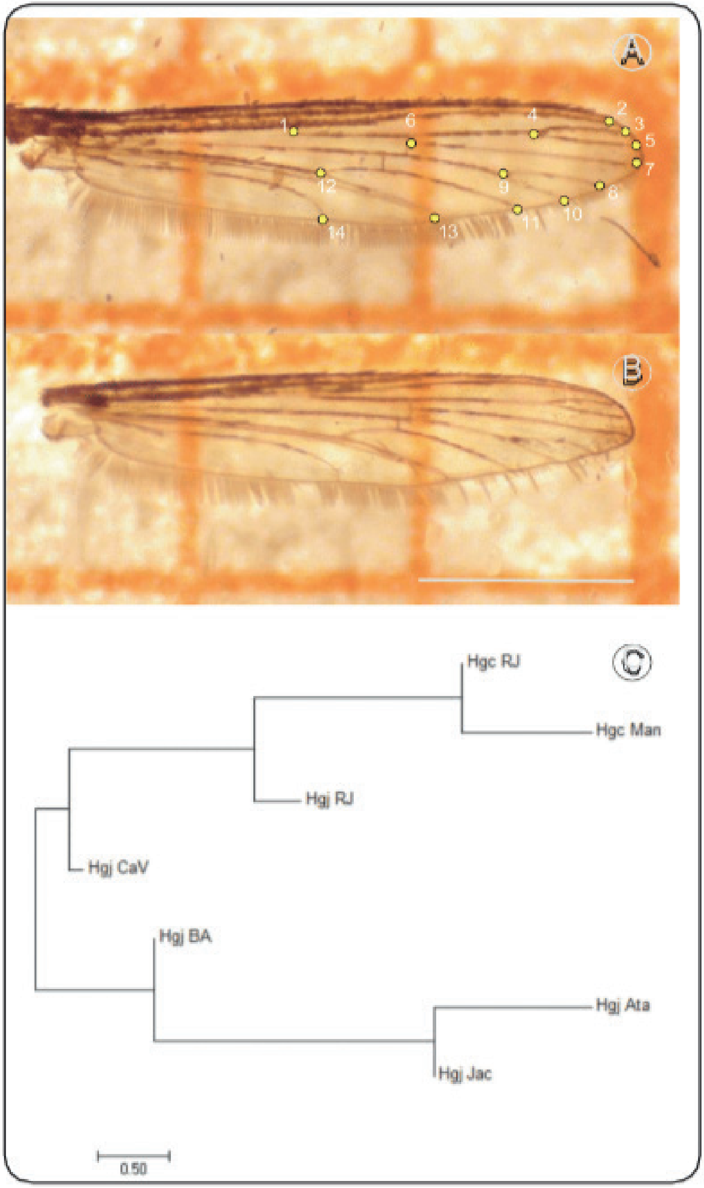

FIGURE 1: Wings of *Haemagogus janthinomys* (A) and *Hg. capricornii* (B) with graph paper in the background. Landmarks (n = 14) are shown in (A). Gray bar = 1 mm. (C) Neighbor-joining trees derived from Mahalanobis distances of shape variables of *Hg. capricornii* and *Hg. janthinomys* females from Brazil. (Populations as in **Table 1**).

The geometric coordinates of each landmark were digitalized using the program tpsDig version 2.09 (available at http://life.bio.sunysb.edu/ee/rohlf/software.html). Centroid size generalized Procrustes analysis, Mahalanobis distances, permutation tests, and allometry were performed using the modules VAR, MOG, PAD, and COV respectively, included in the CLIC98 package, according to Dujardin 2008[10]. The correlation between geographic and Mahalanobis distances was determined by Mantel tests using the PASSaGE 2 software (available at http://www.passagesoftware.net/).

For interspecific comparison, the size variable revealed that the centroid size of *Hg. capricornii* was significantly smaller (Mann-Whitney test, $P = 0.01$) than *Hg. janthinomys*. The permutation test based on the Mahalanobis distances revealed significant differences for shape variables between the two species ($P = 0.01$). The "cross-checked classification" of *Hg. capricornii* and *Hg. janthinomys* individuals showed that 81% and 67%, respectively, of all specimens were correctly assigned.

TABLE 1: Geographical location, coordinates, altitude, origin, and number of wigs ($N = 108$) of the sampled females of *Haemagogus janthinomys* and *Hg. capricornii* populations from Brazil.

Species	Locality/State	Population code	Wings (N)	Latitude	Longitude	Altitude (m)
Hg. janthinomys	Atalaia/Alagoas	Hgj_Ata	9	-9.538056	-36.132778	54
	Jacarandá/Bahía	Hgj_Jac	27	-15.863056	-38.882778	8
	Canavieiras/Bahía	Hgj_BA	4	-15.675000	-38.947222	4
	Campina Verde/ Minas Gerais	Hgj_Cav	18	-19.538611	-49.486389	494
	Duque de Caxias/ Rio de Janeiro	Hgj_RJ	19	-22.785556	-43.311667	19
Hg. capricornii	Duque de Caxias/ Rio de Janeiro	Hgc_RJ	10	-22.578611	-43.314722	24
	Mangarí/Minas Gerais	Hgc_Man	21	-18.587222	-46.514444	950

TABLE 2: Mahalanobis distances for wings of females of *Haemagogus janthinomys* and *Hg. capricornii* from four states in Brazil.

Species	Mahalanobis distances							
	Code	Hgj_Ata	Hgj_Jac	Hgj_BA	Hgj_CaV	Hgj_RJ	Hgc_RJ	Hgc_Man
Haemagogus janthinomys	Hgj_Ata	0.00						
	Hgj_Jac	3.60*	0.00					
	Hgj_BA	5.02	3.30	0.00				
	Hgj_CaV	5.41*	3.74*	4.38	0.00			
	Hgj_RJ	4.54*	1.89	3.54	3.73*	0.00		
Haemagogus capricornii	Hgc_RJ	5.32*	3.86*	4.20	5.44*	3.57*	0.00	
	Hgc_Man	4.55*	2.48*	3.70	4.17*	2.32	3.62*	0.00

Hgj_Ata: Atalaia/Alagoas; **Hgj_Jac:** Jacarandá/Bahía; **Hgj_BA:** Canavieiras/Bahía; **Hgj_CaV:** Campina Verde/Minas Gerais; **Hgj_RJ:** Duque de Caxias/Rio de Janeiro; **Hgc_RJ:** Duque de Caxias/Rio de Janeiro; **Hgc_Man:** Mangarí/Minas Gerais. *Distances were significant at $P < 0.0033$ after Bonferroni correction.

For intraspecific comparison, the size variable, revealed that all populations of *Hg. janthinomys* were significantly different among themselves and bigger than *Hg. capricornii* (Mann-Whitney test, P = 0.01). However, the analysis of populations of *Hg. capricornii* showed no significant differences among them (P = 0.06). The permutation test based on the Mahalanobis distances revealed significant differences for shape variables among some populations (**Table 2**). The contribution of the canonical factors resulted from 38%, 26%, and 15% for the first, second, and third factors, respectively. A "cross-checked classification" of individuals of the seven populations of *Hg. capricornii* and *Hg. janthinomys* showed acceptable and heterogeneous reclassification scores. *Hg. capricornii* populations showed low reclassification scores (30-42%). Although very heterogeneous, populations of *Hg. janthinomys* had better reclassification scores, from low (22% Bahía), to medium (42% Rio de Janeiro), to high (77% Atalaia, Rio de Janeiro). The NJ tree based on the distances of Mahalanobis showed that the two populations of *Hg. capricornii* (Hgc_RJ, Hgc_Man) were the most similar, followed by the *Hg. janthinomys* (Hgj_RJ) population, and morphologically different from the population of Campina Verde (Hgj_CaV) (**Figure 1C**). In addition, the NJ tree showed that the *Hg. janthinomys* populations (Hgj_Ata, Hgj_Jac) were different from the Bahia population (Hgj_BA). The Mantel test revealed a positive and significant association between the geographic distances and distances of Mahalanobis (r = 0.467; P = 0.01). A multivariate regression analysis of shape variables on the size variable showed no significant effect (test after 1000 permutations, P = 0.11).

Our results based on wing geometric morphometry of *Hg. capricornii* and *Hg. janthinomys* indicate that both size and shape variables can differentiate at the interspecific level. However, at the intraspecific level, the results show a relative differentiation. The two populations of *Hg. capricornii* had a smaller centroid size with no significant difference between them, whereas all *Hg. janthinomys* populations showed significant differences. The shape variables were able to separate the two *Hg. capricornii* and *Hg. janthinomys* populations, except for the two originating in Bahia, which were not statistically different.

The importance of taxonomy in biological sciences is undeniable. Biodiversity mapping should focus on limited groups so that research that is more thorough can be carried out effectively. Our results are congruent with the hypothesis that suggests *Hg. capricornii* and *Hg. janthinomys* may constitute a complex of species whose morphological differentiation is complex. To help identify these cryptic species and to study the relationship between them, new tools, such as molecular biology and biochemistry, have been used in addition to morphological methods, such as classical morphology, scanning electron microscopy, and morphometry[11].

Modern molecular tools are available to discriminate between sister species living in sympatry[12]. However, they are expensive to use and require specialized training. Geometric morphometrics have been shown to be highly informative, fast, and affordable. With minimal training, geometric morphometry can be used to answer ecological or taxonomic questions[6]. This study demonstrates that geometric morphometry can discriminate with considerable success *Hg. capricornii* and *Hg. janthinomys* females that cannot be identified by traditional morphological criteria.

Although centroid size is not a good measure to use in species identification because it is affected by environmental factors, our results show that this size variable was able to differentiate between the two species[13]. Thus, conformation is a reasonably good feature to solve identification problems and is merely affected by the environmental factors[14]. Our study was able to differentiate between the two species, as well as between some populations. The correlation analysis between centroid size and the shape variables for *Hg. capricornii* and *Hg. janthinomys* did not show a common allometric slope. The association between the shape of the kites and the geographic distance between the populations suggests that the morphological variation could fit a distance isolation model.

Our study had some limitations. The results were obtained from a limited number of individuals and samples were more abundant for populations of *Hg. capricornii* than *Hg. janthinomys*. This type of problem is frequent in works that present data that involve field collections.

Our results support the use of geometric morphometry in the morphological discrimination of *Hg. capricornii* and *Hg. janthinomys* females. Proper identification of species is the fundamental basis for building knowledge of biodiversity, ecology, and other areas of biology. Failures in species identification may lead to the diffusion and amplification of conceptual and methodological errors in other areas, with implications not only for our knowledge of nature, but also for ecosystem structure functioning, management decisions, and human health vector control programs[15]. Correct species identification using geometric morphometry could contribute to improving vector control strategies.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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