



## Laboratory rearing of dance flies of the genus *Hemerodromia* Meigen, 1822 (Diptera: Empididae) associated with blackfly (Diptera, Simuliidae) pupae from the Brazilian Cerrado and Atlantic Forest

Cría en laboratorio de adultos de moscas danzantes del género *Hemerodromia* Meigen, 1822 (Diptera: Empididae) asociados a pupas de mosca negra (Diptera: Simuliidae) del Cerrado y la Mata Atlántica brasileña

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## ABSTRACT

The Empididae is a family of dipterans with approximately 3,500 recognized species distributed across a wide range of habitats in all biogeographical regions except Antarctica. In recent decades, at least 85 empidid species have been described based on adults of one or both sexes, their respective immatures remaining unknown. Few studies have focused on the methodology for rearing immatures of this family. This study reports the association of immatures of the genus *Hemerodromia* with pupal cocoons of blackflies (Simuliidae) and describes the methodology used to rear Simuliidae, which can be successfully applied to rear Empididae too. Two specimens of dance flies, occasionally reared, were obtained: one from a pupa of a blackfly from the Brazilian Cerrado, representing the first record of this association for the biome; and the other from a pupa from the Atlantic Forest. This work suggests that searching actively for Empididae immatures in blackfly breeding sites may help to record this family in regions with sampling gaps, as well as in breeding sites near the type localities of dance fly species whose immature stages are unknown.

**Keywords:** aquatic insects; predation; biodiversity; biological collection; bionomy; Brazil

## RESUMEN

Empididae es una familia de dípteros con aproximadamente 3,500 especies distribuidas en una amplia gama de hábitats en todas las regiones biogeográficas excepto la Antártida. En las últimas décadas, se han descrito al menos 85 especies de Empididae basadas en adultos de uno o ambos sexos, sin embargo, se desconocen sus respectivas fases inmaduras. Pocos estudios se han centrado en metodologías para criar individuos de esta familia. Este estudio presenta la asociación de inmaduros del género *Hemerodromia* en exuvias pupales de mosca negra (Simuliidae) y describe detalladamente la metodología utilizada en la cría de Simuliidae, la cual puede aplicarse con éxito en la cría de Empididae. Durante el estudio, se obtuvieron dos especímenes de Empididae criados fortuitamente: uno encontrado en asociación con una pupa de un Simuliidae del Cerrado brasileño, el cual representa el primer registro de esta asociación para el bioma, y el otro en asociación con otra pupa de Simuliidae, en este caso, de la Mata Atlántica brasileña. Este trabajo sugiere que buscar activamente las formas inmaduras de Empididae en los criaderos de Simuliidae puede ayudar a registrar esta familia en regiones con vacíos de muestreo, así como en criaderos cercanos a las localidades tipo de especies de Empididae cuyas fases inmaduras son desconocidas.

**Palabras clave:** insectos acuáticos; depredación; biodiversidad; colección biológica; bionomía; Brasil

## INTRODUCTION

Empididae, commonly known as dance flies, is a family of dipterans with approximately 3,500 species distributed throughout a wide range of habitats in all biogeographical regions, except Antarctica (Shamshev & Ivković, 2020). In Brazil, there are 18 genera and 156 confirmed species of Empididae, mainly concentrated in the North (70 spp.), South (40 spp.), and Southeast (26 spp.) regions, with 30 species considered as endemic to the country (Rafael & Câmara, 2024).

In the adult stage, many species of Empididae are predators, while others feed on nectar or pollen (Sinclair & Cumming, 2006). Due to this predatory habit, some species play a key role in the population control of several insects, including blackflies (Diptera: Simuliidae) (Wotton *et al.*, 1993), and there are numerous records of dance fly larvae preying on blackfly immature stages (Vaillant, 1953; Werner & Pont, 2003; Brammer *et al.*, 2009). Larvae and pupae of Empididae can also be found occupying blackfly pupal cocoons, either originally empty or after the predation of the pupa by empidids (Hamada, 1993; Werner & Pont, 2003; Molina & Gil-Azevedo, 2016). There is even a record of oviposition of empidids and simuliids on the same substrate (Hamada, 1993). The predatory action of Empididae on Simuliidae, both immature stages and adults, is common, particularly in species of Hemerodromiinae, subfamily that has been suggested as a potential biocontrol agent against blackflies (Werner & Pont, 2003).

In the last decade, at least 85 species of Empididae have been described (e.g., Câmara *et al.*, 2014; Ivković *et al.*, 2014; Câmara *et al.*, 2015; Plant, 2015; Câmara & Rafael, 2016; Câmara & Rafael, 2017; Kanabalová *et al.*, 2018; Rafael & Marques, 2019, 2021; Shamshev, 2019; Plant, 2020; Shamshev & Ivković, 2020; Ivković *et al.*, 2021; Jonassen *et al.*, 2021; Kerr & Tweet, 2021; Akbar *et al.*, 2022; Bikel, 2023). However, these studies only described adults of one or both sexes, with the immature stages remaining unknown.

Due to their association with aquatic environments, adults of Hemerodromiinae are often collected using Malaise traps installed near flowing

water and are frequently observed on riparian vegetation (MacDonald & Harkrider, 1999). However, the immatures require well-oxygenated water to develop, and only a few species have their immature stages recognized (Câmara, 2016).

Regarding the immature stages of this subfamily, Vaillant (1953) described the larva and pupa, as well as the male and female of *Hemerodromia seguyi*, based on immatures collected from blackfly pupal colonies; MacDonald & Harkrider (1999) provided a morphological description and comparative analysis of the larvae of the genera *Metachela* Coquillett, 1903, and *Neoplasta* Coquillett, 1895; Sinclair & Harkrider (2004) characterized reared larvae and pupae of *Roderiodes wirthi* Chillcot, 1961; Brammer *et al.* (2009) characterized larvae, pupae, and exuviae of *Hemerodromia* Meigen, 1822, *Methachela*, *Chelifera* Macquart, 1823, and *Neoplasta*, based on morphological and molecular data; Sinclair *et al.* (2022) described the final instar larva of the genus *Proclinopyga* Melander, 1928 and presented an updated key for the larvae of genera of Clinocerinae in North America.

Despite the existing knowledge about Empididae in different areas, such as spatial distribution (e.g., Ivković *et al.*, 2017; Shamshev & Ivković, 2020), ecology (e.g., Plant *et al.*, 2011; Chatelain *et al.*, 2018), ethology (e.g., Alcock, 2016; Murray *et al.*, 2022), genetics (e.g., Gao *et al.*, 2020; Liu *et al.*, 2020), and phylogeny (e.g., Sinclair & Cumming, 2006; Rhodén & Wahlberg, 2020), few studies have provide a characterization of their morphological and bionomic features during their immature stages (e.g., Vaillant, 1953; Sinclair, 1994; MacDonald & Harkrider, 1999; Sinclair & Harkrider, 2004). In this study, we report the collection of adult specimens of *Hemerodromia* sp. and their respective pupal exuviae, reared from their association with blackfly pupal cocoons.

Therefore, the primary objective of this work is to describe how the methodology used for rearing Simuliidae pupae can be applied to the rearing of Empididae pupae. Additionally, we report associations between immature stages of Empididae and Simuliidae in Brazilian biomes, marking the first record for the Cerrado and the second for the Atlantic Forest.

## METHODOLOGY

In March 2023, a collection of Simuliidae was carried out in the upper course of the Piabas River, in the Pedra Branca State Park (Field n° JRG2023030301), geographically located at 23°1'58"S 43°31'58"W, at an altitude of 167 m.a.s.l. (Figures 1A and 1B), in the municipality of Rio de Janeiro, Rio de Janeiro State, Brazil. Specimens were collected by José R. Gomes, on 03/03/2023. Simuliid specimens from Goiás were collected at the Rio Formoso waterfall (Field n° APALD1996051202), 13°39'18.9"S 48°53'08.2"W, 361 m.a.s.l., municipality of Formoso, GO, Brazil. The collector was Antônio Paulino Luna Dias, on 12/05/1996.



Figure 1. Collection site: A. General aspect of the phytophysiognomy of the upper Piabas River, Pedra Branca State Park, Rio de Janeiro, RJ, Brazil. B. Detail of the collection point.

The collections followed the Standard Operating Procedure for the collection of blackfly immatures and adults and associated entomofauna (POP-LSO-T002-Rev02) employed by the Integrated Laboratory of Simuliidae, Onchocerciasis, Medical and Forensic Entomology of the Oswaldo Cruz Institute (LSOEMF/IOC-Fiocruz). Pupae and larvae were collected along with their substrates (roots, fallen leaves, and marginal vegetation). The material was

labeled with field numbers and placed in plastic bags, then kept in a cooled thermal box until arrival at the laboratory, where it was identified.

For identification purposes, it is essential to rear adults individually from their pupae, ensuring the correct association with their pupal exuviae. Some Simuliidae pupae were selected for rearing and obtaining adults. Mature or nearly mature pupae (pharate pupae) were chosen for selection, within which the adults were already well developed. Pharate pupae can be recognized by their darker coloration. Pupae were collected and kept attached to cut fragments of their respective substrates, which were still moist. Pupae adhered to rocks should be carefully collected. The colonized substrate should be exposed after water flow is contained with an appropriately sized E.V.A. (Ethylene-Vinyl Acetate) plate, allowing the pupae to be scraped from the substrate with a scalpel blade and placed in containers for adult emergence. To remove excess moisture, substrate fragments with the pupae were rested on absorbent paper before being transferred to microtubes.

Whenever possible, the pupae were individually placed in 1.5 ml cryogenic microtubes with a small strip of absorbent paper to prevent condensation inside the container and kept at room temperature, protected from direct light, for up to five days until adult emergence. Exceptionally, when many pupae were adhered together on the same substrate, they were allowed to emerge in the same container (mass emergence). Sterile microtubes were used as rearing chambers to prevent fungal contamination. During the rearing period, the microtubes were inspected twice a day to check for excess moisture or emerged adults. After obtaining the adults, they were kept alive for a few hours for the hardening of their exoskeleton. The microtubes containing the adults were placed in a refrigerator for 10 minutes for anesthesia and then fixed in 80% ethanol along with their respective exuviae.

The blackflies were identified with the assistance of specialized literature (Gil-Azevedo *et al.*, 2005; Shelley *et al.*, 2010) and by comparison with the reference material deposited in the Simuliidae Collection of the Oswaldo Cruz Institute (CSIOC). For Empididae, Brammer *et al.* (2009) was used for

exuviae identification, and Cumming & Sinclair (2009), Câmara *et al.* (2014) and Câmara *et al.* (2015) were used for adult identification. All the material from this study is kept under the care of CSIOC.

## RESULTS

Two adult specimens of Empididae and their associated exuviae were used in this study, both collected and accidentally reared following the described methodology. One of them was from the aforementioned collection in the Atlantic Forest, and the other was from the Cerrado, found in the CSIOC/Fiocruz backlog.

In the Atlantic Forest of Rio de Janeiro, during the sampling collection conducted at the Piabas River (Field n° JRG2023030301; Figures 1A and 1B), 108 pupae, 24 exuviae, and 197 larvae of five blackfly species were collected (Table 1). Associated with the substrate containing the blackflies, two nymphs of Zygoptera (Odonata) and three larvae of Trichoptera (two from Hydropsychidae and one from Hydroptilidae) were also found. Out

of the total number of collected blackfly pupae, eight were selected for rearing, and only four adult individuals were obtained. In one of the mass rearing tubes containing a pupa, two exuviae, and two empty cocoons of *Simulium incrustatum* Lutz, 1910 (CSIOC22432), a male of *Hemerodromia* sp. was obtained (Figure 2A) along with its respective pupal exuviae (Figure 2B). This empidid was reared accidentally as it was occupying an empty cocoon of *S. incrustatum*.

Regarding the material from Goiás, collected at the Waterfall on the Formoso River (Field n° APALD1996051202), five males and four females with their respective pupal exuviae were collected, belonging to three species of blackflies (Table 1). In addition to the blackflies, a mature pupa of the genus *Hemerodromia* was found occupying an empty cocoon of *S. lutzianum* Pinto, 1932. This specimen was reared accidentally, resulting in an adult (Figure 3A) and its pupal exuviae (Figure 3B). Unfortunately, the specimen was affected by fungi and is poorly preserved. The pupal exuviae of the specimen were crucial for the correct identification of the genus.

Table 1. Specimens of Simuliidae and specimens of *Hemerodromia* collected in the Atlantic Forest of Rio de Janeiro (RJ) and the Cerrado of Goiás (GO).

Field N° and State	Cat. N°	Species	Specimens	Observation
JRG2023030301 - RJ	22431	<i>S. pertinax</i> Kollar, 1832	1♂ + exuvia, 1 pupa	Reared
	22432	<i>S. incrustatum</i> Lutz, 1910	1 pupa, 2 exuviae, 2 cocoons	In this batch, the <i>Hemerodromia</i> sp. emerged
	22433	<i>S. anamariae</i> Vulcano, 1962	1♀, 4 exuviae	Reared
	22434	<i>S. incrustatum</i> Lutz, 1910	1♂ + exuvia	Reared
	22435	<i>S. pertinax</i> Kollar, 1832	12 pupae, 3 exuviae	
	22436	<i>S. incrustatum</i> Lutz, 1910	40 pupae, 3 exuviae	
	22437	<i>S. incrustatum</i> Lutz, 1910	17 pupae, 5 exuviae	
	22438	<i>S. travassosi</i> D'Andretta & D'Andretta, 1947	6 pupae	
	22439	<i>Lutzsimulium hirticosta</i> (Lutz, 1909)	4 pupae, 2 exuviae	
	22440	<i>S. anamariae</i> Vulcano, 1962	2 pupae, 1 exuvia	

	22441	<i>S. incrustatum</i> Lutz, 1910	1 pupa, 5 exuviae	
	22442	<i>Simulium</i> sp.	181 immature larvae	
	22443	<i>S. travassosi</i> D'Andretta & D'Andretta, 1947	3 mature larvae	
	22444	<i>S. pertinax</i> Kollar, 1832	2 mature larvae	
	22445	<i>S. (Psaroniocompsa)</i> sp.	11 mature larvae	
APALD1996051202 - GO	1422	<i>S. nigrimanum</i> Macquart, 1838	2♀ + 2 exuviae	Reared
	1423	<i>S. nigrimanum</i> Macquart, 1838	1♂ + 1 exuvia	Reared
	1431	<i>S. nigrimanum</i> Macquart, 1838	1♀ + 1 exuvia	Reared
	4704	<i>S. luzzi</i> Knab, 1913	1♀ + 1 exuvia	Reared
	4704	<i>S. luzzi</i> Knab, 1913	2♂ + 2 exuviae	Reared
	4705	<i>S. subpallidum</i> Lutz, 1910	2♂ + 2 exuviae	Reared
	23642	<i>S. lutzianum</i> Pinto, 1931	1 cocoon	In this batch, the <i>Hemerodromia</i> sp. emerged

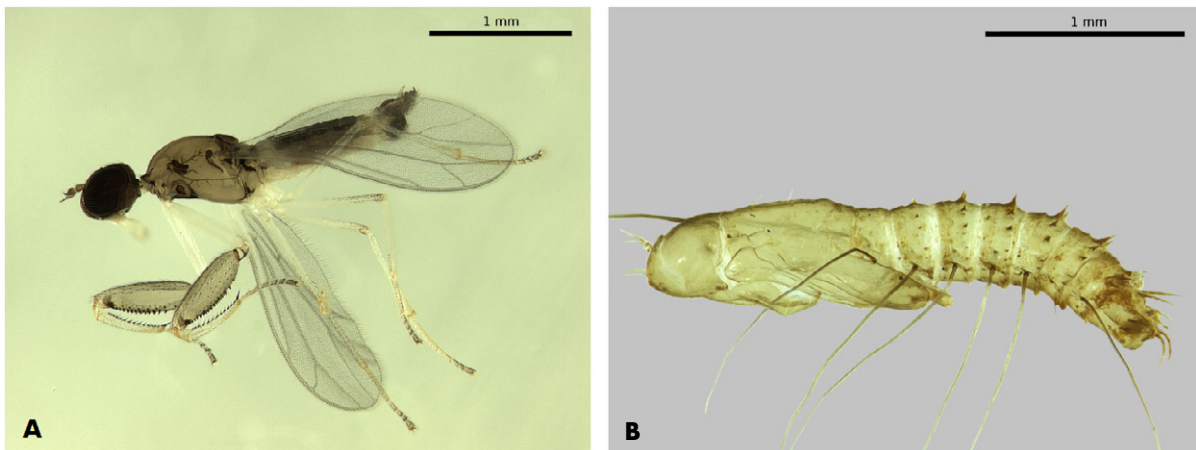


Figure 2. *Hemerodromia* sp. collected from the Piabas River, RJ, Brazil (JRG2023030301#3): A. Male. B. Corresponding pupal exuviae.

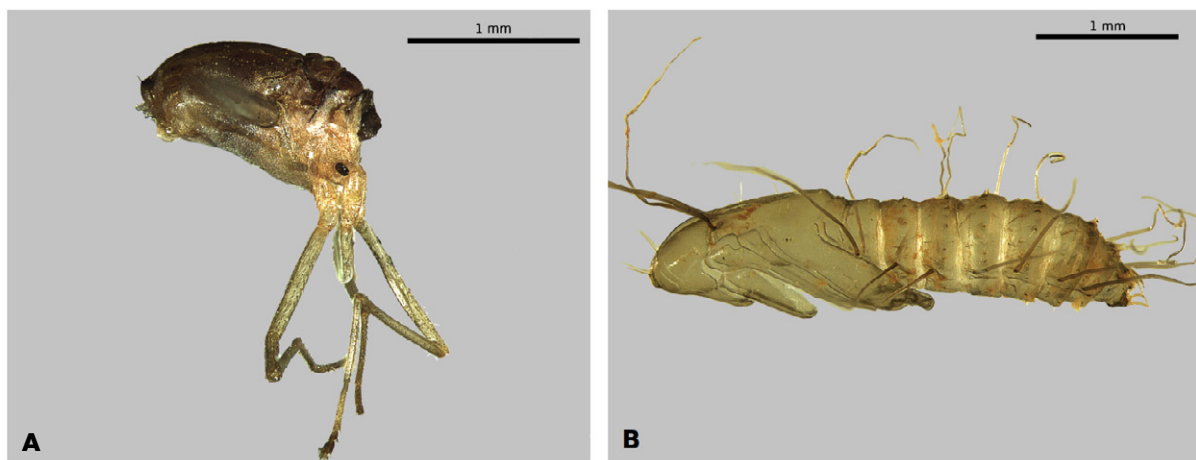


Figure 3. *Hemerodromia* sp. collected from the Formoso River, GO, Brazil (APALD1996051202): A. Thorax and hind legs of the adult. B. Corresponding pupal exuviae.

## DISCUSSION

There are few studies on the rearing of Empididae immatures (e.g., MacDonald & Harkrider, 1999; Sinclair, 1994; Sinclair & Harkrider, 2004). In the Nearctic Region, only immature stages of a few species are known (e.g., Sinclair & Harkrider, 2004; Brammer *et al.*, 2009), and none in the Neotropical Region. Generally, when new species are described, typically only males are described, as the morphology of the male genitalia has relevant characteristics for species recognition. Since female morphology is more uniform, the association between both sexes of the same species can be challenging. Câmara (2016), for instance, mentions the use of molecular techniques using fragments of the mitochondrial gene Cytochrome Oxidase I (COI) to associate males and females, which has allowed the description of previously unknown females.

The fact that species descriptions are based solely on adults may be related to the collection methodology employed to capture these insects, which is mainly based on the installation of flight interception traps (Malaise traps) (Câmara & Rafael, 2017), emergence traps (Harper, 1980; Landry & Harper, 1985), active searching for adults (Ivković *et al.*, 2014), or simultaneous use of active and passive collection methodologies (Câmara *et al.*, 2014; Plant, 2015). Meanwhile, immature stages are manually collected or indirectly captured, as they are associated with insects from other taxonomic groups such as Chironomidae, Simuliidae, Psychodidae, and Trichoptera (e.g., Vinikour & Anderson, 1981; MacDonald & Harkrider, 1999).

Although immature specimens of Empididae can be associated with adults of already described species using molecular analyses, as employed by Brammer *et al.* (2009), they can also be reared for the same purpose. However, literature on the topic is very scarce. Sinclair (1994) reared isolated pupae in hermetic Petri dishes measuring 50 mm in diameter by 9 mm in height, using filamentous algae to preserve humidity, and kept in controlled chambers at 12-15°C. MacDonald & Harkrider (1999) indicated that adults can be reared from larvae and pupae collected in the field and placed in 250 ml containers containing suitable

substrate and aeration. Sinclair & Harkrider (2004) collected Empididae adults and stimulated their oviposition in the laboratory, using Simuliidae eggs as a stimulus. According to the authors, the larvae were reared in 450 ml containers with aerated water and coarse sand as substrate until obtaining some adults. The rearing methodology in cryogenic microtubes described in this study, commonly used for rearing Simuliidae pupae, can be successfully applied for rearing Empididae, enabling the association between the adult and its respective pupal exuviae.

According to Molina & Gil-Azevedo (2016), the incidence of this association is extremely low. Nevertheless, in an initial survey of the fauna associated with Simuliidae constituting the backlog of CSIOC, some isolated larvae and pupae collected along with blackflies were found. For instance, we recorded two pupae and two larvae of *Hemerodromia* sp. collected in association with blackflies from three distinct locations in Goiás in the Tocantins River basin, and one pupa of *Hemerodromia* sp. from Joinville (Santa Catarina State), also associated with blackfly immatures.

Despite dance flies being known as predators of all developmental stages of blackflies, it has not yet been recorded whether their pupae are merely occupants of empty blackfly cocoons or whether they pupate in these sites after the larva has preyed upon the blackfly pupa. Although both scenarios are feasible, as indicated by Molina & Gil-Azevedo (2016), predation was not observed in this study due to the absence of evidence in the exuviae and the pupa of *S. incrustatum* from the same batch.

Regarding the taxonomy of the specimens found in CSIOC, the adult *Hemerodromia* sp. from the Cerrado preserved on a pin is in poor condition, with only the thorax and middle and hind legs remaining, making it impossible to determine the sex or identify the species. However, the exuvia, preserved in glycerin, is intact and matches the diagnosis of the genus. As for the male obtained from the Atlantic Forest, it is in perfect condition and does not fit the diagnosis of any of the nine *Hemerodromia* species recorded for the Neotropical Region (Argentina, Antilles and Bahamas, Costa Rica, Panama, Peru, and Paraguay) (Cumming & Sinclair, 2009), or the 19 species recorded for Brazil, differing in coloration and genital morphology.

## CONCLUSION

This article represents the second record of the association between Empididae and Simuliidae pupae for the Atlantic Forest biome and the first for the Cerrado. It highlights that the methodology of rearing blackfly pupae in microtubes can be successfully applied to rearing Empididae pupae, enabling the association of adults and their respective pupal exuviae.

It appears that actively searching for Empididae immature stages in blackfly breeding sites can assist in recording this family in regions that represent sampling gaps for the group, as well as in breeding sites near the type localities of Empididae species whose immature forms are unknown. It also opens up a favorable scenario for greater scientific collaboration among researchers interested in both families of Diptera. Collaborative studies with Empididae specialists have been initiated, and this new dance fly species is being described in another study. The discovery of a new taxon from reared material will enable the first description for the Neotropical Region of an Empididae species based on two developmental stages: adult male and pupa (pupal exuviae).

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