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Adult emergence of *Phyllocycla* Calvert, 1948 (Odonata: Gomphidae) in artificial environments

Emergência de adultos de *Phyllocycla* Calvert, 1948 (Odonata: Gomphidae) em ambientes artificiais

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Abstract: Aim: This study aimed to analyze aspects of adult emergence of *Phyllocycla* Calvert, 1948 (Odonata: Gomphidae) in artificial environments. Samplings were conducted in Sanharó stream, Caxias municipality, Maranhão State, Brazil. **Methods:** Specimens were examined under a stereomicroscope and identified to the lowest possible taxonomic level. Polystyrene boxes were used as rearing sites, and the following methods were analyzed: a) sand; b) strips of filter paper simulating substrate; c) without substrate. For emergence, the rearing sites had their lids removed and were placed inside larger jars with mesh windows, less water, with most of the substrate exposed above the water level. To verify the difference between treatments, the Log-rank test and Kaplan-Meier survival curves were used. **Results:** The curves indicated that emergences occurred more frequently in the initial days of rearing. There was an absence of statistical difference between groups. Despite that, all treatments showed success in emergence, with larvae without substrate emerging more quickly. **Conclusions:** By exploring the use of different substrates in Odonata rearing, this research provides guidelines for optimizing laboratory conditions, helping to overcome experimental challenges and supporting new studies and inventories.

Keywords: Anisoptera; life cycle; larval rearing; dragonfly.

Resumo: Objetivo: Esse estudo teve como objetivo analisar aspectos da emergência de adultos *Phyllocycla* Calvert, 1948 (Odonata: Gomphidae) em ambientes artificiais. As coletas foram realizadas no riacho Sanharó, Caxias, Maranhão, Brasil. **Métodos:** Os espécimes foram analisados sob estereomicroscópio, sendo identificados ao menor nível taxonômico possível. Foram utilizadas caixas de isopor como criadouros, sendo analisados os seguintes tratamentos: a) areia; b) tiras de papel de filtro simulando substrato; c) sem substrato. Para a emergência, os criadouros tiveram suas tampas retiradas e acondicionados em frascos maiores com janelas de tela, menos água e parte do substrato totalmente fora desta. Para verificar a diferença entre os tratamentos, foram utilizados o teste Log-rank e as curvas de sobrevivência de Kaplan-Meier. **Resultados:** As curvas mostram que as emergências ocorreram mais frequentemente nos primeiros dias de criação. Foi observada ausência de diferença estatística entre os grupos. Apesar disso, todos demonstraram sucesso na emergência, com as larvas sem substrato tendo emergido mais rapidamente. **Conclusões:** Ao explorar o uso de diferentes substratos na criação de Odonata, esta pesquisa fornece diretrizes para otimizar as condições laboratoriais, ajudando a superar desafios experimentais e apoiando novos estudos e inventários.

Palavras-chave: Anisoptera; ciclo de vida; criação de larvas; libélula.

1. Introduction

Insects are one of the most diverse groups of organisms on the planet, considered extremely important as they perform essential ecological functions, and their extinction can collapse entire ecosystems. Despite their significance, our understanding of the biology and natural history of many insect orders remains limited (Del-Claro, 2019). Among the numerous orders, there are the Odonata, composed of insects popularly known as dragonflies and damselflies, with vibrant colors, diverse morphological patterns and behaviors (Koneri et al., 2020). This order is represented by two suborders in the Neotropical region: Anisoptera and Zygoptera. The Zygoptera suborder comprises small species that are largely thermal conformers regarding thermoregulation, while the Anisoptera suborder consists of medium to large species, which can be heliothermic or endothermic (Corbet, 1999).

Within Anisoptera, the genus *Phyllocycla* Calvert, 1948, comprises 31 described species (Garrison & von Ellenrieder, 1991), with 19 of them integrating the Brazilian fauna (Araújo et al., 2020; Pinto, 2024). Insects of this genus inhabit streams in dense forests and rivers, where adults perch on tree leaves and larvae likely inhabit backwaters covered in mud and sand, as they are classified as burrowers, possessing adaptations to bury themselves in the substrate (Carvalho & Nessimian, 1998; Garrison et al., 2006).

Odonata have been widely used as model species in studies of behavioral, evolutionary, ecological, and conservation analysis. Moreover, the significant variation among species in terms of their specific characteristics and requirements throughout their life cycle, with the aquatic larval stage and the aerial adult stage, makes them ideal for analyzing various types of freshwater ecosystems (Bybee et al., 2016). One of the major challenges for studies involving their larval phase is that the description of larvae is based on their final stadium, and identifying early stadia larvae is nearly impossible without rearing specimens (Pessacq et al., 2018).

Larvae identification for species becomes easier through knowledge about adults, as most species definitions are based on the adult stage (Tennessen, 2019a). However, sampling of some adult species is often hindered by their high and rapid flight, while larvae can be encountered periodically, indicating the need to acquire adults through rearing for species-level identification (van Gossum et al., 2003; Carvalho, 2007). Thus, insect rearing is often a fundamental part of laboratory experimental projects because, besides the descriptive value of complete rearing, careful preservation of exuviae allows observation of various structures such as mouthparts, antennas, spines, bristles, and appendages (Tennessen, 2019b). It is noteworthy that field studies are highly effective, enabling a large amount of research to be developed; however, laboratory studies reduce the uncontrollable circumstances related to conventional field studies, which aids and can simplify the understanding and analysis of aspects of the organisms analyzed, whether evolutionary, ecological, or physiological (Piersanti et al., 2015).

One of the main challenges in rearing Odonata larvae lies in reproducing the ideal conditions of the aquatic habitat for the larvae, as maintaining water quality, temperature, and adequate food supply are crucial elements for successful rearing (Lima et al., 2023). The time required for emergence varies among species but generally can range from a few weeks to over a year (Carvalho, 2007). Therefore, it is important to search for larvae in penultimate and last stadia aiming for greater success in emergence. Patience and careful observation are essential for success in rearing Odonata larvae, providing an ideal environment for their development until they reach the adult stage.

The rearing of Odonata larvae emerges as a direct response to Wallacean and Linnean shortfalls. The Linnean shortfall, characterized by the discrepancy between formally described species and the actual number of species, underscores the urgent need to deepen taxonomic studies for Odonata (Whittaker et al., 2005). Larval rearing provides a unique opportunity for detailed observation of different developmental stages, allowing for the identification of specific characteristics and potentially the discovery of new species. Simultaneously, the Wallacean shortfall, related to deficits in knowledge of geographical distribution, can be addressed through studies on the ecology of Odonata in various environments (Bastos et al., 2019; Hortal et al., 2015). Furthermore, in our ecological research, collecting adult Odonata has been challenging due to their fast and agile flight, capable of reaching considerable altitudes, and larval rearing presents itself as a strategic approach to overcome this difficulty.

For larvae rearing, it is necessary to use appropriate substrates, as studies have shown that they prefer specific habitats regardless of biotic or abiotic interactions. Thus, the more similar the substrate used in the artificial environment is to the substrate present in their natural habitat, the more suitable the environment will be for adult emergence (Leipelt & Suhling, 2001; Verdonschot & Peeters, 2012).

Consequently, this study aims to analyze general aspects related to adult emergence of *Phyllocycla* Calvert, 1948 (Odonata: Gomphidae) in artificial environments, considering fundamental premises such as substrate influence on survival and development rates, and necessity of maintaining controlled environmental conditions. To achieve this objective, Odonata larvae were sampled from a stream, followed by rearing under controlled conditions with regular monitoring. It is expected that the different substrates tested will significantly influence adult emergence, as well as larval development and timing required for metamorphosis.

2. Material and Methods

2.1. Study area

Larval sampling was conducted in the Sanharó stream, a tributary of the Itapecuru River, located in the Caxias municipality, Maranhão State, Brazil (Figure 1). Four samplings were made on the following dates: October 3, 2020, October 18, 2020, January 17, 2021, and May 22, 2021. The stream is located in the urban area, and the coexistence between urbanization and the stream is reflected in the presence of household waste along its banks, suggesting a direct interaction with nearby residential activities. Additionally, vehicles pass directly over the stream's water surface, which crosses a road, requiring cars and motorcycles to travel across it.

The presence of a dense canopy cover reveals abundant riparian vegetation, composed of an irregular mix of grass, some pioneer trees, and scattered shrubs. However, numerous breaks in the riparian vegetation are observed, indicative of anthropogenic interventions or local disturbances. Along its length, cutting frequent, undercutting of banks and roots evidencing erosive processes. The adjacent bank reveals significant instability, characterized by loose soil and sand, making it susceptible to disturbances and landslides. The stream has a uniform bottom of sand and silt, stony substrate absent and the aquatic vegetation mainly consists of patches of algae and vascular plants.

2.2. Sampling, rearing, and identification

Samplings were carried out using an aquatic entomological net with a mesh size of 1 mm (D-net) and also through manual samplings from submerged roots, trunks, rocks, leaves, and macrophytes (Figure 2a). Only larvae in penultimate and final stadia were collected (the length of the wing sheets is generally similar to or larger than the width of the head) because after this period, emergence can occur within one to three weeks, approximately (Carvalho, 2007). The larvae were stored in plastic bags containing clean water and substrate (sand) in sufficient quantities to cover them and transported to the Laboratório de Ecologia de Comunidades (LaECO), at the Instituto Federal do Maranhão, Caxias Campus. For identification, specimens were analyzed under a stereomicroscope (Figure 2b), being identified to the lowest possible taxonomic level with the aid of identification keys (Hamada et al., 2014; Costa et al., 2004; Merritt & Cummins, 1996).

For the rearing sites, polystyrene boxes measuring 110×95×75mm were used, prepared with mesh screens glued to their lids, with water levels of approximately 3cm. The following rearing methods were analyzed in this study: 1) with sand substrate; 2) with strips of filter paper simulating a substrate; 3) without any substrate (Figure 2c). In each rearing site, only one larva of *Phyllocycla* was present, and to accelerate emergence, the larvae were not fed (Fulan et al., 2014).

The water was not oxygenated but was changed every 2-7 days to prevent anoxia. For emergence, the rearing sites had their lids removed and were transferred to larger jars, that were covered with mesh to prevent the insects from escaping. The amount of water in the jars was reduced, exposing part of the substrate above the water level. This substrate serves as a support for the larvae to leave the water and for wing drying, creating a suitable environment for the larvae to emerge and metamorphose into

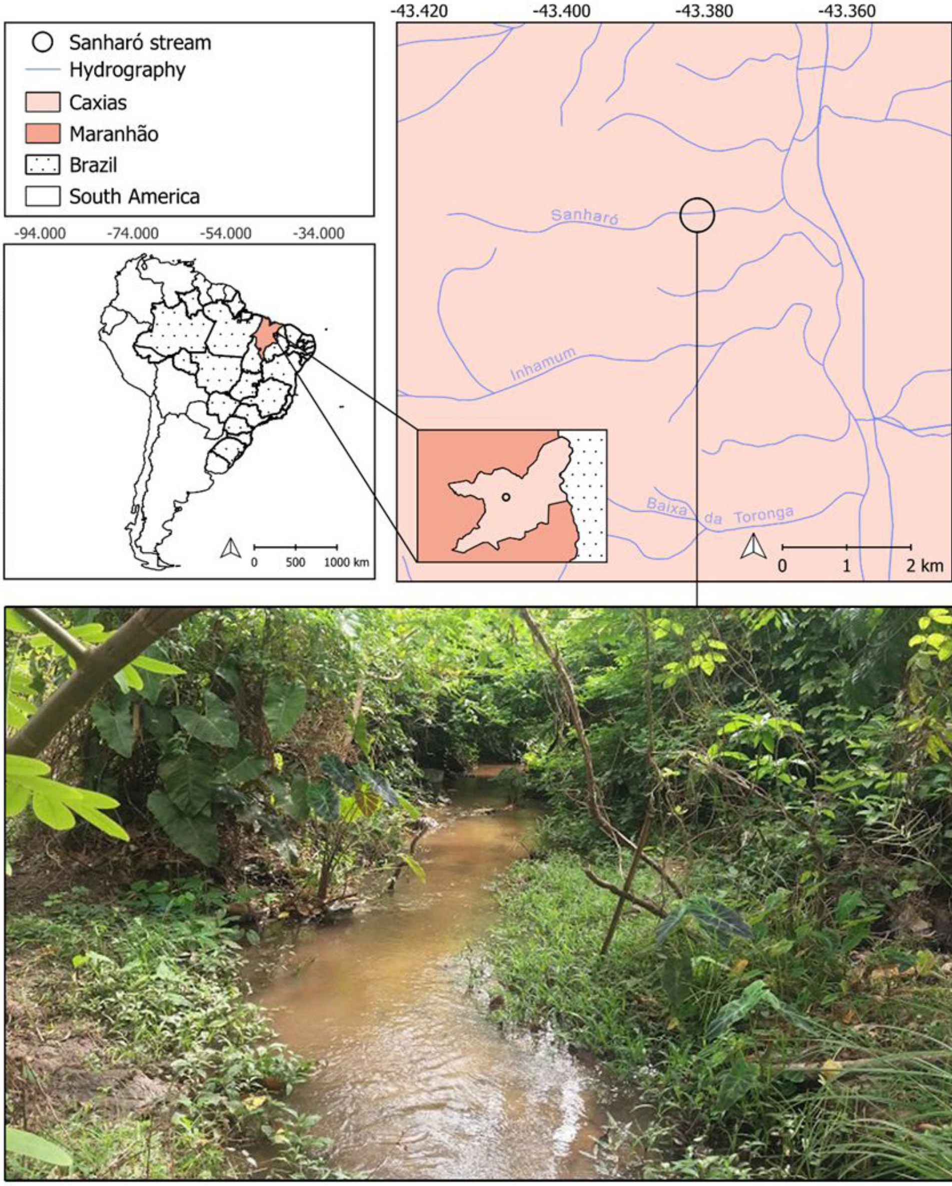

Figure 1. Larvae sampling area located at Sanharó stream, a tributary of the Itapecuru River, in the municipality of Caxias, Maranhão, Brazil.

adults (Fulan et al., 2014; Carvalho, 2007; van Gossum et al., 2003; Corbet, 1999).

Data regarding the stream were recorded, measuring aspects such as temperature, pH, conductivity, and dissolved oxygen using an Akso multiparameter sensor (model AK88). The exuviae resulting from the emergences were stored and preserved in 70% alcohol. Among the adults that emerged, there were males and females, identified using a stereomicroscope and specific taxonomic keys (Fraser, 1947; Belle, 1970, 1975, 1988, 1990). For the identification of adults, characters such as S-10, caudal appendages and seminal vesicle were considered (Figure 3). To confirm the identifications, the specimens were transferred to a taxonomic expert, Dr. Diogo Silva Vilela.

To verify the difference between treatments, were used the Log-rank test and the Kaplan-Meier survival curves (Fulan et al., 2014). KM-curves are used to compare survival durations between two or more groups, offering users a specific estimate of the probability of survival at a given point in time, while the Log-rank test is typically used to conduct statistical inference on survival durations between groups (Creed et al., 2020). The analyses were conducted using the statistical environment R 3.5.1 (R Development Core Team, 2015).

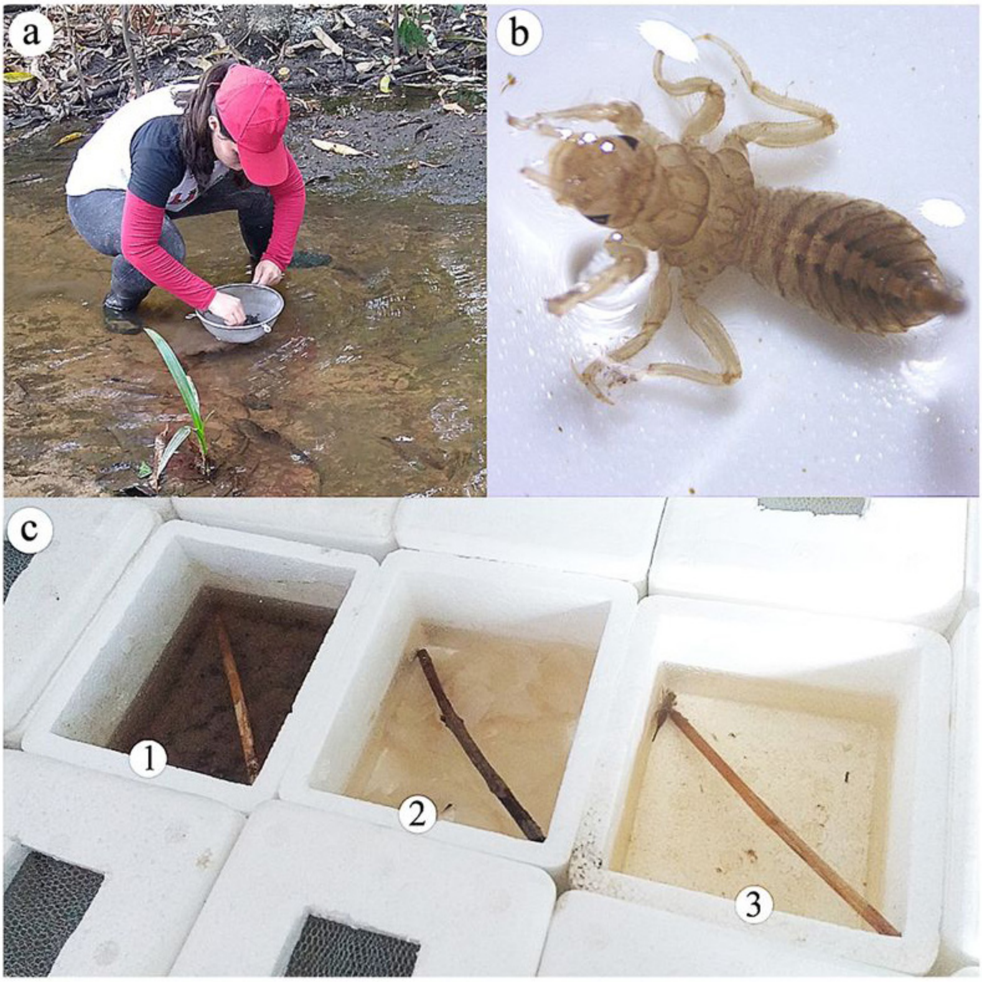

Figure 2. (a) Larvae sampling with sieve; (b) Larva view through a stereomicroscope; (c) Polystyrene boxes containing the analyzed substrates.

Subsequently, the material was deposited in the Zoological Collection of IFMA Caxias Campus.

3. Results

A total of 56 Odonata larvae in penultimate and ultimate stadia were sampled, with 17 being analyzed in the treatment with natural substrate (sand), 18 in the treatment with filter paper, and 21 in the treatment without substrate. From these, 19 adults were obtained, corresponding to 33,92% of the collected larvae. Only three could be identified to species level. Of these, one belongs to the species *Phyllocycla pallida* Belle, 1970, while the other two were classified as *Phyllocycla viridipleuris* (Calvert, 1909) (Table 1).

Survival curves allow for the comparison of time to first emergence of larvae reared with sand and fine gravel substrate, with filter paper strips simulating a substrate, and without any substrate (Figure 4). The curves indicate that emergences occurred more frequently in the initial days of rearing. The Log-rank test shows a p-value > 0,05, indicating a lack of statistical difference among the groups based on the data obtained. The mean days to emergence for each group were 9,16 days for sand substrate, 12,57 days for filter paper strips simulating a substrate, and 7,66 days for absent substrate. In this study, none of the examined treatments were significantly superior, although larvae reared without substrate underwent a shorter period until emergence.

4. Discussion

The results showed a successful rate of transformation of larvae into adults. However, it is crucial to note that while the substrate may have slightly influenced the average emergence time of larvae, all substrates showed relevance and can be utilized, as there was not statistical significance

Table 1. List of species identified after emergence; (m) male specimen, (f) female specimen.

ID	Sampling date	Emergence Date	Species	Substrate
SD009	January 17, 2021	January 26, 2021	*Phyllocycla sp. (f)*	Sand
SD010	January 17, 2021	January 27, 2021	*Phyllocycla viridipleuris (m)*	Sand
SD011	January 17, 2021	January 19, 2021	*Phyllocycla sp. (f)*	Sand
SD012	January 17, 2021	January 17, 2021	*Phyllocycla sp. (m)*	Sand
SD013	January 17, 2021	February 5, 2021	*Phyllocycla sp. (m)*	Sand
SD017	January 17, 2021	January 19, 2021	*Phyllocycla sp. (m)*	Sand
FP010	January 17, 2021	January 25, 2021	*Phyllocycla pallida (m)*	Filter paper
FP012	January 17, 2021	March 7, 2021	*Phyllocycla viridipleuris (m)*	Filter paper
FP014	January 17, 2021	January 22, 2021	*Phyllocycla sp. (f)*	Filter paper
FP015	January 17, 2021	January 30, 2021	*Phyllocycla sp. (m)*	Filter paper
FP016	January 17, 2021	January 19, 2021	*Phyllocycla sp. (m)*	Filter paper
FP017	January 17, 2021	January 19, 2021	*Phyllocycla sp. (f)*	Filter paper
FP018	January 17, 2021	January 24, 2021	*Phyllocycla sp. (m)*	Filter paper
NS013	January 17, 2021	January 24, 2021	*Phyllocycla sp. (m)*	No substrate
NS015	January 17, 2021	January 26, 2021	*Phyllocycla sp. (f)*	No substrate
NS016	January 17, 2021	January 19, 2021	*Phyllocycla sp. (f)*	No substrate
NS017	January 17, 2021	January 19, 2021	*Phyllocycla sp. (f)*	No substrate
NS019	January 17, 2021	February 3, 2021	*Phyllocycla sp. (m)*	No substrate
NS020	January 17, 2021	January 26, 2021	*Phyllocycla sp. (f)*	No substrate

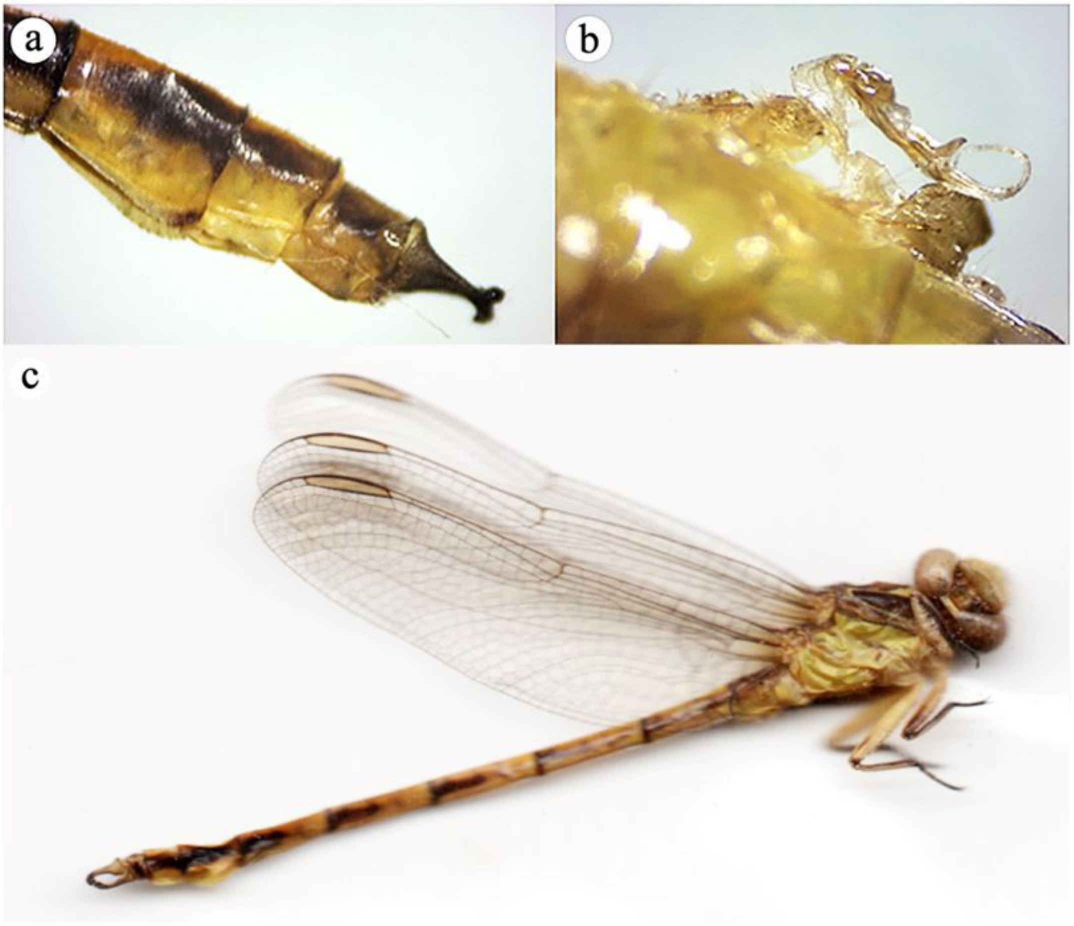

Figure 3. (a) Lateral view of S-10 and caudal appendages; (b) Right view of the seminal vesicle; (c) *Phyllocycla* adult.

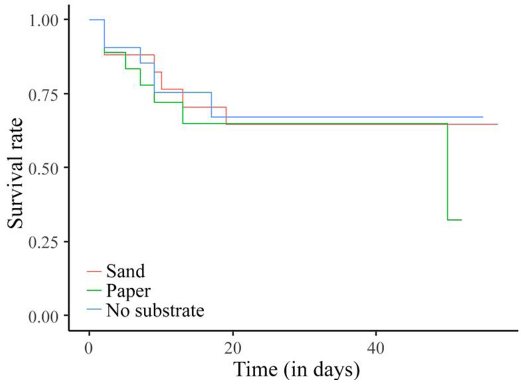

Figure 4. Survival curves of time to emergence, according to rearing substrate.

to claim that any specific substrate significantly outperformed others in promoting dragonfly emergence.

The species *P. pallida* and *P. viridipleuris*, hitherto unreported in Maranhão State, have their occurrences recorded in different regions of Brazil. *P. pallida* has been previously observed in Rio de Janeiro and Santa Catarina, while *P. viridipleuris* has distribution records in Goiás, Minas Gerais, Rio de Janeiro, São Paulo, Rio Grande do Sul, and Santa Catarina, spanning the central-west, southeast, and south regions of Brazil (Marco Júnior et al., 2023a, b). The fact that these species have been identified in a new locality such as Maranhão adds significant information to the geographic distribution of these insects. The uncommon presence of these species in the study region emphasizes the complexity of dragonfly geographic distribution and the influence of different biomes on their ecology.

Given the swiftness and evasiveness of adults, efficient capture often becomes challenging in the natural environment. Therefore, rearing emerges as a crucial tool in addressing both Linnean and Wallacean shortfalls, allowing not only for more detailed observation of developmental stages but also for identifying new species, confirming the presence of previously unknown species in specific areas, and understanding the specific adaptations of these species in regions where their presence is less evident (Whittaker et al., 2005).

There was some difficulty in the precise identification of certain specimens of dragonflies emerged during the course of the study, given that upon emergence, adults are in a morphologically vulnerable state, characterized by the malleability and sensitivity of various body parts, which may result in damage to anatomical structures, complicating their species-level identification (Lima et al., 2023). However, it is important to note that not all specimens were affected, and significant efforts were made to minimize the impact of these difficulties on identification. Given that the quantity of adults that emerged was considerably small, we also emphasize that the general applicability of our results may be limited and should be considered preliminary. Nevertheless, we believe they are valuable, as basic biological information of this kind is scarce for tropical Odonata.

It is noteworthy that in all tested treatments, there was success in emergence, and all individuals that emerged were collected on the same date (January 17, 2021). The successful occurrence of adult Odonata emergences in different types of substrates, including sand, paper, and even without substrate, suggests remarkable adaptability and flexibility in the emergence strategies of these insects. This capacity may be associated with specific larval behaviors during the emergence process, where they can adapt to environmental conditions. In all rearing sites, anchoring points were provided for the larvae, facilitating fixation during emergence. Additionally, the environmental conditions maintained in the rearing environments may have been favorable under all substrate conditions tested.

In nature, the importance of substrate for Odonata larvae is observed, as its presence and type are determinants in the occurrence, distribution, and abundance of immatures (Hanauer et al., 2014) due to its relationship with opportunities for predation, shelter, and protection (Fulan et al., 2011). Moreover, the substrate is used as a support for leaving the water, for wing drying, and also for the emergence and oviposition process (Corbet, 1999). Thus, the influence and relevance of this factor for development and emergence occurrence are evident. However, studies also demonstrate the effectiveness of artificial substrates, such as the filter paper substrate used in this research. Various types of artificial substrates benefit species when the requirements regarding the physical conditions of the habitat match those provided by the substrate, explaining the efficiency in emergence in this paper substrate treatment. Artificial substrates reduce variations in physical conditions relative to the substrate, enabling organisms to live in environments resembling their natural habitat (Wantzen & Pinto-Silva, 2006). In addition, the emergence in the control treatment (without substrate) should also be considered. Since larvae have a low dispersal capacity, they sometimes need to face harsh environments, developing under high levels of stress. Stressors such as competition, predators, or food scarcity during the larval phase can directly and profoundly impact development (Roux & Robert, 2019), supporting the fact that the absence of substrate is a stressful element and consequently was determinant in adult emergence.

The genus *Phyllocycla* tends to prefer sandy substrates, which are generally considered simple in ecological terms due to their homogeneous and relatively uniform structure (Carvalho & Nessimian, 1998; Garrison et al., 2006). Although sand can vary in grain composition and size, it tends to offer less diversity of microhabitats and resources for organisms compared to other substrates (Bueno et al., 2003; Ferreira et al., 2020). In our study, despite the simplicity of the substrates, these insects were able to complete their metamorphosis to the adult form, indicating that substrate complexity is not a limiting factor for their development. This adaptability suggests that, as long as general environmental conditions are within acceptable limits, *Phyllocycla* larvae can thrive.

It was observed that in the initial samplings (October 3, 2020, and October 18, 2020), there was a high abundance of larvae; however, none of them emerged or survived. One possible explanation for the lack of emergence could be the larval phase of dragonflies, as according to the literature, there are situations where rearing periods in the laboratory, particularly of the last stadia, can take from months to over a year (Carvalho, 2007). During the field visit on May 22, 2021, after the successful emergence sampling, no larvae in penultimate and ultimate stadia were found, only tiny larvae were encountered. Hence, it can be considered that there is a specific period for emergence, as all specimens that emerged were collected on the same date (January 17, 2021). However, further studies are necessary to confirm this, along with analyzing the relative and determining aspects of oviposition.

Overall, this research not only expands our knowledge about Odonata but also underscores the crucial importance of considering the adaptive variability of these species to understand ecological and geographical patterns more comprehensively. Despite not observing statistically significant superiority among the examined treatments, it is relevant to highlight that all treatments demonstrated successful emergence. Additionally, species not previously recorded in the region were identified, showcasing the importance of rearing larvae for species recording where adults are not easily observed in the field. Such research serves as a guide for future studies, indicating possible challenges in conducting experiments and proposing recommendations for laboratory conditions to achieve a higher success rate in Odonata adult emergence, thus serving as a basis for new inventories of individuals belonging to the order.

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Data availability

The data that support the findings of this study are available from the corresponding author, CGV, upon reasonable request.

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