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Protocol & Techniques

Some practical and biological information useful for Zoraptera (Insecta) studies

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Abstract. The data presented here summarizes essential information to enhance the collections and breeding of Zoraptera. We present our experience collecting specimens, primarily in the Amazon Basin, ranging from the precarious use of soft paintbrushes to the highly effective method of using a lightweight 9-volt portable aspirator when exploring under the bark of rotting wood and on the sheaths of live plants. The portable aspirator transformed the Instituto Nacional de Pesquisas da Amazônia (INPA) Zoraptera collection into the largest in the world, with over 4,500 specimens. We use a headlamp better to see the dark adult specimens against the dark substrate. We report on a mixed colony comprising more than 1,000 specimens on the same fallen trunk. We provide an easy method for growing specimens in the laboratory to obtain eggs, nymphs, and adults. We also present a rapid identification method for live specimens manipulated over a small amount of water using a soft paintbrush. Based on more than 3,000 specimens collected for *Brazilozoros huxleyi* Bolivar y Pieltain & Coronado, 1963 and *Brazilozoros weidneri* New, 1978 we obtained only 2% of the alated specimens in the field. The sex ratio for both species was close to 1:1.

Keywords: Angel insects, field collection, growing colonies, portable aspirator, sex ratio.

At the beginning of our experience collecting zorapterans, we used a soft paintbrush to look for specimens under the bark of rotting wood. In our first three years of field experiences, we collected nymphs of blattarians, nymphs of dermapterans, and nymphs and adults of psocodeans. We learned how to differentiate zorapterans from other orders of insects, but the collections were not so efficient because we used soft paintbrushes to collect each specimen individually. We collected only two or three specimens when we found a colony of several specimens; the remaining specimens were running inside small holes in the wood or below the surrounding loose bark. Over time, a homemade, lightweight (250 gr) 9-volt portable aspirator was developed, a miniaturized version of the domestic aspirator. This equipment, together with a headlamp, made the collections much more efficient for collecting live nymphs and adults. With the specimens alive, we established an efficient method for cultivating them in the laboratory and a reliable technique for identifying living adults. We aim to share our experiences with those interested in this order of insects and encourage more people to work globally.

The practical pieces of information presented here are our experience of 22 years collecting zorapterans in Brazil and some neighboring countries (Colombia and Peru).

From soft paintbrushes to a lightweight 9-volt portable aspirator. Collecting zorapteran specimens was tricky initially, but our skills improved over time. The zorapteran specimens were collected at the beginning of our first three years of field collection, according to Choe (1992), by sweeping them into a collecting jar with a soft paintbrush moistened with alcohol. This method allowed us to collect only a few specimens when we found a colony of several specimens. The collections were not so efficient because we used a soft paintbrush to collect each specimen. Most specimens disappeared inside small holes in the wood or the surrounding loose bark. Buccal aspirators collect live specimens (Choe 1992) and are still used today (Kočárek & Horká 2023). At INPA, the late INPA technician João Ferreira Vidal developed a miniaturized, lightweight (250 gr) 9-volt portable aspirator (Figs. 1-4). This equipment enabled the collection of live specimens, nymphs and adults, to be much more efficient. The mounted aspirator (Fig. 1) measures 270 mm in length. It is made of a polyvinyl chloride (PVC) tube and consists of two main components: the collector pieces (Fig. 2) and the engine pieces (Fig. 3). The collector pieces are 90 mm long and consist of two parts. Part 1. A cap for a 50 mm diameter tube with a 20 mm diameter suction tube mounted (glued) in the middle of the cap (Figs. 1A, 2A); the suction tube goes 3 cm inside through the cap (Fig. 2A) to prevent the specimens from going out when the aspirator is down-turned. Part 2. The screened compartment is 50 mm in diameter by 60 mm long (Figs. 1B, 2B, 4B). The screened compartment with a plankton net (Fig. 4B) mesh size of 50 µm (enough to retain, for example, the smallest free-living insect of the world, the mymarid *Kikiki huna* Huber, 2000 (Huber & Beardsley 2000) as well as juveniles of psocopterans and zorapterans. The plankton net is fitted onto a homemade, narrow PVC ring inside the chamber. The engine pieces are 150 mm long by 50 mm in diameter (Fig. 1C, 2C) and consist of two parts. The motor and 9-volt battery compartment (Figs. 1C, 3C, 4C) and the cap on the bottom (Figs. 1D, 3D). The top end of the engine compartment is perforated (Fig. 4C) to permit air flow. The engine compartment receives a micro DC motor, which is connected to a replaceable 9V alkaline battery. An external switch bottom (Fig. 1C) activates the motor. The aspirator can collect a wide range of taxa and is particularly helpful for tiny insects that are difficult to collect using other methods. It is ideal for anyone requiring a lightweight portable aspirator for sampling purposes. The use of this aspirator transformed the INPA Zoraptera collection into the largest in the world, currently comprising more than 4,500 specimens, primarily from Amazonia.

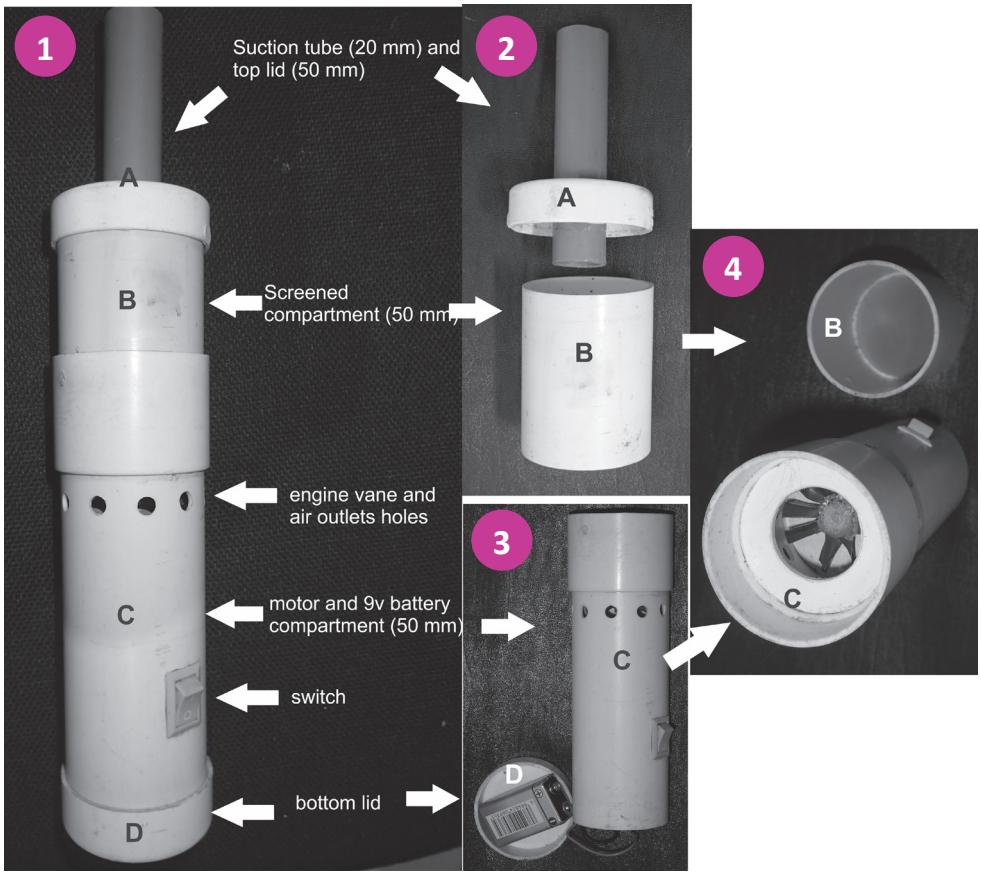
Field collections. The Zorapterans specimens have been collected in soil (Caudell 1927), under rock (Matsumura et al. 2023), in decaying sawdust piles (Riegel 1963); in termite galleries of decayed logs (Shetlar 1978), in logs in decomposition (Aberlenc 1995), mainly under the bark of rotting wood (Bolivar y Pieltain 1940; Bolivar y Pieltain & Coronado 1963; New 1978; Choe 1989; 1992; Rafael & Engel 2006; Kočárek & Horká 2023; Kaláb et al. 2025) and on rotting banana stems (Choe 1992). Our Brazilian field collection efforts concentrated on exploring the cryptic habitat, where zorapteran specimens are more easily found under the bark of rotting wood in shaded, high-humidity environments. When the trunk is dry on the upper side, we explore the underside when possible.

Zorapteran specimens do not occur only in decomposing substrates. In drier areas, we found zorapterans in the sheath of some plants (Rafael & Lima 2024), as older leaves of a young, alive *Mauritia flexuosa* Linnaeus (Arecaceae) (Fig. 5) and older leaves of an alive arbustive bird of paradise (in Brazil commonly called wild banana or queen banana) (Strelitziaceae, Zingiberales) (Fig. 6). In the sheath of a young buriti palm, we found an undescribed *Brazilozoros* Kukalova-Peck & Peck, 1993 species. In the sheath of the Strelitziaceae, we collected specimens of *Brazilozoros huxleyi* Bolivar y Pieltain & Coronado, 1963. From then on, live plant stems that accumulate organic matter were explored. In drier environments, the zorapterans are more challenging to find. It is necessary to look for them near small streams along gallery forests, where some species of plants with sheathed leaves are found. Both plants cited above retain organic material in the sheaths.

Using a headlamp to visualize the Zoraptera specimens better. We always used a headlamp (Fig. 5) to see the brown/black adult specimens against the dark substrate. The light is useful mainly for collecting specimens on the underside of the rotting trunks or near sunset or at night, if necessary.

Colony size. In hundreds of field collections, we found zorapterans in small numbers, ranging from 1 to 10 individuals, with a generally higher proportion of nymphs than adults. Twice, we found over 100 specimens.

In Tabatinga, Brazil, we discovered a colony of over 1,000 specimens



Figures 1-4. Portable aspirator made of polyvinyl chloride (PVC), developed by the late INPA technician J. F. Vidal (1953-2019). 1, mounted aspirator; 2, disconnected collector pieces; 3, disconnected engine pieces; 4, upper view of screened compartment (B) and engine vane (C).

Figures 5-6. Collection of Zoraptera in live stems. 5, Stem of *Mauritia flexuosa* (Arecaceae); 6, Stem of Strelitziaceae (Zingiberales). The white arrows indicate the locations where zorapterans were found. Photos by the authors.

Table 1. The sex ratio for *Brazilozoros* Kukalova-Peck & Peck, 1993 species.

Species	Apterous males	Apterous females	Dealate males	Dealate females	Winged males	Winged females	Total of males	Total of females	Sex ratio M:F
Brazilozoros huxleyi	1047	943	183	248	81	115	1311	1306	1:1
Brazilozoros weidneri	366	259	27	53	16	42	409	354	1.15:1

in rotting wood with loose bark. Unfortunately, we were using soft paintbrushes to collect them on that occasion.

Other collection methods for Zoraptera. The best way to find zorapteran specimens is to be active, walk on trails, and check the fallen and standing trunks in shaded and humid areas. When finding a rotting wood substrate, use a small knife or machete to lift the loose bark to collect nymphs and adults. Besides the active collection, a few zorapteran specimens can be collected passively from litter using a Berlese funnel, Winkler extractor, or pitfall traps. These apparatuses primarily collect juvenile specimens, which are often inadequate for identification. Winged forms were collected by Kukalová-Peck & Peck (1993) using large-area flight intercept traps (window traps) and by Villamizar & González-Montana (2018) with Malaise traps. We also collected twice (two and one specimens) using Townes' model, and once with sticky traps, respectively. These methods yield few alate specimens. We never saw any specimens attracted to the light trap.

Keeping specimens alive. Most specimens, nymphs, and adults can be collected alive using aspirators. After finding the colony, cut two small pieces of bark from where the specimens live and place the small pieces of bark inside a transparent Ziplock plastic bag. Arrange the bark pieces with the inner side facing each other. As soon as they are collected, put the specimens inside the plastic bag. Soon, the specimens entered between the pieces of bark. Seal the bag to maintain the proper humidity level of the bark. We usually collect organic materials from the trunk where they were found. In this bag, the specimens can stay alive for a long time. Protect the bags inside a polystyrene box from light and heat. The nymphs and adults can be carried by hand to the laboratory. In the laboratory, the colony samples must be checked regularly to collect adults as soon as they emerge and before the wings of alates dehisce (Rafael et al. 2008).

Laboratory colony maintenance. As soon as the colonies arrive at the laboratory, each sample must be checked, and the adults must be monitored to determine whether to continue the colony or removed and fixed in a labeled vial for taxonomic purposes. Older nymphs quickly reach adulthood. Growing colonies are the best way to obtain eggs, young nymphs (juveniles), and winged forms with wings still attached to their bodies. The time for each instar can be annotated. It is possible to separate the nymphs that will transform into winged specimens by the presence of ocelli and the wing bottoms. After detecting a juvenile with compound eyes, check the colony daily to have the winged specimen as soon as possible after the ecdysis. Soon after the ecdysis, the specimen is somewhat unsclerotized and whitish; 24 hours later, it is sclerotized and darkened, with the two pairs of wings fully extended. The wings drop soon, and the dealate or other specimen eats the detached wings. For further investigation, the specimens can be fixed in absolute ethanol or any other convenient fixative at different ages. We have already had a colony culture of *B. weidneri* in the laboratory for over a year. They do not need to be fed. The pieces of bark contain enough food for the development of the nymphs.

Rapid identification of live specimens for pure colonies. Adults and juveniles of two or more species can be collected during field collection. Each sample must be checked in the laboratory to ensure the growth of pure colonies. Nymphs are challenging to identify. Adults can be identified alive in the laboratory to achieve different goals as a pure colony. Select the sample from inside the transparent plastic bag and place each bark sample in a larger dry Petri dish. Put just a few drops of water in a smaller Petri dish. From the larger Petri dish, set apart each piece of bark to check for adult specimens (the specimens run to small holes, to the underside of the bark, or to the bottom of the plate). Then, using a soft paintbrush, merge it in water and pick up one specimen at a time, passing the moistened brush quickly and laterally over each specimen. Quickly transfer the specimen to the smaller Petri dish over the water drop. Repeat the procedure with other adults in the sample. The specimens can remain in place for a while, with minimal movement over the water, and then they can be identified under a microscope. If needed, each specimen can be positioned to observe ventral characteristics using the soft paintbrush. After identifying and using the same paintbrush, put the specimens of each species in the bark substrate to continue with them alive. This procedure is suitable for combining sexes of the same species.

Apterous x alate/dealate ratio. Apterous and dealate forms are predominant in the field. The two more common species collected in the Brazilian Amazon Basin are *B. huxleyi* and *B. weidneri* (Tab. 1). For both species, the proportion of apterous forms is 70% and 80%, respectively. The 20% of the winged form is usually found without wings (dealate). Only 2% of the winged forms were found with wings in the field.

Sex ratio. Riegel & Eytalis (1974) and Choe (1992) reported an even sex ratio in four Neotropical species: *Latinozoros barberi* (Gurney, 1938), *Usazoros hubbardi* (Caudell, 1918), *Centrozoros gurneyi* (Choe, 1989), and *Centrozoros neotropicus* (Silvestri, 1916). We obtained the same proportion based on more than 3,000 specimens in both species mentioned above. The sex ratio for both taxa, as field-collected, was close to 1:1, with a slight advantage for *males of B. weidneri* (Tab. 1). Laboratory conditions yielded the same proportion.

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Authors' Contributions

JAR: Conceptualization, Investigation, Methodology, Writing – original draft; Writing – review & editing, Supervision, Validation; FFXF: Investigation, Writing – original draft, Writing – review & editing; JFV (*in memorian*): Methodology.

Conflict of Interest Statement

The authors declare no competing interests.

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