





## Protocol & Techniques

# Method for rearing *Cladomorphus phyllinus* Gray, 1835 (Phasmatodea: Phasmatidae) under laboratory conditions

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**Abstract.** *Cladomorphus phyllinus* Gray, 1835 (Phasmatodea: Phasmatidae) is a species of stick bug native to Brazil and considered one of the largest in the southern hemisphere. The rearing method described uses rectangular cages for the maintenance of nymph and adult insects, with feeding carried out with guava leaves. This method promotes the multiplication of insects, which have been used for educational purposes.

**Keywords:** Stick bug, rearing method, guava.

The insects of the order Phasmatodea stand out for their camouflage, they are morphologically similar to sticks, branches, leaves and hence are popularly known as stick bug or leaf bug (Bedford 1978). These insects are mostly phytophagous and have a wide geographic distribution with the exception of polar regions, with the largest number of species being found in tropical and subtropical regions (Bedford 1978; Aliaga 2015).

These insects are poorly studied (Bradley & Galil 1977; Zompro 2001; Chiquetto-Machado & Albertoni 2017), and most of the studies involve taxonomic approaches and species description (Costa Lima 1938; Toledo Piza 1944; Zompro & Domenico 2005; Hennemann et al. 2016; Kumagai & Fonseca 2009; Heleodoro et al. 2017), others assess biological and ecological aspects (Zapata & Torres 1970; Dorval et al. 2003; Sottoriva et al. 2008; Alvarenga et al. 2018). In addition we have some researches on the digestive system (Azevedo et al. 2013; Monteiro et al. 2014).

*Cladomorphus phyllinus* Gray, 1835 (Phasmatodea: Phasmatidae) is native to Brazil and considered one of the largest species in length in the southern hemisphere, with females reaching up to 22 cm (Brock 1992). Males are shorter, reaching up to 15 cm in length and are winged, differing from females that are apterous (Fig. 1) (Costa Lima 1938; Kumagai & Fonseca 2009). This phasmid feeds on leaves of several fruit trees (Brock 1992), however it prefers plants of the Myrtaceae family (Sottoriva et al. 2008). The incubation period for *C. phyllinus* is over 100 days, while nymph development can take up to 180 days. In adulthood, males live around 127 days and females 208 days, with a fecundity of 390 eggs (Monteiro et al. 2014).

The method for rearing *C. phyllinus* started for educational purposes, in the Laboratory of Biology and Insect Rearing (LBIR), Department of Agricultural Production Sciences (Crop Protection), São Paulo State University (Unesp), Jaboticabal, São Paulo. However according to Cohen (2015) and De Bortoli et al. (2017) an adequate rearing method facilitates the multiplication of insects and studies in several areas. The rearing started with nymphs obtained from the Department of Entomology and Acarology, of the “Luiz de Queiroz” College of Agriculture (ESALQ), Piracicaba, São Paulo. The development of all phases occurs under laboratory-controlled conditions at  $25 \pm 1$  °C,  $70 \pm 10\%$  RH and 12:12 h (light:dark) photoperiod.

The phasmid is kept in rectangular glass cages (40.0 cm length  $\times$  30.0 cm wide  $\times$  31.0 cm height) (Fig. 2) which has one side open and are

closed with voile fabric, fastened with elastic or paper clip, allowing for a better aeration. About 40 insects are kept in this cage at the beginning of the nymphal phase, decreasing during development and reaching 10 insects in the adult phase. The cages are lined with brown paper to facilitate the removal of excrement and the collection of eggs.

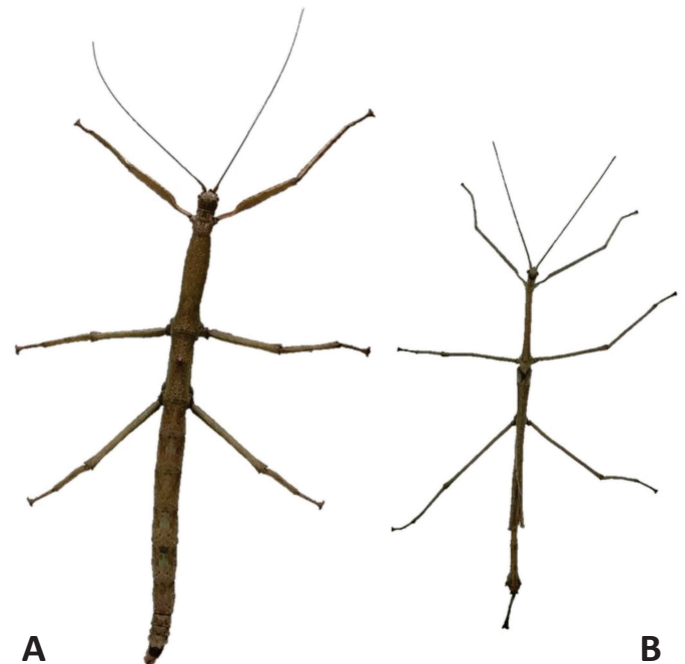
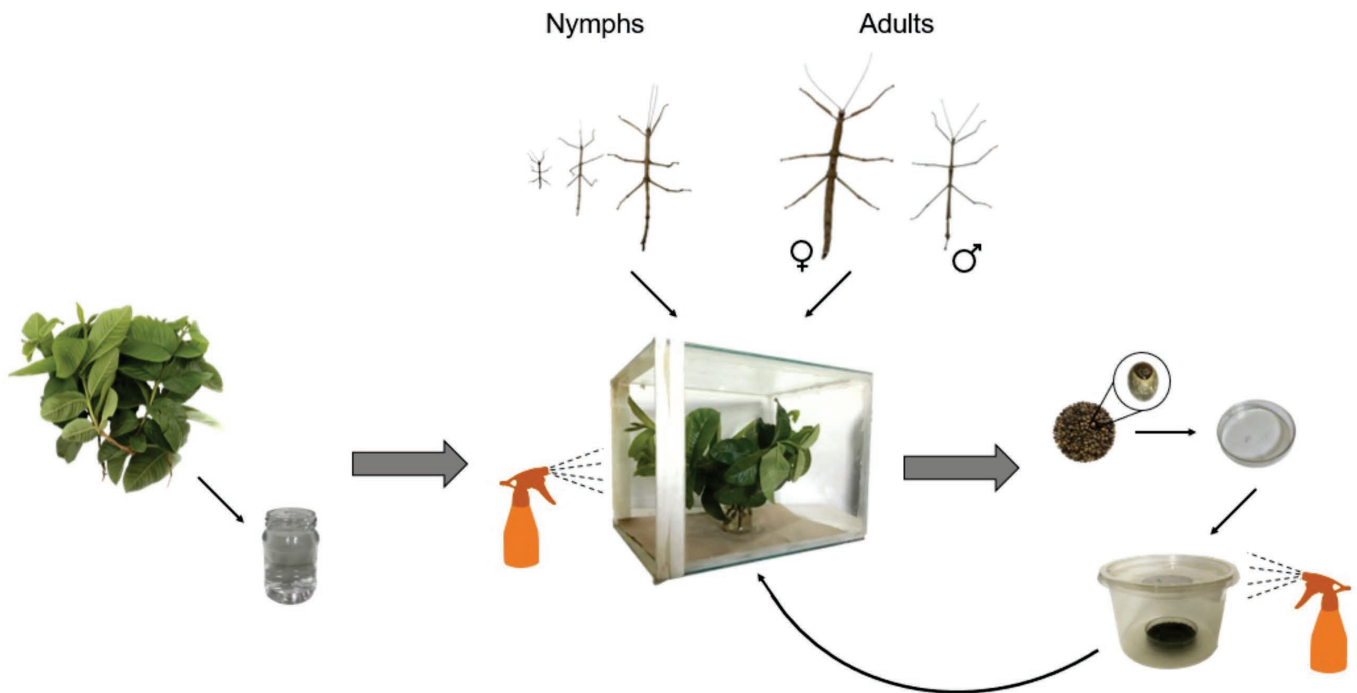


Figure 1. *Cladomorphus phyllinus* adults. A) Female; B) Male.

*C. phyllinus* are fed with guava leaves, a plant of the Myrtaceae family. The branches with leaves are collected, washed with water and placed in a 200 mL container, filled with water, placed in the center of the rearing cage. The water in the container is topped up after 3-4 days in order to maintain the leaves turgid for a longer period of time, while the leaves are replaced after 7 days or when they begin to dry. When this time period is completed, the eggs are collected and the cages are cleaned up (Fig. 2).

Eggs are collected with tweezers and transferred to Petri dishes left without lids (6.0 cm diameter  $\times$  2.0 cm height), which are arranged in



**Figure 2.** Scheme of the method for rearing *Cladomorphus phyllinus* under laboratory conditions.

transparent plastic containers (15.0 diameter × 9.0 cm height) (Fig. 2). The top of the container has a hole (5.0 cm diameter) covered with voile fabric for better internal aeration. After the first nymph hatch, the Petri dish containing the eggs is transferred to the rearing cages. Every 2 days, water is sprayed both in the cages and in containers with eggs, for maintaining humidity and also providing water to insects (Fig. 2).

The order Phasmatodea comprises, for the most part, nocturnal insects that stay in shaded trees (Bedford 1978). In this sense, a black fabric is used to cover the cage top to simulate the common habitat and improve the insect development (Fig. 3). Alternatives to the rearing glass cage are ones crafted with acrylic or wood. However it is important that at least one wall is substituted by voile fabric to allow a proper aeration. This method has been carried out for more than 2 years, allowing the multiplication of stick bugs that are used in events of pedagogical purpose, where children and teenagers have the opportunity to observe and touch these insects.



**Figure 3.** Rearing cage of *Cladomorphus phyllinus* covered with black fabric.

## Authors' Contributions

CCT conducted the rearing and wrote the manuscript; NFV, JMS and BF conducted the rearing; SAB revised the manuscript.

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