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Longevity, fertility, and average eggs viability of parthenogenetic females of *Cladomorphus phyllinus* Gray (Phasmatodea - Phasmatidae)

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Abstract. The order Phasmatodea includes insects known as stick insects. In Brazil, few taxonomic, ecological or evolutionary studies have been published in recent years, the reason is related to the few number of researchers dedicated to this particular group. *Cladomorphus phyllinus* Gray is one of the largest Brazilian insects and perhaps one of the most studied species of Phasmatodea in the country. It is considered as a phytophagous, generalist, and feeds mainly on guava leaves (*Psidium guajava*), powder-puff (*Calliandra sp*) or Angico (*Piptania* sp.). Females of this species reproduce in a sexual and asexual manner, by the production of diploid daughters from unfertilized eggs (thelytokous parthenogenesis). The absence of records on the reproductive capacity of virgin parthenogenetic females of *C. phyllinus* led us to record the longevity, fertility, and eggs viability of ten specimens. The results obtained were compared to those found in the scientific literature for mated females of the same species. The fertility and eggs viability were observed to be much lower for the parthenogenetic females when compared to the mated females; however, the longevity for virgin females was longer than that recorded in the literature for mated females.

Keywords: Bionomy; Phasmida; Stick animals; stick insect.

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Stick insects, from the order Phasmatodea, are the largest Brazilian insects, which present multiple forms of morphological and behavioral camouflage like sticks and leaves, including 3,423 species, of which 852 occur in the neotropical region, with 224 being recorded in Brazil (Brock *et al*. 2021; Crispino *et al*. 2021). Due to the few Brazilian specialists in Phasmatodea, studies related to taxonomy, biology, ecology, and evolution have grown and new initiatives have emerged to contribute to the knowledge of this order and its state of the art (Costa *et al*. 2019; Chiquetto-Machado *et al*. 2020; Heleodoro & Rafael 2020, 2021; Madeira-Ott *et al.* 2020; Chiquetto-Machado & Cancello 2021; Ghirotto 2021; Heleodoro *et al.* 2021).

In this group, which includes insects that have been poorly studied, the information available to researchers and the general population is also restricted. As result of the lack of knowledge, specimens of stick insects are often eliminated when found near residences. Nevertheless, scientific dissemination initiatives have been carried out to inform that phasmatodeans are harmless and, therefore, must be protected, respected and, if possible, reintroduced into the environment when eventually found inside or around the houses (Gomes *et al*. 2010). In this line of scientific dissemination, Torres & Costa (2019, 2020) published educational books presenting the insects as fun characters to bring young audiences closer to biodiversity. Another channel of information about this group to the general population is the "*Ciência Cidadã* from the "*Projeto Phasma*" (Citizen Science of the Phasma Project), which can be accessed on social networks, with various information and photographic records of the different Brazilian species. This project, which has been launched over a year, has even resulted in the publication of some studies (see the results of the project here: https://www.facebook.com/projetophasma).

In Brazil, published records for stick insects are mainly distributed in the North (Pará, Amazonas), Northeast (Ceará, Bahia, Pernambuco, Rio Grande do Norte), Southeast (Espírito Santo, Minas Gerais, São Paulo, Rio de Janeiro) and South (Rio Grande do Sul, Paraná) (Heleodoro & Rafael 2021). They are considered animals of low biotic potential and, to date, few records in the literature categorize Phasmatodeans as a Brazilian agricultural pests or as animals of economic importance. However, some species have been considered harmful to agriculture. Baker (2015) provides a summary of the situation of damage caused by Phasmas in some parts of the world.

Cladomorphus phyllinus (Phasmatidae: Cladomorphinae: Cladomorphini) is one of the most common and known stick insect species in Brazil, since it is often found in scientific collections, museums, schools, and universities for educational purposes. The studied species feeds mainly on guava leaves (*Psidium guajava*), Angico (*Piptania* sp.) and powder-puff (*Calliandra* sp.), has nocturnal habits, and reproduces sexually and

asexually by thelytokous parthenogenesis (Costa Lima 1938). In the literature, some studies on *C. phyllinus* mainly address aspects of its biology, such as the work of Alvarenga *et al*. (2018) that analysed bred mated females fed on guava leaves (*P. guajava*), aiming to survey the biological cycle, number of eggs and their average viability, while also comparing their results with those of Dorval *et al*. (2003). The latter authors had the same objective and observed mated females fed on leaves of Angico (*Piptania* sp.).

Basic knowledge about this species has been gradually increasing. The morphology of *C. phyllinus* is relatively well known (Costa Lima 1938; Kumagai & Fonseca 2009) including the review of the tribe by Hennemann *et al*. (2016). Adult individuals in this species show marked sexual dimorphism, and the winged males, significantly smaller in size than females, can reach up to 13 cm, while the females can reach 23 cm in length and are apterous (Costa Lima 1938).

Recently, predation on *C. phyllinus* was registered where individuals were found to be sucked by the bug *Supputius cincticeps* Stäl (Heteroptera: Pentatomidae) which is known for its eclectic feeding pattern, requiring both insect haemolymph and vegetable juices to complete its biological cycle. This record also served to elaborate a hypothesis about the evolution of camouflage by means of a possible horizontal transfer of genes (Costa *et al*. 2019).

Due to the lack of studies on the reproductive potential of virgin parthenogenetic females of *C. phyllinus*, a study was conducted on its bionomic aspects including longevity, fertility, and egg viability. The data obtained were compared to those in the literature for mated females of the same species.

MATERIALS & METHODS

Four females and three males collected in green areas of the city of Petrópolis\Rio de Janeiro, Brazil, were kept in a cage with a wooden structure and base (80x60x50 cm) and nylon screen on all sides. The specimens were identified as *Cladomorphus phyllinus* according to the criteria proposed by Otte & Brock (2005) and Zompro (2012).

The mated females laid eggs that originated nymphs. From the developed specimens, 10 preimaginal nymphs were randomly selected. The virgin females were separated and kept, individually, in plastic containers (45x30x30 cm) which were drilled on all sides and at the lid to allow for ventilation. The females were bred under uncontrolled environmental conditions, with temperatures ranging from 12° to 33° C, with an average of 25° C, and relative humidity of 50% to 89%, with an average of 78%. The containers were placed in a well-ventilated area covered by a roof and without walls. The containers did not receive direct sunlight but were exposed to natural light cycles. All specimens were fed with guava leaves (*P. guajava*) and sprayed with water weekly.

During the longevity observation period and fertility of the females, the eggs were collected weekly from each of the containers, transferred to a common area placed in another container that was in the same covered area and under the same climatic and environmental conditions previously described. The total number of eggs laid was quantified and the average fertility calculated. Hatched eggs were also quantified, and the mean viability calculated after one year of observation following the last recorded laying. The nymphs that emerged were transferred to another container and then stored in 70% alcohol.

All the specimens observed were deposited in the entomological collection of the Oswaldo Cruz Institute, Manguinhos-RJ, in section J. Costa & Lima Neiva, according to the species registration table below. The females were assembled according to appropriate techniques and deposited in entomological drawers. The eggs and nymphs were grouped into two glass flasks containing 70% alcohol and were also deposited in the same collection (Costa *et al.* 2008; Cerri *et al.* 2014).

RESULTS

The virgin females of *C. phyllinus* presented a longevity with a minimum period of 181 and maximum 306 days with an average of 255.3 (Table 1).

All 10 parthenogenetic females were found to laid eggs. The total number of eggs laid was 2,524 with an average of 252.4 eggs per female. A total of 70 viable eggs were registered, with an average of seven eggs per female, representing 2.77% of hatched eggs.

DISCUSSION

The following research provides new evidence of the life history of the Brazilian native *C. phyllinus*. Still, it presents many questions related to its ecology and biology that need to be clarified. In this study, the longevity, fertility, and eggs viability of parthenogenetic females were recorded for the first time.

Data obtained by Alvarenga *et al*. (2018) showed that the longevity of mated females had a survival average of 163.3 days at 27º C and 50% RH for 12 hours of photophase and laid an average of 392.3 eggs with a 22.25% of viability. In comparison to our results, the average longevity of virgin females of *C. phyllinus* was 255.3 days with a minimum period of 181 and maximum 306 days.

Table 1. Registration number at CEIOC and longevity data of the parthenogenetic virgin females of *Cladomorphus phyllinus*. The observed specimens were kept under uncontrolled environmental conditions, with temperatures ranging from 12° to 33° C, with an average of 25° C, and relative humidity of 50% to 89%, with an average of 78%, and were fed with guava leaves (*Psidium guajava*)

Specimen (registration number)	Date of imaginal moulting	Date of death	Longevity (days)
CEIOC 46342	02/xi/2017	02/v/2018	181
CEIOC 72328	10/xi/2017	20/v/2018	191
CEIOC 46380	10/xi/2017	03/vii/2018	235
CEIOC 44527	10/xi/2017	03/ix/2018	297
CEIOC 46381	10/xi/2017	11/ix/2018	305
CEIOC 28607	12/xi/2017	26/vii/2018	256
CEIOC 57851	15/xi/2017	10/viii/2018	268
CEIOC 57852	18/xi/2017	20/ ix/2018	306
CEIOC 57853	20/xi/2017	16/vii/2018	238
CEIOC 57854	23/xi/2017	26/viii/2018	276

Dorval *et al.* (2003), which studied mated females fed on Angico (*Piptania* sp.) under the same climatic conditions as Alvarenga *et al*. (2018), found an average longevity of adult females of 159.25 days; the average of eggs laid per female was 369.3 and the average viability was 57.14 eggs per female (15.47%)

Our results obtained on virgin parthenogenetic females complement data from the literature, adding a record on the reproductive potential of thelytokous parthenogenesis present in *C. phyllinus*. These data suggest that in the absence of males, despite the low percentage of hatched parthenogenic eggs, this process still represents a viable strategy for the survival of the species.

Parthenogenesis and sexual reproduction have long been studied in different species of the stick insects (Schwander & Crespi 2009; Schwander *et al*. 2010; Morgan-Richards *et al*. 2019). It is known that the maintenance conditions (feeding, temperature, humidity, population density, among other variables) may lead to noticeable influences in the bionomy of the insects resulting in significant biological differences for the same species. The results obtained in this study suggest that virgin females laid fewer eggs than the coupled ones (Dorval *et al.* 2003; Alvarenga *et al.* 2018); however, they had a longer longevity than the mated females recorded by those authors, with the average being of about five months, while in this study the average was approximately of eight months of longevity for the parthenogenetic females. These results are in accordance with Maynard Smith (1958) who presented the first experiment demonstrating the "costs of reproduction" showing the existence of a trade-off between reproduction and survival. It is broadly demonstrated that reproduction negatively affects the lifespan in a wide range of organisms, as for example, in *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) which was detailed analysed and discussed in the review elaborated by Flatt (2011). Interestingly, Burke *et al.* (2015), studying the Australian species of stick insect, *Extatosoma tiaratum* (Macleay) (Phasmatodea: Phasmatidae), observed that an earlier hatching and higher offspring viability are positively correlated with the sexual reproduction, nevertheless the occurrence of facultative parthenogenesis may be explained by the sexual reproduction cost to females. In addition to that, the authors call attention that parthenogenetic females frequently refuse mating, and that switching the females from parthenogenetic to sexual reproduction increased female mortality and lowered egg production. Therefore, the reproductive strategy might have distinct ways for different species and organisms and much more work on the genetics and physiology is necessary to uncover on such trade-offs and their mechanistic basis (Flatt 2011). On the other hand, still in the Phasmatodea some species present populations known to be exclusively parthenogenetic (Morgan-Richards *et al.* 2010; Blackmon *et al.* 2017). The parthenogenetic reproduction is also the main strategy for several other organisms as for example, *Tityus serrulatus* Lutz & Mello (Arachnida: Buthidae) known as the yellow scorpion native in the Northeast of Brazil, with high egg hatching percentages (Lourenço 2008).

Our results also indicate the parthenogenesis as an effective survival strategy, however, the sexual reproductive form, comparing our results to the literature, is the main kind of reproduction for *C. phyllinus.* Observations from field captures and specimens from entomological collections (JC data not published), show that for *C. phyllinus* males are very common and available to keep sexual reproduction in nature and under captivity, at least in several areas of Rio de Janeiro State. The relevance of the asexual reproduction and possible transitions between sexual and asexual reproduction in natural environment are still to be clarified. More detailed studies are needed to better understand and evaluate the importance of the asexual reproduction in the *C. phyllinus* species assessing distinct natural populations.

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