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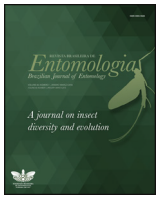

Bionomic notes on parthenogenetic females and a record of parasitism by *Forcipomyia* Meigen (Diptera: Ceratopogonidae) in the stick insect *Cladoxerus cryphaleus* (Westwood) (Phasmatodea: Phasmatidae)

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ABSTRACT

Stick insects (Phasmatodea) are among the least studied insect groups in Brazil. Although in recent years there has been an increase in the number of published studies on taxonomy and morphology of Brazilian stick insects, they remain very little-known concerning biological and bionomic aspects. From five females of *Cladoxerus cryphaleus* (Phasmatidae: Cladomorphinae) collected in the Iguaçu National Park, Paraná, Brazil, a captive colony of parthenogenetic females was started, based on which data on longevity, fertility, and viability of eggs of this species were recorded for the first time. After imaginal molting, the 21 females studied had an average longevity of 103.7 days. Each female produced on average 125.3 eggs, which had a hatching rate of 34.2%. In addition, we recorded a *C. cryphaleus* male nymph being parasitized by a biting midge of the subgenus *Forcipomyia* (*Microhelea*) (Diptera: Ceratopogonidae). The male, in pre-imaginal instar and already parasitized, was collected in the municipality of Petrópolis, Rio de Janeiro.

Stick and leaf insects (Phasmatodea) are medium- to large-sized phytophagous insects. Most representatives of the group are nocturnal, remaining motionless during the day, camouflaged as twigs, leaves or other plant parts (Bradler and Buckley, 2018). Besides the cryptic appearance, they also exhibit behavioral adaptations for camouflage, such as swaying the body from side to side when blown by wind or while walking (Bedford, 1978; Bian et al., 2016). Many phasmids are also capable of secreting an irritating substance from a pair of prothoracic glands, a defense mechanism which in a few species is combined with aposematic coloration (Dossey, 2010; Bradler and Buckley, 2018).

Phasmid females employ different oviposition strategies, which were studied from an evolutionary perspective by Robertson et al. (2018). According to their results, the widespread strategy of dropping or flicking the eggs to the ground represents the ancestral oviposition technique for the group, whereas the also relatively common strategies of gluing the eggs to the substrate and inserting them into the soil or crevices have arisen multiple times throughout the evolution of Phasmatodea. Egg production varies considerably among species, from less than 100 to more than 1,000 eggs laid during the life of a female (Bedford, 1978). In many species the females are able to reproduce parthenogenetically, what may be related to the low dispersal ability of most phasmids (Bradler and Buckley, 2018).

Phasmatodea comprises approximately 3,350 valid species, of which around 230 are recorded for Brazil (data compiled from Brock et al., 2020). This number, however, represents less than half of the estimated diversity of Brazilian stick insects (Zompro, 2012), which is a result of the historical scarcity of researchers investigating the group in the country. Throughout the 20th century, Dr. Salvador de Toledo Piza was the only researcher in Brazil who devoted some attention to the study of these insects, having described a total of 46 Brazilian species of stick insects (see e.g. Toledo Piza, 1936, 1938, 1944).

In the 21st century, and especially in the last five years, the study of Phasmatodea has been intensified in Brazil with the publication of works addressing mainly taxonomic and morphological aspects (see e.g. Chiquetto-Machado and Albertoni, 2017; Chiquetto-Machado, 2018; Heleodoro and Rafael, 2019, 2020; Crispino et al., 2020). Madeira-Ott et al. (2020) detailed the state of knowledge about Brazilian stick insects, synthesizing the main researches that have been carried out on the group in the country and drawing attention to aspects on

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which there is still very little study, such as the biology and bionomy of Brazilian species.

The genus *Cladoxerus* Le Peletier de Saint Fargeau & Serville (Phasmatidae: Cladomorphinae) is composed of large and slender stick insects with accentuated sexual dimorphism. It includes 13 valid species, nine of which are recorded for Brazil (Brock et al., 2020). As in many Brazilian genera of Phasmatodea, bionomic aspects of *Cladoxerus* are little known, and the information available in the literature is mostly restricted to the original descriptions of the species. From recently collected specimens of *Cladoxerus cryphaleus* (Westwood), we present here a study on longevity and fertility of this species, for which information on bionomy has never been published.

Stick insects collected in the Iguaçu National Park, Paraná (five females), and in the municipality of Petrópolis, Rio de Janeiro (one male), were identified by comparison with photos of the holotype of *C. cryphaleus* (available in Brock et al., 2020) and with specimens in the entomological collection of the Museum of Zoology of the University of São Paulo (MZUSP). The exact data of the collection events are: Brazil, Paraná, Iguaçu National Park, 25°22'24"S, 54°02'33"W, 7.VII.2017, O. Conle and F. Hennemann col.; Brazil, Rio de Janeiro, Petrópolis, 22°30'17"S, 43°10'56"W, 22.XII.2019, J. Costa col. The specimens were deposited at the Entomological Collection of the Oswaldo Cruz Institute (CEIOC), in the J Costa & Lima Neiva section (Costa et al., 2008; Cerri et al., 2014). The collections performed are in accordance with the license procedures of SISBio n 12123.

Eggs produced by the females from the Iguaçu National Park were kept for hatching, and the nymphs, all females, were reared until the pre-imaginal instar. At this stage, 21 nymphs were randomly selected and kept individually in open plastic boxes (45 x 30 x 30 cm) (Fig. 1A)

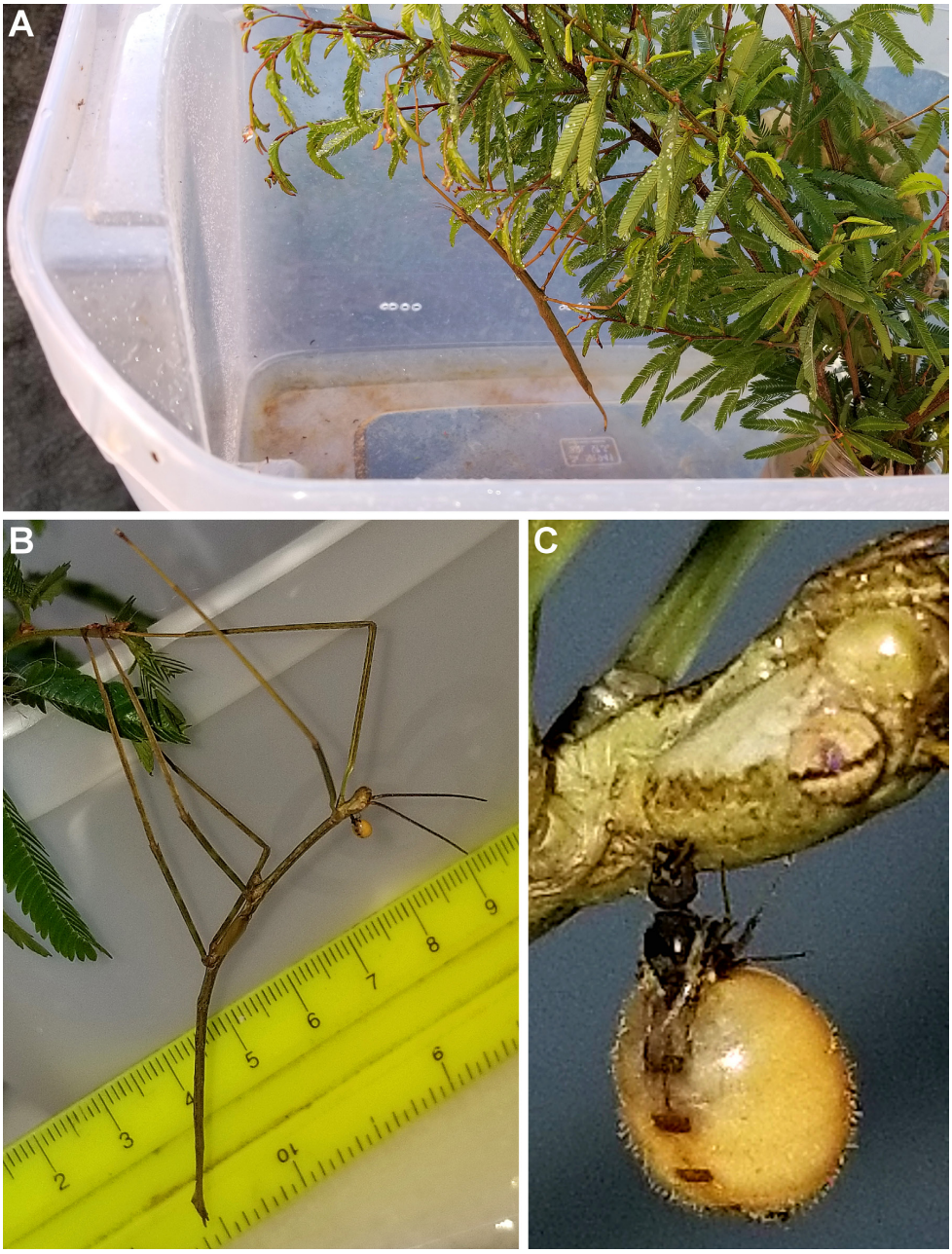

Figure 1 *Cladoxerus cryphaleus*. **A.** Adult female in a branch of *Calliandra* sp., inside a box, illustrating how the specimens were kept in captivity. **B–C.** Male in pre-imaginal instar parasitized by a specimen of *Forcipomyia* (*Microhelea*) sp. (Ceratopogonidae), whole body (**B**) and detail of the parasite attached to the head (**C**).

at temperature from 12°C to 33°C (mean = 25°C) and relative humidity from 60% to 80% (mean = 78%). Branches of *Calliandra* sp. (Fabaceae), popularly known as powder-puff, whose leaves the females fed on, were kept in the boxes. For each female, the date of the imaginal molt was recorded, and, later, the date of death as well. The eggs were collected weekly and kept in another box, with the same dimensions, to be counted and for the calculation of the hatching rate.

At the end of the study all females were placed in isopropyl alcohol and formaldehyde solution, and then pinned and left in a ventilated place for drying and preservation of the exoskeleton (Zompro, 2012). The specimens were then labeled, given registration numbers, stored in entomological drawers with mothballs and deposited at CEIOC, in the Jane Costa & Lima Neiva section, drawers 49 and 52 (Costa et al., 2008; Cerri et al., 2014).

The average longevity of the females of *C. cryphaleus* was 103.7 days after imaginal molting, with a minimum span of 47 days and a maximum of 142 days (Table 1). The eggs produced by the 21 females added up to a total of 2,631, with an average of 125.3 eggs per female. The total number of hatched eggs was 901, representing a hatching rate of 34.2%. The average number of hatched eggs per female was 43.

Although the specimens that started the colony studied here were collected in the Iguaçu National Park, during the present study the authors also recorded *C. cryphaleus* in an Atlantic Forest area in the municipality of Petrópolis, Rio de Janeiro. As the maintenance of the colony was performed in this same municipality, the results presented here were obtained under environmental conditions typical of the area of occurrence of the species, with a considerable thermal amplitude. However, aspects such as longevity and fertility may vary according to environmental conditions, so it is possible that populations of *C. cryphaleus* in other areas of occurrence of the species (e.g., further south in Brazil) present differences in relation to the data obtained by us.

This is the first work on bionomic aspects of *C. cryphaleus*. In fact, bionomy data were recorded in very few Brazilian Phasmatodea species, since most studies on the order conducted in the country focused on taxonomic aspects. Information on life cycle, longevity, and fertility was published only for *Tithonophasma tithonus* (Gray) (Lima et al., 2013) and *Cladomorphus phyllinus* Gray (Dorval et al., 2003; Sottoriva et al., 2007; Vargas et al., 2008; Alvarenga et al., 2018; Costa et al., 2019; Costa and Torres, 2020), the latter being the best known Brazilian species in biological and bionomic terms.

Table 1
Registration number at CEIOC and longevity data of the parthenogenetic virgin females of *Cladoxerus cryphaleus*.

Specimen (registration number)	Date of imaginal molting	Date of death	Longevity after imaginal molting (days)
58997	25/IX/2018	18/I/2019	116
58998	25/IX/2018	10/XI/2018	47
58999	25/IX/2018	10/XI/2018	47
60748	25/IX/2018	23/I/2019	121
60749	25/IX/2018	23/I/2019	121
64789	25/IX/2018	22/I/2019	120
64790	25/IX/2018	24/I/2019	122
64791	05/X/2018	30/I/2019	118
64792	02/XI/2018	15/II/2019	106
64793	24/X/2018	12/II/2019	112
64794	30/X/2018	15/II/2019	109
64795	10/XI/2018	18/II/2019	101
64796	05/XI/2018	18/II/2019	105
65114	10/XII/2018	06/II/2019	58
65115	22/XI/2018	23/II/2019	94
65116	25/XII/2018	23/II/2019	92
72320	27/X/2018	03/III/2019	128
72321	30/X/2018	01/III/2019	123
72322	23/XI/2018	28/II/2019	98
72323	29/XI/2018	06/III/2019	98
72324	10/XI/2018	01/IV/2019	142

The reproductive potential of *C. cryphaleus* was recorded here based on parthenogenetic females. It is possible that the fecundity of the species is higher considering mated females, as observed for *Tithonophasma tithonus* and *Cladomorphus phyllinus*, which had higher egg hatching rates for mated females than for parthenogenetic ones (Lima et al., 2013; Costa and Torres, 2020). Burke et al. (2015), in a study of the Australian spiny leaf insect, *Extatosoma tiaratum* (Macleay) (Phasmatidae), suggested that, although sexual reproduction apparently leads to earlier hatching of the eggs and higher offspring viability, the occurrence of facultative parthenogenesis in this and other species could be explained by the costs of sexual reproduction to females. Accordingly, the authors observed that parthenogenetic females tended to avoid mating, and that switching the females from parthenogenetic to sexual reproduction resulted in increased female mortality and lower egg production.

The specimen of *C. cryphaleus* collected in Petrópolis was a male nymph, in pre-imaginal instar, which was parasitized by a biting midge, attached to the posterior region of its head (Figs. 1B and 1C). The midge, which was preserved in 70% alcohol and deposited at CEIOC, was identified as belonging to the subgenus *Forcipomyia* (*Microhelea*) Kieffer (Diptera: Ceratopogonidae: Forcipomyiinae), which has more than 100 described species, 14 of which are recorded for Brazil (Falaschi et al., 2014). A species of this subgenus, *F.* (*M.*) *paulista* Falaschi, Albertoni & Fusari, was described based on two specimens found parasitizing the stick insect *Prexaspes paulense* (Rehn) (Pseudophasmatidae: Xerosomatinae), in an Atlantic Forest region in the Serra do Mar of the State of São Paulo, Brazil (Falaschi et al., 2014). A more detailed morphological study of the specimen collected by us may reveal whether it belongs to *F.* (*M.*) *paulista* or even to a species not yet described of *Forcipomyia* (*Microhelea*).

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Conflicts of interest

The authors declare no conflicts of interest.

Author contribution statement

JC and PICM conceived the study; JC and LT carried out the experiment; PICM, JC and LT wrote the text and revised the final version of the manuscript.

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