Research Article

Predation strategies of *Harpactor angulosus* (Lepeletier & Serville, 1825) (Hemiptera: Reduviidae) on *Cladomorphus phyllinus* Gray, 1835 (Phasmatodea: Phasmatidae) in captivity

Estrategias de depredación de *Harpactor angulosus* (Lepeletier y Serville, 1825) (Hemiptera: Reduviidae) sobre *Cladomorphus phyllinus* Gray, 1835 (Phasmatodea: Phasmatidae) en cautiverio

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Abstract. Based on the observation of specimens kept in captivity for 69 days, here we describe in detail for the first time the predatory behavior of an assassin bug *Harpactor angulosus* (Lepeletier & Serville, 1825) (Hemiptera: Heteroptera: Reduviidae: Harpactorinae), on a stick insect *Cladomorphus phyllinus* Gray, 1835 (Phasmatodea: Phasmatidae: Cladomorphinae). The behavior of the generalist predator *H. angulosus* as well as the evasive reactions of *C. phyllinus* were detailedly described and illustrated. Because both species occur in the Atlantic Forest biome and were recorded in the same geographic coordinates, in an urbanized green area in the city of Petrópolis, Rio de Janeiro State, Brazil, the ecological relationship here described is also plausible of occurring in nature and opens new venues to be explored. The unprecedented phytophagy of *H. angulosus* was also recorded.

Key words: Ecological relationships; Harpactorini; predatory behavior; prey behavior.

Resumen. A partir de la observación de ejemplares mantenidos en cautiverio durante 69 días, se describe por primera vez en detalle el comportamiento de depredación de la chinche asesina *Harpactor angulosus* (Lepeletier y Serville, 1825) (Hemiptera: Heteroptera: Reduviidae: Harpactorinae), sobre el fásmido *Cladomorphus phyllinus* Gray, 1835 (Phasmatodea: Phasmatidae: Cladomorphinae). El comportamiento del depredador generalista *H. angulosus*, así como las reacciones evasivas de *C. phyllinus* fueron detalladamente descritas e ilustradas. Debido a que ambas especies habitan en el bioma del Bosque Atlántico y fueron registradas en las mismas coordenadas geográficas, en un área verde urbanizada en la ciudad de Petrópolis, Estado de Río de Janeiro, Brasil, la relación ecológica aquí descrita también es plausible que ocurra en la naturaleza y abre nuevos escenarios por explorar. También se registró la fitofagia de *H. angulosus* sin precedentes.

Palabras clave: Comportamiento depredador; comportamiento de presa; Harpactorini; relaciones ecológicas.

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Introduction

Reduviidae (Hemiptera: Heteroptera), whose representatives are known as assassin bugs, is the largest family of predaceous terrestrial Heteroptera. Almost all predatory reduviids are polyphagous, whereas some species are commonly associated with plant crops, and have been studied as biological control agents for crop pests (Gil-Santana *et al.* 2015). Although reduviids are primarily predators, phytophagy by some species, most of which belonging to the tribe Harpactorini (Reduviidae: Harpactorinae), has been recorded (Gil-Santana and Keller 2022). Harpactorini is the most diverse group within the family, with about 53 genera in the Neotropics (Gil-Santana 2022). The latter author and Gil-Santana *et al*. (2015) summarized the publications containing descriptions and revisions of Neotropical Harpactorini.

Harpactor Laporte, 1832 currently includes four species, which can be identified by consulting Wygodzinsky (1947). *Harpactor angulosus* (Lepeletier & Serville, 1825) is a somewhat common species in remnants of Brazilian Atlantic Forest, mainly in states of Southeast and South regions of Brazil (third author, HRG-S, pers. obs.). Pereira *et al.* (2009) recorded *H. angulosus* as a predator of caterpillars of *Hylesia* spp. (Lepidoptera: Saturniidae: Hemileucinae), moths that may cause agricultural damages and dermatological lesions by contact with both the caterpillars and scales of the female adults (Specht *et al*. 2008). Pereira *et al.* (2009) also recorded details of the biology of *H. angulosus*, including the generalist predatory habit of the species. They stated that the potential of the species as a natural enemy in biological control of defoliating caterpillars should be further evaluated. Additionally, Gil-Santana and Keller (2022) recorded oviposition by females and the predation of caterpillars on leaves of *Oxypetalum erianthum* Decne. (Apocynaceae) by *H. angulosus* in Argentina.

The order Phasmatodea is composed of phytophagous, predominantly nocturnal insects, which are popularly known as stick or leaf insects due to their remarkable morphological and behavioral adaptations for camouflage (Bradler and Buckley 2018). It is one of the least explored insect orders in Brazil and currently encompasses approximately 230 species described in the country (data compiled from Brock *et al.* 2022). This number of species is expected to be underestimated, representing less than half of the diversity of the Brazilian Phasmatodea (Zompro 2012) as a consequence of a small number of researchers studying stick insects in Brazil. The state of art of this group in the country was recently reviewed by Madeira-Ott *et al.* (2020) and new initiatives have been raising efforts in order to broaden the knowledge of the Brazilian Phasmatodea, especially regarding taxonomy, morphology and biology (Chiquetto-Machado 2018; Heleodoro and Rafael 2019, 2020; Chiquetto-Machado *et al*. 2020, 2022; Crispino *et al.* 2020; Chiquetto-Machado and Cancello 2021; Ghirotto 2021; Heleodoro 2022).

Cladomorphus phyllinus Gray, 1835 (Phasmatidae: Cladomorphinae) is a large Brazilian stick insect with pronounced sexual dimorphism that is commonly raised in captivity for educational purposes. This species is recorded in the Brazilian Atlantic Forest and also other areas in Brazil and Argentina (Heleodoro and Rafael 2021; Brock *et al*. 2022). However, records from Argentina and the Brazilian north and northeast regions may not truly correspond to *C. phyllinus* but to different species which are currently regarded as synonyms of *C. phyllinus* (second author, PICM, pers. obs.). Its life cycle was studied, including several bionomical characteristics which were analyzed under laboratory conditions (Dorval *et al.* 2003; Alvarenga *et al.* 2018). Like in many species of stick insects, *C. phyllinus* females are able to reproduce parthenogenetically. Torres *et al*. (2022) found that the hatching rate of eggs laid by parthenogenetic females was much lower than the egg hatching rate of females that had previously copulated. However, the parthenogenetic females lived approximately three months longer than the mated ones, with an average longevity of 255.3 days and maximum of 306 days. Although the biology of *C. phyllinus* is

reasonably well-known, diverse aspects of its morphology, ecology and behavior are still to be recorded.

The prey/predator behavior in insects is markedly studied and involves several mechanisms and behavioral patterns in both sides (Virant-Doberlet *et al.* 2019). Specifically, the predation of stick insects by heteropterans is not commonly reported in the literature, but some records were made by Berger and Wirth (2004) and Costa *et al.* (2019), and a few others were compiled by Liu (2021). Here we present a detailed report of the predator assassin bug *Harpactor angulosus* and its predation strategies on *Cladomorphus phyllinus.*

Material and Methods

The predatory behavior here reported was recorded based on three specimens of *H. angulosus* (one third, one fourth and one fifth instar nymph) captured in a green and urbanized area near the center of Petrópolis city, in the State of Rio de Janeiro, Brazil (22°30'18" S; 43°10'44" W). The specimens were found near each other in a green spot of about 30 cm^2 when they were manually collected by the first author (JC). The assassin bugs were initially identified according to previous observations of the third author (HRG-S) and later, when the specimens reached the adulthood, they had the identification confirmed as *H. angulosus* by consulting Wygodzinsky (1947). After the initial identification, the three *H. angulosus* specimens were placed in the same cage as 12 nymphs (eight of first and four of fifth instar) and five adults (one female and four males) of *C. phyllinus*. The individuals of *C. phyllinus* were identified accordingly to Hennemann *et al.* (2016) and Brock *et al.* (2022). The specimens belonged to the F1 generation of a colony kept for educational and scientific purposes that was started with four females captured in the field, in the same mentioned geographic coordinates. The plastic container where the insects were observed and reared measured 60X40X40 cm, with translucent walls, which were perforated to ventilate the cage. The container was placed in an external area, under a roof, exposed to natural light cycles but no direct sunlight. The temperature varied from 20° to 28 °C with an average of 25 °C and the relative humidity oscillated from 65% to 85% with an average of 77%. The specimens of *C. phyllinus* were fed on leaves of guava (*Psidium guajava* L.; Myrtaceae) and powder-puff (*Calliandra* sp.; Fabaceae). The plants were kept fresh since the stems were maintained in a jar with water inside the cage. The observations were carried out for 69 days, at least three times a day (in the morning at 9 AM, in the afternoon at 2 PM and in the evening at 7 PM) and for at least 15 minutes each time. Images were recorded by JC with a Samsung Note 8 camera.

All *H. angulosus* and *C. phyllinus* specimens were deposited in the Entomological Collection of the Oswaldo Cruz Institute, in the J. Costa and V. Lima-Neiva section (Costa *et al.* 2008; Cerri *et al.* 2014).

All collections were authorized by the Brazilian Ministry of the Environment (MMA), through the Biodiversity Authorization and Information System (SISBIO), process number 12123.

Results

During the 69 days of observation, the three *H. angulosu*s nymphs (one of third, one of fourth and one of fifth instar) preyed and fed on the *C. phyllinus* specimens and molted until reaching adulthood (Figs. 1-3).

It was possible to record their preying behavior on eight specimens of *C. phyllinus*, all of which died during the observation period, including the adult female, which presented the most remarkable size difference between prey (22 cm in length) and predator (a fifth instar nymph measuring 1.9 cm in length) (Fig. 4).

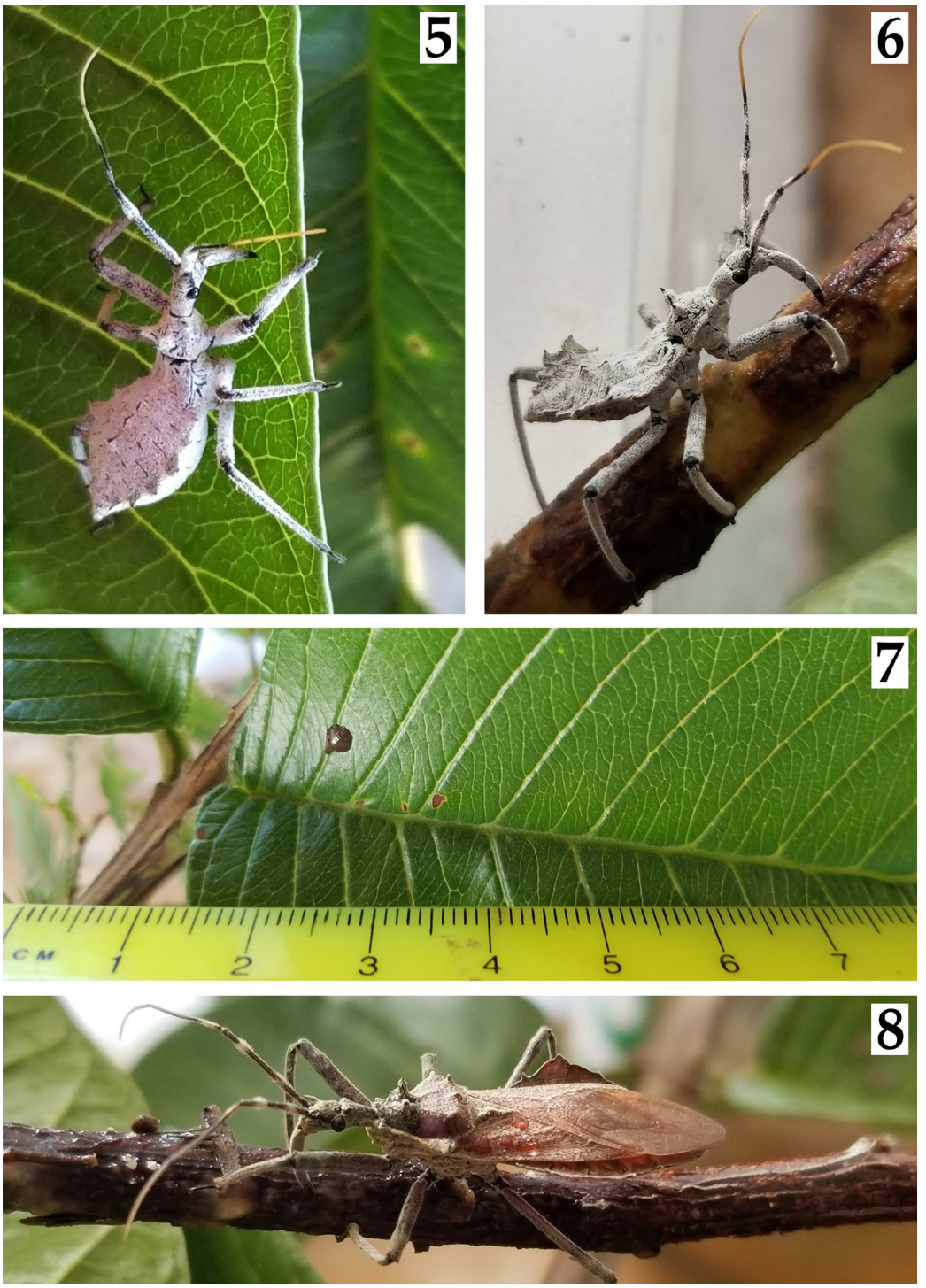
Figures 1-3. *Harpactor angulosus*. **1-2.** Specimens just after moulting, near its exuvia and during the imaginal moulting, respectively. **3.** Male in dorsal view, reared since third instar and fed on *Cladomorphus phyllinus* specimens. / **1-2.** Espécimenes recien mudados, cerca de su exuvia y durante la muda imaginal, respectivamente. **3.** Macho en vista dorsal, criado desde el tercer estadio y alimentado con ejemplares de *Cladomorphus phyllinus*.

Figure 4. Female of *Cladomorphus phyllinus* being preyed by a fifth instar nymph of *Harpactor angulosus*. / Hembra adulta de *Cladomorphus phyllinus* presa de una ninfa de quinto estadio de *Harpactor angulosus*.

Below, the behavior of the assassin bugs while chasing, approaching, attacking and feeding on the stick insects is detailedly described and illustrated. The evasive movements of the stick insects were also registered. In addition to the constant chasing movements and active predatory behavior most of the day, adults and nymphs of *H. angulosus* were recorded several times feeding on guava leaves and powder-puff branches. After these events, drops of excrement were observed near the points where the leaves had been pierced, confirming this unusual reduviid feeding habit (Figs. 5-8).

Harpactor angulosus specimens were always seen walking in very slow movements throughout the cage. The chasing was observed only during daytime, when the predators walked around very smoothly and, once they recognized a prey, they started a very slow and careful approach toward the target. This behavior was never observed during the evening when the predators used to be motionless under a leaf. After an assassin bug got close to a stick insect (0.5 cm or less), sometimes slightly touching the prey several times before the attack, with the fore legs in position to embrace and usually under the legs of the stick insect, it suddenly performed a very rapid movement and attacked the prey. These attempts were not always successful, as the *C. phyllinus* specimens were able to run away, especially the first instar nymphs. The fifth instar nymphs and adults sometimes also moved away to escape, but they were more prone to energetically shake their bodies, throwing the predator away and avoiding being pierced. In other opportunities, a specimen of *H. angulosus* was observed trying to pierce in the arolium between the pretarsal claws of a fifth instar *C. phyllinus* nymph. However, as the assassin bug tried to pierce, the stick insect pulled the leg abruptly and the predator stopped trying. This behavior was repeatedly observed and soon the phasmid did not react anymore, and the assassin bug took advantage and started feeding (Fig. 9).

After successful approaches, *H. angulosus* nymphs were able to pierce the *C. phyllinus* specimens. After about ten seconds of resistance the stick insects showed no more energetic movements to get rid of the predator. Details of the feeding behavior of the assassin bugs on an adult male and an adult female of *C. phyllinus* are presented below (Figs. 4, 9-14).



Figures 5-8. *Harpactor angulosus*. **5-6.** Fourth and fifth instar nymphs feeding in a leaf and in a branch of guava, respectively. **7.** Aspect of the leaf after having been pierced by *H. angulosus* showing the yellow spots and the brown excrement drop. **8.** Male in a powder-puff branch. / **5-6.** Ninfas de cuarto y quinto estadio alimentándose en una hoja y en una rama de guayaba, respectivamente. **7.** Aspecto de la hoja después de haber sido perforada por *H. angulosus* mostrando las manchas amarillas y la gota de excremento marrón. **8.** Macho en una rama de borlas.

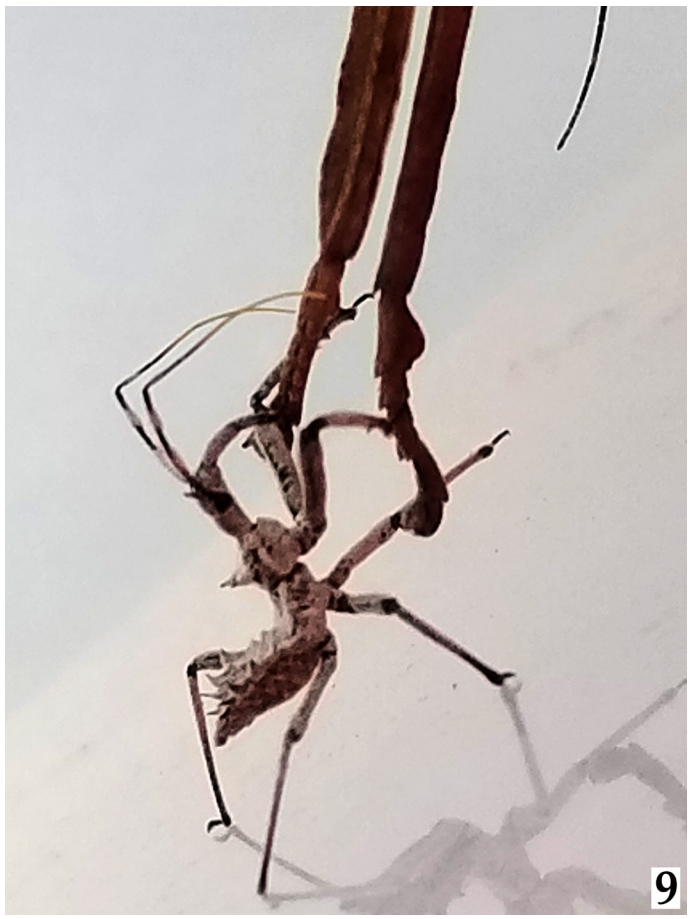


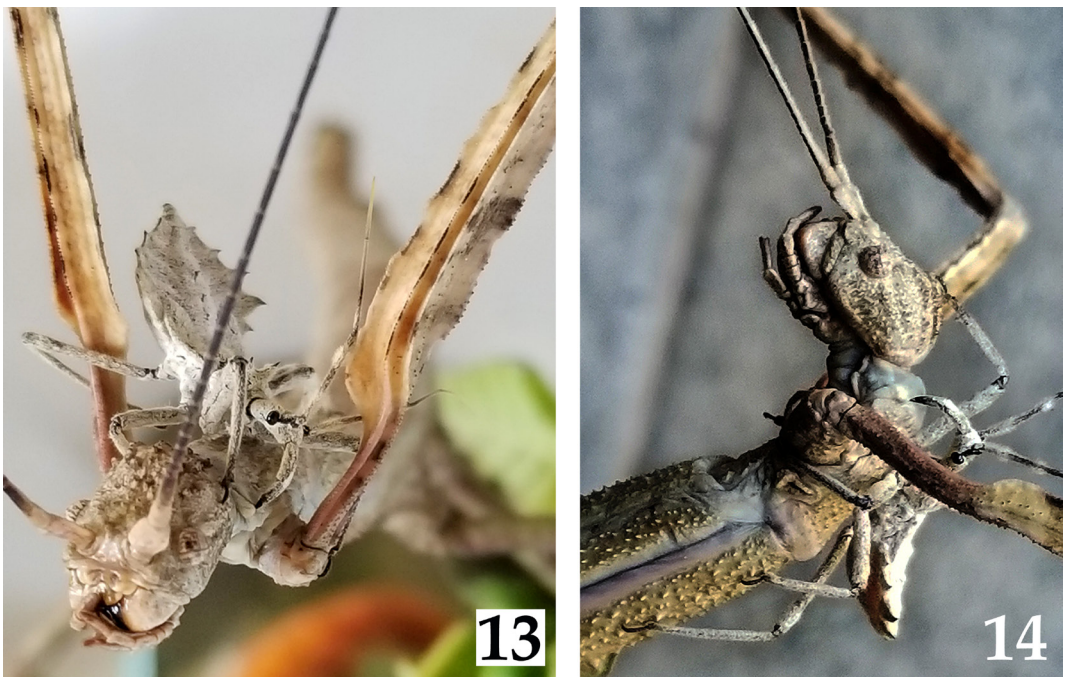
Figure 9. Fourth instar nymph of *Harpactor angulosus* feeding on the arolium of a *Cladomorphus phyllinus* specimen. / Ninfa de cuarto estadio de *Harpactor angulosus* alimentándose del arolio de un espécimen de *Cladomorphus phyllinus*.

After a nymph of *H. angulosus* successfully attacked an adult male of *C. phyllinus* (Figs. 10-11), the phasmid was found hanging by the legs of the assassin bug making some light movements but in a visibly abnormal posture. After feeding for 10 to 15 minutes on the phasmid, the assassin bug's abdomen started to get progressively engorged. The assassin bug was observed feeding on distinct parts of the stick insect: on the legs, specifically in the membranous joint of the femur with the tibia, on the membranous joints between the legs and the thorax and on lateral abdominal sutures (Fig. 12). The assassin bug took one hour and forty minutes until reaching complete repletion. During this time, it was observed exploring several points to feed (at least 27 different points were counted), always on the sutures and joints where membranous areas of the exoskeleton could be pierced. As the individual of *H. angulosus* proceeded in feeding, the stick insect showed no more movements. The assassin bug only stopped sucking when completely ingurgitated. It is noteworthy that the *H. angulosus* nymph was 1.6 cm long while the *C. phyllinus* male was 14.5 cm long. In another opportunity, a third instar nymph of *H. angulosus* was observed feeding on an abdominal suture of an adult male of *C. phyllinus* until repletion. Eight hours after being preyed, this stick insect showed no vital signals: the antennae and legs were completely static and no abdominal movements were noticeable. Similar behavior patterns were repeatedly recorded for other preyed specimens.

Figures 10-12. Nymphs of *Harpactor angulosus* feeding on *Cladomorphus phyllinus*. **10-11.** Fourth instar nymph on a male of *C. phyllinus*. **12.** Third instar nymph feeding on the lateral abdominal suture of a *C. phyllinus* male. / Ninfas de *Harpactor angulosus* alimentándose de *Cladomorphus phyllinus*. **10-11.** Ninfa de cuarto estadio en un macho de *C. phyllinus*. **12.** Ninfa del tercer estadio alimentándose de la sutura abdominal lateral de un macho de *C. phyllinus*.

The most remarkable record involved a fifth instar nymph of *H. angulosus* preying on an adult female of *C. phyllinus*. In the very first attempt, the assassin bug tried to pierce a midleg of the stick insect. However, the female of *C. phyllinus* energetically reacted, pulling its leg away from the nymph of *H. angulosus*. The latter stayed nearby and insisted. In a second approach, the assassin bug advanced very smoothly, coming near the head of the stick insect, and then suddenly it strongly pierced the membranous neck of the phasmid, which was energetically shaking the whole body, unsuccessfully trying to get rid of the predator (Fig. 4). Once the nymph of *H. angulosus* got fixed on the dorsal part of the prey's neck, several stings were performed in close spots in about two minutes (Figs. 13-14). During this time, the female of *C. phyllinus* was less resistant, first unsuccessfully attempting to remove the assassin bug from its body with its fore legs and then getting progressively weaker and slower until it made only slight movements with the antennae and legs. Two minutes after being preyed, the female of *C. phyllinus* performed no more defensive movements. The assassin bug stood on the stick insect sucking and/or injecting toxins for more than three hours and during this time its abdomen got visibly engorged. After completely fed, the assassin bug left the body of the stick insect which was still hanging from the guava branch and visibly alive, showing abdominal movements and slowly moving its legs. The stick insect was not in its natural posture and showed difficulties to support its body hanging from the branch. Twenty-four hours after the beginning of the predatory behavior, the *C. phyllinus* female was still alive. However, after six more hours the stick insect showed no more vital signals, and the *H. angulosus* specimen presented its abdomen less engorged and was observed chasing a fourth instar nymph of *C. phyllinus*.

The assassin bugs were also frequently observed chasing first instar nymphs of *C. phyllinus*, but the latter usually escaped by running away from the predators. No signs of cannibalism and competition for space or prey were observed among the *H. angulosus* specimens. While any assassin bug was feeding on a stick insect, the others were never observed approaching to take advantage of the situation.



Figures 13-14. Fifth instar nymph of *Harpactor angulosus* feeding on the membranous neck of a *Cladomorphus phyllinus* female. / Ninfa de quinto estadio de *Harpactor angulosus* alimentándose del cuello membranoso de una hembra de *Cladomorphus phyllinus*.

Discussion

Harpactor angulosus, despite its potential use for biological control (Pereira *et al.* 2009), needs further studies in terms of biology, ecology and behavior. In this report, an apparent phytophagy by *H. angulosus* was recorded for the first time and this aspect should be further investigated to ascertain its importance as a food source and/or for the developmental cycle of the species. If confirmed, it would be in accordance with previous observations of use of plant resources by several other Harpactorinae species (Gil-Santana and Keller 2022). As mentioned above, the humidity in the cage where the insects were reared was kept by the plants transpiration and also from the water evaporation from the jar where the guava and the powder-puff stems were maintained fresh. Therefore, the observed phytophagy could not be considered as a consequence of a dry atmosphere inside the cage.

Harpactor angulosus is known by its aggressive predatory behavior. Recently a fifth instar nymph was observed in the field, feeding on an adult female of *Cladoxerus cryphaleus* (Westwood, 1859) (Phasmatodea: Phasmatidae) on a *Calliandra* sp. branch, in a green area of Petrópolis city, Rio de Janeiro, Brazil (JC, pers. obs.). This record was previous to this study, which was conducted to allow a more detailed observation of the prey/predator relationship between assassin bugs and stick insects. It is also important to mention that several other phasmid species have been recorded in the geographic area where the *H. angulosus* and *C. phyllinus* specimens were collected (JC, pers. obs.). These species include *Exocnophila* sp. (Diapheromeridae), *Cladoxerus cryphaleus* and *Pygirhynchus* sp. (Heteronemiidae), besides *C. phyllinus* which seems to be the more abundant and/or easiest seen species in the area. For this experiment, we used *C. phyllinus* because there were specimens available in the colony and also because several specimens of this species had been already recorded in the same geographic area where the assassin bugs were collected.

The digestion of *H. angulosus* seems to be very fast, at least in the nymphs. About 48 hours after the specimens preyed on *C. phyllinus* and got fed until completely engorged, it was possible to observe their abdomens getting flat again. This apparently fast digestion probably stimulates the intense predatory activity of this assassin bug, as recorded here. It is important to mention that *C. phyllinus* and most phasmid species are known by their nocturnal activity. *Harpactor angulosus*, as most harpactorines, was observed to be active during the day. In fact, the assassin bugs were seen moving in the cage during all day, most of the time exploring the environment in slow movements. These distinct biological rhythms between the two species may represent an advantage for the predator because even when the stick insects were motionless, they were targeted and chased by the assassin bugs. Therefore, it seems that there were other stimuli than the prey movements being perceived by the predators which probably triggered the chasings and the attacks.

In this report of prey/predator behavior, the size difference between the assassin bugs and the stick insects called attention especially when *H. angulosus* specimens preyed on the fifth instar nymphs, adult males and the adult female of *C. phyllinus*. These remarkable size differences did not impair the predation, stressing the efficacy of the predatory behavior and the toxins injected by the assassin bug to weaken the phasmid's movements and its capacity to react.

Although birds are by far the most commonly reported predators of Phasmatodea (Bedford 1978; Liu 2021), some records of heteropterans preying on phasmids are also available in the literature, including species of both Pentatomidae and Reduviidae (Berger and Wirth 2004; Costa *et al.* 2019; Liu 2021). Here we report the predatory behavior of *H. angulosus* on *C. phyllinus* under captivity. However, it is important to stress that both species occur in the same area and can be found in the same geographic coordinates. Also, *C. phyllinus* can be observed in different layers of the forest (JC, pers. obs.) and *H. angulosus* is known by its generalist feeding habits. These evidences, in addition to the fact that the

three specimens of *H. angulosus* were able to reach adulthood in the rearing conditions, suggest that *H. angulosus* can feed on *C. phyllinus* in nature.

Due to the biological characteristics of *H. angulosus* and the presence of other phasmid species in the geographic area where the assassin bugs were collected, the ecological interactions here described may be of broader occurrence in nature. Future field works are planned to better understand the synecology between *H. angulosus* and stick insect species.

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