First Report of Predation by a Stink Bug on a Walking-Stick Insect with Reflections on Evolutionary Mechanisms for Camouflage

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Research note

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Abstract

Objective: The stink bug Supputius cincticeps is a well-known neotropical, generalist predator. However, in contrast to other predators, S. cincticeps also need to feed on plants to complete its life cycle. The aim of this report is to describe predation by S. cincticeps on Cladomorphus phyllinus, a walking-stick insect that feeds on leaves of several fruit trees, and is considered one of the largest walk-stick insect species in the Southern Hemisphere. Also a new hypothesis for camouflage is discussed. Results: Suputius cincticeps, recently brought from nature, was accidentally introduced into a colony of C. phyllinus, while feeding them with guava leaves. Once the nymph of S. cincticeps detected the C. phyllinus female it displayed immediately feeding behavior. Both adults and nymphs of C. phyllinus were observed to be predated by S. cincticeps. This new predatory interaction offers a novel evolutionary hypothesis for camouflage based on horizontal transfer of genes a process that might be occurring in nature. Keywords: Phasmatodea, Asopinae, predation, camouflage

Introduction

The predatory stink bugs (subfamily Asopinae) is the only group of Pentatomidae that needs to feed on other arthropods to complete their life cycle, contrasting with other groups of the family that are mostly phytophagous [1, 2]. Asopinae is considered and recognized as a natural taxon, distributed worldwide and comprising approximately 63 genera and 303 species [3, 4]. Predatory stink bugs are also important tools in biological control: several species are used to control populations of other insects in agricultural systems, reducing need for some pesticides [2, 5, 6].

The predatory stink bug *Supputius cincticeps* (Stål, 1860) is a well-known, generalist predator that feeds mostly on larvae of Lepidoptera [7] and other insects under lab conditions [8]. However, in contrast to other predators, *S. cincticeps* also need to feed on plants to complete its life-cycle (R. Brugnera, unpublished data). This insect inhabits several ecosystems, and is widely distributed across the Neotropical region [9]. *Supputius cincticeps* is considered important in biological control in crops and forests, particularly in Brazil, which has led to detailed studies of its biology, behavior, morphology and reproduction [6, 2, 10].

Cladomorphus phyllinus Gray, 1835 is a walking-stick insect (Order Phasmatodea). The Order includes 13 families, 523 genera and 2.822 species. Of these species, 591 occur in the Neotropical region [11], and more than 200 in Brazil [12]. *Cladomorphus phyllinus* feeds on leaves of several fruit trees, and is considered one of the largest walk-stick insect species in the Southern Hemisphere, where female can reach 22 cm long [13]. The entire group is little studied [14, 15], particularly the South American species [11].

Presenting a biological cycle of around 280 days from egg to adult, *C. phyllinus* can be reared under conditions of captivity [16].

Materials And Methods

In 1917, we captured four females and two males, from secondary fragments of the Atlantic Forest near Petrópolis, Rio de Janeiro, Brazil (22º 30' 18" S; 43º 10' 43" W; 809m); these individuals were used to establish a robust colony of more than 200 individuals. In order to record bionomical aspects, individuals are being kept in captivity. The colonies are being maintained under environmental conditions ranging in temperature from 15-32°C and 65-93% relative humidity. Adult specimens are kept in enclosures housing from 10 to 20 individuals and provided with leaves from *Psidium guajava* (guava trees) as a primary food source.

The *C. phyllinus* specimens mentioned in this paper were identified [17, 11] and deposited in the collection Jane Costa & Lima Neiva of the Oswaldo Cruz Institute Entomological Collection , Fiocruz [18, 19].

Results

While providing recently collected guava leaves to one of the colonies, a fifth instar nymph of *S. cincticeps* was introduced accidentally into the one of the enclosures housing 20 adult female of *C. phyllinus*. Once the nymph detected an adult female of *C. phyllinus*, it began immediately to display feeding behavior. It first chose to attempt feeding at the pulvilo, which resulted in the repulsive reaction of the adult female showing some sensitivity, manifested as movements of the leg in a manner for scraping off as irritant (Fig 1A). The *S. cincticeps* nymph persisted, appeared to succeed at sucking the pulvilo for approximately 3 min. The *S. cincticeps* nymphmoved to another region of the leg and commenced to feed on the mid-tibia (Fig 1B).

The asopine nymph was then transferred to a small pot together with five first-instar nymphs of *C. phyllinus,* along with some leaves from *Calliandra brevipes* Benth,1840, a food source *C. phyllinus* nymphs. Two days later, *S. cincticeps* was recorded feeding on *C. phyllinus* nymphs; *S. cincticeps* nymph fed upon all five nymphs of *C. phyllinus* over the succeeding nine days, leaving the completely dry, dead bodies of the *C. phyllinus* nymphs in the bottom of the pot. The nymph of *S. cincticeps* did not survive to adulthood: died three days after its last meal, feeding on *C. phyllinus* first-instar nymphs (Fig 2)

Discussion

This report is the first to document predation of *S. cincticeps* on *C. phyllinus*. *Supputius cinctipes* is known for its eclectic feeding behavior, attacking a variety of insects. It has been evaluated carefully as a potential candidate for natural control of agricultural pests. This group of insects can also feed on plant sap when insect prey is scarce [20-23]; this alteration in food source, referred to as zoophytophagous behavior, facilitates increased survival of nymphs and extends the longevity and fecundity of adult insects [24, 25]. Some species need plants for completing the developmental cycle. *Supputius cincticeps* following the rule, dies in the nymphal stage if some vegetal is not available for feeding.

These stink bugs can be phytozoophagous or zoophytophagous: phytozoophagous insects are herbivores that occasionally feed on small insects; and vice versa for zoophytophagous insects [21, 26]. These feeding habits elucidate how pentatomid predators use different food sources as well as ecological and evolutionary changes in feeding [27, 28]. The zoophytophagous *S. cincticeps* can be an important component of biological control programs of soybean pests, and pests of other beans, and cotton in Brazil [29], especially pest species of Lepidoptera, Coleoptera, Diptera, and Hemiptera [30- 32]. They apparently see improved development and reproduction of zoophytophagous predators when they feed on both plants and insects [33].

Possible mechanisms into how morphological changes arise that provide camouflage to insects for escaping predators have long been discussed [34]. The most prevalent concept is that new morphologies evolved through the traditional combination of germ-line mutations and natural selection [35]. However, one can easily speculate that other mechanisms could be used to generate different camouflage strategies. Give the diverse predatory feeding behavior of *S. cincticeps*, we propose that the camouflage of *C. phyllinus* could be facilitated by horizontal or lateral transfer of plant-derived genetic material leading to development of a form resembling a tree stem.

While unprecedented and speculative, a hypothesis based on transfer of genetic material is plausible since the predatory Pentatomidae feed by inserting their stylet into the body of the prey, and injecting toxins, enzymes, or both, before sucking prey tissues [30, 36]. Asopines are known to use the plant juices for producing saliva, which will be used for predation activity [1]. This behavior could also provide a conduit by which to introduce plant genes, or any bacteria or virus that coexists with the plant.

Horizontal transfer of genes was first reported in 1928 [37] and, is now accepted as an important mechanism for evolutionary processes. Horizontal transfer is known to have occurred among prokaryotes [38, 39] and the phenomenon appears to have had significance for unicellular eukaryotes as well [40]. Still, the prevalence and importance of horizontal transfer in the evolution of multicellular-eukaryotes remain unclear [41]. Some clear examples have been documented, such aspea aphids (*Acyrthosiphon pisum*) which have multiple genes from fungi [42]. Plants, fungi, and microorganisms can synthesize carotenoids, but torulene made by pea aphids is the only carotenoid known to be synthesized by an organism in the animal kingdom [43]. Also, HhMAN1, a gene in the genome of the coffee borer beetle (*Hypothenemus hampei*), resembles bacterial genes, and is thought to be transferred from bacteria in the beetle's gut [44].

Here we report a generalist predator *S. cincticeps* feeding on *C. phyllinus*, the latter of which presents a camouflage in the form of an intriguing phenotype, the “walking stick” body form. We propose a novel hypothesis: that the camouflage could have been arisen via of a plant morph gene to the insect genome by an insect vector.

Deep molecular studies at the level of genome sequencing are needed to test this hypothesis opening new models and interesting possibilities in the universe of the genome interactions and dynamics.

Limitations

-The new interaction between a stink bug and a walk-stick insect here reported was not recorded in natural environment. However, *S. cincticeps* presented its predatory behavior immediately after its accidental introduction in one of the enclosures, housing *C. phyllinus* specimens.

- The report of the predation by *S. cincticeps* on *C. phyllinus* offer a new evolutionary hypothesis on camouflage based on horizontal transfer of genes however, genome sequencing analyses to test this hypothesis are required.

Declarations

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Authors’ contributions

JC identified walking-stick insect, kept the insect colonies, recorded the predation and wrote the paper. LC reared the insects, wrote the paper. DWPJr wrote the paper. RB and JG identified stink bug and wrote the paper. All authors read and approved the final manuscript.

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Availability of data and materials

Studied specimens were deposited at the collection Jane Costa & Lima Neiva of the entomological collection of the Oswaldo Cruz Institute, Fiocruz, Brazil.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

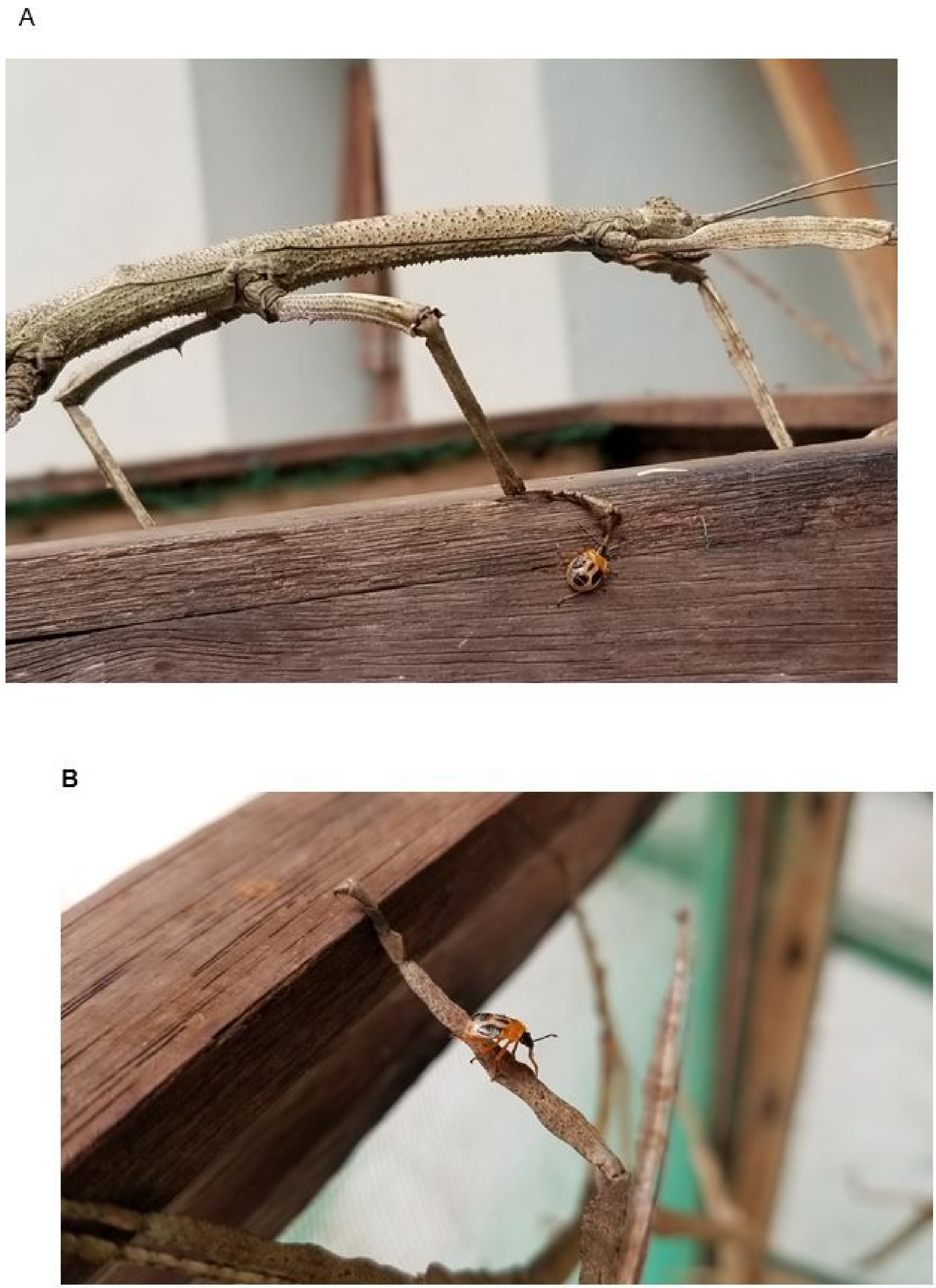

Figure 1

Predation by a fifth-instar nymph of Supputius cincticeps (Stål, 1860) on a female of Cladomorphus phyllinus Gray, 1835: A- feeding the pulvilo; B- feeding the mid-tibia.

Figure 2

Predation by a fifth-instar nymph of Supputius cincticeps (Stål, 1860) on a first instar nymph of Cladomorphus phyllinus Gray, 1835: