Mating behavior and parental care in the neotropical pseudoscorpion *Americhernes bethaniae* Mahnert, 1979 (Arachnida: Chernetidae)

Comportamento de acasalamento e cuidado parental no pseudoescorpião neotropical *Americhernes betaniae* Mahnert, 1979 (Arachnida: Chernetidae)

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Abstract: Pseudoscorpions reproduce indirectly, transferring a spermatophore deposited on the substrate. In Cheliferoidea, males perform an elaborate ritual involving pedipalps vibrations and a mating 'dance' before depositing the spermatophore in the presence of the female. Thus, this study investigated the reproductive behavior and parental care of the neotropical pseudoscorpion *Americhernes bethaniae*, from populations collected in the Brazilian *Cerrado*. Pseudoscorpions were collected from live tree trunks and stored in the laboratory for behavioral observations. In the case of *A. bethaniae*, courtship involved ritual vibration of the pedipalps, followed by eight distinct reproduction stages: approach, sex identification, fixation, dance, spermatophore formation, spermatophore transfer, and then either a fight or resumption of the process. Females built silk nests and produced an embryonic sac about 15 days after mating. Three days after the first ecdysis of the juveniles, the mother left the silk chamber. The male remained with the female until she sealed herself inside the silk nest, but did not contribute to the offspring care. This guarding behavior may suggest a strategy to ensure paternity.

Keywords: Spermatophore transfer. Courtship. Reproductive behavior. *Cerrado*. Pseudoscorpiones.

Resumo: Os pseudoescorpiões se reproduzem de forma indireta, por meio da transferência de espermatóforo depositado no substrato. Em Cheliferoidea, os machos realizam um elaborado ritual envolvendo vibrações dos pedipalpos e uma 'dança' de acasalamento antes de depositarem o espermatóforo na presença da fêmea. Assim, este estudo investigou o comportamento reprodutivo e o cuidado parental do pseudoescorpião neotropical *Americhernes bethaniae*, a partir de populações coletadas no Cerrado brasileiro. Os pseudoescorpiões foram coletados em troncos de árvores vivas e acondicionados em laboratório para observações comportamentais. O cortejo em *A. bethaniae* envolveu um ritual de vibração dos pedipalpos, seguido por oito estágios distintos de reprodução: aproximação, identificação de sexo, fixação, dança, formação de espermatóforo, transferência do espermatóforo e, em seguida, luta ou retomada do processo. As fêmeas construíram seus ninhos de seda e produziram uma bolsa embrionária cerca de 15 dias após o acasalamento. Três dias após a primeira ecdise dos juvenis, a mãe abandonou a câmara de seda. O macho permaneceu junto da fêmea até que ela se selasse no interior no ninho de seda, porém não contribuiu com o cuidado à prole. Este comportamento de guarda pode sugerir uma estratégia para assegurar a paternidade.

Palavras-chave: Transferência de espermatóforo. Cortejo. Comportamento reprodutivo. Cerrado. Pseudoscorpiões.

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INTRODUCTION

The order Pseudoscorpiones is one of the oldest and most diverse groups of arachnids (Shear et al., 1989; Schawaller et al., 1991; Harms & Dunlop, 2017), represented by 25 families, 474 genera, and 4,272 species (World Arachnida Catalog, 2024). Pseudoscorpions are small (2-8 mm), solitary animals that are generally aggressive upon intraspecific contact (Weygoldt, 1969; Zeh & Zeh, 1997). Despite their diversity, the biology of many species is still poorly studied (Weygoldt, 1969; Zeh & Zeh, 1997). Most studies on the behavior and natural history of pseudoscorpions have focused on the superfamily Cheliferoidea (Harvey, 1992; Zeh & Zeh, 1997).

Reproduction in the pseudoscorpions of the superfamily Cheliferoidea is characterized by an elaborate courtship display, in which the male performs a series of rituals before depositing the spermatophores (Zeh & Zeh, 1997). During the courtship phase, the male engages in a series of vibrational and pedipalpal movements, which can be described as a kind of 'dance' (Zeh, 1987; Andrade & Gnaspini, 2003). The courtship and behavior vary according to species or even between families (Weygoldt, 1969; Zeh & Zeh, 1997; Kirchmair & Raspotnig, 2021).

The Chernetidae family is very diverse, with more than 733 described species, distributed in different habitats around the world, from tropical forests to temperate regions. The reproductive behavior of the pseudoscorpions in this family is particularly interesting and complex (Zeh & Zeh, 1997; Palen-Pietri et al., 2019). Males often perform an elaborate courtship ritual that may include mating dances to attract females. During mating, the male deposits a spermatophore on the substrate, which is then collected by the female. Understanding these reproductive interactions is crucial for the conservation and study of the ecology of the Chernetidae, revealing important aspects of their biology and evolution.

Chernetidae represent a large part of the diversity of pseudoscorpions in South America. Approximately 70 species of Chernetidae are known from Brazil (World Arachnida Catalog, 2024), with the genus *Americhernes*, being widely distributed with seven species (Mahnert, 1979; Adis & Mahnert, 1985; Aguiar & Bührnheim, 1998). Of these, at least four have been documented in the Brazilian Central Amazon region, with *A. bethaniae* Mahnert, 1979 being the most abundant (Mahnert, 1979). Subsequent studies have identified this species in other regions of the Amazon (Adis & Mahnert, 1985; Aguiar & Bührnheim, 1998). Nevertheless, *A. bethaniae* is often found under the bark of living trees in the *Cerrado* region (Del-Claro & Tizo-Pedroso, 2009; Tizo-Pedroso & Del-Claro, 2014). Therefore, the aim of this study was to investigate courtship, reproduction, parental care and post-embryonic development of *A. bethaniae* under laboratory conditions.

MATERIAL AND METHODS

The *Cerrado* is the second-largest biome in Brazil, characterized by predominantly savanna vegetation with grasses, scattered shrubs, and trees adapted to poor and acidic soils. This highly diverse biome harbors numerous endemic plant and animal species, and plays a crucial role in regulating the hydrological and climatic regimes of the central region of the country (Oliveira & Marquis, 2002; Colli et al., 2020). Pseudoscorpions were collected from August to October 2017 and 2018 in two seasonal semi-deciduous forests separated by approximately 115 km (Morrinhos municipality, 17° 43' 39" S, 49° 07' 54" W, and Araras Farm, Ipameri municipality, 17° 66' 99" S, 48° 16' 13" W) in the state of Goiás, Brazil.

We searched for pseudoscorpions on live tree trunks. In each area, we searched 60 trunks (15 trunks in 2017 for each area and 15 in 2018) at a height between 30cm and 150cm from the ground. The bark of these trees was manually removed, and individuals were collected (comprising 3 tritonymphs, 29 males, 32 nonpregnant females, and 6 pregnant females) using a thin brush, which prompted the pseudoscorpions to grasp it. Captured individuals were then placed in 10 mL transparent plastic vials for transportation to the laboratory.

The pseudoscorpions were transported to the Laboratory Diversity, Behavior and Conservation of Arachnids at the State University of Goiás. Upon arrival, they were transferred to 5 cm diameter petri dishes, each containing a fragment of tree bark and a piece of moist cotton (Tizo-Pedroso & Del-Claro, 2005). These dishes were covered with red cellophane. Subsequently, the sex of each individual was determined through direct observation using a stereomicroscope.

The pseudoscorpions were individually housed in Petri dishes for a period of 20 days to ensure that the females did not produce brood sacs. Live termites (*Armitermes* sp.) were provided as food twice a week. Following this period, a randomly selected male was transferred from its original dish and introduced into the Petri dish with a resident female. Each dish was assigned a code indicating the animals' origin, collection date, and sex. In total, 18 dishes were prepared, each containing one male and one female, while 6 dishes contained only one pregnant female at the time of collection.

Observations of reproductive behaviors were conducted within one to three days after the formation of pairs in the Petri dishes, between 6 pm-9 pm. The initial step involved transferring one male from its original dish to a dish containing one female. Ten minutes after the introduction of the male, the dish was placed under a stereomicroscope for observation of the subsequent behavioral interactions. The observation of courtship and agonistic behaviors was conducted over the course of two 20-minute sessions, with a 10-minute interval between each session. This approach was employed using the sequential sampling method (Altmann, 1974; Del-Claro, 2010). Over the subsequent 30 days, the dishes were monitored every three days for behavioral changes related to reproduction, with 10 minutes of observation per day. The various developmental milestones, including the period until the formation of the brood sac, the establishment of the silk chamber, embryonic development within the sac, the hatching of protonymphs, and dispersal time, were recorded.

RESULTS

In general, we found one or two individuals of *Americhernes bethaniae* per tree. On one occasion, we found 14 individuals on the same tree in Morrinhos, but without the formation of aggregations. The individuals were scattered along the trunk.

MALE–FEMALE IDENTIFICATION AND THE BEGINNING OF COURTSHIP

The reproductive behavior of *A. bethaniae* was investigated under laboratory conditions, and this species was shown to be an easily manageable model organism. Adults survived well under laboratory conditions, with courtship behavior initiating promptly upon introducing a male to a dish containing a female. Post-manipulation, males typically begin exploring the dish in search of hiding spots, often encountering females in the process.

The male's approach to the female was cautious and deliberate. Upon detecting the female, the male paused for three to five minutes before slowly advancing toward her, extending his pedipalps with chelae open. This deliberate movement continued until the male made contact with the female or until the female exhibited aggression. Every time a male initiated a contact, it swiftly grasped one or both female pedipalps in an attempt to immobilize her or deflect her attacks (Figures 1A-1B). In cases where the male failed to restrain the female, it quickly retreated but resumed the approach shortly after, repeating the process until he successfully grasped her with his pedipalps.

Once the female was immobilized, a ritual resembling a struggle ensued, likely a test of strength. The female attempted to draw her pedipalps close to her cephalothorax, pulling the male toward her chelicerae, while he sought to retreat and find refuge in a crevice.

Given the larger size and robustness of females, they often manage to break free or release one of their pedipalps from the male's grasp. In response, the male promptly resumed control to prevent retaliation. This interaction persisted for an average of seven minutes (±2.5 SD; n = 12), ceasing only when the female inflicted injury, dragged the male into a crevice, or intimidated him with aggressive displays. Eventually, the male succeeded in engaging the female in a courtship dance, coaxing her away from the crevice by 2 to 4 cm. Subsequently, the female's aggression waned, and she remained near the male, receptive to courtship signaled by his pedipalp vibrations.

COURTSHIP AND SPERM TRANSFER

After a brief rest and pedipalp grooming lasting two to three minutes, courtship commenced. The male positioned himself approximately 2 mm from the female and initiated a series of pedipalp vibrations. No discernible pattern or variation was observed in the sequence of vibrations among different males; instead, the form and duration appeared contingent upon the female's receptiveness. At the conclusion of the pedipalp-based courtship, the female either rejected the male, retreated to her previous hiding spot, or progressed to the next stage of the reproductive process.

The subsequent stage commenced with renewed pedipalp interactions. The male again secured the female, who exhibited reduced aggression and led her aimlessly around the dish. At times, the male released one of the female pedipalps, vibrating his pedipalp in her direction, almost making contact, before reseizing it, repeating this sequence several times (Figure 1D). When he finished vibrating one of his pedipalps, the male moved away about 1 cm, vibrating both pedipalps many times. In response, the female approached him, and the two pseudoscorpions reconnected (Figure 1E). However, the male adjusted his grasp, holding one of the female's chelae with one pedipalp and the femur-tibial joint of her pedipalp with the other, as they moved across the bark fragment surface (Figure 1F). The male then deposited its spermatophore without releasing the female, engaging in a back-and-forth 'dance' until she was successfully positioned over the spermatophore (Figure 1G). Once the spermatophore was inserted into

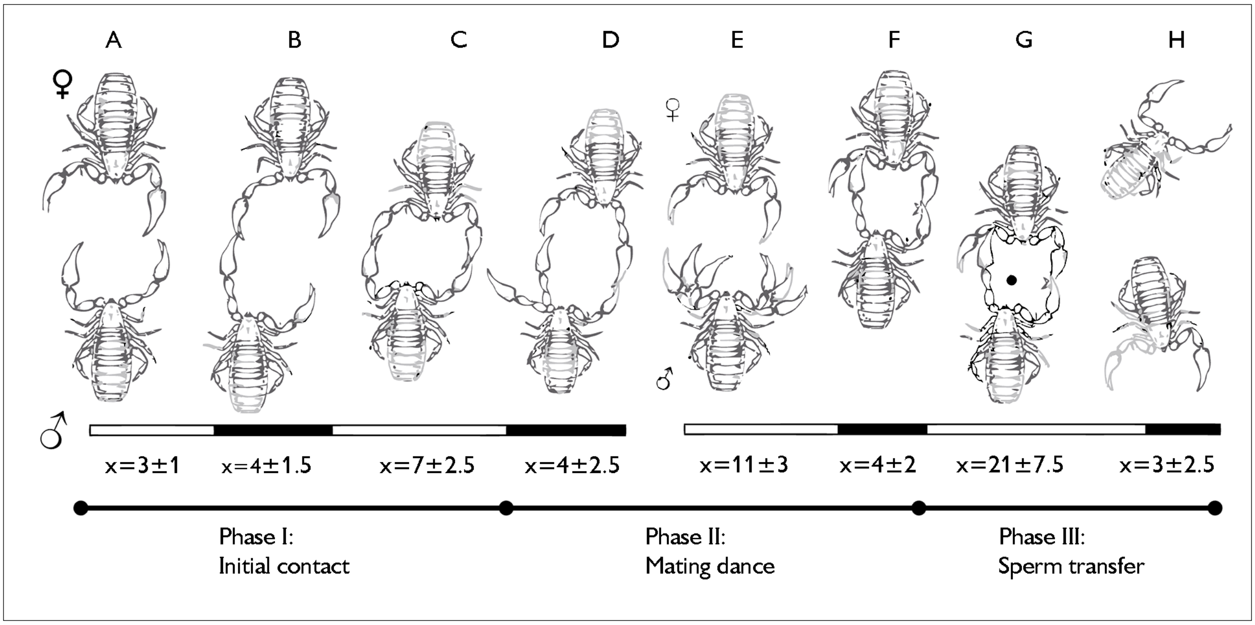

Figure 1. Stages of the courtship process in the pseudoscorpion *Americhernes bethaniae*: A) placement of the individuals and approximation; B) contact of the pedipalps and sex recognition; C) attachment, strength competition, and pushing; D) male signaling with one pedipalp; E) confirmation of female receptivity and vibration of both pedipalps; F) dance position and possible detection of the place for spermatophore deposition; G) spermatophore deposition (black dot) and sperm transfer; H) separation of the pair or fight. The lower bar indicates the average ± standard deviation time (in minutes) spent in each stage of courtship. Photos: Illustrations by the authors.

the female's genital opening, the male held her over it, presumably until she absorbed its entire sperm content. Following sperm transfer, the pair separated, either fleeing or initiating the courtship and spermatophore transfer process anew (Figure 1H). The number and specific shape of spermatophores transferred during the reproductive process were not recorded. On average, the whole mating behavior took about 57 minutes (Figure 1). The sequence of behaviors and possible modifications was organized in a flowchart (Figure 2).

CONSTRUCTION OF THE SILK CHAMBER AND POSTEMBRYONIC DEVELOPMENT

The fertilized females were left undisturbed in their dishes for further observation of reproductive behavior, postembryonic development, and maternal care. Similarly, the males were retained in the dishes, except in cases where the female exhibited aggression toward them. Approximately two weeks following sperm transfer, the females (consisting of 10 females retained in the dishes and six females collected already pregnant) commenced the construction of the silk chamber. The formation of the brood sac occurred approximately six days (±2) after the onset of construction, with the completion of silk chamber building typically taking between four and eight days (±3) (Figure 3). Upon completion, the female rested inside the chamber, providing nourishment to her embryos through a nutritive fluid until the protonymphs hatched. The embryos within the sac reached the second embryonic stage within approximately seven days (±1). The average total duration of development through the first and second embryonic stages within the brood sac was six days (±1). Protonymph hatching occurred approximately five days later (±1 day; n = 13). *Americhernes bethaniae* females exhibited relatively high fecundity, although the exact number of embryos was not accurately recorded. Following hatching, each female produced an average of 24 protonymphs (±4; n = 13).

PARENTAL CARE AND DISPERSAL

After the protonymphs hatched, the female remained in the nest, tending to clean the juveniles for three days. The protonymphs fed on the remaining nutritive fluid in the brood sac until it was fully depleted. During this time, the juveniles moved actively within the chamber, which, although relatively small for the female and the protonymphs, provided enough space for the juveniles to avoid constant contact. On the third day (±1 day) within the chamber, the female used her pedipalps and chelicerae to break the silk wall, creating a small exit hole. This event likely marked the dispersal of females, as some remained nearby, while others ventured farther from the nest, depending on the dish.

Juveniles remained inside the chamber for two more days after the female's departure before dispersing. However, they were kept in Petri dishes for observation of their development and interactions with adult females. This stage proved unsuccessful, as the juveniles constantly moved around the dish and rarely fed until exhaustion and death ensued. They seemed to actively avoid proximity to the adult(s). Only a few number of nymphs reached the second developmental stage (around three nymphs per brood), and none reached to the third stage.

No further parental care was provided after the female left the nest. Female showed no interest in the nymphs, did not touch them, did not bring prey near the nest, and did not allow the juveniles to share it prey. However, no instances of maternal cannibalism or aggressive rejection of the juveniles were observed. By the end of the observation period, it was decided to leave the females in their dishes, resulting in 50% (8 out of 16 females) producing additional broods, with one female even producing a third brood sac. The reproductive season was noted for this species, with pregnant or brood-sac-guarding females observed in *Cerrado* trees between October and April (wet season) (for 2017 and 2018). Reproduction occurred in the laboratory between

October and July. The animals were provided food and kept in Petri dishes until they reached their senility and died. This provided data on the adult lifespan of this species, with adults surviving for approximately one and a half years on average in the laboratory. Voucher specimens were deposited in the arachnid collection of the Arachnid Diversity, Behavior and Conservation Laboratory (State University of Goiás).

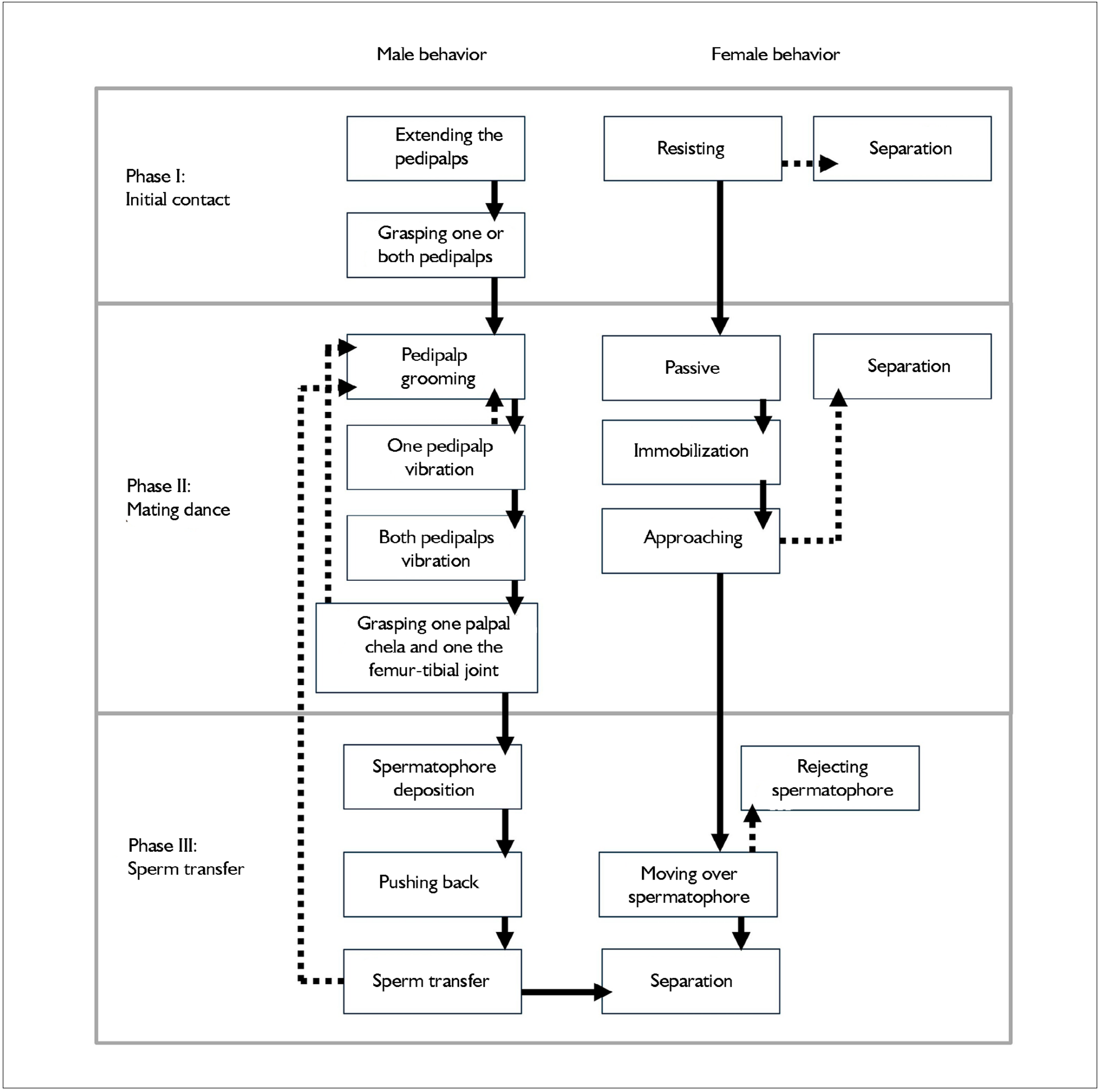

Figure 2. Sequence of behaviors during courtship and sperm transfer in *Americhernes bethaniae*. The phases indicate the three stages of transfer, the boxes indicate the behavioral types, and the lines indicate the sequences. Solid lines indicate the successful sequences in the sperm transfer process, while dotted lines indicate modifications in the sequence. The organization of the flowchart and behavioral types followed Palen-Pietri et al. (2019) and Kirchmair and Raspotnig (2021).

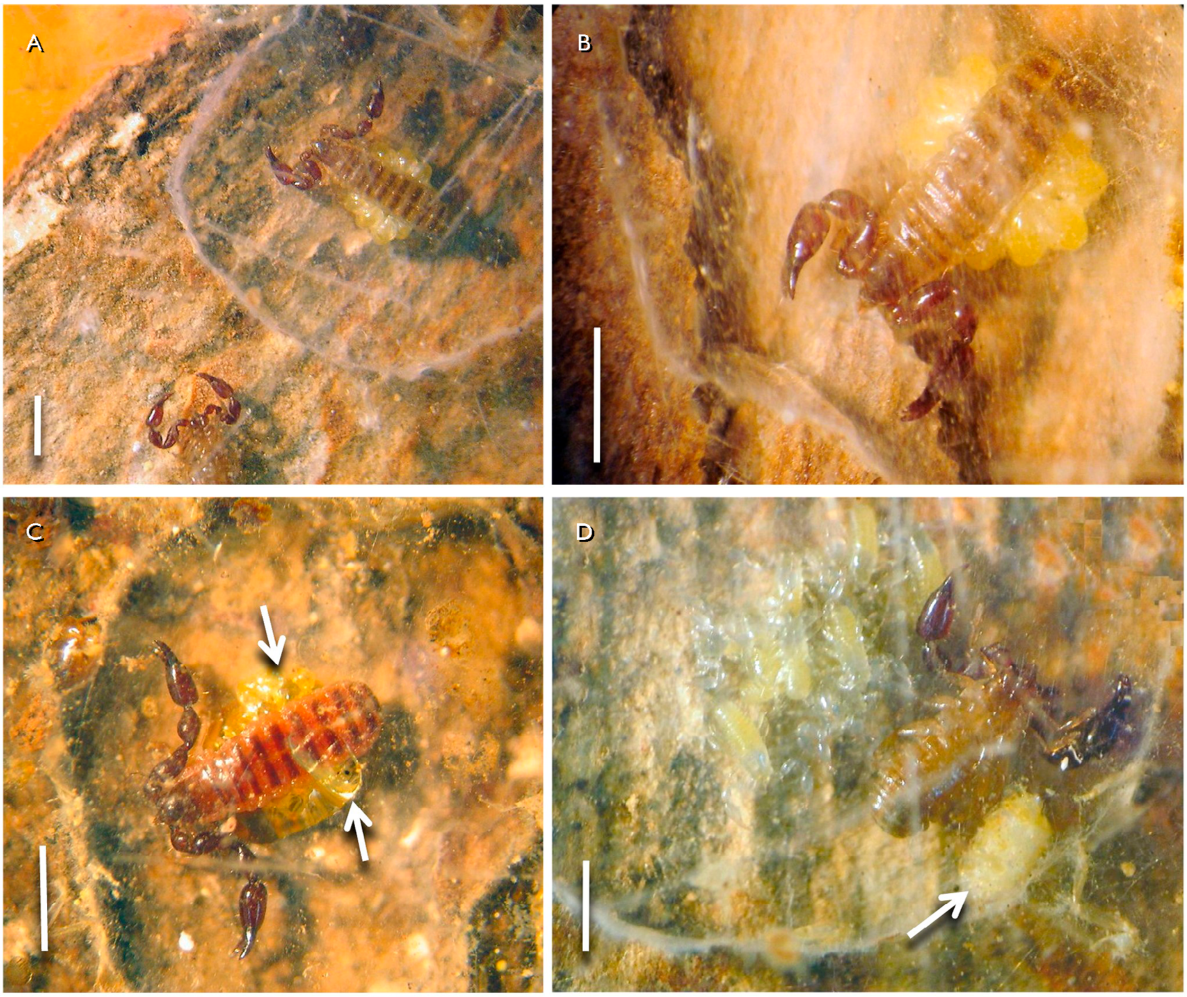

Figure 3. Females of *A. bethaniae* inside the silk chamber after sperm transfer. The images show three different females: A) female 1 resting inside the silk chamber carrying the brood sac with second-instar embryos attached to the genital opening; B) female 1 with a brood sac one day before hatching; C) female 2 inside the nest, with the arrow indicating the nutritive fluid secreted to feed the juveniles; D) female 3 inside the nest with her protonymph two days before dispersal, with the arrow indicating the remains of the brood sac after nymphal hatching and feeding. Scale bars: 1 mm.

DISCUSSION

Sex identification behaviors and female receptivity vary among pseudoscorpion species, even within the Chernetidae family. In *Americhernes bethaniae*, adults can detect and locate conspecifics at approximately 3 cm distances. The relative location between pseudoscorpions during these behaviors appears to be influenced by their movement through the substrate, although the exact distance between pseudoscorpions remains inconclusive based on the present study. Similar occasional perceptions of other individuals with minimal physical contact have been observed in other Chernetidae species (Andrade & Gnaspini, 2003) and Neobisiidae (Weygoldt, 1969). However, such identification behaviors are absent in Chthoniidae species, possibly due to their asexual reproduction (Weygoldt, 1969).

Although intersexual encounters in *A. bethaniae* are cautious and not highly aggressive, agonistic interactions have been observed in other species within the same family (Weygoldt, 1969; Zeh & Zeh, 1997). Nonetheless, non-receptive *A. bethaniae* females may attack and injure males to repel them. Instances of aggressive pedipalp vibrations and attacks by non-receptive females have also been observed in other Chernetidae species, such as *Maxchernes iporangae,* though without resulting in male fatality (Andrade & Gnaspini, 2003). The precise significance of these signaling forms during individual encounters remains unclear, but they likely signal to the other individual who is a potential reproductive partner rather than prey.

The method through which pseudoscorpions identify the sex of conspecifics remains unclear, although it may involve chemical or tactile cues. In some Chernetidae and Cheiridiidae species, males identify only females during leg or pedipalp vibrations (Weygoldt, 1969). However, certain Chernetidae species exhibit marked sexual dimorphism, such as *Dinocheirus arizonensis* (Banks, 1901) and *Cordylochernes scorpioides* (Linnaeus, 1758), where males possess more robust pedipalps than females, and sex identification occurs upon pedipalp contact or after competition from spermatophore deposition (Zeh & Zeh, 1997). In *Lustrochernes argentinus* (Thorell, 1877), females have larger pedipalps than males (Palen-Pietri et al., 2019). The observed behaviors in *A. bethaniae* suggest that sex identification occurs upon pedipalp contact. However, attachment, akin to strength competition, transpires only between individuals of different sexes and not between males. This behavior in *A. bethaniae* is distinct from that in other species, where it seems to determine female receptivity. Overall, females consistently responded covertly to male approaches and only accepted sperm transfer or attacked males post-attachment dancing, indicating that strength competition might serve as a mechanism of sexual selection preceding courtship and sperm transfer.

Courtship and spermatophore transfer behaviors vary uniquely among pseudoscorpion species, with each stage having distinct significance. In *A. bethaniae*, the reproductive process consists of eight stages: approximation, sex identification, attachment, dance, spermatophore formation, sperm transfer, and either fight or continuation. These stages differ among species, and there is no consensus on whether any patterns exist across families, although Andrade and Gnaspini (2003) reviewed the presence or absence of different stages. Attempting to identify a pattern may not be the most effective strategy for understanding these small animals; rather, it may simply underscore their individual differences. Notably, the components of pseudoscorpion reproductive behaviors are strongly influenced by environmental conditions, particularly habitat characteristics (Zeh & Zeh, 1997).

Despite the diversity in sexual behaviors among pseudoscorpion species, there are minimal variations in their nest-building behaviors and embryonic development. Pregnant *A. bethaniae* females construct a silk chamber a few days before the formation of the brood sac. Juveniles are deposited into the brood sac while the chamber is still partially built, consistent with observations in other species and families (Weygoldt, 1969). The most significant differences lie in the number of embryos produced and the timing of juvenile dispersal. For instance, species in the genus *Chthonius* C.L. Koch, 1843 (Chthoniidae) typically produce fewer than ten embryos, while *Neobisium* Chamberlin, 1930 (Neobisiidae) and *Pselaphochernes scorpioides* (Hermann, 1804) (Chernetidae) produce 15 to 25 embryos. The most notable deviation in behavior is observed in some species of the genera *Apocheiridium* Chamberlin, 1924, and *Cheiridium* Menge, 1855 (both Cheiridiidae), where chamber construction does not occur. Instead, females produce a few (3-5) large embryos and guard them until the protonymphs hatch. In Cheliferidae species, the number of embryos ranges from 20 to 40 (Weygoldt, 1969).

Minor variations are also evident among species in the juvenile dispersal stage and in maternal care. For instance, in *Chthonius tetrachelatus* (Preyssler, 1790), the nymphs disperse as deutonymphs after hatching. In contrast, in other Chthoniidae species, females wait for juveniles to hatch, consume the remains of the brood sac, and then disperse. The dispersal pattern in *A. bethaniae* mirrors that of another Chernetidae species, *Pselaphochernes scorpioides*, where the protonymphs disperse four days after the mother abandons the nest. However, in species such as *Serianus carolinensis* Muchmore, 1968, and *Atemnus politus* (Simon, 1878) (Atemnidae), the female disperses only after all the nymphs have left the chamber (Weygoldt, 1969). In these cases, maternal care is limited to the embryonic development period. However, an elaborate form of parental care is found in the family Atemnidae, where females of the social pseudoscorpion *Paratemnoides nidificator* (Balzan, 1888) provide extended care by guarding the nymphs and offering them food until they reach adulthood (Tizo-Pedroso & Del-Claro, 2007).

The findings of the present study revealed that *A. bethaniae* reproduces during the hot and rainy seasons when climatic conditions are more favorable and food is abundant. Pseudoscorpions in the superfamily Neobisioidea, associated with permanent environments such as litter, reproduce year-round. Conversely, reproductive efforts of Cheliferoidea species are concentrated in the most favorable season (Zeh & Zeh, 1997). The existence of a reproductive season is believed to be linked to species inhabiting ephemeral environments such as tree trunks, a pattern broadly applicable to Cheliferoidea pseudoscorpions (Weygoldt, 1969; Zeh & Zeh, 1997). However, in the social pseudoscorpion *P. nidificator*, seasonal reproduction is regulated by food availability and energy allocation during the dry season. Reproductive cycles may deviate from seasonal patterns when food is abundant (Tizo-Pedroso & Del-Claro, 2007). Nevertheless, in *A. bethaniae*, reproduction remains seasonal even in the presence of abundant food. Our understanding of pseudoscorpion reproduction remains fragmented, with reproductive behaviors still unknown in 11 out of 24 families (Harvey, 1992). However, understanding reproductive behavior is crucial for understanding the ecology (Zeh & Zeh, 1997) and evolution (Harvey, 1992; Proctor, 1993) of pseudoscorpions in a given environment, warranting further study within this group.

CONCLUSION

The diverse spectrum of reproductive behaviors observed among pseudoscorpion species, particularly within the Chernetidae family, underscores the complexity of reproductive strategies within this intriguing group of arachnids. Through meticulous observation and analysis, this study elucidated key aspects of sex identification, courtship rituals, and spermatophore transfer behaviors in *A. bethaniae*, shedding light on the intricacies of pseudoscorpion reproductive biology. The findings highlight the significance of environmental factors in shaping reproductive patterns and emphasize the need for further investigation into the mechanisms driving these behaviors across different taxa.

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T. N. Gonçalves contributed to formal analysis, data curation, investigation, and writing (original draft); A. F. S. Tizo contributed to formal analysis, data curation, investigation, and writing (original draft and edition); and E. Tizo-Pedroso contributed to acquisition of funding, conceptualization, data curation, investigation, supervision, validation, and writing (review and edition).