Sex-based defensive behavior influenced by threat level in the scorpion Tityus pusillus (Scorpiones: Buthidae)

Authors: de Albuquerque, Kamila Bento Cavalcanti, and de Araujo Lira, André Felipe

Source: The Journal of Arachnology, 49(3) : 402-406

Published By: American Arachnological Society

URL: https://doi.org/10.1636/JoA-S-20-074

2021. Journal of Arachnology 49:402–406

SHORT COMMUNICATION

Sex-based defensive behavior influenced by threat level in the scorpion *Tityus pusillus* (Scorpiones: Buthidae)

Kamila Bento Cavalcanti de Albuquerque[1] and **André Felipe de Araujo Lira**[2]: [1]Centro de Ciências Biológicas, Universidade Federal de Pernambuco. Rua Prof. Moraes Rego S/N, Cidade Universitária, 50570-420, Recife, PE, Brazil; [2]Programa de Pós-Graduação em Ciência Animal Tropical, Departamento de Morfologia e Fisiologia Animal, Universidade Federal Rural de Pernambuco, Rua Dom Manoel de Medeiros, 52171-900, Recife, PE, Brazil; E-mail: andref.lira@gmail.com

Abstract. Scorpion venom is composed mainly of peptides and proteins and has high metabolic cost. Thus, individuals need to be economic in its use. This study aimed to evaluate the reactions of *Tityus pusillus* Pocock, 1893 scorpion to threats of different intensities. For the experiments, 28 males and 26 females were used; animals were gently touched five times on their mesosoma at different time intervals. Touches with five second intervals were considered as the high-intensity threats, while those with five-minute intervals were considered as the low-intensity threats. We found that scorpions, independently of sex, stung more often under high threat compared to low threat treatments. However, no significant differences in the defensive behavior of males and females were observed according to threat intensity. We can thus infer that regardless of sex, *T. pusillus* uses its stings and consequently venom only in situations of high threat.

Keywords: defensiveness, ethology; venom optimization hypothesis

https://doi.org/10.1636/JoA-S-20-074

Scorpions are preyed upon by more than 150 species of vertebrates and invertebrates (Mullen & Sissom 2019). Therefore, behaviors and mechanisms determining the survival of these arachnids are extremely important (Coelho et al. 2017). To reduce the likelihood of being preyed upon, scorpions employ different strategies. Of these, camouflage, which is facilitated by their similar color to the surrounding environment and avoidance of activities at times when chances of encountering predators are the highest, are classified as primary defense mechanisms (McCormick & Polis 1990; Coelho et al. 2017). If the primary mechanisms fail and the scorpion is forced to directly confront the predator, secondary mechanisms are used, such as thanatosis, metasomal autotomy, clamping with pedipalp pincers, venom spraying and finally stinging (Mattoni et al. 2015; Nisani & Hayes 2015; Coelho et al. 2017; Lira et al. 2019).

Morphological differences, such as those related to sexual dimorphism, may influence the defensive responses in these animals (Carlson et al. 2014; Lira et al. 2019). For example, female *Centruroides vittatus* (Say, 1821), are known to behave more defensively, and sting more often than males, while males, with their elongated appendages, have a higher escape velocity and reduced sting speed (Carlson et al. 2014). In addition, previous studies have shown that scorpions have control over venom use. For example, some species use venom very restrictively, using this feature only against resistant prey or when threat from a potential enemy is high (Nisani & Hayes 2011; Lira et al. 2017; Silva et al. 2019). The production and storage of venom requires high metabolic investment (Nisani et al. 2007; Morgenstern & King 2013; Evans et al. 2019). Thus, scorpions are extremely cautious in using their sting, and consequently the venom is metered through behavioral control (Inceoglu et al. 2003; Nisani et al. 2012; Silva et al. 2019). This type of mechanism in venomous animals, where venom is used only when necessary, has led to the venom optimization hypothesis. According to this hypothesis, venom has a high metabolic cost and animals use it economically (Morgenstern & King 2013; Evans et al. 2019).

A previous study on *Tityus pusillus* Pocock, 1893 behavior showed that this species exhibits a sex-based defensive strategy, with males performing more thanatosis and stinging while females performed fleeing, metasoma wagging, and stinging (Lira et al. 2019). These authors suggest that sexual dimorphism or different life strategies may explain their results. However, these authors performed their experiments with a similar threat intensity. Therefore, for expanding our knowledge about defensive strategies of scorpions, the present study aims to evaluate the defensive response of *T. pusillus* to threats of different intensities. We investigated the differences in the responses of males and females to these stimuli. We tested the following predictions: (1) during low-intensity threats, scorpions use their sting less frequently than during high-intensity threats; (2) females are more defensive than males, especially when subjected to high-intensity threats.

The scorpion species studied in this work, *T. pusillus*, is widely distributed across northeastern Brazil, and is considered to be the most abundant scorpion in the Atlantic Forest, where it predominantly occurs in the litter layers (Lira et al. 2013, 2018a). Individuals are approximately 30–35 mm long, with a reddish yellow color with brown spots over the entire body (Lourenço 2002; Lira et al. 2018b). This species exhibits sexual dimorphism (Fig. 1), with females being larger than males, while males have robust pedipalp chelae and elongated metasomal segments (Lira et al. 2018b). Females of this species can generate up to three litters per year, each consisting of 4–12 juveniles, which undergo 4–5 molts before reaching adulthood (Albuquerque & Lira 2016).

We collected 60 adult scorpions (30 males and 30 females) from an Atlantic forest remnant located in the municipality of Moreno (8°06′38.1″S; 35°06′56.4″W), Pernambuco state, Brazil. In the laboratory, the animals were maintained in individual plastic terraria (14 cm × 10 cm × 8 cm), provided with cardboard for shelter and moistened cotton wool as a water source. They were quarantined for 30 days before the start of the experiments to avoid sick animals and to detect signal of pregnancy (embryos visible through the ventral mesosoma). Scorpions were housed at 24°C ± 2°C and 80% ± 5% relative humidity, under a 12:12 h light:dark photoperiod. They were

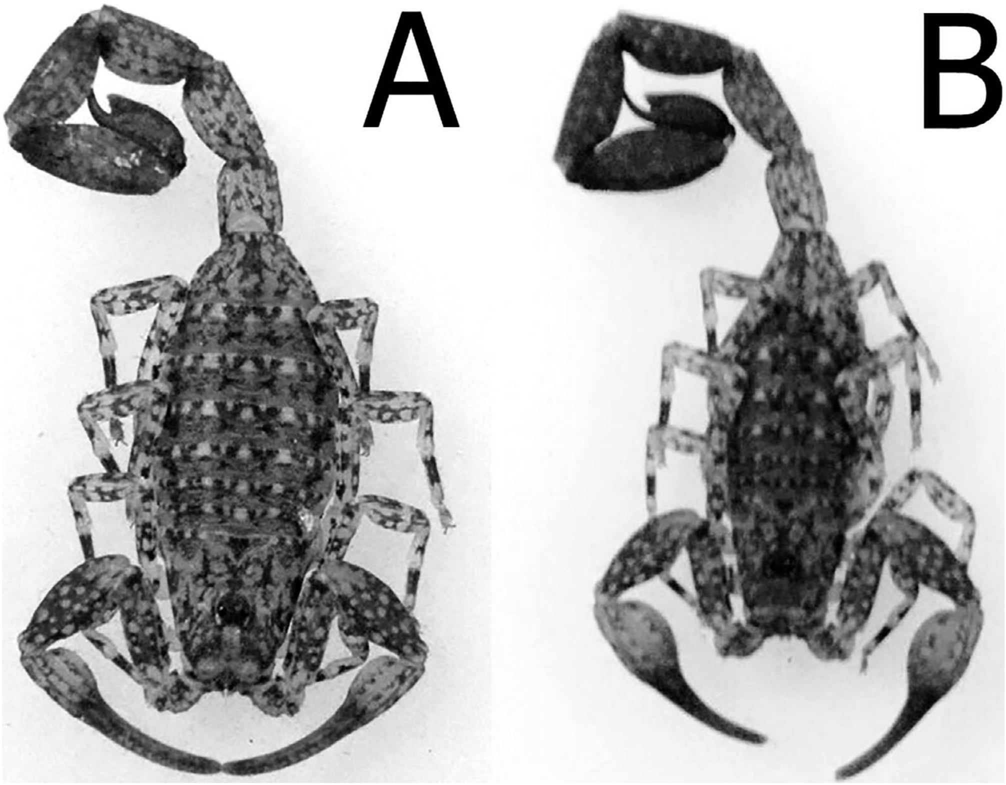

Figure 1.—Adult female (A) and male (B) individuals of *Tityus pusillus* Pocock, 1893. Photo by Hugo Neves.

fed weekly with *Nauphoeta cinerea* (Oliver, 1789) cockroach nymphs. The test animals were not fed for 7 days before the experiments.

The tests were conducted at night, between 19:00–22:00, under red light. Red light was used because scorpions cannot see this spectrum, and thus, do not exhibit any behavioral changes (Machan 1968). Individuals were first separated based on sex, and then divided into four groups, with 15 individuals each (males under high threat, females under high threat, males under low threat, and females under low threat). The experiments were performed simultaneously for each threat level (high and low). All the procedures were performed by two researchers (KBCA and AFAL). Each scorpion was used only once to avoid bias in behavior and pregnant females were excluded. For each test, the scorpions were transferred to individual circular plastic terraria (9 cm high and 14 cm in diameter) for acclimatization for 15 minutes. Behavioral tests under high and low threat were then conducted, following Nisani & Hayes (2011). The high-threat tests consisted of 5 consecutive touches (1 second of duration) on the scorpion's mesosoma within 5 seconds of each other, while in the low-threat tests, 5 mesosomal touches were performed with 5-minute intervals. The stimuli were delivered on the mesosoma, using metal tweezers with rubber on the tips. At the end of each touch, the reaction of each individual was observed. These reactions included stinging and metasoma wagging, described as the defensive behaviors of *T. pusillus* by Lira et al. (2019).

To evaluate the effects of the threat level (low vs. high and the frequency of stimulus one to five) on the defensive behavior of male and female *T. pusillus*, we used a 2×5 repeated measures ANOVA. For this analysis, the behaviors were ranked as follows: 1 = no reaction or cessation of action, 2 = metasoma wagging, and 3 = sting. They were considered as sting when the scorpion managed to insert its stinger into the rubber. The level of defensiveness between the sexes in response to the stimulus level was analyzed using a contingency table G test. In this analysis, we considered individuals that exhibited at least one sting across all five of their trials as being in the "sting" category; all other individuals were grouped in a "no sting" category. All analyses were performed using BioEstat 5.3 software (Ayres et al. 2007).

In total, behaviors of 54 individuals were observed: 30 (15 males and 15 females) under high threat, and 24 under low threat (13 males and 11 females). Six scorpions (2 males and 4 females) died during the quarantine period. Both sexes were more defensive in the high threat tests, with 86% of individuals stinging, while in the low threat tests, just over half (58.3%) of the individuals resorted to stinging. It was also observed that stinging was preceded by metasoma wagging in most cases, especially in high-intensity situations (Fig. 2), with individuals showing an increase in their defensive behaviors (males: $F_{1,120} = 32.17$; $P < 0.0001$ and females: $F_{1,100} = 10.59$; $P = 0.0019$) between high- and low-intensity stimuli. Both sexes stung more in high intensity (males = 2.92 ± 1.44 and females = 3 ± 1.67) than low-intensity (males = 1.07 ± 1.18 and females = 1.90 ± 1.92) situations.

Scorpions under high intensity situations escalated their sting behavior compared to low intensity stimuli (males: $F_{4,120} = 6.04$; $P = 0.0004$ and females: $F_{4,100} = 7.35$; $P = 0.0001$). Around 46% of the males stung on the second stimulus under high-intensity threat, while under low threat stimuli, the animals resorted to stinging only on the third trial (Fig. 2). A similar pattern was observed for females, with 53% of individuals stinging on the second trial under high-intensity threat, and 45% stinging on the third stimulus under low-intensity threat (Fig. 2). Defensive behavior levels of males and females did not differ significantly in either low threat intensity ($G_{2x2} = 0.73$; $P = 0.3901$) or high threat intensity ($G_{2x2} = 0.45$; $P = 0.4979$), where both sexes used stinging in similar way (Fig. 3).

In this study, we investigated the defensive behavior of *T. pusillus* in high- and low threat situations. Our results indicated that this species regulates the use of stinging according to the threat level to which the animal is exposed, since there was a significant difference in the defensive behavior of individuals with escalation to stinging more

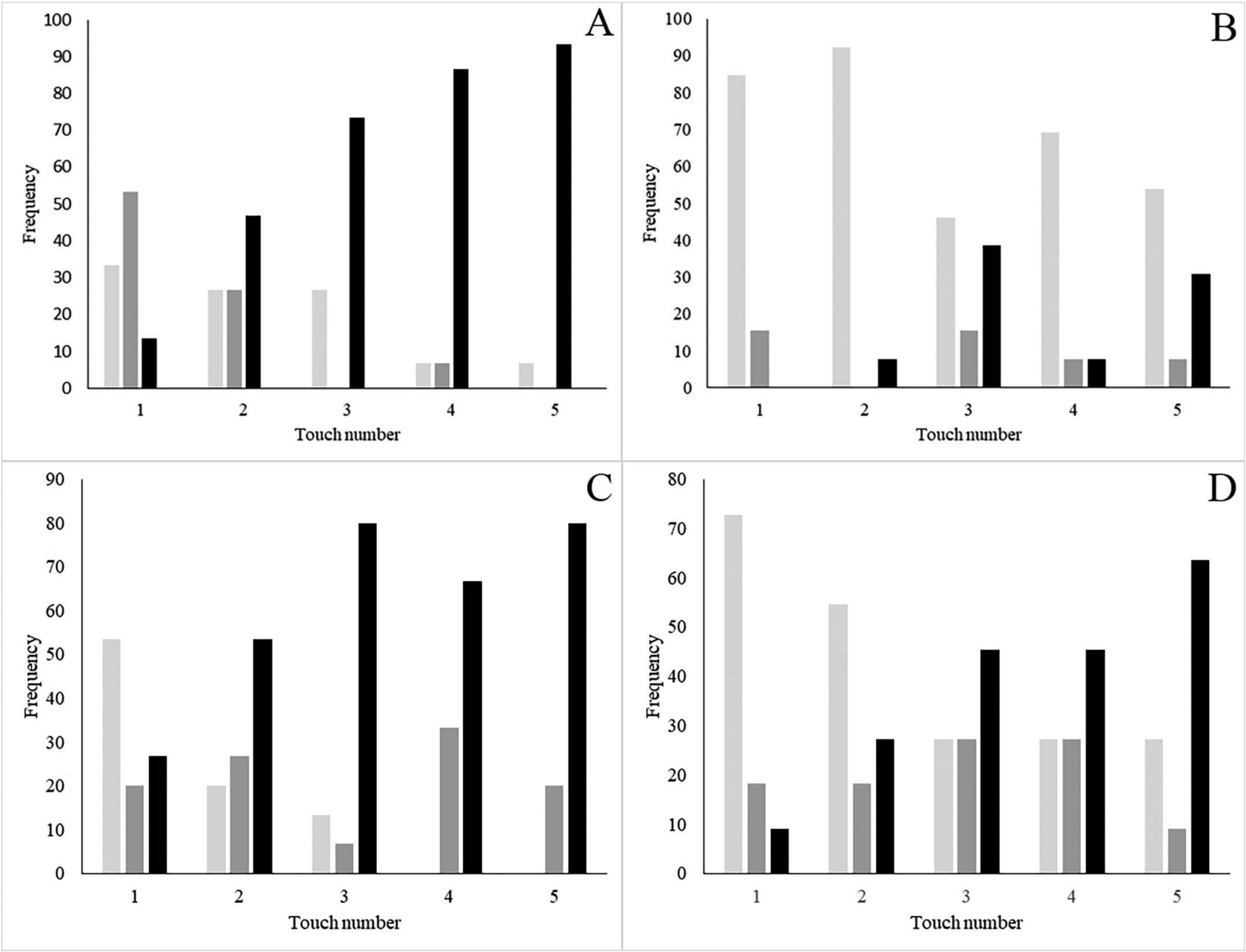

Figure 2.—Reactions of male (A, B) and female (C, D) *Tityus pusillus* to successive stimuli of high (A, C) or low (B, D) intensities. Light gray = no reaction, dark gray = metasoma wagging, black = sting.

likely during high-threat encounters. Previous studies have already reported such behavioral characteristics of scorpions exhibiting a more rapid escalation of defensive behaviors with a more persistent threat (Nisani et al. 2011; Lira et al. 2017; Rasko et al. 2018).

Tityus pusillus individuals were more defensive in the high-intensity tests, with most of the scorpions stinging at least once. Lira et al. (2017) obtained similar results for high threat tests in *Tityus stigmurus* (Thorell, 1876), where more than half of the individuals (83%) resorted to stinging. This more defensive response observed in *T. pusillus*, with a larger number of stinging individuals, is quite common in species of the Buthidae family, as they have less robust pedipalps than members of other families, such as Liochelidae and Scorpionidae, which may explain the greater use their stinger-tipped metasoma (Warburg 1998; van der Meijden et al. 2013).

Based on the sex-related defensive responses, our results showed that both male and female *T. pusillus* react in similar way, independently of threat. This reaction was previously reported for these species during prosoma touches (Lira et al. 2019). According to these authors, *T. pusillus* mostly reacted with stings, independently of sex. Therefore, our findings suggest that sexual dimorphism may not influence this species' reaction to imminent danger. This is in contrast to the observation made in *C. vittatus*, where females were found to be more defensive than males (Carlson et al. 2014). Interestingly, before stinging, individuals of both sexes performed metasoma wagging. This type of behavior has commonly been associated with reproduction (e.g., Melville et al. 2003; Gaffin & Brownell 2010; Taylor et al. 2012; Pordeus et al. 2019), although recently, Lira et al. (2019) linked such behavior to self-defense. According to these authors, this behavior can act as a warning to potential predators. Our results showing that metasoma wagging occurred during simulated encounters with predators supports this earlier suggestion.

In addition, the reluctance of *T. pusillus* to escalate to stinging under low-threat situations may be supported by the venom optimization hypothesis, where scorpions appear to use their stings only when required by the attacks of a persistent predator (Nisani et al. 2011; Lira et al. 2017; Rasko et al. 2018). In conclusion, our results suggest that *T. pusillus* individuals have the ability to modulate their reaction according to level of threat. Although animals reacted defensively at both threat levels (high and low), they exhibited this behavior more often, and stung more often in high-threat situations.

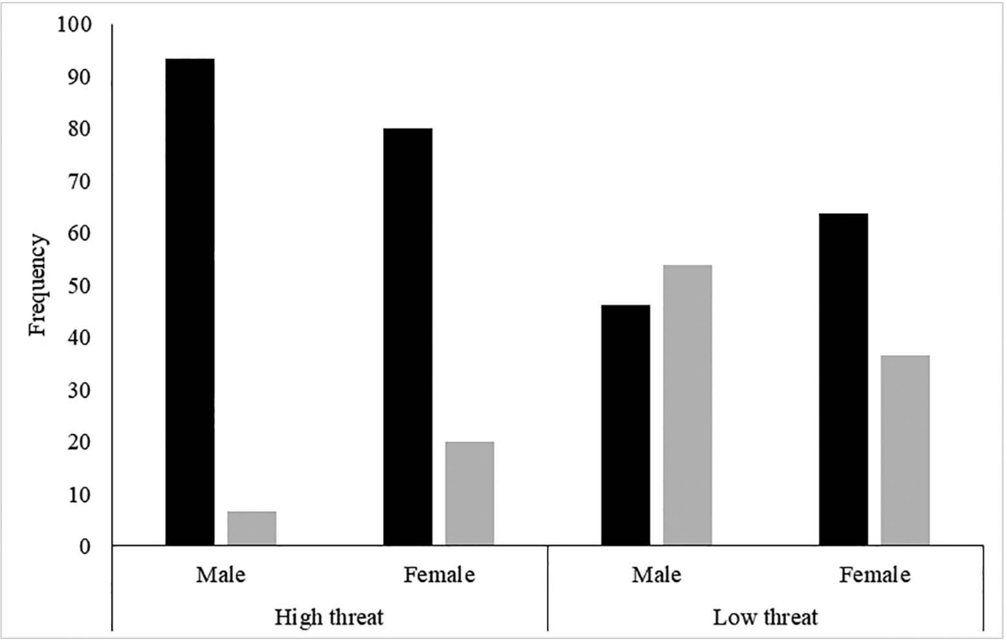

Figure 3.—Defensiveness level (%) of male and female *Tityus pusillus* under stimuli of different intensities (high or low). Sting corresponds to % of individuals exhibiting at least one sting across all five of their trials. Black = sting and gray = no sting.

ACKNOWLEDGMENTS

We are grateful to Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco (FACEPE) for granting a postdoctoral scholarship (BFP-0010-2.05/19) to AFA Lira. We are also indebted to the editor and anonymous reviewers for their comments to improve the presentation of the manuscript. We declare no conflict of interest.

LITERATURE CITED

Albuquerque CMR, Lira AFA. 2016. Insights into reproductive strategies of *Tityus* (*Archaeotityus*) *pusillus* Pocock, 1893 (Scorpiones, Buthidae). *Comptes Rendus Biologies* 339:179–184.

Ayres M, Ayres Júnior M, Ayres DL, Santos ADA. 2007. Aplicações estatísticas nas áreas das ciências biomédicas. Instituto Mamirauá, Belém, 364 p.

Carlson BE, McGinley S, Rowe MP. 2014. Meek males and fighting females: sexually-dimorphic antipredator behavior and locomotor performance is explained by morphology in bark scorpions (*Centruroides vittatus*). *PLoS ONE* 9:e97648.

Coelho P, Kaliontzopoulou A, Rasko M, van der Meijden A. 2017. A 'striking' relationship: scorpion defensive behaviour and its relation to morphology and performance. *Functional Ecology* 31:1390–1404.

Evans ERJ, Northfield TD, Daly NL, Wilson DT. 2019. Venom costs and optimization in scorpions. *Frontiers in Ecology and Evolution* 7:196.

Gaffin DD, Brownell PH. 2010. Evidence of chemical signaling in the sand scorpion, *Paruroctonus mesaensis* (Scorpionida: Vaejovida). *Ethology* 91:59–69.

Inceoglu B, Lango J, Jing J, Chen L, Doymaz F, Pessah IN, et al. 2003. One scorpion, two venoms: Prevenom of *Parabuthus transvaalicus* acts as an alternative type of venom with distinct mechanism of action. *Proceedings of the National Academy of Sciences* 100:922–927.

Lira AFA, Almeida FMF, Albuquerque CMR. 2019. Reaction under the risk of predation: effects of age and sexual plasticity on defensive behavior in scorpion *Tityus pusillus* (Scorpiones: Buthidae). *Journal of Ethology* 30:13–19.

Lira AFA, DeSouza AM, Albuquerque CMR. 2018a. Environmental variation and seasonal changes as determinants of the spatial distribution of scorpions (Arachnida: Scorpiones) in Neotropical forests. *Canadian Journal of Zoology* 96:963–972.

Lira AFA, Pordeus LM, Rego FNAA, Iannuzzi K, Albuquerque CMR. 2018b. Sexual dimorphism and reproductive behavior in the Brazilian scorpion *Tityus pusillus* (Scorpiones, Buthidae). *Invertebrate Biology* 137:221–230.

Lira AFA, Santos AB, Silva NA, Martins RD. 2017. Threat level influences the use of venom in a scorpion species, *Tityus stigmurus* (Scorpiones, Buthidae). *Acta Ethologica* 20:291–295.

Lira AFA, Souza AM, Silva FilhoAAC, Albuquerque CMR. 2013. Spatio-temporal microhabitat use by two co-occurring species of scorpions in Atlantic rainforest in Brazil. *Zoology* 116:182–185.

Lourenço. R. 2002. Scorpions of Brazil. Les editions de l'If, Paris.

Machan L. 1968. Spectral sensitivity of scorpion eyes and the possible role of shielding pigment effect. *Journal of Experimental Biology* 49:95–105.

Mattoni CI, García-Hernández S, Botero-Trujillo R, Ochoa JA, Ojanguren-Affilastro AA, Pinto-da-Rocha R, et al. 2015. Scorpion sheds 'tail' to escape: consequences and implications of autotomy in scorpions (Buthidae: *Ananteris*). *PLoS ONE* 10:e0116639.

McCormick SJ, Polis GA. 1990. Prey, predators, and parasites. Pp. 294–320. *In* The Biology of Scorpions (Polis GA, ed.). Stanford University Press, Stanford, California.

Melville JM, Tallarovic SK, Brownell PH. 2003. Evidence of mate trailing in the giant hairy desert scorpion, *Hadrurus arizonensis* (Scorpionida, Iuridae). *Journal of Insect Behavior* 16:97–115.

Morgenstern D, King GF. 2013. The venom optimization hypothesis revisited. *Toxicon* 63:120–128.

Mullen GR, Sissom WD. 2019. Scorpions (Scorpiones). Pp. 489–504. *In* Medical and Veterinary Entomology. 3rd ed. (Mullen GR, Durden LA, eds.) Academic Press.

Nisani Z, Hayes WK. 2011. Defensive stinging by *Parabuthus transvaalicus* scorpions: risk assessment and venom metering. *Animal Behaviour* 81:627–633.

Nisani Z, Hayes WK. 2015. Venom-spraying behavior of the scorpion *Parabuthus transvaalicus* (Arachnida: Buthidae). *Behavioral Processess* 115:46–52.

Nisani Z, Boskovic DS, Dunbar SG, Kelln W, Hayes WK. 2012. Investigating the chemical profile of regenerated scorpion (*Parabuthus transvaalicus*) venom in relation to metabolic cost and toxicity. *Toxicon* 60:315–323.

Nisani Z, Dunbar SK, Hayes WK. 2007. Cost of venom regeneration in *Parabuthus transvaalicus* (Arachnida: Buthidae). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* 147:509–513.

Pordeus LM, Lira AFA, Albuquerque CMR. 2019. Male courtship behavior is triggered by female chemical cues in the scorpion *Tityus pusillus* (Scorpiones: Buthidae). *Canadian Journal of Zoology* 97:1122–1125.

Rasko M, Coelho P, Simone Y, van der Meijden A. 2018. How to attack a scorpion: venom metering during a repeated attack. *Animal Behavior* 145:125–129.

Silva MA, Silva NA, Lira AFA, Martins RD. 2019. Role of venom quantity in the feeding behavior of *Jaguajir rochae* (Scorpiones: Buthidae). *Acta Ethologica* 22:99–104.

Taylor MS, Cosper CR, Gaffin DD. 2012. Behavioral evidence of pheromonal signaling in desert grassland scorpions *Paruroctonus utahensis*. *Journal of Arachnology* 40:240–244.

van der Meijden A, Coelho PL, Sousa P, Herrel A. 2013. Choose your weapon: defensive behavior is associated with morphology and performance in scorpions. *PLoS ONE* 8: e78955.

Warburg M. 1998. Qualitative and quantitative analysis of intra and interspecific behavioural patterns among scorpions. *Journal of Ethology* 16:115–121.

Manuscript received 1 October 2020, revised 31 October 2020.