SHORT COMMUNICATION

Comparison of desiccation resistance in the litter-dwelling scorpion Tityus pusillus Pocock, 1893 (Scorpiones: Buthidae) from dry and wet tropical forests

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Abstract. Water conservation is one of the major challenges encountered by terrestrial arthropods; species inhabiting dry forests are hypothesized to have adapted to dry conditions. *Tityus pusillus* Pocock,1893 is one of the most abundant scorpion species in northeastern Brazil, occurring in dry and tropical rainforests. Considering the wide distribution of *T. pusillus*, we aimed to investigate differences in desiccation resistance between populations originating from the Atlantic rainforest and the Caatinga dry forest. In this study, 40 individuals of *T. pusillus* from each of the two ecosystems were used. The two groups were separated into control and treatment groups (individuals without a water supply). Scorpions from the Atlantic rainforest had a shorter lifespan than those from the Caatinga dry forest, both in the control and treatment groups. However, the weight loss rate was higher in scorpions from the Caatinga dry forest than those from the Atlantic rainforest. In addition, Atlantic rainforest scorpions presented a lower resistance to desiccation, exhibiting a higher mortality rate than the Caatinga dry forest individuals. These results suggest that *T. pusillus* can exhibit, via phenotypic plasticity or local adaptation, a broad range of tolerances that allow it to persist in different habitats. Furthermore, our findings suggest that *T. pusillus* individuals from the Caatinga dry forest have physiological attributes that allow them to resist prolonged desiccation, which may be related to adaptations that are intrinsic to the population from the dry forest.

Keywords: Arachnida, hydric stress, ecophysiology, water deficit

https://doi.org/10.1636/JoA-S-22-018

Terrestrial arthropods are particularly vulnerable to water loss because of their relatively small body sizes (e.g., Bujan et al. 2016; Nervo et al. 2021). Therefore, desiccation resistance is a fundamental trait required for their survival, especially considering the predicted increase in the frequency and severity of droughts (IPCC 2014). Arthropods possess several mechanisms to reduce the desiccation rate, such as actively slowing water loss by closing spiracles or increasing rectal water reabsorption (Harrison et al. 2012). Another trait is body size, with larger species tending to have a lower surface area to volume ratio, more water storage, and more fat that can be converted to metabolic water (Hadley 1994).

In addition, desiccation resistance is influenced by habitat type (Chown & Klok 2011). In general, metabolic rates in populations from dry environments are lower than those in populations from more mesic environments, which results in reduced water loss under xeric conditions (e.g., Warburg et al. 1980; Robertson et al. 1982; Blanckenhorn 2018). Gefen & Ar (2004) compared four scorpion species of two families, Scorpionidae Latreille, 1802 and Buthidae C.L. Koch, 1837, from humid and dry environments in Israel and found significant differences in osmotic regulation and water control between individuals from those environments. The dynamics underlying species establishment in different ecosystems can be better understood by describing the water loss rate.

Studies on desiccation resistance in scorpions have been conducted in different species, mainly in dry regions (Hadley 1970; Withers & Smith 1993; Woodman 2008; Gefen et al. 2009). However, intraspecific analyses using species that occur in contrasting environments can provide insights into the development of behavioral or physiological adaptation strategies. In this regard, the scorpion *Tityus pusillus* Pocock, 1893 has a broad geographic distribution, occurring both in dry and wet ecosystems in the Neotropics (Lira et al. 2018), and thus is a good model for studies on desiccation resistance. In Brazil, *T. pusillus* is one of the most abundant species in the northeastern Atlantic rainforest (Lira et al. 2018), which has annual rainfall ranging from 1,800 to 3,600 mm (Brown et al. 2018). In this region, *T. pusillus* inhabits leaf litter layers (Lira et al. 2018). Additionally, this species occurs in the Caatinga dry forest vegetation, a seasonally tropical dry forest (STDF) with an annual rainfall of approximately 1,000 mm (Brazil & Porto 2010; Rito et al. 2017).

Considering the wide distribution of *T. pusillus*, we predicted that populations occurring in dry forests would have physiological adaptations to prevent desiccation compared to those living in tropical rainforests. Hence, this study compared the desiccation resistance of *T. pusillus* individuals from the Atlantic rainforest and Caatinga dry forest. Specifically, we investigated the effect of water stress on weight loss and survival. We designed an experiment to test the hypothesis that scorpions from the dry forest will present greater desiccation resistance, with a stronger capacity to control water loss, than those from the tropical rainforest. Our experimental design excluded any behavioral explanations for water loss resistance.

METHODS

Scorpion collection and maintenance.—In the Atlantic rainforest, *T. pusillus* individuals were collected from Campo de Instrução Marechal Newton Cavalcante (CIMNC) (07°50′S, 35°06′W), a semi-deciduous second-growth fragment of the Atlantic rainforest. In the Caatinga dry forest, individuals were collected from Vale do São José

Figure 1.—Sampled sites from the Caatinga dry forest (circle) and Atlantic rainforest (triangle) in Pernambuco state, Brazil.

(VSJ) (08°46′S, 36°43′W), an STDF vegetation remnant. Both sampling sites were in the Pernambuco state, Brazil (Fig. 1). The annual mean precipitation was 1,856 mm in the CIMNC and 755 mm in the VSJ (Climate-Data 2020). The scorpions were kept individually in circular plastic terraria (9.5 cm of diameter x 7.5 cm of depth) containing shelter (cardboard) and water. Each scorpion was fed cricket nymphs (*Gryllus assimilis* (Fabricius, 1775)) once prior to the experiments. Laboratory conditions were maintained at 24 ± 2 °C, 80 ± 5% relative humidity, and a 12:12 h light:dark photoperiod. The scorpions were observed daily for a week before the beginning of the trials to avoid possible sick individuals. To avoid biased results due to age, only adult individuals were used, and pregnant females were avoided (determined by the presence of visible embryos in the mesosoma).

Desiccation resistance.—We conducted experimental trials to assess the effect of hydric stress on survival and weight loss in scorpions from the Atlantic rainforest and the Caatinga dry forest. Individuals from each ecosystem were separated into the control group ($n = 20$) with water availability *ad libitum* and the treatment group ($n = 20$) without water supply. During the experiment, scorpions were kept individually in plastic terraria and were not fed to avoid them obtaining water from food. The individuals were weighed (Mettler ae 260, Delta Range®, accurate to 0.0001 g) daily until the death of all the individuals in the test group of each ecosystem. The animals were maintained under the laboratory conditions described above.

Data analysis.—Kaplan–Meier survival curves were utilized to compare the population survivorship curves (i.e., number of days those scorpions survived) in each ecosystem (Stalpers & Kaplan 2018). Since scorpions from the control groups of both ecosystems showed no mortality during the trial duration (30 days), we only compared survivorship curves between the treatment groups. Statistical significance was assessed using the Mantel–Cox test. Data were analyzed using SPSS software version 26 (IBM Corp 2019).

To test the effect of ecosystem type on weight loss, linear models (LMs) were used. Ecosystem type and scorpion groups were the predictor variables; thus, we analyzed four categorical variables: Caatinga dry forest control, Caatinga dry forest treatment, Atlantic rainforest control, and Atlantic rainforest treatment. The relative weight loss (between the initial weight and final weight) and the daily weight loss (as a proxy for water loss rate) of the individuals were the response variables. Normality of the residuals was visually analyzed with normal quantile-quantile (QQ) plots; outliers were analyzed using Cook's distance, with none found (Cook's distance < 1). The data were analyzed using R software version 4.0.3 (R Core Team 2015).

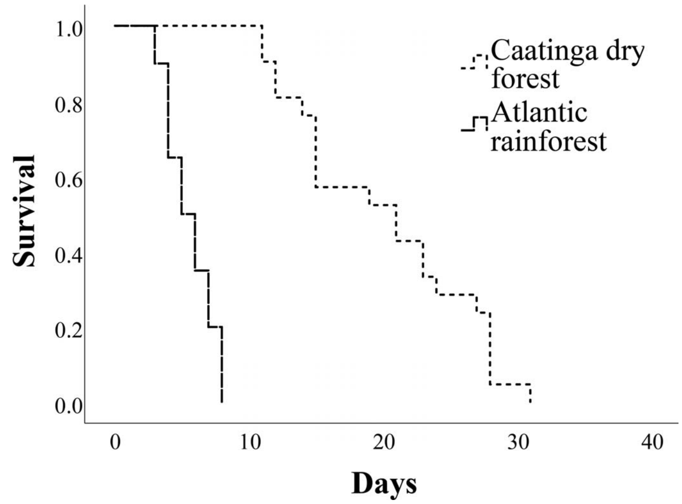

Figure 2.—Kaplan–Meier analysis of survivorship curves of *Tityus pusillus* from the Caatinga dry forest and Atlantic rainforest in the experimental treatments, in which no water was provided.

RESULTS

The lifetime of the treatment group from the Atlantic rainforest was approximately four times shorter (5.6 ± 1.7 days) than that of the Caatinga dry forest group (20 ± 6.6 days). The Caatinga dry forest scorpions had significantly higher survivorship than the Atlantic rainforest scorpions (Mantel-Cox $p < 0.01$, Fig. 2). Individuals from both the control groups exhibited no mortality during the trial. The initial weight of scorpions from the Atlantic rainforest scorpions was 0.18 ± 0.02 g, while those from the Caatinga dry forest had an initial weight of 0.14 ± 0.02 g. Comparing the initial and the final weight of the individuals analyzed, scorpions from the Caatinga dry forest had

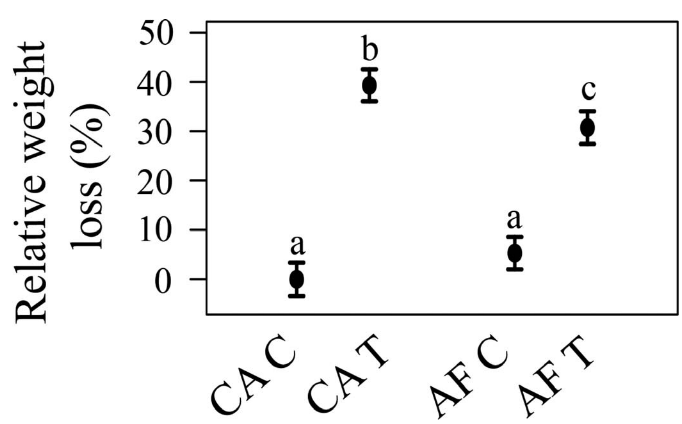

Figure 3.—Relative weight loss (mean ± 95% confidence intervals) of *Tityus pusillus* scorpions recorded at the Caatinga dry forest (CA) and Atlantic rainforest (AF) under control (C) and treatment (T) groups. Different letters indicate significant differences.

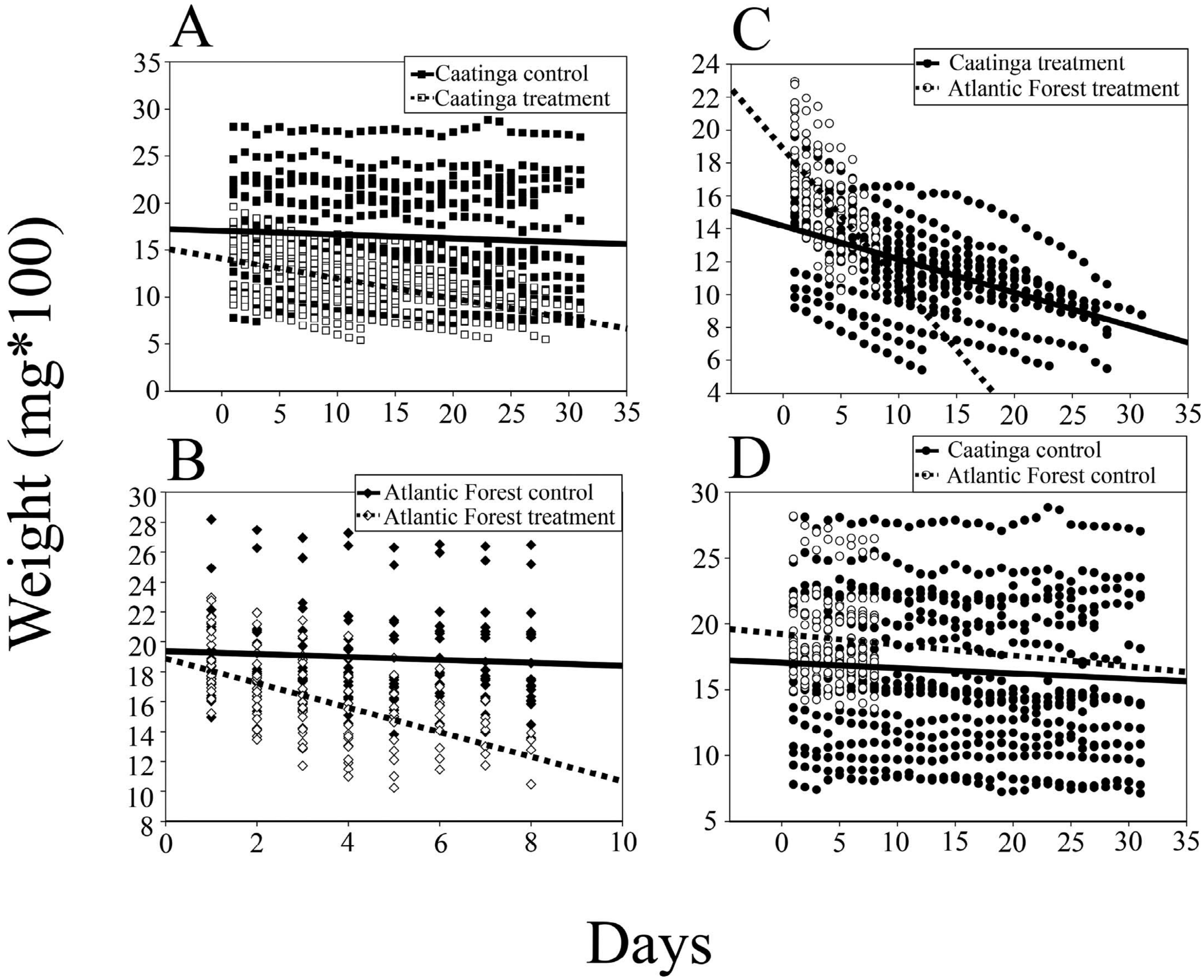

Figure 4.—Significant differences in weight loss of *Tityus pusillus* between control and treatment groups recorded from the Caatinga dry forest (A) and Atlantic rainforest (B); significant differences between the Caatinga dry forest and Atlantic rainforest in treatment (C) and control (D) groups. In each graph, symbols (squares, circles, diamonds) represent the weight of one individual. The summary lines plot the mean weight of control and treatment indibiduals (A, B) or Caatinga and Atlantic Forest individuals (C, D) each day of the experiment.

a relative weight loss approximately 10% higher (39.30 ± 6.38 %) than those from the Atlantic rainforest (30.72 ± 4.14 %). Relative weight loss did not vary between scorpion control groups of the Caatinga dry forest and Atlantic rainforest, which were lower than the weight loss of the treatment groups from both ecosystems (Fig. 3). However, the relative weight loss of the Caatinga dry forest treatment group was significantly higher than that of the Atlantic rainforest treatment ($F_{3,76} = 133.60$, $P < 0.01$, Fig. 3B). In general, all groups showed decreased individual weight throughout the experiment. Analysis of the four treatments revealed a significant difference ($F_{3,1249} = 138.29$, $P < 0.01$) in the daily weight loss of scorpions, with a steeper decline in weight in the treatment groups (Caatinga dry forest: $R^2 = 0.28$; Atlantic rainforest: $R^2 = 0.30$) than in the control groups (Caatinga dry forest: $R^2 < 0.01$; Atlantic Forest: $R^2 < 0.01$; Fig. 4A and 4B). In the statistical analysis comprising only treatment groups, a significant difference was detected between Caatinga dry forest and Atlantic rainforest scorpions ($F_{1,528} = 75.40$, $P < 0.01$), with Atlantic rainforest individuals presenting a steeper daily decrease in weight than the Caatinga dry forest individuals (Fig. 4C). The same pattern was observed in the control group ($F_{1,717}= 13.36$, $P < 0.01$; Fig. 4D).

DISCUSSION

Our study assessed desiccation resistance through weight loss and survival in *T. pusillus* individuals from contrasting habitats: the Atlantic rainforest and Caatinga dry forest. We found that individuals from the Caatinga dry forest exhibited higher desiccation resistance than the Atlantic rainforest scorpions. Individuals from the Caatinga dry forest survived without water for four times longer and lost approximately 10% more weight than those from the Atlantic rainforest. The daily weight loss related to the water balance of scorpions from the Atlantic rainforest was more distinct than that of scorpions from the Caatinga dry forest. This suggests that *T. pusillus* individuals from the Caatinga dry forests are more capable of avoiding water loss to the environment. Our results are consistent with those of previous studies showing that individuals from sites with marked climatic fluctuations (such as the Caatinga dry forest) require broader tolerance ranges and acclimation abilities than individuals from more stable habitats (such as the Atlantic rainforest) (Tieleman & Williams 2000; Cavieres & Sabat 2008).

The physiological adaptations of scorpions in response to dehydration are well known (Hadley 1970; Gefen 2008, 2011; Woodman 2008). Several species can control metabolic water production, for example, by increasing metabolic water through the catabolism of lipids and carbohydrates (Loveridge & Bursell 1975). Another strategy is to relocate water from other organs such as the hepatopancreas, the water content to compensate for the effects of desiccation (Gefen & Ar 2005). Another physiological method to avoid desiccation is to reduce water loss to the environment (Hadley 1974). During prolonged water stress, the consumption of fuel to maintain homeostasis by means of carbohydrate catabolism is known to occur in scorpions (Kalra & Gefen 2012). The conversion of fuel to produce metabolic water during prolonged dehydration is a physiological strategy found in several terrestrial arthropods, such as lipid catabolism in locusts (Hadley & Quinlan 1993), carbohydrate catabolism in *Drosophila* Fallén, 1823 flies (Marron et al. 2003), and glycogen catabolism in the scorpion *Deccanometrus bengalensis* (C. L. Koch, 1841) (Sinha & Kanungo 1967; Chown et al. 2004). These fuel sources may be used by organisms under different degrees of water stress.

The mechanistic differences found in our study may be caused by phenotypic plasticity, which allows an organism to respond quickly to environmental conditions (Merilä & Hendry 2014). Phenotypic plasticity occurs when individuals of a given genotype adjust their phenotypes according to the conditions they experience (West-Eberhard 2003). Therefore, phenotypic plasticity is a plausible mechanism for *T. pusillus*, given their wide distribution in contrasting environments (Lira et al. 2018), such as those sampled in this study. However, another non-exclusive explanation is that the differences observed arose via local adaptation, if the populations are genetically distinct. Individuals from dry forests survived with a weight lower than the minimum weight threshold needed for survival than those from the Atlantic rainforest, suggesting that the population from dry environments can use the fuel from storage tissues more efficiently to create metabolic water. This suggests that the ability to resist prolonged desiccation may be related to adaptations intrinsic to the population of dry forests. Although there is evidence suggesting that differences in water loss conditions may be phylogenetically related in scorpions (Gefen & Ar 2004), we provide evidence of intraspecific variations to resist desiccation in *T. pusillus*. In summary, our study demonstrates the effects of habitat (rainforest vs. dry forest) on desiccation resistance in arthropods, using *T. pusillus* as a model species. Individuals from the Atlantic rainforest exhibited less resistance to water loss than those from the Caatinga dry forest.

ACKNOWLEDGMENTS

We are grateful to M.Sc. Alexandre Teixeira for collection permission on his property (Sítio Serrote, Brazil), Hugo Neves for technical assistance during fieldwork and Dr. Luciana Iannuzzi by authorizing the balance use at her laboratory. We also thanks to Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco (FACEPE) to a postdoctoral scholarship (BFP 0121-2.05/20) to AFA Lira. RP Salomão was supported by Programa Nacional de Pós-doutorado/Capes (Government funds PNPD/CAPES, Brazil).

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Manuscript received 21 March 2022, revised 8 May 2022, accepted 25 July 2022.