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Insights into reproductive strategies of *Tityus* (*Archaeotityus*) *pusillus* Pocock, 1893 (Scorpiones, Buthidae)

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ABSTRACT

A remarkable diversity of life history strategies has evolved among species for achieving reproductive success, including adaptive growth, protandry, iteroparity, and extra molting. Here, we report on the reproductive strategies of the litter-dwelling scorpion, *Tityus (Archaeotityus) pusillus*, the most abundant and widespread scorpion species in the Atlantic Forest of northeastern Brazil. We observed both iteroparity and protandry reproductive strategies in this species. Females were competent to produce up to three broods after a single insemination, and no correlation between female size and litter size was observed. Most males reached adulthood 1 month before females following four molts, characterizing protandry. Nevertheless, an extra molt was observed to occur in some males ($n = 4$) and females ($n = 1$). These findings highlight the life history traits of *T. (A.) pusillus*, which may imply in reproductive success and adaptation to changes in environmental conditions.

1. Introduction

Numerous reproductive strategies have evolved in animals to maximize reproductive success, with the form of reproduction usually considered to be a characteristic of a given species [1]. To mitigate reproduction costs, females of many species have evolved specialized modes of parity, including semelparity, in which death follows a single reproduction event, or iteroparity, in which females allocate resources between several reproductive attempts [1,2]. Although semelparity and iteroparity are expressed to various degrees, offspring development appears to play a major role in determining which strategy to follow. Life-history theory postulates that low mortality of juveniles throughout development would favor semelparity, whereas high rates of juvenile mortality will lead to iteroparity [3–5]. It has also been suggested that semelparous organisms may have opportunities to produce a second brood if they should lose the first set of offspring [6–8] or owing to the high cost of female investment, for instance, due to maternal care [9–15]. Moreover, in some animal populations, both modes of parity have been maintained within populations [7,16]. Therefore, the distinction between semelparity and iteroparity is often ambiguous, and dependent on such factors as availability of food resources [6] and environmental stability [17], time of hatching, season in species, particularly in those that can reproduce twice or more per year [18].

To reduce the reproductive cost, females reproducing through iteroparity face an additional conflict about how to increase offspring fitness while maintaining a low metabolic cost, which is essential for the survival of females. Numerous studies have shown that to reduce reproductive costs, females tend to produce either large

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litters of small young or small litters of large young [19–28]. Such complexity makes it difficult to discern the female reproductive strategy in many animal groups. Males have also evolved specialized reproductive strategies. In many species, males emerge before females, a phenomenon known as protandry [29,30], the opposite of which is protogyny (females emerging before males). The prevalence and adaptive significance of protandry has been the subject of a long-standing debate [29,31,32], with many authors assuming that males may benefit from earlier adult emergence by increasing their potential to fertilize virgin females [33–36] or by increasing overall reproductive success mating with young and old females [37]. Such concern is particularly important for species with low-density populations, given that population growth can be negatively impacted due to the difficulties of locating mates in both space and time [38].

Scorpions are ancient arthropods that can be found in most terrestrial habitats. They are viviparous, with females providing maternal care until the offspring's first molt or longer [3,39,40]. Iteroparity is the predominant mode of parity in scorpions [2,41–43], and a special type of iteroparity in which females give birth numerous times without need of additional inseminations has also been observed in many species [44–47], but semelparity and protandry have also been observed [2,48–57], although semelparity has been recorded only once, for the species *Bothriurus bonariensis* (C.L. Koch, 1842) [48]. On the other hand, studies on the life histories of scorpions, such as offspring development time, which may influence reproduction strategy [7], is usually problematic due to the relatively long lives of these animals compared to other terrestrial arthropods, as well as the difficulty of rearing them in the laboratory, as scorpions often suffer high rates of mortality at times of molt [49–51]. Most of the information on scorpion reproduction and development has been collected from species inhabiting sub-tropical areas; comparatively, little is known about the reproductive strategies of scorpions inhabiting tropical forests from South America. Although often neglected, reproductive strategies of species in tropical forest ecosystems have equally important consequences, given that, as important predators, scorpions can influence the structure of natural communities in terrestrial ecosystems and therefore influence the energetic flow through a system [37]. As in other animals, for most scorpions, the reproductive strategies of both females and males determine reproductive success, and together with offspring development times and fitness, contribute to shape population patterns and abundance [2,52,53].

Here, we examine the reproductive strategies of both male and female *Tityus* (*Archaeotityus*) *pusillus* Pocock, 1893, the most abundant and widespread scorpion species in the northeast Brazilian Atlantic Forest [54–56], with the goal of obtaining insights into the life history of this species. Reproductive strategies were focused on the mode of parity (semelparity/iteroparity), reproductive investment (relationship between female size and offspring size) and sexual timing of emergence (protogyny/protandry). To elucidate patterns associated with postembryonic development, the number of molts, intermolt interval, and synchrony/asynchrony molting were analyzed. In addition, growth increments in successive molts were observed, considering Dyar's rule [57], a model in which the growth of the sclerotized structures assumes geometric progression, and this has been effectively applied in many arthropods, such as moths and butterflies [58–60], beetles [61–63], spiders [64–66], and scorpions [67–69]. Knowledge of the biology of species inhabiting the Brazilian Atlantic Forest may help to understand the ecological patterns of the organisms and serves as a basis for future research on species conservation.

2. Materials and methods

2.1. Reproductive strategies

2.1.1. Mode of parity and female reproductive investment

Eighteen pregnant *T.* (*A.*) *pusillus* (Fig. 1) females were collected in November and December 2009 in the "Centro de Instrução Marechal Newton Cavalcanti", a military area composed of a 6,280-ha fragment of semi-deciduous seasonal Atlantic Forest (07°46'55" S, 35°09'02" W), in the state of Pernambuco, in the northeast of Brazil.

All scorpions were kept individually in plastic terrariums (14 cm × 10 cm × 8 cm) and observed over a year for single (semelparity) or multiple (iteroparity) offspring events. Animals were kept in the laboratory at a mean temperature of 24 °C ± 2 °C and 70% ± 5% relative humidity, and a 12:12 h light/dark photoperiod. Cardboard was provided for shelter and cotton wool as a source of water to each terrarium, and females were fed weekly with nymphs of the cockroach *Periplaneta americana* (Linnaeus, 1758), except when carrying their offspring. The terrariums were checked daily 5 days a week, for the occurrence of births.

Offspring number was determined by counting live individuals on the female's dorsum. Offspring number was correlated with female size as a measure of reproductive investment. For female size, the length of the carapace was measured using a digital caliper at the end of the experiments.

2.1.2. Sexual timing of emergence

The number of molts, the intermolt interval and synchrony/asynchrony molting were recorded for 77

Fig. 1. Adult female of the scorpion *Tityus* (*Archaeotityus*) *pusillus*.

laboratory-reared juveniles from the 18 females, which were transferred to separate transparent plastic boxes (15 cm × 15 cm × 20 cm) upon molting to the second instar. Juveniles were fed three times weekly with *P. americana* nymphs reared in the laboratory, and water and shelter were made available to the juvenile scorpions. Sex identification was performed after reaching adulthood, based on the method described by Lourenço [70].

2.1.3. Growth increments

Size variation was evaluated at successive instars to estimate the ratio of growth increments. Developmental growth was estimated based on the growth factor (Dyar's constant), calculated by dividing the linear size measure of one instar by the size measure of the previous instar in each individual [57]. Measurements were performed using dead individuals or exuvia. The length of heavily sclerotized structures, such as carapace (CL), metasomal segment V (Met V), and movable finger (MF) [70–72], were recorded by a single researcher using digital calipers under a stereomicroscopic. These factors were used as the parameters for growth estimation. Voucher specimens were deposited in the Arachnological Collection of the Universidade Federal de Pernambuco (Curator Dra. Cleide Albuquerque), Brazil.

2.2. Data analyses

Linear correlation (Pearson's correlation) was performed to examine the relationship between offspring size and female size, with significance tested via a *t*-test. All statistical analyses were performed with Biostat v. 5.3 software [73].

3. Results

3.1. Mode of parity and female reproductive investment

Tityus (*Archaeotityus*) *pusillus* most commonly exhibited iteroparity as the mode of parity, with 72% of females giving birth once per year and 28% giving birth a second time 4 to 7 months after the first brood. One female produced three litters within 6 months, with a shorter (one month) interval between the second and third litter (Table 1). Offspring size averaged 7.61 ± 1.74 individuals, ranging from four to 12 specimens, with no significant correlation between female size and offspring number (Pearson's correlation, $r = 0.2676$, $P > 0.05$). Despite scorpion females use to lay unfertilized eggs, in our observation this event was not observed and if it happened, eggs were not considered in our sample. Based on carapace length, female size ranged from 3.55 mm to 4.19 mm (mean: 3.85 mm ± 0.22 mm). Females giving birth once in a year were slightly shorter in length (3.84 mm ± 0.24 mm) than females producing multiple broods (3.88 mm ± 0.18 mm). Litter size was similar amongst broods (Table 1).

3.2. Sexual timing of emergence

Two reproductive strategies, protandry and extra molt, were recorded in *T.* (*A.*) *pusillus* specimens. Most males (85.71%) reached adulthood one month (217.50 days ± 108.80 days) before females (231.16 days ± 106.32 days) (Table 2) following four molts, with five individuals performing an extra molt (4 males and 1 female). Postembryonic development time averaged 424.66 days ± 172.30 days for males that performed five molts before reaching adulthood. One female also reached maturity following five molts, 656 days after birth (Table 2). The overall mortality rate was 20.77%, with the majority of deaths occurring in the fourth instar (14.28%); mortality rates for the second and third instars was 5.19% and 1.29%, respectively. The sex-ratio was close to 1:1 (33 females and 28 males).

3.3. Growth increments

Morphological measurements and growth factor (Dyar's ratio) for all instars, with the exception of first instars, for both males and females are presented in Table 3. An overlap in the extremes of measurements between instars was observed for all structures analyzed in both sexes (Table 3). Among the five juveniles that underwent five molts before adulthood, males were larger (3.47 mm ± 0.35 mm) than four-molt males (3.23 mm ± 0.30 mm).

4. Discussion

Both iteroparity and protandry were observed to be reproductive strategies of the litter-dwelling scorpion *Tityus* (*Archaeotityus*) *pusillus*. Despite being able to mate several times in their lifetimes, females produced up to three separate broods following a single insemination by storing sperm. Most males required a shorter developmental time to reach adulthood than females did.

Table 1
Offspring litter size and birth interval of sequential broods produced by different *Tityus* (*Archaeotityus*) *pusillus* females.

Females	1st brood		2nd brood		3rd brood	
	Litter size	Date	Litter size	Date	Litter size	Date
I	7	11.22.2009	10	03.20.2010	–	–
II	8	11.23.2009	8	04.02.2010	7	05.28.2010
III	6	11.30.2009	5	03.26.2010	–	–
IV	8	12.09.2009	4	03.26.2010	–	–
V	8	12.09.2009	6	07.14.2010	–	–

Table 2
Difference in the development period of male and female *Tityus* (*Archaeotityus*) *pusillus*.

Developmental stage	Duration (Days)	
	Males	Females
Instar I ($n = 77$)	3.17 ± 0.77	3.34 ± 0.96
Instar II ($n = 73$)	52.75 ± 20.19	56.92 ± 21.53
Instar III ($n = 72$)	61.85 ± 36.54	61.63 ± 31.77
Instar IV ($n = 61$)	93.76 ± 66.16	120.93 ± 80.43
Instar V (adult) ($n = 5$)	173.00 ± 93.48	439

Table 3
Variation in growth rate of different instars of male and female *Tityus* (*Archaeotityus*) *pusillus*. Mean ± sd (mm) of carapace, metasoma V segment and movable finger.

	Structures measured			
	Carapace	Movable finger	Seg. V metasoma	Dyar's constant
Males				
Instar II	1.61 ± 0.19	1.70 ± 0.20	1.66 ± 0.17	–
Instar III	2.17 ± 0.18	2.30 ± 0.18	2.26 ± 0.17	1.36/1.32/1.36
Instar IV	2.70 ± 0.22	3.00 ± 0.32	2.88 ± 0.32	1.25/1.30/1.28
Instar V(adult)	3.23 ± 0.30	3.56 ± 0.28	4.00 ± 0.48	1.19/1.19/1.38
Instar VI(adult)	3.47 ± 0.35	3.75 ± 0.36	4.35 ± 0.23	1.19/1.16/1.35
Females				
Instar II	1.71 ± 0.18	1.75 ± 0.14	1.63 ± 0.15	–
Instar III	2.17 ± 0.15	2.31 ± 0.19	2.24 ± 0.18	1.28/1.32/1.32
Instar IV	2.76 ± 0.25	2.95 ± 0.24	2.94 ± 0.26	1.28/1.29/1.32
Instar V(adult)	3.31 ± 0.33	3.63 ± 0.31	3.71 ± 0.38	1.21/1.23/1.27
Instar VI (adult)	3.60	3.50	4	–

Iteroparity is a common reproductive strategy in scorpions [2,37,74] as it is an advantageous approach for species whose breeding success is influenced by environmental changes [37]; for example, the ability to produce broods repeatedly protects against reproductive failure that may result when a resource becomes limiting [37]. In addition to iteroparity, deferred fertilization through sperm storage may increase female fitness, allowing for the generation of new lineages throughout the year, as suggested for other buthid scorpion species within the genera *Tityus*, *Centruroides*, *Isometrus*, and *Rhopalurus* [41,43,44,70]. This phenomenon may have evolved in some species within those genera because mature males are relatively less frequent than females [37], as is also the case for *T.* (*A.*) *pusillus*, where males are outnumbered by females in ratios of 1:4 in their natural habitat [75]. Higher mortality rates associated with the behavior of males, which are more active than females, and therefore are exposed to higher predation, may contribute to the fact that females are outnumbered [37]. Thus, deferred fertilization provides additional opportunities to produce offspring when the chances of a new mating are low due to the small number of males.

Instead, differential sex ratios may favor protandry by reducing competition among males for mating opportunities, as predicted by the mate opportunity hypothesis [76]. The shorter development times for males than for females characterizing the mechanism of protandry in *T.* (*A.*) *pusillus* has not been properly described in scorpions, although in other arthropods, such as insects and spiders, it is a common event [77,78]. A shorter development time involves more rapid morphological and physiological changes that may occur during any stage of development before reaching adulthood; in *T.* (*A.*) *pusillus*, these changes mainly occurred during the subadult instar.

The number of molts necessary to reach adulthood in *T.* (*A.*) *pusillus* (four or five) was the same as for other *Tityus* species (four to six) [67,79–84]. Although the majority of the specimens reached adulthood after four molts, 8.19% individuals become adults following an extra molt, resulting in larger than normal individuals. This phenomenon was observed for both sexes, but was more frequent in males than in females. The presence of both small and large adults has previously been observed in the savannicolous species *Tityus* (*Tityus*) *fasciolatus* Pessôa, 1935 and the Amazonian species *Tityus* (*Atreus*) *neblina* Lourenço, 2008 [71,83]. To our knowledge, however, this is the first report of an extra molt in scorpions from the Atlantic Forest.

The extra molt in *T.* (*A.*) *pusillus* is likely to be the main factor influencing the wide variation in postembryonic development time (7–14 months) compared to other species of similar size from the genus *Tityus*, such as *Tityus* (*Archaeotityus*) *mattogrossensis* Borelli, 1901 (13 months), *Tityus* (*Archaeotityus*) *bastosi* Lourenço, 1984 (11–12 months), *Tityus* (*Archaeotityus*) *ocelote* (7.5 months) Francke and Stockwell, 1987, and *Tityus* (*Archaeotityus*) *columbianus* (12 months) (Thorell, 1876) [67,71,85,86]. As for other taxa, an extra molt may be representative of adaptive growth, assuming that individuals make strategic decisions about how much to grow given the essential tradeoffs between growth and mortality risk, and between achieving large size and early emergence [77]. In scorpions, the extra molt is particularly evident in buthids, although it has been reported for others families as well [37]. In the euscorpiid *Euscorpius flavicaudis* De Geer, 1778, mature males occur in two size classes, small and large, depending on their molt number. Small males mature earlier than larger individuals and experience an extra mating season, whereas larger males display greater reproductive success [87]. Thus, it is probable that *T.* (*A.*) *pusillus* males that performed an extra molt have a reproductive advantage over males that do not. However, given that male scorpions must actively search out females during the breeding season, placing them at greater risk of predation [37], larger males would be at greater risk than smaller males, and therefore the costs and benefits of an extra molt depend on environmental conditions, as in some years, large males may do outperform small males and vice versa. Thus, the low rate of individuals undergoing an extra molt may be explained by the higher mortality risk of males and shorter developmental time of smaller males when competition (others males) is low, thereby maximizing the chances of finding one or more partners during the breeding season.

The size of females is usually a key factor in determining the number of offspring produced in a single litter, with, in general, larger females producing a higher number of juveniles [43,52,53,88–92]. However, this does not appear to be the case for *T.* (*A.*) *pusillus*, for which no significant relationship between litter size and female body size was observed, in contrast to *Tityus* (*Tityus*) *bahiensis* (Perty, 1833) and *Tityus* (*Archaeotityus*) *silvestris* Pocock, 1897 [43]. According to Warburg [93], in such cases, the physiological condition of the individual would be a more relevant factor in determining litter size than would be body size. Lourenço [94] reported a litter size of 10 for *T.* (*A.*) *pusillus*, but only observed a single litter of a single female. Our results expand on this knowledge by reporting data for 18 females, with average litter sizes of 4–12 offspring, lower than those observed in most *Tityus* species, but similar to the values observed for species of the subgenus *Archaeotityus* [43]. Similarity in morphometric growth values for male and female scorpions has been described for various *Tityus* species [67,70–72,83,94]. Although there was an overall similarity between the sexes of *T.* (*A.*) *pusillus*, the Met V in the adult stage (after 4 or 5 molts) was larger in males than in females. According to Lourenço [70], the Met V is a sexually dimorphic characteristic for this species.

In conclusion, the present study describes the iteroparity and protandry reproductive strategies of the litter-dwelling scorpion *Tityus* (*Archaeotityus*) *pusillus*, in addition to the deferred fertilization that has been recorded in this scorpion species. These reproductive strategies may have evolved in response to the uneven sex ratios of *T.* (*A.*) *pusillus* populations, in which females greatly outnumber males, as a means of ensuring offspring production. An extra molt stage was also observed for *T.* (*A.*) *pusillus*, and as this was more common in males than females, it suggests that growth rate plays a role in maintaining protandry and sexual size dimorphism as adaptative phenotypic plasticity in *T.* (*A.*) *pusillus* populations, representing on the phenotypic level, the capacity of an organism to adjust to environmental changes.

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