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Growth and developmental time in the parthenogenetic scorpion *Tityus stigmurus* (Thorell, 1876) (Scorpiones: Buthidae)

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ABSTRACT. This study describes variation in ontogenetic development of the parthenogenetic scorpion *Tityus stigmurus* based on variations in size, intermolt period, and number of instars. Individuals were created under laboratory conditions (28 ± 3ºC) and subjected to similar diet regime. Comparative measurements of carapace, segment V of the metasoma, and the movable finger are used to estimate variations in size among instars. The application of Dyar rule shows a growth factor from 1.14 to 1.38 depending on the parameter analysed with intense variations in size between and within the different instars. The duration of the period from birth to adulthood was 871 days, after six moults. These data suggest that *T. stigmurus* has the longest post-embryonic development of *Tityus* genus and the existence of an extra molt to reach maturity.

Keywords: post-embryonic development, size, intermolt period, parthenogenesis.

Crescimento e tempo de desenvolvimento do escorpião partenogenético *Tityus stigmurus* (Thorell, 1876) (Scorpiones: Buthidae)

RESUMO. Este estudo descreve a variação no desenvolvimento ontogenético do escorpião partenogenético *Tityus stigmurus* com base nas variações de tamanho, período intermuda e número de instars. Os indivíduos foram criados em condições ambientais de laboratório (28 ± 3ºC) e sob o mesmo regime alimentar. Medidas comparativas do prossoma, quinto segmento do metassoma e dedo móvel foram utilizadas para estimativa de variações no tamanho entre os instars. A aplicação da lei de Dyar apresentou um fator de crescimento de 1,14 a 1,38 dependendo do parâmetro analisado, com intensas variações de tamanho entre e dentro dos diferentes instars. A duração do período compreendido entre o nascimento até a idade adulta foi de 871 dias, após seis ecdises. Esses dados sugerem que *T. stigmurus* possui o desenvolvimento pós embrionário mais longo dentre as espécies do gênero *Tityus* e a existência de uma muda extra para alcançar a maturidade.

Palavras chave: desenvolvimento pós-embrionário, tamanho, período intermuda, partenogênese.

Introduction

To understand the dynamics of arthropod populations, it is important to determine life-history characteristics that contribute to reproductive success. Juvenile growth rate and development time are key determinates of organism survival, size, age of maturity and adult reproduction. These factors are also important for understanding the dynamics of arthropod populations (Kleinteich & Schneider, 2011; Roff, 1992; Stearns, 1992). For most animals, variations in growth rates and developmental time have mainly been related to differences in the interaction between the individual genome and in sensitivity to environmental changes (Dmitriew, 2011; Fusco & Minelli, 2010).

Amongst the 1900 scorpion species (Stockmann & Ythier, 2010), parthenogenesis has been reported in approximately eight species (Francke, 2007; Ross, 2010). Seven of these species belong to the genus *Tityus* C. L. Koch, 1836 (Ross, 2010; Schneider & Cella, 2010), which is the largest genus of the Buthidae family, widely distributed across the Americas, from Costa Rica (Francke & Stocwell, 1987) to Central Argentina (Ojanguren Affilastro, 2005) and the Dominican Republic (Armas & Abud Antun, 2004).

Despite medical interest in the genus *Tityus*, which includes scorpions responsible for many deaths and injuries in the tropics each year (Albuquerque, Santa Neto, Amorim, & Pires, 2013; Chippaux & Goyffon, 2008; Stockmann & Ythier, 2010), little information is available about the life history of many species (Lourenço, 1979a, b, 2002, 2007, 2008; Lourenço & Eickstedt, 1988; Lourenço & Cloudsley-Thompson, 1998, 2010; Matthiesen, 1971; Rouaud, Huber, & Lourenço, 2002), especially with regard to growth pattern. Scorpions grow at different rates, which means that adults are capable of producing offspring at highly variable times. Post-birth development (PBD) has been studied in only 22 species, and a large variation in PBD duration (6 to 85 months), with four to eight moltings prior to adulthood was observed (Francke, 1984; Lourenço, Andrzejewski & Cloudsley-Thompson, 2003; Lourenço, Huber & Cloudsley-Thompson, 1999; Lourenço, Ythier & Cloudsley-Thompson, 2008; Polis & Sissom, 1990; Quijano-Ravell, Ponce-Saavedra, & Francke, 2011; Rouaud et al., 2002; Seiter, 2012).

Tityus stigmurus (Thorell 1876) is a parthenogenetic scorpion (Ross, 2010) widely spread across urban areas of northeastern Brazil (Lira-da-Silva, Amorim, & Brazil, 2000; Ministério da saúde [Brasil], 2009), a region inhabited by over 53 million people (Instituto Brasileiro de Geografia e Estatística. Brasília [IBGE], 2015). It is the scorpion that possess the most significant health threat in this region, being responsible for most scorpion stings, including fatal cases (Albuquerque et al., 2013). The aim of this study was to determine the life cycle and intermolt period of *T. stigmurus*. Knowledge of the natural history of this scorpion is fundamental to gaining a better understanding of the eco-epidemiology of human accidents.

Material and methods

Post birth developmental time and intermolt period

Female scorpions were collected at pre-adult stage from the urban area of the city of Recife (08º03'03"S 34º56'54"W), in Pernambuco state, which has around 1.5 million inhabitants (IBGE, 2015). *Tityus stigmurus* females were reared individually in plastic boxes (15 ×15 × 20 cm). Instars were obtained from the offspring of six females, kept in the laboratory. The juveniles were reared at 28 ± 3°C mean temperature, 80 ± 5% relative humidity and a 12:12h light/dark photoperiod, following Aguiar, Santana-Neto, Souza, and Albuquerque (2008).

The total post-birth developmental time (duration from birth to the beginning of the adult stage), number of instars, and intermolt period at each instar were initially determined using 36 specimens. Each scorpion was fed American cockroach *Periplaneta americana* (Linnaeus, 1758) nymphs reared in the laboratory, three times per week. Plastic boxes were checked once a day for five days per week to monitor moulting. Water and shelter were made available to the scorpions.

Morphometric growth

To assess growth rate, specimens from each instar were used. Measurements were performed in exuvia and live subjects were immobilized in a Petri dish with cotton and isoprene. Lengths of heavily sclerotized structures, such as carapace (CL), metasomal segment V (Met V), and movable finger (MF) were measured by Lira, AFA using a digital calliper under a stereo-microscope to avoid discrepancies in parameter measurements, and these were used as a basis for growth estimation (Lourenço, 1979a, b; 2002). The soft exoskeletons of the first instar specimens were very difficult to measure precisely; therefore, measurements were performed using animals after their first instar.

Due to a high mortality rate and consequently small sample size, additional measurements were obtained using scorpions reared in similar conditions but with uncontrolled feeding regimes: 20 specimens in the third instar, 13 in the fourth instar, four in the fifth instar, three in the sixth instar, and 20 adults.

Statistical analysis

Developmental growth was estimated based on the growth factor (Dyar's constant), calculated by dividing the linear size measurement of one instar by the size measurement of the previous instar for each specimen (Dyar, 1890). An Analysis of Covariance (Ancova) was performed to confirm difference in slope (experimental and colony). The slope of each line was used as an index for the growth rate of each individual nymph. Statistical analysis was performed using *Statistica v.7* software, Data analysis software system (2004). The available voucher material was deposited in the Arachnology Collection of the Universidade Federal de Pernambuco, Brazil.

Results

The only *T. stigmurus* specimen that reached the adult stage underwent six moults and became an adult 871 days after birth. The average durations of each postembryonic instar (first to sixth) are presented in Table 1.

Table 1. Intermolt period in days and cumulative age (mean ± SD) of the parthenogenetic scorpion *T. stigmurus* (Thorell, 1876) reared in controlled conditions.

Developmental Stage	Duration	Cumulative Age
First instar (n=40)	3.90 ± 0.70	3.90 ± 0.70
Secund instar (n=16)	60.80 ± 14.19	64.40 ± 14.00
Third instar (n=10)	103.80 ± 19.24	165.60 ± 17.37
Fourth instar (n=6)	216.33 ± 94.24	382.83 ±107.22
Fifth instar (n=6)	261.00 ±78.68	672.25 ± 68.00
Sixth instar (n=1)	186	871

The first molt took place on the dorsum of the mother three to four days after birth, and this juveniles starting dispersion in the next one or two days. Mortality was high in the second instar for juveniles, totalling 44.4% of the sample. Unlike for the first instar, there was a noticeable variation in the duration of the second instar (36 to 89 days). Variation in the total duration of the third instar (Coefficient of variation = 18.5%) was lower than in the second instar (Coefficient of variation = 23.3%), ranging from 87 to 138 days. The mortality rate in the third instar was approximately 38%.

Only five of the 10 specimens that reached the fourth instar survived to the fifth instar. The time spent in the fourth instar for three of the five specimens averaged 148 days, and the remaining two moulted approximately 180 days later. Four of the five surviving specimens reached the sixth instar; one matured during the seventh instar, after spending 186 days in the sixth instar.

Morphological measurements and the growth factors (Dyar's ratio) for all the instars are presented in Table 2.

Table 2. Morphological measurements in millimetres (mean ± SD) and growth factors (mean ± SD) for the three corporeal body structures during in different instars of *T. stigmurus* (Thorell, 1876) during in different instars.

Developmental stages	Structures measured		
	Carapace length	Metasomal segment V	Movable finger
Second instar	2.39 ± 0.08	2.39 ± 0.16	2.82 ± 0.24
Growth fator	1.15 ± 0.06	1.18 ± 0.08	1.23 ± 0.08
Third instar	2.79 ± 0.14	2.91 ± 0.21	3.51 ± 0.17
Growth fator	1.02 ± 0.30	1.08 ± 0.16	1.01 ± 0.30
Fourth instar	3.71 ± 0.38	4.04 ± 0.41	4.68 ± 0.39
Growth fator	1.02 ± 0.19	1.03 ± 0.19	1.01 ± 0.20
Fifth instar	4.31 ± 0.38	4.79 ± 0.45	5.53 ± 0.30
Growth fator	1.07 ± 0.18	1.04 ± 0.16	1.03±0.18
Sixth instar	5.07 ± 0.23	5.54 ± 0.27	6.44 ± 0.25
Groth fator	0.90 ± 0.22	0.93 ± 0.27	0.97 ± 0.18
Adult	6.36 ± 0.30	7.41 ± 0.29	7.62 ± 0.35

Overlapping in measurement extremes between instars was registered for all structures analysed from both populations (Figure 1).

Analyses of covariance for the three structures measured (Met V (F = 1.5098, d.f. = 1.75, p = 0.2209), MF (F = 1.4911, d.f. = 1.74, p= 0.2239 and CL (F = 0.0028, d.f.= 1.75 p = 0.9571)) showed that scorpions from the colony and the experimental group did not differ, therefore permitting their use as complementary data.

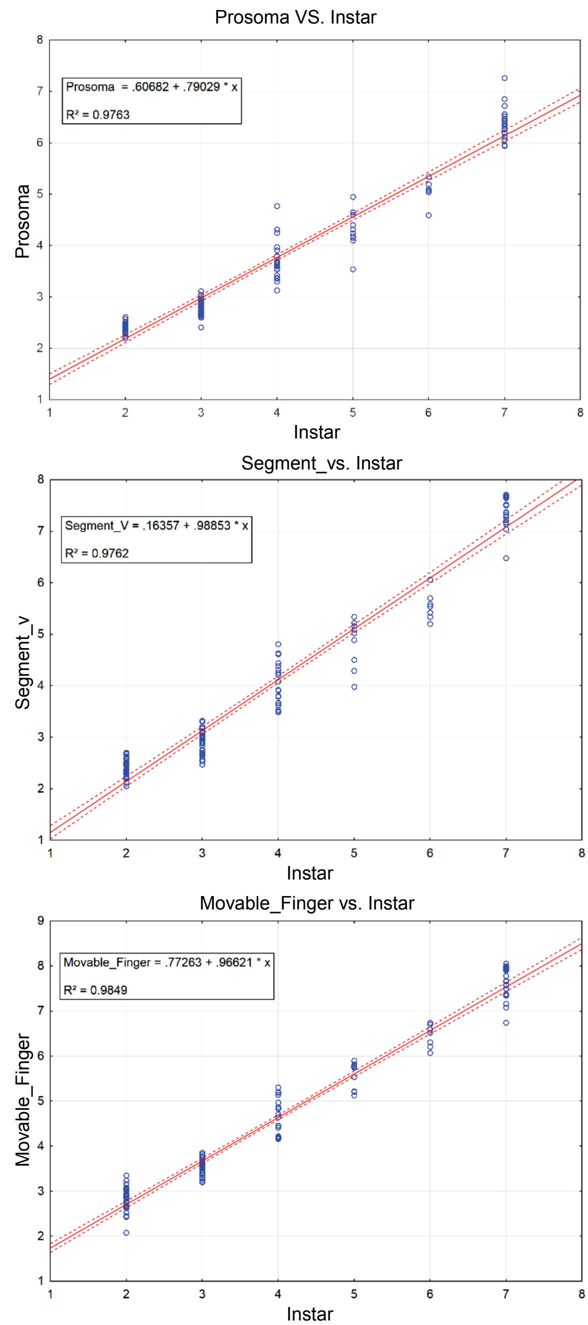

Figure 1. Overlapping size (in mm) in on carapace length, movable finger, V metasomal segment, and instars among individuals of *T. stigmurus* specimens (Thorell, 1876).

Discussion

Studies on scorpion life cycles have shown that their post-embryonic development is usually long (Lourenço, 2002; Polis & Sissom, 1990) with a relatively high juvenile mortality rate (Brown, 1997; Francke & Jones, 1982; Sissom & Francke, 1983). Specimens of the genus *Tityus* reached adulthood within six (Polis & Sissom, 1990) to 25 months (Lourenço, 2002). In the current study, *T. stigmurus* reached adulthood a mean of 29 months after birth, contrasting with previous research which presented developmental duration as between 10 (Ross, 2010) and 17 months (Matthiesen, 1971). This is probably the longest variation in PBD described for the genus *Tityus*. However, because of the limited information available on scorpions' rearing conditions, it is difficult to make a more detailed comparison. In addition, in this study, adulthood was reached after six moults, one moult more than found by other authors.

In this study, the duration of instars 2-5 was considerably shorter than reported by Matthiesen (1971) (148 days, n = 5; 281 days, n = 4; 362 days, n = 2 and 520 days, n = 1) for the same species. However, the author did not provide data on the experimental conditions in which animals were kept, such as their food supply and temperature. These factors may well affect developmental parameters. In addition, the scorpions used by Matthiesen were from Pesqueira, a city with a semi-arid climate, 215 km west of the coastal city of Recife from which our sample was taken. Therefore, population differences may also partly account for the differences between the two studies.

To distinguish between different juvenile instars, morphometric studies of different parts of the scorpion body have been made on several species, forming an index (Francke, 1979; Francke & Sissom, 1984; Lourenço, 1979a, b; Lourenço & Goodman, 2006; Lourenço, Ythier & Cloudsley-Thompson, 2007; Lourenço & Cloudsley-Thompson, 1999; Lourenço et al., 2003; Lourenço et al., 2008; Rouaud et al., 2002; Sissom & Francke, 1983). In the genus *Tityus*, Dyar's rule has been applied to only five species: *T. mattogrossensis* (Borelli, 1901), *T. fasciolatus* (Pessôa, 1935), *T. neblina* (Lourenço, 2008), *T. ocelote* (Francke & Stockwell 1987), and *T. confluens* (Borelli, 1899), and ranged from 1.13-1.57, depending on the species and parameters analysed (CL, MF, and MetV) (Lourenço, 1979a, b; Lourenço & Cloudsley-Thompson, 2010; Seiter, 2012). Variation (1.14-1.34) was also found in a study of *T. stigmurus*, with the size distributions overlapping to some extent between the different instars. However, despite the observed variation, the different instars could be distinguished using the CL, Met V, and MF lengths. Although an overlap in body structures is not common in arthropods, it has also been found in coleopterans *P. darlingensis* (Carter, 1919), *O. discicolle* (Brullé 1840) and in lepidopteran *Helicoverpa armigera* (Hubner 1805) (Allsopp & Adam, 1979; Mohammadi, Abad, Rashidi, & Mohammadi, 2010; Velásquez & Viloria, 2010).

Conclusion

In summary, by monitoring individual juveniles for more than two years, we have observed variation in individual size at each instar and in growth rate for the parthenogenetic scorpion *T. stigmurus*. Our findings indicate that this species may have the longest post-embryonic development period in the genus *Tityus*, reaching adulthood after an average of 29 months and six moults.

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