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SOME ASPECTS OF THE REPRODUCTIVE BIOLOGY OF PANAMANIAN SCORPIONS (SCORPIONES)

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Abstract: Data are provided on some aspects of the reproductive biology of 13 of the 16 species of scorpions known from Panama, Central America, belonging to the genera *Ananteris*, *Centruroides* and *Tityus* (Buthidae), *Chactas* (Chactidae) and *Opisthacanthus* (Hormuridae). The information involves the number of births, the time elapsed between births, litter size and parental care, and are the first details known in the case of several of the studied species.
Key words: Scorpiones, *Ananteris, Centruroides, Chactas, Opisthacanthus, Tityus*, reproduction, Central America, Panama.

Algunos aspectos de la biología reproductiva de los escorpiones panameños (Scorpiones)
Resumen: Se aportan datos sobre algunos aspectos de la biología reproductiva de 13 de las 16 especies de escorpiones conocidas de Panamá, Centroamérica, pertenecientes a los géneros *Ananteris*, *Centruroides* y *Tityus* (Buthidae), *Chactas* (Chactidae) y *Opisthacanthus* (Hormuridae). Esa información se refiere al número de nacimientos, el tiempo transcurrido entre nacimientos, el tamaño de la camada y el cuidado parental, siendo los primeros detalles que se conocen en el caso de varias de las especies estudiadas.
Palabras clave: Scorpiones, *Ananteris*, *Centruroides*, *Chactas*, *Opisthacanthus*, *Tityus*, reproducción, Centroamérica, Panamá.

Introduction

Scorpions are poisonous arachnids widely distributed, mainly in tropical and subtropical regions (Polis, 1990; Borges *et al.,* 2012), with over 2,680 described species (Rein, 2022). These nocturnal predators have an extremely varied diet (McCormick & Polis, 1990; Dupré, 2015). They are equipped with sensory organs that allow them to effectively detect their prey (Stockmann, 2015), whereas their venom facilitates the process of obtaining them, as well as survival by using it to defend themselves against potential predators (Brownell & Polis, 2001; Lourenço, 2018b).

These arachnids have one of the lowest metabolic rates among arthropods (Polis & Sissom, 1990) with a long-life expectancy, from four years in Buthidae species to 25 years in Scorpionoidea ones (Brownell & Polis, 2001; Lourenço, 2002). Furthermore, scorpions are iteroparous, meaning that they can reproduce several times throughout their lives (Warburg, 2011). In the case of the Buthidae, females can store sperm in their genital tract, increasing their reproductive capacity in the prolonged absence of males (Lourenço, 2018a).

After a long gestation period, the females give birth to their young (Polis & Sissom, 1990), called pro-juveniles, neonates, "pulli" or larvae, which receive parental care on the back of the mother until they move to juveniles (Outeda-Jorge *et al.*, 2009; Stockmann, 2015; Lourenço, 2018a). Juveniles, also called nymphs, are capable of hunting and feeding on their own, so they must move away from the mother and begin their life alone, to avoid predation among members of the same litter (Polis & Sissom, 1990). Also, some species show variable degrees of social behaviour (Polis & Lourenço, 1986; Warburg, 2000).

Studies on Panamanian scorpions are mainly devoted to taxonomy (Pocock, 1898; Borelli, 1899; Lourenço & Méndez, 1984; Lourenço, 1986, 1996, 1998; Quintero & Miranda, 2007; Armas *et al.,* 2011; Teruel & Cozijn, 2011; Teruel, 2011; Quintero & Esposito, 2014; Miranda & Armas, 2020; Miranda *et al.,* 2020a), local diversity (Montoya & Armas, 2002; Quintero, 2005; Armas & Miranda, 2019; Murgas *et al*., 2021), scorpionism (Borges *et al.,* 2012; Miranda *et al.*, 2018; Murgas *et al.*, 2020) and the study of toxins produced by buthid species (Salazar *et al.,* 2018; Borges *et al.,* 2020). Nevertheless, there is a notable scarcity of studies on the natural history and biology of these species (Heatwole, 1967; Miranda *et al.*, 2015, 2020b, 2021; Armas *et al.*, 2021).

The objective of this work is to present data on some aspects of the reproductive biology, under laboratory conditions, of several species of scorpions from Panama. Information is included on the number of births, the time elapsed between birth in some species of *Centruroides* and *Tityus*, as well as litter size and parental (maternal) care of the species of Buthidae, Chactidae and Hormuridae.

Methods

The Republic of Panama is in Central America and has a tropical climate that is divided into two seasons throughout the year: dry season (mid-December to April) and rainy season (May to mid-December) influenced by the Caribbean Sea and the Pacific Ocean (Empresa de Transmisión Eléctrica [ETESA], 2021). The latitude/longitude coordinates were taken in degrees, minutes, and seconds (hdddºmm'ss.s") using GPSmap 62s GARMIN with datum WGS84.

Study areas and scorpion species. The scorpions were collected between May 2017 and October 2019, in 12 localities belonging to eight provinces of the Republic of Panama (Figure 1).

PANAMA PROVINCE: El Llano Cartí, sendero Río Paja (9°19'01.2"N, 78°59'30.5"W, alt.: 337 m), *Tityus festae* Borelli, 1899; *Tityus ocelote* Francke & Stockwell, 1987; *Ananteris platnicki* Lourenco, 1993 and *Chactas exsul* (Werner, 1939).

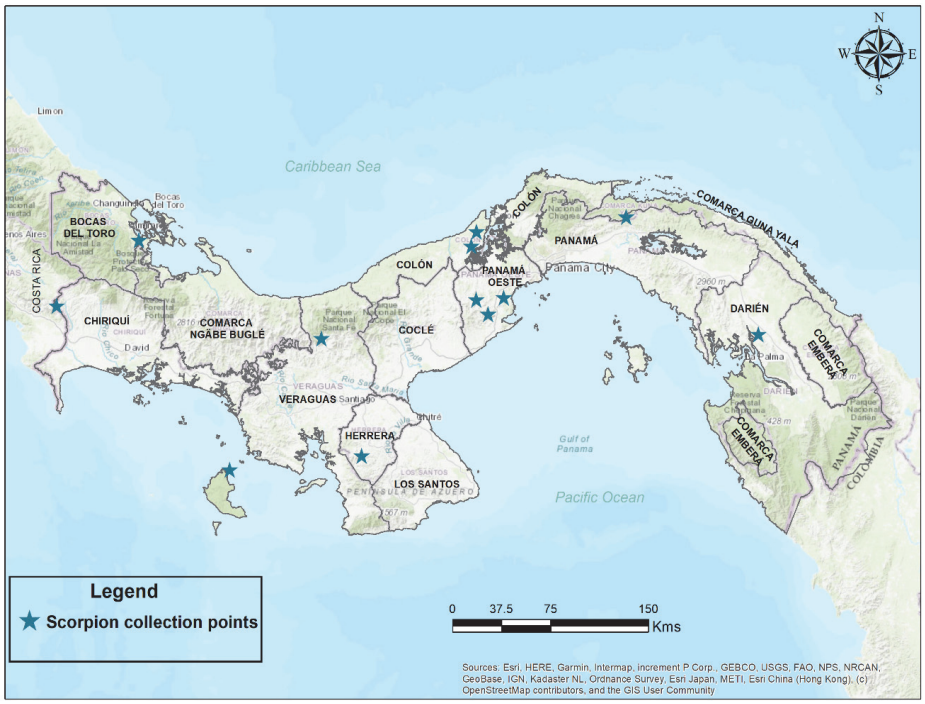


Fig. 1. Scorpion collection points in the Panamanian provinces and an island, between May 2017 - October 2019.

PANAMA OESTE PROVINCE: (1) Parque Nacional Altos de Campana (P.N.A.C) (8°40'32.9"N, 79°55'48.2"W, alt.: 883 m): *Tityus cerroazul* Lourenço, 1986; *Tityus jaimei* Miranda, Bermúdez, Flórez & Armas, 2020 and *A. platnicki.* (2) Trinidad de las Minas (8°46'21.7"N, 80°00'41.7"W, alt.: 193 m): *Tityus tayrona* Lourenço, 1991; *Ananteris canalera* Miranda & Armas, 2020; *T. jaimei* and *T. ocelote*. (3) El Polanco (8°47'12.8"N, 79°49'24.6"W, alt.: 68 m): *Centruroides granosus* (Thorell, 1876) and *A. canalera*.
COLON PROVINCE: (1) El Plátano (9°07'39.0"N, 80°02'46.3"W, alt.: 63 m): *T. tayrona.* (2) Achiote, sendero Café (9°13'24.2"N, 80°00'40.5"W, alt.: 53 m): *T. jaimei; A. platnicki* and *Ch. exsul.*
CHIRIQUI PROVINCE: Alto Quiel (8°43'48.5"N, 82°53' 55.3"W, alt.: 1036 m): *Tityus championi* Pocock, 1898.
BOCAS DEL TORO PROVINCE: Loma Azul (9°10'05.8"N, 82°20'00.9"W, alt.: 378 m): *Centruroides limbatus* (Pocock, 1898) and *T. jaimei*.
DARIEN PROVINCE: Arretí (8°32'31.7"N, 78°04'16.0"W, alt.: 17 m): *T. festae*.
HERRERA PROVINCE: Reserva Forestal El Montuoso (R. F. El Montuoso) (7°43'57.3"N, 80°48'04.5"W, alt.: 595 m): *T. jaimei*.
VERAGUAS PROVINCE: (1) Rancheria Island (7°38'19.6"N, 81°42'31.9"W, alt.: 61 m): *Centruroides bicolor* (Pocock, 1898) and *T. ocelote* (2) Carretera El Alto (8°30'53.4"N, 81°04'36.5"W, alt.: 474 m): *C. bicolor* and *T. jaimei*.

Active searches were conducted between 7:30 pm - 11:30 pm, using UV lamps (Bioquip model 2921, 400 nm) and 12-inch metal tweezers for handling and capture. The scorpions were placed in labelled plastic containers for transport to the laboratory. The specimens captured from field trips were acquired with scientific permit No. SC / A-31-18 from the Ministry of the Environment of Panama (MiAMBIENTE).

Laboratory observations. The observations were made from July 2017 to November 2020 in the Poisonous Arthropods Laboratory of the Department of Medical Entomology Research, Gorgas Memorial Institute for Health Studies, Panama City. The scorpions were kept in plastic containers with pieces of cardboard that served as shelter, but without substrate (sand or soil). They were fed with *Acheta domestica* (Orthoptera, Gryllidae), which was given once every seven days and water was supplied by means of moistened cotton. In the case of females, feeding was discontinued as soon as the start of parturition was observed until the dispersal of the juveniles. Temperature and relative humidity were controlled with averages of 24.7 ± 0.37°C and 49 ± 7.68%, respectively.

For each of the birth, the specimen's species, date of parturition, number of offspring per litter and the day the juveniles were taken off the mother's back were recorded. The data for the time of parental care on the mother were not segregated and were taken as one value (pro-juvenile + first juvenile stage).
Data analysis was performed using descriptive statistics and box plots for litter size using the Microsoft Excel 2010 program.

Results

Litter size, parental care and breeding seasonality. During the study period, 278 parturitions belonging to 210 females of 13 of the 16 species of scorpions reported from Panama were registered those species; belong to the families Buthidae (11), Hormuridae and Chactidae (one species each) (Table I). The only non-studied species were *Centruroides panamensis* Quintero & Esposito, 2014, *Centruroides margaritatus* (Gervais, 1841) and *Tityus* sp. ("*asthenes*").

The litters size in the scorpions of the genus *Tityus* (subgenus *Atreus*) (Buthidae) obtained a count higher than 20 offspring (Figures 2 and 3, Table I). Medium-sized females (60-85 mm), such as *T. jaimei* presented litters of 22.45 ± 5.95 offspring with parental care of 16.84 ± 2.62 days (Table II), with parturitions throughout the year. *T. festae* had an average of 23.94 ± 5.64 offspring, a parental care of 15.75 ±

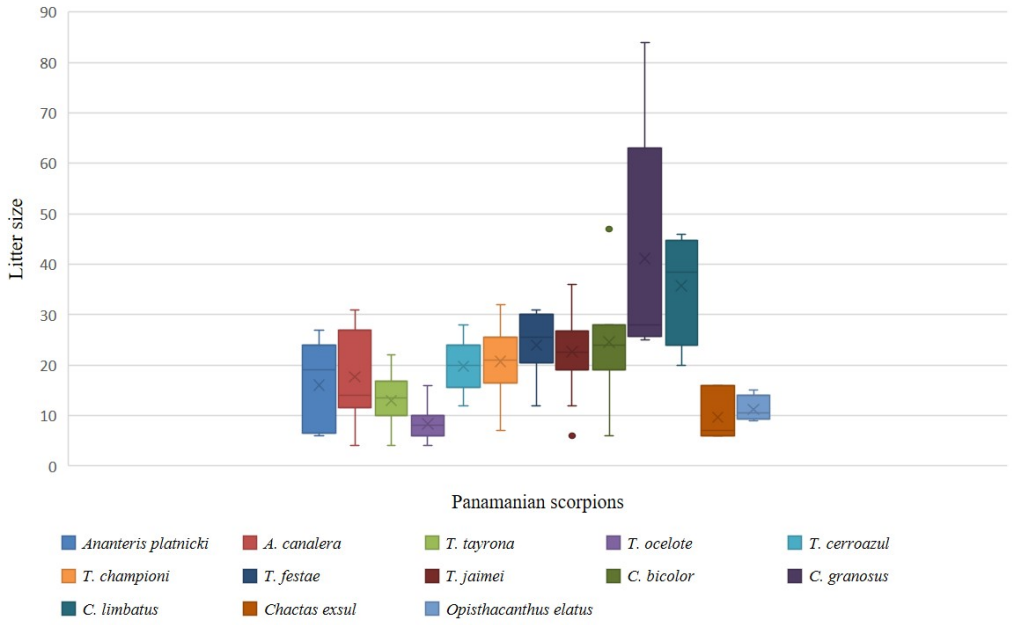


Fig. 2. Litter size among Buthidae, Chactidae and Hormuridae from Panama.

Table I. Birth, litter size, range and date of birth of 13 scorpions species from Panama in laboratory conditions. n = sample, S.D.= standar deviation. *Species associated with more accidents in Panama. ** Important species causing fatal poisoning in Panama

Species by family	Births (n)	Litter size (mean ± S.D.)	(min. - max.)	Birth dates (months)
Buthidae				
Ananteris canalera	12	17.92 ± 7.93	4-31	5-6, 11-1
Ananteris platnicki	7	19.43 ± 11.43	6-39	1, 7, 10-11
Tityus ocelote	30	8.19 ± 2.07	4-12	3-7
Tityus tayrona	24	13 ± 4.63	4-22	3-5, 8-12
*Tityus cerroazul***	5	19.8 ± 5.67	12-28	1, 7, 10-11
*Tityus festae***	18	23.94 ± 5.64	12-31	3-4, 6-12
*Tityus championi***	38	20.82 ± 6.25	7-32	3-4, 6-8, 10-1
*Tityus jaimei***	114	22.45 ± 5.95	6-36	1-12
*Centruroides granosus**	9	39.33 ± 21.78	25-84	1, 3, 4, 6, 9, 12
Centruroides bicolor	7	24.57 ± 12.25	6-47	3, 7-8, 10
Centruroides limbatus	7	33.71 ± 13.05	12-46	5, 12
Chactidae				
Chactas exsul	3	9.67 ± 5.51	6-16	7, 8, 12
Hormuridae				
Opisthacanthus elatus	4	11.25 ± 2.63	9-15	9-11
Total	**278**			

Table II. Parental care of 13 Panamanian scorpions in laboratory conditions. n = sample; S.D.= standar deviation.

Species by family	Days on the mother	
	Range	Mean ± S.D.
Buthidae		
Ananteris canalera (n = 12)	13-17	15.25 ± 1.54
A. platnicki (n = 7)	17-26	20.14 ± 3.24
Tityus ocelote (n = 30)	11-19	16 ± 2.32
T. tayrona (n = 24)	12-20	15.95 ± 2.46
T. cerroazul (n = 5)	17-26	20.4 ± 3.51
T. festae (n = 18)	14-21	15.75 ± 2.02
T. championi (n = 38)	11-24	15.91 ± 2.54
T. jaimei (n = 114)	13-21	16.84 ± 2.62
Centruroides granosus (n = 9)	16-27	20.83 ± 4.22
C. bicolor (n = 7)	14-22	19 ± 2.97
C. limbatus (n = 7)	20-26	22.6 ± 2.41
Chactidae		
Chactas exsul (n = 3)	26-27	26.5 ± 0.71
Hormuridae		
Opisthacanthus elatus (n = 4)	22-24	23.25 ± 0.96

2.02 days and parturitions in the months of March-April and June-December. *T. championi* presented an average litter size of 20.82 ± 6.25 offspring, with an average of 15.91 ± 2.54 days on the mother and synchrony in parturitions on the month of August. Inside the subgenus *Tityus*, *T. cerroazul* (~75 mm) had litters of 19.8 ± 5.67 offspring, with parental care of 20.4 ± 3.51 days and parturitions during the months of January, July, and October-November.

For the species of the genus *Centruroides* (70-100 mm), *C. bicolor* presented an average of 24.57 ± 12.25 offspring and parental care of 19 ± 2.97 days, with parturitions in the months of March, July-August, and October. *C. limbatus* had an average of 33.71 ± 13.05 offspring, with parental care of 22.6 ± 2.41 days and parturitions in May and December. Other species, *C. granosus* recorded an average litter size of 39.33 ± 21.78 offspring (Figures 2 and 4; Table I), parental care of 20.83 ± 4.22 days, with parturitions in January and early April.

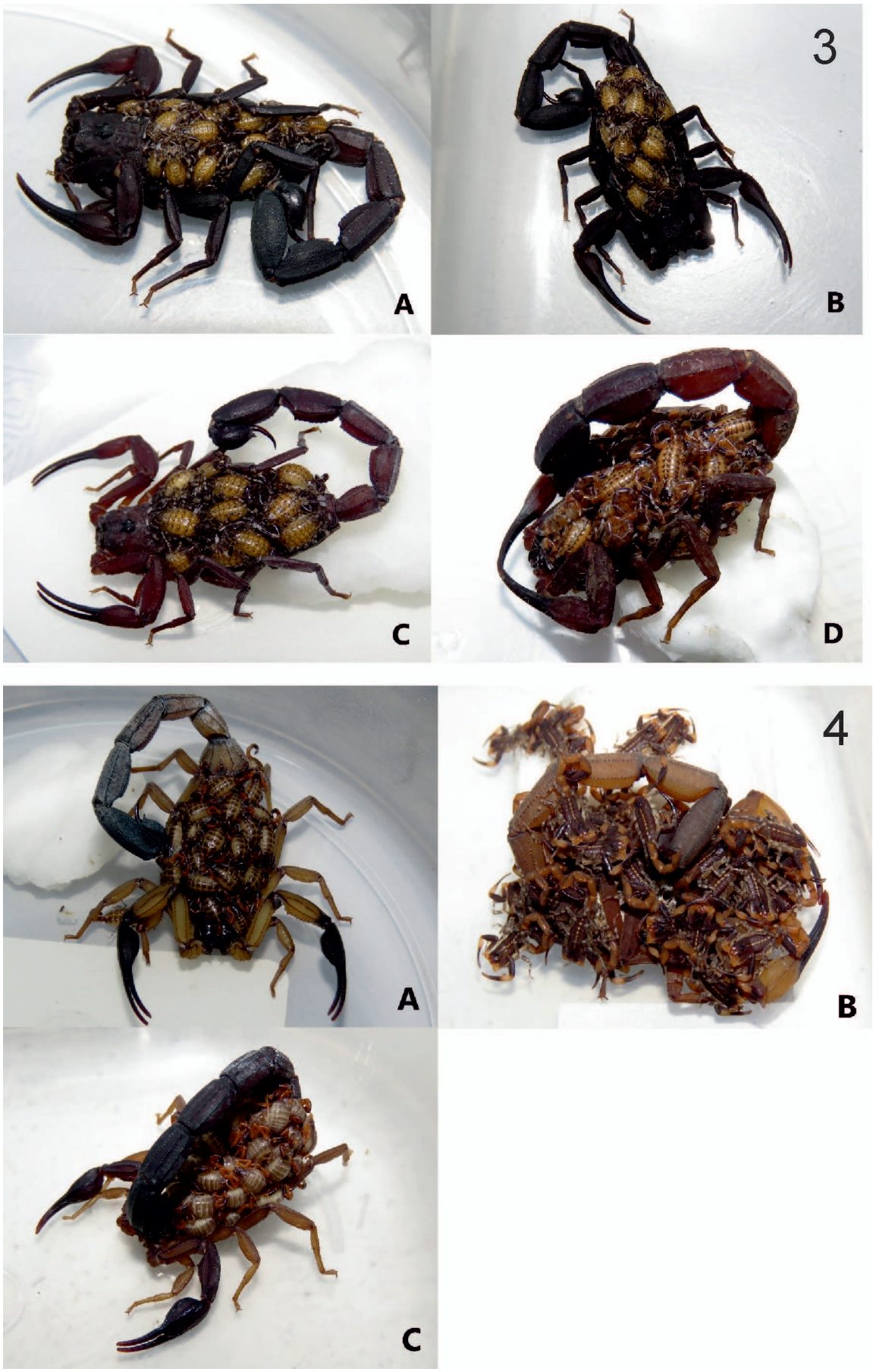


Fig. 3. Scorpions of the genus *Tityus* (Buthidae) with pro-juveniles on the back. **A**. *T. (Atreus) jaimei*, **B**. *T. (Atreus) festae*, **C**. *T. (Atreus) championi*. **D**. *T. (Tityus) cerroazul*. **Fig. 4.** Scorpions of the genus *Centruroides* (Buthidae) with pro-juveniles (**A**, **C**) and first juvenile instar (**B**) on the back: A. *C. bicolor*. B. *C. limbatus*. C. *C. granosus*.

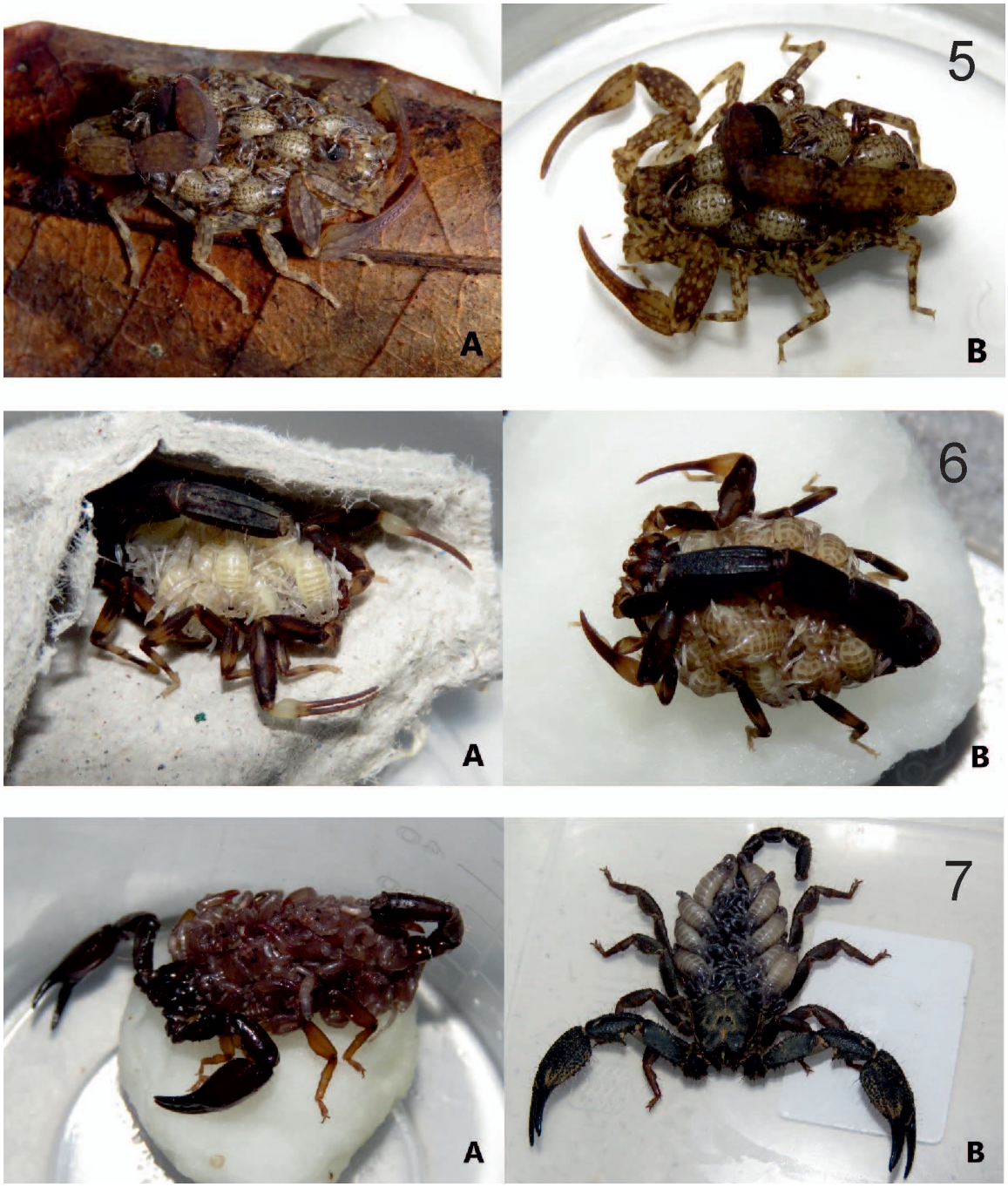


Fig. 5. Buthid scorpions of the genus *Tityus* (subgenus *Archaeotityus*) with pro-juveniles on the back. **A**. *T. tayrona*. **B**. *T. ocelote*. **Fig. 6.** Buthid scorpions of the genus *Ananteris*, with young on the back. **A**. *A. platnicki*. **B**. *A. canalera*. **Fig. 7.** Scorpions with prejuveniles on the back. **A**. *Chactas exsul* (Chactidae); **B**. *Opisthacanthus elatus* (Hormuridae).

The small species (30-40 mm) belonging to the genus *Tityus* (subgenus *Archaeotityus*), presented litters size of 13 ± 4.63 in *T. tayrona* and 8.19 ± 2.07 offspring in *T. ocelote*, with parental care of 15.95 ± 2.46 and 16 ± 2.32 days, respectively (Figures 2 and 5, Table I and II). Parturitions in *T. tayrona* occurred between March-May and August-December and in *T. ocelote* between March-July. In the case of *T. ocelote*, birth synchrony in April-May occurred in population from Rancheria Island, Coiba (Veraguas province).

The genus *Ananteris* (Buthidae) was represented by two species of approximately 30 mm each: *A. platnicki* with average litters of 19.43 ± 11.43 offspring and 20.14 ± 3.24 days on the back of the mother; whereas *A. canalera* had average litters of 17.92 ± 7.93 offspring with parental care of 15.25 ± 1.54 days (Figures 2 and 6, Table I and II).

On the other hand, *Ch. exsul* (Chactidae) of approximately 40 mm, had an average of 9.67 ± 5.51 offspring and 26.5 ± 0.71 days on the back of the mother. The only Panamanian member of the family Hormuridae, *O. elatus* (~85 mm), had average litters of 11.25 ± 2.63 offspring (Figures 2 and 7, Table I), and up to 23.25 ± 0.96 days on the back of the mother. The parturitions in *O. elatus* occurred during September to November. In the case of *O. elatus*, juveniles of all stages may live together prolonged without predation occurring between them, once dispersed from the dorsum of the mother, they can share food together. Noting most of the

young of the litter molt at the same time near the mother and another behavior is that the mother first holds with the robust pedipalps the prey and then the juveniles feed on them (Figure 8).

During the study period, 20 abortions of complete litters and three cases of maternal cannibalism were observed, mainly in the Buthidae (Table III).

Table III. Ocurrence of abortions and maternal cannibalism (m. can.) in scorpions of captivity conditions (May 2017 – Nov 2020). NA = does not apply.

Species by family	Abortions	m. can.
Buthidae		
Centruroides granosus	10	2
Tityus ocelote	6	1
T. jaimei	1	NA
Ananteris platnicki	1	NA
A. canalera	1	NA
Hormuridae		
Opisthacanthus elatus	1	NA

Iteroparity following single insemination. Multiple parturitions were observed during the captivity period in five species of Buthidae: 29 females of *T. jaimei* presented an average of three births per species, of which three of these females had six parturitions each; *T. championi* presented an average of three parturitions, with a female having four parturitions; *T. festae* had an average of two parturitions (n = five) and the record of a female with four parturitions; a female of *T. cerroazul* had three parturitions; *C. limbatus* presented an average of two parturitions (Table IV) and decrease in litter size between parturitions (Table V).

In the case of *T. jaimei,* gestation time tends to decrease between births, with 167.72 ± 42.1 days (births one-two) and 148.1 ± 26.4 days (births two-three). On the other hand, *T. cerroazul* gestation is longer than 260 days (births one-two) and 293 days (births two-three) (Table IV).

Orientation of the pro-juveniles on the mother's back. On all the herein studied buthid and chactid species, the pro-juveniles formed one or two layers, adopting random distribution on the mother's back (Figures 3–6 and 7A). On *O. elatus* the offspring formed a single layer, with the prosoma oriented towards the mother prosoma, but having the body parallel to slightly transversal to the mother body (Figure 7B).

Table IV. Multiple broods in Panamanian scorpions of the family Buthidae. n = sample, S.D. = standard deviation, NA = does not apply.

Variable	*Centruroides limbatus*	*Tityus cerroazul*	*T. festae*	*T. championi*	*T. jaimei*
Number of females (n)	1	1	5	8	29
Number of births	2	3	12	22	90
Litter size mean ± S.D.	28 ± 11.31	20 ± 8	22.5 ± 6.1	19.82 ± 6.62	22.22 ± 6.26
Litter size range	20-36	12-28	12-30	7-30	6-36
Total scorpions born	56	60	270	436	2000
days between births 1-2	214	260	151 ± 29.6	176 ± 40.2	167.72 ± 42.1
days between births 2-3	NA	293	130	146.8 ± 40.1	148.1 ± 26.4
days between births 3-4	NA	NA	136	160	141 ± 44.7
days between births 4-5	NA	NA	NA	NA	129.75 ± 16.5
days between births 5-6	NA	NA	NA	NA	128.33 ± 6.7

Table V. Decrease in litter size between multiple parturitions. n = sample, NA = does not apply.

Buthid scorpions	Birth 1	Birth 2	Birth 3	Birth 4	Birth 5	Birth 6
T. jaimei	24.76 ± 6.86 (n = 29)	22.55 ± 4.83 (n = 29)	22.53 ± 5.22 (n = 17)	17 ± 6.05 (n = 8)	18.5 ± 1.73 (n = 4)	11.7 ± 2.52 (n = 3)
T. championi	23.5 ± 5.98 (n = 8)	20.25 ± 5.28 (n = 8)	14.8 ± 6.69 (n = 5)	12 (n = 1)	NA	NA
T. festae	26.8 ± 3.70 (n = 5)	21.4 ± 5.22 (n = 8)	17 (n = 1)	12 (n = 1)	NA	NA
T. cerroazul	28 (n = 1)	20 (n = 1)	12 (n = 1)	NA	NA	NA
C. limbatus	36 (n = 1)	20 (n = 1)	NA	NA	NA	NA

Discussion

Scorpions have been grouped according to their embryonic development into apoikogenic and kaitogenic. In apoikogenic development, the embryos develop within the ovarian tubules and the eggs have a variable amount of yolk; on the other hand, in species with kaitogenic development, the embryos develop in the diverticula that emerge from the walls of the ovariuterus, and the eggs lack yolk (Francke, 1982; Warburg, 2010; Jędrzejowska *et al.,* 2016). Most of the scorpions included in this study show apoikogenic development (Buthidae, Chactidae) and only one representative (Hormuridae) presents kaitogenic development.

Breeding seasonality. The occurrence of an annual discrete breeding season has been reported in several non-tropical scorpions' (Polis & Sissom, 1990; Warburg, 2012). On the other hand, in tropical species parturitions can occur uniformly throughout the year without showing a preferred seasonality, whereas synchronization of parturitions can be associated with the availability of food for the juveniles (Benton, 2001) or at warmer temperatures (Outeda-Jorge *et al.*, 2009; Dehghani *et al.,* 2018).

The parturitions in our study occurred mostly during the rainy season. On the Pacific side of Panama, this season runs from May to mid-December, while on the Caribbean side it rains all year, even from January to April sporadically. In those species with few obtained births data, it is difficult to determine preferences in the months in which parturitions occur, however, in *T. jaimei* 114 parturitions were got from populations from both slopes, which occurred in all months.

Synchronization cases were observed in populations of *T. championi* and *T. ocelote*. According to Francke (1979) this may be due to a synchronized mating season, embryonic diapause, or chance. This synchronization event has been recorded in species of Bothriuridae, Vaejovidae, and Euscorpiidae (William, 1969; Francke, 1979; Polis & Sissom, 1990).



Fig. 8. Subsocial behavior and cooperative feeding observed on *O. elatus* in conditions of laboratory. **A**. Juveniles near the mother after dispersal. **B**. Most juveniles molt near their mother at the time. **C**. Feeding cooperative in this specie.

Litter size. Previous data on litter size for *Opisthacanthus* species include records of more than 10 offspring. African species *Opisthacanthus capensis* Thorell, 1876 and *Opisthacanthus asper* (Peters, 1861) present ranges between 13-20 and 12-22 pro-juveniles, respectively. In the South American species, *Opisthacanthus cayaporum* Vellard, 1932 and *O. elatus*, the litters are constituted between 15-25 and 15-20 pro-juveniles, respectively (Lourenço, 2002, Lourenço *et al.,* 2003). For *Opisthacanthus maculatus* Lourenço & Goodman 2006, from Madagascar, Ross (2010) reported litters of 11 pro-juveniles, similar to our findings.

There is scarce information regarding the reproductive aspects of scorpions of the Chactidae. Lourenço *et al.,* (2003), recorded for the Colombian species *Chactas reticulatus* Kraepelin, 1912 and *Chactas lepturus* Thorell, 1876 litters of 15-16 and 14-15 pro-juveniles, respectively. These authors provide data on the maternal care of these species (~24 days), corresponding to our observations on *Ch. exsul* (Table I). Outeda-Jorge *et al.,* (2009), reported litters of 8-21 pro-juveniles for an undetermined species of *Brotheas*, which is slightly higher than that observed in the *Chactas* genus.

For the genus *Ananteris*, little is known about the reproductive biology of their species (Lourenço, 2007). Litters of 15-16 pro-juveniles have been reported for *Ananteris coineaui* Lourenço, 1982 from French Guyana (Lourenço & Cuellar 1999; Lourenço, 2007) and 10-34 for *Ananteris balzanii* Thorell, 1891 from Brazil (Outeda-Jorge *et al.*, 2009). On the other hand, Kovařík (2006), report a litter of 13 pro-juveniles for *Ananteris terueli* Kovařík, 2006 from Bolivia. Miranda & Armas (2020), mentioned ranges between 4-31 pro-juveniles for *A. canalera*. Additional information on *A. canalera* and the first for *A. platnicki* is presented in Table I, the latter with litters between 6-39 pro-juveniles, similar to *A. balzanii* (Outeda-Jorge *et al.,* 2009).

The genus *Centruroides* is one of the most diverse with 100 species (Rein, 2022), distributed from the southern United States to the north of South America, including the Caribbean islands and Galapagos (Esposito & Prendini 2019). Information regarding litter size is known for 28 species, mainly from the Antilles and Mexico (12 and 7 species, respectively) (for references, see Dupré 2020). The litters range from five pro-juveniles in *Centruroides hentzi* (Banks, 1900) to 105 in the Jamaican *Centruroides insulanus* (Thorell, 1876), similar to that found in the Panamanian species, with six pro-juveniles in *C. bicolor* up to 84 in *C. granosus* (Table I).

Among the order Scorpiones, *Tityus* is the largest genus, with 224 described species (Rein, 2022), separated into five subgenera (Lourenço, 2006). There are data on the litter size for 28 species: nine for the subgenus *Tityus*, 10 for *Atreus*, seven for *Archaeotityus*, one species for *Brazilotiyus* and *Caribetityus* (Francke, 1982; Polis & Sissom, 1990; Lourenço, 2002, 2007; Outeda-Jorge *et al*., 2009). These collections represent 12.5% of the total known species for this genus, including some species of medical importance.

In our study, species of the subgenera *Tityus* and *Atreus* presented litters between 12-28 and 6-36 pro-juveniles, respectively (Table I). In general, measures in species of the subgenera *Tityus* and *Atreus* range between 50-70 and 65-100 mm in length, respectively. According to Lourenco (2018a), the litter size for most scorpions of medical importance varies

between 15-50 pro-juveniles, although there are records of litters of *Tityus bahiensis* (Perty, 1833) with up to 82 pro-juveniles (Polis & Sissom, 1990).

The species of the subgenus *Archaeotityus* have a size between 18-40 mm in length and litters size that generally does not exceed 20 pro-juveniles, corresponding to what was observed in our study for *T. ocelote* and *T. tayrona*. The size of the litters obtained in *T. ocelote* had a range between 4-12 young, similar to that reported by Seiter (2012). The time spent on the mother, however, was greater compared to Seiter's data, averaging 16 days. In general, it has been suggested that litter size may be influenced by their nutritional status, female size, and the age of the scorpion (Francke, 1981; Lourenço, 2002).

The occurrence of abortions in Buthidae species could be associated with different factors, such as stress, food shortage, presence of ecto- or endoparasites, changes in temperature and photoperiod. It has also been suggested that maternal cannibalism occurs due to stress, normal postpartum maternal behavior, ascension parameters, and mechanochemical properties of the offspring (Mahsberg, 2001; Outeda-Jorge *et al.,* 2009). However, more studies on these parameters will be necessary to test these hypotheses.

Parental care. In our study, parental care was greater than two weeks (15-26 days), similar to those reported by Lourenço (2018a), where scorpions provided care for their young from 5-30 days.

In the case of *O. elatus*, the mother takes care of their young for a larger extended period of time, sharing and cooperating with each. This mother-offspring relationship, as well as the cooperative feeding displayed by juveniles involves social behaviors (Polis & Lourenço, 1986; Polis & Sissom, 1990; Lourenço, 2002; Miranda *et al.,* 2015), and has also been associated to the long-term care behavior (Lourenço, 2000). As pointed out by Mahsberg (2001), long-term care behavior occurs in less than 1% of scorpions and likely it improves offspring survival under adverse conditions. For *O. maculatus*, from Madagascar, Ross (2010) recorded parental care of approximately 24-26 days, similar to our observations *on O. elatus*.

Iteroparity following single insemination. Scorpions can have more than one parturition through a single mating, separated by time intervals of several months or years (Armas & Hernández Contreras, 1981; Warburg, 2011; Stockmann, 2015). Outeda-Jorge *et al.,* (2009) suggested the term delayed fertilization to describe multiple parturitions observed within the Buthidae.

Our observations of multiple parturitions occur with females who have not had contact with males within the laboratory. Consequently, we can associate sperm storage, reported for the genus *Tityus* and *Centruroides*, as multiple fertilization of ovules in the female from single insemination with a male (Lourenço, 2002; Warburg, 2012), increasing the ability to have more offspring when the chance of another mating is unfavourable during the year (Albuquerque & Lira, 2016).

In most cases, as observed in buthid species during the present study, there is a decrease in litter size between multiple parturitions, caused by the end of the reproductive stage, a decrease in the number of ovarian follicles or degeneration of female oocytes (Outeda-Jorge *et al.,* 2009; Warburg, 2012; Lourenço, 2018a; Dehghani *et al.,* 2018).

Since scorpions live many years, iteroparity is likely to be widespread throughout the order (Polis & Sissom, 1990). However, in our study multiple parturitions were not observed neither in *Ananteris* nor *Chactas* species.

We observed multiple parturitions within the Buthidae, mainly in the genus *Tityus* (*T. jaimei*, *T. championi*, *T. festae*, *T. cerroazul*) and *Centruroides* (*C. limbatus*). This reproductive strategy is advantageous when males are scarce and there is a reproductive insufficiency (Warburg, 2011). Females require only one insemination to produce multiple parturitions (Warburg, 2012; Lourenço, 2018a). This event has been reported as frequent or common among species of the genera *Tityus*, *Centruroides* and *Isometrus* with the ability to give birth three to five times when isolated under laboratory conditions (Armas & Hernandez Contreras, 1981; Lourenço *et al.,* 2003; Lourenço, 2018a), and rarely for *Heteroctenus* species (Rodríguez-Cabrera *et al.,* 2015).

We do not know the beginning of insemination (fertilization of eggs) and we only know part of the gestation (the end of parturitions and birth of the offspring). Warburg (2012) suggests the gestation period cannot be determined unless the female is dissected.

The time between in the species with multiple births was 4-5 months in the subgenus *Atreus* and up to nine months in the subgenus *Tityus*, whereas in *Centruroides* it was seven months.

Gestation time in tropical scorpions is 1.3 to 24 months and in 173 species of Buthidae it ranging from 1.3 to 22 months, with mean of 3.5 months (Dupré, 2020). Although these results are under laboratory conditions, they must be evaluated until they can be confirmed by field observations. Scorpion behavior is influenced by laboratory conditions and may differ at the time of their reproductive season compared to species in their natural habitat (Polis & Sissom, 1990; Benton, 2001).

Gestation exhibits high reproductive costs associated with the estimation of nutrients and the development of the embryos. However, there is little information regarding the size of the litters in their natural habitat, whereas in laboratory conditions it is difficult to raise them (Polis & Sissom, 1990).

Orientation of the pro-juveniles on the mother's back. For the studied species belonging to the buthid genera *Centruroides*, *Ananteris* and *Tityus*, the random orientation adopted by the offspring on the mother's back are typical for the family (Savary, 1996; Lourenço *et al.,* 1999). In this regard, available data on chactid species is meagre, but as with most buthid species, the pro-juveniles of *Ch. exsul* showed a random pattern on the mother back, too (Figure 7A). In the case of the hormurid *O. elatus*, the orientation of most offspring was almost transversal (Figure 7B), clearly differing from the random orientation recorded for some congeneric (Ross, 2010 and references therein).

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