

SHORT COMMUNICATION

Sexual behavior of *Bothriurus buecherli* (Scorpiones: Bothriuridae) and comparison with the *B. prospicius* group

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Abstract. This paper describes the sexual behavior of *Bothriurus buecherli* San Martín 1963 and compares it with the rest of the *B. prospicius* group. The mating behavior was very similar to other species of the group, but *B. buecherli* presented some differences in the initial stages, one being a long period of inactivity after the male grasps the female pedipalps. Information presented includes reference to the sexual sting, post-partum copulation, and an analysis of a case of cannibalism by the female.

Keywords: Scorpions, sexual sting, *prospicius* group, Uruguay

Scorpions exhibit a complex and ritualized sexual behavior during which sperm transfer occurs indirectly through a sclerotized spermatophore (Polis & Sissom 1990). However, our knowledge of sexual behavior is very limited. In-depth studies have only been published for thirty species of seven families (Polis & Sissom 1990; Tallarovic et al. 2000). Within the Bothriuridae, published documentation exists for five genera of 176 known so far (Fet et al. 2000): *Bothriurus* Peters 1861; *Brachistosternus* Pocock 1893; *Timogenes* Simon 1880 (Peretti 1995a, 1995b, 1996); *Urophonius* Pocock 1893 (Maury 1968) and *Thestylus* Simon 1880 (Machado & Vasconcellos-Neto 2000). In the current note, I describe the sexual behavior of *Bothriurus buecherli* San Martín 1963, a small bothriurid in the *B. prospicius* group endemic to Uruguay.

Bothriurus buecherli is distributed along the highland areas of Southeast Uruguay in the Departments of Montevideo, Canelones, Maldonado, Rocha, and Lavalleja (San Martín 1963; Toscano-Gadea 2002). The *B. prospicius* group includes four other species endemic to the Republic of Argentina: *B. cordubensis* Acosta 1995, *B. pampa* Ojanguren-Affilastro 2002, *B. noa* Maury 1984, and *B. prospicius* Mello-Leitão 1932 (Ojanguren-Affilastro 2002).

I collected adult *B. buecherli* under stones at Piedras de Afilar (31°24'42.7"S, 55°33'10.6"W) in the Department of Canelones, Uruguay from December to April in 2006, 2007, and 2008. Voucher specimens were placed in the Entomology collection of the Facultad de Ciencias, Montevideo, Uruguay. In the laboratory, I kept them individually in petri dishes (9 cm diameter × 1.5 cm high), with soil as substrate and cotton absorbed in water. I fed them larvae of *Tenebrio molitor* (Coleoptera: Tenebrionidae) ad libitum. I made observations from January to May in 2006 ($n = 3$), 2007 ($n = 1$), and 2008 ($n = 3$) at an average temperature of 19.5° C (range = 14.5–26° C). The females were used only once. I used glass containers of 19 cm diameter and 10 cm height as arenas for these observations, with soil and sand as substrate and some stones for shelter. I carried out the observations at night (after 19:00 h) under red light (40 watts) located 30 cm from the container, which does not alter the behavior of the animals (Peretti 1993).

I placed the females in the arena 24–48 h before the observations and the males immediately before initiating the observations, introducing them extremely carefully and far from the female. For the identification of the behavioral units, I followed the methods and terminology of Polis & Sissom (1990), Benton (1992, 2001), Peretti et al. (2000) and Peretti & Carrera (2005). I recorded the trials with a SONY DCR-SR40 digital video camera, equipped with night shot, registering the beginning and end of each behavioral unit. Also, I

estimated the time required by males to regenerate the hemispermatophores and by females to accept mating after leaving their offspring. Likewise, I analyzed two naturally interrupted courtships during the Promenade phase, as well as one case of cannibalism by a female.

Sexual behavior.—In total, I analyzed seven mating sequences (five with sperm transfer) and recognized 26 behavioral units (Fig. 1). A description and the duration (mean ± SD) of the most relevant units are shown in Table 1.

The Introductory phase began when the male grasped the female by any part of the body, depending on how he found her at the beginning. Afterward, the male holding different part of the female's body (*reorientation* unit), tried to reach the pedipalps (*pedipalp grip* unit). During the entire process, the female maintained the *submissive female* unit with the pedipalps next to the body, the metasoma supported completely on the mesosoma, and the legs withdrawn towards the body. The duration of this phase averaged 1.52 ± 0.88 min (range = 1–3.5).

The mating dance or “*promenade a deux*” is carried out during the Promenade phase. The total duration of this phase averaged 129.5 ± 98.8 min (range = 85–371). The couple passed through the *pause* unit, remaining motionless (while still in *pedipalp grip*) from 4 to 28 min, representing from 4% to 12% of the total mating time. During this phase, the male performed *sexual sting*, the unit where he introduced the sting almost completely into the female's body. Although this behavior can be very intense, emission of hemolymph was not observed at the site either when the *sting* occurred during the experience or afterward when analyzed with a stereoscopic magnifying glass. In three cases, the male introduced the aculeus almost completely into the pleura of the prosoma (between the coxae of the legs II and III) of the female and in the remaining four cases, the male pierced the female between the pedipalps and legs I, near the lateral eyes (Fig. 2). In all the cases, the female stayed next to the male with no evident resistance.

During the spermatophore deposition phase, the couple stopped their movements, and the male carried out the *spermatophore deposition* unit. The average duration of this phase was 14.3 ± 6.6 min (range = 9.3–260). In two cases, the transference did not occur: in one of them the couple carried out the *telson rubbing* unit until they separated, while in the other case, the male tried to repeat the *sexual sting* unit close to the lateral eyes but without achieving it. In this case, the female tried to sting the male near the chelicerae (*female sting*). No females were observed eating the spermatophore upon finalizing this phase.

Cannibalism.—During one experience carried out 22 January 2008, a male and a female captured individually 72 h before, faced one

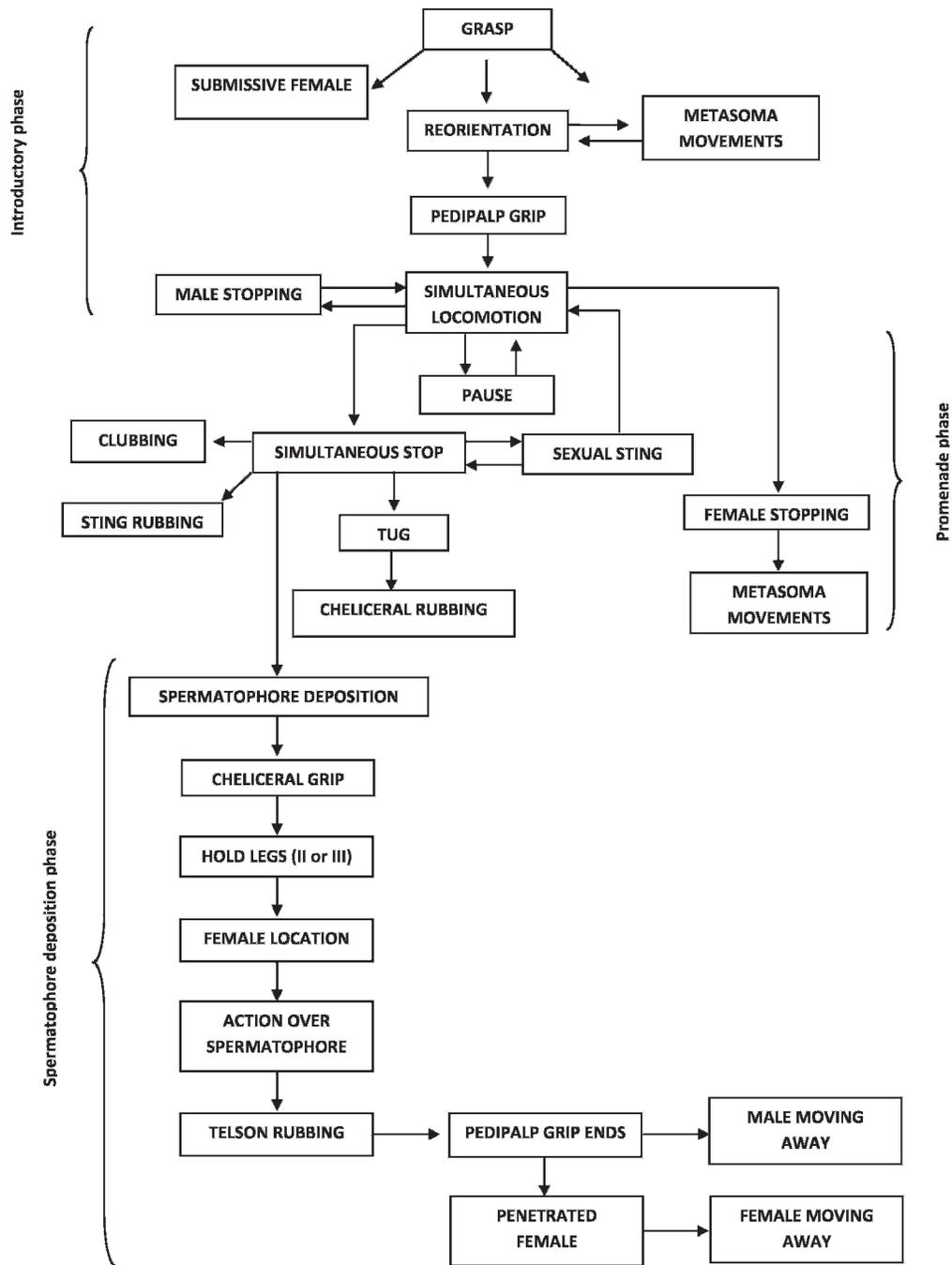


Figure 1.—Schematic diagram of the sexual behavior of *Bothriurus buecherli*.

another. After touching the female two times, the male held the female's right pedipalp. The female held the male's left pedipalp, elevated its mesosoma, and stung the male on its right side, near leg IV. The male tried to sting the female, without success. The female withdrew its stinger 20 min after, grasped the male with its chelicerae, and dragged it under a stone, beginning to eat him, beginning with the right pedipalp. The female exhibited no confrontation or aggressive behavior before or during the interaction. I removed the remainder of the male to evaluate the presence of hemispermatothores. Twenty-four h later, the female was fixed and examined, and a transparent gelatinous substance was found in the genital atrium.

Birth period-new matings.—Since the females live beyond one reproductive period, at least in laboratory conditions (Toscano-Gadea, pers. obs.), I was able to use two females captured in the field in 2007, which had descendants in the laboratory, in January 2008. In one case, the female copulated 54 days after having offspring and in the remaining case, after 48 days.

The mating behavior of *B. buecherli* exhibited similarities to the rest of the species of the *prospicius* group. Almost all of the behavior units detailed by Peretti et al. (2000) were found, although I observed some differences. The total duration of mating time for *B. buecherli* was greater than that published for other species of this group. This

Table 1.—Average (\pm SD) duration of the most relevant behavioral units present during mating in *B. buecherli*.

Behavioral unit	Phase	Duration
Reorientation	Introductory	68.8 \pm 24.6 s
Pause	Promenade	11.6 \pm 8.14 min
Graze with chelicerae	Promenade	1.27 \pm 0.11 min
Sexual sting	Promenade	35.2 \pm 68.2 min
Spermatophore deposition	Spermatophore deposition	1.4 \pm 0.10 min
Action over spermatophore	Spermatophore deposition	44.2 \pm 6.02 s
Penetrated female	Spermatophore deposition	28.4 \pm 3.6 s
Telson rubbing	Spermatophore deposition	22.9 \pm 12.3 s

difference was primarily due to the time taken up by the units *simultaneous locomotion*, *pause*, and *sexual sting*. The unit *simultaneous locomotion* (together with *simultaneous stop*) is hypothesized to reflect the degree of sexual excitation (Peretti et al. 2000). In *B. buecherli*, the duration of this unit varied between 1.51 and 3.33 min, whereas in the other species of the group, it did not surpass 1 min (Peretti et al. 2000).

The extended *pause* unit observed in the *B. buecherli* couples has not been described for any of the species of the *prospicius* group. This unit could be related to *pedipalp grip* and the mutual recognition of the couple. Peretti (1995b) indicates that males of *Bothriurus bonariensis* and *Timogenes elegans* (Bothriuridae) are able to stay motionless and carry out the unit *pedipalp grip* for several minutes even though the female is dead. According to this author, *B. buecherli* could possibly perform this unit by means of sexual recognition through a chemical channel or by means of recognizing the shape of the female's pedipalp. Although I tried not to interfere with the behavior of the scorpions in these experiments, it is possible that the duration of this behavior was altered by the laboratory conditions.

The occurrence of "*sexual sting*" during the Promenade phase correlates with observations in *B. cordubensis* and *B. noa*; the site where the *sexual sting* unit occurs is similar to the one observed in *B. noa*. The duration of this unit in *B. buecherli* (35.2 min) is noticeably greater than that observed in the rest of the *prospicius* species (range = 77–142 s according to Peretti et al. 2000). Unlike the injuries noted by Francke (1979), hemolymph was never observed after the termination of this unit.

I observed no malformations of the spermatophores from the two experiments in which sperm transfer was not achieved; these spermatophores were similar to the ones described by San Martín & Gambardella (1967). Alexander (1957); Peretti (1996) and Tallarovic et al. (2000) indicate that unsuccessful mating sequences are frequent in scorpions either due to the male's inability to locate the spermatophore correctly or due to the female's resistance. In both cases, no female resistance was detected.

If the period of latency between the birth and new mating sequences of *B. buecherli* females (48–54 days) observed in the laboratory is comparable to that in natural conditions, the number of matings during the same reproductive period could be between three and four, similar to what Castelvetri & Peretti (1999) observed in *B. bonariensis*. This number of matings suggests the existence of strong sperm competition between the males. It would be necessary to carry out new experiments at different time intervals to determine the precise moment at which the female becomes receptive.

Castelvetri & Peretti (1999) and Peretti (2001) indicate that at the beginning of every reproductive season, adult females can present different degrees of sexual receptivity: positive, intermediate, and negative. The female *B. buecherli* that attacked and killed the male did



Figure 2.—Detail of the sexual sting unit in *B. buecherli*. (Photo C.A. Toscano-Gadea).

not present the unit *submissive female*, suggesting negative receptivity (common after several inseminations or when the female is gestating). Analysis of the female genitalia revealed a slimy and transparent substance that could be a membranous genital plug similar to that of *B. flavidus* (Mattoni & Peretti 2004). Nevertheless, Castelvetri & Peretti (1999) and Contreras-Garduño et al. (2005) indicate that the presence of a genital plug does not prevent the females from accepting a new mate. Considering that this attack happened before the male started the courtship, this could be a case of an antagonistic sting, when the female is not receptive and tries to kill the male to obtain food. Also, considering the reduced size of the containers used, we cannot dismiss the possibility that this behavior results from laboratory conditions.

The sexual behavior observed in *B. buecherli* is similar to that described in other species of the *prospicius* group. Considering both allopatry and the morphological and behavioral similarities of *B. buecherli* to the other species of the group, it would be interesting to attempt interspecies matings between them, following previous analyses by Peretti et al. (2000). Furthermore, new experiments testing possible causes for the long sexual sting under male coercion or female choice hypotheses would further enlighten researchers about the sexual tactics used by these fascinating scorpions.

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