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Sperm Transfer Behavior in the Neotropical Scorpion *Thestylus glazioui* (Bertkau) (Scorpiones: Bothriuridae)

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In the evolutionary sequence of sperm transfer species of the order Scorpiones show the plesiomorphic condition with males and females forming pairs and sperm transfer occurring indirectly through spermatophore. In this study we describe the mating behavior of the Neotropical scorpion *Thestylus glazioui* (Bertkau) (Bothriuridae). The courtship behavior was characterized by the male's movement forward and backward, conducing the female attached by the pedipalps. During the promenade male showed intense pecten movement and accomplished sexual stings in the female's pedipalps. When the promenade behavior finished, the couple stopped on a dead stick and the male deposited the spermatophore on it. Immediately after the deposition the male brought the female in direction to the spermatophore. The female remained on the spermatophore for 90 seconds and after this period, the female stung the male that fled and abandoned the place quickly. Female left the mating site taking the opposite direction of the male and the spermatophore was not consumed. Post-insemination spermatophore is lamelliform, reddish chestnut in color and very similar to those of the other bothriurids. The reproductive period of *T. glazioui* is strongly seasonal occurring only in the wet-warm season (October-February).

Index terms: Reproductive behavior. Courtship. Spermatophore. Scorpions.

Transferência espermática no escorpião neotrpical *Thestylus glazioui* (Bertkau) (Scorpiones:Bothriuridae). Na seqüência evolutiva do comportamento de transferência espermática os escorpiões apresentam a condição plesiomórfica que corresponde a formação de casais e transferência indireta de espermatozóides através de espermatóforos. Nesse trabalho nós descrevemos o comportamento reprodutivo do escorpião Neotropical *Thestylus glazioui* (Bertkau) (Bothriuridae). O cortejo foi caracterizado pelo movimento do macho para frente e para trás, conduzindo a fêmea presa pelos pedipalpos. Durante a promenade o macho apresentou intenso movimento de pécten e realizou picadas sexuais nos pedipalpos da fêmea. Ao fim da promenade o casal parou sobre um graveto onde o macho depositou o seu espermatóforo. Imediatamente após a deposição o macho trouxe a fêmea em direção ao espermatóforo. A fêmea permaneceu sobre o espermatóforo por 90 segundos e, após esse período, a fêmea picou o macho que fugiu abandonando rapidamente o local. Em seguida, a fêmea também deixou o sítio de acasalamento tomando direção oposta à do macho e não consumiu o espermatóforo. O espermatóforo desativado é lameliforme, castanho-avermelhado e muito parecido ao de outros Bothriuridae. O período reprodutivo de *T. glazioui* é altamente sazonal ocorrendo somente durante a estação quente e úmida (Outubro-Fevereiro).

Descritores: Comportamento reprodutivo. Corte. Espermatóforo. Escorpiões.

Mating behavior and methods of sperm transfer are remarkably diverse in the class Arachnida, ranging from spermatophore deposition irrespective of the female's presence to insemination with liquid sperm through copulatory organs (Thomas & Zeh, 1984). In the

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evolutionary sequence of sperm transfer species of the order Scorpiones show the plesiomorphic condition with males and females forming pairs and sperm transfer occurring indirectly through spermatophore (Alexander, 1964; Thomas & Zeh, 1984). Mating and courtship have been described for six of the seven scorpion families (see review in Polis & Farley, 1979). In the family Bothriuridae there are data for the genus *Bothriurus* Peters, *Urophonius* Pocock, *Brachistosternus* Pocock and *Timogenes* Simon (Peretti, 1992, 1995, 1996a, 1996b, 1997).

On 11 November 1997, a mating pair of *Thestylus glazioui* (Bertkau) was found at daylight under a fallen trunk in the Serra do Japi (23°17' S, 47°00' W; 1250m elevation), near Jundiaí, São Paulo State, southeastern Brazil. The local vegetation consists of semideciduous mesophytic forest (Leitão-Filho & Morellato, 1997) and the climate has two well-defined seasons: one wet-warm from October to March and other dry-cold from April to September (Pinto, 1993). The specimens were collected and placed in a tray (40 x 30 cm and 7 cm high) with soil, stones, sticks and a wet cotton to maintain the moisture. Male (1.42 cm of prosoma) did not untie the pedipalps of the female (1.29 cm of prosoma) even during the transport to laboratory and the couple remained together when placed in the terrarium. The male started the *promenade à deux* (*sensu* Polis & Sissom, 1990) at night (about 2300h) and the observations were carried out with a flashlight covered by a red filter to avoid disturbing the organisms. After the observations the individuals were preserved in 70% ethanol and deposited at Museu de Zoologia da Universidade de São Paulo (MZUSP) as voucher specimens.

The courtship behavior was characterized by the male's movement forward and backward, conducing the female attached by the pedipalps. During the promenade male showed intense movement of the pecten that was maintained in contact with the substratum. When being moved back the male also used the telson to fumble the substratum. One hour after the beginning of this kind of movement the male interrupted the promenade and stung the fixed finger of the female's chela - twice in the right chela and twice in the left chela, alternately. The female reacted only once releasing its pedipalp of the male's pedipalp. However, few seconds later the male got to seize the mating female again and restarted the promenade. This initial sequence of courtship lasted 73 minutes.

When the promenade behavior finished, the couple stopped on a dead stick and then, the male accomplished intense movement with the telson in contact with a stone beside the stick for 12 minutes. The male stung the female's chela again (once in each chela) and in any moment there was reaction of the female. A posterior observation at the stereomicroscope revealed the presence of six perforations in the female's tegument (three in each chela). After sting his mate, the male began to press the gonopore against the substratum. This process lasted five minutes and it culminated with the deposition of the spermatophore on the stick. Immediately after the deposition the male brought the female in direction to the spermatophore. When she was close to the spermatophore the male also grasped with his chelicerae the female's chelicerae (cheliceral massage, sensu Polis & Sissom 1990). When she was positioned over the spermatophore the male released her pedipalps and seized her third pair of legs, compressing the female against the spermatophore. The female remained on the spermatophore for 90 seconds. After this period, the female stung the male's medium portion of the prosoma that fled and abandoned the place quickly. Female left the mating site taking the opposite direction of the male. The spermatophore was not consumed by the female as opposite to occurs with some bothriurid, such as *Bothriurus flavidus* Kraepelin (Abalos & Hominal, 1974) and *Urophonius brachycentrus* (Thorell) (Maury, 1968; see also Peretti, 1993). The whole courtship process and sperm transfer lasted about two hours. Five minutes after the end, male and female were sheltered separately under stones.

Post-insemination spermatophore is lamelliform, reddish chestnut in color (Figure 1) and very similar to those of the other bothriurids (Peretti, 1992, 1993). The lamina is shorter than the trunk and the evaginated capsule bears a rounded foramen at the tip.

The reproductive period of *T. glazioui* could begin in the early wet-warm season (October) since one gravid female of this species was found in November. In the dry-cold season these scorpions are very rare in the forest leaf litter or it may be concentrate in specific sites that show optimal conditions of temperature and moisture. During July 1995 a leaf litter area of 3,072 m^2 were sampled at different sites at Serra do Japi and no individual of *T. glazioui* was found (G. Machado unpublished data).

The fact of the mating pair have been found at daylight can be interpreted in two ways. In the first, the male could have found the female in the previous night in a not receptive state but, to try mating, he maintained the potential partner grasped waiting for the appropriated time, such as occurs with *B. prospicuus* Mello-Leitão (A. V. Peretti personal communication). Second, scorpion males request time to produce a new spermatophore (Polis & Sissom, 1990). The male that we found could have copulated at little time but to obtain an another mating maintained the partner grasped until possessing conditions of depositing a new spermatophore. This behavior have been recorded to *B. bonariensis* (C. L. Koch) in which males that court females without having the hemi-spermatophore well formed obtain higher reproductive success than those that wait to have a ready hemi-spermatophore (Peretti, 1996b).

In several species of Bothriuridae the male has glands of external secretion in the metasoma (see Peretti, 1997 and included references). This glands are located in dorsal side of the telson (as in *Bothriurus* and *Urophonius*) or of the fifth metasomal segment (as in *Brachistosternus* and some *Timogenes* species) (De la Serna de Steban, 1978; Peretti, 1997). During the courtship males rub this glands against some parts of the female's body. According to Peretti (1997) the secretion of those glands could have a role of increasing the female's receptivity during the promenade. We inspected three males of *T. glazioui* (including the studied male) and in none of them we found external openings of glands in the metasoma.

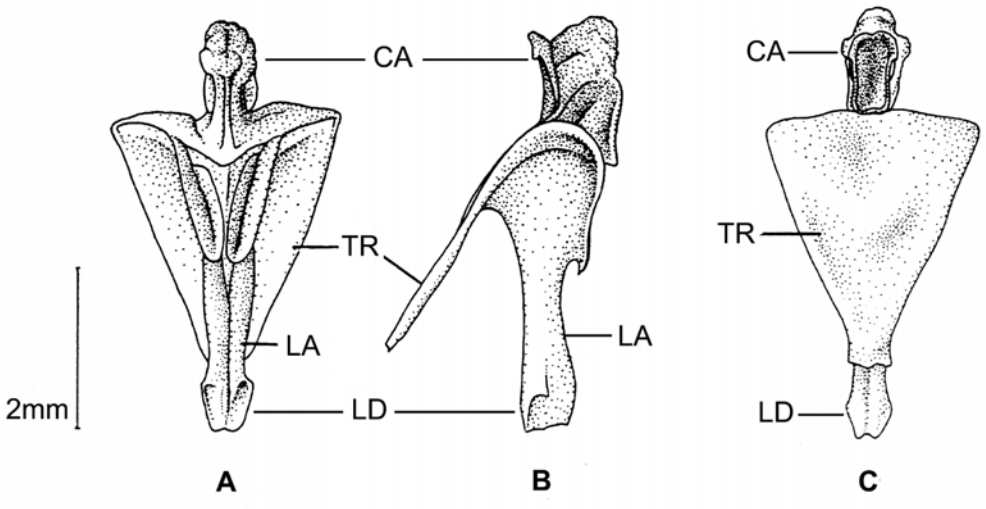

Figure 1. Post-insemination spermatophore of *Thestylus glazioui*: (A) dorsal view; (B) lateral view; (C) ventral view - CA: capsule (evaginated); TR: trunk (collapsed); LA lamina; LD: distal end of the lamina. The foramen at the tip of the capsule is not visible in the drawing.

The absence of this gland could explain the absence of the behavior of rubbing with the telson in this species. On the other hand, the male accomplished sexual stings (*sensu* Polis & Sissom, 1990) in the female during the courtship. This behavior has been observed during the courtship of *B. bonariensis* (in which male stings the female's metasoma), in B. *flavidus* and in *Timogenes elegans* Mello-Leitão (stung in the pedipalp) (Peretti, 1993). The sexual sting is a widespread behavior among the families of the order Scorpiones and does not correspond to an act of intersexual aggression. In the contrary, the male's stung in his mate seems to have an appeasement function during the courtship (Polis & Sissom, 1990).

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