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# SHORT COMMUNICATION

# Copulatory behavior of Microstigmatidae (Araneae: Mygalomorphae): a study with *Xenonemesia platensis* from Argentina

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**Abstract.** Microstigmatidae are small ground-dwelling and free-living spiders. The present study reports on the copulatory behavior of *Xenonemesia platensis* Goloboff 1989, constituting the first report on sexual behavior of the Microstigmatidae. Our findings in *X. platensis* did not show evidence of pheromones associated with silk. The courtship behavioral units of males was comprised of quivers by legs I and II, brusque movements of the palps, and leg tapping with legs II. During mating, a novel courtship behavior by males was observed that consisted of tapping and scraping with legs II on the female legs. The present study not only gives a description of mating behavior in Microstigmatidae for the first time, but also reports strong evidence of nongenital copulatory courtship activity in mygalomorph spiders.

**Keywords:** Argentinean spider, South America, courtship, mating, reproductive biology

Many spider species could be compelling targets for evolutionary studies due to their unusual reproductive biology (Eberhard 2004); it appears that a species of microstigmatids provides just such a target. Microstigmatidae are small ground-dwelling and free-living spiders (Griswold 1985) restricted to habitats offering constant high humidity and even temperature (Lawrence 1953). This family comprises 15 species, nine of them distributed in the New World (Platnick 2011). Members of this family are characterized by rounded book-lung openings and extremely shortened posterior lateral spinnerets (Goloboff 1995). Microstigmatid species, in particular, have long been overlooked, both because of their rarity in collections and their extremely small size (adult males are 1–3 mm in total length) (Raven & Platnick 1981). The spiders are not known to construct burrows or retreats and are supposed to make minimal use of silk. They readily attack and feed upon small insects (Griswold 1985). There are few published records of either the natural history or the ecology of microstigmatid species (Griswold 1985; Dippenaar-Schoeman et al. 2006: Old World species; Indicatti et al. 2008: Brazilian species; Ferretti et al. 2010: Argentinean and Uruguayan species).

Here we report on the copulatory behavior of *Xenonemesia platensis* Goloboff 1989, constituting the first report on sexual behavior of a microstigmatid. We collected three adult males and three adult females at Martín García Island, Buenos Aires, Argentina (34°11′25″S, 58°15′38″W), in August 2009. Voucher specimens are still alive and will be deposited in the Museo de La Plata, Division Entomología, La Plata, Buenos Aires, Argentina. All the females molted before we made observations, so they did not have stored sperm. In the laboratory we kept them individually in plastic Petri dishes (9 cm diameter × 1.5 cm high), with soil as substrate and wet cotton wool moistened daily. These containers allowed us to follow their behavior as they constructed their burrows. We fed all individuals weekly with cockroaches (*Blattella germanica*) of approximately 10 mm length. We used a 12 h light/dark cycle, and the room temperature during breeding and observations was 26.7°C ± 1.52 SD.

In order to observe mating, we placed each female dish inside a larger glass cylindrical container (19 cm diameter and 10 cm high) with a layer of soil approximately 6 cm deep. A depression excavated in the center of the larger container for the female's Petri dish avoided the destruction of the female's shelter during the transfer. The mating arena was illuminated with artificial fluorescent light. For each encounter, we removed the male from his Petri dish and carefully introduced him into the larger container housing the female's dish, and at quite a distance from the female.

We performed nine male-female pairings of *X. platensis* in all combinations, and both males and females were given three possible mating opportunities. We considered only the first pairing for description of behavioral units during courtship and mating sequences because female behavior in particular may change after a first successful insemination, and since these spiders are very rare, they probably never encounter potential mates at such high frequencies. We recorded copulations with a Handycam Panasonic SDR-S7 and analyzed the video records with a PC program (Sony Vegas 9.0) in order to describe behavioral patterns accurately. We used slow motion and single frame advance modes. Durations and frequencies are given as averages ± standard deviations.

We present the frequency and duration of behavioral units during the three mating exposures and five copulations in Table 1. When *X. platensis* engaged in courtship and mated, a common pattern occurred (Fig. 1a). All males began the courtship when they directly contacted the female's body. During this initial contact, females remained largely motionless. The male did not start courtship when he contacted female silk, but did so only after contacting the female herself. Early studies proposed that mygalomorph spiders lacked chemical cues in sexual communication (Baerg 1958; Platnick 1971). However, more recent studies have reported the presence of pheromones associated with female silk threads (Costa & Pérez-Miles 2002; Ferretti & Ferrero 2008). Our findings in *X. platensis* could indicate the absence of pheromones associated with silk, but obviously more detailed studies are needed to confirm this.

After initial contact, the male quivered with the first and second pair of legs, followed by fast upward and downward movement of the pedipalps. The male made nine behavioral bouts with an average duration of 0.52s ± 0.06 SD (range = 0.44−0.60). At first glance, the quivers observed in the courtship of *X. platensis* could be similar to the body vibrations observed in some theraphosids (Costa & Pérez-Miles 2002; Ferretti & Ferrero 2008), but in *X. platensis* the quiver is generated by the first and second pair of legs instead of pair III as observed in theraphosids. After approximately 46 s, the female raised her body up to an angle of almost 60° relative to the substrate, with the first pair of legs elevated and legs III and IV over the substrate. At

Table 1.—Frequencies and durations of behavioral units during the three mating exposures of *X. platensis*. M = male, F = female, N = number, D = duration. Mean values ± SD are presented. No matings occurred in the third set of pairings, thus producing no additional observations.

| | First mating | | | | | | Second mating | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Pair 1 (M1 - F1) | | Pair 2 (M2 - F2) | | Pair 3 (M3 - F3) | | Pair 1 (M1 - F2) | | Pair 2 (M2 - F3) | | Pair 3 (M3 - F1) | |
| Behavioral units | N | D | N | D | N | D | N | D | N | D | N | D |
| **Courtship phase** | | | | | | | | | | | | |
| Quivering | 9 | 0.52s ± 0.06 | 21 | 0.53s ± 0.03 | 9 | 0.56s ± 0.08 | 24 | 0.53s ± 0.05 | 39 | 0.54s ± 0.04 | | |
| Palpal boxing | 6 | 1.92s ± 0.95 | 11 | 2.27s ± 0.99 | 3 | 5.13s ± 3.17 | 5 | 5.34s ± 0.90 | 4 | 3.06s ± 1.04 | | |
| Leg tapping | 7 | 1.00s ± 0.39 | 7 | 1.02s ± 0.47 | 4 | 2.46s ± 1.69 | 11 | 0.90s ± 0.36 | 14 | 1.63s ± 1.42 | | |
| **Mating phase** | | | | | | | | | | | no mating | |
| Palpal insertions | 3 | 25.25s ± 12.97 | 15 | 14.91 ± 10.37 | 6 | 14.13 ± 7.42 | 4 | 14.36 ± 8.70 | 5 | 18.24 ± 13.30 | | |
| Leg beating | 7 | 9.40s ± 5.03 | 2 | 5.08s ± 1.01 | 7 | 8.48s ± 3.54 | 2 | 11.36s ± 2.03 | 6 | 8.53s ± 3.49 | | |
| Mating | 4.61min | | 4.78min | | 2.73 min | | 1.98 min | | 2.01 min | | | |

this instance, the male made alternating movements of the pedipalps, touching the genital zone of the female (palpal boxing). We usually observed palpal boxing alternated with quivers. Palpal boxing occurred six times, with an average duration of 1.92s ± 0.95 SD (range = 0.96 − 3.40 s).

Subsequently, the male vigorously hit legs I and II of the female with the tarsi of his legs II extended. This behavior consisted of high-frequency leg tapping in an alternating or synchronous phase. The male made seven leg tappings with a mean duration of 1.00 s ± 0.39 SD (range = 0.68 − 1.80). The brusque movements of the palps and the scraping with legs II during courtship have not been reported in any other mygalomorph spider. These abrupt palpal movements could be similar to the ''twitching'' observed in a diplurid (Coyle & O'Shields 1990), which consisted of distinct, sudden flexions or extensions of one or more legs or palps. Next, the male clasped the female's palps and chelicerae between his first pair of legs (Fig. 1b). The distal portion of each male tibia without tibial apophyses or megaspines was placed against the prolateral surface of each female pedipalp base. The male placed his second pair of legs against the female's first pair of legs, as if pushing them, and then palpal insertion attempts began.

From the nine encounters, we obtained five successful matings. All of the first copulations were successful during the first three pairings. In the second pairings, we observed two successful copulations, and no matings occurred in the third set of pairings. In one case, the female rejected the male with vigorous lateral abdominal oscillations while raising her body. In three cases, males never initiated courtship. During the copulation, the male positioned himself under the female, facing her sternum. The female's pedicel was flexed upwards so that the cephalothorax-abdomen angle was 30–50°. This mating position continued during the palpal insertion attempts, and the copulation lasted 4.61min. The male made three palpal insertions with a mean duration of 25.25s ± 12.97 SD (range = 11.96−37.88). During palpal insertion attempts, the male continued performing tapping with legs II and quivering.

Afterward, while the male was inserting his palp into the female's genital opening, he added a new behavioral unit. He raised the second pair of legs to an angle of 90° between the femur and patella and quickly moved the legs upward and downward. Male tibia, metatarsi, and tarsi remained extended, and the tarsi beat and scraped the second and third female coxae. The male performed seven repetitions of this leg-beating behavior with a mean duration of 9.40 s ± 5.03 SD (range = 4.36 − 18.88) and a velocity of 14 beats per second. The male's tapping with his second legs during copula could be interpreted as courtship in copula. This behavior, as far as we know, is unique to *X. platensis* and has not been previously reported in mygalomorphs (Costa & Pérez-Miles 1998, 2002; Ferretti & Ferrero 2008; Jackson & Pollard 1990). Finally, when the spiders separated, the male quickly moved backwards. In the observed matings of *X. platensis*, the copulation position achieved was similar to that of most mygalomorphs (Costa & Pérez-Miles 2002), and the behavior displayed by this species during mating is noticeable and unusual among mygalomorph species.

The female's apparent unresponsiveness throughout courtship and copulation may be a test of the male's quality (Eberhard 1985); she may be monitoring his overall performance, not only genital stimulation. The sexual selection by female choice hypothesis predicts selective cooperation in which males perform luring behavior, and females choose a mate according to the male's courtship display (Thornhill 1983; Eberhard 1985, 1996, 1997). One way a male may prevail in this competition is by courting the female during copulation (copulatory courtship) (Eberhard 1994, 1996) and thereby inducing her to use his sperm. Males of hundreds of species of animals perform nongenital behavior, during copulation, that appears to be courtship; this behavior includes biting, tapping, rubbing, squeezing, shaking, vibrating, singing to, and feeding the female (Eberhard 1994, 1996).

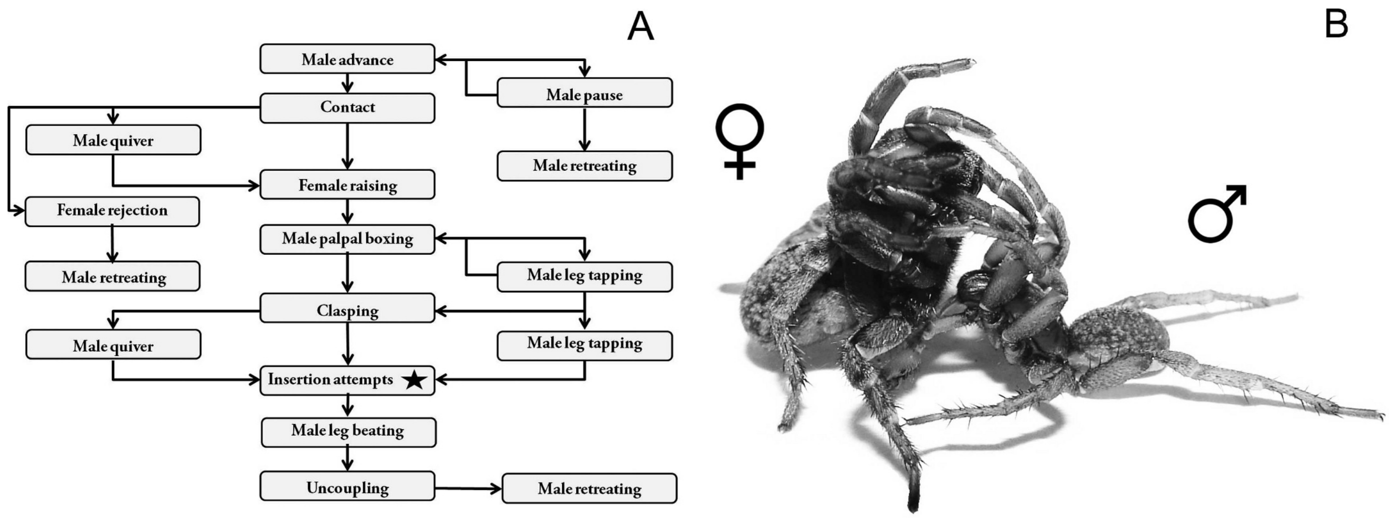


Figure 1.—Courtship and mating of *X. platensis*. a) Ethogram showing the courtship and mating pattern; the black star indicates the instance where photo was taken for Figure 1b. b) Male clasping and tapping female with forelegs during insertion attempts.

Few studies have directly tested the possibility that copulatory courtship affects paternity. In insects, copulatory courtship can result in a decrease in female mobility during copulation (Humphries 1967) and increased resistance to subsequent matings (King & Fischer 2005). These effects could be operating in the mating behavior of *X. platensis*, given the female's largely motionless state during courtship, copulation and post-copulation. They could also lead to some kind of resistance to subsequent mating, given that the three females accepted a first male, two females accepted a second male, and none accepted a third male. Obviously, this work constitutes preliminary observations, and more data are needed to elucidate these hypotheses.

In conclusion, the present study not only gives a descriptive overview of the mating behavior in the Microstigmatidae for the first time, but also reports strong evidence of nongenital copulatory courtship in mygalomorph spiders, both of which offer a promising field of research in the context of sexual selection.

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