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Insights into the courtship and copulation of the worm snake *Xerotyphlops vermicularis*

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The worm snake *Xerotyphlops vermicularis* Merrem, 1820, is a small semi-fossorial snake, distributed in the southern parts of the Balkan Peninsula (along the Adriatic coast, parts of North Macedonia, parts of southern Bulgaria, reaching north to the Central Black Sea coast, and most of Greece including many Aegean islands), Turkey, the Middle East, Caucasus and eastwards to Iran and Afghanistan (Speybroeck et al., 2016; Koynova et al., 2021). Due to its secretive life, the species' biology and ecology have been little studied across its range. In Bulgaria between the end of April and the end of June, *X. vermicularis* is found during the day under stones, and rarely on the surface (only at dusk and at night, or after heavy rains); in July and August it is not usually found under stones, but comes to the surface at night; in September it migrates to the deeper soil layers and it is almost impossible to find (Naumov, 2007; Stojanov et al., 2011). Copulation is in May and June, and the eggs (2–8) are laid in July–August in deep layers of soil (Naumov, 2007; Stojanov et al., 2011; Speybroeck et al., 2016). To date, there are apparently no published descriptions of the mating behaviour of *X. vermicularis* or of other blind snakes of the families Typhlopidae, Anomalepididae and Leptotyphlopidae. In this report, we provide some insights into the courting ritual and the copulation behaviour of *X. vermicularis*.

On 16 May 2022, during an ecological study of snakes in Bulgaria, we found two adult *X. vermicularis* under a stone at 23:44 h (local time), near Dositeevo Village (41° 55'44" N, 26° 0'51" E; 149 m a.s.l.) in south-eastern Bulgaria, aided with an artificial light. We noticed that the two snakes were coiled together and appeared to be conducting courting behaviour, prior to copulation. We carefully removed the stone, then started observing and filming their behaviour without disturbing the snakes. Following copulation, we captured the snakes to measure snout to vent length (SVL) and tail length (TL) (precision 0.1 cm), and weigh (W) them (precision 0.01 g) before releasing them at the site of capture.

During the courtship, the larger snake (SVL = 26.8 cm, TL = 0.5 cm, W = 5.32 g), which we presumed was female, was laying relatively still on a sandy-soil substrate, its body positioned in a W-shape form with the head at one end, hiding in the grass, and the tail exposed on the surface at the other (Fig. 1A, BHS video 2023a). The smaller snake (SVL = 20.5 cm, TL = 0.6 cm, W = 2.43 g), which we presumed was the male, was coiled and moving around the body of the female. The head and the interior 1/3 of the male's body was coiled tightly around the mid-part of the female's body while the other 2/3 of the male's body, including its tail,

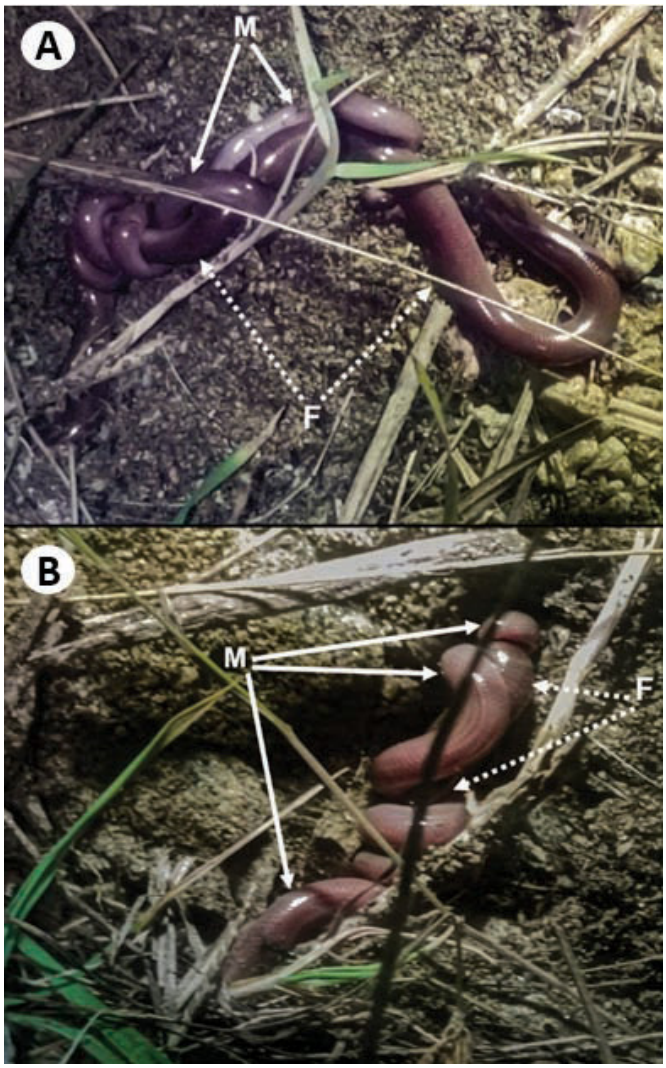

Figure 1. Mating behaviour of *Xerotyplops vermicularis* observed in situ on 16 May 2022 - **A.** Courting behaviour, in which the male coiled its body along that of the female, **B.** Copulating behaviour, during which the two snakes align and press their cloacae together. White dotted arrow indicates the presumed female snake (F); white continuous arrow indicates the presumed male snake (M). For more details see BHS videos 2023a & b.

was coiled more loosely around the female's body. The male was making spiral movements along the axis of the middle part of the female's body, without releasing the tight grip, with the head leading the way and gently rubbing the female's body, while the tail was providing a loose hold and was slowly moving along the female's body (Fig. 1A, BHS video 2023a). This courting behaviour of the male continued for about two minutes. However, since it had already begun at the time the snakes were first observed, its full duration remains uncertain.

After these two minutes, the two snakes started to copulate. During the copulation, the female's body remained in its previous position, although the snake was slowly moving forward. The head and the anterior 1/3 of the male's body was positioned away from the female's body. The middle 1/3 of the male's body was positioned on top of the mid-part of the body of the female, while the posterior 1/3 of the male made two tight coils around the corresponding parts of the female body, followed by a slightly loosened coil, and ending with the cloacae of the two snakes being pressed together. During this phase, the male tried to remain in this fixed position, so he adjusted the posterior part of its body after each movement of the female (Fig. 1B, BHS video 2023b). The cloacae of the two snakes remained pressed together for around 1.2 minutes, after which the male removed his cloaca for 3 seconds, and then pressed again against the cloaca of the female for an additional 10 seconds. Thereafter, the male released the hold on the female's body and moved away from her (BHS video 2023b).

As is generally the case in the Typhlopidae, sexual dimorphism in *X. vermicularis* is very weak, which makes determining the sex of an individual difficult and uncertain without dissection. Because of the species' small size and frail body structure, the usual methods for determining the sex of live snakes, such as probing (Schaefer, 1934) or squeezing the base of tail to reveal the hemipenes (Gregory, 1983) cannot be applied. Males and females differ to a small degree in the tail length and the number of subcaudal scales, with males usually having longer tails with more scales (Perry, 1984). However, because of the small size of those scales, a magnifying glass should be used for accurate counting (Perry, 1984). Additionally, at least in another member of Typhlopidae, *Anilios nigrescens* Gray, 1845, females are larger than males (Shine, 1978). Although sex determination in our case could not be confirmed, based on the difference between the body proportions of the two individuals (the larger snake was approximately 6 cm longer than the smaller one but its tail was 0.1 cm shorter), as well as the clear difference between the behaviours of the two individuals, it is reasonable to assume that the smaller and more active individual was the male.

The mating behaviour of snakes is usually rather complex and includes specific mating rituals where males are much more active and involved than females (Shine, 1978; Andrén & Nilson, 1983; Andrén, 1986; Vitt & Caldwell, 2014). This usually includes three phases: tactile-chase, tactile-alignment, and intromission coitus (Vitt & Caldwell, 2014). The tactile-chase phase includes the initial contact between the snakes, when males often use chemosensory sampling to determine the sex of the other snake. During this phase, the male places his body alongside or over the dorsal surface of the female, contracting segments of his body musculature in a wave-like manner, while often also rubbing his chin on the females back, or even biting her. The tactile-alignment phase begins when the first attempt to copulate occurs, which involves tail-searching and alignment of the male's tail with that of the female, by rapid caudal vibrating movements. The tactile behaviours expressed during the first phase may also continue during the tactile-alignment phase. During the intromission coitus phase, the female gapes her cloaca and allows the insertion of one of the hemipenes of the male (Vitt & Caldwell, 2014). It appears that courtship and copulating behaviour of *X. vermicularis* involves the same three phases. However, because of the species' very short tail, the end of each phase and the respective beginning of the next is hard to distinguish. We suggest that such behaviour with coiled body and tight grip on the female along with the strong pressing of cloacae are required considering the general morphology of the species and males' typical non-ornamented hemipenes (Heyder, 1968).

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