

Mating up in the trees: a new reproductive behaviour in *Bothrops jararaca* (Serpentes: Viperidae)

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Bothrops jararaca (Wied, 1824) is a species of the Viperidae family widely distributed in the southern and southeastern regions of Brazil (Nogueira et al., 2019), where it inhabits forests and is considered a semi-arboreal snake (Sazima, 1992). Juveniles tend to be more arboreal than adults (Martins et al., 2002), but even large adults may occasionally be found on the vegetation (Sazima, 1992). Although there are many studies regarding its reproductive cycle and natural history (Sazima, 1992; Almeida-Santos and Salomão, 2002), little is known about its courtship and copula behaviour in nature. Information on mating periods has been used to infer the onset and development of vitellogenesis, spermatogenesis, and hormonal production (Crews and Gans, 1992). For most Brazilian *Bothrops* species, including *B. jararaca*, the reproductive cycle begins with courtship and mating commonly in late summer and autumn, coinciding with the vitellogenesis, which extends until early spring (September to November) (Almeida-Santos and Salomão, 2002). Herein, we report the first observation of summer mating in *B. jararaca* in the canopy within the “open-door captivity” of Instituto Butantan, São Paulo, Brazil.

The open-door captivity (Fig. 1A) is a semi-natural outdoor enclosure designed to keep different species of snakes in a semi-extensive breeding system (Leloup, 1984). The *B. jararaca* enclosure (Fig. 1B) has an area of 13.65 m x 10.70 m (L x W). The captivity recreates the vegetation of the environment in which the snakes occur in nature. It has an artificial river and natural vegetation

composed of two medium-sized and one small-sized tree. The ground level is covered by natural grass, and there are also logs, rocks, leaves, and an artificial hiding spot where snakes can seek shelter. Because it is an outdoor environment in an urban green area, snakes experience natural climatic variables such as local temperature and humidity. These climatic variables are measured every day by an infrared thermometer (20 cm distance of snakes' microhabitat) and a hygrometer, respectively. Dorsal colour patterns helped identify the individuals, and snout-vent length (SVL) was determined using a measuring tape. The reproductive condition of the female was evaluated by palpation of the abdomen.

On 16 February 2016, at 09:10 h, after a night of heavy rain, a male and a female of *B. jararaca* were observed copulating in the open-door captivity (Fig. 1C, D). Both individuals were lying on a branch of a 3.88 m tall *Tibouchina mutabilis*. The point where the cloacae were in contact was moist with a secretion (Fig. 1E), coming from one or both individuals. The secretion could be extravasated semen or a pheromone secretion produced by the female to attract a male partner. However, the analysis was not possible since the liquid was not collected.

We also observed that the female's cloacal pouch was slightly hanging out (Fig. 1F). By the time that the reproductive event occurred, there were eight *B. jararaca* in the enclosure site accounting for a density of 0.05 snakes per m². We always keep more females than males because females are larger and, consequently, easier to observe. At the enclosure site, at the tree where the copula was observed, the air humidity was 71.20% and the temperature was 26.32 °C. We observed the copula for two continuous hours. At 13:15 h of the same day, both male and female had already separated and were then identified and measured (male: SVL = 93 cm; female: SVL = 115.5 cm).

The copula was observed in February (late summer), during the season when mating is expected in this species (Almeida-Santos and Salomão, 2002). In the first week of August, we recorded the presence of

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Figure 1. (A) View of Instituto Butantan’s open-door captivity; (B) View of the *B. jararaca* enclosure; (C) Mating in the canopy; (D) Mating in the canopy; (E) Hemipenis introduced in the female’s cloacal pouch. Note the secretion moist; (F) Hemipenis inserted in the female’s cloacal pouch. Note that the female’s cloacal pouch is slightly protruding from the body.

Table 1. Mating strata observed in nature for *Bothrops* species.

Species	Habitat use	Mating strata	Authors
<i>B. alternatus</i>	Terrestrial	Ground	Höggren and Rehák, 2020
<i>B. atrox</i>	Semi-arboreal	Logs of a bridge (over the stream)	Sanaïotti et al., 2005
		Ground	Silva et al., 2019
		Submerged (inside Igarape)	Silva et al., 2019
<i>B. bilineatus</i>	Arboreal	Tree	Turci et al., 2009
<i>B. fonsecai</i>	Terrestrial	Above ground (roots of a fallen tree)	Menezes et al., 2019
<i>B. insularis</i>	Arboreal	Tree and ground	Marques et al., 2013
		Ground	Amorim et al., 2019
<i>B. jararaca</i>	Semi-arboreal	Tree	This work
<i>B. moojeni</i>	Terrestrial	Ground	Zacariotti et al., 2011
<i>B. muriciensis</i>	Terrestrial	Above ground (fallen log)	Campbell and Lamar, 2004

vitellogenic follicles in the ovary of the *B. jararaca* female by palpation, indicating that the conditions in the open-door captivity did not affect the individual's reproductive cycle.

Considering snakes of the genus *Bothrops*, previous studies report mating behaviour in natural habitats for eight species, including the present work (Table 1). Reproductive activities above ground, including copulation or courtship, have been recorded for two terrestrial species (*B. fonsecai*, Menezes et al., 2019; *B. muriciensis*, Campbell and Lamar, 2004), two semi-arboreal species (*B. atrox*, Sanaïotti et al., 2005; *B. jararaca*, this work), and two arboreal species (*B. bilineatus*, Turci et al., 2009; *B. insularis*, Marques et al., 2013).

Because *B. jararaca* is semi-arboreal, it can be encountered in low arboreal strata in the open-door captivity. Occasionally, it is even observed preying on birds on tree branches (unpubl. observations of the authors). However, we never witnessed males and females in courtship activity before. The fact that this copula was registered at 3.88 meters from the ground can be indicative that these animals can also opt for copula at high heights in nature. It is possible that the couple searched for a high place to avoid being disturbed by other individuals. However, due to the low density of snakes in the enclosure site (0.05 snakes/m²), we find this unlikely.

Bothrops insularis, the golden lancehead, is a species closely related to *B. jararaca* (Marques et al., 2002;

Grazziotin et al., 2006; Fenwick et al., 2009) and inhabits the Queimada Grande Island. It is known that *B. insularis*, an arboreal snake, copulates on branches or in tops of trees (Marques, et al., 2013). According with our results, we confirm that this copula behaviour also occurs in *B. jararaca*.

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