

SHORT COMMUNICATION

Sexual maturity of *Bothrops asper* (Serpentes: Viperidae) from Costa Rica

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Palavras-chave: Biologia reprodutiva, Segmento sexual renal, Terciopelo, Torção muscular uterina.

Bothrops asper (Garman, 1883), a large, dietary generalist viper, is the only lancehead species widely distributed in the humid lowlands of Middle America and northwestern South America (Sasa *et al.* 2009, Saldarriaga-Córdoba *et al.* 2017). The species exhibits cryptic habits and adapts very well to disturbed environments such as agricultural areas (Sasa *et al.* 2009). These features, added to its high prolificacy, make *B. asper* responsible for the highest snakebite incidence throughout its distribution (Sasa *et al.* 2009). It is not surprising that it is a snake of great medical importance that has monopolized epidemiological and toxicological studies in the region (Saldarriaga *et al.* 2003, Alape-Girón *et al.* 2008, Bravo-Vega *et al.* 2019). Some studies have found an ontogenetic

change in the venom of the species (Saldarriaga *et al.* 2003, Alape-Girón *et al.* 2008). Because of this change, knowledge of reproduction and sexual maturity in this species is necessary (Freitas-de-Sousa *et al.* 2020).

Although some studies have included a large number of individuals of *B. asper* and excellent macroscopic descriptions (Solórzano and Cerdas 1989), knowledge of sexual maturity of *B. asper* based on microscopic morphological changes of the male and female urogenital tract is still lacking. Microscopy allows the accurate elucidation of sexual maturity due to the visualization of several microscopic indicators such as the presence of spermatozoa in the male or female reproductive tract, hypertrophy of epithelial cells of the sexual segment of the kidney (SSK), and hypertrophy of uterine and infundibular glands (Krohmer *et al.* 2004, Siegel and Sever 2008). Often, sexual maturity is assessed simply by macroscopic analyses, with males considered adults when they present

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enlarged testes or opaque ductus deferens and females considered adults by the macroscopic follicular state and whether they are ovigerous (Mathies 2011). These natural history data are necessary for designing and implementing more specific studies that rely on characterizing maturity, such as changes in venom (Freitas-de-Sousa *et al.* 2020), changes in diet (Martins *et al.* 2002), and activities related to reproduction (e.g., minimum age to start a semen collection; Silva *et al.* 2015).

Herein we report sexual maturity of *B. asper* from Costa Rica and provide macroscopic and microscopic descriptions of the male and female urogenital tracts. We also investigate whether body sizes of males and females that reach sexual maturity are similar among sister species (*Bothrops atrox* and *Bothrops jararacussu* groups).

We examined 51 individuals of *B. asper* from Costa Rican collections (19 adult females, 23 adult males, and nine juveniles) (Appendix I). We measured snout–vent length (SVL) of each specimen before performing dissections. Females were considered sexually mature if they had vitellogenic ovarian follicles (Manes *et al.* 2007), oviductal embryos, corpora lutea and empty oviducts (postpartum), uterine muscular twisting (UMT; Silva *et al.* 2019a), or the presence of sperm in the oviduct. Males were considered mature if they had convoluted ductus deferentia with sperm in the lumen, seminiferous tubules in the spermatogenic cycle, or a hypertrophied sexual segment of the kidney (SSK; Silva *et al.* 2021).

Samples of the urogenital system from a subset of adult or juvenile individuals were removed for histological examination. We excised the posterior portions of the right non-glandular uterus, posterior infundibulum, and ovarian follicles for females. For males, we collected the mid-region of the testis, the ductus deferens (portion between the kidney and the cloaca), and the proximal region of the kidneys. Samples were stored in 70% ethanol until examination by light microscopy. We used a rotary microtome to produce transverse and

longitudinal sections and stained them with hematoxylin and eosin (Junqueira and Carneiro 2013).

Adult females averaged 1147 ± 166 mm SVL (range 890–1600 mm, $N = 19$), and adult males averaged 930 ± 216 mm SVL (range 560–1360 mm, $N = 23$). The smallest sexually mature male, SVL 560 mm, presented regressed testes (Figure 1A), hypertrophied SSK (Figure 1B), and convoluted ductus deferentia with sperm in the lumen (Figure 1C). The smallest mature female, SVL 890 mm, presented UMT without sperm in the lumen (Figure 2A), a posterior infundibulum with many receptacles of sperm (Figure 2B), and ovarian follicles measuring 5 mm in diameter. Another female measuring 920 mm SVL had ovarian follicles initiating vitellogenic processes (vacuolated ooplasm) with a diameter of 7 mm (Figure 2C), presence of UMT, and infundibulum with receptacles of sperm (Figure 2D).

Six adult females (previtellogenic or initiating vitellogenic processes) of SVL > 890 mm had UMT. Pregnant females ($N = 4$; > 1120 mm SVL) and some previtellogenic adults ($N = 6$; > 1020 mm SVL) did not show UMT. No immature females ($N = 8$; < 940 mm SVL) showed UMT. The immature females had very attenuated oviducts.

Female *B. asper* reached sexual maturity at a larger body size than males, an observation that can be explained by selection for fecundity: larger females achieve higher levels of fecundity (Stearns and Hoekstra 2001). This condition, in which females are larger and more massive than males, has been termed inverse body dimorphism and has been reported for different organisms (Stearns and Hoekstra 2001) and also observed in other species of the genus *Bothrops* (Sazima 1992, Silva *et al.* 2020).

In several related species [*B. asper*; *B. atrox* (Linnaeus, 1758), *B. leucurus* Wagler, 1824, *B. moojeni* Hoge, 1966, and *B. jararacussu* Lacerda, 1884], males reached sexual maturity at 560, 537, 520, 545 and 460 mm SVL, respectively (Barros *et al.* 2014a, Almeida-Santos *et al.* 2017, Silva *et al.* 2019b, 2020), while females of these

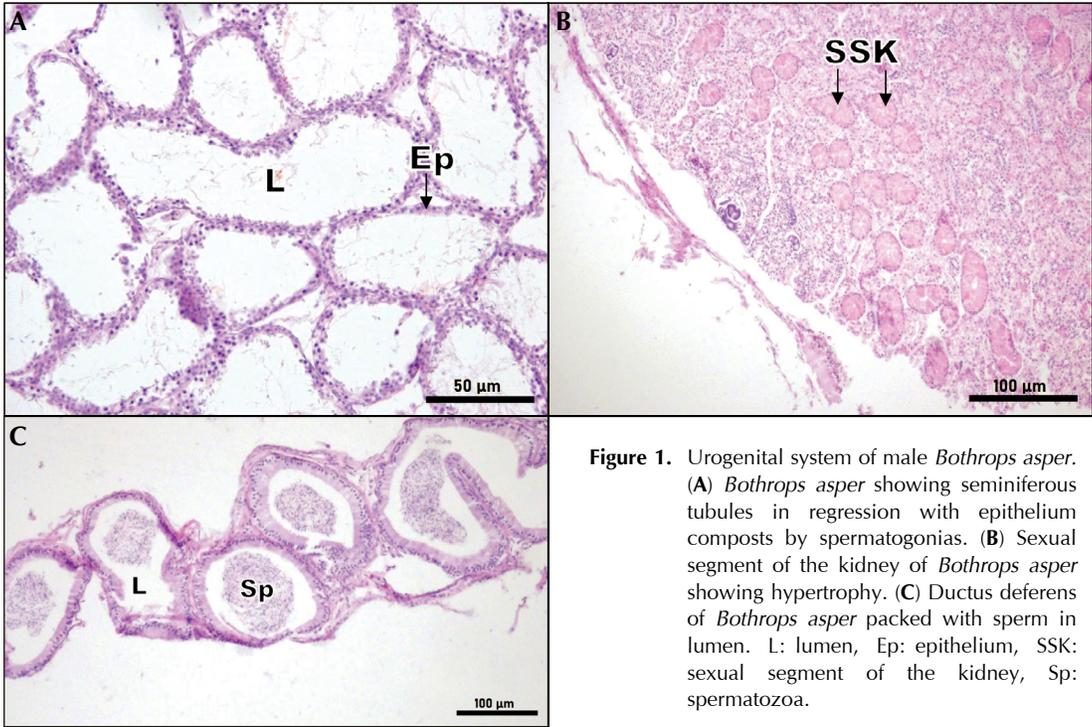


Figure 1. Urogenital system of male *Bothrops asper*. (A) *Bothrops asper* showing seminiferous tubules in regression with epithelium composts by spermatogonias. (B) Sexual segment of the kidney of *Bothrops asper* showing hypertrophy. (C) Ductus deferens of *Bothrops asper* packed with sperm in lumen. L: lumen, Ep: epithelium, SSK: sexual segment of the kidney, Sp: spermatozoa.

same species reached sexual maturity at 862, 665, 555, 580, 800 mm SVL, respectively (Solórzano and Cerdas 1989, Barros *et al.* 2014a, Almeida-Santos *et al.* 2017, Silva *et al.* 2019b, 2020).

The larger or more robust females of the *B. atrox* and *B. jararacussu* groups, *B. asper* and *B. jararacussu* (Martins *et al.* 2001), reached maturity at much larger sizes than other sister species, which favors larger litter size (Solórzano and Cerdas 1989, Silva *et al.* 2020). The great size of these species of *Bothrops* needed to reach sexual maturity should be evaluated by molecular and hormonal techniques or by monitoring these species in captivity to elucidate the intrinsic factors related to the growth rate of these animals. In contrast, females of *B. atrox*, *B. leucurus*, and *B. moojeni* attain sexual maturity at relatively small body sizes compared to *B. asper* and *B. jararacussu*. The males of these

three species perform male-male combat (Almeida-Santos *et al.* 2017). So far, no male-male combat has been reported in *B. asper* and *B. jararacussu*.

The morphology of the male reproductive tract of *B. asper* seems to be conservative among species of *Bothrops*, in which the testes of adult individuals can completely regress during some period of the year (Barros *et al.* 2014b, Silva *et al.* 2020). Thus, collecting the entire urogenital tract (testis, ductus deferens, and kidney) is necessary to characterize maturity. Since SSK hypertrophy is closely related to the production of the hormone testosterone, hypertrophy, when present, is a reliable indication that the individual is an adult (Schuett *et al.* 2002, Krohmer *et al.* 2004).

The morphology of the female reproductive tract of *B. asper* follows the same pattern described for viperids (Siegel and Sever 2008,

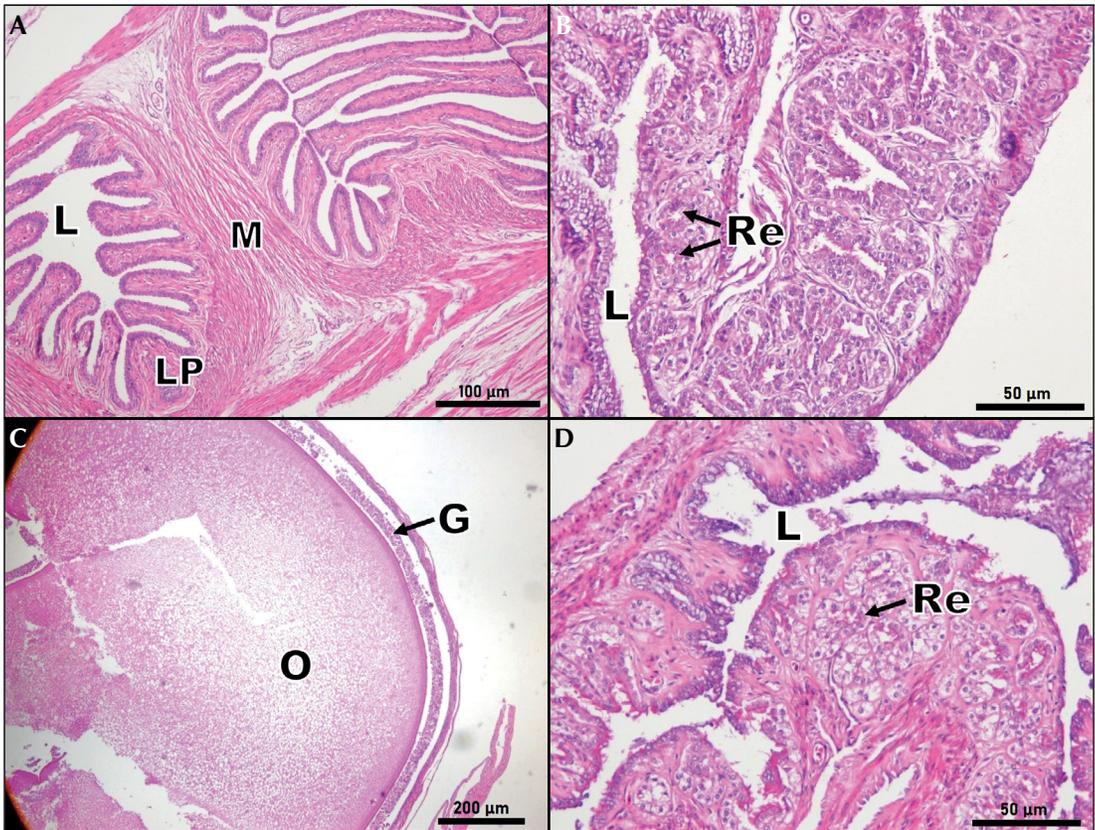


Figure 2. Reproductive tract of female *Bothrops asper*. (A) Non-glandular uterus. (B) Posterior infundibulum of previtellogenic female. (C) Ovarian follicles at the onset of vitellogenesis. (D) Posterior infundibulum of vitellogenic females. L: lumen, LP: lamina propria, M: musculature, Re: receptacle, O: ooplasm, G: granulosa.

Barros *et al.* 2014b, Silva *et al.* 2019a). The non-glandular uterus has a convolution called UMT (Yamanouye *et al.* 2004). The UMT in *Crotalus durissus* Linnaeus, 1758 is seen only in adult females and usually during plasma estradiol increase and development of ovarian follicles (Yamanouye *et al.* 2004, Muniz-da-Silva *et al.* 2018). Thus, the presence of UMT in viperids is indicative of sexual maturity. In *Bothrops*, UMT has been observed in adult females, including previtellogenic, vitellogenic, and pregnant females (Silva *et al.* 2020). The presence of UMT is just one more indication of sexual maturity because adult individuals (mainly with

low estradiol) may not have UMT (Yamanouye *et al.* 2004).

In addition, the non-glandular uterus has been described for viperids as a sperm storage region, and sperm survival is probably favored by UMT formation (Yamanouye *et al.* 2004, Silva *et al.* 2019a). Another storage region in viperids is the posterior infundibulum, a region with many infundibular glands and receptacles. In *Bothrops* the sperm receptacles are tubular and composed of ciliated cells (Barros *et al.* 2014b, Silva *et al.* 2019a).

Males of *B. asper* reach sexual maturity at sizes that are much smaller than 860 mm SVL,

while females can reach maturity at sizes larger than 890 or 860 mm SVL, which is the size observed by Solórzano and Cerdas (1989). In addition, the determination of sexual maturity through histology, especially for young males, found that some males became mature at 560 mm SVL, which indicates that small males may be selected for or can court large females.

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Appendix I. Specimens examined.

Museo de Zoología de la Universidad de Costa Rica (MZUC): 8, 9, 1441, 6234, 6241, 7227, 11436, 11508, 12336, 12337, 13846, 14860, 15560, 16088, 16171, 16191, 16196, 16198, 16492, 16681, 20157, 20358, 20572, 21772, 22051, 22191.
 Instituto Clodomiro Picado (ICP): 1789, 1790, 1791, 1792, 1793, 1794, 1795, 1802, 1864; (LIAP) 23, 115, 116, 117, 118, 120, 121, 122, 150, 151, 157, 161, 170, 171, 172, 184.