

The sexual attractiveness of the corpse bride: unusual mating behaviour of *Helicops carinicaudus* (Serpentes: Dipsadidae)

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Abstract. Natural history studies on Neotropical snake fauna are still scarce. Even though reproductive data for the aquatic snake *Helicops carinicaudus* is available, the lack of information on courtship, copula and reproductive behaviour in the field is noteworthy. Copulation of *H. carinicaudus* and necrophilia in snakes in nature is reported for the first time. Data was collected during a fieldwork survey on the herpetofauna at the São Camilo environmental station, Atlantic Forest area along the coast in São Paulo State. We observed a young male copulating, with the hemipenis fully inserted into a headless female. Specimens were collected, dissected and measured. Histological sections of male and female genital tract and male kidney were prepared in order to assess reproductive status. The observation was made during the vitellogenic period of females of *H. carinicaudus*, which occurs from July to December, and is probably associated to the production of sex pheromones. Spermatogenesis was observed in male, with testosterone production characterized by the hypertrophy of the sexual segment of the kidney (SSK). In this case, vitellogenic condition of the female *H. carinicaudus* favoured necrophilia, as chemical cues, trails and pheromones were still being released, permitting courtship and copulation by the young reproductive male.

Introduction

Mechanisms used by snakes for detecting pheromone trails through the vomeronasal organ, especially for finding partners and recognizing potential mates during the breeding season, are crucial to understand the courtship behaviour and copulation of these animals (Parker and Mason, 2011). In some cases, pheromone communication can trigger unusual behaviours in males, such as necrophilia (Costa *et al.*, 2010; Sazima, 2015).

Necrophilia or Davian behaviour was reported in several species of lizards and snakes (Amaral, 1932; Lambiris, 1966; Sharrad *et al.*, 1995; How and Bull, 1998; Vitt, 2003; Fallahpour, 2005; Brinker and Bucklin, 2006; Costa *et al.*, 2010; Sazima, 2015). These cases are often associated with the approaching reproductive period (Costa *et al.*, 2010; Sazima, 2015). Males were presumably attracted by chemical cues secreted by recently dead reproductive females, and mated with the stationary and seemingly “receptive” females by mistake (Costa *et al.*, 2010). However, since necrophilia offers no advantage to the reproductive success of the individuals, it is necessary to clarify the role of the pheromones on the attraction of the males.

Helicops carinicaudus (Wied, 1825) is a viviparous snake with aquatic habits and it is distributed along the Serra do Mar range, in southeastern and southern Brazil (Marques *et al.*, 2001; Marques and Sazima, 2004). Despite the availability of biological data on the reproduction of this snake (Scartozonni, 2009), information on its courtship, copula and reproductive behaviour remains scarce. Could pheromones related to the reproductive condition of the dead female elicit male mating behaviour?

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Figure 1. A) Copulation between individuals of *Helicops carinicaudus* (MZUSP 22322 and MZUSP 22323); B) Left hemipenis inserted into the cloaca; C) Moment of copulation, focusing on the male head.

In the present work, we describe the mating of an adult male *H. carinicaudus* with a dead conspecific female in the field, and report for the first time the actual reproductive status of both sexes after copulation.

Materials and methods

The observation took place during fieldwork, at São Camilo environmental station, Itanhaém municipality, at the southern coast of São Paulo State, Brazil. The site is a very anthropized swamp, with predominance of shrub vegetation, grasses and herbs, and it is located in a transition area between the Atlantic Forest and Restinga (24 ° 8'34.85 "S 46 ° 45'47.00" W).

A local resident reported that on November 2nd, 2012, around 9:30 p.m., a fisherman using chunks of *Geophagus brasiliensis* (Quoy and Gaimard, 1824) as bait had accidentally caught two water snakes. Because

he considered them to be dangerous, he cut off their heads.

The next morning, at 9:30 am, with sunlight (24°C), two dead females close to each other, and a male snake were observed at the reported site. The specimens were identified as *Helicops carinicaudus*.

Specimens were photographed with a digital camera and observed for 30 minutes before being collected. Three specimens were collected, the couple in copula and another headless female. After collection, the specimens were stored in a clear plastic container for observation. Afterwards, all snakes were fixed in 10% formalin and deposited at the Museu de Zoologia de São Paulo (MZUSP) under the labels MZUSP 22323 (male), MZUSP 22322 and MZUSP 21254 (females). In this study only the specimens corresponding to the couple in copulation (MZUSP 22323 and MZUSP 22322) were analysed. Snout-vent length (SVL) and tail length (TL)

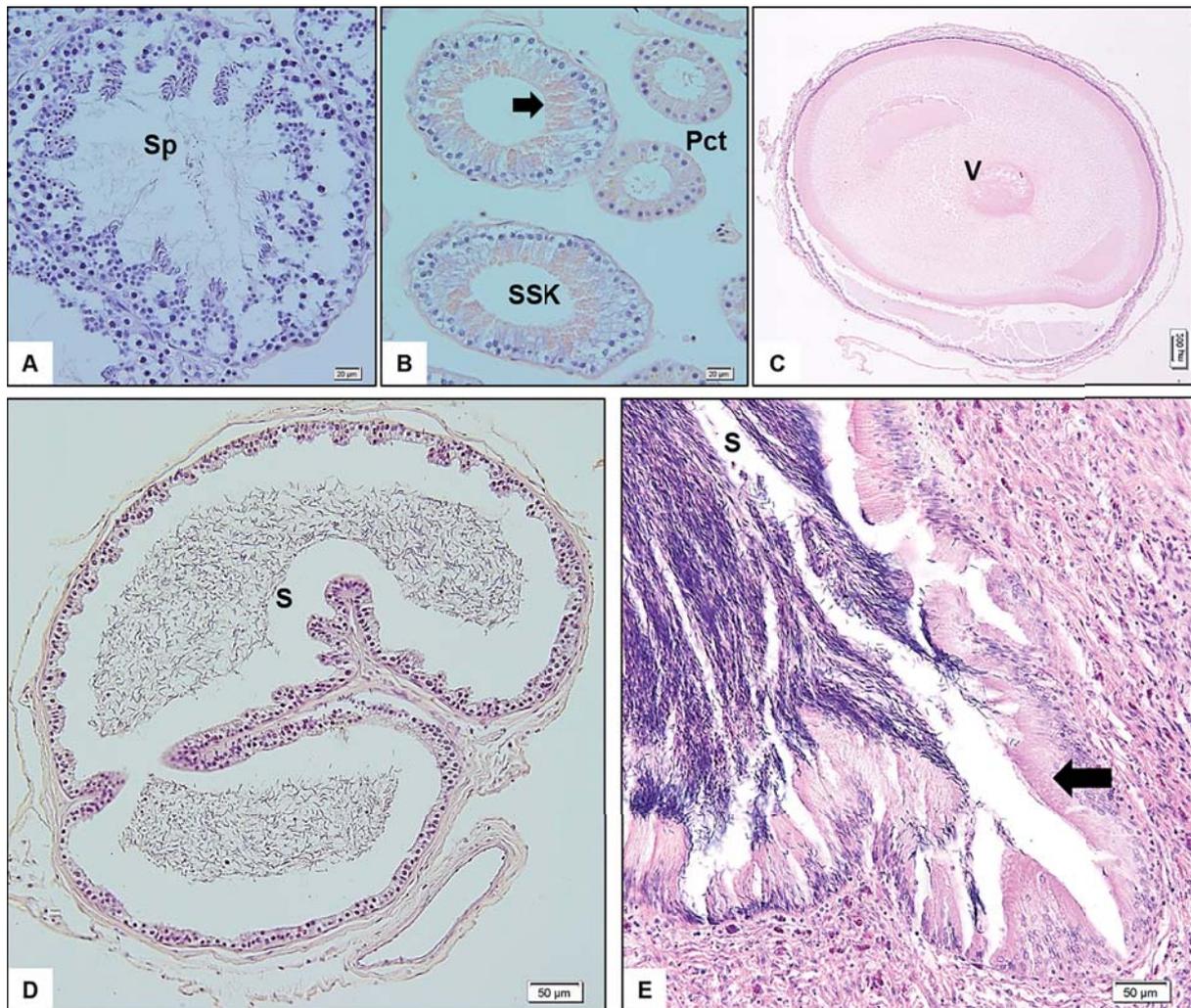


Figure 2. Cross sections (5- μ m thick; H/E stain) of male and female reproductive organs A) Testis with spermatogenic stage (Sp). B) Male kidney with the sexual segment of the kidney (SSK), the proximal convoluted tubule (Pct) and the secretory granules (arrow). C) Follicle in secondary vitellogenesis and vitellus (V). D) Ductus deferens with sperm (S) in the lumen. E) Vaginal epithelium (arrow) and vaginal sperm (S).

of the couple were measured using a caliper rule to the nearest millimeter.

To assess the reproductive condition, samples of the reproductive organs were dissected and measured. The diameter of the copulated female's largest ovarian follicle and the width of the male's right and left testis were measured, as described by Almeida-Santos *et al.* (2014). Small fragments of female organs (ovary and vagina) and male organs (sexual segment of the kidney - SSK, testes and vas deferens) were used for histological analyses (Rojas *et al.*, 2013). Histological sections were 5 μ m thick, and stained with hematoxylin and eosin (H/E) (Junqueira *et al.*, 1995).

Results

The adult male was performing copulation movements in one of the headless females (Figure 1A and Figure 1C), entering his left hemipenis fully into the cloaca of this female (Figure 1B). Even after intense handling, the male copula behaviour did not change for another 14 hours, until 11 p.m. of the same day.

The male measured 450 mm SVL. The right and left testicles measured 17.41 and 12.25 mm in length, respectively. The histological sections of the testicle indicated a spermatogenic stage (Figure 2A). Small segment fragments evidenced the SSK which was showing hypertrophy of the distal convoluted tubule

Table 1. Morphological traits of the couple of *Helicops carinicaudus*. SVL = snout-vent length, TL = tail length, R = right, L = left; Testes; Sexual Segment of the Kidney (SSK) and size of follicle.

| Male (MZUSP 22323) | Testes (mm) | SSK | Female (MZUSP 22322) | Size of follicle |
|--------------------|-------------|-----------------------------------|----------------------|------------------|
| 450 mm (SVL) | 17.41(R) | Hypertrophy with granules evident | 697 mm (SVL) | > 7.28mm |
| 17 mm (TL) | 12.25 (L) | | 16.5 mm (TL) | |

with high density of granules (Figure 2B). The vas deferens contained sperm in the lumen (Figure 2D).

The female measured 697 mm SVL, and the largest ovarian follicle measured 7.28 mm. Histological sections showed that the follicle was sexually mature, and the specimen showed vitellogenic follicles (Figure 2C). Sections of the vaginal region showed sperm clusters in the light of the epithelium (Figure 2E).

Discussion

Courtship and mating behaviour of male reptiles in the presence of freshly dead females is probably related to sexual pheromones still emitted by the carcasses (Vitt, 2003; Costa *et al.*, 2010; Sazima, 2015). Female snakes can signal estrus during the vitellogenesis period by releasing pheromone cues that attract males (Mason, 1992; Parker and Mason, 2011). Since snakes are strongly influenced by pheromones during their sexual activity (Garstka and Crews, 1981; Shine and Mason, 2011), males were likely guided by these pheromones and unable to notice the immobility of the females being courted. Thus, during the reproductive period and the presence of pheromones the female had “sex appeal” despite being dead. Similarly to other cases of necrophilia (Brinker and Bucklin, 2006; Costa *et al.*, 2010; Sazima *et al.*, 2015), the vitellogenic condition of the female *H. carinicaudus* favoured Davian behaviour.

Although the body length of the male *Helicops carinicaudus* corresponds to that of a young male (Scartozzoni, 2009), the sperm in the ducts shows that it was sexually active. Likewise, the sperm in the female’s vagina proves that the copulation occurred. However, since the female was dead, the mating was a waste of energy and resources for the male.

Our results confirm the data of Scartozzoni (2009), about the vitellogenic period of females of *H. carinicaudus* ranging from July to December. This period

is probably related to the production of sex pheromones (Garstka and Crews, 1981; Lemaster and Mason, 2002). Spermatogenesis was observed in the male and together with the hypertrophied SSK indicates the production of testosterone (Rojas *et al.*, 2013). Therefore, potentially both male and female would be reproductive. The fact that, by the time of our observation, the male copulated using the left hemipenis is unusual, since a study by Shine *et al.* (2000) reports that colubrid snakes preferentially use their right hemipenis.

Thus, this report shows that even a dead female may be sexually attractive and elicit courtship behaviour, encouraging young and “naive” males to copulate.

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