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Intersexuality in *Helicops infrataeniatus* Jan, 1865 (Dipsadidae: Hydropsini)

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Herein, we describe the first case of intersexuality in the Hydropsini tribe. After examination of 720 specimens of *Helicops infrataeniatus* Jan, 1865, we discovered one individual that presented feminine and masculine reproductive features. The specimen was 619 mm long, with seven follicles in secondary stage, of different shapes and sizes, and a hemipenis with 13.32 and 13.57 mm in length. The general shape of this organ is similar to that observed in males, although it is smaller and does not present conspicuous spines along its body. Deformities found in feminine and masculine structures suggest that this specimen might not be reproductively functional.

Key words: Follicles, hemipenis, hermaphroditism, water snake.

As all amniotes, lepidosaurian reptiles have internal fecundation and, with the exception of Tuataras, which have no copulatory organ, male specimens have hemipenes (Vitt & Caldwell, 2013). However, some squamate species evolved to break free of sexual reproduction, being the only vertebrates that truly reproduce by parthenogenesis (Kearney et al., 2009; Vitt & Caldwell, 2013). Such mechanism is predominant in lizards (e.g. Darevski, 1966; Hardy & Cole, 1981; Schmidtler, 1993; Schmidtler et al., 1994), but it also occurs in snakes (e.g. Wynn et al., 1987; Groot et al., 2003; Booth et al., 2012).

Another condition reported for reptiles is intersexuality, which is defined as a condition in which reproductive structures in a given sex are also found in the opposite sex (Goldschmidt, 1917). The condition may include both hermaphroditism (presence of both ovarian and testicular tissues) and pseudohermaphroditism (presence of gonadal tissue of one single sex) (Forbes, 1964). Intersexual individuals in squamates have been recorded for snakes only (Hardy, 1970). In lizards, hermaphroditic males are not viable and occur in hybridogenic rock lizards (genus *Darveskia*, see Darvesky 1966).

The Hydropsini tribe currently encompasses a total of 22 valid species allocated in the following genera: *Helicops*, *Hydrops* and *Pseudoeryx* (Uetz et al., 2017). While *Hydrops* and *Pseudoeryx* are oviparous, most species of *Helicops*

are viviparous, and interestingly, *H. angulatus* exhibits both reproductive modes (Rossman, 1984; Aguiar & Di-Bernardo, 2005; Braz et al., 2016). *Helicops infrataeniatus* has a wide distribution that encompasses south-southeastern Brazil, southern Paraguay, North-eastern Argentina and Uruguay (Deiques & Cechin, 1991; Giraudo, 2001; Carreira & Maneyro, 2013). At the coastal zone of southernmost Brazil, *H. infrataeniatus* is among the most abundant species in many types of limnic and estuarine environments (Quintela & Loebmann, 2009; Regnet et al., 2017). In October 2015 at the Laranjal beach, municipality of Pelotas, state of Rio Grande do Sul, Brazil (31°46'S, 52°13'W), a remarkable aggregation of reptiles and caecilians occurred after a flood event associated to an El Niño event (Regnet et al., 2017). This event provided an outstanding opportunity to investigate aspects of the reproductive biology of several species with a large sample size. When such specimens were examined, one showed follicles in advanced vitellogenesis as well as a hemipenis, characterising a rare case of intersexuality, which is here described.

The intersexual specimen was deposited in the Herpetological Collection of the Universidade Federal do Rio Grande - FURG (CHFURG3946). Although the individual presented female and male sexual characteristics, it presents a number of subcaudal scales corresponding to the females of the species (64) (Giraudo, 2001). For comparison purposes, we everted and inflated a total of four males hemipenes as well as follicles in secondary vitellogenesis from a female. For each specimen studied, we measured the hemipenis length as well as snout-vent length (SVL). For standardisation, we used only the length of the right hemipenis. Description of reproductive organs follows Zaher (1999) for hemipenis and Almeida-Santos et al. (2014) for follicles.

Snout-vent length of the intersexual specimen (Fig. 1) was 537 mm, tail length was 82 mm and weight was 103.87 g. The left ovary contained seven follicles in secondary stage, with different shapes and sizes, and follicles of the right ovary were all in the primary stage (Fig. 1). Right and left hemipenes were 13.32 and 13.57 mm long, respectively. Males had SVL ranging from 380 to 432 (mean = 410.5 mm) and right hemipenes length

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Figure 1. (A) General view of reproductive organs of the intersexual specimen of *H. infrataeniatus*; (B) intersexual hemipenis; (C) male hemipenis; (D) follicles of the intersexual specimen with different shapes and sizes and (E) follicle of a normal female.

ranging from 13.79 to 14.61 (mean = 14.17 mm). Relative measures (organ length/SVL) of right hemipenes were 0.025 and 0.035 on average, respectively. General shape of the intersexual hemipenis in both sides (assulcate and sulcate) is similar in both structures, although is smaller (ca. 71.43% in relation to the mean length of hemipenes measured). Both structures are bilobated, but only male hemipenes have conspicuous spines along their body (Fig. 1).

The intersexual specimen here described was detected in a large sample of 720 individuals (327 females and 376 males), that is, it represents only 0.14% of the studied population. Therefore, the condition should be considered rare for this species. Aguiar & Di-Bernardo (2005) provide biological data on the reproduction of *H. infrataeniatus* and, when comparing those data to the observation of the intersexual specimen, the number of follicles found is to be expected. However, follicles from the intersexual specimen were highly variable in size, shape and color as well, so it is possible that intersexual follicles may not be viable.

Intersexual condition in squamates is usually evidenced by the presence of erectile organs in females

similar to the male hemipenis (Forbes, 1964). It is important to emphasise that this condition differs from individuals that exhibit hemiclitoris, a homologous structure to the hemipenis in females, but with smaller size (Böhme, 1995). Examples of females with hemiclitoris seem to be more frequent in squamates than intersexuality (e.g. Böhme, 1995; Ziegler & Böhme, 1997; Kasperovicz et al., 2011), which has been reported only for snakes (e.g. *Bothrops insularis* (Hoge et al., 1954), *Bothrops moojeni* (McClean, 1968), *Pseudoficimia frontalis* (Hardy, 1970), and *Pareas stanleyi* (Pope, 1935)). Interestingly, *B. insularis* females with hemipenes are frequently found in populations and individuals without this structure seem to be sterile (Hoge et al., 1959).

Seminiferous tubules or testicles were absent in the intersexual specimen and its hemipenis was smaller, more translucent and did not present spines along their body in comparison with males hemipenes. A notorious case regarding confusion in the interpretation of this structure can be found in the description of *Pseudoficimia pulcherrima*, where two females with smaller hemipenes and without spines were described as males of a new species (Taylor & Smith, 1942). After the mistake

was discovered, the species was synonymised with *Pseudoficimia frontalis* (Hardy, 1970).

This is the first report of an intersexual individual in the Hydropsini tribe. The presence of intersexual individuals emphasises the importance to check other sexual features concomitantly (Ziegler & Böhme, 1996) to avoid erroneous sex determination. Although cases of intersexuality are apparently rare in most snake species, we recommend that sexual determination based on the presence of a structure similar to a hemipenis might not be conclusive and, therefore, it should be used other morphological sexual features whenever is possible.

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