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Communication

[*Comunicação*]

Identification of microchips through diagnostic imaging helps to determine the longevity of the golden lancehead (*Bothrops insularis*) in captivity

[*Identificação de microchips por meio de diagnóstico por imagem auxilia a determinar a longevidade da jararaca-ilhoa (Bothrops insularis) em cativeiro*]

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The golden lancehead *Bothrops insularis* is endemic to the Queimada Grande Island, located 32km from the city of Itanhaém, state of São Paulo, southeastern Brazil (Marques *et al.*, 2012). This species was intensively collected on the Queimada Grande Island between 1914 and 1924, and during this period, about 450 specimens were incorporated into the herpetological collection and laboratories of the Butantan Institute. These specimens were brought to the institute by lighthouse keepers who lived on the island and by the institute's researcher Afrânio do Amaral (Duarte *et al.*, 1995).

No further expeditions occurred for over 20 years until the institute's researcher, Dr. Alphonse Richard Hoge, visited the island between 1947 and 1970 (Hoge *et al.*, 1959). The island was visited again for research purposes only fourteen years later, during the 1980s (Kasperoviczus and Almeida-Santos, 2012). At the beginning of the 21st century, Professor Marcio Martins (University of São Paulo) obtained financial support from the Boticário Foundation for Nature Protection, and the snakes found on the island began to be marked with transponders (microchips) allowing advances in long-term population studies (Marques *et al.*, 2012). From 2007 to 2009, several expeditions were carried out by researcher Otavio Marques (Butantan Institute) funded by FAPESP (São Paulo Research Foundation).

These expeditions enabled the collection of about 20 individuals of *B. insularis*. These individuals were brought to the Laboratory of Ecology and Evolution of the Butantan Institute, and since then they have been kept in captivity for biological and reproductive studies, as well as for *ex situ* conservation (IBAMA License no. 25.650-1; Kasperoviczus and Almeida-Santos, 2012). During the expeditions, snakes were measured, weighed, sexed, palpated for the presence of follicles or developing embryos, and marked with implantable microchips.

Diagnostic imaging has been of great importance in reptile medicine. A precise physical exam is difficult to perform, but radiography and ultrasonography have allowed more detailed studies aiming at a better knowledge of organs, bones, and tissues of several snake species (Garcia *et al.*, 2015). This work reports the use of diagnostic imaging (radiography and ultrasound) to identify the presence of microchips and, consequently, estimate the age of a captive *B. insularis*.

In July 2003, researchers from the Butantan Institute visited the Queimada Grande Island to collect biological data and ascertain environmental conditions (Duarte *et al.*, 2005).

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In this expedition, researchers captured a female *B. insularis* containing eight ovarian follicles. This specimen measured 700mm snout-vent length (SVL), 100mm tail length (TL), and weighed 140g (Table 1). After examination, the animal was implanted with a microchip (identification number 0001268792) and released at the capture site. In 2009, about 20 individuals of *B. insularis* were collected (with permission from SISBIO and IBAMA) and brought to the Laboratory of Ecology and Evolution of the Butantan Institute (São Paulo, Brazil).

Table 1. Biological data of an individual of *Bothrops insularis* (number 13) observed between 2003 and 2018

Date	Snout-vent length+tail length (mm)	Body mass (g)	Age (years)
July 2003	700+100	140	≅ 3-4
October 2009	818+108	275	≅ 9-10
February 2018	1000+120	579	≅ 18-19

Once in the lab, snakes were quarantined and implanted with subcutaneous microchips. In 2015, some captive snakes received new microchips because the old ones were no longer being detected with the universal microchip reader (Global Mark). In December 2017, radiographic and ultrasonographic examinations were performed on the snakes to investigate their physical conditions. We found that an individual of *B. insularis* (number 13) had three microchips. Imaging procedures were performed at the Diagnostic Imaging Service of the School of Veterinary Medicine and Animal Science of the University of São Paulo. Microchip 1 (0001268792) was read using a pocket reader (Trovan, Ltd.), and microchip 3 (FDXB982000364507461) was read using a Global Mark reader. Microchip 2 was not readable and was identified by retrieving its information from the animal's record database (Table 2).

Table 2. Location of the microchips in the snake *Bothrops insularis* (number 13)

Microchip	Microchip number	Location/Radiography
1	0001268792	Left side – 380 mm above the cloaca (ventral - midbody)
2	6361AD7	Left side – 200 mm above the cloaca (dorsal – caudal third)
3	FDXB982000364507461	Right side – 90 mm above the cloaca (dorsal – caudal third)

Through radiography, we identified subcutaneous structures compatible with microchips. These structures were located in the ventral region of the mid-body and the dorsal region of the caudal third of the snake (Figure 1A). Through ultrasonography, the microchips were visualized as hyperechogenic subcutaneous structures located in the coelomic cavity (Figure. 1B) and ventral to the vertebral column (Figure. 1C-D). From these data, we conclude that this individual was the same one collected and marked on the island in July 2003. When this individual arrived at our laboratory in October 2009, it had 818mm SVL, 108mm TL and weighed 275g.

In February 2018, the snake measured 1000mm SVL, 120mm TL, and weighed 579g (Table 1). The SVL of this individual in 2003 was similar to that observed in captive *B. insularis* at 3-4 years old (Araoz *et al.*, 2018). According to Mansano *et al.* (2013), radiography allows us to find the correct location of the implanted microchips. Thus, diagnostic imaging helped us to identify the microchips in an individual of *B. insularis* and, consequently, estimate its age at about 18-19 years, suggesting a generation of old snakes.

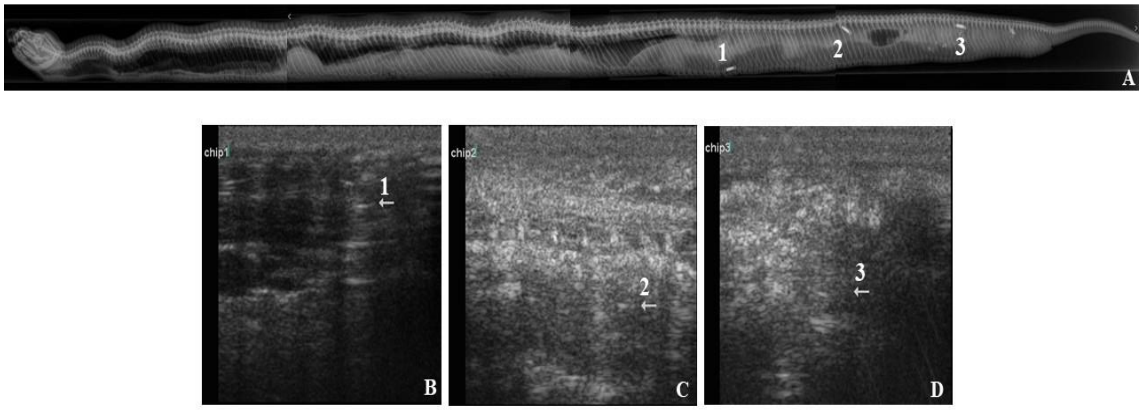

Figure 1. A- Radiographic evaluation of an individual of *Bothrops insularis* (number 13) performed in February 2018. The radiographic image shows structures compatible with microchips in the ventral region of the mid-body (microchip 1) and the dorsal region of the caudal third of the snake (microchips 2 and 3). B-D - Ultrasound evaluation showing subcutaneous hyperechogenic structures measuring 1 cm in the (B) coelomic cavity (microchip 1) and (C-D) ventral to the vertebral column (microchips 2 and 3). Images provided by the Diagnostic Imaging Service of the School of Veterinary Medicine and Animal Science of the University of São Paulo (FMVZ - USP).

Keywords: snake, radiology, ultrasound, body size, age

RESUMO

As serpentes vindas da natureza e encaminhadas para centros de reabilitação ou de pesquisa têm uma idade incerta. Na natureza, esses animais, muitas vezes atingem um tamanho corpóreo menor do que os indivíduos cativos devido a uma menor frequência de alimentação. Assim, a idade de uma cobra recém-chegada da natureza é geralmente estimada com base em seu tamanho corpóreo, o qual é comparado com dados em cativeiro. A utilização dos meios de diagnóstico por imagem tem auxiliado a medicina na análise das serpentes em cativeiro, mediante avaliação da estrutura óssea, dos órgãos e de alterações. Este trabalho relata o uso de diagnóstico por imagem (radiografia e ultrassonografia) para identificar a presença de microchips e, consequentemente, estimar a idade de uma Bothrops insularis em cativeiro.

Palavras-chave: serpente, radiologia, ultrassom, tamanho corpóreo, idade

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