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Short communication

First report of parasitism by *Hexametra boddaertii* (Nematoda: Ascaridae) in *Oxyrhopus guibei* (Serpentes: Colubridae)

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ABSTRACT

The current study summarizes the *postmortem* examination of a specimen of *Oxyrhopus guibei* (Serpentes, Colubridae) collected in Iguazu National Park (Argentina), and found deceased a week following arrival to the serpentarium of the National Institute of Tropical Medicine (Argentina). Although the snake appeared to be in good health, a necropsy performed following its death identified the presence of a large number of roundworms in the coelomic cavity, with indications of peritonitis and serosal adherence. Additional observations from the necropsy revealed small calcifications in the mesothelium of the coelomic cavity; solid and expressive content in the gallbladder; massive gastrointestinal obstruction due to nematodes; and lung edema and congestion. Histopathological analyses of lung sections also showed proliferative heterophilic and histiocytic pneumonia. Parasites isolated from both the intestine and coelomic cavity were identified as *Hexametra boddaertii* by a combination of light and scanning electron microscopic examination. Results from this necropsy identify *O. guibei* as a new host for *H. boddaertii*, and is the first report of a natural infection by *Hexametra* in Argentina. Since *Hexametra* parasites may contribute to several pathological conditions in humans, and with the recent availability of *O. guibei* specimens through the illegal pet trade, it is necessary to consider the possibility of zoonotic helminth transmission of *Hexametra* from snake to human.

Despite the diversity of parasites observed in reptiles, studies examining the helminth fauna of snakes native to the Neotropic ecozone have been relatively scarce (Mati et al., 2015). In particular, there are only a few examples of parasitism affecting snakes in Argentina (Boero and Led, 1971; Lamas and Lunaschi, 2009; Mati et al., 2015; Poumarau, 1968), and there is a limited understanding of endoparasites in wild caught snakes (Mati et al., 2015).

Among serpents from the Neotropics, Guibe's false coral snake, *Oxyrhopus guibei* (Hoge and Romano, 1976), inhabits regions of southeastern Brazil, and west into Bolivia, and Paraguay, and throughout the northernmost provinces of Argentina (Uetz et al., 2015). Like many other terrestrial colubrid snakes, this generalist predator feeds on lizards and rodents, and exhibits a primarily crepuscular to nocturnal activity pattern (Alencar et al., 2009; Sazima and Abe, 1991). *O. guibei* displays a colorful banding pattern that mimics the highly venomous Coral Snake *Micrurus frontalis* (Elapidae) (Giraudo, 2001), and therefore is often referred to as the False Coral Snake. It is also worth mentioning that, like so many other colorful snakes, *O. guibei* is a part of the illegal pet trade (Souza et al., 2007).

The current study summarizes the results of a *postmortem* examination of an adult female *O. guibei* specimen captured in Iguazu National Park (Misiones, Argentina). The snake was captured on April 28th, 2015, in accordance with authorization from the National Park Administration (APN) N° 335/13, transported to the serpentarium of the National Institute of Tropical Medicine (INMeT) (Argentina), and found unexpectedly dead one week later.

Upon arrival to INMeT, the snake weighed 370 g with a snout-vent length of 92.5 cm and total length of 101 cm. It entered into a quarantine area where regular veterinary examinations and

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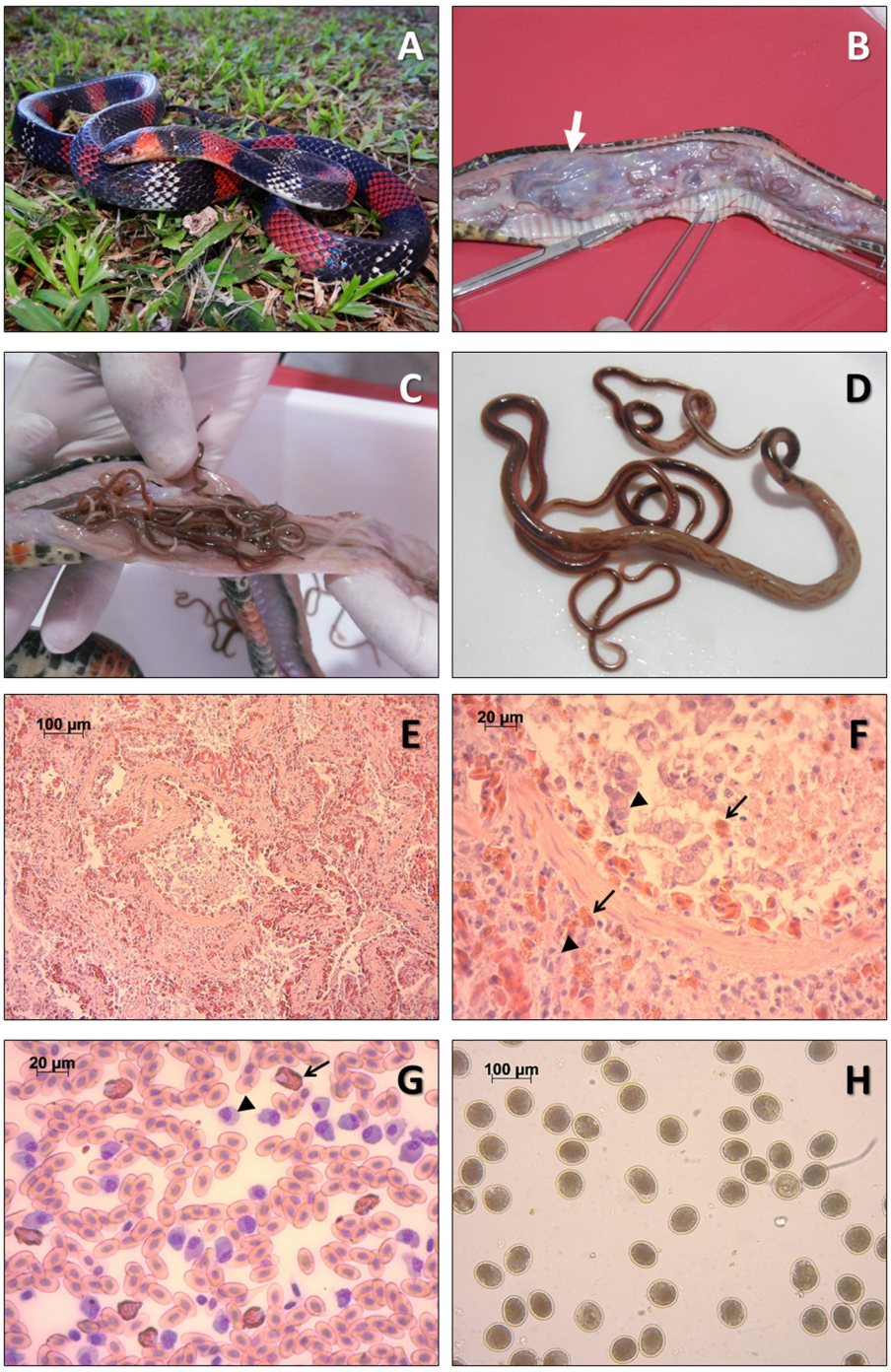

Fig. 1. A – Specimen of *Oxyrhopus guibei* a few days after its arrival at the serpentarium. B – Nematodes found in the coelomic cavity of *O. guibei* during necropsy (arrow indicates a large bolus of worms). C – Roundworms causing obstruction of the bowel lumen. D – Some large nematodes removed from the gastrointestinal tract of *O. guibei*. E and F – Photomicrographs showing the histopathology of *O. guibei* lung. In F note mixed inflammatory infiltrate of heterophils (arrow) and monocytes (arrowhead) in the alveolar septa and air spaces. Sections were stained with haematoxylin and eosin. Images were taken at 10× and 40× magnifications, respectively. G – Photomicrograph of peripheral blood cells of *O. guibei* snake stained with May Grunwald and Giemsa. Note the relative high numbers of heterophils (arrow) and monocytes (arrowhead). Image was taken at 40× magnification. H – Photomicrograph of *Ascarididae*-type eggs in feces of *O. guibei*. Note their abundant presence in a microscopic field of × 10.

screens were systematically performed. Although the specimen was in captivity for only a short period of time, activity levels appeared normal, and there were no signs of behavioral abnormalities, or detectable disease-related symptoms. Nevertheless, it is worth mentioning that during initial observations, the snake appeared to have an enlarged mass near the middle region of its body, which at the time was assumed to be a distension in the stomach caused by consumption of a natural prey item. Although the specimen appeared to be in good health (Fig. 1A), it was unexpectedly found dead with its mouth agape a week after its arrival to the INMeT serpentarium. Visual observation of the deceased snake identified fluid draining from the mouth and there were no detectable signs of bleeding. Additional external evaluation suggested that the snake was in a good nutritional state, however, immediately following the start of the necropsy a significant abundance of roundworms were seen in the body cavity. A total of 68 nematodes were collected and found throughout the whole extension of the coelomic cavity (Fig. 1B), ranging from the upper

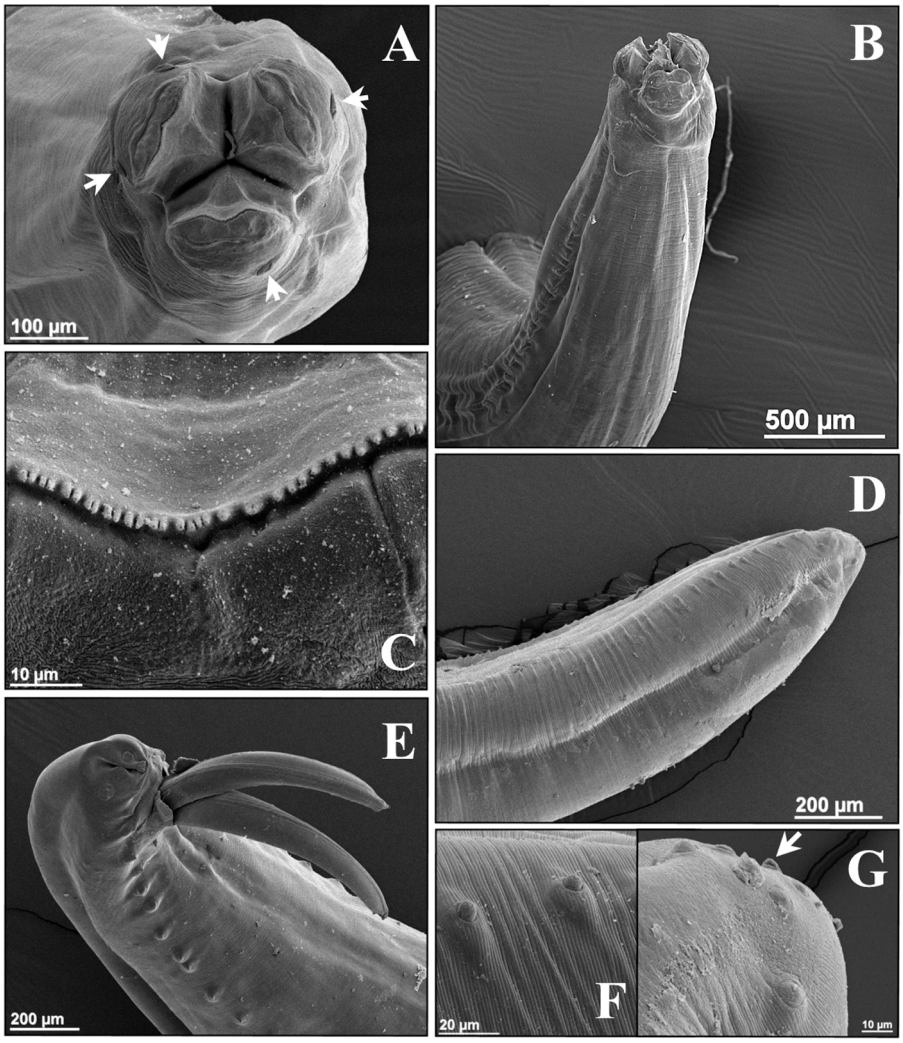

Fig. 2. Scanning electron micrographs of *Hexametra boddaertii* (male) from *Oxyrhopus guibei*. A – Cephalic extremity, apical view (double labial papillae arrowed). B – Anterior region of the body, dorsal view showing the lateral ala. In A and B note the presence of three large lips, a characteristic of ascarids. C – Detail of dentigerous ridge. D – Posterior end of the body, dorsolateral view showing precloacal papillae. E – Posterior end of the body, ventral view showing precloacal papillae and spicules. F – Detail of precloacal papillae. G – Tail, dorsolateral view (postcloacal papillae, terminal mucron arrowed).

gastrointestinal tract to the pericloacal region. Larger nematodes contributed to a significant gastrointestinal obstruction (Fig. 1C and D), with a total of 52 individual nematodes collected from the whole digestive tract. Other significant findings of the necropsy were: peritonitis with serosal adherence; small calcifications in the mesothelium of the coelomic cavity; solid and expressive content in the gallbladder (which is strongly suggestive of obstruction); and lung edema and congestion.

Histopathological analyses of lung sections (5 µm) revealed proliferative heterophilic and histiocytic pneumonia, with images showing heterophils and monocytes broadening the alveolar septa and filling the faveolae. In addition, necrotic cellular debris resulting from the destruction of the basement membrane (Fig. 1E and F) was also observed. These findings are in accordance with the inflammatory patterns observed on the microscopic examination of smears from the oral exudate. Regarding the gastric and intestinal tissue sections, the mucosa was congested and inflamed, and rounded structures compatible with parasitic forms were surrounded by fibrotic and/or inflammatory tissue in the submucosa (data not shown).

During the necropsy, peripheral blood was collected by aortic puncture, however, due to limited resources, white blood cell (WBC) counts were not performed. Nevertheless, based on a subjective blood smear evaluation, the concentration of WBCs appeared to be significantly elevated and consisted predominantly of heterophils and monocytes (Fig. 1G), which is indicative of infection and/or stress leukocytosis. Biochemical analyses of serum was conducted using VetScan® Avian/Reptilian Profile Plus (Abaxis, Inc.), and revealed high levels (>200 µmol/L) of bile acids, which is in accordance with the gallbladder obstruction verified during the necropsy. All other biochemical parameters examined were within the normal ranges for captive colubrid snakes (Coz-Rakovac et al., 2011): aspartate aminotransferase 461 U/L, creatine kinase 935 U/L, uric acid 5.7 mg/dL, glucose 13 mg/dL, total calcium 10 mg/dL, phosphorus 8.3 mg/dL, total protein 6.1 g/dL, albumin 1.1 g/dL, globulin 5.0 g/dL, potassium 4.5 mmol/L, sodium 148 mmol/L.

A parasitological analysis of a fecal sample collected three days prior to the snake's death revealed abundant Ascarididae-type eggs (Fig. 1H) by both sedimentation and flotation concentration techniques. Unfortunately, all veterinary efforts attempting to de-worm the snake – therapy with 10% fenbendazole suspension (Panacur, Intervet) orally at a single dose of 25 mg/kg body weight, and cleaning and disinfection of its cage frequently – were unsuccessful to keep the individual alive. It was even not possible to collect a fecal sample after treatment. It should be noted that serpent fatalities due to severe ascaridid infections have been previously reported

(Rataj et al., 2011). Following the necropsy, some nematodes collected from both the gastrointestinal tract and the coelomic cavity were cleared with lactic acid and prepared for microscopic examination, while other nematodes were gold coated and examined by scanning electron microscopy (SEM) using a JEOL 5800 LV SEM at an accelerating voltage of 15 Kv (SEM Service from the University of Northeastern Argentina). Both intestinal and coelomic parasites were identified as *Hexametra boddaertii* (Fig. 2A-F) based on size and morphological characteristics as described by Vicente et al. (1993) and Sprent (1978). Body length of *H. boddaertii* isolated from the gastrointestinal tract ranged from 52 to 159 mm (mean = 81.45 ± 22.93 mm, n = 30), whereas those collected from the coelomic cavity exhibited a smaller body length and ranged from 18 to 72 mm (mean = 42.32 ± 11.03 mm, n = 30). The body measurements determined in the current study are in agreement with those reported by Sprent (1978). *H. boddaertii* egg sizes ranged from 72.39 to 90.82 μm (mean = 80.54 ± 4.36 μm, n = 60) in length, and 59.48–79.40 μm (mean = 71.25 ± 5.04 μm, n = 60) in width. These measurements are in accordance with those reported by Sprent (1978) and Bursey et al. (1995).

H. boddaertii was originally isolated and described by Baird (1860), who removed a single female specimen from a Boddaert's tropical racer (*Mastigodryas boddaerti*), a colubrid snake found throughout much of South America. Since then, several neotropical colubrid snakes have been identified as host for this parasite including: *Oxyrhopus trigeminus* and *Philodryas patagoniensis* from Brazil (Sprent, 1978), *Spilotes pullatus* and *Trimorphodon biscutatus* from Costa Rica (Bursey and Brooks, 2011), and a *Philodryas baroni* specimen from an unknown locality (Hartdegen and Gamble, 2002). The current study reports the presence of *H. boddaertii* in an individual *O. guibei* captured in Argentina, which not only identifies a new host for this parasite, but also reports the first country record for *H. boddaertii*. It is important to note that some nematodes removed from the gastrointestinal tract exhibited a body length greater than 10 cm, suggesting that most, if not all of the nematodes found during the necropsy were in the adult phase of their life cycle. This strongly indicates that the severe parasitism seen in this individual snake was established well before its capture and arrival to the INMeT serpentarium, and that the transmission of *H. boddaertii* likely occurred through the consumption of infected frogs/rodents/lizards in the forest (see Anderson (2000) for review of the life cycle of *H. boddaertii*). Likewise, Vrcibradic et al. (2000, 1999) reported the presence of immature ascarids which were identified as *H. boddaerti* from the coelomic cavity of several Brazilian lizards. Thus, this report certainly contributes to the current knowledge of the parasitic fauna of wild squamates from Argentina.

Human handling of snakes infected with *H. boddaertii* may permit transmission of the parasite and pose a risk to human health. In fact, the transmission of *Hexametra* larvae from fecal excretion of the snake *Bitis arietans* to the primate *Macaca fascicularis* has been documented (Petter et al., 1967), and further suggest that, if encountered, *Hexametra* could be transmitted to humans. It has also been suggested that *Hexametra* may be the causative nematode contributing to the ocular disease known as diffuse unilateral subacute neuroretinitis (DUSN) in Brazil (Souza et al., 2005). *Hexametra* has also been shown to exhibit life cycle characteristics similar to those of *Toxocara canis*, one of the major causative parasites that contributes to visceral larva migrans (VLM) in humans (Bowman, 1987). Therefore, it may be safe to assume that *Hexametra* parasites may contribute to VLM and other potentially harmful pathological conditions (Bowman, 1987). The recent popularity of *O. guibei* in the pet trade, although being illegally sold (Souza et al., 2007), may cause possible zoonotic helminth transmission of *Hexametra* from snake to human, and special care must be taken while manipulating this snake.

The snake reported here was deposited in the Herpetological Collection of the National Institute of Tropical Medicine, Puerto Iguazú, Argentina (code INMeT 017). Representative male and female specimens of *H. boddaertii* have been maintained separately in different preservative solutions and deposited in the Helminthological Collection of the National Institute of Tropical Medicine, Puerto Iguazú, Argentina (code INMeT ES_001:15).

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