Spargana in the Neotropical frog Hypsiboas pulchellus (Hylidae) from Uruguay

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Abstract. We report the first record of parasitic larval tapeworms of the genus Spirometra (sparganum) in the frog Hypsiboas pulchellus. A total of 139 frogs were collected in Uruguay, and two of them presented a single sparganum (1.4%). One sparganum was found free into the coelomic cavity, and the other was located under the skin and in the coelomic cavity. The relevance of this finding in Uruguayan amphibians is discussed along with a review of available data.

Key words: amphibians, parasitic disease, Spirometra, sparganum, zoonoses.

Sparganosis is a cosmopolitan parasitic disease caused by plerocercoid larvae of tapeworms belonging to the genus Spirometra, currently included in the family Diphyllobothriidae (Platyhelminthes: Eucestoda; see Kuchta et al. 2008). These larvae are called sparganum. Adult tapeworms inhabit the intestine of carnivore mammals (like cats and dogs), their eggs are spread with faeces and produce ciliated forms called coracidia, which once ingested by copepods (primary intermediate host) develop into procercoid larvae. The procercoid may develop the plerocercoid larval form in skin or viscera after ingestion by secondary intermediate vertebrate hosts, usually amphibians and reptiles (Mueller 1974, Bowman et al. 2002). Sparganosis is an uncommon zoonosis that could be lethal, acquired by consumption of water with infected copepods, raw amphibian and reptile meat or close contact with them, being particularly important in Asia (Chi et al. 1980, Ausayakhun et al. 1993, Hou et al. 2012).

In Uruguay, the occurrence of plerocercoid larvae of Spirometra was first reported by Vogelsang (1925) in anuran amphibians of the species Leptodactylus latrans that beared spargana encysted in forelimb musculature. Also in Uruguay, Wolffhügel and Vogelsang (1926) found sparganum in native marsupial mammals (Didelphis albiventris and Lutreolina crassicaudata parnalis). These larvae were identified as Sparganum reptans, and then dogs were experimentally infected using them for completion of the parasitic life cycle, identifying the adult form as Dibothriocephalus decipiens, currently in the genus Spirometra. Dei-Cas et al. (1976) communicated also the finding of sparganum in some species of reptiles from Uruguay and in specimens of L. latrans collected in south of the country, at the Departments of Montevideo, Maldonado and Rocha. Spontaneous cases of Spirometra in domestic cats were more recently reported from Montevideo by Sampaio et al. (1987).

In this work we communicate the finding of sparganum in anuran amphibians of the species Hypsiboas pulchellus (Hylidae) collected in Uruguay. Between September 2012 and April 2014 a total of 139 specimens of Hypsiboas pulchellus were collected at La Paloma, Department of Rocha, in southeastern Uruguay, as part of a monitoring protocol of amphibian diseases. The specimens were captured at temporary and permanent ponds close to the Atlantic Ocean used for reproduction; they were apparently in good condition and did not present weakness, abnormal postures or behaviours. They were transported to the laboratory, euthanized by cutaneous application of lidocaine (20%) and dissected for inspection of the coelomic cavity. They were fixed in formalin 10%, preserved in ethanol 70% and deposited in the herpetological collection of Museo Nacional de Historia Natural de Montevideo (MNHN). Histological preparations of worms were done to confirm diagnosis; samples from the studied specimens were embedded in parafin, sectioned at 5 µm and stained with haematoxylin and eosin.

Two male frogs (1.4%) were parasitized by a single worm each, with typical external characters of sparganum, a flattened, whitish and flexible body
Figure 1. Sparganosis in the frog Hypsiboas pulchellus from Uruguay. A, sparganum extracted from the coelomic cavity of an adult male frog MNHN 9503, La Paloma, Rocha (August 2013). Bar = 1 mm. B, adult male frog MNHN 9504 from the same site (April 2013). Notice the dorsal swelling (arrow). C, specimen MNHN 9504 with the swelling dissected, a large sparganum can be seen under the skin and extending to the coelomic cavity through the dorsal musculature (arrow). Bar = 2 mm. D, view of the parasite from the coelomic cavity (arrows), covered by a thin membrane. (i- intestine; k- kidney. Bar = 2 mm).

presenting an enlarged end with evident transverse wrinkles (Fig. 1A). The frogs, MNHN 9503 and MNHN 9504, measured 32.8 and 36.4 mm of snout-vent length, and were collected on 17 August and 17 April 2013 respectively. They were collected at a permanent pond used for cattle production in a grassland area (34°38′29″ S, 54°12′39″ W). In MNHN 9503, the sparganum measured 25 mm, and was found free in the coelomic cavity. In the second case a sparganum of 47 mm was found in a swelling of approximately 4 x 2 mm on the posterior part of the back (Fig. 1B). The parasite was partially located at this site under the skin, but about a half of the worm was into the coelomic cavity through a communication between the dorsal muscles. This sparganum was covered by a thin and translucent membrane (Fig. 1B-D). Examination of worm histologic preparations showed some characteristic anatomical features of cestodes such as a thick external cuticle, lack of digestive tract, groups of muscle fibers in the parenchyma and the presence of calcareous corpuscles.

In the present study, the frog Hypsiboas pulchellus is reported as an intermediary host of Spirometra for the first time. This frog species has mostly terrestrial habits and fed mainly on terrestrial prey (Maneyro & da Rosa 2004). The acquisition of plerocercoid larvae in non-aquatic amphibians like H. pulchellus probably occurs during the anuran larval phase, by the accidental consumption of infested copepods (Venturini 1989). The prevalence of sparganum in the studied sample was low (1.4%) as also the parasitic load, one larva in each host. Some reports on amphibian sparganosis documented low parasitic loads (Vogelsang 1925, Gomez-Puerta et al. 2010). However, the parasitic load and incidence of sparganosis in amphibians could be high (Cui et al. 2011, Bezerra et al. 2012), causing a severe disease condition (Berger et al. 2009). It must be noticed that the observation of rather low parasitic loads is strongly influenced by sample size because high concentrations of parasites are unusual events in host populations (Poulin 2013). In our case, studied frogs seemed to be not severely affected by relatively large spargana. Most of previous reports in amphibians described small spargana, measuring between of 2 and 32 mm (Vogelsang 1925, Gomez-
Puerta et al. 2010, Bezerra et al. 2012, Berger et al. 2009 found spargana of Spirometra erinacei in amphibians of Australia with size up to 50 mm. The location of spargana in adult amphibians is variable, these migrant parasitic larvae can be present in the subcutaneous tissue, muscles, coelomic epithelium, liver or they are just free in the coelomic cavity (Rego & Schäffer 1992, Berger et al. 2009, Bezerra et al. 2012). As far as we know, the double location of a single larva we observed in the specimen MNHN 9504, subcutaneous and coelomic, is novel.

In Uruguay there are only two recorded cases of human sparganosis, probably caused by the ingestion of water contaminated with copepods (Osimani & Peyrallo 1954, Sakamoto et al. 2003). The risk of acquiring the disease in this country by the use of amphibians is extremely low as amphibians are not used in folk medicine and human consumption of amphibian meat is limited to Leptodactylus latrans, but it is extraordinary.

The species of Spirometra affecting amphibians in South America remains to be further characterized. The incidence and pathogenicity of its plerocercoid larvae in amphibian populations also merit more studies, as Spirometra has a broad host range and many species could be potentially affected (Berger et al. 2009). Coastal Uruguay is being rapidly urbanized and an increment of domestic dogs and cats that are final hosts of Spirometra may enhance infestations in intermediate hosts, like amphibians. This effect on wildlife was documented for other parasitic diseases as sarcotic mange and toxoplasmosis (Dickman 1996, Skerrat et al. 1999).

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