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## PROTOZOA AND NEMATODES INFECTING *ODONTOPHRYNUS OCCIDENTALIS* (ANURA, ODONTOPHRYNIDAE) FROM THE MONTE DESERT OF ARGENTINA

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**Protozoa and Nematodes Infecting *Odontophrynus occidentalis* (Anura, Odontophrynidae) from the Monte Desert of Argentina.** Piñeiro, M., Sanabria, E. & González, C. — The parasitofauna of amphibians from the province of San Juan has been studied very little. In particular, only one helminthological study has been reported on *Odontophrynus occidentalis* (Berg, 1896). The aim of the present study was to describe the endoparasites of *O. occidentalis*, including protozoa and nematodes. Twenty-one adults (13 males and 8 females) of *O. occidentalis* were analysed for parasites. Collected parasites were examined using standard parasitological techniques. Parasitological descriptors such as prevalence and mean intensity were calculated. Specimens belonging to the phyla Ciliophora and Nematoda were found in the intestinal tract. Protozoa of the genus *Nyctotheroides* and two nematode species, *Aplectana hylambatis* and *Falcaustra sanjuanensis*, were identified. This study provides new information on the new parasitic infection of amphibians for the province of San Juan. We emphasize the need to increase the knowledge of the amphibian parasitofauna, mainly related to protozoa in these vertebrates, since the literature presents few reports.

**Key words.** *Aplectana hylambatis*, cururu lesser escuerzo, *Falcaustra sanjuanensis*, *Nyctotheroides*, San Juan province.

## Introduction

The genus *Odontophrynus* Reinhardt & Lütken, 1862 is comprised of 11 species of amphibians distributed in the Neotropical Region, 4 of which are present in Argentina, covering a latitudinal range from 5° S to 21° S and a longitudinal range from 58° W to 70° W, and an altitudinal range from sea level to 2200 m (Martino et al., 2019). *Odontophrynus occidentalis* (Berg, 1896) is found in the provinces of Catamarca, La Rioja, San Juan, Mendoza, San Luis, Neuquén, La Pampa and Buenos Aires (Turazzini et al., 2016). Regarding the biology of the species, the advertisement call was studied by Salas & di Tada (1988), the diet is preferably composed of arthropods (Cocilio et al., 2020), and the thermal biology was studied by Sanabria et al. (2012; 2013). *O. occidentalis* is classified as Least Concern (LC) by the IUCN (2023) and as Not Threatened in the latest classification of the conservation status of amphibians and reptiles of Argentina (Vaira et al., 2012). Among the parasites of *O. occidentalis*, only the nematode *Falcaustra sanjuanensis* González, Sanabria & Quiroga, 2013 has been described from specimens collected in the province of San Juan, Argentina (González et al., 2013 a). The amphibian diversity of San Juan province consists of 13 species of anurans (Vaira et al., 2012). So far, only the parasitofauna of *Leptodactylus luctator* (Hudson, 1892) (= *L. latrans*), *Lithobates catesbeianus* (Shaw, 1802) (introduced species), *Pleurodema nebulosum* (Burmeister, 1861) and *Rhinella arenarum* (Hensel, 1867) have been studied (González et al., 2013 b; González et al., 2014; Ramallo et al., 2016; Piñeiro et al., 2017). Therefore, there is a clear need to continue this type of study in order to increase the knowledge of parasitic diversity in this host group. Here we report the new record of protozoa of the genus *Nyctotheroides* Grassé, 1928 and the nematode *Aplectana hylambatis* (Baylis, 1927) Travassos, 1931 parasitising *O. occidentalis* from the Monte Desert, Argentina.

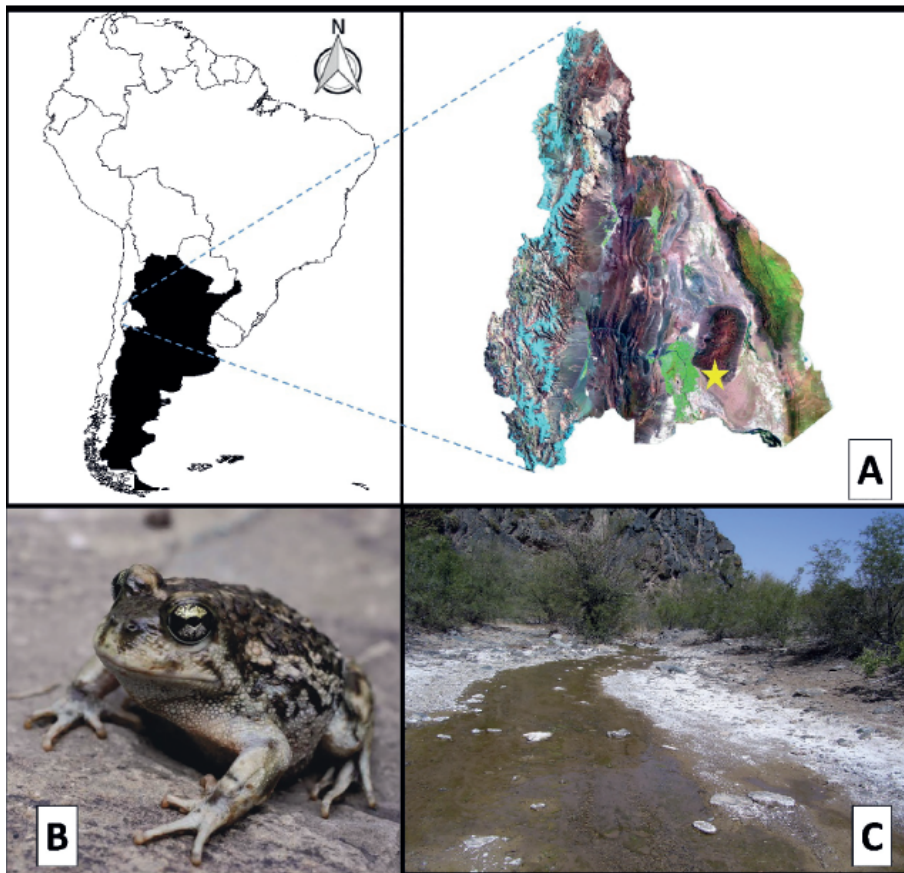


Fig. 1. The collection site and study species from the Monte Desert, San Juan, Argentina. A — collection site “Quebrada de las Flores” (Atlas socioeconómico de San Juan. 1° Ed. San Juan: Editorial UNSJ., after 2017); B — adult male *O. occidentalis*; C — habitat used by *O. occidentalis*.

## Materials and Methods

### Study area and animal collection

The “Quebrada de las Flores” is located 55 km East of the City of San Juan, in the Caucete Department (31.700604 S, 68.095190 W; elevation: 765 m; fig. 1, A). This region is a part of the Monte Desert ecoregion, characterized by its arid climate with a mean annual temperature of 17.3 °C, a mean maximum temperature of 25.7 °C, and a mean minimum temperature of 10.4 °C, with a mean annual rainfall of 89 mm during the summer months (wet season) (Cabrera, 1976; Warner, 2004). Climatologically it belongs to the climatic group BWw (desert with summer rainfall) of the Köppen climatic classification. In this area, we collected by hand 21 specimens (13 males and 8 females) of *O. occidentalis* (snout-vent length mean = 5.75 ± 0.93 cm; weight mean = 18.39 ± 2.07 g; fig. 1, B) from October 2015 to November 2016.

### Analytical procedure

Amphibians were transported alive to the laboratory and euthanized in freezer, following the recommendation of Lillywhite et al. (2017). At necropsy, the alimentary canal, lungs, liver, kidneys, urinary bladder, coelomic cavity, musculature, and integument were examined for parasites by dissection. Parasites were observed and counted. Nematodes were preserved in 70 % ethyl alcohol and cleared in glycerine, while protozoa were conserved in 10 % formaldehyde. Illustrations were made using a Leica DM 2500 microscope with the aid of a camera lucida. Prevalence and mean intensity were calculated according to Bush et al. (1997) for nematode parasites, for protozoan only the prevalence of infection was calculated.

Parasites were deposited in the Colección Helmintológica of the Centro de Ecología Aplicada del Litoral, Corrientes, Argentina (CECOAL). The specimen of *O. occidentalis* was deposited in the Colección Herpetológica of the collection of vertebrates of the Instituto de Ciencias Básicas, Facultad de Filosofía, Humanidades y Arte, Universidad Nacional de San Juan; accession number ECRA1086-1107.

## Results

In the amphibians analyzed, protozoan and nematode parasites were found in the intestinal tract; a species from the genus *Nyctotheroides* from the phylum Ciliophora, was identified. From the phylum Nematoda, species of two genera, namely *Aplectana* Railliet and Henry, 1916 and *Falcaustra* Lane, 1915 were identified.

### Ciliophora

#### Order Clevelandellida

#### Family Nyctotheridae Amaro, 1972

#### *Nyctotheroides* sp. (fig. 2)

Prevalence: 57.14 % (12/21)

Site of infection: Large intestine

Specimens deposited: CECOAL 17021403 (10 specimens)

Remarks: The genus *Nyctotheroides* have very close morphological and genetic similarities with the genus *Nyctotherus* Leidy, 1849, making its distinction difficult. However, until now, there is consensus that the individuals belonging to *Nyctotheroides* are found only parasitizing amphibians, while *Nyctotherus* can parasitize arthropods, reptiles and fish, among others, but not anurans (Albaret, 1975; Affa'a et al., 2004; Lynn & Wright, 2013; Suzuki et al., 2020). Specimens found in this study present an oval completely ciliated body, with well-defined peristome and infundibulum, presence of a kidney-shaped macronucleus, a small micronucleus, a posterior contractile vacuole and a cytoproct.

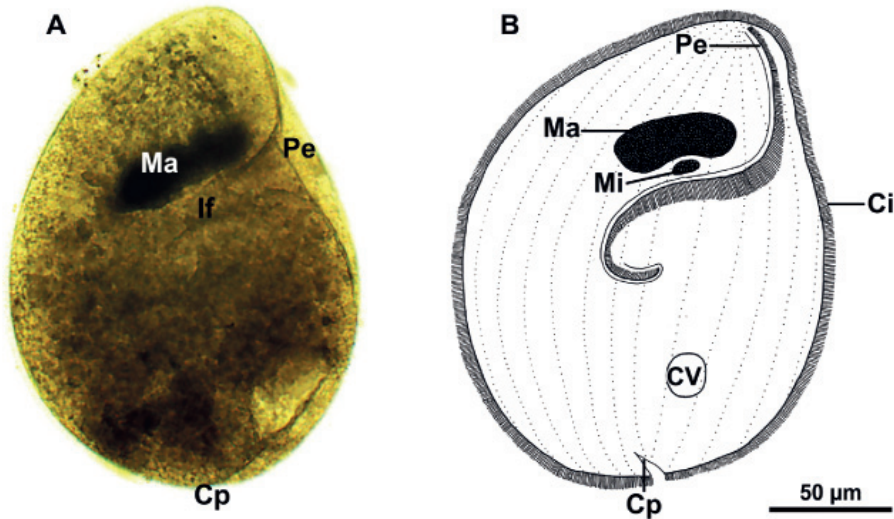


Fig. 2. *Nyctotheroides* sp. A — Light microscopic image; B — Schematic drawing; Abbreviations: Ci — cilia; CV — contractile vacuole; Cp — cytoproct; If — infundibulum; Ma — macronucleus; Mi — micronucleus; Pe — peristome.

## Nematoda

### Order Ascaridida

#### Superfamily Cosmocercoidea

#### Family Cosmocercidae Railliet, 1916

#### *Aplectana hylambatis* (Baylis, 1927) Travassos, 1931 (fig. 3)

Prevalence; mean intensity; total number of parasite: 9.52 % (8/21);  $0.42 \pm 0.70$ ; 9.

Site of infection: Large intestine.

Specimens deposited: CECOAL 17021401 (6 females, 3 males)

Remarks: *A. hylambatis* is a cosmocercid species that presents considerable intraspecific variation. However, is easily identifiable by two morphological characters: the specific shape of spicule when extruded out of the body — in its last portion it possess an articulation that gives the appearance of a sickle or a hockey stick, and the possession of three pairs of adcloacal papillae on the anterior lip of the cloaca, with a large unpaired papilla between them (González et al., 2019). Both these characters were clearly observed in the specimens of this study (fig. 3, D). In males, the number of pairs of cloacal papillae and their arrangement coincide with the specimens of *A. hylambatis* collected in *Rhinella major* (Müller & Hellmich, 1936) from Chaco and Formosa provinces, Argentina: 4 : 5 : 5 + 1; likewise, in females, the mamelon-like structures anterior to the vulva coincides, in size, number and disposition, with the specimens collected in *R. major* from Chaco: two considerable protuberances separated and covering partially the vulva (fig. 3, G) (González et al., 2019).

#### Family Kathlaniidae Lane, 1914

#### *Falcaustra sanjuanensis* González, Sanabria & Quiroga, 2013 (fig. 4)

Prevalence; mean intensity; total number of parasite: 38.09 % (8/21);  $0.85 \pm 1.27$ ; 18.

Site of infection: large intestine

Specimens deposited: CECOAL 17021402 (8 females, 6 males, 4 juveniles)

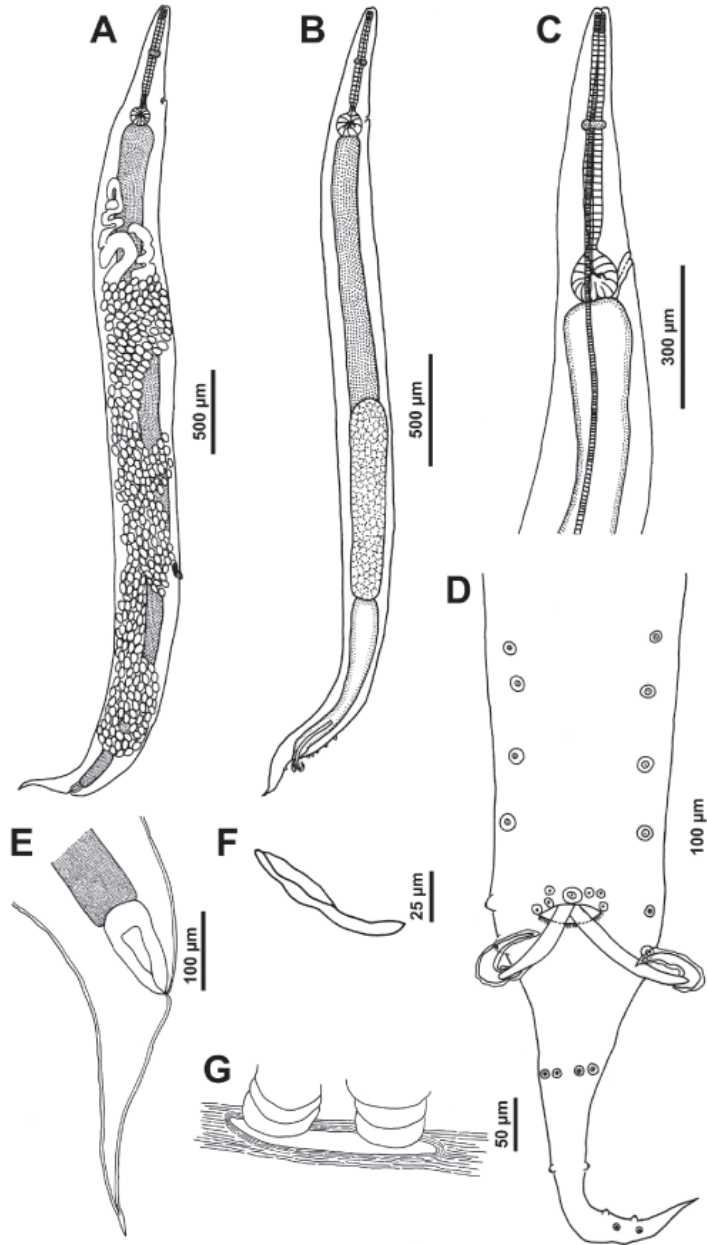


Fig. 3. *Aplectana hylambatis*. A — female, general view; B — male, general view; C — male, anterior extremity, lateral view; D — male, posterior extremity, ventral view; E — female, posterior extremity, lateral view; F — gubernaculum, lateral view; G — vulva, ventral view.

Remarks: Of the 104 species of *Falcaustra* described so far (Burse et al., 2020), this is the only species described from specimens collected from Argentinean amphibians. *F. sanjuanensis* differs from the rest of the known species by a set of characters such as: presence of pseudosucker, distribution pattern of caudal papillae (6 precloacal, 5 adcloacal, 12 postcloacal), unpaired papilla anterior to cloaca located on a protuberance, and triangular gubernaculum. All of these characters were observed in the specimens examined in the present study (fig. 4, B, C, F).



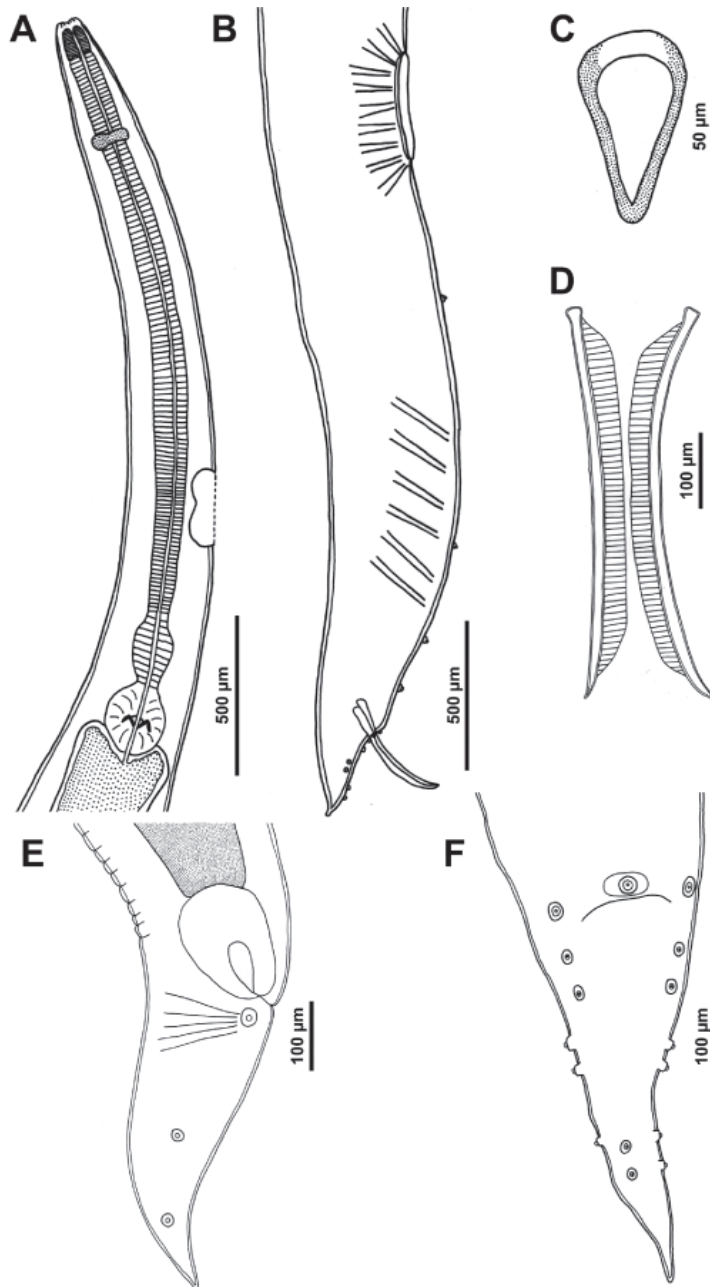


Fig. 4. *Falcaustra sanjuanensis*. A — female, anterior extremity, lateral view; B — male, posterior extremity, lateral view; C — gubernaculum, ventral view; D — spicules, ventral view; E — female, posterior extremity, lateral view; F — male, posterior extremity, ventral view.

## Discussion

The genus *Odontophrynus* have 11 species in the actuality (Frost, 2023), and only three have been the subject of parasitological studies both in the tadpole and in adult stage (table 1). *Odontophrynus americanus* (Duméril & Bibron, 1841) was the most studied species, from which such endoparasites as myxozoans, protozoans, helminths, and ectoparasites (leeches) have been recorded in Argentina, Brazil, and Uruguay. Nematode parasites have

been found in *O. lavillai* Cei, 1985 from Formosa province, Argentina (González et al., 2021).

The Nyctotheridae family consists of 15 genera, being *Nyctotheroides* and *Nyctotherus* the most studied (Li et al., 2020). Albaret (1975) proposed that *Nyctotheroides* can be distinguished from *Nyctotherus* by the presence of one (*Nyctotherus*) or two (*Nyctotheroides*) kinetal suture systems in the anterior part of the cell. *Nyctotheroides* is restricted to amphibian hosts, while *Nyctotherus* may be present in invertebrates and non-amphibian vertebrates such as fish and reptiles (Albaret, 1975). So far, more than 70 species belonging to the genus *Nyctotheroides* have been reported in amphibian host (Li et al., 2020). Due to the aforementioned, it is possible that *Nyctotherus* sp. reported in *O. americanus* by Cabagna Zenkluzen et al. (2009) actually belong to the genus *Nyctotheroides*.

In their review of South American amphibian helminths, Campião et al. (2014) expressed that *A. hylambatis* is one of the species with the greatest geographic range: it was found in five countries from 26 amphibian species. Specifically, in Argentina it was found in *Rhinella achalensis* (Cei, 1972), *R. arenarum*, *R. dypticha* (Cope, 1862), *R. major* (Bufoni-

**Table 1. Parasites found in species of *Odontophrynus* from South America**

<i>Odontophrynus</i> spp.	Locality	Parasite species	Developmental stage of the host	References
<i>O. americanus</i>	Uruguay	<i>Cystodiscus immersum</i> Lutz, 1889	Not established	Cordero (1928)
	Montevideo, Uruguay	<i>Aplectana membranosa</i> (Schneider, 1866) <i>Aplectana meridionalis</i> Lent and Freitas, 1948 <i>Cosmocerca uruguayensis</i> Lent and Freitas, 1948 <i>Rhabdias</i> sp.	Adult	Lent & Freitas (1948)*
	Mato Grosso do Sul, Brazil	Ascarididae gen. sp. Cosmocercidae gen. sp. <i>Rhabdias</i> sp. (cf. <i>elegans</i> ) <i>Glythelmins palmipedis</i> (Lutz, 1928) Travassos, 1930 Glossiphoniidae gen. sp.	Adult	da Silva et al. (2018)
	Corrientes, Argentina	<i>Rhabdias elegans</i> Gutiérrez 1945 <i>Cosmocerca podicipinus</i> Baker and Vaucher, 1984 <i>Cosmocerca parva</i> Travassos, 1925	Adult	González & Hamann (2009)
	Corrientes, Argentina	<i>Travtrema</i> aff. <i>stenocotyle</i> (Cohn, 1902) <i>Styphlodora</i> sp. <i>Opisthogonimus</i> sp. Echinostomatidae gen. sp.	Tadpole	Hamann & González (2009)
<i>O. lavillai</i>	Entre Ríos, Santa Fe, Argentina	<i>Zelleriella</i> Metcalf 1920, <i>Nyctotherus</i> <i>Trichodina</i> Ehrenberg, 1838	Tadpole	Cabagna Zenkluzen et al. (2009)
	Formosa, Argentina	<i>Aplectana hylambatis</i>	Adult	González et al. (2021)
<i>O. occidentalis</i>	San Juan, Argentina	<i>Falcaustra sanjuanensis</i>	Adult	González et al. (2013 a)**
	San Juan, Argentina	<i>Nyctotheroides</i> sp. <i>A. hylambatis</i> <i>F. sanjuanensis</i>	Adult	This study

\*Amphibian host cited in Lent & Freitas (1948) as *Ceratophrys americana*.

\*\*Amphibian host cited in González et al. (2013 a) as *O. cf. barrioi*.

dae), *Chacophrys pierottii* (Vellard, 1948), *Ceratophrys cranwelli* Barrio, 1980 (Ceratophryidae), *O. lavillai* (Odontophrynidae), *Trachycephalus typhonius* (Linnaeus, 1758) (Hylidae), *Leptodactylus latinasus* Jiménez de la Espada, 1875, *L. macrosternum* Miranda-Ribeiro, 1926 (= *L. chaquensis*), *L. bufonius* Boulenger, 1894, *Physalaemus santafecinus* Barrio, 1965 (Leptodactylidae), and *Dermatonotus muelleri* (Boettger, 1885) (Microhylidae), from Buenos Aires, Corrientes, Córdoba, Chaco, Formosa, Salta and San Juan provinces (González & Hamann, 2015; González et al., 2019; 2021).

*F. sanjuanensis*, since its description, was found in different localities of the province of San Juan, in both native and introduced amphibians, *L. luctator* (= *L. latrans*) and *L. catesbeianus*, respectively (González et al., 2014; Ramallo et al., 2016).

The present study is the second report about parasites carried out throughout its geographical range for *O. occidentalis*, and this is the first record of *A. hylambatis* and *Nyctotheroides* sp. in this amphibian hosts.

Parasitological studies in Argentinean amphibians have been carried out for the most part in the Dry Chaco and Humid Chaco ecoregions and deal mainly with helminths (see Hamann et al., 2013; González et al., 2019; 2021); in other regions, there are few records so far. Regarding studies of protozoa in amphibians, these are extremely scarce compared to the helminths group; protozoa of both blood (Mazza et al., 1927; Cabagna Zenklusen et al., 2009) and intestinal forms (Otamendi, 1945; Villegas Ojeda et al., 2020) were reported in Argentinean amphibians.

In conclusion, we identify a protozoan of the genus *Nyctotheroides* and two species of nematodes, namely *A. hylambatis* and *F. sanjuanensis* in *O. occidentalis*. This study represents the sixth record for parasites from amphibians for San Juan province and the second record of *O. occidentalis* parasites. Therefore, with this study we highlight the need to increase the knowledge of the amphibian parasitofauna in other regions of the country, and, on the other hand, the need to increase the studies related to protozoans in these vertebrates.

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