

First report of *Lernaea cyprinacea* (Copepoda: Lernaeidae) in tadpoles and newly-metamorphosed frogs in wild populations of *Lithobates catesbeianus* (Anura: Ranidae) in Argentina

Zulma A. Salinas^{1,2}, Fernanda G. Biolé^{1,2}, Pablo R. Grenat^{1,2}, Favio E. Pollo^{1,2}, Nancy E. Salas¹, and Adolfo L. Martino¹

¹ Ecología, Departamento de Ciencias Naturales, Facultad de Ciencias Exactas, Físico-Químicas y Naturales, Universidad Nacional de Río Cuarto, Ruta Nacional N° 36, Km 601, X5804BYA, Río Cuarto, Argentina. E-mail: zlm.salinas@gmail.com.

² Consejo Nacional de Investigaciones Científicas y Técnicas, CONICET, Argentina.

Abstract

First report of *Lernaea cyprinacea* (Copepoda: Lernaeidae) in tadpoles and newly-metamorphosed frogs in wild populations of *Lithobates catesbeianus* (Anura: Ranidae) in Argentina. *Lernaea cyprinacea* is an ectoparasitic copepod that can result in the mortality of the host by causing hemorrhages, ulcerations, and secondary infections. *Lernaea cyprinacea* is widely distributed in Argentina. Previous reports are restricted almost exclusively to cases of parasitism in fishes; copepod parasitism of anuran larvae is rarely documented. This is the first record and description of the parasitic infestation of *L. cyprinacea* on tadpoles of the exotic and invasive species *Lithobates catesbeianus* in Argentina. A total of 15 tadpoles and 21 newly-metamorphosed individuals of *L. catesbeianus* were collected from the mountain town of Río de los Sauces, and 12 tadpoles from the Toledo Stream. A maximum of two parasites per host was found, principally in the cloaca. Clinical symptoms of inflammation hemorrhage and ulcers in the skin with mucus formation in the attachment area of parasites were observed.

Keywords: American Bullfrogs, copepod, infections, inflammation, parasitism.

Resumo

Primeiro relato de *Lernaea cyprinacea* (Copepoda: Lernaeidae) em girinos e rãs recém-metamorfoseadas em populações selvagens de *Lithobates catesbeianus* (Anura: Ranidae) na Argentina. *Lernaea cyprinacea* é um copépode ectoparasita que pode causar a morte do hospedeiro

Received 15 December 2015
Accepted 22 February 2016
Distributed June 2016

devido às hemorragias, ulcerações e infecções secundárias que provoca. *Lernaea cyprinacea* está amplamente distribuída na Argentina. Os relatos anteriores estão restritos quase que exclusivamente a casos de parasitismo em peixes; o parasitismo de larvas de anuros por copépodes tem sido raramente documentado. Este é o primeiro registro e descrição da infestação parasitária de *L. cyprinacea* em girinos da espécie exótica e invasora *Lithobates catesbeianus* na Argentina. Um total de 15 girinos e 21 indivíduos recém-metamorfoseados de *L. catesbeianus* foi coletado na região serrana do Rio de los Sauces, e 12 girinos, no córrego Toledo. Foi encontrado um máximo de dois parasitas por hospedeiro, principalmente na cloaca. Foram observados sintomas clínicos de hemorragia, inflamação e úlceras na pele, com formação de muco na área de fixação dos parasitas.

Palavras-chave: copépode, infecções, inflamação, parasitismo, rã-touro norteamericana.

Introduction

Lernaea cyprinacea is an ectoparasitic copepod of low host specificity (Kabata 1979, Nagasawa et al. 2007, McAllister et al. 2011); it has been reported to parasitize a variety of freshwater fishes (Hoffman 1999, Mancini et al. 2008, Plaul et al. 2010), adult and larval anurans (Martins and Souza 1996, Alcalde and Batistoni 2005, Kupferberg et al. 2009), and larval stages of axolotls (Carnevia and Speranza 2003). Infestations of these copepods can result in high pathogenicity and mortality by causing hemorrhages, ulcerations (Carnevia and Speranza 2003, McAllister et al. 2011), blood loss (Silva-Souza et al. 2000), and secondary infections (Schäperclaus 1991). However, the severity and significance of traumatic lesions and associated effects depend on the parasite load (Densmore and Green 2007), the host species, and the characteristic of host-parasite system (Idris and Amba 2011).

Lernaea cyprinacea occurs naturally in Africa, central Asia, the southern region of Siberia, and also has been reported in Europe, Japan, and Israel (Schäperclaus 1991, Thatcher and Williams 1998). *Lernaea* was introduced into North and South America by importation of tropical fishes (Figueira and Ceccarelli 1991); the copepod is widely distributed in Argentina. However, most reported findings of this parasite are restricted almost exclusively fishes (Mancini

et al. 2008, Plaul et al. 2010). Copepod parasitism by of anuran tadpoles is rarely documented, but it has been reported on field-caught (Baldauf 1961) and laboratory-reared *Lithobates catesbeianus* (Martins and Souza 1996), and on field-caught *Hylarana chalconota* (Leong 2001). In Argentina, Alcalde and Batistoni (2005) reported parasitism of *Lernaea* sp. on larvae of the hylid frog *Hypsiboas cordobae* from Tanti, Córdoba Province.

The American bullfrog, *Lithobates catesbeianus* (Shaw 1802), is an exotic and invasive species worldwide, and in the last decade, wild populations of this species have been found in Argentina (Sanabria et al. 2005, Pereyra et al. 2006, Barrasso et al. 2009, Akmentis and Cardozo 2010, Sanabria et al. 2011), particularly in Córdoba Province (Akmentis et al. 2009, Nori et al. 2011). This is the first report and description of the parasitic infestation of *Lernaea cyprinacea* in early developmental stages of *L. catesbeianus* in Argentina.

Materials and Methods

Our study area is located in the southwest of Cordoba Province, Argentina, at two localities from which *Lithobates catesbeianus* has been recorded: Río de los Sauces (32°31'53" S, 64°35'27" W) and the Toledo Stream (32°27'8" S, 64°33'21" W). The montane study area is characterized by a humid, temperate climate that

transitions to semi-wet or dry to the west (di Tada and Bucher 1996). The maximum temperature is 34°C with an average minimum of 9°C. Maximum rainfall occurs from October–March, with an annual average of 901 mm.

The sampling period was December 2013–March 2014. A series of water quality parameters (pH, temperature, conductivity, total dissolved solids and salinity) were measured *in situ* with a digital instrument, Tests TM Multiparameter 35-Series 35425-10.

Tadpoles and recently metamorphosed (staged fide Gosner 1960) were captured with a net. The specimens were examined in the laboratory under stereo microscope Zeiss SR to determine the parasite presence, number, location, and lesions produced by *L. cyprinacea* and then deposited in the Herpetological Collection of Ecology, Department of Natural Sciences, Faculty of Exact, Physical-Chemical and Natural Sciences, National University of Río Cuarto, Córdoba Province, Argentina.

Parasites were dissected from the host tissue with needles and preserved in 70% alcohol (Mancini *et al.* 2008). Our identification of the parasites is based on Kabata (1979), Huys and Boxhall (1991), and Eiras *et al.* (2003). The parasitological parameters applied were prevalence (*P*), calculated as proportion of individual hosts infested in relation to the total sample analyzed, and mean abundance (MA) calculated as average number of parasites recorded across

all hosts examined (Margolis *et al.* 1982, Bush *et al.* 1997). Snout-vent length (SVL) and weight (*W*) of each individual *L. catesbeianus* were recorded with a digital caliper Mahr 16 (0.01 mm) and an Ohaus digital balance (0.01 g), respectively.

Results

A total of 15 tadpoles and 21 recently metamorphosed individuals of *L. catesbeianus* from the mountain town of Río de los Sauces and 12 tadpoles from the Toledo Stream were collected from December 2013 to March 2014. Río de los Sauces had lower pH values than did the Toledo Stream, but the water temperatures were similar. Conductivity, total dissolved solids, and salinity were higher in the Toledo Stream. There were no statistically significant differences in any variable between the sites (Table 1).

Snout-vent length (SVL), weight (*W*) and parasitological parameters of each specimen of *L. catesbeianus* (tadpoles and recently metamorphosed) are shown in Tables 2 and 3. Statistical comparison of the sites showed that prevalence of the parasites did not differ significantly (Test Binomial. $Z_{AT-RS} = 0.05$, $P > 0.05$).

Lernaea parasites (Figure 1A) were usually found in the cloaca, with a maximum of two parasites per individual (Figure 1C, D). We observed clinical symptoms of inflammation,

Table 1. Water variables for the sampling sites (RR = Río de los Sauces, TS = Toledo stream). Letters show results of post-hoc tests. Mean values with the same letter are not significantly different ($p > 0.05$).

	RR	TS
pH	7.96 ± 0.38 ^a	8.27 ± 0.06 ^a
Temperature (°C)	18.34 ± 0.54 ^a	18.40 ± 0.69 ^a
TDS (ppm)	138.64 ± 54.28 ^a	192 ± 23.64 ^a
Conductivity (µS)	197.06 ± 78.71 ^a	257.67 ± 53.00 ^a
Salinity (ppm)	93.78 ± 37.9 ^a	133.67 ± 12.58 ^a

Table 2. Snout-vent length (SVL), weight (W) and larval stage of each *L. catesbeianus* individual (tadpoles and recently metamorphosed). RR = Río de los Sauces, TS = Toledo stream.

		RR	TS
Tadpoles	SVL (mm)	90.53 ± 8.47	53.55 ± 6.91
	W (g.)	6.73 ± 1.67	1.33 ± 0.50
	larval stage	31, 36, 37, 40, 41	27, 28, 29, 30, 34
Recently metamorphosed	SVL (mm)	49.90 ± 12.39	-
	W (g.)	4.77 ± 0.50	-
	larval stage	43, 44, 45, 46	-

Table 3. Parasitological parameters (P = prevalence, MA = mean abundance) of each *L. catesbeianus* individual (tadpoles and recently metamorphosed). RR = Río de los Sauces, TS = Toledo stream.

		RR	TS
Tadpoles	P	53.33	58.33
	MA	0.60	0.58
	larval stage parasitized	31, 36, 37, 40	27, 28
Recently metamorphosed	P	38.10	-
	MA	0.38	-
	larval stage parasitized	43, 44, 45, 46, 47	-

hemorrhage, and ulcers in the skin with mucus formation in the attachment area of the parasites (Figure 1D). In some cases, only lesions produced by the attachment of copepods were observed (Figure 1B). Recently metamorphosed frogs rarely had parasites, but when present, they were attached to the cloaca (Figure 1E, F).

Discussion

The environmental conditions of sample sites in our study seem suitable for *Lernaea cyprinacea* parasitism. Growth of *Lernaea* sp. is negatively associated with salinity (Noga 1996, Shepherd and Bromage 1999, Rodríguez-

Gutiérrez *et al.* 2001, Klinger and Floyd 2002). Both, Río de los Sauces and Toledo Stream are lotic environments with low salt concentrations, thereby favoring parasite occurrence.

Reproduction of *Lernaea cyprinacea* is affected by high water temperature (Schäperclaus 1991, Stoskopf 1993, Pavanelli *et al.* 1998). According to Mancini *et al.* (2008), there is a direct relationship between temperature and parasitic intensity. Temperature values recorded in this study were low for the season (summer). However, according to Plaul *et al.* (2010), these copepods are adapted to large variety of habitats offered by different hosts and different geographical locations.

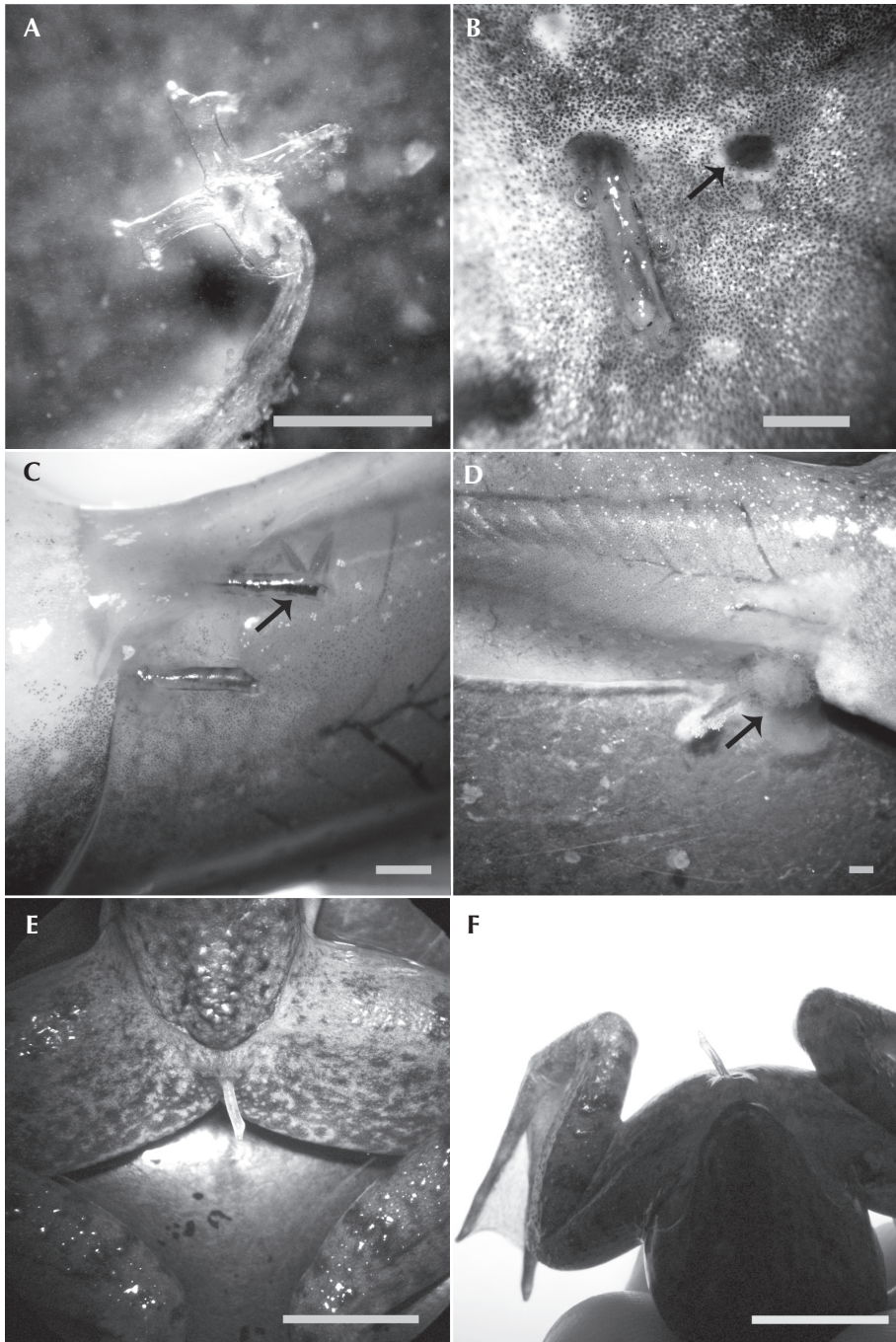


Figure 1. (A) Cephaloma of a female *Lernaea cyprinacea*. (B) Lesion with and without (arrow) the parasite. (C) Female *Lernaea cyprinacea* with egg sacs (arrow). (D) Characteristic lesion. (E-F) Recent metamorph with parasites attached to the cloaca. (A-D scale bar in mm, E-F scale bar in cm.)

Lernaea cyprinacea shows a high affinity for early larval stages of *Lithobates catesbeianus*. Although host preferences for parasites are complex, and in the case of generalists, exposure to parasites depends on larval development stages (Johnson and Hartson 2009). First, the epithelial lining of larvae compared to adults is more sensitive and wet, facilitating adhesion of the parasite. Second, large host size favors more parasitic infestation. Our work corroborated these observations, because larvae of *L. catesbeianus* are much larger than recent metamorphs and have higher prevalence of parasites (Sears et al. 2012).


We recorded a maximum of two parasites per host and Alcalde and Batistoni (2005) reported a maximum of four parasites on the hylid frog *Hypsiboas cordobae* in natural conditions. However, in wild fishes such as *Cyphocharax voga* and *Oligosarchus jenynsii*, maximum parasite loads of 19 and 16 parasites, respectively, were reported by Mancini et al. 2008. In our study, copepods were located preferentially near the cloaca; this agrees with reports by Martins and Souza (1996). The low rate of attachment in other areas of tadpole body may reflect the absence of structures that can act as physical support for the parasites (Martins and Souza 1996).

Despite the low parasite load, previous infestation by copepods was evident by scaring, as reported by Mancini et al. (2008) and Martins and Souza (1996). Lesions observed in the skin of fishes are mainly caused by secondary bacterial infections (Barson et al. 2008) that significantly affect survival of the hosts (Ming 2001, Kupferberg et al. 2009). Low survival owing to parasite loads also may occur in bullfrog tadpoles; however, further studies would be needed to substantiate this. The presence of hemorrhage and ulcers in the absence of copepods may be the result of an immune host reaction (Shariff and Roberts 1989).

Although little is known about the specificity of *Lernaea cyprinacea* on bullfrog tadpoles, copepods have a low host specificity according

to Plaul et al. (2010). *Lithobates catesbeianus* is considered one of the most invasive species in the world (Lowe et al. 2000). Our study of parasitism of *L. catesbeianus* by *L. cyprinacea* suggests that copepod parasitism might act as a natural biological control agent.

Acknowledgments

We thank the anonymous reviewers and the editor for their helpful comments and Linda Trueb for English revision of the manuscript. Financial support was provided by SECyT-UNRC (Grant PPI 18/C416) and FONCyT (Grant PICT 2012-0932). In addition, CONICET (Consejo Nacional de Investigaciones Científicas y Tecnológicas), Argentina, provided a fellowship. We are indebted to Dr. Rubén Tanzona (Universidad Nacional del Sur, Departamento de Biología, Bioquímica y Farmacia, Bahía Blanca, Argentina) for the taxonomic identification of *Lernaea cyprinacea*. The investigation was conducted according to the state law “Protection and Conservation of Wild Fauna” (Argentina National Law N° 22.421). Our study was authorized by Agencia Córdoba Ambiente (A.C.A.S.E.) and Secretaría de Ambiente del Gobierno de Córdoba. 

References

- Akmentins, M. S. and D. E. Cardozo. 2010. American bullfrog *Lithobates catesbeianus* (Shaw, 1802) invasion in Argentina. *Biological Invasions* 12: 735–737.
- Akmentins, M. S., L. C. Pereyra, and J. N. Lescano. 2009. Primer registro de una población asilvestrada de rana toro (*Lithobates catesbeianus*) en la provincia de Córdoba, Argentina. Notas sobre la biología de la especie. *Cuadernos de Herpetología* 23: 25–32.
- Alcalde, L. and P. Batistoni. 2005. *Hyla pulchella cordobae* (Córdoba Tree frog) parasitism. *Herpetological Review* 36: 302.
- Baldauf, R. J. 1961. Another case of parasitic copepods on amphibians. *Journal of Parasitology* 47: 195.
- Barrasso, D. A., R. Cajade, S. J. Nenda, G. Baloriani, and R. Herrera. 2009. Introduction of the American bullfrog

- Lithobates catesbeianus* (Anura: Ranidae) in natural and modified environments: an increasing conservation problem in Argentina. *South American Journal of Herpetology* 4: 69–75.
- Barson, M., A. Mulonga, and T. Nhwatiwa. 2008. Investigation of a parasitic outbreak of *Lernaea cyprinacea* Linnaeus (Crustacea: Copepoda) in fish from Zimbabwe. *African Zoology* 43: 175–183.
- Bush, A. O., K. D. Lafferty, J. M. Lotz, and A. W. Shostak. 1997. Parasitology meets ecology on its own terms: Margolis *et al.* revisited. *Journal of Parasitology* 83: 575–583.
- Carnevia, D. and G. Speranza. 2003. First report of *Lernaea cyprinacea* L., 1758 in Uruguay, introduced by goldfish *Carassius auratus* (Linnaeus, 1758) and affecting axolotl *Ambystoma mexicanum* (Shaw, 1798). *Bulletin European Association of Fish Pathologists* 23: 255–256.
- Densmore, C. L. and D. E. Green. 2007. Diseases of Amphibians. *ILAR Journal* 48: 235–254.
- di Tada, I. E. and E. H. Bucher. 1996. *Biodiversidad de la Provincia de Córdoba. Algunos Aspectos de la Geografía de Córdoba*. Río Cuarto. Universidad Nacional de Río Cuarto. 155 pp.
- Eiras, J. C., R. M. Takemoto, and G. C. Pavanelli. 2003. *Métodos de Estudio y Técnicas Laboratoriales en Parasitología de Peces*. Zaragoza. Editorial Acribia. 133 pp.
- Figueira, L. B. and P. S. Ceccarelli. 1991. Observações sobre a presença de ectoparasitas em pisciculturas tropicais de interior (CEPTA e região). *Boletim Técnico do CEPTA* 4: 57–65.
- Gosner, K. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* 16: 183–190.
- Hoffman, G. L. 1999. *Parasites of North American Freshwater Fishes*. Second edition. Ithaca. Comstock Publishing Associates, Cornell University Press. 543 pp.
- Huys, R. and G. A. Boxshall. 1991. *Copepod Evolution*. Volume 159. London. The Ray Society. 370 pp.
- Idris, H. B. and M. A. Amba. 2011. A note on *Lernaea cyprinacea* parasitizing the cultured marble goby *Oxyeleotris marmorata* and their control with salinity modification. *Advances in Environmental Biology* 5: 817–820.
- Johnson, P. T. J. and R. B. Hartson. 2009. All hosts are not equal: explaining differential patterns of malformations in an amphibian community. *Journal of Animal Ecology* 78: 191–201.
- Kabata, Z. 1979. *Parasitic Copepoda of British Fishes*. Volume 152. London. Ray Society. 468 pp.
- Klinger, R. and R. F. Floyd. 2002. *Introduction to Freshwater Fish Parasites*. Florida. CIR 716, IFAS Extension, University of Florida. 13 pp.
- Kupferberg, S. J., A. Catenazzi, K. Lunde, A. J. Lind, and W. J. Palen. 2009. Parasitic copepod (*Lernaea cyprinacea*) outbreaks in foothill yellow-legged frogs (*Rana boylei*) linked to unusually warm summers and amphibian malformations in Northern California. *Copeia* 2009: 529–537.
- Leong, T. M. 2001. Parasitic copepods responsible for limb abnormalities? *Froglog* 46: 2–3.
- Lowe, S., M. Browne, S. Boudjelas and M. De Poorter. 2000. *100 of the World's Worst Invasive Alien Species: a Selection from the Global Invasive Species Database*. Auckland. Hollands Printing Ltd. 12 pp.
- Mancini, M., C. Rodríguez, M. Ortiz, V. Salinas, and R. Tanzola. 2008. Lerneosis en peces silvestres y cultivados del centro de Argentina. *Biología Acuática* 24: 33–41.
- Margolis, L., G. W. Esch, J. C. Holmes, A. M. Kuris, and G. A. Schad. 1982. The use of ecological terms in parasitology (report of an ad hoc Committee of the American Society of Parasitologists). *Journal of Parasitology* 68: 131–133.
- Martins, M. L. and F. L. D. J. Souza. 1996. Experimental infestations of *Rana catesbeiana* Shaw tadpoles by copepodids *Lernaea cyprinacea* Linnaeus (Copepoda, Lernaeidae). *Revista Brasileira de Zoologia* 12: 619–625.
- McAllister, C. T., C. R. Bursey, and S. D. Martin. 2011. *Lernaea cyprinacea* (Crustacea: Copepoda: Lernaeidae) anchorworms from two larval aquatic insects (Ephemeroptera: Baetidae, Trichoptera: Hydropsychidae) in Northeastern Oklahoma. *Proceedings of the Oklahoma Academy of Science* 91: 37–40.
- Ming, L. T. 2001. Parasitic copepods responsible for limb abnormalities? *Froglog*. 46: 3.
- Nagasawa, K., A. Inoue, S. Myat, and T. Umino. 2007. New host records for *Lernaea cyprinacea* (Copepoda), a parasite of freshwater fishes, with a checklist of the Lernaeidae in Japan (1915–2007). *Journal of the Graduate School of Biosphere Science, Hiroshima University* 46: 21–33.
- Noga, E. J. 1996. *Fish Disease. Diagnosis and Treatment*. Saint Louis. Mosby-Year Book Inc. 367 pp.
- Nori, J., M. S. Akmentins, R. Ghirardi, N. Frutos, and G. C. Leynaud. 2011. American bullfrog invasion in

- Argentina: where should we take urgent measures? *Biodiversity and Conservation* 20: 1125–1132.
- Pavanelli, G. C., J. C. Eiras, and R. M. Takemoto. 1998. *Doenças de Peixes: Profilaxia, Diagnóstico e Tratamento*. Maringá. Editora da Universidade Estadual de Maringá. 264 pp.
- Pereyra, M. O., D. Baldo, and E. R. Krauczuk. 2006. La “rana toro” en la selva Atlántica interior argentina: un nuevo problema de conservación. *Cuadernos de Herpetología* 20: 37–40.
- Plaul, S. E., N. García-Romero, and C. G. Barbeito. 2010. Distribution of the exotic parasite, *Lernaea cyprinacea* (Copepoda, Lernaeidae) in Argentina. *Bulletin European Association of Fish Pathologists* 30: 65–73.
- Rodríguez-Gutiérrez, M. C. M., D. G. Rodríguez-Cázares, Y. Monroy-García, and J. A. Mata-Sotres. 2001. Manual de Enfermedades de Peces., México. *Boletín del Programa Nacional de Sanidad Acuicola y Red de Diagnóstico, Conapesca* 3: 1–14.
- Sanabria, E. A., L. B. Quiroga, and J. C. Acosta. 2005. Introducción de *Rana catesbeiana* Shaw (rana toro), en ambientes pre-cordilleranos de la provincia de San Juan, Argentina. *Multequina* 14: 67–70.
- Sanabria, E., G. Debandi, L. Quiroga, F. Martínez, and V. Corbalán. 2011. First record of the American bullfrog *Lithobates catesbeianus* (Shaw, 1802) in Mendoza province, Argentina. *Cuadernos de Herpetología* 25: 43–46.
- Schäperclaus, W. 1991. *Fish Diseases*. Vol. 2. Berlin. Fischkrankheiten Akademie-Verlag. 911 pp.
- Sears, B. F., A. D. Schlunk, and J. R. Rohr. 2012. Do parasitic trematode cercariae demonstrate a preference for susceptible host species? *PLoS One* 7: e51012.
- Shariff, M. and R. J. Roberts. 1989. The experimental histopathology of *Lernaea polymorpha* Yu, 1938 infection in naïve *Aristichthys nobilis* (Richardson) and a comparison with the lesion in naturally infected clinically resistant fish. *Journal of Fish Diseases* 12: 405–414.
- Shepherd, C. J. and N. R. Bromage (eds.). 1999. *Piscicultura Intensiva*. Zaragoza. Editorial Acribia. 405 pp.
- Silva-Souza, A. T., S. C. Almeida, and P. M. Machado. 2000. Effect of the infestation by *Lernaea cyprinacea* Linnaeus, 1758 (Copepoda, Lernaeidae) on the leucocytes of *Schizodon intermedius* Garavello & Britski, 1990 (Osteichthyes, Anostomidae). *Revista Brasileira de Biologia* 60: 217–220.
- Stoskopf, M. K. 1993. *Fish Medicine*. Philadelphia. Saunders Company. 882 pp.
- Thatcher, V. E. and E. H. Williams Jr. 1998. Comparative morphology of three native lernaeids (Copepoda: Cyclopoida) from Amazonian fishes and descriptions of two new genera. *Journal of Aquatic Animal Health* 10: 300–308.

Editor: Stephen Goldberg