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Eye trematode infection in small passerines in Peru caused by *Philophthalmus lucipetus*, an agent with a zoonotic potential spread by an invasive freshwater snail

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ABSTRACT

Until now, four species of eye trematodes have been found in South America. Of them, *Philophthalmus lucipetus* (synonymized with *Philophthalmus gralli*) displays a broad host spectrum, with at least 30 bird species (prevalently large water birds), five mammal species and humans serving as definitive hosts, and with snails *Fagotia* (*Microcolpia*) *acicularis*, *Amphimelania holandri*, *Melanopsis praemorsa* and *Melanoides tuberculata* serving as intermediate hosts. When examining a total of 50 birds of ten species in the wetland of Pantanos de Villa, Lima, Peru in July 2011, eye trematodes were identified visually in the edematous conjunctival sac of 11 (48%) out of 23 resident many-colored rush tyrants *Tachuris rubrigastra*. Based on morphometric characteristics, the trematodes were identified as *P. lucipetus*. ITS2 and CO1 gene of the examined specimens combined showed a 99% similarity to an Iranian isolate of *Philophthalmus* sp. from the intermediate host *Melanoides tuberculata*, an invasive freshwater snail, suggesting that these two isolates represent the same species with a wide geographical range. Moreover, the prevalence of infection with the philophthalmid cercariae was 31% in 744 *Melanoides tuberculata* examined in Pantanos de Villa in 2010. It is evident that *P. lucipetus* occurs throughout the world as well as locally, including Eurasia and South America. Here we report this trematode for the first time in Peru, and we were the first to sequence any of the South American eye trematodes. Low host specificity of *P. lucipetus* and the invasive character of *Melanoides tuberculata* as a competent intermediate host suggest that eye trematodosis caused by *P. lucipetus* may emerge frequently in various parts of the world, especially in the tropics. Increase of the zoonotic potential of the *P. lucipetus* associated with this invasive snail spreading across the world is predictable and should be of interest for further research.

1. Introduction

Eye trematodes (Echinostomata: Philophthalmidae Loos, 1819) of the genus *Philophthalmus* Loos, 1899 (including recently proposed but already questioned genus *Natterophthalmus* Radev, Kanev, Nollen and Sattmann, 1996) are cosmopolitan parasites, occurring as adults mostly in conjuctival sacs in various birds and mammals [1]. Human cases of philophthalmosis have previously been reported in Europe, Asia, and North America (i.e., former Yugoslavia, Sri Lanka, Japan, Israel, Mexico, and the United States); for a review cf. J. Waikagul et al. [2].

Until now, four species of the eye trematodes have been found in South America. *Philophthalmus zalophi* Dailey, Ellin and Paras, 2005 has recently been described from a mammalian host, the Galapagos fur seal *Arctocephalus galapagoensis* at the Galapagos Islands, Ecuador [3]. Other species of eye trematodes are known from continental South America. *Philophthalmus lachrymosus* Braun, 1902 (also frequently spelled out as *P. lacrymosus* or *P. lacrimosus*) was described on the basis of Brazilian trematode samples recovered from eyes of brown-hooded gulls *Chroicocephalus maculipennis* [4]. All other findings of *P. lachrymosus* in birds originated also from Brazil: J. F. T. Freitas [5] revealed great egret *Ardea alba*, yellow-crowned night heron *Nyctanassa violacea* and royal tern *Thalasseus maximus* as final hosts for the parasite. The species was also found in the conjunctival sacs of kelp gull *Larus dominicanus* [6]. Moreover, adult *P. lachrymosus* was found in human eyes in Mexico [7], and in the eyes of capybaras

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Hydrochaeris hydrochaeris in Brazil [8]. *Philophthalmus semipalmatus* Nasir and Diaz, 1972 was synonymized with *Philophthalmus lachrymosus* by R. M. Pinto et al. [8], but is noted as a valid species by H. U. Pinto and A. L. de Melo [9]. *P. semipalmatus* was described in Venezuela based on the finding of the trematode in willet *Tringa semipalmata* [10]. *P. lucipetus* (Rudolphi, 1819) identified as *P. gralli* Mathis and Leger, 1910 was found in white-cheeked pintail *Anas bahamensis* and Brazilian teal *Amazonetta braziliensis*, and in an intermediate snail host red-rimmed melania *Melanoides tuberculata* in Brazil [9,11]. *P. gralli* was synonymized with *P. lucipetus* from Eurasia and the Americas [1]. At least 30 different bird species (mostly large water birds of the families Anatidae, Ardeidae, Laridae or Scolopacidae), five mammal species and humans living in different parts of Europe were found as definitive hosts of *P. lucipetus*. Snails *Fagotia* (*Microcolpia*) *acicularis*, *Amphimelania holandri*, and *Melanopsis praemorsa* were revealed as intermediate hosts of *P. lucipetus* [12]. Recently, *P. lucipetus* infection was reported from captive greater rheas *Rhea americana*, where *Melanoides tuberculata* were reported as intermediate hosts [13].

Here we focus on the highly prevalent eye trematode infection of a small resident passerine bird species in the wetland of Pantanos de Villa within the agglomeration of Lima, Peru, which we observed in 2011. Here we report the first record of a passerine bird as a host of eye trematodes in South America. We identified the species of eye trematode that caused this infection using morphometric features and provide supplemental molecular data for the species. Regarding a discussion of this case, we used the results of the research which characterized the trematode community found in a population of *Melanoides tuberculata* in Pantanos de Villa in 2010.

2. Materials and methods

2.1. Study area

Birds were examined in a wetland at sea level in Refugio de Vida Silvestre Los Pantanos de Villa, Lima, Peru (12°13′S, 76°59′W). This protected area was established in 1989 and since 1997 the RAMSAR Convention has recognized it as an internationally important area for aquatic birds. It spans across 396 ha and it is the only protected area which exists within the urban agglomeration of Lima. Los Pantanos de Villa is an integral part of the hydrological system of the Rimac River, whose underground waters appear in a natural depression.

2.2. Study of bird species

A total of 50 birds of 10 species were examined from 19 to 22 July 2011. The species and numbers of birds examined were as follows: amazilia hummingbird *Amazilia amazilia* (1), Trochilidae; striated heron *Butorides striata* (2), least bittern *Ixobrychus exilis* (2), Ardeidae; band-tailed gull *Larus belcheri* (1), Laridae; American kestrel *Falco sparverius* (1), Falconidae; wren-like rushbird *Phleocryptes melanops* (9), Furnariidae; many-colored rush tyrant *Tachuris rubrigastra* (23), Tyrannidae; house wren *Troglodytes aedon* (7), Troglodytidae; grassland yellow finch *Sicalis luteola* (2), chestnut-throated seedeater *Sporophila telasco* (2), Thraupidae. After examination, all the birds were released back into the wild.

2.3. Sampling of eye trematodes

Eye trematodes were visually identified in the edematous conjunctival sac of a number of *T. rubrigastra*. Eye trematodes were removed from conjunctival sacs using tweezers and preserved in 96% ethanol and further analyzed in a laboratory. Trematodes were stained in Semichon's carmine, dehydrated by alcohol series, and mounted in Canada balsam. All measurements are in mm, with the range followed by the mean in parentheses. All the measures provided are based on 10 boiling-ethanol-fixed specimens. Drawings were made using a drawing tube. Voucher specimens of *P. lucipetus* were deposited in the Museo de Historia Natural, Facultad de Ciencias Biologicas, Universidad Ricardo Palma, Av. Benavides 54440, Lima 33, Peru; Natural History Museum, Cromwell Road, London SW7 5BD, UK (voucher no. 2012.4.11); Comenius Museum, Moravian Ornithological Station, Horní náměstí 1, 751 52 Přerov, Czech Republic (voucher no. P-P-1870/1), DNA specimens of *P. lucipetus* are deposited with P. Heneberg at the Third Faculty of Medicine, Charles University in Prague, Czech Republic.

2.4. Study of red-rimmed melanias Melanoides tuberculata

M. tuberculata were collected in Pantanos de Villa on 20 January, 22 February and 3 March 2010. They were collected in shallow water from the western edge of the estuary and transported to the laboratory at the Universidad Ricardo Palma (Museum of Natural History) and isolated in small containers with water from the wetland or with tap water. Tap water had no impact on snail or trematode mortality. Containers with snails were subjected to either natural or fluorescent light for 1–2 days to encourage release of cercariae. Containers were then examined using a dissecting scope for snails with patent infections (those infected snails exhibiting cercarial release). Snails were crushed and the shell removed. The snail body was then dissected and examined for larval trematodes. Larvae were examined using dissecting and compound microscopy and identified to the lowest taxonomic level possible according to S. C. Schell [14].

2.5. DNA extraction

To extract DNA, the adult trematodes stored in 96% ethanol were washed twice for 5 min using 1 ml of buffer containing 10 mM Tris–HCl (pH 7.5) and 5 mM EDTA. Following the wash, the specimens were incubated at 65 °C overnight in 2 ml of lysis buffer according to L. J. Smith et al. [15] with some modifications (10 mM Tris–HCl pH 7.5, 5 mM EDTA, 100 mM NaCl, 0.8 mg/ml proteinase K, 10 mg/ml sodium dodecyl sulfate). Following complete dissociation of the specimens, the tubes were centrifuged for 1 min at 16,000 ×*g*. The supernatant was then precipitated for 15 min at −20 °C following addition of one volume of isopropanol. The precipitate was pelleted by centrifugation for 15 min at 16,000 ×*g*, the pellet was washed using one volume of 75% ethanol, then centrifuged for 5 min at 7600 ×*g*. The pellet was air-dried for 15 min at 60 °C to remove residual ethanol, and then dissolved in 100 μl of H_2O for 15 min at 65 °C. Two aliquots of DNA obtained were stored at −20 °C.

2.6. DNA amplification and sequencing

The extracted DNA was amplified using the following polymerase chain reaction (PCR) mix: 10 mM Tris–HCl (pH 8.8), 50 mM KCl, 1.5 mM $MgCl_2$, 0.1% Triton X-100, 0.2 mM dNTP (each), 1 μM 5′ primer, 1 μM 3′ primer, 0.5 U Taq DNA polymerase (Top-Bio, Prague, Czech Republic), and 300 ng of extracted genomic DNA. Total volume was 50 μl. Primers were identical with those used by P. Heneberg and I. Literák [16] (Table S1). PCR was carried out for 36 cycles with 15 s denaturation at 94 °C, 2 min annealing at 53–57 °C, followed by 1–3 min extension at 72 °C (depending on the length of the amplified region). Cycling was started by 2 min denaturation at 94 °C and terminated by 5 min incubation at 72 °C. The experiments were performed using the Bioer XP Thermal Cycler (Bioer Technology Co., Hangzhou, China). Mouse genomic DNA (strain C57BL/6J) was used as a negative control. Following electrophoresis in 1% agarose gel, the PCR products were excised and purified using the Wizard SV Gel and PCR Clean-Up System (Promega, Madison, WI) according to the