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Preliminary Report of the Gastrointestinal Helminths of *Rhinella marina* (Anura: Bufonidae) in Metro Manila, Philippines

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When colonizing new areas, invasive alien species (IAS) have the potential to introduce parasites, which may be perilous to native species. In the Philippines, however, parasite communities of IAS have received little attention. Here, we provide the first report of the gastrointestinal helminths parasitizing *Rhinella marina*, one of the most common and widespread IAS anurans in the Philippines. A total of 318 individuals of helminths classified as trematodes (*Glypthelmins* sp. and *Mesocoelium* sp.) and nematodes (*Falcaustra* sp. and *Physaloptera* sp.) were isolated from the gastrointestinal tract of 31 out of 67 individuals (46%) of *R. marina* collected from four selected urban green spaces within Metro Manila. *Glypthelmins* sp. was the most prevalent (P = 20.9%), dominant (D = 62.3%), and had the highest mean intensity (14.1 ± 15.9) among the identified helminths. Our results contribute knowledge on diversity and infection patterns of gastrointestinal helminths associated with the invasive toad *R. marina* in the Philippines. Further studies are needed to investigate the helminth fauna of other IAS anurans present in the country and to accurately assess the possible transmission of these parasites between colonizers and native species of anurans.

Keywords: anurans, helminths, IAS, Metro Manila, *Rhinella marina*, urban areas

The cane toad (*Rhinella marina*) is a widespread invasive species that has a native range from South and Central America to Mexico and the southern portion of Texas (CABI 2020). In the Philippines, this species was initially introduced as a biological control for insect and rodent pests in agricultural fields (Merino 1936). However, it has spread uncontrollably and is now the most widespread among the six IAS anurans recorded in the Philippines, occupying all major islands of the country except the Palawan and Batanes group of islands (Pili *et al.* 2019).

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But while its tolerance to disturbance and wide range of diet have made it successful in areas where it was introduced, these traits also made *R. marina* more exposed and susceptible to helminth infection (Ruiz-Torres *et al.* 2017). Currently, 135 helminth parasites were recorded for *R. marina* across its global range (Drake *et al.* 2014; Ruiz-Torres *et al.* 2017). In the Philippines, however, the helminth fauna of *R. marina* has never been documented.

Here we report the gastrointestinal helminths of *R. marina* collected in urban green spaces within Metro Manila, Philippines. Furthermore, we also quantify the parasite load of this species.

From 10 Feb to 16 Mar 2019, 67 individuals of *R. marina* were hand-collected at night (from 18:00–20:00) in four urban green spaces located within Metro Manila: University of the Philippines Diliman Lagoon (UPD) in Quezon City, Arroceros Forest Park (AFP) in Manila City, Las Piñas-Parañaque Critical Habitat and Ecotourism Area (LPPCHEA), and Navotas Marine Natural Park (NMNP) in Navotas City (Figure 1). Field sampling, sample collection, and processing was covered by Wildlife Gratuitous Permit No. WGP-NCR 2019-02. Prior Institutional Animal Care and Use Committee (IACUC) clearance was not needed since samples were not kept on a facility and immediately dissected the following morning. Gastrointestinal tract organs from the collected samples were isolated, cut longitudinally, then examined for endoparasites. Helminths were isolated and rinsed with saline solution and then fixed and preserved by immersing in 70% ethanol. Specimens were deposited in Biodiversity Research Laboratory, Institute of Biology, University of the Philippines Diliman. Specimens were mounted on slides and micrographed using an Olympus CX-23 microscope, then identified using parasitological keys (Anderson *et al.* 1974; Velasquez and Eduardo 1994; Razo-Mendivil *et al.* 2006; Dronen *et al.* 2012).

Select descriptive ecological parameters of parasite load were computed following Bush *et al.* (1997) using R software (https://www.r-project.org/). Prevalence (P) was calculated by dividing the number of infected hosts over the total number of host samples while mean intensity (MI) was computed by dividing the number of parasite individuals over the number of hosts infected. Dominance (D) values were computed by dividing the mean abundance (MA) value of a parasite taxon over the sum of MA values of all parasite taxa.

Thirty-one (31) out of the 67 individuals of *R. marina* were infected with helminths (overall P = 46.27%; overall MI = 4.7 ± 12.1). A total of 318 individuals of helminths were isolated representing four taxa and two phyla: *Glypthelmins* sp. and *Mesocoelium* sp. (Platyhelminthes: Trematoda: Plagiorchiida), and *Falcaustra* sp. and *Physaloptera* sp. (Nematoda: Chromadorea: Rhabditida) (Figure 2).

Trematodes *Glypthelmins* sp. and *Mesocoelium* sp. were identified by having a subterminal oral sucker, a ventral sucker smaller than the oral sucker and positioned at the anterior 1/3 of the soma, and an I-shaped excretory bladder (Figure 2); *Glypthelmins* sp. differs from the latter by

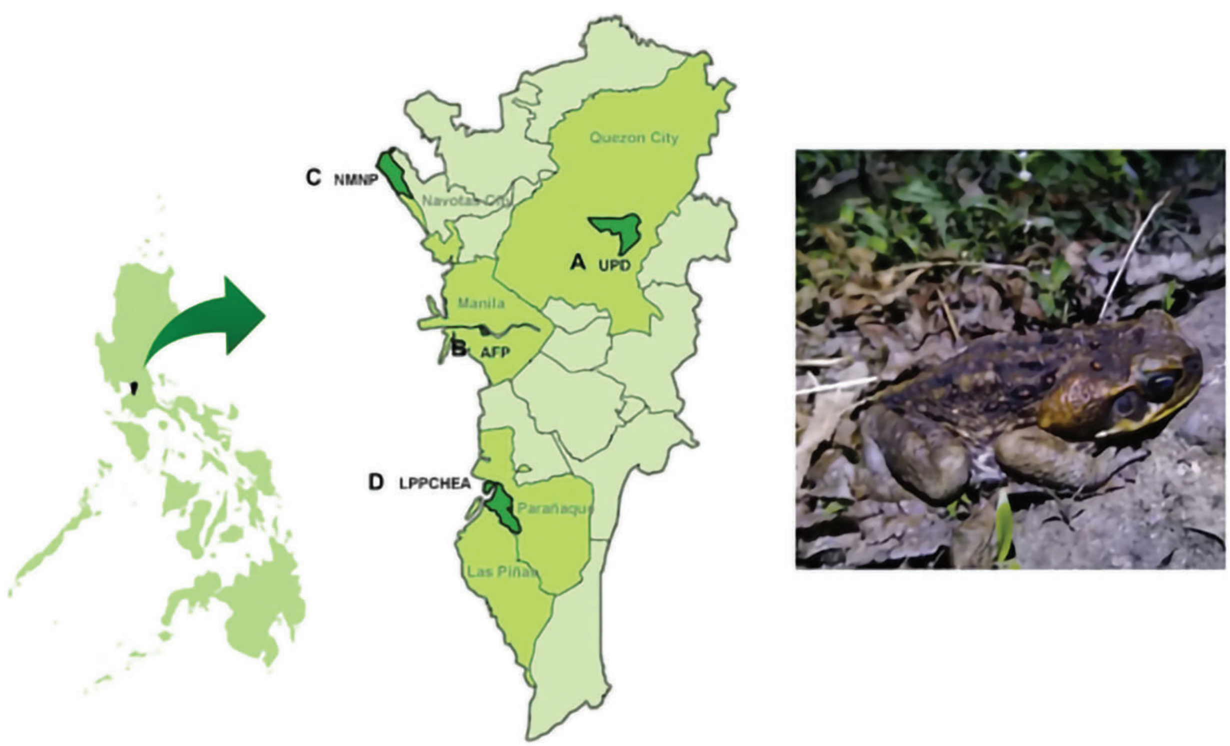

Figure 1. Location of sampling sites within Metro Manila, Philippines (left): A) University of the Philippines Diliman Lagoon (UPD); B) Arroceros Forest Park (AFP); C) Navotas Marine Natural Park (NMNP); D) Las Piñas–Parañaque Critical Habitat and Ecotourism Area (LPPCHEA). *Rhinella marina* (right) found in UPD lagoon (photo by CJ Pueblo) Map generated using QGIS (Quantum Geographic Information System; https://www.qgis.org/).

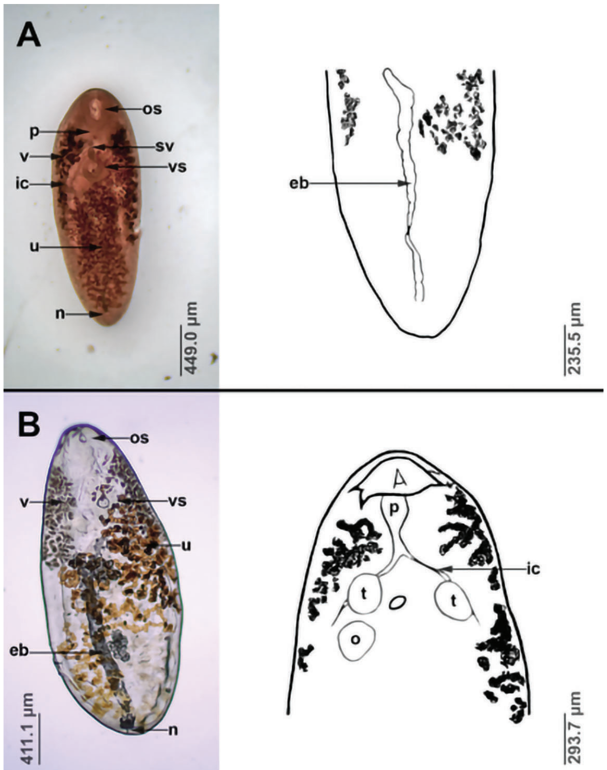

Figure 2. Trematodes retrieved from *R. marina* in four urban green spaces of Metro Manila – namely, A) *Glypthelmins* sp. and B) *Mesocoelium* sp. os – oral sucker; vs – ventral sucker; p – pharynx; ic – intestinal ceca; t – testis; sv – seminal vesicle; o – ovary; v – vitellaria; u – uterus; eb – excretory bladder; n – nephridiopore.

having a fusiform soma, a subterminal nephridiopore, and vitellaria flanking the anterior 2/3 of the soma (Razo-Mendivil *et al.* 2006) while *Mesocoelium* sp. has an ovoid soma, a terminal nephridiopore, an oval ovary situated posterior to the dextral testis, and vitellaria flanking the anterior half of soma (Dronen *et al.* 2012). For nematodes, while *Falcaustra* sp. and *Physaloptera* sp. both lack a cuticular collar and have a relatively short tail (Figure 3), *Falcaustra* sp. differs by having an esophagus comprised of a corpus, an isthmus, a valved bulb, and multiple preanal suckers and *Physaloptera* sp. by having pseudolabia, eversible esophageal lobes, an esophagus comprised of distinct muscular and glandular regions, and a didelphic uterus (Anderson *et al.* 1974).

Among the helminths identified, *Glypthelmins* sp. was the most prevalent (P = 20.9%) and dominant (D = 62.3%) (Table 1). Likewise, infected *R. marina* samples were most heavily parasitized with *Glypthelmins* sp. (MI = 14.1 ± 15.9).

The helminths reported here were also documented previously by other studies done in other anuran host species present in the country. For instance, *Glypthelmins* sp. was isolated from the gastrointestinal tract of *Rana* sp. collected from the Laguna and Manila areas, while *Mesocoelium* sp. was reported from *Ingerophrynus philippinicus*, an endemic toad from the island of Palawan (Velasquez and Eduardo 1994). *Physaloptera amphibia* was also previously observed in the esophagus and heart of *Limnonectes macrodon* collected in Luzon Island (Velasquez and Eduardo 1994). Lastly, a species of *Falcaustra* was isolated from *Megophrys stejnegeri* in

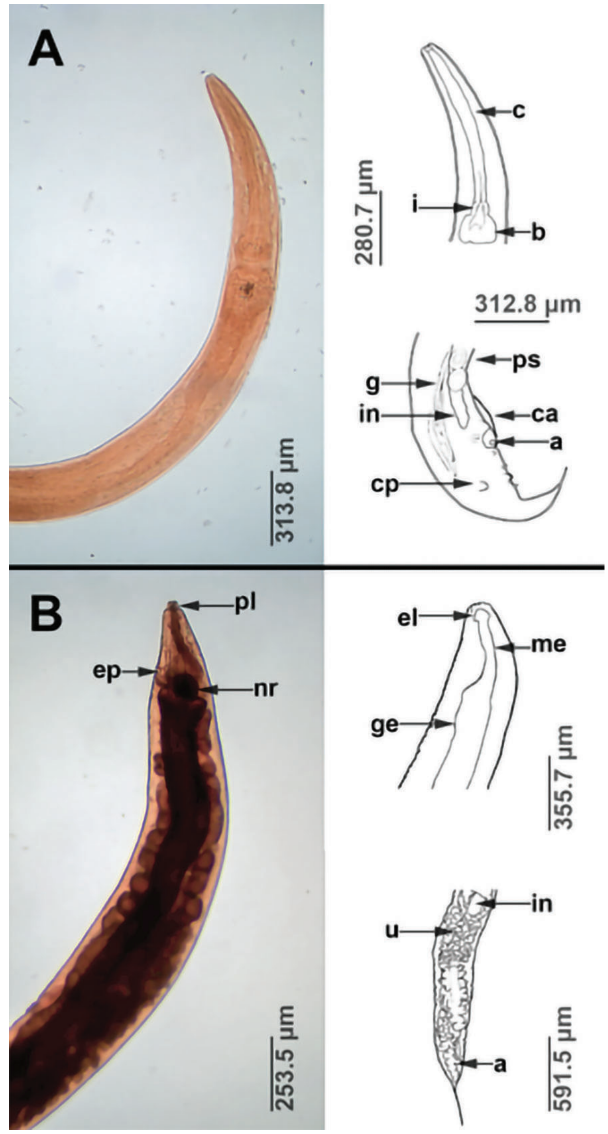

Figure 3. Nematodes retrieved from *R. marina* in four urban green spaces of Metro Manila – namely, A) *Falcaustra* sp. and B) *Physaloptera* sp. pl – pseudolabia; el – esophageal lobes; me – muscular esophagus; ge – glandular esophagus; c – corpus; I – isthmus; b – bulb; in – intestine; ps – preanal sucker; ca – caudal ala; cp – caudal papilla; g – gubernaculum; u – uterus; nr – nerve ring; ep – excretory pore; a – anus.

Table 1. Parasite burden values and site of infection of helminths isolated from *Rhinella marina* in urban green spaces of Metro Manila, Philippines.

Helminth species	%P (n)	%D	MI ± SD	R (min–max)	Site of infection
Platyhelminthes					
Trematoda					
***Glypthelmins* sp.**	20.9 (14)	62.3	14.1 ± 15.9	55 (1–56)	Small intestine
***Mesocoelium* sp.**	3.0 (2)	3.1	5.0 ± 4.2	6 (2–8)	Small intestine
Nematoda					
Chromadorea					
***Falcaustra* sp.**	14.9 (6)	19.5	6.2 ± 5.7	19 (1–20)	Large intestine
***Physaloptera* sp.**	9.0 (10)	15.1	8.0 ± 8.7	22 (2–24)	Large intestine

%P – percent prevalence; n – number of infected individuals; %D – percent dominance; MI – mean intensity; SD – standard deviation; R – range

Samar Island and was described as a new species recently (Bursey *et al.* 2019).

Although there have been previous records of helminths infecting native anuran species of the Philippines (Velasquez and Eduardo 1994; Maglangit *et al.* 2020), to the best of our knowledge, this study is the first report of the helminth fauna of *R. marina* in the country. The majority of studies involving ecology and identification of helminth communities of this invasive toad has been concentrated in countries of its native range such as Mexico (Ruiz-Torres *et al.* 2017; Espínola-Novelo *et al.* 2017), Peru (Toledo *et al.* 2017), and Brazil (e Silva *et al.* 2014).

While the helminths we isolated from *R. marina* are yet to be identified to species level, our survey nonetheless established a baseline parasitofaunal inventory for *R. marina* in the Philippines and contributed to the understanding of endoparasite diversity among IAS anurans in the country. We, therefore, recommend that additional surveys involving *R. marina* employ ultrastructural microscopy and molecular methods. Furthermore, we also suggest that when parasitofaunal surveys are done involving IAS anurans, it must be with the additional intention of learning potential ramifications to species conservation and public health.

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