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Research Note

Parasitic helminths of alien invasive anurans in Butuan City, Northeastern Mindanao, Philippines

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Summary

This study aimed to identify the helminth parasites of invasive anuran species in selected barangays in Butuan City, Philippines. In urbanized areas, invasive species dominate anuran diversity, and one of the primary threats they pose to native wildlife is the transmission of diseases and parasites. Out of the 91 collected individuals of invasive anuran species, *Rhinella marina* was the most abundant (88 %), followed by *Hoplobatrachus rugulosus* (12 %) and *Kaloula pulchra* (3 %). The study identified five species of parasites, with *Spirometra* sp. being the most prevalent (17.58 %), followed by *Echinostoma* sp. (16.5 %), *Rhabdias bufonis* (14.3 %), *Cosmocerca* sp. (6.6 %), and *Strongyloides stercoralis* (3.30 %), respectively. *Spirometra* sp. also had the highest intensity (7.67), followed by *Cosmocerca* sp. (5), *Strongyloides stercoralis* (3.33), *Rhabdias bufonis* (3.30), and *Echinostoma* sp. (2.73). This parasitological survey revealed that *H. rugulosus* had the highest prevalence and infection of parasites, and residential areas had the highest parasite prevalence among the habitat types. Adult hosts were found to harbor a higher prevalence and intensity, and male hosts had a higher prevalence. The results highlight the high risk of parasite transmission from anurans to other animals and emphasize the need for the community to control the population of invasive anuran species for the safety of native anurans and to prevent zoonotic transmission to other animals and humans.
Keywords: Invasive anurans; habitat types; helminth parasites; prevalence; zoonosis

Introduction

The ecological impacts of invasive species, mainly the disease, and parasite transmission are important to control the infection and prevent future outbreaks. The interspecific transmission of parasites from invasive to native species is unlikely due to its host specificity (Paulin, 2007; Lettoof *et al.,* 2013). However, some parasites with shorter generation times than their host allows them a quick counter-adaptation to new host resistance strategies (Kaltz & Shaykoff, 1998). Amphibians are susceptible to parasite infections, such as cestodes, nematodes, and trematodes; some of these are zoonotic, namely *Echinostoma* spp., *Gnathostoma* spp., and *Spirometra* spp., given the fact that these are food-borne parasites (Hallinger *et al.*, 2020). In Australia, cane toads *Rhinella marina,* harboring a diverse fauna of parasites, has raised the concern that they may increase the parasitism rate in endemic anurans. Contrary to the prediction, cane toads do not transfer novel parasites to endemic anurans; instead, they reduce the parasitic burden in native frogs by taking up native parasites and killing them with their immune defense (Lettoof *et al.,* 2013).

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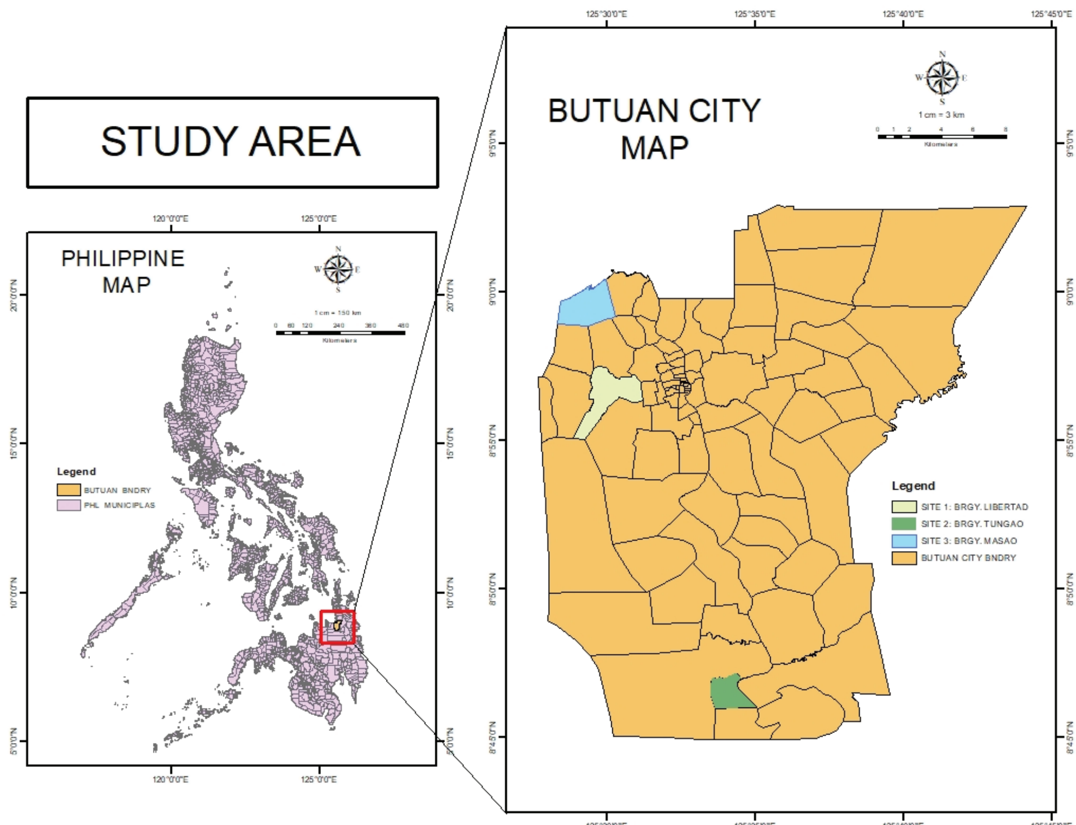

Fig. 1. Map showing the sampling sites in Butuan City, Northeasthern Mindanao, Philippines.

The *Rhinella marina* is one of the most widespread invasive anuran species (IAS) in the Philippines and was initially introduced for agricultural pest control. This species competes with three native frog species (*Rana cancrivora, Polypedates leucomystax,* and *Kaloula conjucta negrosensis*) for breeding sites (Joshi, 2011). *Hoploatrachus rugulosus* was commercially farmed for the food market, and *Kaloula pulchra* was introduced commercially for the pet trade. Studies in various Asian countries have reported zoonotic parasite infections in these invasive anuran species. In Manila, Philippines, gastrointestinal parasite infection was detected from *R. marina* (Duco *et al.,* 2020), while in Myanmar, 75 % of *H. rugulosus* obtained from the local market were found to have sparganosis and gnathostomes (Chai *et al.,* 2020). However, there are currently no reported infections in *K. pulchra.*

In the Caraga region, Philippines, two invasive species have been identified, which are the commercially edible *H. rugulosus* and the failed biological control agent, *R. marina* (Solania & Fernandez-Gamalinda, 2018; Solania *et al.,* 2020; Torralba *et al.,* 2022). It is worth noting that anuran invasion and parasitic infections are more commonly observed in highly urbanized areas, such as Butuan City in the Caraga Region of the Philippines. The dominant presence of these invasive anurans in highly urbanized areas threatens the health of native wildlife and the local population due to their harmful ecological impacts. The present study provides relevant data on the parasitological survey of these three invasive alien species (IAS).

Materials and Methods

Sampling Site (Fig. 1)

Site 1, located in Brgy. Libertad (8°56'55.0"N and 125° 30' 44.9"E) is an urbanized barangay with numerous commercial buildings, subdivisions, and a few agricultural areas. Site 2 is situated in Brgy. Tungao (8°46'47.6"N and 125°30'40.0"E), a small rural barangay that serves as an agroforestry site with vegetation that includes falcata *(Paraserianthes falcataria)*, rubber tree *(Havea brasiliensis)*, coconut (*Cocos nucifera*), rambutan (*Nephelium lappaceum*), and lanzones *(Lancium parasiticum*). Site 3, Brgy. Masao (9°00'10.2"N and 125°29'23.0"E) is a coastal area with human settlement, beach resorts, and an adjacent river system (Torralba *et al.,* 2022). Within each barangay, extensive and opportunistic sampling was employed in collecting invasive anurans from 7:00 to 9:00 P.M., using handling equipment such as gloves and wooden sticks, then placed inside a net bag (DENR-BMB 2017; Jabon *et al.,* 2019). Available taxonomic keys, published articles, and other online sources were utilized for identifying the captured IAS (Solania & Fernandez-Gamalinda, 2018; Diesmos *et al.,* 2015).

Collection of samples

The collected samples were sourced from three barangays in Butuan City, Agusan del Norte, Philippines. Butuan City is a coastal, highly urbanized area in the Caraga region. As of 2020, Butuan City has a population of 372,910 and covers an area of 81,662 hectares. It serves as the region's administrative, commercial, and industrial center and is located in the northeastern part of the Agusan Valley, Mindanao (Mapa, 2021).

Dissection, Isolation, and Identification of Parasitic Helminths

The methods of Akani *et al.* (2011) and Imasuen *et al.* (2012) were utilized to identify the parasites obtained from the collected IAS. Individuals were first pitted to immobilize. It is a technique used to destroy the brain and is relatively painless to the frog (Back *et al.,* 1915). All anurans dissected were processed humanely. The samples were then eviscerated by opening a longitudinal incision from its snout to the vent, and internal organs were collected. The heart, lungs, liver, stomach, intestine, and body cavities were sliced open in the longitudinal section to examine endoparasites. The parasitic helminths were taken from samples and kept in a 0.96 % NaCl solution before being cleaned in PBS and preserved in 70 % ethanol. Before being stored in 70 % ethanol, collected parasitic helminths were placed and given a brief bath in warm water to soothe and bolster the worms. In order to allow the specimens to discharge eggs that could otherwise cover some organs before they are preserved in 70 % ethanol, trematodes were additionally relaxed in warm water. Parasitic helminths were collected and kept in vials with the appropriate labels. For identification, helminth parasites were put on slides. A trinocular stereo zoom microscope (Leica, China) and a compound microscope (Nikon, Tokyo) were used to examine all processed permanent slides. Stage, ocular micrometers, and an optic camera (OptixCam, OCS-SK2-5.2X, China; ToupView software) were used to measure the specimens. Typical reference sources were used to identify the parasites (Khalil *et al.*, 1994; Imasuen *et al.,* 2012).

Data Analysis

Collected data on endo-parasites were tested using SPSS v. 20.0 software, applying a 95 % confidence level. Differences in prevalence among age, sex, and habitat of the collected invasive anuran species were analyzed using the chi-square test of independence. Parasites' mean intensity among invasive anuran species was also computed. The Shapiro–Wilk test was used to determine the normality of the data collected in the study.The Kruskal- Wallis test was used to compare the intensity of parasite infection among anuran species and across habitat types of selected barangays. Pearson correlation test was used to associate the parasite's infection intensity with the host's weight and length. Mann–Whitney U-test was used to compare the parasite intensity between sex and ages of invasive anuran (Estaño *et al.,* 2020).

The following formula were also used in this study:

$$\text{Prevalence} = \frac{\#\ of\ infected\ samples}{total\ number\ of\ samples} \times 100$$

$$\text{Mean Intensity} = \frac{\#\ of\ parasite\ in\ anurans}{total\ number\ of\ infected\ anuran\ samples}$$

Ethical Approval and/or Informed Consent

All applicable national and institutional guidelines for the care and use of animals were followed. A gratuitous permit from the Department of Environment and Natural Resources in the Caraga Region (DENR-13) was obtained before collecting samples.

Results and Discussion

Invasive anuran species

Three invasive anuran species were obtained from the three selected barangays of Butuan city (Table 1), namely the cane toad (*Rhinella marina,* Linnaeus 1758), Chinese tiger frog (*Hoplobatrachus rugulosus,* Weigmann 1834), and Banded Bullfrog (*Kaloula pulchra*, Gary 1831). The chi-square test of independence revealed a significant relationship between species and age (*P = 0.002*) among habitat types.

In the Philippines, invasive species like *R. marina, H. rugulosus,* and *K. pulchra* have proliferated rapidly in all habitat types. The distribution of these species in the study area was due to patchy environmental conditions, dispersal along environmental corridors, anthropogenic activities, and water bodies in all habitat types. *R. marina* is the commonly found species reported to compete with native frogs in breeding sites (Joshi, 2006). It is now widely distributed from Luzon to Mindanao. Climate is one of the factors

Table 1. Prevalence of parasites and its associated in diseases, recovered from invasive anurans in selected areas in Butuan City, Philippines.

Parasite	Number of infected samples (n=91)	Number of parasite	Prevalence (%) (CI**)	Associated Diseases
Spirometra sp.	16	123	17.77 (11.12 – 26.67)	Sparganosis
Strongyloides stercoralis	3	10	3.30 (1.12 – 9.24)	Strongyloidiasis
Cosmocerca sp.	6	30	6.6 (3.057 – 13.65)	Nematodiasis
Rhabdias bufonis	13	43	14.3 (8.543 – 22.92)	Veterinary Important
Echinostoma sp.	15	41	16.5 (10.25 – 25.43)	Echinostomaiasis

that affect anuran activity. Rainfall and temperature influence the reproductive activity and the abundance and species richness of the anurans due to the quality and availability of breeding sites (Garcia, 2019; Luna-Gomez *et al.,* 2017).

Parasitic helminths recovered in invasive anurans

Rhinella marina and *Hoplobatrachus rugulosus* were the only observed species with parasite infection, and a total of 247 parasites were recovered from their organs. However, no recorded parasite infection in the examined samples of *K. pulchra* is supported by the findings of Zhang *et al*. (2020). This suggests that *K. pulchra* may be insensitive to parasite infection. Among the identified parasite species, *Spirometra* sp. was the most prevalent 17.58 % (95 % CI 11.12-26.67), followed by *Echinostoma* sp. 16.5 % (95 % CI 10.25-25.43), *Rhabdias bufonis* 14.3 % (95 % CI 8.543-22.92), *Cosmocerca* sp. 6.6 % (95 % CI 3.057-13.65), and *Strongyloides stercoralis* 3.30 % (95 % CI 1.12-9.24), respectively.

The most prevalent parasite among all the collected parasites from various habitat types was found to be *Spirometra* sp. This finding is consistent with a recent report from the Hainan Province of China (Fu *et al.,* 2022). The presence of *Spirometra* sp. in all habitat types in this study is attributed to the presence of water bodies where the parasites deposit eggs inhabited by copepods, including plankton. Consequently, copepods become infected with *Spirometra* sp. plerocercoid larvae, which are then consumed as part of the anuran diet (Box *et al.,* 2021). The second most prevalent parasite recovered from the examined samples was *Echinostoma* sp. High rates of *Echinostoma* infection have been reported among Filipinos, which is believed to be caused by the consumption of secondary hosts, such as freshwater snails and fish, known to contain metacercariae (Belizario *et al.,* 2007). The prevalence of *Echinostoma* sp. in this study was attributed to the snails found in the agroforest and freshwater habitats near the residential area. *Rhabdias bufonis*, the third most prevalent parasite detected, has also been reported in several studies to have a prevalence among Bufonidae family members (Morsy *et al.,* 2018). The prevalence of *R. bufonis* in both the agroforest and residential areas is due to the availability of snails, the intermediate host of the parasites that serve as food items of invasive anurans in these areas. *Cosmocerca* sp., the fourth most prevalent parasite, was found to have a high intensity and prevalence in agroforests. *Cosmocerca* sp. is a bioindicator and bioaccumulator of pesticides that inhabits moist soil and dry areas with a direct life cycle through skin penetration of its host (Okeagu *et al.,* 2022 & Saad *et al*., 2009). *Strongyloides stercoralis*, the least prevalent parasite recovered from invasive anuran species, is a soil-transmitted parasite that has been shown to affect people's health worldwide (Buonfrate *et al.,* 2020). The first recorded case of Strongyloides is in anurans (Petterson-Kane *et al.,* 2001). In this study, the recorded *S. stercoralis* was manifested in anurans captured in an agricultural area due to the chemicals used in farming, which aided its free-living life cycle (Hendrix *et al., 1987).*

Prevalence and intensity of parasitic helminths

The parasitological survey involved collecting and examining 91 specimens of invasive anuran species, as summarized in Table 2. The results showed an overall prevalence of 46.2 % of parasitic helminths, with an average intensity of 5.9 ± 1.8 worms per host. Among the invasive species of anurans, *H. rugulosus* harbors the highest prevalence with 70 % (95 % CI 39.68-89.22) and a mean intensity of 9.14 ± 0.81, and *R. marina* 43.75 % (95 % CI 33.41-54.66) with a mean intensity of 5.23 ± 0.12. Statistical analysis

Table 2. Parasite infections observed in the collected invasive anuran species.

Group	No. of collected samples	No. of infected	Prevalence (%) (CI**)	Mean intensity (Parasite/host) ±S.E.M	P value
Host species					0.001
R. marina	80	35	43.75 (33.41 – 54.66)	5.23 ± 0.12	
H. rugulosus	10	7	70 (39.68 – 89.22)	9.14 ± 0.81	
Habitat					0.214
Agriculture	31	11	35.5 (21.12 – 53.05)	7.72 ± 0.47	
Agroforest	30	14	46.7 (30.23 – 63.86)	6 ± 0.20	
Residential	30	17	56.7 (39.2 – 72.62)	4.59 ± 0.17	
Sex					0.911
Male	35	17	48.5 (33.01 – 64.43)	4.5 ± 0.20	
Female	56	25	44.6 (32.39 – 57.59)	6.76 ± 0.18	
Age					0.096
Adult	68	35	50.8 (39.84 – 62.95)	5.41 ± 0.15	
Juvenile	23	7	33.3 (15.6 – 50.86)	7.87 ± 0.32	

showed a significant difference in prevalence among invasive anuran species ($P = 0.001$). *H. rugulosus* is an edible frog that plays an economic role in several Asian countries. However, intensive farming causes poor environmental conditions and causes health problems. A study from Thailand revealed that all stages of *H. rugulosus* (91.72 %) were affected by parasites (Phadee & Julawong, 2019). Similarly, a recent study from Metro Manila, Philippines, reported a parasite infection prevalence of 46 % in *R. marina* (Duco *et al.,* 2020). The dispersal of invasive anurans also contributes to their susceptibility to parasitic infection, as frequent movement compromises their immune system and exposes them to a wider range of pathogens (Brown & Shine, 2014).

In terms of habitat type comparison, the highest prevalence was observed in residential areas with 56.7 % (95 % CI 39.2-72.62) and mean intensity of 4.59 ± 0.17, followed by agroforests with 46.7 % (95 % CI 30.23-63.86) and mean intensity of 6 ± 0.20, and then agriculture 35.5 % (95 % CI 21.12-53.05) and mean intensity of 7.72±0.47. However, statistical analysis revealed no significant difference in prevalence and mean intensity at p=*0.990* and p=*0.214*, respectively, among the three habitat types. Residential development has been identified as a leading cause of land-use change, which poses significant implications for biodiversity conservation (Pejchar *et al.,* 2015). In the urban areas of the Philippines, high prevalence of gastrointestinal parasites was reported in anurans (Ybanez *et al.,* 2018). The process of urbanization also reduces the number of hosts and increases the vector population of anurans (Aisen *et al.,* 2021).

In contrast, agroforestry is a land management strategy that involves the integration of woody vegetation and livestock to diversify production systems (Brown *et al.,* 2013). In Brazil, a prevalence of 52.14 % helminth parasites has been reported in cocoa agroforestry (Kersul *et al.,* 2020). In this study, agroforest had a recorded 46.7 % (95 % CI 30.23-63.86) parasite infection due to the high biodiversity in the area, where there is close interaction between anurans, reptiles, and rodents. Agroforestry helps to maintain biodiversity by creating diverse habitats and reducing environmental disruptions caused by agricultural activities (Rosenstock *et al.,* 2019). However, agricultural practices that involve pesticides and herbicides can alter the environment and contribute to the development of free-living parasite forms due to their immunosuppressive and inhibitory effects (Aisien *et al.,* 2021). In the Philippines, farm areas in Luzon have recorded a prevalence of 40 % to 72 % soil-transmitted parasites. Also, the use of manure as fertilizer and wash water has been found to be significantly associated with soil parasite contamination (Paller & Babia-Abion, 2019; De Guia & Flores, 2019).

Prevalence and intensity between sexes and ages

Mann-Whitney U-test showed that the recorded parasitic helminth infection between sexes was insignificant (p= *0.911*). The high prevalence of the parasitic helminths recovered in male hosts with 49 %, (95 % CI 33.01-64.43) compared to females with 45 % (95 % CI 32.39-57.59). The highest infection rate may be due to their higher dispersal activity. However, the female host had a higher mean intensity (6.76 ± 0.18) than male (4.5 ± 0.20) invasive anurans. This report agrees with the study of Kia *et al.* (2018), where a 92 % prevalence rate of gastrointestinal parasites from male *Hoplobatrachus* sp. and 50 % in females were reported. Another reason is that male frogs are more malnourished than females, especially during the dry season. It results in high activity, making them susceptible to parasitic burden (*Kia et al.,* 2018). The test also showed that the distribution of parasites is the same among ages (p=0.096). As the age of the host increases, its vulnerability against infection also increases. It increases the burden of parasite infection, especially in males, due to their shorter life expectancy than females. In juveniles, the low prevalence of infection is due to their less exposure to pathogens and acquired immune response as they grow and develop (Bradley *et al.,* 2019).

In this study, adult hosts have a 48.5 % (95 % CI 33.01-64.43) prevalence rate higher than juvenile hosts with a 33.3 % (95 % CI 15.6-50.86) prevalence rate. The results of age influence on the adult showed a higher prevalence and mean intensity compared to juvenile. The higher infection is attributed to the exposure and vulnerability of adult hosts to parasitic helminths. The high infection in adult invasive anurans has more prolonged exposure to acquired parasitic helminths infective stage in the environment compared to juveniles living for a short time than adults. Adult invasive anurans have a more extensive niche availability in most parasites due to their behavior and ability to travel, seeking food accessibility (Tujan *et al.,* 2016; Estaño *et al.,* 2020).

Correlation of infection to weight and length of the host

The statistical analysis using Pearson's correlation test revealed no significant correlation between the prevalence of parasite infection and the weight ($r = 0.024$, $P = 0.821$) and length ($r = -0.031$, $P = 0.771$) of the host in various habitats. However, a weak positive correlation was observed between the body weight of the host and *Cosmocerca* sp. ($r= 0.224$, $p= 0.033$), and a negative weak correlation was found between the length of the host and *Strongyloides stercoralis* ($r = -0.247$, $p= 0.018$). The recorded correlation of *Cosmocerca* sp. and *S. stercoralis* to the weight and length of the host is that the larger the host, the more space and resources it can provide to parasites (Poulin, 1997). Nematodes are also the most common anuran parasites, and it was associated with a larger host that harbors the richest parasite fauna (Campiao *et al.,* 2015). The correlation between invasive anuran's body weight, body length, and parasitic helminth intensity will increase as the body length and weight of the invasive anuran hosts increases. The findings of this survey concur with the study of parasitic nematodes in rats by Castillo and Paller (2018) and Estaño *et al.* (2020) that large-bodied hosts provide more niches and nutrients for parasitic helminths. The occurrence of parasitic helminths in invasive anurans might exhibit a high possibility of anurans to human transmission of parasitic helminths that threaten public health. The existence of these invasive anuran species threatens both the native wildlife and other animals with its parasite infection. Areas with high anthropogenic activities exhibit high prevalence and infection. Therefore, authorities must implement strategies to control its fast-growing population, spread awareness to the locals about these invasive anuran species and their parasites, and improve their sanitation practices. Due to the lack of published information on the parasitic helminths in invasive anurans in the Philippines, monitoring and surveys should be done in other areas to assess the potential impact of parasitic helminths on public health and veterinary importance.

Conflict of Interest

Authors state no conflict of interest.

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