*Biogeography* **19.**
25–30 . Sep. 20, 2017

# Host utilization by the anuran acanthocephalan *Pseudoacanthocephalus bufonis* (Echinorhynchidae) on two subtropical islands, southern Japan

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**Abstract.** Both Iriomote-jima Island and Ishigaki-jima Island belong to the Ryukyu Islands and are located in the subtropical region of Japan. The echinorhynchid acanthocephalan *Pseudoacanthocephalus bufonis* (Shipley, 1903) was found to parasitize two species of frogs, *Fejervarya sakishimensis* Matsui, Toda & Ota, 2007 (Dicroglossidae) and *Buergeria japonica* (Hallowell, 1861) (Rhacophoridae), on Iriomote-jima Island, and one species of toad, *Rhinella marina* (Linnaeus, 1758) (Bufonidae), on Ishigaki-jima Island. No *P. bufonis* parasitized *Rhacophorus owstoni* (Stejneger, 1907) (Rhacophoridae) and *Microhyla okinavensis* Stejneger, 1901 (Microhylidae) on Iriomote-jima Island. Importance of frogs as the host of *P. bufonis* differs between host species on this island: *F. sakishimensis* was very frequently infected and is considered to be the most important host of *P. bufonis*, but since *B. japonica* was rarely infected and none of *R. owstoni* and *M. okinavensis* was parasitized, the role of these three frogs as the hosts of *P. bufonis* appears very low. This study also suggests that *P. bufonis* is native to Iriomote-jima Island.

**Key words:** *Pseudoacanthocephalus bufonis,* Acanthocephala, anuran parasite, host utilization, Iriomote-jima Island, Ishigaki-jima Island, Ryukyu Islands

## Introduction

*Pseudoacanthocephalus bufoni*s (Shipley, 1903) is an intestinal parasite of frogs and toads (Anura) (*e.g.*, Amin *et al*., 2008; Bush *et al*., 2009; Tkach *et al*., 2013). In Japan, this acanthocephalan is known to infect various anurans of the Ryukyu Islands in the subtropical region of Japan: the species was reported and described by Hasegawa (1984) from *Bufo gargarizans miyakonis* Okada, 1931 (Bufonidae) (as *B. gargarizans*) on two islands, Kitadaito-jima Island and Miyako-jima Island; again from *B. gargarizans miyakonis* on the latter island (Hasegawa & Iwatsuki, 1993); from *Fejervarya sakishimensis* Matsui, Toda & Ota, 2007 (as *Rana limnocharis* Gravenhorst, 1829) (Dicroglossidae) and *Polypedates leucomystax* (Gravenhorst, 1829) (Rhacophoridae) on three other islands, Ishigaki-jima Island, Iriomote-jima Island, and Okinawa-jima Island (Hasegawa *et al*., 1987; Hasegawa, 1994); from *Buergeria japonica* (Hallowell, 1861) (Rhacophoridae) and *Microhyla okinavensis* Stejneger, 1901 (as *M. ornata* (Duméril and Bibron, 1841)) (Microhylidae) on Okinawa-jima Island (Hasegawa, 1993); and from *P. leucomystax* on Miyako-jima Island (Hasegawa

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& Ota, 2017). The acanthocephalan was also found as pseudoparasitism from the intestine of wild Iriomote cat, *Prionailurus bengalensis iriomotensis* (Imaizumi, 1967) (as *Felis iriomotensis*) (Mammalia: Felidae), on Iriomote-jima Island (Hasegawa, 1992). Recently, we had the opportunity to examine four species of frogs, *i.e.*, *F. sakishimensis*, *Rhacophorus owstoni* (Stejneger, 1907) (Rhacophoridae), *B. japonica*, and *M. okinavensis*, from Iriomote-jima Island, and one species of toad, *Rhinella marina* (Linnaeus, 1758) (Bufonidae), from Ishigaki-jima Island for the occurrence of *P. bufonis*. Of these anurans, *Rha. owstoni* and *Rhi. marina* were examined for the first time in Japan for *P. bufonis*. As the four species of frogs were collected on the same island, we herein compare the data on prevalence and intensity of *P. bufonis* in those frogs.

## Materials and Methods

A total of 87 individuals of four species of frogs, representing *F. sakishimensis* (n=50), *Rha. owstoni* (n=10), *B. japonica* (n=7), and *M. okinavensis* (n=20), were collected at six sites (Midara [24°22′16″N, 123°45′06″E], Urauchi [24°24′53″N, 123°46′45″E], Uehara [24°24′57″N, 123°47′43″E], Nadara [24°23′52″N, 123°49′41″E], Omija [24°23′43″N, 123°51′37″E], and Takana [24°22′49″N, 123°54′39″E]) on Iriomote-jima Island from April 2016 to May 2017 (Table 1). Seven individuals of *Rhi. marina* were also collected at two sites (Tonoshiro [24°20′59″N, 124°10′14″E] and Hirae [24°22′51″N, 124°11′34″E]) on Ishigaki-jima Island in May of 2016 and 2017 (Table 2). All anurans were road-killed animals and sampled from roads in the late evening or early morning. Some of these anurans were examined on the day of collection at the laboratory of Iriomote Station, Tropical Biosphere Research Center, University of the Ryukyus, Taketomi, where they were measured for body length (BL) and examined for endohelminths including *P. bufonis*, whereas the others were frozen and transported to the laboratory of Hiroshima University, Higashi-Hiroshima, where they were thawed and similarly examined. In both laboratories, the number of acanthocephalans was recorded for each anuran, and many of them were flattened and fixed in 70% ethanol with coverslip pressure, but some of them were fixed in 99.5% ethanol for future molecular analysis. The specimens fixed in 70% ethanol were later stained with alum carmine or Heidenhain's iron hematoxylin, dehydrated, and mounted in Canada balsam. They were identified as *P. bufonis* based on the description of the species given by Hasegawa (1984) from *B. gargarizans miyakonis* on Kitadaito-jima Island and Miyako-jima Island near our study area. Voucher specimens of *P. bufonis* are deposited in the Aschelminthes (As) collection of the National Museum of Nature and Science, Tsukuba, Ibaraki Prefecture (NSMT-As 4299 from *F. sakishimensis* on Iriomote-jima Island; NSMT-As 4300 from *Rhi. marina* from Ishigaki-jima Island). Three terms, *i.e.*, prevalence, intensity, and mean intensity, follow those defined by Bush *et al.* (1997). The scientific names of anurans used in this paper are those recommended by the Herpetological Society of Japan (2017).

## Results

There was a marked difference in prevalence and intensity of *P. bufonis* between the four species of frogs examined from Iriomote-jima Island (Table 1): *F. sakishimensis* was very frequently infected (overall prevalence, 86.0%) and up to 42 worms of *P. bufonis* were found from an infected individual (Fig. 1), whereas only one individual was parasitized by one worm in *B. japonica* and none of *Rha. owstoni* and *M. okinavensis* was infected.

*Rhinella marina* was found to be infected by *P.*

*bufonis* on Ishigaki-jima Island (Table 2): its overall prevalence was high (71.4%) and up to 83 worms were collected from an infected individual.

## Discussion

The observed difference in the occurrence of *P. bufonis* may be caused by differences in amount of intake of the acanthocephalan's intermediate hosts between frog species. The intermediate hosts of *P. bufonis* are yet unknown but ground isopods may serve for such a role because a closely related acanthocephalan *Pseudoacanthocephalus toshimai* Nakao, 2016 uses the ground isopod *Ligidium japonicum* Verhoeff, 1918 as its intermediate host in Japan (Nakao, 2016). While *Rha. owstoni* often occurs on

Table 1. Occurrence of *Pseudoacanthocephalus bufonis* in four species of frogs on Iriomote-jima Island, the Ryukyu Islands, southern Japan.

| Family and species | Sampling site | Sampling date | No. of individuals examined | Body length (mean) in mm | Prevalence (%) | Intensity | Mean intensity |
|---|---|---|---|---|---|---|---|
| Dicroglossidae | | | | | | | |
| *Fejervarya sakishimensis* | Midara | 6–14 June 2016 | 7 | 48–65 (57) | 42.9 | 0–16 | 7.3 |
| | | 8 July 2016 | 8 | 45–65 (56) | 100 | 2–18 | 7.8 |
| | Urauchi | 6 August 2016 | 1 | 57 (57) | 100 | 1 | 1.0 |
| | Uehara | 6–18 July 2016 | 6 | 52–64 (56) | 83.3 | 0–17 | 5.0 |
| | | 6–10 August 2016 | 14 | 50–63 (56) | 92.9 | 0–17 | 7.6 |
| | Nadara | 16 August 2016 | 3 | 61–66 (63) | 100 | 3–7 | 4.7 |
| | Omija | 8 April 2016 | 1 | 69 (69) | 100 | 5 | 5.0 |
| | | 18 May 2016 | 1 | 58 (58) | 100 | 5 | 5.0 |
| | | 22 May 2017 | 7 | 53–72 (61) | 85.7 | 0–42 | 15.2 |
| | Takana | 23 May 2017 | 2 | 56–72 (64) | 100 | 3–12 | 7.5 |
| | | Total | 50 | 45–72 (58) | 86.0 | 0–42 | 7.9 |
| Rhacophoridae | | | | | | | |
| *Rhacophorus owstoni* | Uehara | 18 July 2016 | 1 | 32 | 0 | 0 | – |
| | | 10 August 2016 | 1 | 45 | 0 | 0 | – |
| | | 23–30 November 2016 | 7 | 43–67 (50) | 0 | 0 | – |
| | | 20 January 2017 | 1 | 57 | 0 | 0 | – |
| | | Total | 10 | 32–67 (48) | 0 | 0 | – |
| *Buergeria japaonica* | Uehara | 18 July 2016 | 2 | 32–35 (34) | 50.0 | 1 | 1.0 |
| | | | 3 | 28–35 (31) | 0 | 0 | – |
| | | 8 September 2016 | 1 | 26 | 0 | 0 | – |
| | | 13 March 2017 | 1 | 27 | 0 | 0 | – |
| | | Total | 7 | 26–35 (30) | 14.3 | 1 | 1.0 |
| Microhylidae | | | | | | | |
| *Microhyla okinavensis* | Midara | 8 July 2016 | 2 | 29–31 (30) | 0 | 0 | – |
| | Uehara | 18 July 2016 | 6 | 22–31 (28) | 0 | 0 | – |
| | | 10 August 2016 | 11 | 25–32 (28) | 0 | 0 | – |
| | | 8 September 2016 | 1 | 28 | 0 | 0 | – |
| | | Total | 20 | 22–32 (28) | 0 | 0 | – |

Table 2. Occurrence of *Pseudoacanthocephalus bufonis* in *Rhinella marina* (Bufonidae) on Ishigaki-jima Island, the Ryukyu Islands, southern Japan.

| Sampling site | Sampling date | No. of individuals examined | Body length (mean) in mm | Prevalence (%) | Intensity | Mean intensity |
|---|---|---|---|---|---|---|
| Hirae | 20 May 2016 | 2 | 117–138 (128) | 100 | 2–83 | 42.5 |
| | 25 May 2017 | 3 | 72–140 (111) | 66.7 | 0–8 | 6.5 |
| Tonoshiro | 20 May 2016 | 2 | 118–142 (133) | 50.0 | 0–2 | 2.0 |
| | Total | 7 | 72–142 (122) | 71.4 | 0–83 | 19.6 |

Fig. 1. *Pseudoacanthocephalus bufonis* infecting the intestine of *Fejervarya sakishimensis* caught at Omija, Iriomote-jima Island, southern Japan, on 22 May 2017. Scale bar: 5 mm.

trees, the other frogs are ground dwellers, but all of these frogs prey on small ground invertebrates at night (Takada & Ohtani, 2011). *Fejervarya sakishimensis* appears to prey most abundantly on ground isopod intermediate hosts, which has probably resulted in the observed most frequent and heaviest infection of *P. bufonis* in this frog among the four host species.

In this study, we collected our samples of *F. sakishimensis* at all of the six sites on Iriomote-jima Island. This indicates that the frog is widely and abundantly distributed on the island. In contrast, despite the same sampling effort, only a small number of individuals were sampled for the three other frogs, which implies that their abundance is much lower than that of *F. sakishimensis* on the island, especially along the roads sampled. In addition to these implications, the fact that *F. sakishimensis* was very frequently infected (Table 1) suggests that this frog is the most important host of *P. bufonis* on Iriomote-jima Island, and based on another fact that *B. japonica* was rarely infected and none of *Rha. owstoni* and *M. okinavensis* was parasitized (Table 1), the role of these three frogs as the hosts of *P. bufonis* appears very low or almost none.

The fauna of Iriomote-jima Island has been strictly conserved by the Government of Japan, and it is important to discuss the native status of *P. bufonis* on this island because two alien anurans *Rhi. marina* and *Polypedates leucomystax* were found there in 2000 and 2015, respectively (Naha Office of the Nature and Environment, Ministry of the Environment, 2017). Fortunately, however, *P. bufonis* was discovered on Iriomote-jima Island as early as 1985 (Hasegawa *et al*., 1987), and it is evident that the acanthocephalan was not introduced by the two alien anurans into the island. In other words, *P. bufonis* is native to Iriomote-jima Island.

*Rhinella marina* was found to be very frequently infected by *P. bufonis* on Ishigaki-jima Island (Table 2). A similar heavy infection by *P. bufonis* was also recorded from *Bufo gararizans miyakonis* on Kitadaito-jima Island (up to 210 worms, Hasegawa, 1984) and Miyako-jima Island (up to 150 worms, Hasegawa & Iwatsuki, 1993). There is a previous record of *P. bufonis* from Ishigaki-jima Island (Hasegawa *et al*., 1987), where only *F. sakishimensis* (as *R. limnocharis*) has been reported as a natural

host of the acanthocephalan. *Rhinella marina* was introduced from Minamidaito-jima Island, one of the Ryukyu Islands, to Ishigaki-jima Island in 1978 and since has been established there (Naha Office of the Nature and Environment, Ministry of the Environment, 2017). Another alien frog *P. leucomystax* also has been found on this island since 2007 (Naha Office of the Nature and Environment, Ministry of the Environment, 2017). As we examined only *Rhi. marina* from Ishigaki-jima Island in this study, it is desirable to examine a variety of wild frogs and *P. leucomystax* for *P. bufonis* on Ishigaki-jima Island for assessing the present status of the acanthocephalan infection in these frogs.

Recently, morphological (Amin *et al*., 2008; Bush *et al*., 2009) and both morphological and molecular studies (Tkach *et al*., 2013; Nakao, 2016) have been conducted on the taxonomy of the species of *Pseudoacanthocephalus*. Eighteen nominal species are currently known, and nine species of them occur in East and Southeast Asia: *P. bufonis*, *P. xenopeltidis* (Shilpley, 1903), *P. lucidus* (Van Cleave, 1925), *P. elongatus* (Van Cleave, 1937), *P. nguyenthileae* Amin, Van Ha & Heckman, 2008, *P. reesei* Bush, Duszynski & Nickol, 2009, *P. nickoli* Tkach, Lisitsyna, Crossley, Binh & Bush, 2013, *P. smalesi* Tkach, Lisitsyna, Crossley, Binh & Bush, 2013, and *P. toshimai*. In this study, we identified our specimens as *P. bufonis* based on the description of the species given by Hasegawa (1984), but the previous and our specimens of *P. bufonis* from the Ryukyu Islands (Hasegawa 1984, 1992, 1993, 1994; Hasegawa *et al*., 1987; Hasegawa & Iwatsuki, 1993; Hasegawa & Ota, 2017; this paper) may need re-identification based on the current taxonomy of the genus.

## Acknowledgments

We thank Toru Naruse, Iriomote Station, Tropical Biosphere Research Center, University of the Ryukyus, for providing laboratory facilities. We are also grateful to an anonymous reviewer for useful comments to improve the manuscript.

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(Received June 29, 2017; Accepted August 18, 2017)