Further records of *Pseudoacanthocephalus* cf. *bufonis* (Acanthocephala: Echinorhynchidae) infecting anurans in the Ryukyu Islands, southern Japan

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■ Abstract

Echinorhynchid acanthocephalans, reported as *Pseudoacanthocephalus* cf. *bufonis* in this paper, were found in the small intestine of *Polypedates leucomystax* (Gravenhorst, 1829) (Rhacophoridae) on Okinawa-jima Island and *Bufo gargarizans miyakonis* Okada, 1931 (Bufonidae) on Miyako-jima Island, the Ryukyu Islands, southern Japan. This acanthocephalan species was previously reported as *P. bufonis* (Shipley, 1903) in Japan. The known anuran hosts and collection localities of *P.* cf. *bufonis* in the Ryukyu Islands are listed. A comment is also made on the previous identification of *P. bufonis* found in Hokkaido, northern Japan.

■ Introduction

One species of echinorhynchid acanthocephalan is found in the small intestine of anurans in the Ryukyu Islands, southern Japan. Hasegawa (1984) first identified the acanthocephalan as *Pseudoacanthocephalus bufonis* (Shipley, 1903) and this scientific name was adopted by him and his colleagues (Hasegawa et al., 1987; Hasegawa and Iwatsuki, 1993; Hasegawa, 1993, 1994; Hasegawa and Ota, 2017). Later, based on Hasegawa's (1984) paper, Nagasawa and Yoshida (2017) identified their specimens of echinorhynchid acanthocephalan from anurans in the Ryukyu Islands as *P. bufonis*. However, for exact identification of the acanthocephalan, these authors suggested to re-examine it using morphological and molecular data. Most currently, Hasegawa et al. (2018) commented the taxonomic status of the acanthocephalan and treated it as *Pseudoacanthocephalus* sp. because molecular data (unpublished) of their specimens did not match those of *P. bufonis*. Under these situations, we here provisionally use *Pseudoacanthocephalus* cf. *bufonis* for the acanthocephalan based on the fact that it has not yet been exactly identified at species level but is morphologically similar to *P. bufonis*.

Previously, we reported the occurrence of the acanthocephalan in five species of anurans on Iriomote-jima Island and Ishigaki-jima Island, the Ryukyu Islands (Nagasawa and Yoshida, 2017). Currently, we had another chance to examine some anurans from two adjacent islands, Okinawa-jima Island and Miyako-jima Island, and the results of this examination are here reported.

Since the 1980s, some investigations into the parasites of anurans have been conducted in the Ryukyu Islands, and data on the prevalence and intensity of *P.* cf. *bufonis* in various species of anurans have been reported (Hasegawa, 1984; Hasegawa et al., 1987; Hasegawa and Iwatsuki, 1993; Hasegawa, 1993, 1994; Hasegawa and Ota, 2017; Nagasawa and Yoshida, 2017; Hasegawa et al., 2018). Based on the results from those investigations, this paper also provides a list of the known anuran hosts and collection localities of *P.* cf. *bufonis* in the Ryukyu Islands.

■ Materials and Methods

Eighteen individuals of five species of frogs, representing *Odorrana narina* (Stejneger, 1901) (n=4), *Rana ulma* Matsui, 2011 (n=1), *Lithobates catesbeianus* (Shaw, 1802) (n=1) (all Ranidae), *Buergeria japonica* (Hallowell, 1861) (n=4), and *Polypedatus leucomystax* (Gravenhorst, 1829) (n=8) (both Rhacophoridae), were

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Fig. 1. *Pseudoacanthocephalus* cf. *bufonis* infecting the small intestine of *Bufo gargarizans miyakonis* collected in the Ono forest area, Hirara, on Miyako-jima Island, southern Japan, on 14 June 2012. In total, 156 worms of *P.* cf. *bufonis* were found in the intestine. Scale bar: 5 mm.

collected from a mountain road in Nuuha (26°41'44"N, 128°08'34"E to 26°40'36"N, 128°08'55"E), Ogimi on Okinawa-jima Island. The first three species were found road-killed on 4 November 2012, and the other two species were hand-caught on 5 August 2017. Five road-killed individuals of toad, *Bufo gargarizans miyakonis* Okada, 1931 (Bufonidae), were also collected in the Ono forest area, Hirara on Miyako-jima Island on 14 June 2012. All these anurans were frozen after collection and transported to the laboratory of Hiroshima University, Higashi-Hiroshima, where they were thawed, dissected, and examined for intestinal parasites. Acanthocephalans taken from the small intestine 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 stained with Heidenhain's iron hematoxylin, dehydrated, and mounted in Canada balsam. Voucher specimens of *P.* cf. *bufonis* have been deposited in the Aschelminthes (As) collection of the National Museum of Nature and Science, Tsukuba, Ibaraki Prefecture, Japan (NSMT-As 4450 from *Polypedatus leucomystax*; NSMT-As 4451 from *B. gargarizans miyakonis*). The scientific names of anurans mentioned in this paper are those recommended by the Herpetological Society of Japan (2017).

■ Results and Discussion

Of the six species of anurans examined from the two islands, only two species, *Polypedatus leucomystax* from Okinawa-jima Island and *B. gargarizans miyakonis* from Miyako-jima Island, were found infected by *P.* cf. *bufonis*. Two (25%) of eight *Polypedatus leucomystax* were infected each by one and 15 worms, and four (80%) of five *B. gargarizans miyakonis* by a total of 184 worms (2–156 [mean 46] worms per infected host). The acanthocephalan species has been reported from the same host species on the same islands: *Polypedatus leucomystax* on Okinawa-jima Island (Hasegawa, 1994) and *B. gargarizans miyakonis* on Miyako-jima Island (Hasegawa, 1984; Hasegawa and Iwatsuki, 1993).

As many as 156 worms of *P.* cf. *bufonis* were found from *B. gargarizans miyakonis* in this study (Fig. 1). The same host species has been reported to have a similar heavy infection on Kitadaito-jima Island (up to 210 worms per infected host, Hasegawa, 1984) and Miyako-jima Island (up to 150 worms, Hasegawa and Iwatsuki, 1993). A heavy infection (up to 86 worms) of the acanthocephalan was also recorded from *Rhinella marina* (Linnaeus, 1758) (Bufonidae) on Ishigaki-jima Island (Nagasawa and Yoshida, 2017).

Pseudoacanthocephalus cf. *bufonis* has so far been reported from two species of toads (Bufonidae) and five species of frogs (Dicroglossidae, Rhacophoridae, Microhylidae) in the Ryukyu Islands (Table 1), which indicates that the acanthocephalan species shows low host specificity and can use various species of anurans. Nevertheless, a marked difference in prevalence and intensity of *P.* cf. *bufonis* between four species of anurans from Iriomote-jima Island was observed by

Nagasawa and Yoshida (2017): *Fejervarya sakishimensis* Matsui, Toda and Ota, 2007 (Dicroglossidae) was most frequently and heavily infected by *P.* cf. *bufonis*. The authors considered that *F. sakishimensis* is the most important host of *P.* cf. *bufonis* on the island and the observed difference in occurrence of the acanthocephalan may be caused by differences in amount of intake of its intermediate hosts between anuran species. As a case of pseudoparasitism by *P.* cf. *bufonis*, Hasegawa (1992) reported its occurrence in the Iriomote cat, *Prionailurus bengalensis iriomotensis* (Imaizumi, 1967) (as *Felis iriomotensis*) (Mammalia: Felidae), endemic to the island.

In Japan, in addition to the records of *P.* cf. *bufonis* from anurans in the Ryukyu Islands, there are three records of "*Pseudoacanthocephalus bufonis*" from *Bufo japonicus formosus* Boulenger, 1883 (Bufonidae) from Hokkaido, a northern subarctic island of Japan (Nishikawa et al., 2012a, 2012b, 2014). This bufonid species was introduced from Honshu, a major island of Japan, to Hokkaido and has established there (e.g., Tokuda, 2010; Sarashina and Yoshida, 2015; Naito and Shiga, 2016). The acanthocephalan reported as "*P. bufonis*" from Hokkaido was poorly studied for its morphology (Nishikawa et al., 2012a, 2014), and, recently, *Pseudoacanthocephalus toshimai* Nakao, 2016 was described as a new species from *Rana pirica* Matsui, 1991 (Ranidae) and *Hynobius retardatus* Dunn, 1923 (Caudata: Hynobiidae) in Hokkaido (Nakao, 2016). Thus, the previous identification of "*P. bufonis*" from Hokkaido needs verification, and it is important to make a comparative study using specimens of "*P. bufonis*" and *P. toshimai*.

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Table 1. Known anuran hosts and collection localities of *Pseudoacanthocephalus* cf. *bufonis* in the Ryukyu Islands, southern Japan. The scientific names of anurans are adopted from the Japanese Society of Herpeology (2017).

Host	Locality	Island	Reference
Bufonidae			
Bufo gargarizans miyakonis	— *	Kitadaito-jima Island	Hasegawa (1984)
	—	Miyako-jima Island	Hasegawa (1984)
	Tropical Botanical Garden, Hirara		Hasegawa and Iwatsuki (1993)
	Ono forest area, Hirara		This paper
Rhinella marina	Hirae	Ishigaki-jima Island	Nagasawa and Yoshida (2017)
	Tonoshiro		Nagasawa and Yoshida (2017)
Dicroglossidae			
*Fejervarya kawamurai***	Onna	Okinawa-jima Island	Hasegawa et al. (1987)
	Haneji, Nago		Hasegawa (1994)
*Fejervarya sakishimensis***	Arakawa	Ishigaki-jima Island	Hasegawa et al. (1987)
	Komi	Iriomote-jima Island	Hasegawa et al. (1987)
	Midara		Nagasawa and Yoshida (2017)
	Urauchi		Nagasawa and Yoshida (2017)
	Uehara		Nagasawa and Yoshida (2017)
	Nadara		Nagasawa and Yoshida (2017)
	Omija		Nagasawa and Yoshida (2017)
	Takana		Nagasawa and Yoshida (2017)
Rhacophoridae			
Buergerya japonica	Haneji, Nago	Okinawa-jima Island	Hasegawa (1993)
	Uehara	Iriomote-jima Island	Nagasawa and Yoshida (2017)
	—	Ishigaki-jima Island	Hasegawa et al. (2018)
Polypedates leucomystax	Haneji, Nago	Okinawa-jima Island	Hasegawa (1994)
	Nuuha, Ogimi		This paper
	—	Miyako-jima Island	Hasegawa and Ota (2017)
	—	Ishigaki-jima Island	Hasegawa et al. (2018)
	—	Iriomote-jima Island	Hasegawa et al. (2018)
Microhylidae			
Microhyla okinavensis	Haneji, Nago	Okinawa-jima Island	Hasegawa (1993)

* No information on detailed collection locality.

** *Rana limnocharis* was used for the species by Hasegawa et al. (1987) and Hasegawa (1994), but currently, its population of Okinawa-jima Island and those of both Ishigaki-jima and Iriomote-jima islands have been identified as independent species, *Fejervarya kawamurai* and *F. sakishimensis*, respectively (see Matsui et al., 2007; Djong et al., 2011).

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