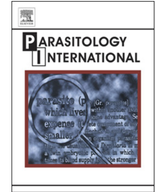
Parasitology International 66 (2017) 240–249

Contents lists available at ScienceDirect

Parasitology International

journal homepage: www.elsevier.com/locate/parint



Brachylaima ezohelicis sp. nov. (Trematoda: Brachylaimidae) found from the land snail *Ezohelix gainesi*, with a note of an unidentified *Brachylaima* species in Hokkaido, Japan

Minoru Nakao [a,*], Tsukasa Waki [b], Mizuki Sasaki [a], Jason L. Anders [c], Daisuke Koga [d], Mitsuhiko Asakawa [e]

[a] Department of Parasitology, Asahikawa Medical University, Asahikawa, Hokkaido 078-8510, Japan
[b] Meguro Parasitological Museum, Shimomeguro, Meguro-ku, Tokyo 153-0064, Japan
[c] Graduate School of Environmental Earth Science, Hokkaido University, Sapporo, Hokkaido 060-0808, Japan
[d] Department of Microscopic Anatomy and Cell Biology, Asahikawa Medical University, Asahikawa, Hokkaido 078-8510, Japan
[e] School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido 069-8501, Japan

ARTICLE INFO

Article history:
Received 30 November 2016
Received in revised form 21 January 2017
Accepted 23 January 2017
Available online 25 January 2017

Keywords:
Brachylaima ezohelicis sp. nov
Ezohelix gainesi
Hokkaido

ABSTRACT

In the Japanese Archipelago, *Ezohelix gainesi*, a member of bradybaenid land snails, is endemic mainly to the island of Hokkaido. During July to August of 2016, a survey to detect trematode infections from *E. gainesi* was carried out at a forest city park in Asahikawa, Hokkaido. Systemic infections of the snails with sporocysts containing short-tailed cercariae were found in 5.3% of 94 individuals examined. Furthermore, most of them (90.4%) harbored non-encysted metacercariae within their kidneys. A DNA sequence identification revealed that both of the sporocyst and the metacercaria belong to an unknown species of the family Brachylaimidae. The metacercariae showed a genetic diversity with 6 haplotypes of mitochondrial DNA (mtDNA) even in the limited sampling area. A definitive host of the unknown species could not be determined, although 34 field mice (*Apodemus speciosus*) and 21 voles (*Myodes rufocanus*) from the city park were examined for intestinal parasites. To examine the adult stage, the metacercariae were perorally administrated to mice, together with anti-inflammatory treatment with methylprednisolone. Fully matured adult worms were recovered from the intestinal ileum 8 and 14 days postinfection. The gravid adults showed typical features of the genus *Brachylaima*. A morphological and biogeographical evaluation prompted us to propose *Brachylaima ezohelicis* sp. nov. for the parasite from *E. gainesi*. The autochthony of the first intermediate host and the spatial heterogeneity of mtDNA suggest that the new species found in the city park is not a recently expanded population of immigrant origin.
© 2017 Elsevier B.V. All rights reserved.

1. Introduction

Land snails are terrestrial gastropod mollusks with or without shells, and the majority are hermaphroditic pulmonates. They have been highly diversified in the Japanese Archipelago, and the resulting fauna now includes approximately 800 species in spite of its small land area [Biodiversity Center of Japan (biodic.go.jp)]. Many of them are endemic species to Japan. Such a unique situation provides advantages to study evolutionary ecology in a geographic context. However, there have been few studies done concerning host-parasite ecological relationships between Japanese land snails and their internal organisms.

As concerns trematode parasites, members of the families Brachylaimidae [1] and Dicrocoeliidae [2] exclusively use land snails as the first intermediate host for asexual proliferation of cercarial larvae, which occurs within sporocysts grown in the hepatopancreas [3]. Land snails are also required as intermediate hosts in maintaining the life cycle of the Leucochloridiidae [4]. Especially in brachylaimid species, another land snail individual is further needed as the second intermediate host. That is, cercariae released from an infected snail invade another snail where they metamorphose into metacercariae in the kidney or the pericardial cavity [3]. Birds and mammals become definitive hosts by feeding on the second intermediate hosts. Exceptionally, amphibians and reptiles can serve as definitive hosts for a few brachylaimid species belonging to the subfamily Zeylanurotrematinae [5]. Ingested metacercariae develop into adult worms in the alimentary canal of birds and mammals, and resulting fecal eggs are fed on by snails [3]. Brachylaimids are thus strictly linked to land snails under ecological and evolutionary forces.

The family Brachylaimidae is divided into 3 or 4 subfamilies [1,5,6]. The subfamily Brachylaminae is the largest group, including the genera *Brachylaima*, *Glaphyrostomum*, *Postharmostomum*, *Ectosiphonus*, *Parabrachylaima*, and *Renylaima* [1,6]. Several species of this subfamily have been recorded from birds and mammals in Japan. They are *Brachylaima syrmatici* from the copper pheasant [7], *Brachylaima eophonae* from the Japanese grosbeak [8], *Brachylaima ishigakiense*

* Corresponding author.
E-mail address: nakao@asahikawa-med.ac.jp (M. Nakao).

http://dx.doi.org/10.1016/j.parint.2017.01.015
1383-5769/© 2017 Elsevier B.V. All rights reserved.

from the black rat [9], *Glaphyrostomum soricis* from the long-clawed shrew [10], *Postharmostomum gallinum* from the domestic fowl [11], and *Brachylaima tokudai* and *Ectosiphonus orientalis* from the Japanese shrew mole [12,13]. Records of undetermined brachylaimids are as follows: *Brachylaima* spp. from the large Japanese field mouse [14], the brown rat [15], the domestic dog [16], and the raccoon (an alien introduced into Japan) [17] and *Ectosiphonus* sp. from the long-clawed shrew [10]. Moreover, *Panopistus japonicus* of another subfamily Panopistinae has been found again from the Japanese shrew mole [13]. Nevertheless, there are only a few early reports on detection of larval brachylaimids from land snails in Japan [18,19].

In this study, a small number of immature brachylaimids were found from intestines of Japanese toads (*Bufo japonicus formosus*) captured in a forest city park in Asahikawa, Hokkaido, the northernmost island of Japan. The infection seems to be accidental because the gravid adult worms were never seen in the other toad samples. The toad is an alien species from the Kanto region of Honshu island where there are no records of brachylaimid infections in amphibians [20]. A subsequent snail survey in the city park showed that land snails of *Ezohelix gainesi* (Bradybaenidae) are heavily infected with both sporocysts and metacercariae. A DNA sequence identification revealed that all the immature parasites detected from the toads and the snails belong to the same species. Fully matured adults were obtained through an experimental infection of immunosuppressed mice with metacercariae from *E. gainesi*. Based on morphological features of the adult specimens, we propose a new species of the genus *Brachylaima*. In this study, an additional description of *Brachylaima* sp., which has already been reported from the large Japanese field mouse *Apodemus specious* in Hokkaido [14], was also made for comparison with the new species.

2. Materials and methods

2.1. Field surveys

Field surveys were mostly conducted at Kaguraoka Park (43°44′52″ N, 142°22′03″E), a forest city park located in the uptown of Asahikawa, Hokkaido. The park consists of a riverside (artificial zone) and a hillside (natural forest zone), surrounded by residential housing. Its total land area is 40.99 ha.

An amphibian survey was carried out to examine the parasitic infections of *B. japonicus formosus* in September 2015 and May 2016. The captured toads were subjected to necropsy to detect parasites from internal organs. A dissection of the organs was done in Dulbecco's phosphate-buffered saline (PBS) under a binocular microscope.

The discovery of immature brachylaimids from the toads prompted a subsequent snail survey to confirm larval brachylaimids from land snails. A preliminary collection of land snails showed that *Hemipoma hakodadiense* (Helicinidae), *Succinea lauta* (Succineidae), *Cochlicopa lubrica* (Cochlicopidae), *Discus pauper* (Discidae), *Zonitoides arboreus* (Gastrodontidae), and *Acusta despecta sieboldiana* (Bradybaenidae) are thinly distributed in the riverside of the city park, but a large population of *E. gainesi* inhabits the hillside section. The snail survey, therefore, focused on *E. gainesi*. The snails were collected during July to August of 2016. Shell dimensions (height and width) were measured prior to dissection. After removing shells, each snail was dissected in PBS, focusing mainly on the hepatopancreas and the kidney. When metacercariae were found, the number of individuals were counted. To compare the prevalence of brachylaimids, *E. gainesi* was further collected from the suburbs of Asahikawa, namely Asahiyama Park (43°45′56″N, 142°28′ 40″E) and Arashiyama Park (43°47′36″N, 142°18′09″E). Linear distances from the city park to Asahiyama and Arashiyama are 9.3 km and 6.6 km, respectively.

Definitive hosts of brachylaimids were examined through a rodent survey in August of 2016. Rodents were captured by Sherman box traps baited with plain oatmeal (Quaker Oats Company) or small pieces of fish sausage (Nissui Company). During 5 consecutive nights, a total of 424 traps were set in the underbrush. Intestines of captured rodents were opened in PBS to examine parasites.

Most of the parasites collected from the field surveys were used for morphological observations and experimental infections. The remaining parasites were kept in 70% ethanol for later DNA analyses.

2.2. Experimental infections of mice and rats

To obtain adult worms of the unknown species, 20 to 30 metacercariae were perorally administrated to each of 7 female ICR mice. Simultaneously, 0.2 ml Depo-Medrol® (Pfizer) (corresponding to 8 mg methylprednisolone acetate) was subcutaneously injected into each of 6 mice to prevent inflammatory reactions. After 8, 14, and 21 days postinfection, two mice each were killed with ethyl ether to recover adult worms from the intestine. One mouse without methylprednisolone treatment was killed 8 days postinfection. A formalin-ether sedimentation technique [21] was used to confirm parasite egg outputs into feces.

Wild rodents of the genus *Rattus* serve as definitive hosts for many species of *Brachylaima*. Laboratory rats were used for experimental infections to check for their susceptibility to the unknown species. Three young female SD rats (6-week-old) were similarly infected with 20 to 30 metacercariae. One of the rats was simultaneously treated with subcutaneous injection of 0.3 ml Depo-Medrol®. The immunosuppressed rat and the others were killed 11 and 14 days postinfection, respectively.

2.3. DNA analyses

Templates of PCR (polymerase chain reaction) were simply prepared without purification of genomic DNA. Using a PCR thermal cycler, the small piece (approximately 1 mm^3) of sporocysts or adults was lysed in 25 μl of 0.02 N NaOH at 99 °C for 30 min. The whole body was lysed in the case of metacercariae used. One μl of each of the crude lysates was used as a template for PCR.

Both nuclear 28S ribosomal DNA (rDNA) and mitochondrial cytochrome *c* oxidase subunit 1 (*cox1*) were selected as target genes. The primer sets used are as follows: digl2 and 1500R for 28S rDNA [22] and JB3 and CO1-R trema for *cox1* [23]. A Tks Gflex DNA polymerase (TaKaRa) and the manufacturer-supplied reaction buffer, which are standardized for crude templates, were used for PCR. The PCR was run in 25 μl reaction volume including 0.25 μM each primer for 35 cycles (98 °C for 10 s, 50 °C for 20 s, and 68 °C for 90 s). When the extra bands appeared, the annealing temperature was set to 55 °C. The amplicons were read using BigDye terminator cycle sequencing kit and ABI genetic analyzer 3500 (Applied Biosystems). Each of the PCR primers was used as a sequencing primer. The 28S rDNA and *cox1* sequences determined were 1268 and 786 bases in length, respectively. A molecular identification of parasites was done through a similarity search for the sequences by the BLAST algorithm (ddbj.nig.ac.jp/blast).

Nucleotide data sets of 28S rDNA and *cox1* were prepared using the multiple aligner MAFFT [24]. Sequences of related taxa were retrieved from DDBJ/EMBL/GenBank databases. All gaps of the 28S rDNA alignment were removed. Phylogenetic trees were made by the neighbor-joining (NJ) method using the genetic software MEGA6 [25]. Population genetics indices were calculated using Arlequin 3.5 [26], and a network figure of *cox1* haplotypes was illustrated by TCS1.21 [27].

2.4. Morphological observations

A calibrated optical microscope with a digital camera (Axio Imager, Zeiss) was used for morphological observation. Sizes of objects were measured via their digital images using the accessory software (AxioVision).

Histological sections of infected snails were prepared to observe the internal structure of affected organs. The hepatopancreas and the kidney were kept in 10% neutral-buffered formalin, and later embedded

Download English Version:

https://daneshyari.com/en/article/5674275

Download Persian Version:

https://daneshyari.com/article/5674275

Daneshyari.com