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New records of an alien digenean *Glypthelmins quieta* (Plagiorchiidae) infecting the American bullfrog, *Lithobates catesbeianus*, in western Japan

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Abstract. An alien plagiorchiid digenean, *Glypthelmins quieta* (Stafford, 1900), is newly reported from the American bullfrog, *Lithobates catesbeianus* (Shaw, 1802), in Hiroshima Prefecture (western Honshū) and Saga Prefecture (northern Kyūshū), western Japan. In Japan, this digenean has been found previously only from Saitama, Ibaraki, and Chiba prefectures, central Honshū. Thus, the present collections represent its new prefectural records and extend its geographical distribution westward to northern Kyūshū. The digenean may have a similar distribution pattern as its first intermediate gastropod host in central-western to southern Japan.

Key words: *Glypthelmins quieta,* Digenea, *Lithobates catesbeianus*, American bullfrog, new prefectural records, alien species, Japan.

Introduction

The non-native American bullfrog, *Lithobates catesbeianus* (Shaw, 1802) (Ranidae), is presently distributed throughout Japan (Hasegawa, 1999; Ota, 2002). Its first introduction to Japan was made in 1918 from New Orleans, the U.S.A. to Tōkyō, and then bullfrogs were transported for culture to and also released into many sites in Japan (Hasegawa, 1999). In addition to some domestic parasites (see Uchida, 1975, 1976; Hasegawa and Asakawa, 2004), three species of helminth parasites of North American origin, *i.e.*, *Glypthelmins quieta* (Stafford, 1900) (Digenea: Plagiorchiidae), *Magalodiscus temperatus* (Stafford, 1900) (Digenea: Paramphistomidae), and *Falcaustra catesbeianae* Walton, 1926 (Nematoda: Kathlaniidae), have been reported from Japan (Hasegawa, 2006; Hasegawa *et al.*, 2013). The latter three parasites have been found so far only in the Kantō District, including three prefectures (Saitama, Ibaraki, and Chiba), central Japan (Hasegawa, 2006; Hasegawa *et al.*, 2013). When we conducted a parasitological survey of freshwater fishes in Hiroshima Prefecture (western Honshū) and Saga Prefecture (northern Kyūshū), western Japan, in the late winter and early spring of 2014 and 2015, we incidentally found three freshly-dead American bullfrogs, from which specimens of *G. quieta* were collected. In this paper, we report these collections as new records of this digenean from western Japan.

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Materials and Methods

Three individuals of *L. catesbeianus* were found dead at two sites in western Japan: two in a reservoir called Okuda-Ōike (34°24′21.2″N, 132°43′43.1″E), Higashi-Hiroshima City, Hiroshima Prefecture each on 2 February and 2 March 2014; and one in an irrigation canal of the Ushitsu River (33°15′45.1″N, 130°10′27.7″E), Taku City, Saga Prefecture on 30 March 2015. These frogs were brought on ice to the

laboratory of Hiroshima University, and examined for metazoan parasites. Live trematodes were picked up using forceps under the dissecting microscope: some specimens were fixed in hot 70% ethanol, while others were flattened under slightly coverslip pressure and fixed in AFA (acetic acid-formalin-alcohol). One of the ethanol-fixed specimens was dehydrated through a graded ethanol series, critical point dried, coated with gold, and examined in a scanning electron microscope (SEM). AFA-fixed, flattened specimens were stained in Heidenhain's iron hematoxylin, dehydrated through a graded ethanol series, cleared in xylene, and mounted permanently in Canada balsam. Drawings were made with the aid of a drawing tube fitted on a light microscope. Measurements, in micrometers, are expressed as the range. Prevalence and intensity of infection are as defined by Bush *et al.* (1997). Permanent preparations are deposited in the Platyhelminthes collection of the National Museum of Nature and Science, Tsukuba City, Ibaraki Prefecture, Japan (NSMT-Pl).

Glypthelmins quieta (Stafford, 1900)

[New Japanese name: Ushi-gaeru shakō kyūchū]

(Fig. 1)

Material examined. Ten stained specimens (NSMT-Pl 6174–6175) from the two sites were used for measurements and observations of the internal anatomy, and one specimen from Hiroshima Prefecture was used for the SEM observation.

Description. Body (Fig. 1A) elongate, length 2768–3764, width in mid-body 921–1246. Body surface covered scales (Fig. 1E) developed as serrated. Oral sucker subterminal, rounded, 250–343×298–492. Prepharynx particularly short; pharynx 118–170×156–292 connected developed pharyngeal glands each anterior side; esophagus length 90–298, width 114–233; intestinal bifurcation anterior of cirrus pouch; intestinal caeca extends 70–77 % of body length. Ventral sucker (Fig. 1F) 174–235×121–202, located posterior of cirrus pouch, 30–35% of body length with papillae. Oral sucker: pharynx: ventral sucker ratio, length 1: 0.4–0.6: 0.6–0.8, width 1: 0.5–0.7: 0.4–0.6. Pair of testes subspherical, slightly oblique; anterior testis dextral, 297–400×230–353; posterior testis sinistral, 292–426×270–373. Cirrus pouch (Fig. 1B) including seminal vesicle, prostatic cells, ejection duct, cirrus anterodorsal to ventral sucker, 280–411×147–198. Ovary round to ovate, 251–357×161–325, anterior of left testis. Reproductive system shown in Fig. 1C. Oviduct arising from lower right side of ovary, connecting seminal receptacle, receiving vitelline reservoir, and entering Mehlis' gland. Seminal receptacle posterior left to ventral sucker, subspherical, 61–179×82–200. Laurer's canal present, opening dorsally. Uterus occupying hindbody in inter-intestinal region. Eggs (Fig. 1D) ovate, 41–49×21–25. Vitelline follicles developed each side, top 25–36% of body length, end 65–75% of body length.

Site of infection. Small intestine.

Prevalence and intensity. All of the three American bullfrogs examined were found infected by a total of 27 individuals of *G. quieta* (intensity: 6–12, mean: 9).

Remarks. The morphology of the specimens collected in this study corresponds to the description of the genus *Glypthelmins* emended by Razo-Mendivil *et al.* (2006). This genus currently contains 10 species (Razo-Mendivil *et al.*, 2006; Razo-Mendivil and Pérez-Ponce de León, 2008). The presence of large pharyngeal glands is one of the major characteristics to distinguish *G. quieta* from its congeners (Stafford, 1900; O'Grady, 1987; Razo-Mendivil and Pérez-Ponce de León, 2008; Hasegawa *et al.*, 2013).

Glypthelmins quieta is a common parasite of anurans in North America (O'Grady, 1987; Razo-Mendivil *et al.*, 1999). Currently, Hasegawa *et al.* (2013) suggested that this digenean was co-introduced with

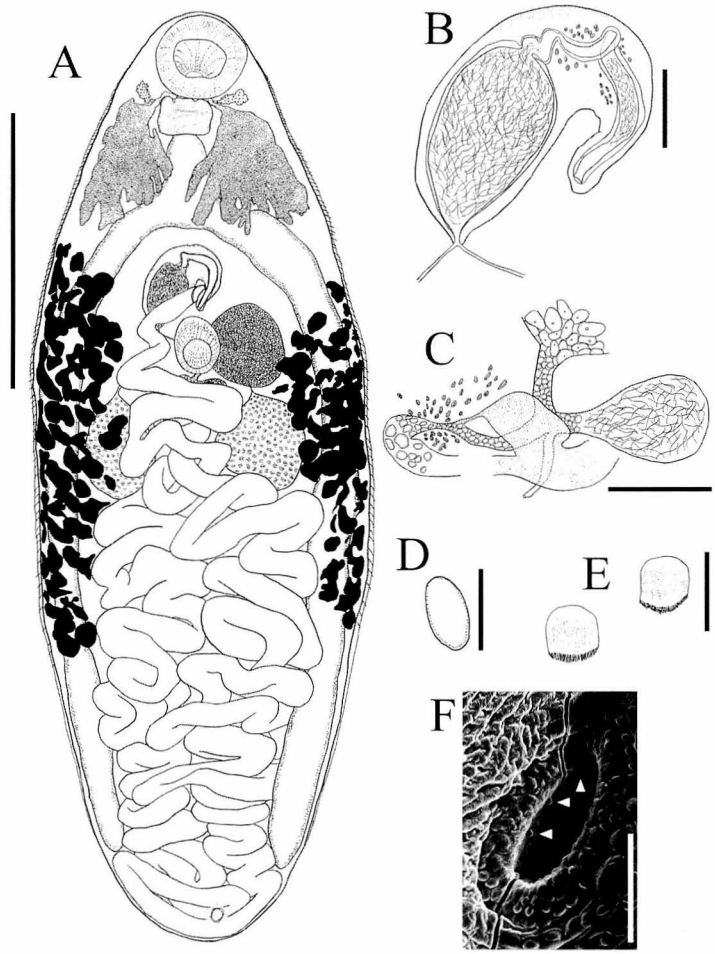

Fig.1 . *Glypthelmins quieta* (Stafford, 1900), ventral view, NSMT-Pl 6174. A, whole mount; B, terminal genitalia; C, reproductive system; D, egg; E, scales; F, scanning electron microscope photo of ventral sucker. Scale bars: 1 mm in A; 100 μm in B, C; 10 μm in D, E; 20 μm in F.

American bullfrogs to Japan, where it occurs in three prefectures (Saitama, Ibaraki, and Chiba), central Honshū. The present collections represent its new prefectural records in Japan and extend its geographical distribution westward to northern Kyūshū.

Discussion

While only *G. quieta* was found from the American bullfrogs examined in this study, some other helminths, such as cestodes, nematodes, and acanthocephalans as well as digeneans, have been reported from this host species in other localities of Japan (Hasegawa, 2006; Hasegawa *et al.*, 2013; Takaki *et al.*, 2013; see Uchida, 1975, 1976; Hasegawa and Asakawa, 2004 for the earlier literature). The examination of more American bullfrogs from Hiroshima and Saga prefectures will find out various helminth parasites. Moreover, because the geographical distribution in Japan of alien parasites co-introduced with American bullfrogs is still poorly known, it is desirable to examine individuals of the species from a wide area of Japan.

Glypthelmins quieta is known to infect anurans of the families Bufonidae, Hylidae, and Ranidae in its native habitats (North, Central, and South America) (Razo-Mendivil and Pérez-Ponce de León, 2008). In Japan, however, this digenean has been observed only from American bullfrogs (Hasegawa *et al.*, 2013). Although about 100 years have passed since 1918 when American bullfrogs were introduced into Japan, *G. quieta* has never been reported from any anuran in Japan (see Uchida, 1975, 1976). In contrast, Japanese bufonids and ranids are known to harbor a native plagiorchiid digenean, *Rauschiella rugocaudata* (Yoshida, 1916) (see Uchida, 1975, 1976), but this digenean has never been reported from American bullfrogs in Japan. It appears that *G. quieta* and *R. rugocaudata* are specific each to American bullfrogs and Japanese frogs in Japan regardless of their wide host ranges in their native countries, and more work is necessary to clarify the host utilization of these digeneans.

The life cycle of *G. quieta* was reported by Miller (1935), Leigh (1937), and Rankin (1944): the freshwater physid gastropod of *Physella* (as *Physa*) serves as the first intermediate host, and shedding cercariae penetrate the skin of amphibians as the second intermediate hosts and become metacercariae; infected frogs are then shed and feed on their own skin with the metacercariae, and ingested metacercariae become mature in the frog's intestine. As shown by Hasegawa *et al.* (2013) and in this study, the prevalences of *G. quieta* in American bullfrogs caught in central and western Japan are quite high, ranging from 71–100%. This may be caused by the availability of both intermediate and final hosts of this digenean. In Japan, *Physella acuta* (Draparnaud, 1805) (=*Physa acuta*), a possible first intermediate gastropod host in Japan, is commonly distributed in central-western to southern Japan, ranging from central Honshū in the north to the Ryūkyū Islands in the south (Anonymous, 2010), and American bullfrogs to serve as the possible second intermediate as well as the final hosts also have a wide distribution. It is thus likely that the digenean occurs in central-western to southern Japan as its possible first intermediate gastropod host.

Takaki *et al.* (2013) did not find any specimen of *G. quieta* in the American bullfrogs caught around Lake Ōnuma, Hokkaidō, northern Japan: this is most likely attributed to the fact that *P. acuta* occurs only in limited locations of northern Japan (Anonymous, 2010). The optimal water temperature of *P. acuta* is between 20° and 30°C (Brackenbury and Appleton, 1991; Seeland *et al.*, 2013), and increased water temperature under global climate change has been suggested to give a great advantage for the adaptation of the species (Seeland *et al.*, 2013). Therefore, following a future northward extension of the distributional range of *P. acuta* affected by predicted climate change, *G. quieta* may invade northern Japan.

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References

Anonymous, 2010. Physidae *Physa acuta*. In: Biodiversity Center of Japan, (Ed), *The National Survey on the Natural Environment Distributional Survey of Japanese Animals. Animal Distribution Atlas of Japan*: 806. Nature Conservation Bureau, Ministry of the Environment, Fuji-Yoshida. (in Japanese)

Brackenbury, T. D. & Appleton, C. C., 1991. Effect of controlled temperatures on gametogenesis in the gastropods *Physa acuta* (Physidae) and *Bulinus tropicus* (Planorbidae). *J. Moll. Stud.*, **57**:

461–469.

Bush, A. O., Lafferty, K. D., Lotz, J. M. & Shostak, A. W., 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. *J. Parasitol.*, **83**: 575–583.

Hasegawa, H., 2006. First record of *Falcaustra catesbeianae* Walton, 1929 (Nematoda, Cosmocercoidea, Kathlaniidae) from the bullfrog, *Rana catesbeiana*, in Japan. *Biogeography* **8**: 1–5.

Hasegawa, H. & Asakawa, M., 2004. Parasitic nematodes recorded from amphibians and reptiles in Japan. *Curr. Hepetol.*, **23**: 27–35.

Hasegawa, H., Sato, A. Kai, M. & Uchida, A., 2013. Helminth parasites of bullfrogs, *Lithobates catesbeianus* (Shaw, 1802), in Kanto District, Japan, with special reference to those introduced from North America. *Jpn. J. Vet. Parasitol.*, **12**: 1–10.

Hasegawa, M., 1999. [Untold History of American bullfrog]. *In*: Ozaki, K. & Hasegawa, M. (Eds), *Kaeru no Kimochi*: 100–107. Natural History Museum and Institute, Chiba, Chiba. (in Japanese)

Leigh, W. H., 1937. The life cycle of a trematode of frogs. *Science*, **86**: 423.

Miller, E. L., 1935. Studies on North American cercariae. *Ill. Biol. Monogr.* **33**: 1–125.

O'Grady, R. T., 1987. *Phylogenetic Systematics and the Evolutionary History of Some Intestinal Flatworm Parasites (Trematoda: Digenea: Plagiorchioidea) of Anurans*. Ph.D. dissertation, University of British Columbia, Vancouver, British Columbia.

Ota, H., 2002. *Rana catesbeiana*. *In*: The Ecological Society of Japan (Ed), *Handbook of Alien Species in Japan*: 106. Chijin Shokan, Tokyo. (in Japanese)

Rankin, J. S. Jr., 1944. A review of the trematode genus *Glypthelmins* Stafford, 1905, with an account of the life cycle of *G. quieta* (Stafford, 1900) Stafford, 1950. *Trans. Amer. Micr. Soc.*, **63**: 30–43.

Razo-Mendivil, U. J., & Pérez-Ponce de León, G., 2008. Taxonomic revision of the genus *Glypthelmins* Stafford, 1905 (Platyhelminthes: Digenea: Plagiorchiida), parasites of anurans in the Americas. *Zootaxa*, **1882**: 1–45.

Razo-Mendivil, U. J., Laclette, J. P. & Pérez-Ponce de León, G., 1999. New host and locality records for three species of *Glypthelmins* (Digenea: Macroderoididae) in anurans of Mexico. *J. Helminthol. Soc. Wash.*, **66**: 197–201.

Razo-Mendivil, U. J., León-Règagnon, V. & Pérez-Ponce de León, G., 2006. Monophyly and systematic position of *Glypthelmins* (Digenea), based on partial lsrDNA sequences and morphological evidence. *Org. Divers. Evol.*, **6**: 308–320.

Seeland, A., Albrand, J., Oehlmann, J. & Müller, R., 2013. Life stage-specific effects of the fungicide pyrimethanil and temperature on the snail *Physella acuta* Draparnaud, 1805) disclose the pitfalls for the aquatic risk assessment under global climate change. *Environ. Pollut.*, **174**: 1–9.

Stafford, J., 1900. Some undescribed trematodes, *Zool. Jahrb., Syst.*, **13**: 399–414.

Takaki, Y., Sarashina, M., Yoshida, T. & Asakawa, M. 2013. Preliminary report on parasitic helminthes from bull frogs (*Lithobates catesbeianus*) introduced to Hokkaido, Japan. *Bull. Biogeogr. Soc. Japan*, **68**: 99–101. (in Japanese with English abstract)

Uchida, A., 1975. Check list of the helminth parasites of Japanese amphibians. *Bull. Azabu Vet. Coll.*, **30**: 63–81. (in Japanese with English abstract)

Uchida, A., 1976. Check list of the helminth parasites of Japanese amphibians (supplement). *Bull. Azabu Vet. Coll.*, **31**: 23–27. (in Japanese with English abstract)

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