Herpetology Notes, Volume 18: 1159–1162 (published online on 12 December 2025)

New records of non-native digeneans (*Haematoloechus floedae* and *Glypthelmins quieta*) in invasive American Bullfrogs, *Aquarana catesbeiana* (Shaw, 1802), in Japan

Karin Tsuchida[1,2,*], Junya Nakaichi[3], Misako Urabe[2], and Kazuyoshi Nakata[3]

Parasites are one of the keys to the increasing number of biological invasions (Prenter et al., 2004). They have direct effects on ecosystems, for instance, positive or negative impacts on their hosts, responses in altering the food web, and changes in the physical and chemical properties (Hatcher et al., 2012). Parasites also have indirect effects on ecosystems, which include not only density-mediated effects (caused by reduction in host reproduction and survival) but also trait-mediated indirect effects (caused by changes in host phenotype, behaviour or life history) (Dunn et al., 2012). Thus, parasites are thought to play an important role in invasion success, and parasitological surveys allow us to understand the impact of biological invasions.

The American Bullfrog, *Aquarana catesbeiana* (Shaw, 1802), is one of the most widespread invasive anurans in the world today, originating from North America. In the Japanese archipelago, they were imported from the U.S.A. in the early 1900s and have since then invaded almost every island (Yanai, 2003).

In previous studies, 15 species of digenean parasites have been recorded from American Bullfrogs invading Japan, eight of which were reported as adults (Uchida et al., 2022). Two species, *Glypthelmins quieta* (Stafford, 1900) (Glypthelminthidae) and *Megalodiscus temperatus* (Stafford, 1905) (Cladorchiidae), have been reported as introduced digenean species from North America (Hasegawa et al., 2013). Ansai et al. (2024) suggested that *G. quieta* was able to establish its population in Japan from the 1970s to the 2000s and continued to use the Acute Bladder Snail, *Physella acuta* (Draparnaud, 1805), and American Bullfrogs as its hosts in the new regions. Although *M. temperatu*s has a two-host life cycle in which planorbid snails serve as its intermediate hosts in North America (Krull and Price, 1932; Smith, 1967), it is unclear whether these original intermediate hosts have been introduced into Japan. These non-native digeneans have only been reported from the Kanto District, Hiroshima, and Saga prefectures in Japan (Hasegawa et al., 2013; Nitta and Nagasawa, 2015). However, since American Bullfrogs have already established a wide range in the Japanese archipelago, they could introduce additional non-native parasite species and adversely affect native communities through parasite mediation.

Here, we report two digenean species from the internal organs of American Bullfrogs from Okayama City, Western Japan. These were recovered during the examination of the stomach contents to help to understand the invasion impact of American Bullfrogs. We also provide the DNA sequences of the digenean specimens examined.

We examined the stomach contents of seven dead American Bullfrogs (snout-vent length mean ± SD: 113.84 ± 21.33 mm) collected from a pond at the Okayama City Handayama Botanical Garden of Okayama Prefecture, Japan (34.6919°N, 133.9303°E, elevation 12 m), and collected flukes from their internal organs in August 2024. We fixed flukes in 90% ethanol, and they were refixed in the alcohol formalin glacial acetic fixative (AFA), stained with alum carmine, dehydrated in ethanol series (70%, 90%, and 100%), cleared in creosote, replaced in xylene, and mounted in Canada balsam for morphological identification. We have deposited the examined specimens in the Zoological

---

[1] Sustainable Design for the Global Environmental Infrastructure (Ueda Memorial Foundation), Graduate School of Global Environmental Studies, Kyoto University, Kyoto, Sakyo, Kyoto 606-8501, Japan.

[2] Faculty of Environmental Sciences, The University of Shiga Prefecture, Hikone, Shiga 522-8533, Japan.

[3] Graduate School of Environmental Life, Natural Science and Technology, Okayama University, Kita-ku, Okayama 700-8530, Japan.

[*] Corresponding author. E-mail: tsuchida.karin.2d@kyoto-u.ac.jp

Collection of Kyoto University (KUZ) (catalog numbers KUZ Z6276 – Z6277: paragenophore of LC878069 and PV769064; KUZ Z6278: hologenophore of LC878068 and PV769063).

Genomic DNA was extracted from the tissues of the flukes using the alkaline lysis method (Bimboim and Doly, 1979). We amplified the partial region of the nuclear 28S ribosomal DNA and the mitochondrial cytochrome c oxidase subunit I (*cox1*) gene from trematode specimens using a polymerase chain reaction (PCR). For the 28S rDNA fragment, the primer set LSU-5 (5′-TAGGTCGACCCGCTGAAYTTAAGC-3′) and 1500R (5′-GCTATCCTGAGGGAAACTTCG-3′) was used (Tkach et al., 2003), and the primer set JB3 (5′-TTTTTTGGGCATCCTGAGGTTTAT-3′) and COI R-Trema (5′-CAACAAATCATGATGCAAAAGG-3′) was used for *cox1* (Bowles et al., 1995; Miura et al., 2005). PCR and purification protocols were performed according to Tsuchida et al. (2024) and sequenced directly (DNA sequencing service of FASMAC Co., Ltd., Kanagawa, Japan). We have deposited the obtained sequences in the DNA Data Bank of Japan (DDBJ) (accession numbers 28S: LC878068–LC878069, *cox1*: PV769063–PV769064). The obtained sequences were confirmed for similarity with registered sequences in GenBank by BLAST search and for genetic deviation with related species by *p*-distance of MEGA 7 (Kumar et al., 2016) as necessary.

Adults of two plagiorchioid species, *Glypthelmins quieta* and *Haematoloechus floedae* Harwood, 1932 (Haematoloechidae), were recovered from the small intestine and lung, respectively, of the examined American Bullfrogs (Fig. 1). The frogs had no symptoms. *Glypthelmins quieta* was identified morphologically based on the presence of large pharyngeal glands, symmetrical testes, and the absence of tegument spines (Razo-Mendivil and Pérez-Ponce de León, 2008). And *H. floedae* was morphologically identified by the absence of a tegument spine, unlobed testes, longer uterine longitudinal loops, and the presence of numerous small vitelline follicles, which are identical to the description by León-Règagnon and Brooks (2003) and León-Règagnon et al. (2005). *Haematoloechus floedae* differs from its congeners recorded in Japan, *Haematoloechus nanchangensis major* (Yamaguti, 1936) and *H. japonicus* (Yamaguti, 1936), in the absence of tegument spines. This species was distinguished from *H. lobatus* (Seno, 1907) by the more anterior uterine longitudinal loops. Five out of seven frogs were infected with *G. quieta* (prevalence = 0.71) and the mean intensity was 6.6 (range 1–15). One out of seven frogs was infected with *H. floedae* (prevalence = 0.14; intensity = 2).

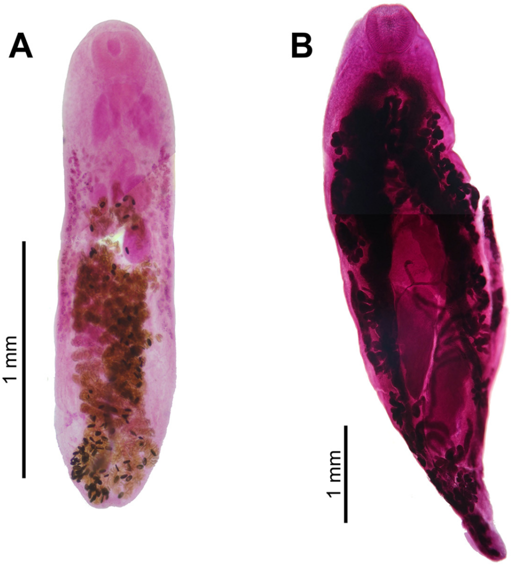

Figure 1. Photomicrographs of digeneans recovered from American Bullfrogs. (A) *Glypthelmins quieta* (KUZ Z6277) parasitic in the intestine. (B) *Haematoloechus floedae* (KUZ Z6278) parasitic in the lung. Photos by Karin Tsuchida.

The obtained sequences of *G. quieta* (28S rDNA: 1286 bp; *cox1*: 777 bp) were 100% identical to those of *G. quieta* registered by Ansai et al. (2024) (LC794204 and LC794206). A total of 1260 bp of 28S sequence was obtained from *H. floedae*, which was 100% identical to the sequence of *H. floedae* recovered from *A. catesbeiana* in the U.S.A. (AF387800; as *H. breviplexus* in Snyder and Tkach, 2001, and revised as *H. floedae* in León-Règagnon and Brooks, 2003). It differed from those of *H. lobatus* (AB818362; Hasegawa et al., 2013) and *H. japonicus* (AB818366; as *H. sibiricus* in Hasegawa et al., 2013) by 0.8% and 7.6% as *p*-distance, respectively. The *cox1* sequence of *H. floedae* (792 bp) was 100% identical to that of *H. floedae* from the U.S.A. (AY672119; León-Règagnon et al., 2005), which differed from those of *H. lobatus* (AB818359; Hasegawa et al., 2013) and *H. japonicus* (AB818363; as *H. sibiricus* in Hasegawa et al., 2013) by 8.3% and 18.9% (*p*-distance), respectively.

These results present an additional record of *Glypthelmins quieta* and the first record of *Haematoloechus floedae* in Japan (Fig. 2). This indicates that *H. floedae* is one of the invasive species

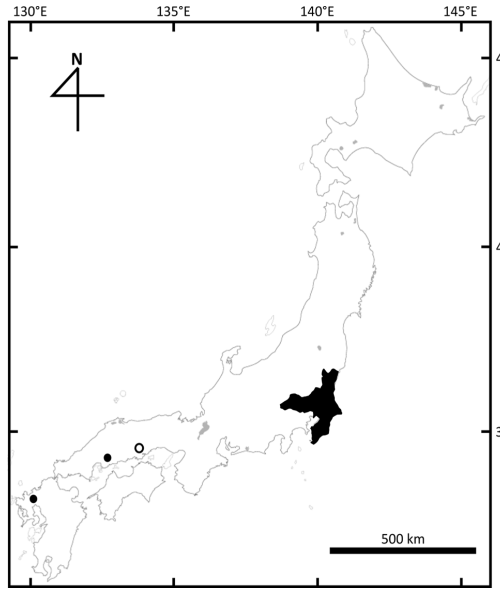

Figure 2. Map of sites where adult *Glypthelmins quieta* and *Haematoloechus floedae* were recorded in the Japanese archipelago. Filled circles and areas indicate previous records of *G. quieta* (Hasegawa et al., 2013; Nitta and Nagasawa, 2015), and the open circle is the reported site of both species in this study.

utilizing American Bullfrogs in Japan, besides the previously recorded *G. quieta*, *M. temperatus*, and *Falcaustra catesbeianae* Walton, 1926 (Nematoda: Kathlaniidae) (Hasegawa, 2006; Hasegawa et al., 2013; Nitta and Nagasawa, 2015). What stage the introduced *H. floedae* had when these arrived into Japan is unclear. León-Règagnon et al. (2005) indicated that its host, the American Bullfrog, was transmitting *H. floedae* to new region of the United States, as well as to Mexico and Costa Rica. In addition, Brooks et al. (2006) estimated that *H. floedae* could complete its life cycle in the presence of any lymnaeoid pond snail and anisopteran dragonfly species. Thus, the invasive American Bullfrogs may play a role in transporting adult *H. floedae* from its native or invaded ranges to Japan, allowing *H. floedae* to establish its population there. We note that we found *P. acuta* and adult odonates whilst examining the stomach contents of American Bullfrogs at the study site (Nakaichi et al., unpublished). The presence of these intermediate hosts likely allowed *G. quieta* and *H. floedae* to establish a population at the study site.

Within the non-native range of American Bullfrogs, their invasive parasites often negatively impact the native amphibian communities. For instance, *Batrachochytrium dendrobatidis* (*Bd*) has been transported by American Bullfrogs to new regions and significantly increases the mortality of native amphibians (Peterson and McKenzie, 2014; Miaud et al., 2016). In addition, *Bd* also has indirect effects on native amphibians, such as reducing foraging efficiency (Venesky et al., 2009). *Glypthelmins quieta* and *H. floedae* appear to use American Bullfrogs as their main definitive hosts in Japan and so far we have not observed any direct effects of these parasites on native species and ecosystem. However, continued monitoring of these introduced parasites is critical to assess the impact of American Bullfrogs on native ecosystems in the Japanese archipelago.

Acknowledgments. We thank Ms. T. Okada and all members of Okayama City Handayama Botanical Garden for obtaining hosts.

References

Ansai, E., Nitta, M., Saito, T., Kojima, Y., Waki, T. (2024): The first intermediate host of the invasive frog trematode *Glypthelmins quieta* in Japan. Diseases of Aquatic Organisms **159**: 9–14.

Bimboim, H.C., Doly, J. (1979): A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Research **7**: 1513–1523.

Bowles, J., Blair, D., Mcmanus, D.P. (1995): A molecular phylogeny of the human schistosomes. Molecular Phylogenetics and Evolution **4**: 103–109.

Brooks, D.R., McLennan, D.A., León-Règagnon, V., Hoberg, E. (2006): Phylogeny, ecological fitting and lung flukes: helping solve the problem of emerging infectious diseases. Revista Mexicana de Biodiversidad **77**: 225–233.

Dunn, A.M., Torchin, M.E., Hatcher, M.J., Kotanen, P.M., Blumenthal, D.M., Byers, J.E., et al. (2012): Indirect effects of parasites in invasions. Functional Ecology **26**: 1262–1274.

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., 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. Japanese Journal of Veterinary Parasitology **12**: 1–10.

Hatcher, M.J., Dick, J.T.A., Dunn, A.M. (2012): Diverse effects of parasites in ecosystems: linking interdependent processes. Frontiers in Ecology and the Environment **10**: 186–194.

Krull, W.H., Price, H.F. (1932): Studies on the life history of *Diplodiscus temperatus* Stafford from the frog. Occasional Papers of the Museum of Zoology, University of Michigan **237**: 1–41.

Kumar, S., Stecher, G., Tamura, K. (2016): MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets.

Molecular Biology and Evolution **33**: 1870–1874.

León-Règagnon, V., Brooks, D.R. (2003): Molecular phylogeny of *Haematoloechus* Looss, 1899 (Digenea: Plagiorchiidae), with emphasis on North American species. Journal of Parasitology **89**: 1206–1211.

León-Règagnon, V., Guillén-Hernández, S., Arizmendi-Espinosa, M.A. (2005): Interspecific variation of *Haematoloechus floedae* Harwood, 1932 (Digenea: Plagiorchiidae), from *Rana* spp. in North and Central America. Journal of Parasitology **91**: 915–921.

Miaud, C., Dejean, T., Savard, K., Millery-Vigues, A., Valentini, A., Gaudin, N.C.G., Garner, T.W.J. (2016): Invasive North American bullfrogs transmit lethal fungus *Batrachochytrium dendrobatidis* infections to native amphibian host species. Biological Invasions **18**: 2299–2308.

Miura, O., Kuris, A.M., Torchin, M.E., Hechinger, R.F., Dunham, E.J., Chiba, S. (2005): Molecular-genetic analyses reveal cryptic species of trematodes in the intertidal gastropod, *Batillaria cumingi* (Crosse). International Journal for Parasitology **35**: 793–801.

Nitta, M., Nagasawa, K. (2015): New records of an alien digenean *Glypthelmins quieta* (Plagiorchiidae) infecting the American bullfrog, *Lithobates catesbeianus*, in western Japan. Biogeography **17**: 37–41.

Peterson, A.C., McKenzie, V.J. (2014): Investigating differences across host species and scales to explain the distribution of the amphibian pathogen *Batrachochytrium dendrobatidis*. PLoS ONE **9**: e107441.

Prenter, J., MacNeil, C., Dick, J.T.A., Dunn, A.M. (2004): Roles of parasites in animal invasions. Trends in Ecology & Evolution **19**: 385–390.

Razo-Mendivil, U., 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.

Smith, R.J. (1967): Ancylid snails as intermediate hosts of *Megalodiscus temperatus* and other digenetic trematodes. Journal of Parasitology **53**: 287–291.

Snyder, S.D., Tkach, V.V. (2001): Phylogenetic and biogeographical relationships among some Holarctic frog long flukes (Digenea: Haematoloechidae). Journal of Parasitology **87**: 1433–1440.

Tkach, V.V., Littlewood, D.T.J., Olson, P.D., Kinsella, J.M., Swiderski, Z. (2003): Molecular phylogenetic analysis of the Microphalloidea Ward, 1901 (Trematoda: Digenea). Systematic Parasitology **56**: 1–15.

Tsuchida, K., Urabe, M., Nishikawa, K., Hoso, M., Wu, S.P. (2024): Addressing the taxonomic confusion of *Mesocoelium* Odhner, 1910 (Trematoda: Plagiorchioidea: Mesocoeliidae) in Japanese urodelan and anuran amphibians. Systematic Parasitology **101**: 11.

Uchida, A., Taira, K., Ooi, H.K. (2022): A review for parasites of amphibians in Japan (3) Digenetic trematodes. Japanese Journal of Veterinary Parasitology **21**: 1–94.

Venesky, M.D., Parris, M.J., Storfer, A. (2009): Impacts of *Batrachochytrium dendrobatidis* infection on tadpole foraging performance. EcoHealth **6**: 565–575.

Yanai, T. (2003): A study about the imported bull frog (*Rana catesbeiana*) of the year and a nationwide distribution (Natural Science). Journal of Kawaguchi Junior College **17**: 89–110. [in Japanese]

Accepted by Russell J. Gray