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The First Survey for Helminths Parasitic in Hybrid and Introduced Giant Salamanders, Genus *Andrias* (Amphibia: Caudata: Cryptobranchidae) in Kyoto, Japan

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Abstract: The first survey was conducted for helminth fauna of hybrid giant salamanders (hybrids between *Andrias japonicus* and other congeneric species), and introduced *A. davidianus* in Kyoto Prefecture, Japan. Three nematode species, *Spiroxys hanzaki*, *Amphibiocapillaria tritonispunctati* and *Falcaustra* sp., and one trematode species, *Liolope copulans*, were recovered from their alimentary canals. These results show that hybrid and introduced *Andrias* species are commonly infected with similar helminth species to those previously reported to infect *A. japonicus*. We conclude that the spillback of native parasites to introduced *A. davidianus* has occurred in Kyoto Prefecture. This study is also the first record of *Falcaustra* species parasitizing *Andrias* species in Japan.

Key words: Cryptobranchidae; 18S rDNA; Helminth; ITS1; 28S rDNA

Introduction

The Japanese giant salamander, *Andrias japonicus* (Temminck, 1836) (Amphibia: Cryptobranchidae), is endemic to the western and central Japanese Archipelago and listed as both species of special natural monument in Japan and a Near Threatened species on the IUCN Red List (Kaneko and Matsui, 2004; Yoshikawa et al., 2012; Matsui, 2014).

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A few surveys have been conducted for the helminths parasitizing *A. japonicus*. In the early 20th century, *Liolope copulans* Cohn, 1902 (Trematoda: Liolopidae) and *Filaria cingula* Linstow, 1902 (Nematoda: Micropleuridae) (now *Kamegainema cingulum* (Linstow, 1902): Hasegawa et al., 2000) were first found from *A. japonicus* transported to Europe (Cohn, 1902; Linstow, 1902). Decades later, Yamaguti (1936, 1939, 1941) reported the following species from *A. japonicus* in Kyoto City, Japan: *Diplodiscus japonicus* (Yamaguti, 1936) (Trematoda: Diplodiscidae), *Pseudoacanthocephalus lucidus* (Van

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Cleave, 1925) (Acanthocephala: Echinorhynchidae) and *Megalobatrachonema nipponicum* Yamaguti, 1941 (Nematoda: Kathlaniidae). Hasegawa et al. (1998) described *Spiroxys hanzaki* Hasegawa, Miyata & Doi, 1998 (Nematoda: Gnathostomatidae) recovered from *A. japonicus* in Hyogo Prefecture, Japan. Hasegawa et al. (2000, 2002) also reported the parasite fauna of *A. japonicus* in Osaka and Hyogo Prefectures, Japan, as follows: *L. copulans*, *S. hanzaki*, *K. cingulum*, *Amphibiocapillaria tritonispunctati* (Diesing, 1851) (Nematoda: Trichuridae), *Dioctophyme renale* (Goeze, 1782) (Nematoda: Dioctophymatidae) and Kathlaniidae gen. sp. (Nematoda). In addition, Physaloptеroidea gen. sp. (Nematoda) was recovered but was considered as a pseudoparasite that was accidentally acquired through ingesting parasitized fish (Hasegawa et al., 2002). Tanaka et al. (2016) documented similar parasite species to Hasegawa et al. (2002) in zoo-bred *A. japonicus* in Hiroshima Prefecture, Japan.

The genetic introgression of Chinese *Andrias* species into the native population of *A. japonicus* has been serious in Japan, particularly in Kyoto Prefecture (Yoshikawa, 2011). A recent molecular study concluded that several *Andrias* species occur in China, including *A. davidianus* (Blanchard, 1871) and *A. sligoi* (Boulenger, 1924) (Turvey et al., 2019). These species were introduced to Japan in 1970s, leading to the ongoing hybridization with *A. japonicus* (Fukumoto et al., 2015).

To fully evaluate the impacts of alien species, it is essential to examine whether parasites are also introduced with novel vertebrate species (e.g., Dunn et al., 2012). Introduced species can increase parasite transmission via spillover or spillback. Spillover occurs when a reservoir host species that was introduced transmits novel parasites to a native species (Hatcher et al., 2012). Alternatively, an introduced species can become a new reservoir for native parasite infection, which can increase infection in native hosts through spillback (Hatcher et al., 2012). In Kyoto Prefecture, the current parasite fauna on *Andrias* is unclear because parasitological surveys have not been conducted in the almost 80 years since Yamaguti (1936, 1939, 1941). In this study, we documented the current parasite fauna of *Andrias*, especially of introduced and hybrid individuals, the latter of which is now dominant in the rivers of Kyoto Prefecture. Based on the results, we discuss whether the introduction of Chinese *Andrias* species affected the parasite fauna of *A. japonicus* via spillover or spillback.

Materials and Methods

A total of 27 *Andrias* were euthanized by the injection or immersion in 2-phenoxyethanol (Fig. 1, Tables 1 and 2). All dissections were approved by the Culture Bureau of Kyoto City. Because each *Andrias* species is difficult to identify by morphology, all collected salamanders were analyzed genetically (Yoshikawa et al., 2012). As a result, we identified 25 "hybrids" between *A. japonicus* and Chinese *Andrias* species (species not identified) and two *A. davidianus*, which is redefined by Turvey et al. (2019) (Nishikawa, unpublished). Parasites were collected from the alimentary canal, liver, lungs and skin of each salamander. All *Andrias* specimens used for this study were deposited to the Graduate School of Human and Environmental Studies, Kyoto University (KUHE; see Appendix).

Collected nematodes were fixed in 70% ethanol, cleared in undiluted glycerin or mounted in glycerin-gelatin. Some of collected trematodes were fixed in 90% ethanol, and the other were pressed between a coverslip and glass slide, fixed in alcohol-formol-acetic fixative, mordanted in 4% ammonium iron (III) sulfate solution, stained with Heidenhain's iron hematoxylin, differentiated in 4% ammonium iron (III) sulfate solution, dehydrated in 95 and 100% ethanol series, cleared in creosote, replaced in xylene and mounted in Canada balsam. These specimens were observed using a light microscope for

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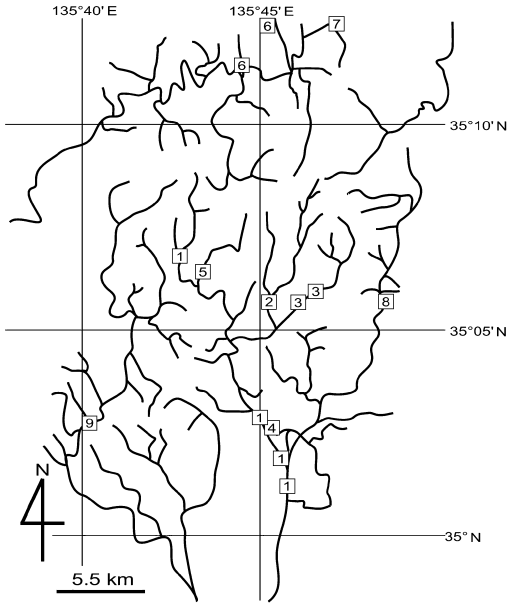


FIG. 1. Map of sampling sites for *Andrias* surveyed for internal parasites in Kyoto City (1, Kamo River; 2, Kurama River; 3, Shizuhara River; 4, Myozin River; 5, Nakatsu River; 6, Katsura River; 7, Teratani River; 8, Takano River and 9, Kiyotaki River).

morphological study. *Liolope copulans*, *Spiroxys hanzaki* and *Amphibiocapillaria tritonispunctati* were identified based on morphological description in Baba et al. (2011), Hasegawa et al. (1998) and Moravec (1982, 1986), respectively. All measurements are given in micrometre (μm) unless otherwise stated, as range followed by mean±standard deviation in parentheses. All specimens studied were deposited in the Zoological Collection of Kyoto University (catalog no. KUZ Z3908–Z3912).

Nematodes and trematodes fixed in 90% ethanol were used for genetic study. Genomic DNA was extracted from the specimens using Wizard® SV Genomic DNA Purification System (Promega Corp., Madison, WI). Polymerase chain reaction (PCR) was performed to amplify the internal transcript spacer (ITS) 1 region of *S. hanzaki*. The PCR was performed using 50 μl PCR reaction mixture containing 5 μl of 10×KOD-Plus-Neo Buffer, 5 μl of dNTPmix (2 mM), 3 μl of $MgSO_4$ (25 mM), 1 μl of KOD-Plus-Neo (TOYOBO Co., Ltd., Osaka, Japan), 1.5 μl of forward primer SSU24HF (5'-AGAGGTGAAATTCG TGGACC-3') (10 mM) and of reverse primer AB28 (5'-ATATGCTTAAGTTCAGCGGGT-3') (10 mM) (Li et al., 2014), and 33 μl of each template. The PCR process was conducted using 2720 Thermal Cycler (Applied Biosystems Inc., Waltham, MA), with thermocycling profile as follows; 30 s at 94°C, 40 cycles of 10 s at 94°C, 30 s at 50°C, 1 min at 72°C, and the final extension for 7 min at 72°C.

To amplify the partial 18S rDNA region of *Am. tritonispunctati*, PCR was performed in 20 μl PCR reaction mixture containing 13.8 μl of Milli-Q water (MQW), 2 μl of 10×Ex Taq Buffer, 1.6 μl of dNTP mixture, 0.1 μl of Ex Taq (Takara Bio Inc., Shiga, Japan), 1 μl of forward primer NSF4/18 (5'-CTGGTTGATCCTGCCAGT-3') (10 mM) and of reverse primer SSU18R (5'-TGATCCT TCYGCAGGTTCAC-3') (10 mM) (Tamaru et al., 2015), and 0.5 μl of each template. Thermocycling profile was as follows: 30 s at 94°C, 40 cycles of 10 s at 94°C, 30 s at 50°C, 1 min at 72°C, and the final extension for 7 min at 72°C.

To amplify the partial 28S rDNA region of *Falcaustra* sp., PCR was performed in 20 μl PCR reaction mixture containing 7.1 μl of MQW, 10 μl of 2×Gflex PCR Buffer, 0.4 μl of Tks Gflex DNA Polymerase (Takara Bio Inc.), 1 μl of forward primer 28S-F (5'-AGCG GAGGAAAAGAAACTAA-3') (10 mM) and of reverse primer 28S-R (5'-ATCCGTGTTTC AAGACGGG-3') (10 mM) (Nadler and Hudspeth, 1998), and 0.5 μl of each template. Thermocycling profile was as follows: 1 min at 94°C, 40 cycles of 10 s at 94°C, 15 s at 50°C, 1 min at 68°C, and the final extension for 7 min at 68°C.

PCR products were visualized on electrophoresis gels with 1 μl Midorigreen Direct (NIPPON Genetics Co., Ltd, Tokyo, Japan) and purified using the Wizard® SV Gel and PCR Clean-up System (Promega Corp.).

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Table 1. Summary for the examined hybrid *Andrias* and their parasites (TL: Total length shown by mm).

Locality	Capturing date	Euthanizing date	Host TL	*S. hanzaki*	*Am. tritonispunctati*	*Falcaustra* sp.	*L. copulans*
Kamo River	2011Nov30	2017Jan24	1075	1		1	149
	2016Dec03		1007			9	1332
	2016Nov05		781			50	95
	2016Nov05		754			19	29
	2010May20	2017Mar14	413			5	1
Kurama River	2011Oct19	2017Mar02	880			3	16
	2011Jul17	2017Apr05	1017	1	2	1	12
Shizuhara River	2016Dec03	2017Jan24	1085			23	262
	2011Jul17	2017Mar14	895			2	150
	2014Sep11	2017Apr11	1139			176	289
Myozin River	2009Oct13	2017Mar14	979			2	4
	2010Jun14		917			6	7
Nakatsu River	2017Jun24	2017Jun28	508			6	
			348		1	69	47
Katsura River	2016Aug31	2017Mar02	931	7		25	358
	2013Oct12		770	7			6
	2014Sep08		993	45		6	88
	2012Aug06	2017Mar24	991			1	495
	2015Apr09		901	10		2	996
	2012Feb09	2017Apr11	1079	6			23
	2014Sep08		1119	11			33
Teratani River	2011Nov04	2017Mar02	880	10		17	557
		2017Mar24	1014			2	705
Takano River	2012Oct18	2017Mar02	812		1	49	25
Kiyotaki River	2011Oct27	2017Mar02	840	3		4	334
			Prevalence (%)	40	12	88	96
			Mean Intensity	10	1	22	251

Table 2. Summary for the examined *Andrias davidianus* and their parasites (TL: Total length shown by mm).

Locality	Capturing date	Euthanizing date	Host TL	*Am. tritonispunctati*	*Falcaustra* sp.	*L. copulans*
Kamo River	2009Jul10	2017Apr05	1131	17	114	8
			1018			32

Sequencing was outsourced to FASMAC Co., Ltd. (Kanagawa, Japan).

The quality of returned sequences was checked using the Applied Biosystems™ Sequence Scanner Software v2.0. All high-quality sequences were aligned using ClustalW implemented in MEGA 7 (Kumar et al., 2016). BLAST searches were performed in GenBank to compare obtained and registered sequences and identify sequences with the lowest E-values and highest similarities.

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Results

Morphological study

Nematoda

Family Gnathostomatidae Railliet, 1895
Subfamily Spiroxyinae Baylis & Lane, 1920
Spiroxys hanzaki Hasegawa, Miyata & Doi, 1998

Description

Male (based on 10 adult specimens): body 13.9–30.0 (21.2±5) mm long and 0.4–0.6 (0.5±0.1) mm wide. Esophagus 2.9–6.6 (4.6±1) mm long and 133–280 (211±54) wide near posterior end. Nerve ring, excretory pore, deirids 534–947 (753±133), 634–1234 (889±176) and 1207–1367 (1309±61), respectively, from anterior extremity. Spicules 760–1234 (1005±124) long and 40–53 (47±5). Tail 227–334 (293±30) long.

Female (based on 12 adult specimens): body 21.9–40.2 (29.9±5) mm long and 0.4–0.9 (0.7±0.1) mm wide. Esophagus 2.7–6.5 (4.8±1) mm long and 173–320 (235±44) wide near posterior end. Nerve ring, excretory pore, deirids 667–1121 (845±157), 867–1254 (1005±118) and 1301–1934 (1431±194), respectively, from anterior extremity. Vulva 12.7–24.2 (19.4±4) mm from anterior extremity. Eggs 76–88 (80±4) by 45–76 (58±6) (n=25). Tail 367–667 (519±93) long.

Taxonomic summary

Host: hybrid *Andrias* between *A. japonicus* (Temminck, 1836) and Chinese *Andrias* species.

Infection site: stomach.

Stage: adults and third stage larvae.

Locality: Kyoto City, Kyoto Prefecture, Japan: Kamo River (35°03'33" N, 135°45'00" E) (site 1, Fig. 1), Kurama River (35°06'23" N, 135°45'52" E) (site 2, Fig. 1), Katsura River (35°12'19" N, 135°44'32" E; 35°14'58" N, 135°45'56" E; 35°15'57" N, 135°44'34" E) (site 6, Fig. 1), Teratani River (35°13'58" N, 135°47'35" E) (site 7, Fig. 1), and Kiyotaki River (35°03' N, 135°46' E) (site 9, Fig. 1).

Studied specimens: KUZ Z3910.

Remarks: general morphology agreed with Hasegawa et al. (1998). This work provides the first measurements of this species parasitizing *Andrias* spp. in Kyoto.

Family Trichuridae (Ransom, 1911)
Subfamily Capillariinae Railliet, 1915
Amphibiocapillaria tritonispunctati (Diesing, 1815)

Description

Female (based on 2 specimens): body 9.2–9.7 mm long and 67–87 wide. Esophagus 203–266 long. Stichocytes and vulva at 3.7–4.2 mm and 4.6–4.9 mm, respectively, from anterior extremity. Nuclei 110–112 in stichosome. Eggs 52–60 (56±3) by 27–30 (30±1) (n=24). Rectum 79–88 long.

Taxonomic summary

Host: *Andrias davidianus* (Blanchard, 1871) and hybrid *Andrias* between *A. japonicus* and Chinese *Andrias* species.

Infection site: intestine and rectum.

Stage: adults.

Locality: Kyoto City, Kyoto Prefecture, Japan: Kamo River (35°06'46" N, 135°43'12" E), Kurama River (35°06'23" N, 135°45'52" E), Nakatsu River (35°06'41" N, 135°43'27" E) (site 5, Fig. 1), and Takano River (35°06'03" N, 135°49'32" E) (site 8, Fig. 1).

Studied specimens: KUZ Z3911.

Remarks: general morphology consistent with Moravec (1982, 1986). This work provides the first measurements of this species parasitizing *Andrias* spp. in Kyoto.

Family Kathlaniidae Lane, 1914
Subfamily Kathlaniinae Lane, 1914
Falcaustra sp.

Description

General: body elongate. Three well-developed lips present. Esophagus consisting of three distinct parts; esophageal corpus, short isthmus and esophageal bulb. Tail tapering.

Male (based on 10 specimens): body 7.8–12.3 (9.7±1) mm long and 250–434 (334±50) wide in midbody. Lips 27–33 (29±2) by 55–67 (61±4). Pharyngeal part 55–79 (71±8) long and 39–52 (46±5) wide. Esophageal corpus

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1.2–1.5 (1.4±0.9) mm long and 67–87 (73±6) wide, short isthmus 100–120 (108±6) long and 73–113 (91±11) wide, esophageal bulb 139–193 (163±17) long and 147–220 (178±21) wide. Nerve ring and excretory pore at 279–349 (318±21) and 1201–1414 (1306±65), respectively, from anterior extremity. Single pseudosucker consisting of 13–15 pairs of muscles, 1.2–2.5 (1.9±0.4) mm from cloaca. Spicules two, elongate, pointed; left spicule 547–727 (614±45) long and 20–40 (31±7) wide, right spicule 600–700 (635±27) long and 21–40 (32±7) wide. Gubernaculum 91–127 (108±10) by 30–47 (37±7). Tail 320–434 (386±32) long.

Female (based on 10 specimens): body 9.8–14.0 (11.6±1) mm long and 313–534 (399±62) wide in midbody. Lips 24–36 (31±4) by 36–70 (59±10). Pharyngeal part 58–82 (72±7) long and 24–58 (47±11) wide. Esophageal corpus 1.3–1.9 (1.5±0.2) mm long and 67–87 (78±6) wide, short isthmus 73–120 (100±13) long and 87–127 (103±12) wide, esophageal bulb 147–193 (172±17) long and 173–220 (194±16) wide. Nerve ring and excretory pore at 306–427 (347±41) and 1234–1581 (1393±123), respectively, from anterior extremity. Vulva 6.2–8.8 (7.3±0.8) mm long from anterior extremity. Eggs oval, with a layer, 61–73 (65±3) by 42–55 (48±3) (n=62). Tail 239–1134 (689±227) long.

Taxonomic summary

Host: *Andrias davidianus* (Blanchard, 1871) and hybrid *Andrias* between *A. japonicus* and Chinese *Andrias* species.

Infection site: intestine and rectum.

Stage: adults and larvae.

Locality: Kyoto City, Kyoto Prefecture, Japan: Kamo River (35°01'16–52" N, 135°46'14–17" E; 35°03'33" N, 135°45'00" E; 35°06'46" N, 135°43'12" E), Kurama River (35°05'52" N, 135°45'47" E; 35°06'23" N, 135°45'52" E), Shizuhara River (35°05'52" N, 135°46'20" E; 35°06'14" N, 135°46'51" E) (site 3, Fig. 1), Myozin River (35°03'27" N, 135°45'17" E) (site 4, Fig. 1), Nakatsu River (35°06'41" N, 135°43'27" E), Katsura River (35°12'19" N, 135°44'35" E; 35°15'57" N, 135°44'34" E), Teratani River (35°13'58" N, 135°47'35" E), Takano River (35°06'03" N, 135°49'32" E), Kiyotaki River (35°03' N, 135°46' E).

Studied specimens: KUZ Z3912.

Remarks: the specimens examined showed morphological features consistent with the genus *Falcaustra* as defined by Chabaud (2009) in the structure of lips and esophagus. Compared to the native congeneric species previously reported in Japan, *Falcaustra* sp. differed as follows: (1) single pseudosucker present instead of plural pseudosuckers present in males of *F. odaiensis* Hasegawa & Nishikawa, 2009, (2) spicules (547–727 long) shorter than those (1.2–1.3 mm long) in *F. japonensis* (Yamaguti, 1935) (Yamaguti, 1935; Hasegawa and Nishikawa, 2009). *Falcaustra* sp. also differed from the introduced congeneric species reported in Japan as follows: (1) spicules (547–727 long) longer than those (277–314 long) in *F. catesbeianae* Walton, 1929, (2) pseudosucker consisting of 13–15 pairs of muscles instead of elongate pseudosucker consisting of 41–44 pairs of muscles in *F. wardi* (Mackin, 1936) (Baker, 1985; Hasegawa, 2006).

Trematoda

Family Liolopidae

Liolope copulans Cohn, 1902

Description

Adult (based on 9 specimens): body 2.3–3.7 (3.1±0.5) mm by 1.4–1.9 (1.7±0.2) mm. Oral sucker 107–200 (160±26) by 173–247 (210±23). Pharynx 73–113 (95±14) by 80–167 (124±23). Ventral sucker 193–260 (230±24) by 280–340 (307±16). Anterior testis 173–567 (387±117) by 334–494 (417±54), posterior testis 273–614 (391±127) by 287–534 (409±74). Cirrus pouch 400–754 (631±107) by 400–714 (588±91). Seminal vesicle 400–700 (594±94) by 160–300 (223±37). Ovary 187–293 (254±29) by 200–293 (253±30). Eggs 12–26 (19±4) in uterus, 140–147 (145±3) by 73–80 (78±3) (n=38).

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Taxonomic summary
Host: *Andrias davidianus* (Blanchard, 1871) and hybrid *Andrias* between *A. japonicus* and Chinese *Andrias* species.
Infection site: stomach and intestine.
Stage: adults.
Locality: Kyoto City, Kyoto Prefecture, Japan: Kamo River (35°01'16–52" N, 135°46'14–17" E; 35°03'01–33" N, 135°45'00–29" E; 35°06'46" N, 135°43'12" E), Kurama River (35°05'52" N, 135°45'47" E; 35°06'23" N, 135°45'52" E), Shizuhara River (35°05'52" N, 135°46'20" E; 35°06'14" N, 135°46'51" E), Myozin River (35°03'27" N, 135°45'17" E), Nakatsu River (35°06'41" N, 135°43'27" E), Katsura River (35°12'19" N, 135°44'32" E; 35°14'58" N, 135°45'56" E; 35°15'57" N, 135°44'34" E), Teratani River (35°13'58" N, 135°47'35" E), Takano River (35°06'03" N, 135°49'32" E), Kiyotaki River (35°03' N, 135°40' E).
Studied specimens: KUZ Z3908–Z3909.
Remarks: general morphology agreed with Baba et al. (2011). This work provides the first measurements of this species parasitizing *Andrias* spp. in Kyoto.

Molecular study

The ITS1 region of *S. hanzaki* was successfully sequenced for 1,551 bp (accession no. LC605542). The BLAST search showed the highest similarity (99%) with a sequence of *S. hanzaki* from *A. japonicus* (Japan) (KF530326: Li et al., 2014).

The partial 18S rDNA of *Am. tritonispunctati* was successfully sequenced for 786 bp (accession no. LC605543). The BLAST search showed the highest similarity (94%) with a sequence of *Aonchotheca putorii* (Rudolphi, 1819) (Nematoda: Trichuridae) (LC052349: Tamaru et al., 2015).

The partial 28S rDNA of *Falcaustra* sp. was successfully sequenced for 596 bp (accession no. LC605539–LC605541). The BLAST search showed the highest similarity (98%) with a sequence of *Megalobatrachonema terdentatum* (Linstow, 1890) (Nematoda: Kathlaniidae) (MN444706, Chen et al., 2020). The haplotype of larval *Falcaustra* sp. differed from those of adult *Falcaustra* sp. by 0.2–0.3% (*p*-distance). Two haplotypes of adult *Falcaustra* sp. differed by 0.2% (*p*-distance).

Discussion

The parasite fauna of *Andrias* populations in Kyoto Prefecture consisted of *Liolope copulans*, *Spiroxys hanzaki*, *Amphibiocapillaria tritonispunctati* and *Falcaustra* sp. *Liolope copulans* and *Falcaustra* sp. were found in specimens at all of the study sites and were the most abundant species in helminth fauna of *Andrias* species in Kyoto Prefecture. No parasite species documented by Yamaguti (1936, 1939, 1941) were found in this study.

Molecular data from the *S. hanzaki* confirmed the species-level identification of the specimens based on morphology. Molecular studies for *Am. tritonispunctati* and *Falcaustra* sp. also supported the subfamily-level identifications based on morphology. Genetic differentiation between the haplotype of larval *Falcaustra* sp. and those of adults were similar to genetic differentiation between those of two adult nematodes; therefore, we concluded that larval *Falcaustra* specimens were the same species as adult *Falcaustra* specimens.

Spiroxys hanzaki and *Am. tritonispunctati* are considered native parasites in Japan, because *S. hanzaki* have been only reported parasitizing *A. japonicus* in Japan (e.g., Hasegawa et al., 2002). *Amphibiocapillaria tritonispunctati* is widely distributed over Holarctic region (Moravec, 1986); however, this species has been recorded from multiple different species of Caudata in Japan for many years (Uchida et al., 2019). These facts permit us to regard them as helminths not derived from other countries. Therefore, it was concluded that introduced *A. davidianus* could act as spillback reservoirs for native parasites in Kyoto Prefecture. It suggests that

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"enemy release" could not be found in introduced *A. davidianus* in Kyoto Prefecture, unlike the case demonstrated in Torchin et al. (2003). It is unclear whether such spillback affects the host-parasite relationship between the native populations of *A. japonicus* and parasites.

This study is the first record of *Falcaustra* sp. found in *Andrias* spp. in Japan. The genus *Falcaustra* is a cosmopolitan group, and some introduced species of this genus have been reported in Japan (Hasegawa et al., 2006; Oi et al., 2012). *Falcaustra* sp. morphologically differs from both native and introduced congeneric species reported to parasitize amphibians and reptiles in Japan. Three congeneric species, *F. andrias* (He, Liu & Ma, 1992), *F. fopingensis* (He, Liu & Ma, 1992) and *F. chengguensis* (He, Liu & Ma, 1992), have been once recovered from Chinese *Andrias* species in China (He et al., 1992). However, these species cannot be compared with *Falcaustra* sp. due to insufficient morphological study and lacking molecular study. Further taxonomic study is necessary to identify *Falcaustra* species parasitic in *Andrias* spp. at species-level.

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Appendix

Summary of examined hosts. TL: total length (mm); KUHE: voucher ID of Graduate School of Human and Environmental Studies, Kyoto University; Hybrid: hybrid between *Andrias japonicus*×Chinese *Andrias* spp.

Locality	Microchip tag ID	Voucher	Species	TL	Captured date	Euthanized date	Depository
Kamo River	968000004887665	KUHE57580	Hybrid	1075	2011Nov30	2017Jan24	Kyoto University
	—	—	Hybrid	1007	2016Dec03	2017Jan24	Kyoto University
	—	KUHE57583	Hybrid	781	2016Nov05	2017Jan24	Kyoto University
	—	KUHE57582	Hybrid	754	2016Nov05	2017Jan24	Kyoto University
	—	KUHE58937	Hybrid	413	2010May20	2017Mar14	Kyoto University
	968000005259849	KUHE58903	*A. davidianus*	1131	2009Jul10	2017Apr05	Kyoto University
	968000005260759	KUHE58902	*A. davidianus*	1018	2009Jul10	2017Apr05	Kyoto University
Kurama River	968000005420797	KUHE57651	Hybrid	880	2011Oct19	2017Mar02	Kyoto University
	968000005423413	KUHE58904	Hybrid	1017	2011Jul17	2017Apr05	Kyoto University
Shizuhara River	392145000068831	—	Hybrid	1085	2016Dec03	2017Jan24	Kyoto University
	968000005263408	KUHE58714	Hybrid	895	2011Jul17	2017Mar14	Kyoto University
	00071E724A	KUHE58925	Hybrid	1139	2014Sep11	2017Apr11	Kyoto University
Myozin River	968000005257094	KUH58715	Hybrid	979	2009Oct13	2017Mar14	Kyoto University
	968000005423597	KUHE58716	Hybrid	917	2010Jun14	2017Mar14	Kyoto University
Nakatsu River	392145000239450*	KUHE59464	Hybrid	508	2017Jun24	2017Jun28	Kyoto University
	392145000231551	KUHE59470	Hybrid	348	2017Jun24	2017Jun28	Kyoto University
Katsura River	392145000233739	KUHE57655	Hybrid	931	2016Aug31	2017Mar02	Kyoto University
	00071E9EE0	KUHE57654	Hybrid	770	2013Oct12	2017Mar02	Kyoto University
	00071EC160	KUHE59037	Hybrid	993	2014Sep08	2017Mar24	Kyoto University
	0006B864DB	KUHE59038	Hybrid	991	2012Aug06	2017Mar24	Kyoto University
	392145000093853	KUHE59039	Hybrid	901	2015Apr09	2017Mar24	Kyoto University
	392145000074730	KUHE58926	Hybrid	1079	2012Feb09	2017Apr11	Kyoto University
	000725907F	KUHE58924	Hybrid	1119	2014Sep08	2017Apr11	Kyoto University
Teratani Rivier	0006B86C91	KUHE57653	Hybrid	880	2011Nov04	2017Mar02	Kyoto University
	0006B85C12	KUHE59036	Hybrid	1014	2011Nov04	2017Mar24	Kyoto University
Takano River	0006B86466	KUHE57647	Hybrid	812	2012Oct18	2017Mar02	Kyoto University
Kiyotaki River	0006B84BF8	KUHE57648	Hybrid	840	2011Oct27	2017Mar02	Kyoto University

*Dead body

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