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Original article

Molecular Phylogenetic and Morphological Problems of the Aki Salamander *Hynobius akiensis*: Description of Two New Species from Chugoku, Japan

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Abstract. Two new species of the genus *Hynobius* are described from the southern part of Hiroshima Prefecture, Japan. *Hynobius akiensis* was divided into three groups (Hiroshima-Ehime group, Higashihiroshima group, Northern Hiroshima group) based on morphological and molecular analyses; thus, the Hiroshima-Ehime and Higashihiroshima groups were described as *Hynobius geiyoensis* sp. nov. and *Hynobius sumidai* sp. nov., respectively. According to morphological comparisons, males of *H. geiyoensis* sp. nov. have significantly larger snout–vent length relative to those of males of the other two species, whereas members of *H. sumidai* sp. nov., unlike *H. akinesis*, have a distinct brownish–yellow stripe on the dorsal side of their tail. Following this description, the distribution area of *H. akiensis* has changed substantially; thus, the habitat status of this species or two new species should be reassessed for its conservation.

Key words: cytochrome b, fragmented habitat, Hiroshima Prefecture, natural monument, Seto Inland Sea

Introduction

The Aki salamander, *Hynobius akiensis*, was described from Mirasakacho Haizuka, Miyoshi-shi, Hiroshima Prefecture, but it is distributed in Hiroshima and Ehime Prefectures across the Seto Inland Sea (Setonaikai) (Matsui *et al.*, 2019). Based on mitochondrial DNA analyses, this species has three genetically distinct groups, namely the Northern Hiroshima, Higashihiroshima, and Hiroshima-Ehime groups; monophyly is strongly supported in the latter two groups, whereas the Northern Hiroshima group is not part of the monophyletic group with the Higashihiroshima, and Southern Hiroshima groups (Matsui *et al.*, 2019). However, these results were obtained from analysis of only 14 samples; thus, the monophyly of the three groups should be reassessed using many populations across the entire distribution range of *H. akiensis*. In addition, the morphological uniformity of the three *H. akiensis* groups is unreliable because Matsui *et al.* (2019) did not make comparisons among them; therefore, such comparisons are still required.

In the present study, we evaluated the species validity of the three aforementioned groups using the morphological, phylogenetic, and evolutionary species concepts, similar to Sugawara *et al.* (2018). To resolve the taxonomic issues related to *H. akiensis*, we performed statistical analyses of morphological characteristics to compare the three groups. We also used additional DNA sequence data to reconstruct the phylogeny of *H. akiensis* collected from the entire distribution range of this species. Finally, we revealed the new distribution ranges of the three *H. akiensis* groups in detail.

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Materials and methods

Molecular analysis

For phylogenetic analysis, we collected DNA samples from personal property or fields from February 2007 to April 2021 (Table 1; Fig. 1). We obtained a single tailbud embryo from each paired egg sac or tissue samples from larvae when tissue sampling from fields; these tissues were preserved in 99.5 % ethanol. The DNA extraction was performed using a DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany). For constructing phylogenetic trees, we sequenced a 630-bp mitochondrial cytochrome b gene (630 bp) using the two primers: L14010 (5′-TAHGGWGAHGGATTWGAWGCMACWGC-3′) and H14778 (5′-AARTAYGGGTGRAADGRRAYTTTRTCT-3′) (Matsui *et al.*, 2007). We deposited the acquired sequences into the DNA Data Bank of Japan (Table 1). DNA sequences were aligned using MEGA X (Kumar *et al.*, 2018) and then phylogenetic analyses of the aligned sequences were conducted using Bayesian inference (BI) and maximum likelihood (ML) estimations with several species of *Hynobius* and *Salamandrella keyserlingii* as the outgroup (Table 1). We estimated the best-fit nucleotide substitution model based on the Bayesian information criterion (Schwarz, 1978) and corrected Akaike's information criterion (AICc) (Sugiura, 1978) via jModelTest 2 (Darriba *et al.*, 2012). We selected the Hasegawa-Kishino-Yano model (gamma distribution) for BI and Tamura-Nei model (invariant sites) for ML and constructed Bayesian and maximum likelihood trees using MrBayes 3.2 (Ronquist *et al.*, 2012) and MEGA X (Kumar *et al.*, 2018), respectively. For Bayesian analyses, we performed two independent MCMC runs for 2,000,000 generations with a sampling frequency of 100; we examined the stationarity of the likelihood scores of sampled trees using Tracer version 1.7 (http://tree.bio.ed.ac.uk/software/tracer/) with the first 25 % of generations discarded as burn-in. Finally, we assessed monophyly using the values of posterior probability (PP) and bootstrap (BS) based on the criteria of Huelsenbeck and Rannala (2004) and Hillis & Bull (1993): monophyletic group = PP ≥ 0.95 and BP ≥ 70.

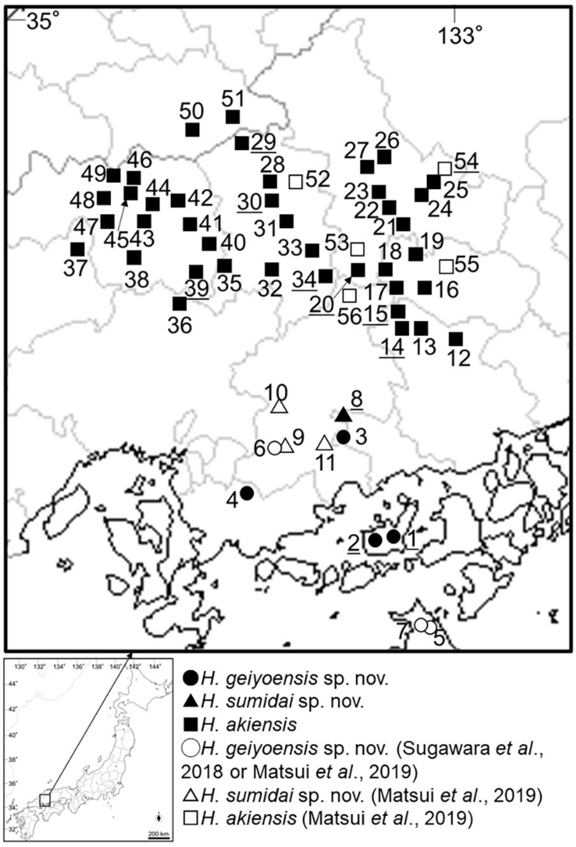

Fig. 1. Sampling map of the three *Hynobius* species used in this study. The enlarged area includes the central to the western part of Hiroshima Prefecture and the northernmost part of Ehime Prefecture. Closed symbols correspond to each of the three species sequenced in the current study. Open symbols correspond to each of the three species sequenced by other studies. The underlined localities show the sampling points of individuals for morphological comparisons: Pops. 1 (18 males) and 2 (1 male) for *H. geiyoensis* sp. nov.; Pop. 8 (7 males) for *H. sumidai* sp. nov.; Pops. 14 (3 males), 15 (7 males), 20 (6 males), 29 (3 males), 30 (1 male), 34 (6 males), 39 (1 male), 54 (1 male) for *H. akiensis*.

Morphological analysis

We sampled 54 individuals of *H. akiensis* from February 2017 to April 2021: 19 individuals of the Hiroshima-Ehime group from 2 populations (Pops. 1, 2), 7 individuals of the Higashihiroshima group from 1 population (Pop. 8), and 28 individuals of the Northern Hiroshima group from 8 populations (Pops. 14, 15, 20, 29, 30, 34, 39, 54) (Table 1; Fig. 1). In this study, we used only male individuals for morphological analyses, as did Matsui *et al.* (2019), because we could not collect enough females for statistical analyses. The collected individuals were measured under anesthesia using ethyl 3-aminobenzoate methanesulfonate salt (Sigma-Aldrich®, St. Louis, MO, USA) diluted 1,000-fold with water (Bennett, 1991). For conservation, measured individuals were subsequently returned to their site of capture except for the candidate individuals of type specimens. Before individuals were returned, photographs were taken of their dorsal, ventral, and lateral sides with a black background, and tissue samples (preserved to 99.5 % ethanol) were collected from the tail tips of all individuals as evidence of collection. All examined individuals were measured using a vernier caliper with the following 22 measurements: snout–vent length (SVL), trunk length

Table 1. List of samples used for molecular analyses

Population	Species	Sampling licality	Accession number / Label in Fig. 2
1	*Hynobius geiyoensis* sp. nov.	Nakano, Osakikamijima-cho, Hiroshima*	LC647835 / GEI01
2	*Hynobius geiyoensis* sp. nov.	Harada, Osakikamijima-cho, Hiroshima	LC647836 / GEI02
3	*Hynobius geiyoensis* sp. nov.	Nikacho, Takehara-shi, Hiroshima	LC647837 / GEI03
4	*Hynobius geiyoensis* sp. nov.	Goharacho, Kure-shi, Hiroshima	LC647838 / GEI04
5	*Hynobius geiyoensis* sp. nov.	Chikamicho, Imabari-shi, Ehime	LC279198 / EIC2018
6	*Hynobius geiyoensis* sp. nov.	Saijocho Goso, Higashihiroshima-shi, Hiroshima	LC436389 / H24
7	*Hynobius geiyoensis* sp. nov.	Namikatacho Hinokuchi, Imabari-shi, Ehime	LC436387 / H22
8	*Hynobius sumidai* sp. nov.	Tamaricho, Takehara-shi, Hiroshima*	LC647839 / SUM01
9	*Hynobius sumidai* sp. nov.	Saijocho Goso, Higashihiroshima-shi, Hiroshima	LC436390 / H25
10	*Hynobius sumidai* sp. nov.	Hachihonmatsucho Yoshikawa, Higashihiroshima-shi, Hiroshima	LC436392 / H27
11	*Hynobius sumidai* sp. nov.	Akitsucho Mitsu, Higashihiroshima-shi, Hiroshima	LC436394 / H29
12	*Hynobius akiensis*	Kuicho Hagura, Mihara-shi, Hiroshima	LC647840 / AKI01
13	*Hynobius akiensis*	Daiwacho Shimotokura, Mihara-shi, Hiroshima	LC647841 / AKI02
14	*Hynobius akiensis*	Daiwacho Ogu, Mihara-shi, Hiroshima	LC647842 / AKI03
15	*Hynobius akiensis*	Daiwacho Kuramune, Mihara-shi, Hiroshima	LC647843 / AKI04
16	*Hynobius akiensis*	Tsukuchi, Sera-cho, Hiroshima	LC647844 / AKI05
17	*Hynobius akiensis*	Yoshiwara, Sera-cho, Hiroshima	LC647845 / AKI06
18	*Hynobius akiensis*	Naka, Sera-cho, Hiroshima	LC647846 / AKI07
19	*Hynobius akiensis*	Nagata, Sera-cho, Hiroshima	LC647847 / AKI08
20	*Hynobius akiensis*	Miwacho Kamiichi, Miyoshi-shi, Hiroshima	LC647848 / AKI09
21	*Hynobius akiensis*	Uedamachi, Miyoshi-shi, Hiroshima	LC647849 / AKI10
22	*Hynobius akiensis*	Miwakamachi, Miyoshi-shi, Hiroshima	LC647850 / AKI11
23	*Hynobius akiensis*	Megurikamimachi, Miyoshi-shi, Hiroshima	LC647851 / AKI12
24	*Hynobius akiensis*	Kisacho Shikiji, Miyoshi-shi, Hiroshima	LC647852 / AKI13
25	*Hynobius akiensis*	Mirasakacho Mirasaka, Miyoshi-shi, Hiroshima	LC647853 / AKI14
26	*Hynobius akiensis*	Minamihatajikimachi, Miyoshi-shi, Hiroshima	LC647854 / AKI15
27	*Hynobius akiensis*	Nishisakeyamachi, Miyoshi-shi, Hiroshima	LC647855 / AKI16
28	*Hynobius akiensis*	Takamiyacho Kurumegi, Akitakata-shi, Hiroshima	LC647856 / AKI17
29	*Hynobius akiensis*	Midoricho Ikeda, Akitakata-shi, Hiroshima	LC647857 / AKI18
30	*Hynobius akiensis*	Midoricho Yokota, Akitakata-shi, Hiroshima	LC647858 / AKI19
31	*Hynobius akiensis*	Yoshidacho Aio, Akitakata-shi, Hiroshima	LC647859 / AKI20
32	*Hynobius akiensis*	Yoshidacho Oyama, Akitakata-shi, Hiroshima	LC647860 / AKI21
33	*Hynobius akiensis*	Kodacho Kamiobara, Akitakata-shi, Hiroshima	LC647861 / AKI22
34	*Hynobius akiensis*	Mukaiharacho Saka, Akitakata-shi, Hiroshima	LC647862 / AKI23
35	*Hynobius akiensis*	Yachiyocho Sasai, Akitakata-shi, Hiroshima	LC647863 / AKI24
36	*Hynobius akiensis*	Kabecho Nabara, Asakita-ku, Hiroshima-shi, Hiroshima	LC647864 / AKI25
37	*Hynobius akiensis*	Inoshiyama, Akiota-cho, Hiroshima	LC647865 / AKI26
38	*Hynobius akiensis*	Tsushimi, Kitahiroshima-cho, Hiroshima	LC647866 / AKI27
39	*Hynobius akiensis*	Minamigata, Kitahiroshima-cho, Hiroshima	LC647867 / AKI28
40	*Hynobius akiensis*	Yorohoyobara, Kitahiroshima-cho, Hiroshima	LC647868 / AKI29
41	*Hynobius akiensis*	Haruki, Kitahiroshima-cho, Hiroshima	LC647869 / AKI30
42	*Hynobius akiensis*	Kawado, Kitahiroshima-cho, Hiroshima	LC647870 / AKI31
43	*Hynobius akiensis*	Kamiishi, Kitahiroshima-cho, Hiroshima	LC647871 / AKI32
44	*Hynobius akiensis*	Motsuna, Kitahiroshima-cho, Hiroshima	LC647872 / AKI33
45	*Hynobius akiensis*	Ikadazu, Kitahiroshima-cho, Hiroshima	LC647873 / AKI34
46	*Hynobius akiensis*	Otuka, Kitahiroshima-cho, Hiroshima	LC647874 / AKI35
47	*Hynobius akiensis*	Mizoguchi, Kitahiroshima-cho, Hiroshima	LC647875 / AKI36
48	*Hynobius akiensis*	Utsunohara, Kitahiroshima-cho, Hiroshima	LC647876 / AKI37
49	*Hynobius akiensis*	Takano, Kitahiroshima-cho, Hiroshima	LC647877 / AKI38
50	*Hynobius akiensis*	Iwaya, Onan-cho, Shimane	LC647878 / AKI39
51	*Hynobius akiensis*	Asuna, Onan-cho, Shimane	LC647879 / AKI40
52	*Hynobius akiensis*	Takamiyacho Bogo, Akitakata-shi, Hiroshima	LC436381 / H16
53	*Hynobius akiensis*	Miwacho Kamiitaki, Miyoshi-shi, Hiroshima	LC436383 / H18
54	*Hynobius akiensis*	Mirasakacho Haizuka, Miyoshi-shi, Hiroshima*	LC436384 / H19
55	*Hynobius akiensis*	Kurobuchi, Sera-cho, Hiroshima	LC436385 / H20
56	*Hynobius akiensis*	Toyosakacho Kiyotake, Higashihiroshima-shi, Hiroshima	LC436386 / H21
	Hynobius abuensis		LC436395 / H. abuensis
	Hynobius hidamontanus		LC225434 / H. hidamontanus
	Hynobius tosashimizuensis		LC436447 / H. tosashimizuensis
	Hynobius utsunomiyaorum		LC436376 / H. utsunomiyaorum
	Salamandrella keyserlingii		NC_026032 / S. keyserlingii

The number of males indicates the individuals used in morphological analyses. Asterisks with sampling localities indicate the type locality of three species.

(TRL), axilla-groin distance (AGD), head length (HL), tail length (TAL), median tail width (MTAW), median tail height (MTAH), vomerine teeth length (VTL), and vomerine teeth wide (VTW), head width (HW), forelimb length (FLL), hindlimb length (HLL), second finger length (2FL), third finger length (3FL), third toe length (3TL), five toe length (5TL), internarial distance (IND), interorbital distance (IOD), upper eyelid length (UEL), snout length (SL), upper eyelid width (UEW), and lower jaw length (LJL). On each individual, we also checked for the presence of the following markings: distinct black dots on the dorsal side of the body (DBDD), distinct white dots on the ventral side of the body (DWDV), distinct white dots on the lateral sides of the body (DWDL), distinct brownish–yellow lines on the dorsal (DTBYLD), and ventral (DTBYLV) sides of the tail, and distinct gular mottling (DGM). The number of costal folds between the adpressed limbs (CFBALN) and the number of costal grooves (CGN) were counted, with the counting method of Matsui *et al.* (2019) used to count CGN. Prior to performing morphological comparisons among the three groups, we tested the data for normality using a Shapiro–Wilk test; when the data followed a normal distribution, we tested for homoscedasticity using Bartlett's test. When the variances among populations were equal, we performed Tukey–Kramer tests; when variances were not equal, we performed Games–Howel tests. When data did not follow a normal distribution and variances among populations were not equal, we performed Steel–Dwass tests. To examine the overall morphological variation among the three groups, we performed canonical discriminant analysis using SVL and standardized values for the 21 measurements: RTRL, RAGD, RHL, RTAL, RMTAW, RMTAH, RVTL, RVTW, RHW, RFLL, RHLL, R2FL, R3FL, R3TL, R5TL, RIND, RIOD, RUEW, RSL, RUEL, RLJL. Statistical analyses were conducted using R with $\alpha = 0.05$ (Ihaka & Gentleman, 1996).

From type specimens, we took 43 measurements as follows: SVL, TRL, AGD, HL, TAL, MTAW, MTAH, basal tail width (BTAW), basal tail height (BTAH), VTL, VTW, HW, left forelimb length (LFLL), left hindlimb length (LHLL), right forelimb length (RFLL), right hindlimb length (RHLL), left first finger length (L1FL), left second finger length (L2FL), left third finger length (L3FL), left fourth finger length (L4FL), right first finger length (R1FL), right second finger length (R2FL), right third finger length (R3FL), right fourth finger length (R4FL), left first toe length (L1TL), left second toe length (L2TL), left third toe length (L3TL), left fourth toe length (L4TL), left fifth toe length (L5TL), right first toe length (R1TL), right second toe length (R2TL), right third toe length (R3TL), right fourth toe length (R4TL), right fifth toe length (R5TL), IND, IOD, left upper eyelid length (LUEL), right upper eyelid length (RUEL), SL, left upper eyelid width (LUEW), right upper eyelid width (RUEW), and LJL. Measurements were taken after specimens were fixed in 10 % formalin and transferred to 70 % ethanol.

Two holotype specimens designated in this study are stored in the Shobara Municipal Hiwa Museum for Natural History: 1119-1, Hiwacho Hiwa, Shobra-shi, Hiroshima Prefecture, 727-0301, Japan. All paratypes of the two new species and a single topotype of *H. akiensis* are stored in the Kanagawa Prefectural Museum: 499 Iryuda, Odawara-shi, Kanagawa Prefecture, 250-0031, Japan. Further details are available only by contacting the corresponding author or the museums for avoiding overcollection of the species.

Results

The monophyly of *H. akiensis* was not supported by PP and BS values (Fig. 2); indeed, the Hiroshima-Ehime, Higashihiroshima, and Northern Hiroshima groups were genetically distinguishable. In contrast, the monophyly of *H. akiensis*, *Hynobius utsunomiyaorum*, and *Hynobius hidamontanus* was strongly supported by both BI and ML (Fig. 2). The transition type of *H. akiensis* was polyphyletic, included in the clade of the Northern Hiroshima group of *H. akiensis* (Fig. 2).

Morphological measurements of the three groups and significant values of all measurements among these groups are shown in Table 2 and Table 3, respectively. Males of the Hiroshima-Ehime and Higashihiroshima groups differed significantly in terms of nine morphological characteristics; males of the Hiroshima-Ehime and Northern Hiroshima groups differed significantly in terms of ten morphological characteristics; males of the Higashihiroshima and Northern Hiroshima groups differed significantly in terms of three morphological characteristics (Table 3). The canonical discriminant analysis also indicated that the three groups were different and that the distribution area of scores did not overlap (Fig. 3).

Morphological observations are given in Table 4. The Hiroshima-Ehime group always had DWDV (19/19 = 100 %), almost always had DTBYLD (17/19 = 89.5 %), 12 CGN (17/19 = 89.5 %), and −2–0 CFBALN (18/19 = 94.7 %), usually had DWDL (16/19 = 84.2 %), and almost always had no DGM (18/19 = 94.7 %), and never had DTBYLV (19/19 = 100 %). The Higashihiroshima group always had DTBYLD (7/7 = 100 %), usually had −1–0 CFBALN (6/7 = 85.7 %), and never had DTBYLV (7/7 =

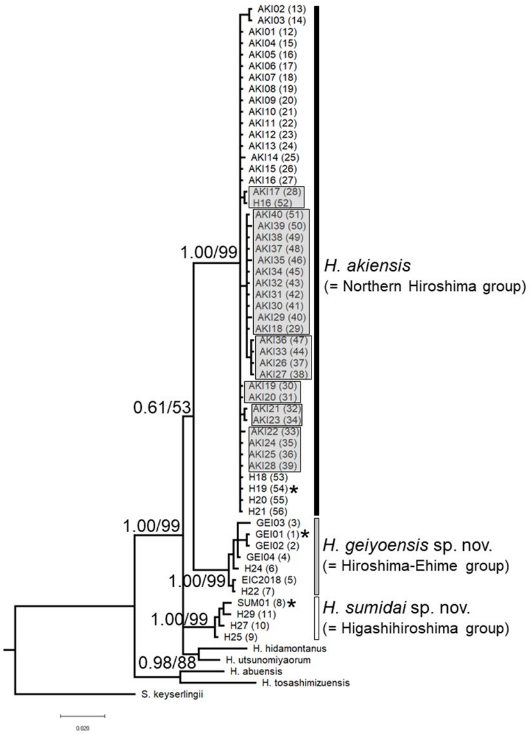

Fig. 2. Phylogenetic tree produced by Bayesian inference using 630-bp cytochrome b genes. *Salamandrella keyserlingii* was used as an outgroup. Scale bar shows genetic distance (expected changes per site). Numbers located near the nodes are posterior probabilities (PP) for Bayesian inference and bootstrap (BS) values for maximum likelihood estimation. Values appearing in parentheses after the haplotype names correspond to population localities as indicated in Table 1 and Fig. 1. Asterisks after the parentheses (Pops. 1, 8, and 54) indicate the type locality of the three species. The labels covered by shaded boxes indicate the transition type of *Hynobius akiensis*.

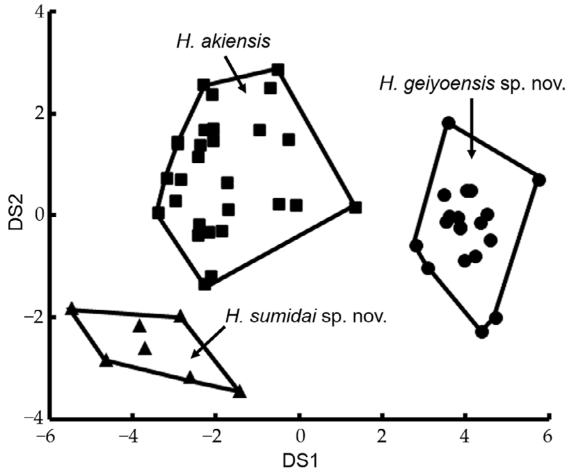

Fig. 3. Two-dimensional plots of canonical discriminant analysis in males. The x and y axes show discriminant score 1 (DS1) and discriminant score 2 (DS2), respectively. The contribution ratios of DS1 and DS2 were 88.03 % and 11.97 %, respectively.

100 %) and DGM (7/7 = 100 %). The northern Hiroshima group usually lacked the DTBYLD (25/28 = 89.3 %), usually had DWDV (23/28 = 82.1 %), 12 CGN (24/28 = 85.7 %), and −1.5–0 CFBALN (89.3 %), but never had DTBYLV (7/7 = 100 %).

According to the results from molecular and morphological analyses, the Hiroshima-Ehime and Higashihiroshima groups of *H. akiensis* should be distinct species based on the three species concepts. Thus, we described them as new species below.

Taxonomy

Hynobius geiyoensis **sp. nov.**

(New standard Japanese name: *Geiyo-sanshouo*)
(Figs. 4–5)

Hynobius nebulosus: Okawa *et al.*, 1990: 50, in part.
Hynobius akiensis Matsui, Okawa and Nishikawa in Matsui *et al.*, 2019: 75, in part.

Etymology. The specific name is derived from "*Geiyo*." In ancient times, the combined area of Aki (= central to the western part of Hiroshima Prefecture) and Iyo (= Ehime Prefecture), where the new species occurred, was called *Geiyo*.

Holotype. An adult male (specimen number: HMNH-AM-101) from Nakano, Osakikamijima-cho, Hiroshima Prefecture, Chugoku, Japan [34° 13′ N, 132° 53′ E; elevation = 150 m above sea level (a.s.l.); in all cases, datum = WGS84], collected by Jun-ichi Naito on February 23, 2019.

Paratype. An adult female (specimen number: KPM-NFA 943) from Harada, Osakikamijima-cho, Hiroshima Prefecture, Chugoku, Japan [34° 14′ N, 132° 52′ E; elevation = 30 m above sea level (a.s.l.); in all cases, datum = WGS84], collected by Jun-ichi Naito on 23 February 2019. An adult male (specimen number: KPM-NFA 944) from the same locality as the holotype, collected by Jun-ichi Naito on February 23, 2019.

Diagnosis. A comparatively large species (with a mean snout–vent length of 60.1 mm in males) within the Japanese lentic *Hynobius*: snout–vent length usually more than 50 mm; distinct white dots on the ventral side of the body always present; distinct brownish–yellow line on the dorsal edge of the tail almost always present; distinct white dots on the lateral side of the body usually present; distinct yellow stripe on the ventral edge of the tail always absent; distinct gular mottling almost always absent in males; fifth toe of hindlimb always present; V-shaped vomerine teeth

Table 2. Measurements (mm) of SVL and character ratios (R = %SVL) of TRL to LJL (ranges are shown in parentheses)

	H. geiyoensis sp. nov.		*H. sumidai* sp. nov.		*H. akiensis*	
Trait	Holotype	*n* = 19	Holotype	*n* = 7	Topotype	*n* = 28
SVL	58.4	60.1±3.23 (50.5-64.5)	48.2	47.2±2.23 (44.9-49.7)	55.3	51.2±4.07 (42.6-60.5)
RTRL	78.3	77.2±0.80 (75.5-78.4)	75.9	76.9±1.26 (75.3-78.3)	77.8	77.0±1.14 (74.6-78.9)
RAGD	54.1	52.5±1.32 (49.8-54.4)	53.9	53.5±1.78 (51.0-56.8)	50.3	52.9±1.41 (49.7-55.3)
RHL	21.4	23.1±0.86 (21.3-24.9)	23.4	24.3±1.54 (22.6-27.4)	23.1	24.2±0.82 (23.1-26.0)
RTAL	85.6	75.7±6.46 (60.5-85.7)	71.8	68.9±7.47 (55.5-76.2)	58.2	68.2±5.91 (57.2-78.4)
RMTAW	6.8	6.0±0.78 (4.7-7.5)	5.0	5.9±0.53 (5.0-6.7)	4.9	6.5±0.86 (4.2-8.1)
RMTAH	14.0	11.7±1.00 (9.5-14.0)	9.8	10.7±1.19 (9.3-12.6)	10.1	10.4±1.18 (7.7-12.7)
RVTL	5.4	4.9±0.48 (3.9-5.9)	5.4	5.2±0.49 (4.3-5.6)	4.7	4.7±0.38 (3.3-5.3)
RVTW	4.8	5.3±0.26 (4.8-5.7)	5.2	5.4±0.24 (5.1-5.7)	5.6	5.5±0.35 (4.9-6.1)
RHW	17.5	16.4±0.60 (15.3-17.8)	16.6	16.6±0.58 (15.7-17.4)	17.7	16.7±0.61 (15.4-17.8)
RFLL	23.6	23.0±1.29 (20.6-25.0)	24.5	25.1±1.42 (23.9-28.1)	22.8	25.1±1.22 (22.7-27.5)
RHLL	30.5	30.6±1.03 (28.4-32.7)	29.3	30.1±1.80 (27.6-32.7)	31.8	30.6±1.29 (27.2-32.9)
R2FL	5.5	5.0±0.54 (4.2-6.1)	4.4	4.8±0.27 (4.4-5.1)	5.8	4.9±0.58 (3.8-5.8)
R3FL	5.3	4.5±0.64 (3.0-5.3)	3.9	3.8±0.21 (3.6-4.2)	5.2	4.2±0.43 (3.4-5.3)
R3TL	9.1	8.2±0.49 (7.4-9.3)	6.8	7.5±0.72 (6.7-8.7)	8.9	7.9±0.69 (6.3-9.5)
R5TL	2.7	2.4±0.44 (1.7-3.4)	1.7	1.3±0.37 (0.8-1.8)	2.2	1.3±0.79 (0.0-2.6)
RIND	5.0	5.4±0.43 (4.7-6.1)	4.8	5.2±0.35 (4.7-5.7)	5.2	5.3±0.47 (4.1-6.2)
RIOD	6.5	5.8±0.31 (5.1-6.6)	6.2	6.4±0.62 (5.6-7.6)	6.0	6.3±0.33 (5.7-7.0)
RUEW	2.7	2.9±0.20 (2.6-3.4)	3.3	3.4±0.19 (3.2-3.8)	2.7	3.4±0.35 (2.6-4.0)
RSL	6.8	6.3±0.36 (5.7-6.8)	7.1	6.2±0.47 (5.7-7.1)	7.2	6.5±0.47 (5.4-7.3)
RUEL	4.3	3.9±0.21 (3.6-4.4)	5.0	4.7±0.35 (4.4-5.1)	4.2	4.7±0.31 (3.8-5.1)
RLJL	12.5	13.4±0.61 (12.5-14.4)	14.3	13.9±0.62 (13.0-14.9)	13.6	14.3±0.73 (13.1-15.8)

See Materials and Methods for definitions of morphological characteristics.

series; 12 (rarely 11) costal grooves; the number of costal folds between adpressed limbs always −2 to 0.5 in males.

Description of holotype. A moderately large individual: HL larger than HW; TAL shorter than SVL; body almost cylindrical; rounded snout; gular fold present; tail gradually compressed toward the tip; clearly expanded cloaca; webbing between digits absent; four fingers on each forelimb, order of length II > III > IV > I on both sides; five toes on each hindlimb, order of length III > IV > II > I > V on both sides; V-shaped vomerine teeth; skin smooth and shiny; DBDD present; DWDV and DWDL present (it became unclear after preservation); DTBYLD present; DTBYLV absent; DGM absent. The holotype had the following measurements (in mm): SVL =58.4, TRL = 45.7, AGD = 31.6, HL = 12.5, TAL = 50.0, MTAW = 4.0, MTAH = 8.2, BTAW = 8.0, BTAH = 7.3, VTL = 3.2,

Table 3. Significant values of the 22 morphological traits in males among three species

		Male	
Trait	GEI vs. SUM	GEI vs. AKI	SUM vs. AKI
SVL	**$P < 0.001$**	**$P < 0.0001$**	$P < 0.05$
RTRL	NS	NS	NS
RAGD	NS	NS	NS
RHL	$P < 0.05$	$P < 0.01$	NS
RTAL	$P < 0.05$	**$P < 0.001$**	NS
RMTAW	NS	NS	NS
RMTAH	NS	**$P < 0.001$**	NS
RVTL	NS	NS	$P < 0.05$
RVTW	NS	NS	NS
RHW	NS	NS	NS
RFLL	$P < 0.01$	**$P < 0.0001$**	NS
RHLL	NS	NS	NS
R2FL	NS	NS	NS
R3FL	$P < 0.01$	NS	$P < 0.01$
R3TL	$P < 0.05$	NS	NS
R5TL	**$P < 0.0001$**	**$P < 0.0001$**	NS
RIND	NS	NS	NS
RIOD	NS	**$P < 0.0001$**	NS
RUEW	**$P < 0.001$**	**$P < 0.0001$**	NS
RSL	NS	NS	NS
RUEL	**$P < 0.001$**	**$P < 0.0001$**	NS
RLJL	NS	**$P < 0.001$**	NS
$P < 0.05$	3	0	2
$P < 0.01$	2	1	1
$P < 0.001$	3	3	0
$P < 0.0001$	1	6	0
Total	9	10	3

GEI, SUM, and AKI are abbreviations for *Hynobius geiyoensis* sp. nov., *H. sumidai* sp. nov., and *H. akiensis*, respectively. Significant differences ($P < 0.001$) are shown in bold. See Materials and Methods for definitions of morphological characteristics.

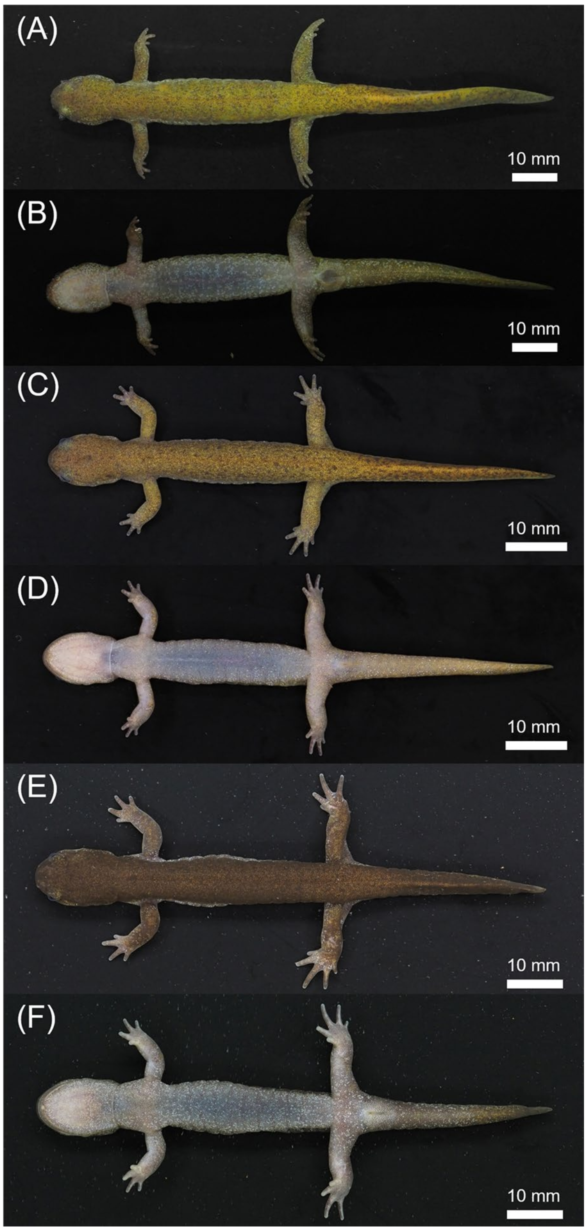

Fig. 4. Holotype of *Hynobius geiyoensis* sp. nov. (HMNH-AM-101, adult male, 58.4 mm SVL) from the (A) dorsal and (B) ventral perspective; holotype of *H. sumidai* sp. nov. (HMNH-AM-102, adult male, 48.2 mm SVL) from the (C) dorsal and (D) ventral perspective; and a specimen from the type locality (topotype) of *H. akiensis* (KPM-NFA 946, adult male, 55.3 mm) from the (E) dorsal and (F) ventral perspective.

VTW = 2.8, HW = 10.2, MXHW = 10.4, LFLL = 13.8, RFLL = 13.7, LHLL = 17.8, RHLL = 18.0, L1FL = 1.5, L2FL = 3.2, L3FL = 3.1, L4FL = 1.7, R1FL = 1.3, R2FL = 3.4, R3FL = 2.7, R4FL = 1.6, L1TL = 1.9, L2TL = 3.9, L3TL = 5.3, L4TL = 4.0, L5TL = 1.6, R1TL = 1.6, R2TL = 3.6, R3TL = 5.0, R4TL = 3.7, R5TL = 1.0, IND = 2.9, IOD = 3.8, LUEW = 1.6, RUEW = 1.4, SL =4.0, LUEL =2.5, RUEL = 2.7, LJL = 7.3, CGN = 12.

Comparisons. The new species statistically differs from *H. akiensis* in terms of the following length measurements in males: SVL, RHL, RTAL, RMTAH, RFLL, R5TL, RIOD, RUEW, RUEL, and RLJL; except for SVL, RTAL, RMTAH, and R5TL, these lengths were significantly shorter in *H. geiyoensis* sp. nov. relative to the lengths in *H. akiensis*. The new species differs from *H. akiensis* by the following characters: SVL > 57.1 mm (18/19 = 94.7 %) vs. SVL < 57.2 mm (26/28 = 92.9 %); RUEW < 3.2 % (18/19 = 94.7 %) vs. RUEW > 3.1 % (23/28 = 82.1 %); RUEL < 4.2 % (17/19 = 89.5 %) vs. RUEL > 4.1 % (27/28 = 96.4 %); usually have DTBYLD (17/19 = 89.5 %) vs. usually lack DTBYLD (25/28 = 89.3 %).

Variation. Morphometric measurements and observations are presented in Tables 2 and 4, respectively. The dorsum is uniformly yellowish brown or darkish brown. In the new species, DBDD are sometimes present (8/19 = 42.1 %), DWDL (3/19 = 15.8 %) and DTBYLD (2/19 = 10.5 %) are rarely absent, DGM is rarely present (1/19 = 5.3 %), CGN rarely 11 (2/19 = 10.5 %), and CFBALN rarely more than 0 (1/19 = 5.3 %). The iris is dark brown. When preserved, the dorsal coloration tends to fade to dark gray and DTBYLD becomes unclear after preservation.

Distribution. This new species is known from Higashihiroshima-shi (including the former Higashihiroshima-shi and Kurose-cho), Takehara-shi, and Kure-shi (including the former Kure-shi) in Hiroshima Prefecture, and Imabari-shi (including the former Imabari-shi and Namikata-cho) in Ehime Prefecture. DNA samples from the former Kurose-cho is not included in this study; however, based on previous field surveys (Okawa *et al.*, 1990), this species may be distributed in this area. This new species may also occur in the former Akitsu-cho (currently part of Higashihiroshima-shi) but there is currently no evidence to support this hypothesis. It was reported in 2018 that the populations of Kure-shi (2018) might already be extinct as. However, sufficient field surveys with objective evidences to support its extinction in Kure-shi have not been performed. Therefore, further field surveys are needed to clarify the current distribution range of the new species in greater detail.

Natural History. The dominant vegetation type of the type locality is a mixed forest of evergreen Fagaceae trees (i.e., *Quercus* and *Castanopsis* spp.) and Japanese cedar (*Cryptomeria japonica*) (Fig. 5). Larvae have distinct black dots on the lateral sides of the tail, whereas claws on the tips of the fingers and toes are absent. One pair of balancers is present during the early developmental stages of the larvae. Egg sacs are coil-shaped and are attached to fallen branches or leaves in ponds, puddles, or swamps at forest edges from February to April.

Remarks. The new species forms a monophyletic group with the Highland and Aki groups (Matsui *et al.*, 2019). The morphology of females is unclear; thus, further studies to clarify female morphology are required.

Hynobius sumidai **sp. nov.**

(New standard Japanese name: *Hiroshima-sanshouo*)
(Figs. 4–5)

Hynobius nebulosus: Okawa *et al.*, 1990: 50, in part.
Hynobius akiensis Matsui, Okawa and Nishikawa in Matsui *et al.*, 2019: 75, in part.

Etymology. The specific name "*sumidai*" is dedicated to Dr. Masayuki Sumida who was the phylogenetic

Table 4. Characteristics among the three species of *Hynobius*

		H. geiyoensis sp. nov.	*H. sumidai* sp. nov.	*H. akiensis*
Character	Condition	*n* = 19	*n* = 7	*n* = 28
DBDD	Absent	11 (57.9 %)	2 (28.6 %)	20 (71.4 %)
	Present	8 (42.1 %)	5 (71.4 %)	8 (28.6 %)
DWDV	Absent	0 (0 %)	3 (42.9 %)	5 (17.9 %)
	Present	19 (100 %)	4 (57.1 %)	23 (82.1 %)
DWDL	Absent	3 (15.8 %)	3 (42.9 %)	6 (21.4 %)
	Present	16 (84.2 %)	4 (57.1 %)	22 (78.6 %)
DTBYLD	Absent	2 (10.5 %)	0 (0 %)	25 (89.3 %)
	Present	17 (89.5 %)	7 (100 %)	3 (10.7 %)
DTBYLV	Absent	19 (100 %)	7 (100 %)	28 (100 %)
	Present	0 (0 %)	0 (0 %)	0 (0 %)
DGM	Absent	18 (94.7 %)	7 (100 %)	22 (78.6 %)
	Present	1 (5.3 %)	0 (0 %)	6 (21.4 %)
CGN	11	2 (10.5 %)	0 (0 %)	0 (0 %)
	12	17 (89.5 %)	4 (57.1 %)	24 (85.7 %)
	13	0 (0 %)	3 (42.9 %)	4 (14.3 %)
CFBALN	1	0 (0 %)	0 (0 %)	2 (7.1 %)
	0.5	1 (5.3 %)	1 (14.3 %)	1 (3.6 %)
	0	5 (26.3 %)	2 (28.6 %)	7 (25.0 %)
	-0.5	8 (42.1 %)	3 (42.9 %)	11 (39.3 %)
	-1	3 (15.9 %)	1 (14.3 %)	6 (21.4 %)
	-1.5	1 (5.3 %)	0 (0 %)	1 (3.6 %)
	-2	1 (5.3 %)	0 (0 %)	0 (0 %)

Values show the number of individuals exhibiting the characteristic (with associated percentages given in parentheses). See Materials and Methods for definitions of morphological trait

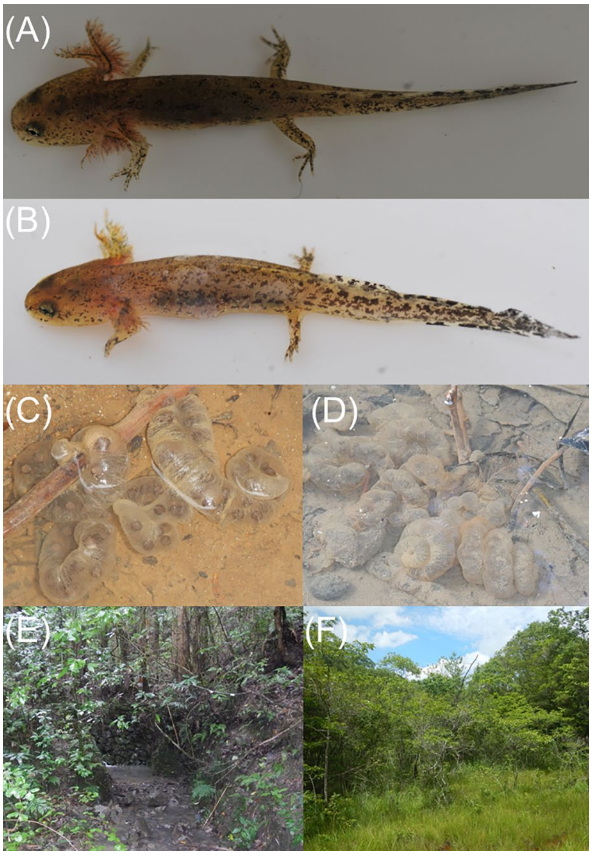

Fig. 5. Larvae, egg sacs, and habitat at the type locality of *Hynobius geiyoensis* sp. nov. (A, C, and E, respectively) and *H. sumidai* sp. nov. (B, D, and F, respectively).

taxonomist of amphibians at Hiroshima University.

Holotype. An adult male (specimen number: HMNH-AM-102) from Tamaricho, Takehara-shi, Hiroshima Prefecture, Chugoku, Japan [34° 24′ N, 132° 47′ E; elevation = 340 m above sea level (a.s.l.); in all cases, datum = WGS84], collected by Jun-ichi Naito on 23 March 2021.

Paratype. An adult female (specimen number: KPM-NFA 945) from the same locality of the holotype, collected by Jun-ichi Naito on 23 March 2021.

Diagnosis. A comparatively small species (mean snout–vent length of 47.2 mm in males) within the Japanese lentic *Hynobius*; SVL usually less than 50 mm; distinct brownish–yellow stripe on the dorsal edge of the tail always present; distinct brownish–yellow line on the ventral side of the tail never present; distinct gular mottling never present; yellowish–brown to blackish–brown on dorsum; the fifth toe of hindlimb always present; V-shaped vomerine teeth series; usually 12 costal grooves (rarely 13); costal folds between adpressed limbs always less than 1.0; coil-shaped egg sacs.

Description of holotype. A moderately large individual with HL larger than HW; TAL shorter than SVL; body almost cylindrical; rounded snout; gular fold present; tail gradually compressed toward the tip; non-expanded cloaca; webbing between digits absent; four fingers on each forelimb, order of length II > III > I > IV on left and II > III > IV > I on right; five toes on each hindlimb, order of length III > IV > II > I > V on both sides; V-shaped vomerine teeth; skin smooth and shiny; DBDD absent; DWDV and DWDL absent; DTBYLD present (it became unclear after preservation); DTBYLV absent; DGM absent. The holotype had the following measurements (in mm): SVL = 48.2, TRL = 36.6, AGD = 26.0, HL = 11.3, TAL = 34.6, MTAW = 2.4, MTAH = 4.7, BTAW = 5.4, BTAH = 4.4, VTL = 2.6, VTW = 2.5, HW = 8.0, MXHW = 8.2, LFLL = 11.8, RFLL = 11.9, LHLL = 14.1, RHLL = 14.0, L1FL = 1.3, L2FL = 2.1, L3FL = 1.9, L4FL = 0.9, R1FL = 0.7, R2FL = 1.6, R3FL = 1.2, R4FL = 0.8, L1TL = 1.1, L2TL = 2.0, L3TL = 3.3, L4TL = 2.3, L5TL = 0.8, R1TL = 1.3, R2TL = 1.7, R3TL = 2.9, R4TL = 1.9, R5TL = 0.7, IND = 2.3, IOD = 3.0, LUEW = 1.6, RUEW = 1.4, SL = 3.4, LUEL = 2.4, RUEL = 2.2, LJL = 6.9, and CGN = 12.

Comparisons. The new species statistically differs from *H. geiyoensis sp.* nov. in the following length measurements: SVL, RHL, RTAL, RFLL, R3FL, R3TL, R5TL, RUEW, and RUEL in males; the lengths of these measurements, except for RHL, RFLL, RUEW, and RUEL, are significantly longer in *H. sumidai* sp. nov. relative to the measurements in *H. geiyoensis* sp. nov.. The new species differs from *H. geiyoensis* sp. nov. by the following characters: SVL < 50 mm (7/7 = 100 %) vs. SVL > 50 mm (19/19 = 100 %); R5TL < 1.9 % (7/7 = 100 %) vs. R5TL > 1.8 % (18/19 = 94.7 %); RUEW > 3.1 % (7/7 = 100 %) vs. RUEW < 3.2 % (18/19 = 94.7 %); RUEL > 4.3 % (7/7 = 100 %) vs. RUEL < 4.3 % (18/19 = 94.7 %). The new species statistically differs from *H. akiensis* in the following length measurements: SVL (shorter), RVTL (longer), and R3FL (shorter) in males. The most distinct characteristic between the two species is the presence of DTBYLD: *H. sumidai* sp. nov. always has this characteristic (7/7 = 100 %), whereas *H. akiensis* usually lacks it (25/28 = 89.3 %).

Variation. Morphometric measurements and observations are presented in Tables 2 and 4, respectively. The dorsum is uniformly yellowish–brown or darkish–brown. The venter is lighter than the dorsum. DBDD rarely absent (2/7 = 28.6 %), DWDV and DWDL sometimes absent (3/7 = 42.9 %), sometimes it has 13 CGN (3/7 = 42.9 %), and rarely has more than 0 CFBALN (1/7 = 14.3 %). The iris is dark brown or light brown. When preserved, the dorsal coloration tends to fade to dark gray and DTBYLD becomes unclear after preservation.

Distribution. This new species is endemic to Hiroshima

Prefecture, and it is known from Higashihiroshima-shi (only former Higashihiroshima-shi and Akitsu-cho) and Takehara-shi. Its distribution is thought to bein the former Kurose-cho and Kure-shi areas, but no supporting evidence is available to confirm this hypothesis. Further phylogenetic studies using more samples from Higashihiroshima-shi and Kure-shi are therefore essential.

Natural History. The dominant vegetation type in the type locality is a mixed forest of evergreen oak (*Quercus* spp.) and Japanese cypress (*Chamaecyparis obtusa*). The larval and egg sac morphologies of this new species are similar to those of *H. geiyoensis* sp. nov. The breeding season of the new species is February to April.

Remarks. The new species forms a monophyletic group with the Highland and Aki groups (Matsui *et al.*, 2019). The morphology of females is unclear; hence, additional studies to clarify the morphology of females are needed.

Hynobius akiensis Matsui, Okawa et Nishikawa, 2019

(Standard Japanese name: *Aki-sanshouo*)

(Fig. 4)

Hynobius akiensis Matsui, Okawa and Nishikawa in Matsui *et al.*, 2019: 75, in part.

Holotype. An adult male (specimen number: KUHE 35925) from Mirasakacho Haizuka, Miyoshi-shi, Hiroshima Prefecture, collected by Yasuchika Misawa and Kanto Nishikawa on 26 March 2005. This specimen is stored in the Graduate School of Human and Environmental Studies, Kyoto University: Yoshidahonmachi, Sakyo-ku, Kyoto-shi, Kyoto Prefecture, 606-8501, Japan.

Diagnosis. A comparatively small species (mean snout–vent length of 51.2 mm in males) within the Japanese lentic Hynobius; distinct white dots on the ventral side of the body usually present; distinct brownish–yellow stripe on the dorsal edge of the tail usually absent; distinct brownish–yellow line on the ventral side of the tail never present; distinct gular mottling usually absent; yellowish–brown to blackish–brown on dorsum; fifth toe of hindlimb always present; V-shaped vomerine teeth series; 12 costal grooves (rarely 13); costal folds between adpressed limbs usually less than 0.5; coil-shaped egg sacs.

Description of a specimen from the type locality (Topotype). An adult male (specimen number: KPM-NFA 946) from the same locality of holotype, collected by Takayuki Iwata on 23 March 2019. A moderately large individual with HL larger than HW; TAL shorter than SVL; body almost cylindrical; rounded snout; gular fold present; tail gradually compressed toward the tip; slightly expanded cloaca; webbing between digits absent; four fingers on each forelimb, order of length II > III > IV > I in left and III > II > IV > I in right; five toes on each hindlimb, order of length III > IV > II > V > I in left and III > IV > II > I > V in right; V-shaped vomerine teeth; skin smooth and matte; DBDD absent; DWDV and DWDV present before preservation (it became unclear after preservation); DTBYLD and DTBYLV absent; DGM absent. The holotype had the following measurements (in mm): SVL = 55.3, TRL = 43.0, AGD = 27.8, HL = 12.8, TAL = 32.2, MTAW = 2.7, MTAH = 5.6, BTAW = 6.7, BTAH = 5.6, VTL = 2.6, VTW = 3.1, HW = 9.8, MXHW = 9.9, LFLL = 12.6, RFLL = 12.7, LHLL = 17.6, RHLL = 16.8, L1FL = 0.6, L2FL = 3.2, L3FL = 2.9, L4FL = 1.5, R1FL = 0.8, R2FL = 3.0, R3FL = 3.2, R4FL = 1.7, L1TL = 1.1, L2TL = 3.2, L3TL = 4.9, L4TL = 3.4, L5TL = 1.2, R1TL = 0.8, R2TL = 3.3, R3TL = 4.9, R4TL = 3.5, R5TL = 0.3, IND = 2.9, IOD = 3.3, LUEW = 1.5, RUEW = 1.7, SL = 4.0, LUEL = 2.3, RUEL = 2.4, LJL = 7.5, and CGN = 12.

Comparisons. This species is parapatrically distributed with *Hynobius iwami* but is distinguishable concerning for the presence of the fifth toe as well as distinct and bright yellow lines on the dorsal and ventral sides of the tail (Matsui *et al.*, 2019). Moreover, *H. utsunomiyaorum* is distributed near the range of *H. akiensis* but *H. akiensis* significantly differs from *H. utsunomiyaorum* by longer vomerine teeth series, fifth toe, and a greater degree of limb separation (Matsui *et al.*, 2019).

Variation. Morphometric measurements and observations were presented in Tables 2 and 4, respectively. The dorsum is uniformly darkish–brown or blackish–brown. The venter is lighter than the dorsum. DBDD (8/28 = 28.6 %), DGM (6/28 = 21.4 %), and DTBYLD (3/28 = 10.7 %) are rarely present. DWDV (5/28 = 17.9 %) and DWDL (6/28 = 21.4 %) rarely absent. CGN rarely 13 (4/28 = 14.3 %), and CFBALN rarely more than 0 (3/28 = 10.7 %). The iris is dark brown. When preserved, the dorsal coloration tends to fade to dark gray.

Distribution. According to Matsui *et al.* (2019), this species is endemic to Hiroshima Prefecture. However, the results of our field survey do not support this finding; we show that the species is known from Hiroshima and Shimane Prefectures as follows: Mihara-shi (only former Kui-cho and Daiwa-cho), Miyoshi-shi (only former Miyoshi-shi and Mirasaka-cho, Kisa-cho, and Miwa-cho), Akitakata (former Takamiya-cho, Koda-cho, Mukaihara-cho, Yoshida-cho, Yachiyo-cho, and Midori-cho), Higashihiroshima (only former Toyosaka-cho), and Hiroshima (only former Hiroshima-shi, Asakita-ku), and Sera-cho (only former Sera-cho and Nishisera-cho), Akiota-cho (only former Togochi-cho), and Kitahiroshima-cho(former Chiyoda-cho, Oasa-cho, Toyohira-cho, and Geihoku-cho), Hiroshima Prefecture, and Onan-cho (only former Mizuho-cho, and Hasumi-mura), Shimane Prefecture.

Remarks. *Hynobius akiensis* from Akitakata-shi and Kitahiroshima-cho is called the transition type of *H. akiensis* (Okawa *et al.*, 2019), but the common morphological character of transition type is not defined and unclear, and a type is a polyphyletic group (Fig. 2).

Discussion

According to Matsui *et al.* (2019), *H. akiensis* and the two new species described in this study can be regarded as the same species. However, the monophyly of *H. akiensis* and the two new species described here was rejected based on the criteria of Huelsenbeck and Rannala (2004) and Hillis & Bull (1993) (Matsui *et al.*, 2019; Fig. 2). Neither mitochondrial data nor phylogenetic analyses from allozyme data supported the monophyletic relationship among the species (Matsui *et al.*, 2006). Therefore, there is no evidence that *H. akiensis* and the two new species are in a monophyletic group as the closest relatives. Moreover, these species are morphologically distinguishable based on our analyses (Tables 2–4; Fig. 3). Thus, *H. akiensis* and the two new species should be distinct species based on three species concepts. Although the monophyly of *H. geiyoensis* sp. nov. and *H. sumidai* sp. nov. was supported by Matsui *et al.* (2019), our analyses did not support it (Fig. 3). The two new species were identified by mitochondrial DNA and morphologically distinct according to their differences (Tables 2-4; Fig. 3). Thus, we suggest that they should be different species based on the morphological species concept.

Okawa *et al.* (2019) suggested that the transition type of *H. akiensis* is a new species based on morphological perspectives. However, this type is included in the clade of *H. akiensis* and it cannot be recognized as a new species based on the phylogenetic and evolutionary species concepts (Fig. 2) (Matsui *et al.*, 2019). Thus, this type should be regarded as one morphotype of *H. akiensis* at present.

Following this description, the distribution area of *H. akiensis* is substantially changed; the species is not distributed in the southern part of Hiroshima Prefecture (Table1; Fig 1). However, the number of samples and localities from Higashihiroshima-shiwas not sufficient in our analyses. In Higashihiroshima-shi, *H. akiensis* has been protected by law since November 5, 1986, so permission must be granted to collect individuals. We applied to collect specimens in Higashihiroshima-shi, but our application was rejected because individuals must not be killed to ensure the conservation of *H. akiensis*. Unlike before, the current populations in Higashihiroshima-shi and the surrounding areas might be decreasing (e.g., Kure-shi, 2018), therefore morphological re-examination of living male and female individuals of *H. geiyoensis* sp. nov. and *H. sumidai* sp. nov. from Higashihiroshima-shi should be performed in the future. The conservation statuses of these three species must be reassessed after our description and management plans for the conservation of these species should be made immediately to ensure they do not become extinct.

Acknowledgements

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摘 要

菅原弘貴・内藤順一・岩田貴之・永野昌博 , 2022. アキサンショウウオ *Hynobius akiensis* における分子系統学的および形態学的問題：日本の中国地方からの 2 新種の記載 . 神奈川県立博物館研究報告（自然科学）, (51): 35–46. [Sugawara, H., J. Naito, T. Iwata & M. Nagano, 2022. Molecular Phylogenetic and Morphological Problems of the Aki Salamander *Hynobius akiensis*: Description of Two New Species from Chugoku, Japan. *Bull. Kanagawa Pref. Mus.* (*Nat. Sci.*), (51): 35–46.]

サンショウウオ属の 2 新種を、日本の広島県南部から記載した。形態学的および分子系統学的解析に基づくと、アキサンショウウオは 3 つのグループ（広島北部グループ、広島 - 愛媛グループ、東広島グループ）に区別された。したがって、広島県南部および愛媛県北部に分布するグループと東広島市を中心に分布するグループを、それぞれ *Hynobius geiyoensis* sp. nov.（和名：ゲイヨサンショウウオ）と *Hynobius sumidai* sp. nov.（和名：ヒロシマサンショウウオ）として記載した。雄個体による形態比較の結果、前者は他の 2 種よりも頭胴長が有意に長かった。一方、後者はアキサンショウウオに似るが、尾の背面に明瞭な黄茶色の線をもっていた。本記載により、アキサンショウウオの分布域は大きく変更となるため、本種ならびに 2 新種の保全に際して、生息状況を再評価する必要がある。