Original article

Taxonomic Re-examination of the Yamato Salamander *Hynobius vandenburghi*: Description of a New Species from Central Honshu, Japan

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Abstract. A new species of the genus *Hynobius* is described from the western part of Aichi Prefecture, Japan. *Hynobius vandenburghi* can be divided into two groups, the Aichi and Kinki groups, based on molecular and morphological analyses; thus, we described the Aichi group of *H. vandenburghi* as a new species, *H. owariensis* sp. nov.. Morphological comparisons revealed that although male *H. vandenburghi* have distinct bright yellow lines on the dorsal and ventral sides of the tail, males of the new species do not. Additionally, in males, the new species usually has fewer costal folds between its adpressed limbs than are observed in *H. vandenburghi*. Other significant differences in several morphological characteristics were also found between *H. vandenburghi* and the new species, and results of discriminant analyses between the two species in both sexes suggested that they are separated in terms of morphological data. The new species is restricted in the western part of Aichi Prefecture, which is threatened with extinction by artificial development or reformation of well-drained paddy fields.

Key words: Chita Peninsula, discriminant analysis, extinction, Kinki District, mitochondrial DNA

Introduction

The Yamato salamander, *Hynobius vandenburghi*, was originally described from Yamato Province (= Nara Prefecture) (Dunn, 1923a), and it is distributed in Kinki (Osaka, Nara, Kyoto, and Shiga Prefectures) and Tokai (Mie, Gifu, and Aichi Prefectures) Districts (Matsui *et al.*, 2019). This species is genetically separated into two distinct groups, the Aichi (excluding populations of Atsumi Peninsula) and Kinki (including populations of Atsumi Peninsula) groups, based on molecular analyses (Matsui *et al.*, 2019). Additionally, the monophyly of the two groups is strongly supported by maximum likelihood (ML) estimations and Bayesian inference (BI) (99/1.00) (Matsui *et al.*, 2019). However, these analyses were performed using only 10 populations; thus, the monophyly of the two groups should be reassessed using a lot of populations across the entire distribution range of *H. vandenburghi*. Furthermore, the morphological similarity of the two *H. vandenburghi* groups is unreliable because Matsui *et al.* (2019) did not compare the Aichi and Kinki groups despite clear genetic evidence that they are separated. Indeed, the Aichi populations of *H. vandenburghi* are morphologically more similar to *Hynobius tokyoensis* than those of *H. vandenburghi* (Sato, 1943; Nakamura & Ueno, 1963); therefore, it is doubtful that the Aichi populations satisfy the diagnosis of *H. vandenburghi*. Consequently, it is necessary to reassess the morphology of the two *H.*

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vandenburghi groups.

In the present study, we evaluated the species validity of the two *H. vandenburghi* groups using the morphological, phylogenetic, and evolutionary species concepts following Sugawara *et al.* (2018). To resolve the taxonomic problems associated with *H. vandenburghi*, we performed statistical analyses on morphological characteristics to compare the two *H. vandenburghi* groups. We also used additional DNA sequence data to reconstruct the phylogeny of *H. vandenburghi* collected from the entire distribution range of this species. Finally, we reveal the distribution ranges of the two *H. vandenburghi* groups in detail.

Materials and methods

Molecular analysis

To reconstruct a molecular phylogeny, we collected DNA samples from *H. vandenburghi* located on personal property or in fields from February 2007 to April 2021 (Table 1; Fig. 1). When sampling in fields, we obtained a single tailbud embryo from each paired egg sac or tissue samples from larvae. The tissues collected from fields were preserved in 99.5 % ethanol. Subsequently, we extracted total genomic DNA from the tissues using a DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany). For molecular analyses, we then amplified a 585-bp fragment of the cytochrome b gene from each individual using the primers L14010 (5′-TAHGGWGAHGGATTWGAWGC MACWGC-3′) and H14778 (5′-AARTAYGGGTGRAAD GRRAYTTTRTCT-3′) (Matsui *et al.*, 2007). Our methods for polymerase chain reaction (PCR) and sequencing analysis followed those of Sugawara *et al.* (2018). The obtained sequences were registered in the DNA Data Bank of Japan (DDBJ) (Table 1). Before phylogenetic analyses, DNA sequences were aligned using MEGA X (Kumar *et al.*, 2018). After alignment, molecular phylogenies were constructed using other *Hynobius* species, *Salamandrella keyserlingii* as the outgroup (Table 1), and with BI and ML estimation. The best-fit nucleotide substitution model was decided based on the Bayesian information criterion (BIC) (Schwarz, 1978) and corrected Akaike's information criterion (AICc) (Sugiura, 1978) using jModelTest 2 (Darriba *et al.*, 2012). The Hasegawa-Kishino-Yano (HKY) model was selected with a gamma distribution in BI and ML. The Bayesian and ML trees were reconstructed 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. In the Bayesian analysis, we examined the stationarity of the likelihood scores of sampled trees using Tracer version 1.7 (http://tree.bio.ed.ac.uk/software/tracer/) and we discarded

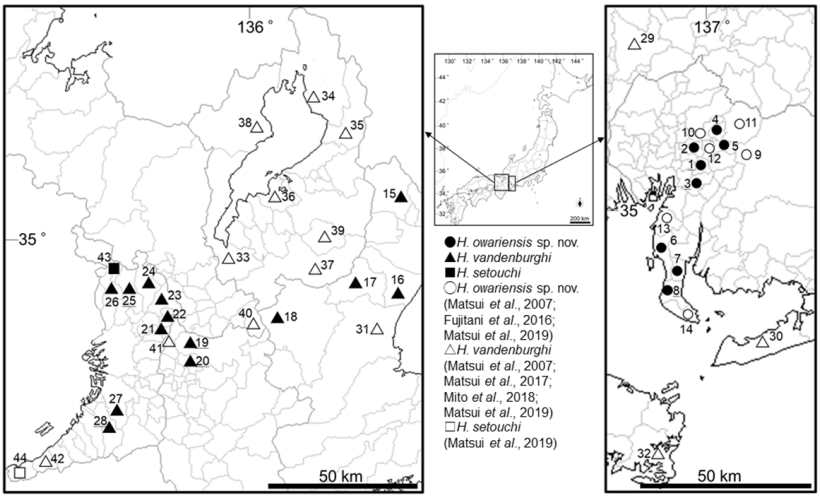

Fig. 1. Localities for populations of two species of *Hynobius* sampled in their distribution areas. Population numbers match those used for molecular analyses (see Table 1 and Fig. 2). The left and right enlarged areas include the central part of Kinki and central part of Tokai, respectively. The closed symbols correspond to each of three species sequenced in this study. The open symbols correspond to each of three species cited from other studies. For the morphological comparisons, individuals of the two species were sampled from the localities that are underlined: Pops. 1 (type locality of *H. owariensis* sp. nov.: 2 males and 1 female), 3 (18 males and 6 females), and 8 (2 males and 3 females) for *H. owariensis* sp. nov.; Pops. 19 (6 males and 3 females), 20 (7 males), 25 (3 females), and 28 (4 males) for *H. vandenburghi*.

Table 1. List of specimens used in molecular analyses

Population	Species	Sampling licality	Accession number / Label in Fig. 2
1	*Hynobius owariensis* sp. nov.	Tenpaku-ku, Nagoya-shi, Aichi	LC644689 / OWA01
2	*Hynobius owariensis* sp. nov.	Chikusa-ku, Nagoya-shi, Aichi	LC644690 / OWA02
3	*Hynobius owariensis* sp. nov.	Midori-ku, Nagoya-shi, Aichi	LC644691 / OWA03
4	*Hynobius owariensis* sp. nov.	Owariasahi-shi, Aichi	LC644692 / OWA04
5	*Hynobius owariensis* sp. nov.	Nagakute-shi, Aichi	LC644693 / OWA05
6	*Hynobius owariensis* sp. nov.	Tokoname-shi, Aichi	LC644694 / OWA06
7	*Hynobius owariensis* sp. nov.	Taketoyo-cho, Aichi	LC644695 / OWA07
8	*Hynobius owariensis* sp. nov.	Mihama-cho, Aichi	LC644696 / OWA08
9	*Hynobius owariensis* sp. nov.	Toyota-shi, Aichi	AB972617 / TYO10
10	*Hynobius owariensis* sp. nov.	Moriyama-ku, Nagoya-shi, Aichi	AB972596 / NagF7
11	*Hynobius owariensis* sp. nov.	Seto-shi, Aichi	LC225431 / H81
12	*Hynobius owariensis* sp. nov.	Meito-ku, Nagoya-shi, Aichi	LC436440 / H78
13	*Hynobius owariensis* sp. nov.	Chita-shi, Aichi	LC436441 / H79
14	*Hynobius owariensis* sp. nov.	Minamichita-cho, Aichi	AB266662 / H80
15	*Hynobius vandenburghi*	Inabe-shi, Mie	LC644697 / VAN01
16	*Hynobius vandenburghi*	Suzuka-shi, Mie	LC644698 / VAN02
17	*Hynobius vandenburghi*	Kameyama-shi, Mie	LC644699 / VAN03
18	*Hynobius vandenburghi*	Iga-shi, Mie	LC644700 / VAN04
19	*Hynobius vandenburghi*	Nara-shi, Nara	LC644701 / VAN05
20	*Hynobius vandenburghi*	Yamatokoriyama-shi, Nara	LC644702 / VAN06
21	*Hynobius vandenburghi*	Shijonawate-shi, Osaka	LC644703 / VAN07
22	*Hynobius vandenburghi*	Katano-shi, Osaka	LC644704 / VAN08
23	*Hynobius vandenburghi*	Hirakata-shi, Osaka	LC644705 / VAN09
24	*Hynobius vandenburghi*	Takatsuki-shi, Osaka	LC644706 / VAN10
25	*Hynobius vandenburghi*	Ibaraki-shi, Osaka	LC644707 / VAN11
26	*Hynobius vandenburghi*	Mino-shi, Osaka	LC644708 / VAN12
27	*Hynobius vandenburghi*	Sakai-shi, Osaka	LC644709 / VAN13
28	*Hynobius vandenburghi*	Izumi-shi, Osaka	LC644710 / VAN14
29	*Hynobius vandenburghi*	Gifu-shi, Gifu	AB972627 / GIF9
30	*Hynobius vandenburghi*	Tahara-shi, Aichi	AB266663 / H72
31	*Hynobius vandenburghi*	Tsu-shi, Mie	AB266665 / Tsu
32	*Hynobius vandenburghi*	Shima-shi, Mie	AB266666 / Shimacho
33	*Hynobius vandenburghi*	Otsu-shi, Shiga	AB266667 / Otsu
34	*Hynobius vandenburghi*	Nagahama-shi, Shiga	LC274713 / Tk2
35	*Hynobius vandenburghi*	Maibara-shi, Shiga	LC274716 / Iso12
36	*Hynobius vandenburghi*	Omihachiman-shi, Shiga	LC274709 / Mt5
37	*Hynobius vandenburghi*	Koka-shi, Shiga	LC274699 / nakahatah10
38	*Hynobius vandenburghi*	Takashima-shi, Shiga	LC274701 / Makinoh3
39	*Hynobius vandenburghi*	Hino-cho, Shiga	LC436436 / H74
40	*Hynobius vandenburghi*	Minamiyamashiro-mura, Kyoto	LC436437 / H75
41	*Hynobius vandenburghi*	Ikoma-shi, Nara	LC436438 / H76
42	*Hynobius vandenburghi*	Hannan-shi, Osaka	LC436439 / H77
43	*Hynobius setouchi*	Toyono-cho, Osaka	LC644711 / SET01
44	*Hynobius setouchi*	Misaki-cho, Osaka	LC436432 / H69
	Hynobius setouchi		LC436426 / H. setouchi (Holotype)
	Hynobius abei		LC225433 / H. abei
	Hynobius lichenatus		AB750782 / H. lichenatus
	Hynobius mikawaensis		LC225429 / H. mikawaensis
	Hynobius nigrescens		AB548378 / H. nigrescens
	Hynobius setoi		LC225432 / H. setoi
	Hynobius takedai		LC225430 / H. takedai
	Hynobius tokyoensis		AB266640 / H. tokyoensis
	Salamandrella keyserlingii		NC_026032 / S. keyserlingii

Population number corresponds to the localities in Fig. 1 from which the individuals were collected.

the first 25 % of generations as burn-in. The assessment of monophyly was performed using posterior probability (PP) and bootstrap (BS) values based on the criteria of Huelsenbeck & Rannala (2004) and Hillis & Bull (1993); thus, a monophyletic group was considered to have PP ≥ 0.95 and BP ≥ 70.

Morphological analysis

We sampled 55 individuals of *H. vandenburghi* from February 2019 to March 2021, including 32 individuals (22 males and 10 females) of the Aichi group from three populations (Pops. 1, 3, and 8) and 23 individuals (17 males and 6 females) of the Kinki group from four populations (Pops. 19, 20, 25, and 28) (Table 1; Fig. 1). The collected adults were measured after being anesthetized using ethyl 3-aminobenzoate methane sulfonate salt (Sigma-Aldrich, St. Louis, MO, USA) diluted 1,000-fold in water (Bennett, 1991). For conservation of *H. vandenburghi*, measured adults were returned to their site of capture except for the candidates of type specimens. Before we returned individuals to their site of capture, we took photos of their dorsal, ventral, and lateral sides against a black background, and we also obtained tissue samples (preserved in 99.5 % ethanol) from their tail tips for evidence of collection. All examined adults were measured using a vernier caliper with 22 measurements as follows: snout-vent length (SVL), trunk length (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 width (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), lower jaw length (LJL). For each individual, we also recorded data for the presence of distinct black dots on the dorsum (DBDD), the presence of distinct white dots on the venter (DWDV), the presence of distinct white dots on the lateral side of the body (DWDL), the presence of a distinct and bright yellow line on the dorsal (DBTYLD) and ventral (DBTYLV) sides of the tail, and presence of distinct gular mottling (DGM). The number of costal folds between the adpressed limbs (CFBALN) and the number of costal grooves (CGN) was counted using the method of Matsui *et al.* (2019).

Before performing morphological comparisons between the two groups, we tested for normality using Shapiro–Wilk tests. When data followed a normal distribution, we tested for homoscedasticity using F tests. When population variances were equal, we performed Student's t-tests; when variances were not equal, we performed Welch's t-tests. When data did not follow a normal distribution, we performed Brunner–Munzel tests. To examine the overall morphological variation between the two groups, we performed discriminant analyses using SVL and standardized values (R = %SVL) for 21 measurements as follows: RTRL, RAGD, RHL, RTAL, RMTAW, RMTAH, RVTL, RVTW, RHW, RFLL, RHLL, R2FL, R3FL, R3TL, R5TL, RIND, RIOD, RUEW, RSL, RUEL, RLJL. All statistical analyses were performed in R with $\alpha = 0.05$ (Ihaka & Gentleman, 1996).

For measurements of the holotype and a specimen from the type locality of *H. vandenburghi* (topotype), 43 characteristics were measured including SVL, TRL, AGD, HL, TAL, MTAW, MTAH, basal tail width (BTAW), basal tail height (BTAH), VTL, VTW, HW, maximum head width (MXHW), 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), LJL.

The holotype of a new species described in this study is stored in the Toyohashi Museum of Natural History: 1-238, Oiwacho Oana, Toyohashi-shi, Aichi Prefecture, 441-3147, Japan. A single topotype of *H. vandenburghi* and two paratypes of the new species described in this study are stored in the Kanagawa Prefectural Museum of Natural History: 499, Iryuda, Odawara-shi, Kanagawa Prefecture, 250-0031, Japan. In order to avoid overcollection of these species, further details would be made available only by either contacting the corresponding author or each museum.

Results

The monophyly of the Aichi and Kinki groups of *H. vandenburghi* was supported (but not strongly supported) by PP, whereas it was rejected by BS (Fig. 2). The monophyly of each group was strongly supported by PP and BS (Fig. 2). Genetically, these groups were not divided

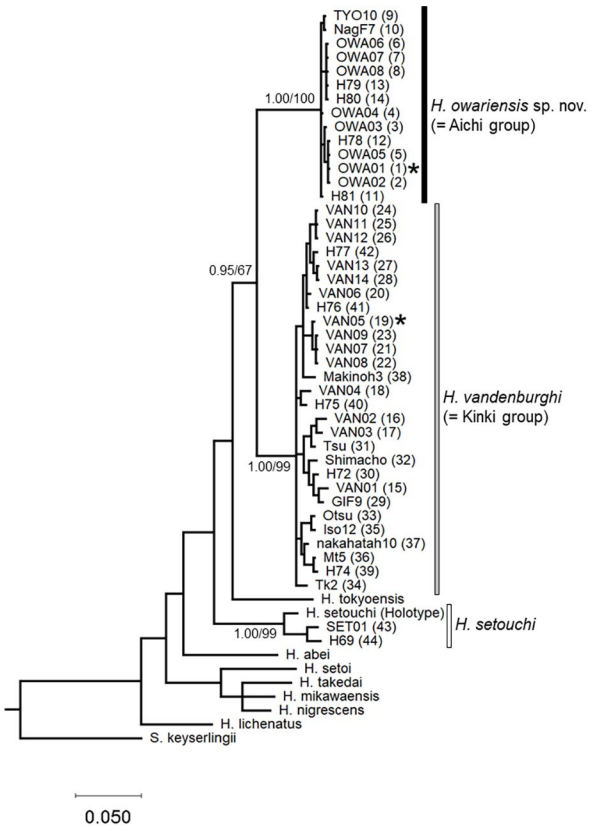

Fig. 2. Phylogenetic tree produced using Bayesian inference (BI) based on 585-bp cytochrome b sequences rooted with *Salamandrella keyserlingii* as an outgroup. Scale represents genetic distance (expect for changes per site). Numbers located near the nodes are posterior probabilities (PP) for BI and bootstrap values (BS) 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 and 19) indicate the type locality of each species.

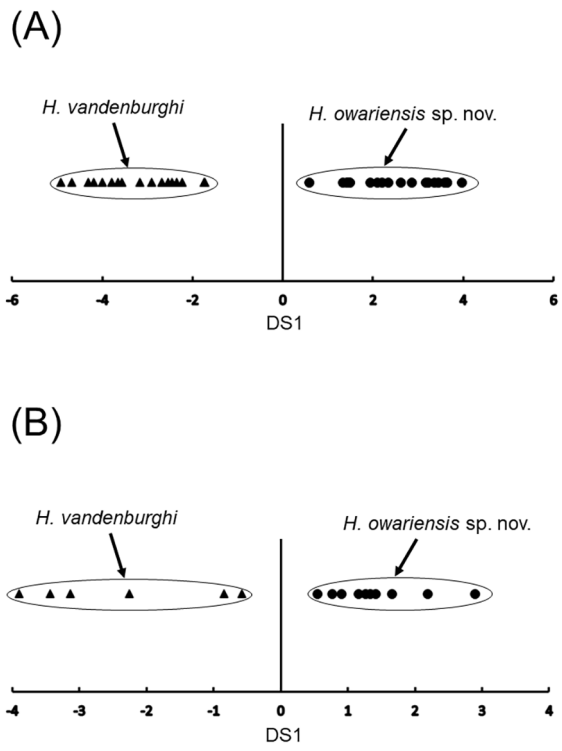

Fig. 3. Results of discriminant analyses of two species for (A) males and (B) females. The x axis indicates "discriminant score 1" (DS1).

into further populations based on PP and BS (Fig. 2).

Morphological measurements of the two groups are shown in Table 2 and the significant values of all measurements between the two groups are listed in Table 3. Males and females of the Aichi and Kinki groups differed significantly in 11 (SVL, RTRL, RAGD, RHL, RMTAH, RVTW, RHLL, R3TL, RUEW, RUEL, and RLJL) and 2 (SVL and RVTL) morphological characteristics, respectively (Table 3). Discriminant analyses indicated that the two groups were different and that the distribution areas of scores did not overlap (Fig. 3). Results of morphological observations are shown in Table 4. Males of the Aichi group almost always had DGM (20/22 = 90.9 %), almost always had no DBDD (20/22 = 90.9 %), usually had no DWDV (18/22 = 81.8 %) and DWDL (19/22 = 86.4 %), usually had 13 costal grooves (18/22 = 81.8 %) and CFBALN ≤ −1.5 (19/22 = 86.4 %), and never had DBTYLD and DBTYLV (22/22 = 100 %). Females of the Aichi group almost always had 13 CGN (9/10 = 90.0 %) and CFBALN ≤ −1.5 (9/10 = 90.0 %), almost always had no DBTYLV (9/10 = 90.0 %), and never had DGM (10/10 = 100 %). Males of the Kinki group always had DBTYLD and DBTYLV (17/17 = 100 %), and usually had 13 CGN (14/17 = 82.4 %) and CFBALN ≥ −1.0 (15/17 = 88.2 %), and usually had no DBDD (15/17 = 88.2 %). Females of the Kinki group always had DBTYLD, DBTYLV, and CFBALN ≤ −1.5 (6/6 = 100 %), usually had 13 CGN (5/6 = 83.3 %), usually had no DBDD (5/6 = 83.3 %), and never had DGM (6/6 = 100 %).

Given the results of molecular and morphological analyses, we described the Aichi group of *H. vandenburghi* as a new species based on three species concepts.

Taxonomy

***Hynobius owariensis* sp. nov.**

(New standard Japanese name: *Owari-sanshouo*)

(Figs. 4–5)

Hynobius nebulosus: Kuzumi and Kakegawa, 1989; Fujitani, 2000; Fujitani *et al.*, 2016: 3, in part.

Hynobius vandenburghi: Matsui *et al.*, 2019: 49, in

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

	H. owariensis sp. nov.			*H. vandenburghi*		
	Holotype	Male	Female	Topotype	Male	Female
Trait		*n* = 22	*n* = 10		*n* = 17	*n* = 6
SVL	58.2	60.6±4.65	59.1±6.46	54.5	53.8±3.30	54.3±1.52
		(51.2-66.7)	(47.3-65.7)		(49.6-60.3)	(52.0-56.0)
RTRL	79.7	77.9±1.31	78.0±3.69	77.1	77.2±0.63	78.0±0.75
		(75.4-82.0)	(69.6-84.5)		(76.2-78.2)	(77.4-79.5)
RAGD	55.7	54.5±1.42	55.7±1.76	53.4	53.4±1.43	56.1±0.78
		(51.7-57.0)	(53.8-59.0)		(50.7-56.1)	(54.6-56.8)
RHL	21.5	22.8±0.89	22.8±0.97	23.3	23.5±0.91	23.0±1.08
		(21.4-24.8)	(21.2-24.0)		(22.2-24.8)	(21.6-24.5)
RTAL	76.5	74.1±7.33	71.1±5.24	74.3	77.8±5.73	71.8±8.30
		(60.9-86.9)	(60.7-77.2)		(65.7-87.3)	(58.1-81.3)
RMTAW	6.9	7.5±0.94	7.3±1.27	5.9	6.9±0.91	7.0±1.53
		(4.9-8.7)	(5.5-9.3)		(5.8-9.0)	(5.1-9.3)
RMTAH	12.0	13.8±1.87	12.5±1.82	10.5	12.2±1.20	12.0±0.79
		(9.8-17.8)	(9.9-15.7)		(10.5-14.0)	(10.5-12.5)
RVTL	4.1	4.8±0.49	4.5±0.42	5	4.8±0.46	5.0±0.31
		(3.8-5.7)	(4.1-5.2)		(3.6-5.6)	(4.6-5.4)
RVTW	5.5	5.5±0.46	5.2±0.27	5.1	5.1±0.47	4.9±0.28
		(4.6-6.3)	(4.7-5.5)		(4.2-5.9)	(4.6-5.3)
RHW	16.8	17.0±0.91	16.7±0.69	15.6	16.7±0.73	15.9±0.70
		(14.9-18.8)	(15.6-17.7)		(15.3-18.1)	(15.3-17.2)
RFLL	21.1	23.1±1.35	21.9±1.95	21.5	23.6±1.53	23.2±1.44
		(21.0-25.6)	(19.1-25.6)		(21.4-26.1)	(21.5-24.9)
RHLL	25.9	28.3±1.56	27.9±2.05	28.6	30.8±1.34	28.6±2.14
		(24.4-30.7)	(25.1-31.1)		(28.5-33.2)	(25.5-30.8)
R2FL	5.0	5.2±0.44	4.5±0.56	5.7	5.2±0.50	4.7±0.28
		(4.4-6.1)	(3.2-5.3)		(4.0-5.7)	(4.4-5.2)
R3FL	3.1	4.2±0.62	4.2±0.49	5.1	4.1±0.71	4.1±0.58
		(2.7-5.3)	(3.2-5.0)		(2.9-5.2)	(3.3-5.0)
R3TL	7.0	7.4±0.62	7.3±0.84	7.2	8.1±0.76	7.5±0.57
		(5.9-8.5)	(6.2-9.0)		(6.8-9.4)	(7.0-8.5)
R5TL	1.2	2.1±0.48	2.0±0.64	2.4	2.1±0.71	2.1±0.21
		(1.1-3.1)	(1.2-3.1)		(1.0-3.3)	(1.8-2.4)
RIND	4.1	4.2±0.52	4.5±0.63	4.6	4.3±0.54	4.4±0.48
		(3.4-5.7)	(3.9-5.7)		(2.9-5.1)	(3.8-5.1)
RIOD	6.4	5.7±0.35	5.8±0.32	6.1	5.7±0.45	5.6±0.47
		(4.9-6.4)	(5.5-6.4)		(4.9-6.9)	(4.9-6.1)
RUEW	2.6	2.8±0.20	3.0±0.31	2.8	3.3±0.31	3.3±0.23
		(2.5-3.2)	(2.5-3.6)		(2.6-3.9)	(2.9-3.6)
RSL	6.4	5.8±0.37	5.8±0.37	6.6	5.9±0.29	5.9±0.29
		(5.2-6.4)	(5.1-6.2)		(5.5-6.6)	(5.4-6.2)
RUEL	4.1	4.2±0.39	4.4±0.30	4.6	4.4±0.25	4.4±0.48
		(3.7-5.6)	(4.1-5.1)		(4.0-4.8)	(3.8-5.1)
RLJL	11.7	12.6±0.60	12.8±0.86	12.8	13.9±0.65	13.4±0.38
		(11.4-13.5)	(11.7-14.2)		(12.8-14.8)	(12.9-13.8)

See Materials and Methods section for definitions of morphological traits.

part; Ichioka *et al.*, 2021

Etymology. The specific epithet "*owariensis*" refers to the old name of the western part of Aichi Prefecture (= Owari) where the new species occurs.

Holotype. An adult male (specimen number: TMNH-AM-78) from Tenpakucho Yagotourayama, Tenpaku-ku, Nagoya-shi, Aichi Prefecture, Japan (35° 08' N, 136° 58' E; elevation = 50 m), collected by Takeshi Fujitani on 18 February 2020. This population is on private land; thus, we obtained permission from the landowner to collect the specimen.

Table 3. Significant values for the 22 morphological characteristics compared between the two species (for both sexes and between sexes of each species)

	OWA vs. VAN		Male vs. Female	
Trait	Males	Females	OWA	VAN
SVL	**P < 0.0001**	P < 0.05	NS	NS
RTRL	P < 0.05	NS	**P < 0.0001**	P < 0.05
RAGD	P < 0.05	NS	P < 0.05	**P < 0.0001**
RHL	P < 0.05	NS	NS	NS
RTAL	NS	NS	NS	NS
RMTAW	NS	NS	NS	NS
RMTAH	P < 0.01	NS	NS	NS
RVTL	NS	P < 0.05	NS	NS
RVTW	P < 0.01	NS	NS	NS
RHW	NS	NS	NS	P < 0.05
RFLL	NS	NS	P < 0.05	NS
RHLL	**P < 0.0001**	NS	NS	P < 0.01
R2FL	NS	NS	P < 0.01	P < 0.01
R3FL	NS	NS	NS	NS
R3TL	P < 0.01	NS	NS	NS
R5TL	NS	NS	NS	NS
RIND	NS	NS	NS	NS
RIOD	NS	NS	NS	NS
RUEW	**P < 0.0001**	NS	**P < 0.0001**	NS
RSL	NS	NS	NS	NS
RUEL	P < 0.01	NS	P < 0.01	NS
RLJL	**P < 0.0001**	NS	NS	NS
P < 0.05	3	2	2	2
P < 0.01	4	0	2	2
P < 0.001	0	0	0	0
P < 0.0001	4	0	2	1
Total	11	2	6	5

OWA and VAN are abbreviations of *Hynobius owariensis* sp. nov. and *H. vandenburghi*, respectively. Larger significant difference values are shown in bold. See Materials and Methods section for definitions of morphological traits.

Fig. 4. Holotype of *Hynobius owariensis* sp. nov. (TMNH-AM-78, adult male, 58.2 mm SVL): (A) dorsal and (B) ventral views.

Paratypes. An adult female (specimen number: KPM-NFA 940) from the same locality of the holotype, collected by Takeshi Fujitani on 28 February. 2020. An adult male (specimen number: KPM-NFA 941) from Odakacho Takayama, Midori-ku, Nagoya-shi, Aichi Prefecture, Japan (35° 03’ N, 136° 56’ E; elevation = 30 m), collected by Takeshi Fujitani on 4 March 2019. This population is also on private land; therefore, we again obtained permission from the landowner to collect specimens.

Diagnosis. A comparatively large species (mean SVL = 60.6 mm in males and 59.1 mm in females) within the Japanese lentic *Hynobius* species: SVL usually > 56 mm in males; ratio of hindlimb length almost always < 30 %SVL in males; distinct and bright yellow stripe on the ventral edge of tail always absent in males and almost always absent in females; distinct and bright yellow stripe on the dorsal edge of tail always absent in males; distinct black dots on the dorsum almost always absent in males; distinct white dots on the ventral and lateral sides of the body usually absent in males; DGM almost always present in males and never present in females; fifth toe of hindlimb always present; U-shaped or V-shaped vomerine teeth series; 13 (rarely 12 or 14) costal grooves; number of costal folds between adpressed limbs usually −3.0 to −1.5 in males and almost always −4.0 to −1.5 in females.

Description of holotype. A moderately large individual: HL slightly larger than HW; TAL shorter than SVL; body almost cylindrical; rounded snout; gular fold present; tail gradually compressed toward the tip; expanded cloaca; webbing between digits absent; four fingers on each forelimb, order of length II > III > IV > I on left and III > II > IV > I on right; five toes on each hindlimb, order of length III > II > IV > I > V on left and III > IV > II > V > I on right; U-shaped vomerine teeth; skin smooth and shiny; DWDV and DWDL absent; DBDD absent; DBTYLD and DBTYLV absent; DGM present. The holotype had the following measurements (in mm): SVL =58.2, TRL = 46.4, AGD = 32.4, HL = 12.5, TAL = 44.5, MTAW = 4.0, MTAH = 7.0, BTAW = 7.6, BTAH = 6.4, VTL = 2.4, VTW = 3.2, HW = 9.8, MXHW = 10.1, LFLL = 12.3, RFLL = 10.7, LHLL = 15.1, RHLL = 14.6, L1FL = 1.3, L2FL = 2.9, L3FL = 1.8, L4FL = 1.5, R1FL = 0.9, R2FL = 1.4, R3FL = 1.8, R4FL = 1.3, L1TL = 1.5, L2TL = 3.2, L3TL = 4.1, L4TL = 2.9, L5TL = 0.7, R1TL = 0.9, R2TL

= 2.9, R3TL = 3.8, R4TL = 3.1, R5TL = 1.2, IND = 2.4, IOD = 3.7, LUEW = 1.5, RUEW = 1.1, SL =3.9, LUEL =2.4, RUEL = 2.6, LJL = 6.8, CGN = 13.

Comparisons. The new species resembles *H. vandenburghi* in morphology but differs statistically from it in the following length measurements: SVL, RTRL, RAGD, RHL, RMTAH, RVTW, RHLL, R3TL, RUEW, RUEL, and RLJL in males and SVL and RVTL in females; the lengths of these measurements, except for SVL, RTRL, RAGD, RMTAH, and RVTW in males and SVL in females, are significantly shorter in *H. owariensis* sp. nov. than in *H. vandenburghi*. In males, *H. owariensis* sp. nov. differs from *H. vandenburghi* by the following combination of characters: SVL > 56 mm (18/22 = 81.8 %) vs. SVL < 56 mm (14/17 = 82.4 %); RHLL shorter than 30 % (20/22 = 90.9 %), vs. RHLL of 30 % or longer (14/17 = 82.4 %); usually have CFBALN ≤ −1.5 (19/22 = 86.4 %) vs. usually have CFBALN ≥ −1.0 (15/17 = 88.2 %); always lack DBTYLD and DBTYLV (22/22 = 100 %) vs. always have DBTYLD and DBTYLV (17/17 = 100 %). In females, *H. owariensis* sp. nov. almost always lacks DBTYLV (9/10 = 90 %), whereas *H. vandenburghi* always have DBTYLV (6/6 = 100 %).

Table 4. Characteristics of skin markings between the two species of *Hynobius*

		H. owariensis sp. nov.		*H. vandenburghi*	
		Male	Female	Male	Female
Character	Condition	*n* = 22	*n* = 10	*n* = 17	*n* = 6
DBDD	Absent	20 (90.9%)	7 (70.0%)	15 (88.2%)	5 (83.3%)
	Present	2 (9.1%)	3 (30.0%)	2 (11.8%)	1 (16.7%)
DWDV	Absent	18 (81.8%)	4 (40.0%)	11 (64.7%)	3 (50.0%)
	Present	4 (18.2%)	6 (60.0%)	6 (35.3%)	3 (50.0%)
DWDL	Absent	19 (86.4%)	7 (70.0%)	11 (64.7%)	3 (50.0%)
	Present	3 (13.6%)	3 (30.0%)	6 (35.3%)	3 (50.0%)
DBTYLD	Absent	22 (100%)	5 (50.0%)	0 (0%)	0 (0%)
	Present	0 (0%)	5 (50.0%)	17 (100%)	6 (100%)
DBTYLV	Absent	22 (100%)	9 (90%)	0 (0%)	0 (0%)
	Present	0 (0%)	1 (10%)	17 (100%)	6 (100%)
DGM	Absent	2 (9.1%)	10 (100%)	10 (58.8%)	6 (100%)
	Present	20 (90.9%)	0 (0%)	7 (41.2%)	0 (0%)
CGN	12	2 (9.1%)	0 (0%)	3 (17.6%)	0 (0%)
	13	18 (81.8%)	9 (90.0%)	14 (82.4%)	5 (83.3%)
	14	2 (9.1%)	1 (10.0%)	0 (0%)	1 (16.7%)
CFBALN	1.0	0 (0%)	0 (0%)	1 (5.9%)	0 (0%)
	0.5	0 (0%)	0 (0%)	1 (5.9%)	0 (0%)
	0.0	0 (0%)	0 (0%)	5 (29.4%)	0 (0%)
	-0.5	1 (4.5%)	1 (10.0%)	1 (5.9%)	0 (0%)
	-1.0	2 (9.1%)	0 (0%)	7 (41.2%)	0 (0%)
	-1.5	10 (45.5%)	2 (20.0%)	2 (11.8%)	3 (50.0%)
	-2.0	4 (18.2%)	2 (20.0%)	0 (0%)	0 (0%)
	-2.5	4 (18.2%)	0 (0%)	0 (0%)	0 (0%)
	-3.0	1 (4.5%)	1 (10.0%)	0 (0%)	3 (50%)
	-3.5	0 (0%)	3 (30.0%)	0 (0%)	0 (0%)
	-4.0	0 (0%)	1 (10.0%)	0 (0%)	0 (0%)

Values indicate the number of individuals exhibiting that characteristic with percentages show for each condition in parentheses. See Materials and Methods section for definitions of morphological characteristics.

Variation. Morphometric measurements are presented in Table 2 and the significant values of all measurements between sexes are listed in Table 3. Males have relatively longer RFLL and R2FL than females, whereas males have relatively shorter RTRL, RAGD, RUEW, and RUEL than those of females. The skin markings of *H. owariensis* sp. nov. are listed in Table 4. The dorsum is uniformly yellowish-brown or darkish-brown. DBDD is rarely present in males (2/22 = 9.1 %) and sometimes present in females (3/10 = 30 %). The venter is lighter than the dorsum. DWDV is rarely present in males (4/22 = 18.2 %) and sometimes absent in females (4/10 = 40 %). DWDL is rarely present in males (3/22 = 13.6 %) and rarely present in females (3/10 = 30.0 %). In females, DBTYLD is sometimes present (5/10 = 50 %) and DBTYLV is rarely present (1/10 = 10 %). DGM is rarely absent in males (2/22 = 9.1 %). CGN is rarely 12 (2/22 = 9.1 %) or 14 (2/22 = 9.1 %) in males and rarely 14 in females (1/10 = 10.0 %). CFBALN is rarely > −1.5 in males (3/22 = 13.6 %) and rarely > −1.0 (1/10 = 10 %) in females. The iris is dark brown. When preserved in 70 % ethanol, the dorsal coloration tends to fade to dark gray. The indistinct yellowish line on the dorsal and ventral sides of the tail (e.g.,

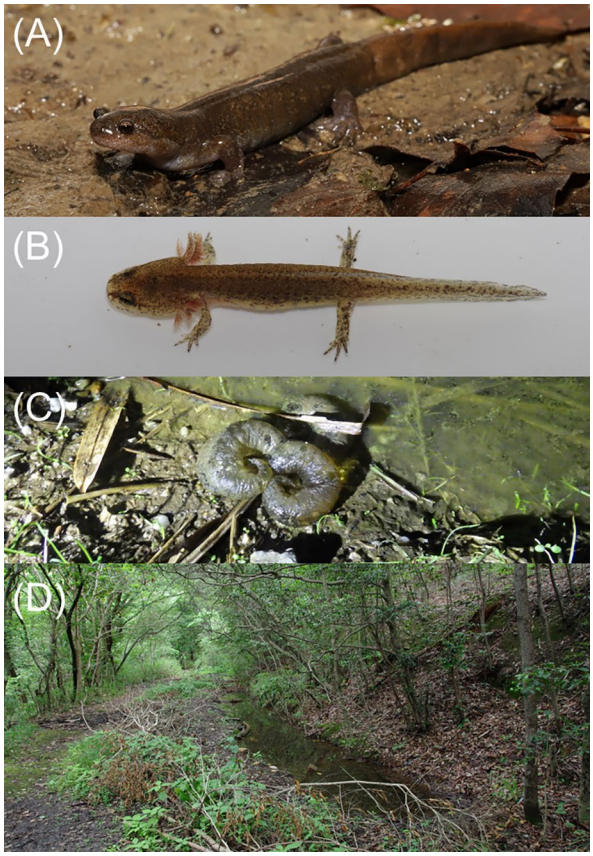

Fig. 5. (A) Live holotype of *Hynobius owariensis* sp. nov. (TMNH-AM-78), and the (B) larva, (C) banana-shaped egg sacs, and (D) type locality of the new species.

Fig. 4) is difficult to confirm after preservation.

Natural History. The main vegetation in the surrounding habitat of the new species is an evergreen forest of Fagaceae trees (i.e., *Castanopsis* and *Quercus* spp.) (Fig. 5). Larvae have black spots on the lateral sides of their body and tail, and they have no claws on the tips of their fingers and toes. In the early developmental stages of the larvae, they have one pair of balancers. Egg sacs are banana-shaped and are attached to fallen leaves or branches in puddles or ponds at forest edges from February to April.

Distribution. It is known from Nagoya-shi (including the Midori-ku, Tenpaku-ku, Moriyama-ku, Chikusa-ku, and Meito-ku), Tokai-shi, Chita-shi, Tokoname-shi, Seto-shi, Toyota-shi (only former Fujioka-cho), Nagakute-shi, and Owariasahi-shi, and Higashiura-cho, Agui-cho, Mihama-cho, Taketoyo-cho, and Minamichita-cho (Matsui *et al.*, 2019) in Aichi Prefecture. In this study, DNA data from Higashiura-cho and Agui-cho were not included, but adult specimens from these towns are stored in the Toyohashi Museum of Natural History (confirmed by T. Fujitani). It is possible that this species may also be distributed in Handa-shi, but reliable records on its inhabitation are not available. Probably, the populations of Tokai-shi, Chita-shi, and Tokoname-shi, as well as Higashiura-cho, Agui-cho, and Taketoyo-cho, were already extinct by 2021 based on our field surveys.

Remarks. The new species forms a monophyletic group with *H. vandenburghi* (Matsui *et al.*, 2019). The results of our study support this hypothesis by BI, but the posterior probability is not high (Fig. 2).

***Hynobius vandenburghi* Dunn, 1923**

(Standard Japanese name: *Yamato-sanshouo*)

(Fig. 6)

Hynobius nebulosus: Fujitani *et al.*, 2016: 3, in part.

Holotype. An adult male (specimen number: CAS 26714) from Nara, Yamato Province, Hondo, collected by Victor Kühne (an alias used by John Cheesman Thompson) (Beolens *et al.*, 2011) in May 1907 (Dunn, 1923a; Dunn, 1923b). This specimen is stored in the California Academy of Sciences: 55 Music Concourse Drive, San Francisco, California, 94118, United States.

Diagnosis. A comparatively small species (with a mean SVL of 53.8 mm in males and 54.3 mm in females) within the Japanese lentic salamander species complex of Hynobius: SVL usually < 56 mm in males; the ratio of hindlimb length usually ≥ 30 %SVL in males; distinct and bright yellow stripe on the dorsal and ventral edges of the tail always present in both sexes; distinct black dots on dorsum usually absent in both sexes; distinct gular mottling never present in females; fifth toe of hindlimb always present; V-shaped or U-shaped vomerine teeth series; 13 (rarely 12 or 14) costal grooves; the number of costal folds between adpressed limbs usually > −1.5 in males and always < −1.0 in females.

Fig. 6. A specimen of *Hynobius vandenburghi* (KPM-NFA 942, adult male) from the type locality: (A) dorsal, (B) ventral, and (C) lateral views.

Description of a specimen from the type locality (Topotype). An adult male (specimen number: KPM-NFA 942) from Nakamachi, Nara-shi, Nara Prefecture, Japan (34° 40’ N, 135° 43’ E; elevation = 230 m), collected by Shota Seguchi on 1 March 2020. This specimen was collected after obtaining collection permission from The Unit of Natural Environment, Division of Landscape and Natural Environment, Department of Water Cycle, Forest, and Landscape Environment, Nara Prefecture. 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; slightly expanded cloaca; webbing between digits absent; four fingers on each forelimb, order of length II > III > IV > I on left and II > III > I > IV on right; five toes on each hindlimb, order of length III > IV > II > I > V on left and III > II > IV > V > I on right; V-shaped vomerine

teeth; skin smooth and shiny; DWDV and DWDL absent; DBDD absent; DBTYLD and DBTYLV present; DGM absent (but indistinct gular mottling present). This specimen had the following measurements (in mm): SVL =54.5, TRL = 42.0, AGD = 29.1, HL = 12.7, TAL = 40.5, MTAW = 3.2, MTAH = 5.7, BTAW = 5.1, BTAH = 5.0, VTL = 2.7, VTW = 2.8, HW = 8.5, MXHW = 8.8, LFLL = 11.7, RFLL = 11.8, LHLL = 15.6, RHLL = 14.9, L1FL = 1.1, L2FL = 3.1, L3FL = 2.8, L4FL = 1.3, R1FL = 1.2, R2FL = 3.4, R3FL = 2.7, R4FL = 1.1, L1TL = 1.5, L2TL = 3.3, L3TL = 3.9, L4TL = 3.4, L5TL = 1.3, R1TL = 1.2, R2TL = 3.3, R3TL = 3.7, R4TL = 3.1, R5TL = 1.7, IND = 2.5, IOD = 3.3, LUEW = 1.5, RUEW = 1.4, SL = 3.6, LUEL =2.5, RUEL = 2.4, LJL = 7.0, CGN = 13.

Variation. Morphometric measurements are presented in Table 2 and the significant values of all measurements between sexes are listed in Table 3. Males have relatively longer RHW, RHLL, and R2FL than those of females, whereas males have relatively shorter RTRL and RAGD than those females. Skin markings are listed in Table 4. The dorsum is uniformly yellowish-brown or darkish-brown. DBDD are rarely present in males (2/17 = 11.8 %) and females (1/6 = 16.7 %). The venter is lighter than the dorsum. DWDV are often lacking in males (11/17 = 64.7 %) and sometimes present in females (3/6 = 50.0 %). DWDL are frequently lacking in males (11/17 = 64.7 %) and sometimes present in females (3/6 = 50.0 %). DGM sometimes lacking in males (10/17 = 58.8 %). CGN rarely 12 in males (3/17 = 17.6 %) and rarely 14 in females (1/6 = 16.7 %). CFBALN rarely < −1.0 in males (2/17 = 11.8 %) and sometimes > 2.0 in females (3/6 = 50 %). Iris is dark brown. When preserved in 70 % ethanol, dorsal coloration tends to fade to dark gray. DBTYLD and DBTYLV can be confirmed after preservation.

Distribution. It is known from Tahara-shi (only former Tahara-cho and Atsumi-cho), Aichi Prefecture (Matsui *et al.*, 2019), Gifu-shi (only former Gifu-shi), Seki-shi (only former Seki-shi), Kakamigahara-shi (only former Kakamigahara-shi), and Kaizu-shi (only former Nanno-cho), and Ibigawa-cho (only former Tanigumi-mura), Gifu Prefecture (Matsui *et al.*, 2019; Sakai *et al.*, 2019; Gifu High School, 2018), Nagahama-shi (only former Nagahama-shi, and Azai-cho and Kinomoto-cho), Maibara-shi (former Maibara, Omi-cho, and Santo-cho), Hikone-shi, Omihachiman-shi (only former Omihachiman-shi), Konan-shi (only former Kosei-cho), Higashiomi-shi (only former Yokaichi-shi and Gamo-cho), Koka-shi (only former Minakuchi-cho, Konan-cho, Koka-cho, and Tsuchiyama-cho), Ritto-shi, Kusatsu-shi, Otsu-shi (only former Otsu-shi), Takashima-shi (only former Adogawa-cho, Shin-asahi-cho, Imazu-cho, and Makino-cho), and Hino-cho and Ryuo-cho , Shiga Prefecture (Tago, 1931; Kokashi-Minakuchi-Kodomonomori-Shizenkan, 2013; Mito *et al.*, 2018; Matsui *et al.*, 2019), Kuwana-shi (only former Tado-cho), Inabe-shi (only former Inabe-cho), Suzuka-shi, Kameyama-shi (only former Kameyama-shi), Iga-shi (only former Ueno-shi), Tsu-shi (only former Tsu-shi and Hisai-shi, and Kawage-cho, Ano-cho, Hakusan-cho, and Ichishi-cho), Matsusaka-shi (only former Matsusaka-shi and Ureshino-cho), and Shima-shi (only former Ago-cho, Daio-cho, and Shima-cho), Mie Prefecture (Miyamoto, 2001; Shimizu, 2014; Matsui *et al.*, 2019), Nara-shi (only former Nara-shi), Yamatokoriyama-shi, and Ikoma-shi, and Oyodo-cho, Nara Prefecture (Matsui *et al.*, 2019), Kyoto-shi (only former Kyoto-shi of Nishikyo-ku, Higashiyama-ku, and Fushimi-ku), Nagaokakyo-shi, Kyotanabe-shi, Kizugawa-shi (only former Kizu-cho and Kamo-cho), Uji-shi and Kameoka-shi, Oyamazaki-cho, Seika-cho, and Ujitawara-cho, and Minamiyamashiro-mura, Kyoto Prefecture (Tanabe & Matsui, 2002; Matsui *et al.*, 2019), Toyonaka-shi, Mino-shi, Ibaraki-shi, Takatsuki-shi, Hirakata-shi, Katano-shi, Shijonawate-shi, Higashiosaka-shi, Tondabayashi-shi, Sakai-shi (only former Sakai-shi), Izumi-shi, and Hannan-shi, Osaka Prefecture (Osaka Prefecture, 1978; Matsui *et al.*, 2019). Populations from Nantan-shi (only former Sonobe-cho) of Kyoto Prefecture (Tanabe & Matsui, 2002) are lacking DNA data. This population is adjacent to the distribution area of *Hynobius setouchi*, so there is a possibility that this is the *H. setouchi* population. DNA analyses including samples from this population are essential for deciding the distribution range of *H. vandenburghi.*

Remarks. We examined a male specimen (specimen number: TMNH-AM-70) from Tahara-shi stored in the Toyohashi Museum of Natural History (1-238, Oiwacho Oana, Toyohashi-shi, Aichi Prefecture, 441-3147), but DBTYLD was not clear. The line of this specimen may have already faded; thus, the presence of DBTYLD still requires confirmation in several living individuals from the area after obtaining *Hynobius vandenburghi* collection permission from Tahara-shi. *H. vandenburghi* is also parapatrically distributed with *H. setouchi*, but it has no DBTYLD and DBTYLV.

Discussion

According to Matsui *et al.* (2019), *H. owariensis* sp. nov. and *H. vandenburghi* are the same species. In our study, the monophyly of the two species was also

supported by BI based on the criteria of Huelsenbeck & Rannala (2004), but the probability was very low (Fig. 2). However, the monophyly was rejected by ML based on the criteria of Hillis & Bull (1993) (Fig. 2). Therefore, the monophyly of the two species was not strongly supported by our molecular analyses. On the other hand, the two species were morphologically distinguishable based on our analyses (Tables 2–4; Fig. 3). Males of *H. vandenburghi* usually have yellow stripes on the edges of their tail based on the diagnosis of *H. vandenburghi* (Matsui *et al.*, 2019); however, all examined males from *H. owariensis* sp. nov. did not have clear yellow stripes on the dorsal and ventral sides of their tail (Table 4). Therefore, *H. owariensis* sp. nov. does not strictly satisfy the diagnosis of *H. vandenburghi* described by Matsui *et al.* (2019). Furthermore, statistical analyses using morphological data suggested that these two groups were distinguishable (Table 3; Fig. 3). Furthermore, they are also genetically distinguishable based on the phylogenetic analyses using allozyme and mitochondrial data (Fig. 2) (Matsui *et al.*, 2006; Matsui *et al.*, 2019). Thus, we conclude that *H. owariensis* sp. nov. should be distinct species based on the morphological, phylogenetic, and evolutionary species concepts.

The distribution area of *H. vandenburghi* was limited to Tokai and Kinki Districts (Matsui *et al.*, 2019); it is not distributed in the Chita Peninsula and surrounding areas of Nagoya-shi, Aichi Prefecture (Figs 1–2). Following this description, the eastern, northern, southern, and western limits of the distribution range for *H. vandenburghi* are as follows: the Tahara-shi (Aichi Prefecture) and Seki-shi (Gifu Prefecture) line, Nagahama-shi (Shiga Prefecture) and Ibigawa-cho (Gifu Prefecture) line, Shima-shi (Mie Prefecture) and Hannan-shi (Osaka Prefecture) line, and Mino-shi (Osaka Prefecture) and Hannan-shi line, respectively. The distribution areas of *H. vandenburghi* and *H. setouchi* are adjacent in the northwestern and southwestern parts of Osaka Prefecture (Fig. 1; Table 1). According to Matsui *et al.* (2019), the boundary of the two species in the southwestern part of Osaka Prefecture is located in Hannan-shi (Pop. 42) and Misaki-cho (Pop. 44) (See Fig. 1). In addition, our analyses suggested that the boundary for the two species' distributions in the northwestern part of Osaka Prefecture is located in Mino-shi (Pop. 26) and Toyono-cho (Pop. 43) (Fig. 1). In our molecular analyses, samples from Kyoto Prefecture only included single sequencing data (Pop. 40) obtained by Matsui *et al.* (2019) because *H. vandenburghi* is protected by law in Kyoto Prefecture and collection is not allowed without permission. According to Tanabe & Matsui (2002), *H. nebulosus* (which currently is *H. vandenburghi*) is distributed in Nantan-shi of Kyoto Prefecture, but this species is not distributed in the adjacent area. Further molecular analyses, including samples from Nantan-shi, are required to clarify the boundary of *H. vandenburghi* and *H. setouchi*.

Hynobius vandenburghi is widely distributed in Tokai to Kinki regions, but our analyses only included individuals from Nara and Osaka Prefecture. Thus, there is a possibility that additional morphological variation exists. However, populations from Aichi (only Atsumi Peninsula), Gifu, Shiga, and Kyoto Prefectures are protected by the laws of the local governments; hence, we were unable to include these populations in our morphological analyses. Additionally, the present study did not include the Mie populations, which are not protected by local government law, because we could not obtain normal individuals from the Mie populations. Further morphological analyses, including individuals from the Mie and other populations, are therefore needed (following *H. vandenburghi* collection permission being granted by each local government) to reach a final decision on the diagnosis of *H. vandenburghi.* Based on our field surveys and previous reports (e.g., Sakai *et al.*, 2018), populations of *H. vandenburghi* are threatened with extinction. Considering that *H. vandenburghi* has a wider distribution range than *H. owariensis* sp. nov., some populations might already be extinct (e.g., former Tahara-cho of Aichi Prefecture, Kuwana-shi of Mie Prefecture, Toyonaka-shi, Tondabayashi-shi, and Higashi-osaka-shi of Osaka Prefecture). To save this species from extinction, reassessment of the conservation status of *H. vandenburghi* should be performed after widely incorporating the opinions of actors who conserve this species locally and without being overly focused on the apparent scale of the distribution area of *H. vandenburghi.*

Hynobius owariensis sp. nov. is found only at the Chita Peninsula and surrounding areas of Nagoya-shi (Fig. 1); however, the introduction of *H. vandenburghi* to Nagoya-shi has been confirmed in a specific park of the city (Fujitani *et al.*, 2016). To avoid a more artificial introduction of *H. vandenburghi*, information on the taxonomic revision of *H. vandenburghi* should be widely and immediately disseminated. This new species exists in very fragmented habitats and all populations are unsustainable because of their small population size or location close to development areas. Therefore, the viability of *H. owariensis* sp. nov. may be strongly affected by catastrophes or human activities such as deforestation and development. As previously mentioned, several populations (e.g., Tokai-shi, Chita-shi, and Tokoname-shi, and Higashiura-cho,

Agui-cho, and Taketoyo-cho) of *H. owariensis* sp. nov. might already be extinct based on evidence from our field surveys. Following this description, the conservation status of the new species must therefore be reassessed immediately to ensure that essential conservation strategies are implemented and *H. owariensis* sp. nov. is saved from extinction.

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摘　要

菅原弘貴・藤谷武史・瀬口翔太・澤畠拓夫・永野昌博 , 2022. ヤマトサンショウウオ *Hynobius vandenburghi* の分類学的再検討：日本の本州中部からの 1 新種の記載 . 神奈川県立博物館研究報告（自然科学）, (51): 47–59. [Sugawara, H., T. Fujitani, S. Seguchi, T. Sawahata & M. Nagano, 2022. Taxonomic Re-examination of the Yamato Salamander *Hynobius vandenburghi*: Description of a New Species from Central Honshu, Japan. *Bull. Kanagawa Pref. Mus.* (*Nat. Sci.*), (51): 47–59.]

サンショウウオ属の 1 新種を、日本の愛知県西部から記載した。分子遺伝学的および形態学的解析の結果、ヤマトサンショウウオは愛知グループと近畿グループの二つに分けられることが示唆された。このため、ヤマトサンショウウオの愛知グループを、新種 *Hynobius owariensis* sp. nov.（和名：オワリサンショウウオ）として記載した。形態比較の結果、調査した雄個体において、ヤマトサンショウウオが尾の上下縁に明瞭かつ鮮明な黄色線をもつのに対して、本新種ではこの形質が確認できなかった。さらに、雄個体において、体側に沿って前肢と後肢を伸ばした時、本新種は多くの個体が肋皺 1 個分よりも離れるが、ヤマトサンショウウオでは多くの個体が肋皺 1 個分以内（個体によっては重複する）に収まっていた。その他、両種間には有意に異なる形質が複数存在していることに加えて、判別分析の結果においても、雌雄共に形態的に区別可能であることが示唆された。本新種は愛知県の西部（知多半島から名古屋市周辺部）に固有であるが、既に絶滅したと考えられる集団も複数存在し、現在も開発や乾田化によって、絶滅の危機に瀕している。