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Systematics of the Widely Distributed Japanese Clouded Salamander, *Hynobius nebulosus* (Amphibia: Caudata: Hynobiidae), and Its Closest Relatives

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Abstract: The abundant clouded salamander from western Japan, *Hynobius nebulosus*, has long been considered a single widespread species, although some authors have suggested the inclusion of several cryptic species. This led to a molecular and morphological analysis of populations from all parts of the known range. Phylogenetic relationships were inferred from complete sequences of the mitochondrial cytochrome b gene, and nuclear genome differentiations were estimated by multiplexed inter simple sequence repeat genotyping by sequencing (MIG-seq). The results suggest that *H. nebulosus* hitherto recognized consists of at least nine species. We applied existing names to two of them, *H. nebulosus* (Temminck et Schlegel) and *H. vandenburghi* Dunn, and described seven others as new species.

Key words: Cryptic species; Japan; New species; Phylogeny; Taxonomy

Introduction

The Japanese clouded salamander, *Hynobius nebulosus*, originally described as *Salamandra nebulosa* from Nagasaki, Kyushu

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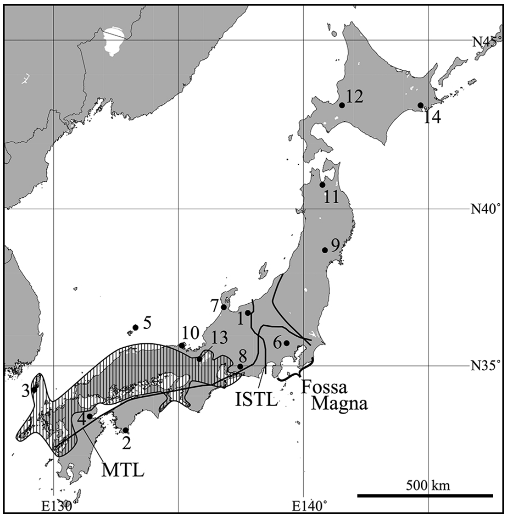

FIG. 1 Range of salamanders hitherto assigned to *Hynobius nebulosus* (hatched) and localities of Japanese congeners genetically compared (1. *H. hidamontanus*, 2. *H. tosashimizuensis*, 3. *H. tsuensis*, 4. *H. dunni*, 5. *H. okiensis*, 6. *H. tokyoensis*, 7. *H. takedai*, 8. *H. mikawaensis*, 9. *H. nigrescens*, 10. *H. abei*, 11. *H. lichenatus*, 12. *H. retardatus*, 13. *H. kimurae,* 14: *S. keyserlingii*). Positions of Major (Median) Tectonic Line (MTL), Itoigawa-Shizuoka Tectonic Line (ISTL), and Fossa Magna are shown (Yanai et al., 2010).

(Temminck et Schlegel, 1838), is the type species of the genus *Hynobius* Tschudi, 1838, which is the type genus of the family Hynobiidae Cope, 1859. The genus *Hynobius* is now very large containing about 40 species from Japan, Taiwan, Korea, and China (excluding the enigmatic *H. turkestanucus* Nikolskii, 1910 from central Asia: Frost, 2018) with the center of speciation in Japan. *Hynobius nebulosus* occurs mainly in the lowlands of western Japan, including the mainland and adjacent small islands (Fig. 1). It is basically a lentic breeder and usually breeds in still waters, such as wetlands and small pools (Sato, 1943), but some populations from the Chugoku District in western Honshu breed in high altitude wetlands and sometimes in small streams (Okawa and Utsunomiya, 1989).

Although *H. nebulosus* is currently viewed as a representative widespread salamander in western Japan, contents of this species experienced complicate taxonomic history. Dunn (1923a) described *H. vandenburghi* from Nara, Yamato Province, Hondo (mainland of Japan), *H. ikishimae* from Iki-shima Is., Tsushima Straits, *H. bicolor* from southern Tsushima Is., and *H. tagoi* from northern Tsushima Is., all as allied to *H. nebulosus* from Kyushu. He (Dunn, 1923b) further proposed the name "*nebulosus* group" to accommodate them, where *H. bicolor* was synonymized with *H. tsuensis* Abe, 1922.

However, Oyama (1930) found the characters listed by Dunn (1923a) to differentiate *H. ikishimae* and *H. nebulosus* are all unreliable. For the taxonomic study of the *H. nebulosus* group, Sato (1934) used skull morphology, which he found useful in interspecific comparisons of *H. kimurae* Dunn, 1923 (Sato, 1933), and completely confirmed Oyama's (1930) taxonomic idea about *H. nebulosus* and *H. ikishimae*. Regarding *H. tsuensis* and *H. tagoi* from Tsushima Is., Sato (1934) closely examined body color, which Dunn (1923a) used to differentiate them, of a large number of specimens, and found them quite variable. He also studied egg sacs and skull morphology and concluded them conspecific, as to be called *H. tsuensis*. Additionally, Sato (1934) doubted the heterospecific status of *H. vandenburghi* and *H. nebulosus*, and because they were differentiated only by subtle differences in coloration and tail shape, he (Sato, 1934) considered them as conspecific.

Another topic related to the taxonomy of *H. nebulosus* is the discovery of a salamander from Nagoya, Chubu District of central Honshu. It was identified by Sato (1934) as *H. tokyoensis* Tago, 1931, which had been known from the Kanto District disjunct from Chubu, and unequivocally considered to be closely related but distinct from *H. nebulosus* (Sato, 1934, 1943). However, Ebitani (1952) found *H. tokyoensis* from Chubu and *H. nebulosus* from Nagasaki to be very similar in skull morphology, and suggested their conspecificity. Additionally, Kawamura (1953) obtained fertile hybrids among some populations of these two species. Nakamura and Uéno (1963) thus treated them as two different subspecies, *H. n. nebulosus* and *H. n. tokyoensis*.

Morphological and ecological studies of *H. nebulosus* from western Japan were initiated in the 1980s by Utsunomiya and Okawa (1987), and presence of several distinct morphotypes has been reported (e.g., Okawa et al., 2009). Similarly in the 1980s, biochemical techniques became applied to salamander systematics in Japan (Matsui and Miyazaki, 1984) and in Matsui (1987), relationships of *H. nebulosus* with some other species were assessed based on allozyme variations. Also, through allozymic comparisons, Matsui et al. (2001) found *H. tokyoensis* from Chubu to be conspecific with *H. nebulosus*, but heterospecific with *H. tokyoensis* from Kanto District. Matsui et al. (2006) made a comprehensive allozymic variation study in 46 populations of *H. nebulosus*, and recognized two major groups (western group from Kyushu to westernmost Chugoku, and an eastern group from Shikoku through Kinki to Chubu) and two less clearly defined groups (montane group from the Chugoku Mountains, and a Chugoku group from northern coastal Chugoku).

Meanwhile, mitochondrial DNA (mtDNA) analyses were applied to the study of genetic variations and phylogenetic relationships of *Hynobius* salamanders (e.g., Matsui et al., 2007a; Aoki et al., 2013). Matsui et al. (2007a) studied complete sequences of cytochrome b (cyt b) and control region (CR) genes among populations of *H. tokyoensis*, where populations of *H. nebulosus* from Chubu (Aichi, Mie) and Kinki regions were included. Confirming Matsui et al. (2001), populations of *H. nebulosus* formed a clade, which was sister to the clade of *H. tokyoensis*. Aoki et al. (2013) also performed mitochondrial cyt b analysis of *H. lichenatus* Boulenger, 1883, and found distinct clades.

Molecular phylogenetic studies based on mtDNA analysis revealed cryptic diversity within species of other Japanese small salamanders (e.g., Tominaga et al., 2006; Yoshikawa et al., 2008), and using mtDNA markers for *H. nebulous* is expected to be effective for elucidating intraspecies cryptic diversity. However, no detailed DNA study of *H. nebulosus* has been done that is equivalent to allozymic study by Matsui et al. (2006) or studies on *H. tokyoensis* (Matsui et al., 2007a) and *H. lichenatus* (Aoki et al., 2013).

Instead, several *H. nebulosus* have been used as reference in the study of other species. In describing *H. mikawaensis* Matsui, Misawa, Nishikawa et Shimada, 2017, Matsui et al. (2017a) used mitochondrial cyt b gene to estimate phylogeny, and included *H. nebulous* from three separate regions (Aichi, Tottori, and Nagasaki). As a result, samples of *H. nebulosus* from three populations did not form a clade with each other, but the Tottori sample was sister species to the clade of *H. mikawaensis*, *H. takedai* Matsui et Miyazaki, 1984 and *H. nigrescens* Stejneger, 1907, Aichi sample to *H. tokyoensis*, and the Nagasaki sample to *H. dunni* Tago, 1931. Genetic divergences between different populations as estimated by uncorrected p-distance were larger than those observed among recognized species. The study demonstrated high variation and cryptic genetic diversity within *H. nebulosus*, which has long been studied since Dunn (1923a, b).

Thus, *H. nebulosus*, currently treated as a single species, is surely a composite of cryptic species, yet the magnitude and pattern of geographical differentiation of this species are still unclear. Therefore, a comprehensive mtDNA and nuclear DNA survey was conducted to elucidate geographical variation and genealogical structure throughout the range of salamanders now called *H. nebulosus* and to define the boundaries of species within them.

MATERIALS AND METHODS

Voucher specimens are stored in the Graduate School of Human and Environmental Studies, Kyoto University (KUHE), and the private collection of Mr. H. Okawa (OU) at Kure, Hiroshima, Japan. Additionally, collections of Dr. T. Seto (Se), Mr. T. Sugahara (Su), and Mr. S. Tanabe (T) were examined. Populations of salamanders treated here are declining and mostly listed in national and local governmental Red Data Books. Thus the locality data are given not in detail so as to prevent salamanders from illegal collection.

Genetic analyses

MtDNA analyses

For genetic comparisons, we obtained the complete sequence data of the gene encoding cyt b of mtDNA from muscle or liver tissue samples preserved in 99% ethanol. Methods for DNA extraction, and amplification and sequencing of the mtDNA fragments are the same as those reported by Aoki et al. (2013). In order to know phylogenetic relationships among populations of *H. nebulosus*, we preliminarily sequenced 286 individuals from localities representing whole of the current distributional range of the species. From the clades recognized, we chose 91 samples and re-analyzed them together with members of the *nebulosus* group of Dunn (1923b) and Sato (1943) (*H. tsuensis*, *H. dunni*, and *H. tokyoensis*) and other primarily lentic-breeding Japanese *Hynobius* (*H. lichenatus, H. abei* Sato, 1934, *H. nigrescens*, *H. mikawaensis, H. takedai*, *H. okiensis* Sato, 1940, *H. tosashimizuensis* Sugawara, Watabe, Yoshikawa et Nagano, 2018, and *H. hidamontanus* Matsui, 1987). We also added GenBank data of a lotic-breeder *H. kimurae* and outgroup species *H. retardatus* Dunn, 1923 and *Salamandrella keyserlingii* Dybowski, 1870 (Table 1) for comparisons. We deposited the newly obtained sequences in GenBank (Accession numbers LC436366–436448, 437992). For tree construction and calculation of genetic distances (uncorrected p-distance), we followed Aoki et al. (2013).

MIG-seq

To estimate the population genetic structure among 91 individuals of *H. nebulosus*, genome-wide SNPs were obtained by multiplexed inter simple sequence repeat (ISSR) genotyping by sequencing (MIG-seq: Suyama and Matsuki, 2015) using Illumina Miseq system. The first PCR step was performed to amplify ISSR regions from genomic DNA with MIG-seq primer set-1. The first PCR products were diluted 40 times with deionized water and subjected to the second PCR. The second PCR step was separately conducted to each sample to construct indexed library for Illumina platform. Then, 3 μl of each second PCR product was pooled as a single mixture, and the mixed library was purified and size-selected (300–800 bp) by BluePippin DNA size selection system (Sage Science, Beverly, MA, USA). The size-selected library was quantified (size range 300–800 bp) using the Agilent 4200 TapeStation (Agilent Technologies, CA, USA), and the mixed library of 10 pM final concentration was used for sequencing on an Illumina MiSeq System (Illumina, San Diego, CA, USA), using a MiSeq Reagent Kit v3 (150 cycle, Illumina).

Obtained raw sequence data were pretreated and quality-filtered by the FASTX-Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/) using the settings of q=30 and p=40. Then the TagDust (Lassmann et al., 2009) was used to remove the reads derived from extremely short library entries and to trim read 1 and read 2 sequences.

The quality-filtered reads were then used as input data for SNP detection with Stacks 1.35 (Catchen et al., 2011). First, using ‘ustacks’ option, a set of identical reads was bundled together in a ‘stack’ in each individual, and several of these stacks were merged to form putative loci, setting minimum depth of coverage (m), maximum distance allowed between stacks (M), and maximum distance allowed to align secondary reads to primary stacks (N) as 3, 2, and 1, respectively. Second, a catalog of stacks observed in entire samples (possible loci and alleles) was created using ‘cstacks’

Table 1. Sample of salamander species used for DNA analysis in this study together with information on vouchers, collection locality, GenBank accession numbers, and literature citations. Asterisks indicate samples used in MIG-seq analysis. Voucher abbreviations: KUHE=Graduate School of Human and Environmental Studies, Kyoto University; UL=unnumbered larva; OU=Mr. Okawa's collection of urodeles; T=Mr. Tanabe's collection; nb=temporal number.

Species/morphotype	Haplotype	n	Group	Locality	Voucher	GenBank	Source
Highland	H1*	4	B2b4	Tottori (Daisen-cho)	KUHE 32704	LC436443	This study
(*H. utsunomiyaorum* sp. nov.)	H2*	2	B2b4	Hiroshima (Shobara-shi)	KUHE OU0513	LC436367	This study
(*H. utsunomiyaorum* sp. nov.)	H3*	2	B2b4	Okayama (Kagamino-cho)	KUHE OU 870516	LC436368	This study
(*H. utsunomiyaorum* sp. nov.)	H4*	1	B2b4	Tottori (Daisencho)	KUHE 21729	LC436369	This study
(*H. utsunomiyaorum* sp. nov.)	H5*	1	B2b4	Shimane (Yasugi-shi)	KUHE OU0503	LC436370	This study
(*H. utsunomiyaorum* sp. nov.)	H6*	1	B2b4	Okayama (Maniwa-shi)	KUHE nb18-5	LC436371	This study
(*H. utsunomiyaorum* sp. nov.)	H7*	1	B2b4	Hyogo (Shiso-shi)	KUHE 11076	LC436372	This study
(*H. utsunomiyaorum* sp. nov.)	H8*	1	B2b4	Okayama (Nishiawakura-mura)	KUHE 26187	LC436373	This study
(*H. utsunomiyaorum* sp. nov.)	H9*	3	B2b4	Shimane (Iinan-cho)	KUHE nb18-6	LC436374	This study
(*H. utsunomiyaorum* sp. nov.)	H10*	1	B2b4	Hiroshima (Shobara-shi)	KUHE OU0515	LC436375	This study
(*H. utsunomiyaorum* sp. nov.)	H11*	1	B2b4	Hiroshima (Shobara-shi)	KUHE nb18-7	LC436376	This study
(*H. utsunomiyaorum* sp. nov.)	H12*	1	B2b3	Shimane (Yasugi-shi)	KUHE OU0541	LC436377	This study
(*H. utsunomiyaorum* sp. nov.)	H13*	3	B2b3	Shimane (Yasugi-shi)	KUHE OU0487	LC436378	This study
(*H. utsunomiyaorum* sp. nov.)	H14	1	B2b3	Shimane (Matsue-shi)	KUHE 24762	LC436379	This study
(*H. utsunomiyaorum* sp. nov.)	H15*	2	B2b3	Shimane (Iinan-cho)	KUHE OU 880422	LC436380	This study
Aki	H16*	1	B2b2	Hiroshima (Akitakata-shi)	KUHE OU0510	LC436381	This study
(*H. akiensis* sp. nov.)	H17	1	B2b2	Hiroshima (Akitakata-shi)	KUHE nb18-8	LC436382	This study
(*H. akiensis* sp. nov.)	H18*	8	B2b2	Hiroshima (Miyoshi-shi)	KUHE OU0301	LC436383	This study
(*H. akiensis* sp. nov.)	H19*	1	B2b2	Hiroshima (Miyoshi-shi)	KUHE 35925	LC436384	This study
(*H. akiensis* sp. nov.)	H20*	4	B2b2	Hiroshima (Sera-gun)	KUHE OU0474	LC436385	This study
(*H. akiensis* sp. nov.)	H21*	1	B2b2	Hiroshima (Higashihiroshima-shi)	KUHE OU0311	LC436386	This study
(*H. akiensis* sp. nov.)	H22*	1	B2b1	Ehime (Imabari-shi)	KUHE OU0343	LC436387	This study
(*H. akiensis* sp. nov.)	H23*	7	B2b1	Ehime (Imabari-shi)	KUHE OU0348	LC436388	This study
(*H. akiensis* sp. nov.)	H24*	1	B2b1	Hiroshima (Higashihiroshima-shi)	KUHE OU0299	LC436389	This study
(*H. akiensis* sp. nov.)	H25*	1	B2b1	Hiroshima (Higashihiroshima-shi)	KUHE OU0298	LC436390	This study
(*H. akiensis* sp. nov.)	H26*	3	B2b1	Hiroshima (Higashihiroshima-shi)	KUHE OU0297	LC436391	This study
(*H. akiensis* sp. nov.)	H27*	1	B2b1	Hiroshima (Higashihiroshima-shi)	KUHE OU0342	LC436392	This study
(*H. akiensis* sp. nov.)	H28*	1	B2b1	Hiroshima (Higashihiroshima-shi)	KUHE nb8-7	LC436393	This study
(*H. akiensis* sp. nov.)	H29*	1	B2b1	Hiroshima (Higashihiroshima-shi)	KUHE OU0319	LC436394	This study
Abu	H30*	2	B2a	Yamaguchi (Yamaguchi-shi)	KUHE OU0271	LC436395	This study
(*H. abuensis* sp. nov.)	H31*	1	B2a	Shimane (Tsuwano-cho)	KUHE OU0542	LC436396	This study
(*H. abuensis* sp. nov.)	H32*	2	B2a	Yamaguchi (Hagi-shi)	KUHE 35887	LC436397	This study
(*H. abuensis* sp. nov.)	H33*	2	B2a	Yamaguchi (Yamaguchi-shi)	KUHE OU0272	LC436398	This study
Kyushu	H34*	1	B1b2	Nagasaki (Sasebo-shi)	KUHE OU0508	LC436399	This study
(*H. nebulosus*)	H35*	1	B1b2	Saga (Taku-shi)	KUHE OU0384	LC436400	This study
(*H. nebulosus*)	H36*	1	B1b2	Saga (Imari-shi)	KUHE 24719	LC436401	This study
(*H. nebulosus*)	H37*	1	B1b2	Nagasaki (Goto-shi)	KUHE 24729	LC436402	This study
(*H. nebulosus*)	H38*	1	B1b2	Nagasaki (Iki-shi)	KUHE 24749	LC436403	This study
(*H. nebulosus*)	H39*	1	B1b2	Fukuoka (Munakata-shi)	KUHE OU0562	LC436404	This study
(*H. nebulosus*)	H40*	1	B1b2	Nagasaki (Iki-shi)	KUHE 24745	LC436405	This study
(*H. nebulosus*)	H41*	1	B1b2	Kumamoto (Uki-shi)	KUHE OU0476	LC436406	This study
(*H. nebulosus*)	H42	1	B1b2	Nagasaki (Tsushima-shi)	KUHE 57643	LC437992	This study
(*H. nebulosus*)	H43*	1	B1b2	Fukuoka (Kitakyushu-shi)	KUHE 22802	LC436407	This study
(*H. nebulosus*)	H44*	1	B1b2	Kagoshima (Izumi-shi)	KUHE OU 951227	LC436408	This study
(*H. nebulosus*)	H45*	1	B1b2	Nagasaki (Isahaya-shi)	KUHE 24693	AB445786	Matsui et al. 2008b
(*H. nebulosus*)	H46*	1	B1b2	Nagasaki (Nagasaki-shi)	KUHE 8978	LC436409	This study

Table 1. (continued)

Species/morphotype	Haplotype	n	Group	Locality	Voucher	GenBank	Source
Yamaguchi	H47*	1	B1b1	Yamaguchi (Shimonoseki-shi)	KUHE OU0264	LC436410	This study
(*H. bakan* sp. nov.)	H48*	2	B1b1	Yamaguchi (Mine-shi)	KUHE OU0481	LC436411	This study
(*H. bakan* sp. nov.)	H49*	1	B1b1	Yamaguchi (Mine-shi)	KUHE OU0259	LC436412	This study
(*H. bakan* sp. nov.)	H50*	1	B1b1	Yamaguchi (Shimonoseki-shi)	KUHE OU0382	LC436413	This study
(*H. bakan* sp. nov.)	H51*	3	B1b1	Oita (Bungotakada-shi)	KUHE OU0320	LC436414	This study
(*H. bakan* sp. nov.)	H52*	2	B1b1	Oita (Usa-shi)	KUHE OU 930206	LC436415	This study
(*H. bakan* sp. nov.)	H53*	1	B1b1	Yamaguchi (Ube-shi)	KUHE OU0393	LC436416	This study
Iwami	H54*	2	B1a	Shimane (Masuda-shi)	KUHE 35900	LC436417	This study
(*H. iwami* sp. nov.)	H55*	2	B1a	Shimane (Masuda-shi)	KUHE nb18-2	LC436418	This study
(*H. iwami* sp. nov.)	H56*	2	B1a	Shimane (Masuda-shi)	KUHE OU0339	LC436419	This study
(*H. iwami* sp. nov.)	H57*	1	B1a	Shimane (Masuda-shi)	KUHE nb18-3	LC436420	This study
(*H. iwami* sp. nov.)	H58*	1	B1a	Shimane (Hamada-shi)	KUHE nb3-1	LC436421	This study
(*H. iwami* sp. nov.)	H59*	1	B1a	Shimane (Oda-shi)	KUHE nb18-4	LC436422	This study
Setouchi	H60*	1	A2b	Okayama (Okayama-shi)	KUHE OU0290	LC436423	This study
(*H. setouchi* sp. nov.)	H61*	4	A2b	Okayama (Ibara-shi)	KUHE OU0305	LC436424	This study
(*H. setouchi* sp. nov.)	H62*	2	A2b	Okayama (Kibichuo-cho)	KUHE OU0295	LC436425	This study
(*H. setouchi* sp. nov.)	H63*	3	A2b	Okayama (Okayama-shi)	KUHE OU0285	LC436426	This study
(*H. setouchi* sp. nov.)	H64*	1	A2b	Tokushima (Tokushima-shi)	KUHE nb18-1	LC436427	This study
(*H. setouchi* sp. nov.)	H65*	1	A2b	Hyogo (Himeji-shi)	KUHE 18527	LC436428	This study
(*H. setouchi* sp. nov.)	H66*	4	A2b	Hyogo (Kobe-shi)	KUHE 11594	LC436429	This study
(*H. setouchi* sp. nov.)	H67*	1	A2b	Wakayama (Tanabe-shi)	KUHE 20523	LC436430	This study
(*H. setouchi* sp. nov.)	H68	1	A2b	Tokushima (Anan-shi)	KUHE T2907	LC436431	This study
(*H. setouchi* sp. nov.)	H69*	1	A2b	Osaka (Misaki-cho)	KUHE 26543	LC436432	This study
(*H. setouchi* sp. nov.)	H70*	1	A2b	Kagawa	KUHE nb12-2	LC436433	This study
(*H. setouchi* sp. nov.)	H71*	2	A2b	Okayama (Okayama-shi)	KUHE OU0287	LC436434	This study
Kinki-Chubu	H72*	1	A2a	Aichi (Tahara-shi)	KUHE 24863	AB266663	Matsui et al. 2007a
(*H. vandenburghi*)	H73	1	A2a	Aichi (Nagoya-shi)	KUHE UL	AB266664	Matsui et al. 2007a
(*H. vandenburghi*)	H74*	3	A2a	Shiga (Hino-cho)	KUHE T2655	LC436436	This study
(*H. vandenburghi*)	H75*	1	A2a	Kyoto (Minamiyamashiro-mura)	KUHE 9300	LC436437	This study
(*H. vandenburghi*)	H76*	1	A2a	Nara (Ikoma-shi)	KUHE 26462	LC436438	This study
(*H. vandenburghi*)	H77*	1	A2a	Osaka (Hannan-shi)	KUHE 18553	LC436439	This study
(*H. vandenburghi*)	H78*	1	A2a	Aichi (Nagoya-shi)	KUHE 8078	LC436440	This study
(*H. vandenburghi*)	H79*	1	A2a	Aichi (Chita-shi)	KUHE 9241	LC436441	This study
(*H. vandenburghi*)	H80*	1	A2a	Aichi (Minamichita-cho)	KUHE 24869	AB266662	Matsui et al. 2007a
(*H. vandenburghi*)	H81*	1	A2a	Aichi (Seto-shi)	KUHE 25292	LC225431	Matsui et al. 2017a
Matsue	H82*	1	A1b	Tottori (Tottori-shi)	KUHE 16881	LC225432	Matsui et al. 2017a
(*H. setoi* sp. nov.)	H83*	1	A1b	Tottori (Tottori-shi)	KUHE 24666	LC436366	This study
(*H. setoi* sp. nov.)	H84*	1	A1b	Shimane (Yasugi-shi)	KUHE OU0558	LC436444	This study
(*H. setoi* sp. nov.)	H85*	3	A1b	Shimane (Yasugi-shi)	KUHE OU0557	LC436445	This study
H. hidamontanus		1	B2b4	Nagano (Hakuba-mura)	KUHE 9484	LC225434	Matsui et al. 2017a
H. tosashimizuensis		1	B2	Kochi (Tosashimizu-shi)	Finger tip	LC436447	This study
H. tsuensis		1	B1b2	Nagasaki (Tsushima-shi)	KUHE 22851	LC436448	This study
H. dunni		1	B1b1	Oita (Oita-shi)	KUHE 24826	LC225435	Matsui et al. 2017a
H. okiensis		1	B1	Shimane (Okinoshima-cho)	KUHE 18917	LC436446	This study
H. tokyoensis		1	A2a	Tokyo (Hachioji-shi)	KUHE 25836	AB266640	Matsui et al., 2007a
H. takedai		1	A1a	Ishikawa (Hakui-shi)	KUHE 24764	LC225430	Matsui et al., 2007a
H. mikawaensis		1	A1a	Aichi (Shinshiro-shi)	KUHE 26917	LC225429	Matsui et al. 2017a
H. nigrescens		1	A1a	Miyagi (Kami-machi)	KUHE 17924	AB548378	Nishikawa et al. 2010
H. abei		1	A	Kyoto (Kyotango-shi)	KUHE 22359	LC225433	Matsui et al. 2017a
H. lichenatus		1	A	Aomori (Aomori-shi)	KUHE 36971	AB750782	Aoki et al. 2013
H. retardatus		1		Hokkaido (Ebetsu-shi)	KUHE 13034	AB363609	Matsui et al., 2008a
H. kimurae		1		Shiga (Otsu-shi)	KUHE 16689	AB266674	Matsui et al. 2007a
S. keyserlingii		1		Hokkaido (Kushiro-shi)	KUHE 13057	AB363573	Matsui et al., 2008a

option, with the number of mismatches allowed between sample loci (n)=2. All stacks created by 'ustacks' were then matched against the catalog produced by 'cstacks', using the 'sstacks' option. Finally, SNP loci were selected using 'populations' option with 'write_single_snp' option. Parameters of minimum percentage of samples in a population (r) and minimum number of populations in a locus (p) were set as 0.75 and 10, respectively. If a locus could not be detected in an individual, the genotype in the individual was treated as missing data.

Genetic structure for entire sample based on the SNP loci obtained by MIG-seq was then assessed using STRUCTURE 2.3.4 (Pritchard et al., 2000) for number of clusters assumed (K) of 1 to 10 with 1,000,000 MCMC iterations following a burn-in period of 200,000, using the admixture model and correlated allele frequencies. Evanno's deltaK value (Evanno et al., 2005) was estimated by STRUCTURE HARVESTER (Earl and von Holdt, 2012). Detailed genetic structure was further estimated dividing data set into three according to clustering at K=3. SNP loci were re-extracted for these divided data sets, using same parameters in Stacks but only parameter 'p' was changed (see RESULTS). Extracted SNP data were analyzed by STRUTURE using same setting described above.

Morphological analyses

Univariate analyses

In morphological analyses, we used only adult males, because some *Hynobius* species are known to exhibit sexual dimorphism in some morphological characters (e.g., Sato, 1933, 1943; Tominaga et al., 2005; Nishikawa et al., 2007), and it is more difficult to collect females than males. For specimens fixed in 10% formalin and later preserved in 70% ethanol, we took the following 21 body measurements to the nearest 0.1 mm with dial calipers, and when necessary, under a binocular dissecting microscope, and two counts (for the definition of characters, refer to Tominaga et al. [2005]): 1) SVL (snout-vent length, from tip of snout to anterior tip of vent); 2) HL (head length); 3) HW (head width); 4) IND (internarial distance); 5) IOD (interorbital distance); 6) UEW (upper eyelid width); 7) UEL (upper eyelid length); 8) AGD (axilla-groin distance); 9) TRL (trunk length); 10) TAL (tail length, individuals with damaged or regenerated tails excluded); 11) BTAW (basal tail width); 12) MTAW (medial tail width); 13) MXTAH (maximum tail height); 14) FLL (forelimb length); 15) HLL (hindlimb length); 16) 2FL (second finger length); 17) 3FL (third finger length); 18) 3TL (third toe length); 19) 5TL (fifth toe length); 20) VTW (vomerine teeth series width); 21) VTL (vomerine teeth series length); 22) CG (number of costal grooves following Misawa [1989]); and 23) LO (number of costal folds between adpressed limbs). For the holotypes, we also measured 24) SL (snout length); 25) LJL (lower jaw length); 26) BTAH (basal tail height); 27) MTAH (medial tail height); 28) UJTN (number of upper jaw teeth); 29) LJTN (number of lower jaw teeth); and 30) VTN (number of vomerine teeth).

Among nine lineages recognized in *H. nebulosus* by genetic analyses (see Results), we compared SVL by one-way analysis of variance (ANOVA) with the Tukey-Kramer test, while we performed Mann-Whitney's U-test and Kruskal-Wallis test (or Dunn's multiple comparison test) for ratio values and detection of the presence or absence of differences in the frequency distributions. Significance level of 5% was used in all statistical tests. All statistical analyses were performed by R 3.4.2 (R Development Core Team, 2017). In the following description, average values are shown with 1 standard deviation (±SD).

Multivariate analyses

We conducted a linear discriminant analysis for all 156 samples using 20 morphometric characters excluding fifth toe length (5TL), which was omitted from the analyses because complete lack of the toe in some individuals strongly affected the results. On the basis of linear discriminant functions thus obtained, we determined each sample with which each of the nine lineages was associated. For examining overall morphological variation among nine lineages, we also conducted multivariate analyses using $\log_e$-transformed metric values of all the 20 characters. We conducted principal components analysis (PCA) and canonical discriminant analysis (CANDISC) using the software package R 3.4.2 (R Development Core Team, 2017).

Finally, we compared the gross pattern of body markings between each sample.

Estimating divergence time

To estimate the age of each mitochondrial lineage, we prepared a data set from 45 cyt b sequences, including *H. nebulosus* and allied taxa, Korean *H. leechii* (AB548375) and Chinese *H. chinensis* (EF076244). The divergence times were estimated using a Bayesian relaxed molecular clock calculated in BEAST ver. 2.5.1 (Bouckaert et al., 2014) using 30 million generations, discarding the first 10 million generations as burn-in. Parameter values were sampled every 1000 generations, under a GTR substitution model and uncorrelated lognormal 'relaxed' clock rate model (Drummond et al., 2006). Parameter estimates and convergence were checked using TRACER ver. 1.5 (Rambaut and Drummond, 2009). Because no reliable calibration points were available, the evolutionary rate for cyt b of 1.28% per MY (see Yoshikawa et al. [2008] for discussion on this rate in hynobiids) was used to estimate the dates of cladogenic events.

RESULTS

Genetic analyses

MtDNA phylogeny

Of 1141 complete sequences of cyt b gene, 469 were variable and 413 were parsimony-informative within the ingroup. The best substitution model for maximum likelihood (ML), derived from Kakusan4 (Tanabe, 2011) was the codon-equal rate model with general time reversible model (GTR: Tavaré, 1986) with a G (gamma shape parameter: 0.136, 0.057, and 2.248 for 1st, 2nd, and 3rd codon positions, respectively) for each codon position. For Bayesian analysis, symmetrical model (SYM: Zharkikh, 1994)+G (0.674)+I (0.562), HKY (Hasegawa et al., 1985)+G (0.415)+I (0.688), and GTR+G (3.570)+I (0.037) were selected for 1st, 2nd, and 3rd codon positions, respectively. The likelihood values (lnL) of the ML and Bayesian inference (BI) trees were −12494.772 and −12436.664, respectively.

Phylogenetic analyses employing two different optimality criteria yielded nearly identical topologies and only the BI tree is presented in Fig. 2. *Hynobius nebulosus* was split into nine lineages mainly corresponding to morphotypes from the sampling localities (Matsue, Setouchi, Iwami, Yamaguchi, Kyushu, Abu, Aki, and Highland) proposed by Okawa et al. (2007) and Kinki-Chubu lineage here defined. Interestingly, these lineages of *H. nebulosus* formed a clade usually not with each other, but with other named species.

1) Monophyly of lentic, and secondarily lotic, breeding Japanese *Hynobius* taxa (*H. nebulous*, *H. lichenatus*, *H. abei*, *H. mikawaensis*, *H. nigrescens*, *H. takedai*, *H. tokyoensis*, *H. okiensis*, *H. dunni*, *H. tsuensis*, *H. tosashimizuensis*, and *H. hidamontanus*) with respect to a primarily lotic breeder, *H. kimurae* was barely supported (ML bootstrap values [BS]=69%/Bayesian posterior probabilities [BPP]=0.99).

2) Although monophyly of lentic breeding *Hynobius* employed was not strongly supported, two clades, A (ML BS=98%/BPP=1.00) and B (ML BS=79%/BPP=1.00) were recognized, and Clades B1 (98%/1.00) and B2 (85%/1.00) were split in Clade B.

3) In Clade A, *H. lichenatus*, *H. abei*, Clade A1 (100%/1.00), and Clade A2 (83%/1.00) including *H. tokyoensis* exhibited unresolved relationships. In Clade A1, Clade A1a

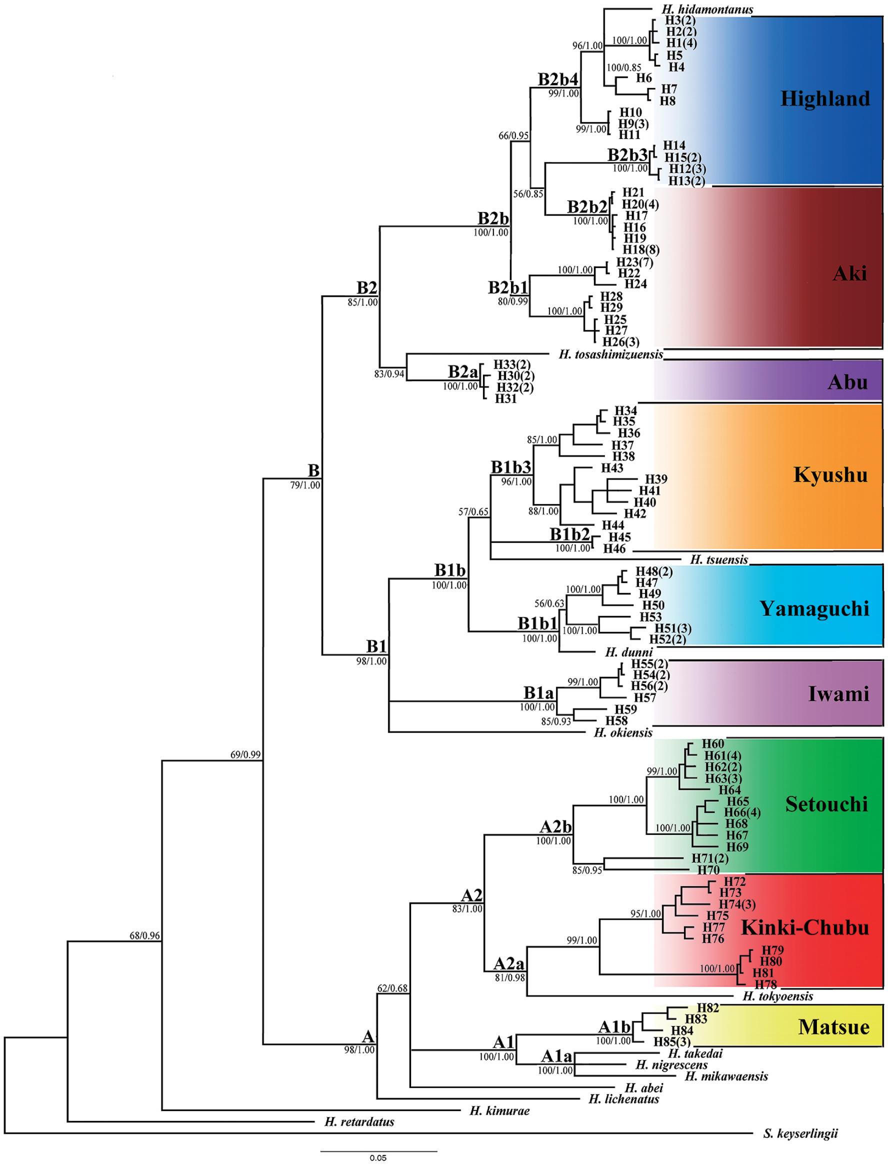

FIG. 2 Bayesian tree based on the complete cyt b gene for samples used (haplotype numbers, with n in parenthesis included in Table 1). Numbers above or below branches represent bootstrap supports for ML inference and Bayesian posterior probability (ML-BS/BPP).

(100%/1.00 including *H. nigrescens*, *H. mikawaensis*, and *H. takedai*) and Clade A1b (100%/1.00 including H [Haplotype] 82–85 [Matsue]) were split. In Clade A2, Clade A2a (81%/0.98) was split from Clade A2b (100%/1.00). In Clade A2a, *H. tokyoensis* was the sister species to the clade including H72–80 (Kinki-Chubu). Clade A2b included H60–71 (Setouchi).

4) In Clade B1, *H. okiensis*, Clade B1a (100%/1.00), and B1b (100%/1.00) exhibited unresolved relationships. Clade B1a inclu-

TABLE 2. Uncorrected p-distances (%) among morphotypes of salamanders for complete cyt b gene region.

	Highland	Aki	Abu	Kyushu	Yamaguchi	Iwami	Setouchi	Kinki-Chubu
Aki	5.5–7.5							
Abu	8.8–10.9	9.9–10.5						
Kyushu	11.8–13.2	12.0–13.2	10.5–10.7					
Yamaguchi	12.8–14.0	13.1–14.0	10.8–11.0	7.8–9.6				
Iwami	11.8–12.8	12.7–14.1	12.2–12.5	10.7–11.7	10.9–11.9			
Setouchi	13.8–15.2	14.9–15.4	13.7–14.3	14.5–15.8	14.2–15.7	13.4–14.9		
Kinki-Chubu	14.4–15.9	14.3–15.8	14.3–14.6	14.2–15.5	14.6–15.8	14.4–15.8	10.6–11.9	
Matsue	13.2–14.0	13.7–14.1	13.4	13.7–15.1	14.5–14.7	13.5–13.8	12.6–13.2	12.9–13.3

ded H54–59 (Iwami), while Clade B1b included Clade B1b1 (100%/1.00), *H. tsuensis*, Clade B1b2 (100%/1.00), and Clade B1b3 (96%/1.00). Clade B1b1 included *H. dunni* and two clades containing H47–53 (Yamaguchi), Clade B1b2 contained H45 and 46 (Kyushu, including topotypic *H. nebulosus*), and Clade B1b3 included two clades containing H34–44 (Kyushu).

5) Clade B2 included Clade B2a (83%/0.94) and Clade B2b (100%/1.00). In Clade B2a, *H. tosashimizuensis* was the sister species to the clade containing H30–33 (Abu), while in Clade B2b, four clades B2b1 (containing H22–28 [Aki]), B2b2 (H16–21 [Aki]), B2b3 (H12–15 [Highland]) and B2b4 (H1–11 [Highland] and *H. hidamontanus*) showed unresolved relationships.

Mean genetic distances ranged from 13.4–15.8% between Clades A and B1, from 13.2–15.9% between Clades A and B2, and from 10.5–14.1% between Clades B1 and B2 (Table 2).

MIG-seq

Among 91 individuals of *Hynobius nebulosus* from mtDNA clades, 324 genomic SNP loci were scored and subjected to estimation of genetic structure. As a result, likelihood values estimated continued to increase until K=8, but Evanno's deltaK was highest at K=3. Clustering at K=3 was nearly concordant with separation of major mtDNA clades, corresponding to separation of Clades A, B1+B2a, and B2b (Fig. 3A). We then divided data set into these three groups, and detailed genetic structure was estimated. In the cluster of mitochondrial Clade A, 406 loci were scored (parameter p=5) and delta K was highest at K=3 (Fig. 3B), corresponding to separation of Clades A1b (Matsue), A2a (Kinki-Chubu), and A2b (Setouchi). For the cluster of mitochondrial B1+B2a clades, 105 loci were scored (parameter p=6) and delta K was highest at K=4 (Fig. 3C), and clusters corresponding to B1a (Iwami), B1b1 (Yamaguchi), B1b2+B1b3 (Kyushu, including topotypic *H. nebulosus*), and B2a (Abu) were recognized. For mitochondrial B2b (B2b1–B2b4) clade, 266 loci were scored (parameter p=6) and delta K was highest at K=2 (Fig. 3D), and B2b1+B2b2 (Aki) and B2b3+B2b4 (Highland) were split.

Although a few individuals in Clades A2a (Kinki-Chubu), A2b (Setouchi), B1b1 (Yamaguchi), B1b2, B1b3 (Kyushu), and B2b2 (Aki) showed probable past hybridization with the neighboring clades (Fig. 3A–D), we considered the separation by MIG-seq as reliable. Thus, the nine lineages recognized by mtDNA analyses, (1) Matsue (A1b), (2) Kinki-Chubu (forming A2a with *H. tokyoensis*), (3) Setouchi (A2b), (4) Iwami (B1a), (5) Yamaguchi (forming B1b1 with *H. dunni*), (6) Kyushu (B1b2+B1b3), (7) Abu (forming B2a with *H. tosashimizuensis*), (8) Aki (B2b1+B2b2), and (9) Highland (forming B2b3+B2b4 with *H. hidamontanus*) were regarded as geneti-

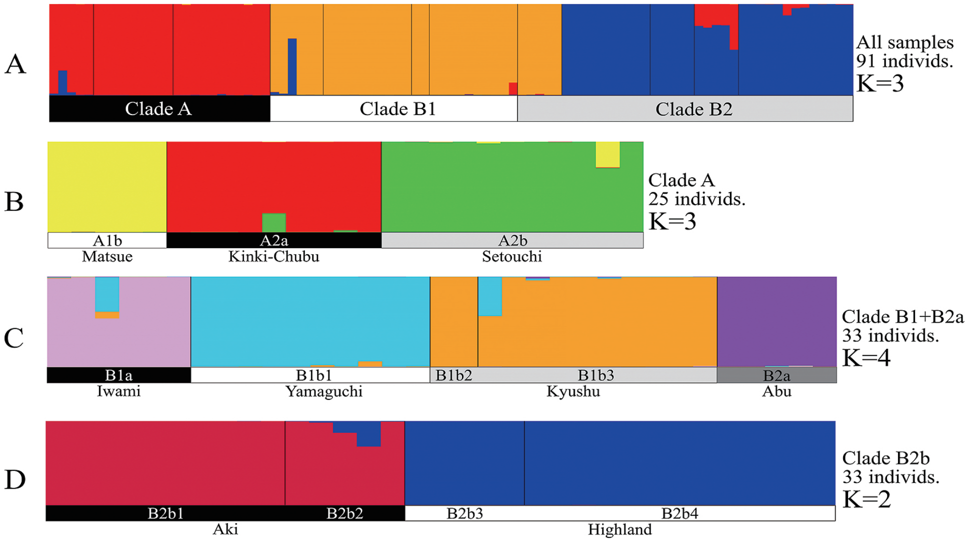

FIG. 3 Barplots of the results of Structure analyses for *H. nebulosus* based on SNP loci obtained by MIG-seq. Mitochondrial clades and morphotypes to which each individual belongs are shown below barplots. (A) Barplot of entire samples (91 individuals) of *H. nebulosus* at K=3. (B) Barplot of Clade A (25 individuals) at K=3. (C) Barplot of Clades B1+B2a (33 individuals) at K=4. (D) Barplot of Clade B2b (33 individuals) at K=2.

cally distinct entities.

Morphological Analyses

Univariate analyses

Unlike the genetic separation, the male specimens of nine lineages of *H. nebulosus* were not well separated from each other in morphometric and meristic characters. Results of ANOVA indicated that the samples somewhat differ from each other in SVL. Some of all combinations were significantly different in SVL ($P<0.05$), and Kyushu was larger than all others except for Abu and Matsue while Aki was smaller than others except for Highland, Yamaguchi, Iwami, Setouchi, and Kinki-Chubu. Each morphotype can be divided into large and small by the boundary of 54.6 mm (mean of the means of each morphotype).

For many characters, significant differences (Kruskal-Wallis tests, multiple comparisons, $P<0.05$) were found among lineages, and greatest numbers of differences were found between Abu and Kinki-Chubu. However, Aki and Yamaguchi, Setouchi and Aki, Yamaguchi and Kyushu, respectively, did not differ in any character. Thus, the nine lineages grouped by the result of mtDNA analyses were recognized as more or less distinct by the univariate analyses.

Multivariate analyses

The posterior probability of group membership serves to classify each specimen to one of nine lineages. In Abu and Aki references, none were misclassified, while some of the reference specimens were misclassified as members of the other species (Table 3). In Setouchi, four of 12 specimens (33.3%) were misclassified (each one as Aki, Kyushu, Kinki-Chubu or Yamaguchi). Similarly, in Yamaguchi, four of 18 specimens (22.2%) were misclassified (one as Abu, one as Iwami, and two as Setouchi). Few of the reference specimens was misclassified as a member of the parapatric lineages, and ruled out possible hybridization.

TABLE 3. The posterior probability of group membership.

	Assigned morphotypes								
	Abu	Aki	Iwami	Matsue	Highland	Kyushu	Setouchi	Kinki-Chubu	Yamaguchi
Abu (n=18)	18	0	0	0	0	0	0	0	0
Aki (n=19)	0	19	0	0	0	0	0	0	0
Iwami (n=14)	0	1	11	0	1	0	1	0	0
Matsue (n=14)	1	0	0	11	0	1	1	0	0
Highland (n=13)	0	0	0	0	11	0	0	2	0
Kyushu (n=22)	0	0	0	0	1	21	0	0	0
Setouchi (n=12)	0	1	0	0	0	1	8	1	1
Kinki-Chubu (n=26)	0	0	1	2	1	0	0	22	0
Yamaguchi (n=18)	1	0	1	0	0	0	2	0	14

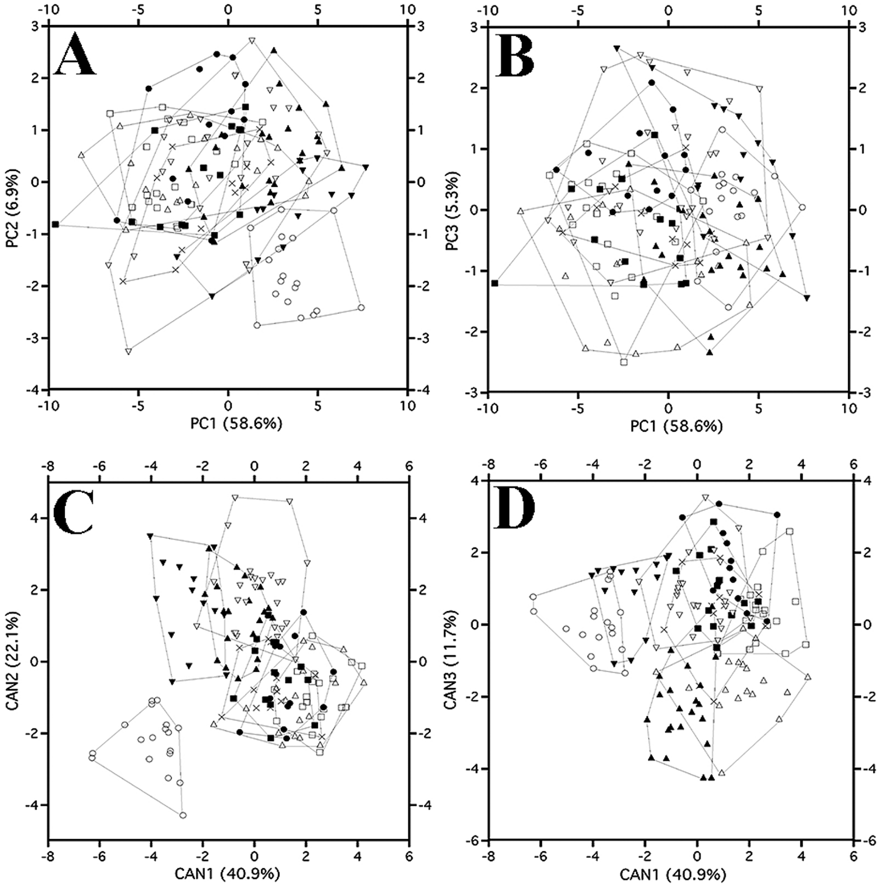

FIG. 4 Principal (A, B) and canonical (C, D) components scores of morphological variables for male samples of salamanders assigned to *Hynobius nebulosus*. Reversed closed triangle=Matsue; Reversed open triangle=Kinki-Chubu; Cross=Setouchi; Closed square=Iwami; Open triangle=Yamaguchi; Closed triangle=Kyushu; Open circle=Abu; Open square=Aki; Closed circle=Highland. Axes show components and their proportion of variance.

PC analysis totally failed to separate the ranges of nine lineages (Fig. 4A, B). The eigenvalues of the first three axes in PCA (PC1–PC3) accounted for 12.30 (58.6%), 1.45 (6.9%), and 1.12 (5.3%) of the total variation, respectively.

Similarly, CANDISC analysis revealed the ranges of nine lineages to mostly overlap with each other. However, one exception was the Abu lineage, the range of which was completely separated from other lineages by the first and second axes (Fig. 4C, D). The eigenvalues of the first three axes (CAN1–CAN3) accounted for 40.9%, 22.1%, and 11.7% of the total variation, respectively. Thus the first three canonical variables accounted for 74.7 % of the total amount of eigenvalue.

In body coloration, the nine lineages are not easily distinguishable from each other. However, the highland lineage is unique in usually having a marked dorsum with light color, which is rarely seen in other lineages. Furthermore, the Setouchi and Aki lineages and the Matsue and Highland lineages, respectively, can be found sympatrically in some regions (see species accounts), suggesting no evidence of hybridization.

From these results of morphological analyses, we concur the separation of nine lineages recognized by genetic analyses (Matsue, Kinki-Chubu, Setouchi, Iwami, Yamaguchi, Kyushu, Abu, Aki, and Highland), and regard each of them as genetically distinct species. Of these, Kyushu, and Kinki-Chubu, respectively, included topotypes of *H. nebulosus* (Nagasaki) and *H. vandenburghi* (Nara), and the remaining seven lineages are considered so far as undescribed.

We thus retain the current taxonomic status of *H. nebulosus* and *H. vandenburghi*, and describe each of the remaining seven geographic populations formerly assigned to *H. nebulosus* as new taxa.

Species Accounts

Concepts and the criteria of species used to implement them are contentious and widely debated (e.g., de Queiroz, 2007; Chan et al., 2018). We adopt a conservative operational criterion of recognizing as species those lineages that are diagnosable on the basis of either morphology or DNA sequence. Genetic distances are provided as heuristic measures of evolutionary isolation, but are not always used as criteria for recognizing species.

In this section morphological data apply only to adult males with light nuptial markings on the throat.

1. *Hynobius **setoi*** Matsui, Tanabe et Misawa sp. nov.

[Japanese name: San'in-sansyou-uwo]
[English name: San'in salamander]
Fig. 5

Hynobius nebulosus (part, as Matsue morphotype) Okawa et Utsunomiya, 1992, 12.
Hynobius nebulosus (part, as Nihonkai morphotype) Okawa et al., 2007, 58.

Holotype

KUHE 7313, an adult male from Takae, Fukube-cho, Tottori-shi, Tottori Prefecture (35°32'31"N, 134°16'42"E, alt. 50 m), collected by M. Matsui, S. Tanabe, and T. Hayashi on 23 December 1983.

Paratypes

KUHE 7308–7312, 7314, six males, data same as the holotype.

Referred specimens

Tottori Prefecture: KUHE 7292–7296, four males and one female from Yaebara, Fukube-cho, Tottori-shi on 23 December 1983; KUHE 7297–7301, four males and one female from Ikedani, Iwami-cho, Iwami-gun on 22 December 1983 all by M. Matsui, S. Tanabe, and T. Hayashi. KUHE 16879–16883, three males and two females from Kyusho-zan, Tottori-shi by Y. Misawa on 8 March 1994. KUHE 16884, one female from Ochidani Shrine, Tottori-shi by Y. Misawa on 8 March 1994. Shimane Prefecture: KUHE

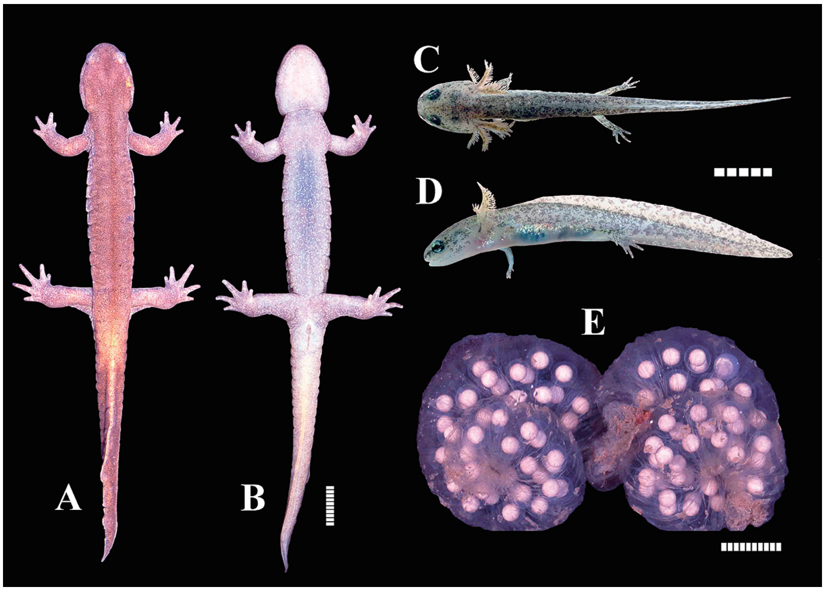

FIG. 5 *Hynobius setoi* sp. nov. Dorsal (A) and ventral (B) views of a male (KUHE 32424), scale bar=10 mm. Dorsal (C) and lateral (D) views of a larva (Stage 63), scale bar=5 mm. Egg sac (E), scale bar=10 mm.

7330–7339, nine males and one female from San'oji, Daito-cho, Unnan-shi by T. Seto and S. Kuzumi on 24 January 1984. KUHE OU0538–0539, two males from Shimoyamasa, Hirose-cho, Yasugi-shi by H. Okawa on 8 January 2011. KUHE OU0742–0744, three males from Shinobuchi, Daito-cho, Unnan-shi by H. Okawa on 2 March 2013.

Etymology

The specific name "*setoi*" is dedicated to Dr. Takeshi Seto, Professor Emeritus of Shimane University, who is the distinguished pioneer of chromosome survey of the Japanese amphibians.

Diagnosis

Body large (male SVL 49–69 mm, mean ±SD=58.8±5.5, n=15), fore- and hindlimbs relatively long (26.7%SVL and 32.3%SVL, respectively), ratio of hindlimb to forelimb large (1.21). The fifth toe almost always present. Tail long (76.8%SVL). Upper and lower edges of the tail usually with clear yellow stripes. The egg sac envelope has no striations. Phylogenetically, *H. setoi* sp. nov. forms a mitochondrial DNA clade with the clade of *H. takedai*, *H. mikawaensis*, and *H. nigrescens*. This clade, the clade including *H. vandenburghi* and *H. setouchi* sp. nov., *H. abei*, and *H. lichenatus* form a clade sister to the one including the remaining species. However, *H. setoi* sp. nov. has larger head, wider interorbital and tail at base, longer hindlimb and third toe, greater limb overlap, and shorter trunk, axilla-groin, and smaller number of costal grooves than *H. vandenburghi*, and has larger SVL and longer head, and shorter trunk and vomerine teeth series, and smaller number of costal grooves than *H. setouchi* sp. nov.

Description of holotype (measurements in mm)

Head-body large for the species (SVL 64.0) and robust; head oval and not depressed, distinctly longer (HL 16.0, 25.0%SVL) than wide (HW 13.0, 20.3%SVL); snout rounded, slightly projecting beyond lower jaw; nostril close to snout tip; labial fold absent; eye moderate, protruded, slightly inset from edge of head in dorsal view; upper eyelid well developed (UEW 2.0, 3.1%SVL), shorter (UEL 3.9, 6.1%SVL) than snout (SL 4.9, 7.7%SVL); gular fold distinct, curving anteriorly; parotoid gland evident, extending from angle of jaw to gular fold; postorbital grooves distinct, branching posterior to angle of jaw, one short and running down to lower jaw, the other long and posterior to parotoid gland; vomerine teeth shallow V-shaped, series nearly touching at midline (Fig. 14A), wider (VTW 3.6, 5.6%SVL) than long (VTL 2.7, 4.2%SVL), posterior to line connecting choanae; tongue broad, both sides free from mouth floor; limbs thick, forelimb long and hindlimb medium for the species (FLL 18.6, 29.1%SVL; HLL 20.7, 32.3%SVL); CG 12; depressed limbs separated by half a costal fold (LO -0.5); relative length of fingers I<IV<III<II, toes I<V<II<IV<III; fifth toe well developed (5TL 1.8, 2.4%SVL); cloaca greatly swollen with longitudinal slit; genital tubercle on anterior cloaca present; tail long for the species (TAL 51.7, 80.8%SVL), cylindrical at base (BTAW 8.6, 13.4%SVL; BTAH 8.8, 13.8%SVL), compressed posteriorly (MTAW 3.6, 5.6%SVL; MTAH 9.4, 14.7%SVL), with dorsal fin (MXTAH 9.9, 15.5%SVL); tip of tail slightly pointed in lateral view.

Additional measurements and counts of the holotype: IND (3.6, 5.6%SVL); IOD (3.8, 5.9%SVL); AGD (32.1, 50.2%SVL); TRL (48.0, 75.0%SVL); 2FL (3.4, 5.2%SVL); 3FL (3.0, 4.7%SVL); 3TL (5.2, 8.1%SVL); UJTN (88); LJTN (80); VTN (48).

Color

In life, dorsum olive without markings (Fig. 5A). Underside of body lighter than dorsum with small white dots (Fig. 5B). Throat covered with white nuptial color. Tail with yellow stripes on posterior three-fourths of dorsal and posterior two-thirds of ventral edges. In alcohol, dorsum changes to gray color, but trace of yellow on tail edges recognizable.

Variation

Morphometric data are summarized in Table 4. In 15 males examined, SVL was 58.8±5.5 (48.6–69.0) mm, and the ratios to SVL were: HL 24.8 (24.0–27.0) %SVL; TRL 75.2 (73.1–76.0) %SVL; HW 17.7 (16.8–20.3) %SVL; IND 5.4 (5.1–6.5) %SVL; IOD 6.3 (5.6–7.4) %SVL; UEW 3.1 (3.0–3.8) %SVL; UEL 5.9 (5.4–6.5) %SVL; AGD 48.2 (44.1–51.4) %SVL; TAL 76.8 (65.6–86.3) %SVL; BTAW 11.7 (10.9–13.4) %SVL; MXTAH 13.4 (10.7–16.1) %SVL; FLL 26.7 (24.9–29.1) %SVL; HLL 32.3 (29.9–35.7) %SVL; MTAW 5.3 (3.6–6.1) %SVL; VTW 4.8 (4.2–5.6) %SVL; VTL 3.7 (3.1–4.7) %SVL; 2FL 5.2 (4.3–6.3) %SVL; 3FL 4.5 (4.0–6.0) %SVL; 3TL 8.0 (6.7–9.6) %SVL; 5TL 2.3 (0.9–3.6) %SVL. VTW/VTL 127.7 (109.8–150.0)%. MTAW/BTAW 0.44 (0.31–0.52). TotL was 103.8 (82.5–117.3) mm; CG 12 (11–12); LO -0.50 (-1.0–0).

Fifth toes were almost always present (96.1% of 127 specimens examined: Okawa et al., 2009). Yellow stripes on the tail were usually present, and of 65 individuals examined, 53 (81.5%) had clear stripes on both edges, one (1.5%) had white stripe on lower edge, six (9.2%) had weak stripes, one (1.5%) had stripe only on lower edge, and three (4.6%) lacked stripes.

Eggs and egg sacs

The egg sacs are string-like in shape and coiling, with a thin, wrinkled envelope lacking notable striations and any distinct whiptail structure (Fig. 5E). The clutch size varied from 34–159, with mean±SD of 86.2±27.5 (n=72). The ova from a female ranged from 2.4–2.9 (mean±SD=2.6±0.2, n=7) mm. The animal pole is dark brown and vegetal pole is light brown in color.

Larva

A larva at Stage 63 of Iwasawa and Yamashita (1991) had SVL of 16.7 and total

TABLE 4. Measurements (in mm) in morphotypes of salamanders. Mean±SD followed by ranges in parentheses. See text for character abbreviations.

	Matsue (n=15)	Kinki-Chubu (n=28)	Setouch (n=13)	Iwami (n=14)	Yamaguchi (n=18)
SVL	58.8±5.5 (48.6–69.0)	56.5±6.4 (43.9–67.3)	52.3±4.3 (44.6–58.3)	52.0±4.8 (42.5–57.9)	51.8±5.7 (44.8–62.7)
HL	14.7±1.2 (13.1–16.6)	13.4±1.3 (11.1–16.9)	12.2±0.9 (10.4–13.4)	12.7±1.0 (11.3–14.2)	11.9±1.3 (9.7–14.1)
TRL	44.1±4.3 (35.5–52.4)	43.1±5.4 (31.7–52.8)	40.1±3.5 (34.2–45.2)	39.3±4.0 (31.2–14.2)	40.0±4.6 (34.0–48.6)
HW	10.7±1.3 (8.6–13.4)	9.4±1.1 (7.1–11.5)	9.1±0.8 (8.0–10.5)	9.1±0.8 (7.4–10.1)	9.5±1.2 (7.6–11.5)
IND	3.3±0.4 (2.6–4.1)	2.8±0.3 (2.3–3.4)	2.8±0.3 (2.4–3.4)	2.7±0.2 (2.2–3.1)	2.8±0.4 (2.2–3.4)
IOD	3.8±0.4 (3.2–4.5)	3.2±0.4 (2.1–4.0)	3.2±0.3 (2.7–3.7)	3.2±0.4 (2.6–3.8)	3.0±0.4 (2.6–4.1)
UEW	1.9±0.2 (1.5–2.2)	1.7±0.3 (1.1–2.3)	1.6±0.2 (1.4–2.0)	1.7±0.2 (1.1–2.0)	1.5±0.2 (1.2–1.9)
UEL	3.5±0.3 (3.0–4.2)	3.1±0.4 (2.6–4.0)	3.2±0.6 (2.7–5.0)	3.0±0.3 (2.2–3.2)	3.0±0.3 (2.6–3.7)
AGD	28.1±3.3 (23.0–33.1)	29.1±4.3 (20.6–37.2)	26.3±2.3 (21.7–29.7)	26.5±2.8 (20.7–31.5)	26.9±3.2 (23.0–32.7)
TAL	44.9±6.3 (33.9–53.4)	39.3±6.7 (29.2–59.3)	39.8±4.9 (33.7–47.5)	38.8±3.5 (32.2–42.5)	38.8±5.2 (31.4–50.3)
BTAW	7.0±0.8 (5.3–8.6)	5.9±1.2 (3.5–8.6)	5.8±1.2 (3.8–7.8)	5.4±0.8 (2.9–6.3)	5.7±1.3 (3.6–8.0)
MXTAH	7.9±1.1 (6.2–9.8)	6.6±1.5 (4.2–10.0)	6.0±1.1 (4.2–7.4)	5.7±1.3 (2.7–7.6)	6.5±1.1 (4.6–9.1)
FLL	15.9±1.2 (13.4–18.6)	14.2±1.2 (12.2–16.6)	13.7±1.1 (11.7–15.3)	13.8±1.0 (11.2–15.2)	13.3±1.7 (10.3–16.8)
HLL	19.1±1.7 (16.2–22.7)	16.6±1.6 (13.7–19.9)	16.3±1.5 (13.9–18.3)	16.0±1.5 (12.3–18.0)	15.6±2.0 (12.2–20.4)
MTAW	3.0±0.6 (2.0–3.7)	2.7±0.9 (1.0–4.2)	2.4±0.7 (1.2–3.5)	2.0±0.6 (1.0–3.3)	2.2±0.9 (0.5–4.0)
VTW	2.8±0.4 (2.2–3.6)	2.5±0.3 (2.2–3.3)	2.7±0.3 (2.2–3.2)	2.6±0.2 (2.4–3.0)	2.8±0.4 (2.1–3.4)
VTL	2.3±0.4 (1.5–3.3)	2.2±0.3 (1.6–3.1)	2.4±0.3 (2.1–3.0)	2.4±0.4 (1.9–3.0)	2.5±0.4 (1.9–3.2)
2FL	3.1±0.3 (2.4–3.6)	2.8±0.4 (2.0–3.6)	2.6±0.3 (2.1–3.0)	2.3±0.4 (2.0–3.2)	2.5±0.4 (1.8–3.1)
3FL	2.7±0.3 (2.2–3.1)	2.4±0.4 (1.3–3.1)	2.4±0.3 (1.8–2.8)	1.9±0.3 (1.5–2.4)	2.3±0.4 (1.9–3.1)
3TL	4.7±0.4 (3.9–5.3)	4.0±0.6 (3.0–5.1)	4.1±0.3 (3.6–4.7)	4.0±0.5 (3.1–4.7)	3.9±0.6 (2.8–5.3)
5TL	1.3±0.5 (0.5–2.5)	1.2±0.4 (0–1.8)	1.2±0.3 (0.6–1.5)	0.1±0.2 (0–0.6)	1.0±0.5 (0–1.7)

	Kyushu (n=31)	Abu (n=20)	Aki (n=21)	Highland (n=15)
SVL	59.3±4.5 (50.9–66.9)	57.3±3.2 (49.6–64.2)	49.6±4.3 (44.4–59.9)	53.6±4.1 (43.4–60.0)
HL	14.0±0.9 (11.8–15.8)	13.7±0.9 (11.3–15.8)	11.5±0.7 (10.0–12.8)	12.4±0.9 (10.2–13.7)
TRL	45.3±3.9 (39.1–52.2)	43.6±2.4 (38.3–48.4)	38.0±3.7 (33.4–47.2)	41.2±3.3 (33.2–46.5)
HW	11.1±0.7 (9.2–12.3)	11.0±0.9 (9.6–13.0)	8.8±0.7 (8.0–10.4)	9.5±0.7 (8.2–10.4)
IND	3.5±0.3 (2.6–4.0)	3.3±0.2 (2.7–3.7)	2.7±0.3 (2.2–3.3)	2.9±0.3 (2.3–3.4)
IOD	3.6±0.4 (2.8–4.4)	3.5±0.2 (3.1–4.1)	3.1±0.4 (2.5–3.8)	3.3±0.3 (2.7–3.7)
UEW	1.7±0.2 (1.2–2.2)	2.0±0.2 (1.3–2.4)	1.6±0.2 (1.2–1.9)	1.7±0.2 (1.2–1.9)
UEL	3.2±0.3 (2.7–4.0)	3.4±0.3 (2.9–3.9)	3.0±0.2 (2.4–3.5)	3.1±0.2 (2.9–3.5)
AGD	30.5±3.0 (25.2–35.2)	27.3±2.1 (23.8–32.0)	25.6±2.5 (21.9–30.5)	27.8±2.3 (22.9–31.6)
TAL	39.5±6.1 (26.8–53.2)	49.6±3.6 (43.7–56.2)	36.5±3.8 (30.0–44.7)	37.3±4.8 (26.4–44.2)
BTAW	6.3±1.0 (4.8–8.6)	6.9±1.1 (4.5–9.2)	5.5±0.9 (3.7–7.4)	5.9±0.9 (4.9–7.7)
MXTAH	7.3±1.2 (5.2–9.6)	7.1±0.9 (5.4–9.0)	5.6±0.7 (4.5–7.5)	5.2±0.7 (4.2–7.0)
FLL	15.2±1.2 (13.2–17.5)	17.4±1.3 (14.8–20.3)	12.5±1.0 (10.7–14.9)	13.3±0.9 (11.4–15.0)
HLL	17.7±1.5 (14.8–20.6)	19.8±1.6 (15.8–22.9)	14.8±1.3 (12.3–17.4)	16.3±1.1 (13.6–18.3)
MTAW	2.7±0.7 (1.5–3.6)	2.5±0.4 (1.9–3.6)	2.5±0.5 (1.9–3.8)	2.9±0.6 (1.7–3.8)
VTW	2.8±0.2 (2.1–3.2)	3.1±0.3 (2.6–3.7)	2.7±0.3 (2.2–3.2)	2.6±0.2 (2.3–3.0)
VTL	2.7±0.3 (2.1–3.3)	2.4±0.2 (2.1–3.0)	2.3±0.3 (1.8–3.1)	2.0±0.2 (1.7–2.3)
2FL	2.9±0.3 (2.2–3.7)	3.5±0.3 (3.1–4.2)	2.5±0.3 (2.0–3.1)	2.5±0.4 (2.0–3.2)
3FL	2.6±0.4 (1.9–3.2)	2.9±0.3 (2.3–3.4)	2.1±0.3 (1.5–2.7)	1.9±0.3 (1.3–2.4)
3TL	4.5±0.6 (3.4–5.5)	4.9±0.6 (3.8–6.2)	3.7±0.4 (2.8–4.3)	3.9±0.5 (2.9–4.5)
5TL	1.4±0.4 (0.6–2.0)	1.7±0.3 (1.1–2.3)	1.0±0.3 (0.3–1.5)	0.3±0.3 (0–0.9)

length of 32.3 mm, head rounded in dorsal view and in profile (Figs. 5C, D); snout short and truncate; eyes slightly protruded, inset from edge of head in dorsal view; labial fold distinct at half of upper jaw; external gills developed; caudal fin higher than head; dorsal fin higher than ventral fin; origin of dorsal fin in anterior one-third of trunk; ventral fin originating from the vent; tail tip weakly pointed; limbs slender; claws on fingers and toes absent. In life, the dorsum was light brown with dark marking; venter whitish and transparent; black spots scattered on tail; silver dots scattered on flank and lower tail fin.

Range

Known so far from mainly lowland areas along the Japan Sea from northwestern Hyogo to eastern Shimane (Fig. 15). Hyogo Prefecture: Shin-onsen-cho, Mikata-gun. Tottori Prefecture: Iwami-cho, Iwami-gun; Daisen-cho (including the former Nawa-cho), Saihaku-gun; Kurayoshi-shi; Tottori-shi; Yonago-shi. Shimane Prefecture: Matsue-shi (including the former Tamayu-cho and Higashi-izumo-cho); Yasugi-shi; Unnan-shi (including the former Kamo-machi and Mitoya-cho); Izumo-shi (including the former Hirata-shi, Taisha-machi, and Hikawa-cho).

Comparisons

Hynobius setoi sp. nov. significantly differs from *H. vandenburghi* by larger head (median RHL 24.8% vs. 23.4%, RHW 17.7% vs. 16.7%), wider interorbital (RIOD 6.3% vs. 5.7%) and tail at base (RBTAW 11.7% vs. 10.5%), longer hindlimb (RHLL 32.3% vs. 29.4%) and third toe (R3TL 8.0% vs. 6.9%), greater limb overlap (LO -0.5 fold vs. -1.5 folds), and shorter trunk (RTRL 75.2% vs. 76.6%), axilla-groin (RAGD 48.2% vs. 51.6%), and smaller number of costal grooves (CG 12 vs. 13); from *H. setouchi* sp. nov. by usual presence of yellow stripes on tail, larger SVL (59.3 mm vs. 52.3 mm) and longer head (RHL 24.8% vs. 23.1%), and shorter trunk (RTRL 75.2% vs. 76.9%) and vomerine teeth series (RVTL 3.7% vs. 4.6%), and smaller number of costal grooves (CG 12 vs. 13).

The new species is different from *H. nebulosus* by its longer head (RHL 24.8% vs. 23.6%) and hindlimb (RHLL 32.3 % vs. 29.4%), larger upper eyelid (RUEW 3.1% vs. 2.8%, RUEL 5.9% vs. 5.3%), and greater limb overlap (LO -0.5 fold vs. -2 folds), and shorter trunk (RTRL 75.2% vs. 76.4%), axilla-groin (RAGD 48.2% vs. 51.0%) and vomerine teeth series (RVTL 3.7% vs. 4.6%), and smaller number of costal grooves (CG 12 vs. 13); from *H. bakan* sp. nov. by larger SVL (58.8 vs. 51.8 mm), longer head (RHL 24.8% vs. 22.5%), and hindlimb (RHLL 32.3% vs. 29.9%), and shorter trunk (RTRL 75.2% vs. 77.6%), axilla-groin (RAGD 48.2% vs. 51.8%) and vomerine teeth series (RVTL 3.7% vs. 4.1%); from *H. iwami* sp. nov. by the possession of the fifth toe, larger SVL (58.8 vs. 52.0 mm), larger medial tail width (RMTAW 5.3% vs. 3.7%), maximum tail height (RMXTAH 13.4% vs. 11.0%), third finger (R3FL 4.5% vs. 3.6%), and fifth toe (R5TL 2.3% vs. 0%), and shorter vomerine teeth series (RVTL 3.7% vs. 4.7%).

It differs from *H. utsunomiyaorum* sp. nov. with which it occurs sympatrically in some localities, by the absence of yellow stripes on tail, longer head (RHL 24.8% vs. 23.1%), forelimb (RFLL 26.7% vs. 24.9%), third finger (R3FL 4.5% vs. 3.6%), and fifth toe (R5TL 2.3% vs. 0%), and deeper tail (RMXTAH 13.4% vs. 9.7%), and shorter trunk (RTRL 75.2% vs. 76.9%) and axilla-groin (RAGD 48.2% vs. 51.7%); from *H. akiensis* sp. nov. by the presence of yellow stripes on tail, larger SVL (58.8 vs. 49.6 mm), longer head (RHL 24.8% vs. 23.2%) and hindlimb (RHLL 32.3% vs. 29.8%), deeper tail (RMXTAH 13.4% vs. 11.1%), greater limb overlap (LO -0.5 fold vs. -2 folds), and shorter trunk (RTRL 75.2% vs. 76.8%), axilla-groin (RAGD 48.2% vs. 51.7%), and smaller vomerine teeth series (RVTW 4.8% vs. 5.8% and RVTL 3.7% vs. 4.9%); from *H. abuensis* sp. nov. by the presence of yellow stripes on tail, narrower vomerine teeth series

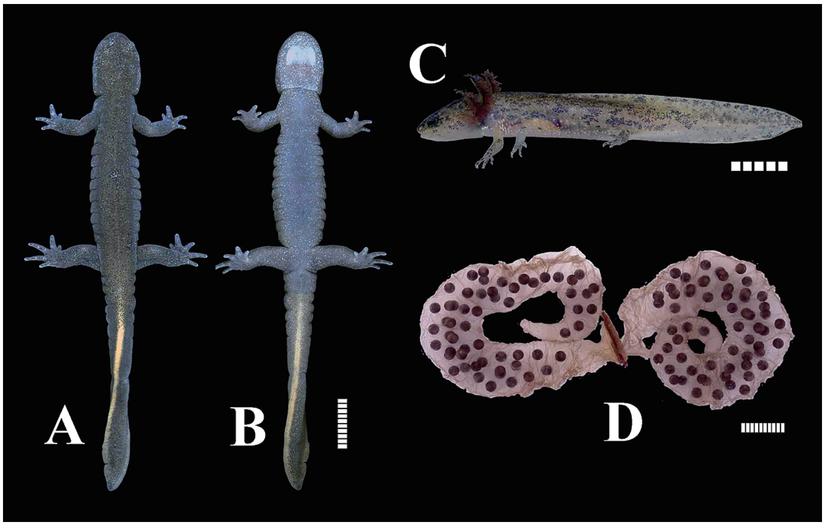

FIG. 6 *Hynobius vandenburghi*. Dorsal (A) and ventral (B) views of a male from Mie (KUHE T2640), scale bar=10 mm. Lateral (C) view of a larva (Stage 63) from Sakai-shi, scale bar=5 mm. Egg sac (D), scale bar=10 mm.

(RVTW 4.8% vs. 5.3%), and shorter second finger (R2FL 5.2% vs. 6.3%).

The mean uncorrected pairwise sequence divergence between *H. setoi* and the other taxa is 12.6–15.1% (Table 2).

Natural history

Breeding occurs from January to March (Okada, 2012). Of 33 cases recorded, ditches (27.3%) were used most frequently as the breeding sites and marshes and abandoned paddies (both 21.2%) and fountains (18.2%) followed them in frequency. The remaining sites were small ponds and water canals (both 6.1%). Egg sacs are attached to debris or roots of water plants at the bottom of the water. Water temperature at the time of breeding is about 9.0–10.5°C. Sympatric with *H. utsunomiyaorum* sp. nov. at 90 m in altitude in Hirose-cho, Yasugi-shi, and at 355 m in altitude, in Daito-cho, Unnan-shi, Shimane Prefecture.

Conservation

The species is in the Red List of Ministry of the Environment Government of Japan (https://www.env.go.jp/nature/kisho/hozen/redlist/RL2018_5_180604.pdf) as VU under the name of *H. nebulosus* with all other taxa here treated. Likewise the species is on the list of Hyogo and Tottori Prefectures as VU, and on the list of Shimane Prefecture as NT under the name of *H. nebulosus*.

2. *Hynobius vandenburghi* Dunn, 1923
[Japanese name: Yamato-sansyou-uwo]
[English name: Yamato Salamander]
Fig. 6

Hynobius nebulosus vandenburghi Mori, 1928, 2.
Hynobius nebulosus (part) Sato, 1934, 218.
Hynobius tokyoensis (part) Sato, 1943, 68.
Hynobius (*Hynobius*) *nebulosus nebulosus* (part) Nakamura and Uéno, 1963, 6.

Holotype

California Academy of Sciences (CAS) 26714, a male from Nara, Province Yamato, Hondo, collected by V. Kühne (alias for Joseph C. Thompson), in May 1907.

Referred specimens

Nara Prefecture: KUHE 12955–12960, six males from Nakamachi, Nara-shi on 2 April 1992 by T. Sugahara. KUHE 18526, 18527, two males from same locality on 6 April 1995 by T. Sugahara. KUHE 26462, one male from Shiraniwadai, Kamimachi, Ikoma-shi on 4 June 1999 by T. Hirai. Shiga Prefecture: KUHE T 2655, one male from Chuzaiji, Hino-cho, Gamo-gun by S. Tanabe. Kyoto Prefecture: KUHE 9799, 9800, 9802, 9808–9811, seven males from Fukakusa Mukaikehara-cho, Fushimi-ku, Kyoto-shi on 8 May 1988 by M. Matsui. KUHE 9300–9310, six males, four females and one juvenile from Tayama, Minamiyamashiro-mura, Soraku-gun on 20 March 1987 by M. Matsui and S. Tanabe. Osaka Prefecture: KUHE 18553, one male from Izumi-Tottori, Hannan-shi on 21 March 1995 by H. Okawa. Mie Prefecture: KUHE 8064–8069, six males from Handa, Tsu-shi on 15 March 1985 by M. Matsui and S. Tanabe. Aichi Prefecture: KUHE 8073–8078, six males from Meito-ku, Nagoya-shi on 15 March 1985 by M. Matsui. KUHE 24862, one male from Moriyama-ku, Nagoya-shi on 8 March 1998 by Y. Misawa. KUHE 25292–25297, six larvae from Higashi-Matsuyama-cho, Seto-shi on 10 August 1998 by M. Otake. KUHE 24863, one male from Kurogawa-marsh, Okubo-cho, Tahara-shi on 9 March 1998 by Y. Misawa. KUHE 9241–9252, six males and six females from Sori-ike, Sori, Chita-shi on 13 March 1987 by M. Matsui. KUHE 24864–24870, seven males from Utsumi, Minamichita-cho, Chita-gun on 23 February 1998 by Y. Misawa.

Etymology

The specific name "*vandenburghi*" is dedicated to John Van Denburgh, curator of the reptile and amphibian collection of the California Academy of Sciences.

Diagnosis

Body large (male SVL 44–67 mm, mean ±SD=56.5±6.4, n=28), fore- and hindlimbs short (24.8%SVL and 29.4%SVL, respectively), ratio of hindlimb to forelimb large (1.19). Tail short (68.2%SVL). Usually with yellow stripes on edges of the tail. Phylogenetically, *H. vandenburghi* forms a mitochondrial DNA clade with *H. tokyoensis*, which is the sister clade of *H. setouchi* sp. nov. The clade including these three species, the clade including *H. setoi* sp. nov. and the clade of *H. takedai*, *H. mikawaensis*, and *H. nigrescens*, together with *H. abei* and *H. lichenatus* form a clade sister to the one including the remaining species. However, *H. vandenburghi* has smaller vomerine teeth series than *H. setouchi* sp. nov., and has longer tail and axilla-groin, larger number of costal grooves, smaller head, narrower interorbital and tail width, shorter hindlimb, and lesser degree of limb overlap than *H. setoi* sp. nov.

Description of topotype (KUHE 12960 from Nara, measurements in mm)

Head-body large for the species (SVL 67.3) and robust; head oval and not depressed, distinctly longer (HL 15.1, 22.4%SVL) than wide (HW 11.0, 16.3%SVL); snout rounded, slightly projecting beyond lower jaw; nostril close to snout tip; labial fold absent; eye moderate, protruded, slightly inset from edge of head in dorsal view; upper eyelid well developed (UEW 2.3, 3.4%SVL), shorter (UEL 4.0, 5.9%SVL) than snout (SL 4.4, 6.5%SVL); gular fold distinct, curving anteriorly; parotoid gland evident, extending from angle of jaw to gular fold; postorbital grooves distinct, branching posterior to angle of jaw, one short and running down to lower jaw, the other long and posterior to parotoid gland; vomerine teeth V-shaped, series nearly touching at midline (Fig. 14B), slightly wider (VTW 3.3, 4.8%SVL) than long (VTL 3.1, 4.6%SVL), anterior margin on line connecting center of choanae; tongue broad, both sides free from mouth floor; fore- and hindlimbs thick, moderate in length for the species (FLL 16.6, 24.7%SVL; HLL 19.9, 29.6%SVL); CG 13; depressed limbs separated by one and half costal folds (LO -1.5); relative length of fingers I<IV<III<II, toes

I<V<II<IV<III; fifth toe well developed (5TL 1.4, 2.1%SVL); cloaca greatly swollen with longitudinal slit; genital tubercle on anterior cloaca present; tail short for the species (TAL 43.8, 65.1%SVL), cylindrical at base (BTAW 8.6, 12.8%SVL; BTAH 7.4, 11.0%SVL), gradually compressed posteriorly (MTAW 4.2, 6.2%SVL; MTAH 6.7, 10.0%SVL), with weak dorsal fin near tip (MXTAH 7.9, 11.7%SVL); tip of tail slightly rounded in lateral view.

Additional measurements and counts of the holotype: IND (2.6, 3.9%SVL); IOD (3.1, 4.6%SVL); AGD (35.2, 52.3%SVL); TRL (52.2, 77.6%SVL); 2FL (3.2, 4.7%SVL); 3FL (3.1, 4.6%SVL); 3TL (5.0, 7.4%SVL); UJTN (75); LJTN (80); VTN (75).

Color

In life, dorsum dark brown without markings (Fig. 6A). Underside of body lighter than dorsum without marking, except for throat covered with white nuptial color (Fig. 6B). Tail with median yellow stripe dorsally and ventrally.

Variation

Morphometric data are summarized in Table 4. SVL of 28 males has the mean of 56.5±6.4 (range=43.9–67.3) mm. Medians and ranges in males: HL 23.4 (21.0–28.9)%SVL; TRL 76.6 (71.1–79.0)%SVL; HW 16.7 (14.5–18.5)%SVL; IND 4.9 (3.9–6.0)%SVL; IOD 5.7 (4.5–7.3)%SVL; UEW 3.0 (2.2–3.8)%SVL; UEL 5.6 (4.3–7.1)%SVL; AGD 51.6 (46.9–56.3)%SVL; TAL 68.2 (57.5–89.6)%SVL; BTAW 10.5 (7.7–13.1)%SVL; MXTAH 11.7 (7.2–15.4)%SVL; FLL 24.8 (21.1–29.4)%SVL; HLL 29.4 (24.8–35.0)%SVL; MTAW 5.0 (1.7–6.5)%SVL; VTW 4.5 (3.6–5.9)%SVL; VTL 3.9 (3.0–4.9)%SVL; 2FL 4.9 (3.4–7.1)%SVL; 3FL 4.2 (2.6–6.4)%SVL; 3TL 6.9 (5.3–9.5)%SVL; 5TL 2.3 (0–3.0)%SVL. VTW/VTL 117.3 (97.7–157.1)%. TotL 94.25 (73.1–125.5) mm. CG 13 (12–13); LO -1.5 (-3–-0.5).

The fifth toe was almost always present. Some specimens have dorsum dotted with black and venter scattered with silver dots.

Eggs and egg sacs

The egg sacs are string-like in shape and coiling, with a thin, wrinkled envelope lacking notable striations and whiptail structure (Fig. 6D). In the population from Kyoto Prefecture, egg sacs had a length (ESL) of 94–154 (mean±SD=129.9±26.6) mm and the width (ESW) of 12–15.8 (mean±SD=14.3±2.0) mm, with ESL/ESW being 9.2–10.5 (mean ±SD=9.9±0.7, n=4), and the clutch size ranged from 65–113 (mean±SD=82.1±16.2, n=7). The ova ranged from 2.4–2.7 mm in 4 females (mean±SD=2.5±0.1). According to Sato (1943, as *H. tokyoensis*), egg sacs are thicker than in the Kyoto population: an egg sac has the length of 100–185 (mean ±SD=131.7±18.7) mm and the width of 15.5–21 (mean±SD=17.4±1.6) mm, with ESL/ESW being 6.8–9.3 (mean ±SD=7.6±0.8, n=28), and the clutch size ranging from 41–88 (mean±SD=65.9±14.6, n=14) in the population from Nagoya-shi, Aichi Prefecture. The ova ranged from 2.1–2.3 mm in a female from Chita, Aichi, and 2.5–3.0 mm (Sato, 1943) in diameter, and are dark brown in color, darker in the animal pole and lighter in the vegetal pole.

Larvae

A larva at Stage 63 of Iwasawa and Yamashita (1991) had SVL of 15.8 and total length of 34.8 mm, head rounded in profile (Fig. 6C); snout short and broadly rounded; eyes slightly protruded, inset from edge of head in dorsal view; labial fold distinct at half of upper jaw; external gills developed; caudal fin higher than head; dorsal fin higher than ventral fin; origin of dorsal fin in middle of trunk; ventral fin originating from the vent; tail tip weakly pointed; limbs slender; claws on fingers and toes absent. In life, their dorsum was light brown dotted with dark brown; larger black spots scattered on side of body and tail; venter whitish and transparent; golden dots scattered on flank and ventral tail fin.

Range

Known so far from areas in eastern Kinki and southern Chubu regions (Fig. 15). Nara Prefecture: Nara-shi; Oyodo-cho, Yoshino-gun; Ikoma-shi. Shiga Prefecture: Otsu-shi; Hino-cho, Gamo-gun; Higashiomi-shi (including the former Gamo-cho and Yokaichi-shi); Koka-shi (including the former Minakuchi-cho); Kusatsu-shi; Nagahama-shi (including the former Kinomoto-cho); Omihachiman-shi; Ritto-shi; Takashima-shi (including the former Adogawa-cho, Makino-cho, and Shin-asahi-cho); Maibara-shi (including the former Maihara-cho, Omi-cho, and Santo-cho); Hikone-shi. Kyoto Prefecture: Kyoto-shi; Uji-shi; Kameoka-shi; Kyotanabe-shi; Minamiyamashiro-mura, Soraku-gun; Kizugawa-shi (including the former Kamo-cho and Kizu-cho); Oyamazaki-cho, Otokuni-gun; Seika-cho, Soraku-gun; Ujitawara-cho, Tsuduki-gun. Osaka Prefecture: Takatsuki-shi; Katano-shi; Hirakata-shi; Ibaraki-shi; Shijonawate-shi; Izumi-shi; Tondabayashi-shi; Higashiosaka-shi; Sakai-shi; Hannan-shi. Mie Prefecture: Inabe-shi; Kuwana-shi; Iga-shi; Matsusaka-shi; Tsu-shi (including the former Hisai-shi); Shima-shi; Suzuka-shi; Kameyama-shi. Aichi Prefecture: Nagoya-shi; Tahara-shi; Chita-shi; Seto-shi; Minamichita-cho, Chita-gun; Mihama-cho, Chita-gun; Toyota-shi; Nagakute-shi. Gifu Prefecture: Gifu-shi; Ibigawa-cho, Ibi-gun; Kaizu-shi.

Comparisons

Hynobius vandenburghi significantly differs from *H. setouchi* sp. nov. by smaller vomerine teeth series (RVTW 4.5% vs. 5.1% and RVTL 3.9% vs. 4.6%); from *H. setoi* sp. nov. by longer trunk (RTRL 76.6% vs. 75.2%) and axilla-groin (RAGD 51.6% vs. 48.2%), larger number of costal grooves (CG 13 vs. 12), and smaller head (RHL 23.4% vs. 24.8%, RHW16.7% vs. 17.7%), narrower interorbital (RIOD 5.7% vs. 6.3%) and tail width (RBTAW 10.5% vs. 11.7%), shorter hindlimb (RHLL 29.4% vs. 32.3% and R3TL 6.9% vs. 8.0%), and lesser degree of limb overlap (LO -1.5 folds vs. -0.5 folds).

It is different from *H. nebulosus* by narrower head (RHW 16.7% vs. 18.9%) and internarial (RIND 4.9% vs. 5.8%), and shorter vomerine teeth series (RVTL 3.9% vs. 4.6%); from *H. bakan* sp. nov. by a narrower head (RHW 16.7% vs. 18.4%) and smaller vomerine teeth series (RVTW 4.5% vs. 5.2% vs. and RVTL 3.9% vs. 4.7%); from *H. iwami* sp. nov. by longer fifth toe (R5TL 2.3% vs. 0%), larger number of costal grooves (CG 13 vs. 12), shorter vomerine teeth series (RVTL 3.9% vs. 4.7%) and lesser degree of limb overlap (LO -1.5 folds vs. -0.5 fold).

It differs from *H. utsunomiyaorum* sp. nov. by higher tail (RMXTAH 11.7% vs. 9.7%), longer fifth toe (R5TL 2.3% vs. 0%), and larger number of costal grooves (CG 13 vs. 12); from *H. abuensis* sp. nov. by longer axilla-groin (RAGD 51.6% vs. 47.1%), larger number of costal grooves (CG 13 vs. 12), narrower head (RHW 16.7% vs. 19.6%), internarial (RIND 4.9% vs. 5.8%), upper eyelid (RUEW 3.0% vs. 3.5%), tail (RBTAW 10.3% vs. 11.4%) and vomerine teeth series (RVTW 4.5% vs. 6.3%), shorter tail (RTAL 68.2% vs. 86.9%) and limbs (RFLL 24.8% vs. 27.4%, RHLL 29.4% vs. 34.3%, R2FL 4.9% vs. 6.3%, R3FL 4.2% vs. 5.1%, R3TL 6.9% vs. 8.9%), and lesser degree of limb overlap (LO -1.5 folds vs. 1 fold); from *H. akiensis* sp. nov. by larger SVL (56.5 mm vs. 49.6 mm) and smaller vomerine teeth series (RVTW 4.5% vs. 5.4% and RVTL 3.9% vs. 4.6%).

The mean uncorrected pairwise sequence divergence between *H. vandenburghi* and the other species is 10.6–15.9% (Table 2).

Natural history

Breeding occurs from February to May and egg sacs are attached to debris or water plants in the bottom of pools, abandoned paddies, ponds, and ditches sometimes with very slow current. Water temperature at the time of breeding is about 4.0–15.0°C in Nagoya, Aichi Prefecture (Fujitani, 2000).

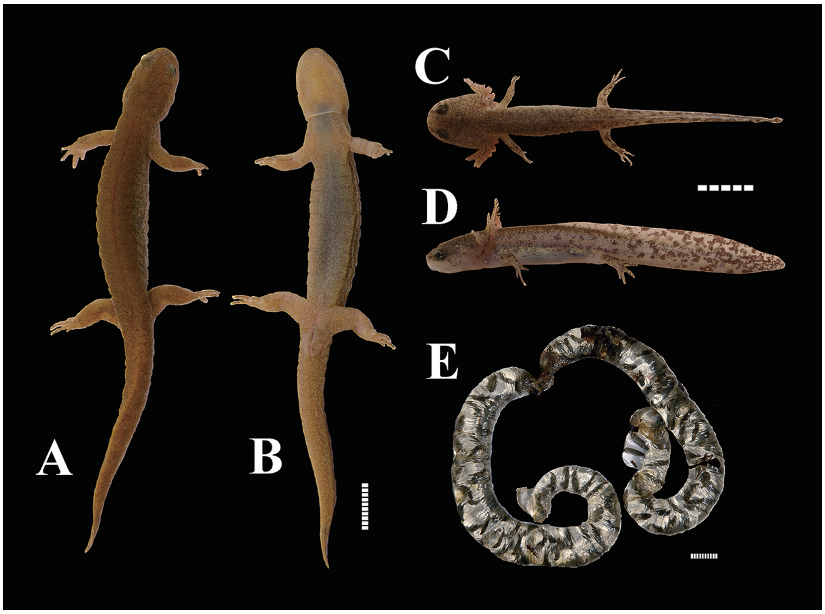

Fig. 7 *Hynobius setouchi* sp. nov. Dorsal (A) and ventral (B) views of male holotype (KUHE OU 0285), scale bar=10 mm. Dorsal (C) and lateral (D) views of a larva (Stage 63) from Okayama-shi, scale bar=5 mm. Egg sac (E), scale bar=10 mm.

Conservation

The species is in the Red List of Ministry of the Environment Government of Japan (https://www.env.go.jp/nature/kisho/hozen/redlist/RL2018_5_180604.pdf) as VU under the name of *H. nebulosus* with all other taxa here treated. Locally, the species is listed on the Prefectural Red List as CR+EN in Osaka, as EN in Gifu, Aichi, Kyoto, and Nara, as VU in Mie, and as NT in Shiga.

3. *Hynobius* ***setouchi*** Matsui, Okawa, Tanabe et Misawa sp. nov.

[Japanese name: Setouchi-sansyou-uwo]
[English name: Setouchi salamander]
Fig. 7

Hynobius nebulosus (part, as Okayama morphotype) Okawa and Utsunomiya, 1989, 142.
Hynobius nebulosus (part, as Setouchi morphotype) Okawa et al., 2007, 58.

Holotype

KUHE OU 0285, an adult male from Shimokoume, Takebe-cho, Kita-ku, Okayama-shi, Okayama Prefecture, (34°53'01"N, 133°55'40"E, alt. 150 m) by H. Okawa on 17 March 2007.

Paratypes

KUHE OU 0286–0289, four males, data same as the holotype. KUHE 6841–6844, four males from Higashichujyo, Kannabe-cho, Fukuyama-shi, Hiroshima Prefecture (34°34'40"N, 133°22'18"E, alt. 40 m) on 14 March 1983 by T. Utsunomiya and Y. Utsunomiya. KUHE 6860, 6861, two males from Nakasanna, Numakuma-cho, Fukuyama-shi, Hiroshima Prefecture (34°24'46"N, 133°19'52"E, alt. 82 m) on 24 March 1983 by T. Utsunomiya and Y. Utsunomiya. KUHE 6862–6864, three males from Minari, Minogo-cho, Onomichi-shi, Hiroshima Prefecture (34°26'02"N, 133°11'52"E, alt 105 m), on 24 March 1983 by T. Utsunomiya and Y. Utsunomiya.

Referred specimens

Okayama Prefecture: KUHE OU0290, KUHE OU0291, two males, Okamidani, Mito-cho, Kita-ku, Okayama-shi by H. Okawa on 17 March 2007. KUHE OU0295, KUHE OU0296, two males, Nishi, Kibichuo-cho, Kaga-gun by H. Okawa on 18 March 2007. KUHE OU0305–0308, four males, Nashinokitoge, Utodani, Bisei-cho, Ibara-shi by H. Okawa on 31 March 2007. Hyogo Prefecture: KUHE 11594, one male, Myodani, Suma-ku, Kobe-shi in March 1991 by M. Matsui. KUHE 18528, one male, Matogata-machi, Himeji-shi by T. Sugahara, on 29 March 1995. Osaka Prefecture: KUHE 26543, one male, Tanagawa, Misaki-cho, Sennan-gun by H. Ashida in July 1999. Wakayama Prefecture: KUHE 20516, one male, Chibayama, Aridagawa-cho, Arida-gun (the former Kibi-cho) by T. Sugahara on 23 March 1996. KUHE 20523, one male, Tenjinzaki, Tanabe-shi by T. Sugahara on 23 March 1996.

Etymology

The specific name "*setouchi*" refers to geographic distribution of the new species, being distributed around the Seto Inland Sea (Setouchi District).

Diagnosis

Body small (male SVL 45–58 mm, mean ±SD=52.3±4.3, n=13), fore- and hindlimbs relatively long (26.7%SVL and 31.0%SVL), ratio of hindlimb to forelimb moderate (1.16). The fifth toe almost always present. Tail long (77.8%SVL). Upper and lower edges of the tail usually lack yellow stripes. The egg sac envelope has no striations. Phylogenetically, *H. setouchi* sp. nov. forms a mitochondrial DNA clade with the clade of *H. tokyoensis* and *H. vandenburghi*. The clade including these three species, the clade including *H. setoi* sp. nov. and the clade of *H. takedai*, *H. mikawaensis*, and *H. nigrescens*, together with *H. abei* and *H. lichenatus* form a clade. However, *H. setouchi* sp. nov. differs from *H. vandenburghi* by larger vomerine teeth series, and from *H. setoi* sp. nov. by smaller SVL, longer trunk and vomerine teeth series, larger number of costal grooves, and shorter head.

Description of holotype (measurements in mm)

Head-body large for the species (SVL 58.3) and robust; head oval and not depressed, distinctly longer (HL 13.4, 23.0%SVL) than wide (HW 9.8, 16.8%SVL); snout rounded, slightly projecting beyond lower jaw; nostril close to snout tip; labial fold absent; eye moderate, protruded, slightly inset from edge of head in dorsal view; upper eyelid well developed (UEW 2.0, 3.4%SVL), shorter (UEL 3.0, 5.1%SVL) than snout (SL 4.5, 7.7%SVL); gular fold distinct, curving anteriorly; parotoid gland evident, extending from angle of jaw to gular fold; postorbital grooves distinct, branching posterior to angle of jaw, one short and running down to lower jaw, the other long and posterior to parotoid gland; vomerine teeth V-shaped, series nearly touching at midline (Fig. 14C), wider (VTW 3.0, 5.1%SVL) than long (VTL 2.8, 4.8%SVL), anterior margin on line connecting choanae; tongue broad, both sides free from mouth floor; limbs thick, forelimb short, but hindlimb medium for the species (FLL 14.5, 24.9%SVL; HLL 18.1, 31.1%SVL); CG 13; depressed limbs separated by one and half costal folds (LO -1.5); relative length of fingers I<IV<III<II, toes V<I<II<IV<III; fifth toe well developed (5TL 1.1, 1.9%SVL); cloaca greatly swollen with longitudinal slit; genital tubercle on anterior cloaca present; tail medium for the species (TAL 45.4, 77.9%SVL), cylindrical at base (BTAW 7.4, 12.7%SVL; BTAH 6.2, 10.6%SVL), gradually compressed posteriorly (MTAW 3.5, 6.0%SVL; MTAH 7.4, 12.7%SVL), without marked dorsal fin near tip (MXTAH 7.5, 12.8%SVL); tip of tail pointed in lateral view.

Additional measurements and counts of the holotype: IND (3.1, 5.3%SVL); IOD (3.5, 6.0%SVL); AGD (28.3, 49.4%SVL); TRL (44.9, 77.0%SVL); 2FL (2.9, 4.9%SVL); 3FL (2.6, 4.5%SVL); 3TL (4.5, 7.7%SVL); UJTN

(84); LJTN (70); VTN (43).

Color

In life, dorsum light brown without markings (Fig. 7A). Underside of body lighter than dorsum, dotted with silvery white (Fig. 7B). Throat covered with pale white nuptial color.

Variation

Morphometric data are summarized in Table 4. SVL of 13 males has the mean of 52.3±4.3 (44.6–58.3) mm. Medians and ranges in males are: HL 23.1 (22.3–24.5)%SVL; TRL 76.9 (75.5–77.7)%SVL; HW 17.8 (14.8–19.9)%SVL; IND 5.5 (4.8–6.1)%SVL; IOD 6.1 (5.3–6.7)%SVL; UEW 3.2 (2.4–3.6)%SVL; UEL 5.9 (5.2–10.6)%SVL; AGD 50.0 (48.7–53.9)%SVL; TAL 77.9 (58.9–88.3)%SVL; BTAW 10.6 (8.5–14.0)%SVL; MXTAH 11.7 (9.3–13.8)%SVL; FLL 26.7 (24.2–28.6)%SVL; HLL 31.1 (29.2–34.0)%SVL; MTAW 4.4 (2.7–6.3)%SVL; VTW 5.1 (4.3–5.5)%SVL; VTL 4.6 (3.9–5.6)%SVL; 2FL 4.9 (3.8–5.6)%SVL; 3FL 4.7 (3.4–5.4)%SVL; 3TL 7.8 (7.0–8.6)%SVL; 5TL 2.4 (1.3–3.1)%SVL; VTW/VTL 118.8 (76.5–135.0)%. TotL 91.75 (78.3–103.7) mm; CG 13 (12–13); LO -1 (-2.5–-0.5).

Yellow stripes on the tail are usually lacking: 67% of 12 individuals lacked stripes, 25% had slightly yellow stripe on upper edge, and 8% had weak stripes on both edges. In many individuals, white nuptial color on throat was clearer than the holotype. The fifth toe was almost always present: in 41 individuals studied, 38 (92.7%) had complete fifth toe on both sides, 1 (2.4%) with rudimentary fifth toe on one side, 1 with complete toe on one side and lacking on another side, and 1 with rudimentary fifth on both sides.

Eggs and egg sacs

The egg sacs are string-like in shape and coiling, with a thin, wrinkled envelope lacking notable striations and any distinct whiptail structure (Fig. 7E). In a population from Okayama, egg sacs have the lengths (ESL) of 110–210 (mean±SD=164.0±56.8) mm and the widths (ESW) of 17–20 (mean ±SD=18.3±1.5) mm, with ESL/ESW being 5.8–12.7 (mean±SD=9.2±3.8, n=4), and the clutch size ranging from 42–155 (mean ±SD=87.4±25.4, n=52). In two populations from Wakayama Prefecture, ESL of 101–169 (mean±SD=133.7±18.5) mm and ESW of 16–20 (mean±SD=17.4±1.2) mm, with ESL/ESW being 5.9–9.9 (mean±SD= 7.7±1.1, n=20), and the clutch size ranging from 48–113 (mean±SD=79.1±19.3, n=10), were reported by Sato (1943). Six ova from a female from Takarazuka, Hyogo Prefecture range from 2.5–2.9 (mean±SD=2.7±0.2) mm in diameter, and are light brown in color, tending to darker in the animal pole than in the vegetal pole.

Larvae

Three larvae from Kobe-shi at Stage 63 of Iwasawa and Yamashita (1991) had SVL ranging from 15.0–22.2 (mean=18.8) and total length of 31.7–40.7 (mean=35.0) mm, head rounded in dorsal view and truncate in profile (Fig. 7C, D); snout short and broadly rounded; eyes slightly protruded, inset from edge of head in dorsal view; labial fold distinct at half of upper jaw; external gills developed; caudal fin higher than head; dorsal fin higher than ventral fin; origin of dorsal fin in middle of trunk; ventral fin originating from the vent; tail tip weakly pointed; limbs slender; claws on fingers and toes absent. In life, dorsum was light brown with darker pigmentations; venter whitish and transparent; large black spots scattered on flunk and tail; golden dots scattered on tail fin.

Range

Known so far from western Kinki, eastern Chugoku, and eastern Shikoku regions (Fig. 15). Hyogo Prefecture: Kobe-shi; Nishinomiya-shi; Ashiya-shi; Takarazuka-shi; Sanda-shi; Inagawa-cho, Kawabe-gun; Akashi-shi; Kakogawa-shi; Nishiwaki-shi; Miki-shi; Takasago-shi; Ono-shi; Kato-shi; Taka-cho, Taka-gun; Himeji-shi; Tatsuno-shi;

Fukusaki-cho, Kanzaki-gun; Taishi-cho, Ibo-gun; Sasayama-shi; Sumoto-shi; Minamiawaji-shi; Awaji-shi. Okayama Prefecture: Ibara-shi (including the former Bisei-cho); Mimasaka-shi (including the former Mimasaka-cho); Okayama-shi (including the former Takebe-cho); Kumenan-cho, Kume-gun; Kibichuo-cho, Kaga-gun (including the former Kayo-cho); Akaiwa-shi (including the former Akasaka-cho); Asakuchi-shi (including the former Konko-cho); Kurashiki-shi; Sojya-shi; Kagamino-cho, Tomata-gun; Wake-cho, Wake-gun (including the former Saeki-cho). Hiroshima Prefecture: Fukuyama-shi (including the former Numakuma-cho and Kannabe-cho), Onomichi-shi; Osaki Kamijima-cho, Toyota-gun (including the former Osaki-cho). Kagawa Prefecture: Takamatsu-shi; Sakaide-shi. Tokushima Prefecture: Tokushima-shi; Anan-shi; Awa-shi (including the former Awa-cho, Ichiba-cho, and Donari-cho); Kamiita-cho, Itano-gun; Komatsushima-shi; Mima-shi (including the former Waki-machi); Mugi-cho, Kaifu-gun; Naruto-shi; Sanagochi-son, Myodo-gun; Yoshinogawa-shi (including the former Misato-son, and Yamakawa-cho). Osaka Prefecture: Misaki-cho, Sennan-gun. Wakayama Prefecture: Wakayama-shi; Aridagawa-cho, Arida-gun; Minabe-cho, Hidaka-gun; Tanabe-shi; Shirahama-cho, Nishimuro-gun.

In Onomichi-shi, Hiroshima Prefecture, the species occurs parapatrically with *H. akiensis* sp. nov. According to Watabe (2018), occurrence of *H. nebulosus* was known around 1967 in a restricted range of eastern Kochi Prefecture, but in 2002, when the salamander was designated as CR+EN, it could no longer be found there. However, it is said that someone witnessed the salamander recently (Watabe, 2018). We have no data about the population, but it may belong to *H. setouchi*, which occurs in Mugi-cho, near the locality in question in Kochi.

Elevations of the known localities extend from 20 to 460 (239.1±136.2, n=10) m.

Comparisons

Hynobius setouchi sp. nov. significantly differs from *H. nebulosus* by smaller SVL (52.3 mm vs. 59.3 mm) and from *H. vandenburghi* by larger vomerine teeth series (RVTW 5.1% vs. 4.5% and RVTL 4.6% vs. 3.9%). The new species differs from *H. utsunomiyaorum* sp. nov. by longer vomerine teeth series (RVTL 4.6% vs. 3.7%) and digits (R3FL 4.7% vs. 3.6% and R5TL 2.4% vs. 0%); from *H. abuensis* sp. nov. by larger number of costal grooves (CG 13 vs. 12) and narrower head (RHW 17.8% vs. 19.4%), shorter forelimb (RFLL 26.7% vs. 30.1% and R2FL 4.9% vs. 6.3%), and lesser degree of limb overlap (LO -1 fold vs. 1 fold); from *H. setoi* sp. nov. by longer trunk (RTRL 76.9% vs. 75.2%) and vomerine teeth series (RVTL 4.6% vs. 3.7%), larger number of costal grooves (CG 13 vs.12), and smaller SVL (52.3 mm vs. 58.8 mm) and shorter head (RHL 23.1% vs. 24.8%); and from *H. iwami* sp. nov. by longer digits (R3FL 4.7% vs. 3.6% and R5TL 2.4% vs. 0%) and larger number of costal grooves (CG 13 vs. 12).

Hynobius setouchi sp. nov. did not show significant morphometric differences from *H. bakan* sp. nov. and *H. akiensis* sp. nov. However, *H. setouchi* sp. nov. differs from *H. bakan* sp. nov. by usual lack of yellow stripes in the tail. Both *H. setouchi* sp. nov. and *H. akiensis* sp. nov. lack the tail stripes, but the latter (mean SVL of 49.6±4.3 mm) tends to be smaller than the former (52.3±4.3 mm).

The mean uncorrected pairwise sequence divergence between *H. setouchi* sp. nov. and the other species is 10.6–15.8% (Table 2).

Natural history

Breeding occurs usually from mid March to early April in Okayama, Hiroshima, and Kagawa Prefectures, but from early January in Wakayama Prefecture. Of 24 cases recorded, ditches and pools in and around paddy fields occupied more than half (63%). Otherwise, breeding occurred in marshes, ponds, ditches along roads, and slowly flowing streams. Egg sacs are attached to debris or

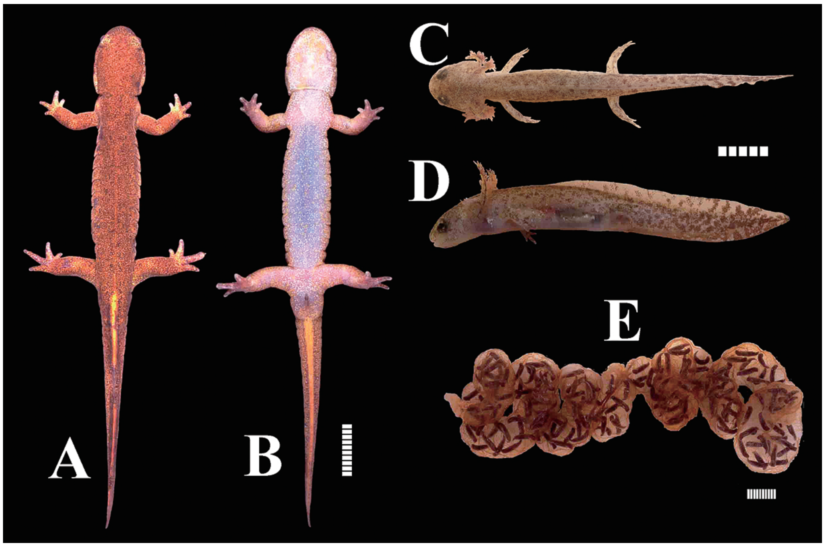

FIG. 8 *Hynobius iwami* sp. nov. Dorsal (A) and ventral (B) views of male holotype (KUHE 35891), scale bar=10 mm. Dorsal (C) and lateral (D) views of a larva (Stage 63), scale bar=5 mm. (E) Egg sac, scale bar=10 mm.

roots of water plants. Water temperature at the time of breeding varies from 6.6 to 14.1°C.

Conservation

The species is in the Red List of Ministry of the Environment Government of Japan (https://www.env.go.jp/nature/kisho/hozen/redlist/RL2018_5_180604.pdf) as VU with all other taxa here treated under the name of *H. nebulosus*. The species is listed as CR+EN on the list of Osaka, as EN on the list of Okayama, and as VU on the lists of Hyogo, Hiroshima, Kagawa, Tokushima, and Wakayama Prefectures.

4. *Hynobius* ***iwami*** Matsui, Okawa, Nishikawa et Tominaga sp. nov.

[Japanese name: Iwami-sansyou-uwo]
[English name: Iwami salamander]
Fig. 8

Hynobius nebulosus (part, as Geihoku morphotype) Okawa et Utsunomiya, 1992, 12.
Hynobius nebulosus (part, as Iwami morphotype) Okawa et al., 2005, 11.
Hynobius nebulosus (part, as Nihonkai morphotype) Okawa et al., 2007, 58.

Holotype

KUHE 35891, an adult male from Toda-cho, Masuda-shi, Shimane Prefecture (34°41'44"N, 131°52'16"E, alt. 38 m), collected by K. Nishikawa and A. Tominaga on 9 March 2005.

Paratypes

KUHE 35892–35894, 35923, 35939, five adult males collection data same as the holotype; KUHE 35900–35903, four males, from the type locality, collected by K. Nishikawa and A. Tominaga on 8 March 2005.

Referred specimens

All from Masuda-shi, Shimane Prefecture: KUHE 35895–35897, three males from Honmataga-cho, collected by K. Nishikawa and A. Tominaga on 8 March 2005. KUHE 35910, one male from Musou-cho collected by K. Nishikawa and A. Tominaga on 8 March 2005. KUHE OU 0339, one male from Kawanobori-cho, collected by H. Okawa on

11 February 2009.

Etymology

The specific name “*iwami*” refers to the old name of the region (=Iwami) of the present western part of Shimane Prefecture, including the type locality of the new species.

Diagnosis

Body small (male SVL 43–58 mm, mean ±SD=52.0±4.8, n=14), fore- and hindlimbs medium (26.6%SVL and 30.3%SVL, respectively), ratio of hindlimb to forelimb small (1.14). Tail medium (74.0%SVL). Always with clear yellow stripes on dorsal and ventral edges of the tail. Phylogenetically, *H. iwami* sp. nov. and *H. okiensis* form a large mitochondrial DNA clade with the clade of *H. bakan* sp. nov., *H. nebulosus* and *H. tsuensis*. However, *H. iwami* sp. nov. differs from all of the others by usual lack of the fifth toe. Additionally, it differs from *H. bakan* sp. nov. by longer head, shorter trunk, and from *H. nebulosus* by longer head, greater limb overlap, smaller SVL, shorter trunk, narrower internarial, and smaller number of costal groove.

Description of holotype (measurements in mm)

Head-body large for the species (SVL 56.6) and robust; head oval and not depressed, distinctly longer (HL 13.9, 24.6%SVL) than wide (HW 10.0, 17.7%SVL); snout rounded, slightly projecting beyond lower jaw; nostril close to snout tip; labial fold absent; eye moderate, protruded, slightly inset from edge of head in dorsal view; upper eyelid well developed (UEW 1.6, 2.8%SVL), shorter (UEL 3.1, 5.5%SVL) than snout (SL 3.9, 6.9%SVL); gular fold distinct, curving anteriorly; parotoid gland evident, extending from angle of jaw to gular fold; postorbital grooves distinct, branching posterior to angle of jaw, one short and running down to lower jaw, the other long and posterior to parotoid gland; vomerine teeth U-shaped, series nearly touching at midline (Fig. 14D), wider (VTW 2.8, 4.9%SVL) than long (VTL 2.4, 4.3%SVL), anterior margin anterior to line connecting anterior tips of choanae; tongue broad, both sides free from mouth floor; fore- and hindlimbs medium for the species and thin (FLL 15.1, 26.7%SVL; HLL 16.9, 29.9%SVL); CG 12; depressed limbs separated by one costal fold (LO –1); relative length of fingers IV<I<III<II, toes I<IV<II<III; fifth toe rudimentary (5TL 0, 0%SVL); cloaca greatly swollen with longitudinal slit; genital tubercle on anterior cloaca present; tail medium for the species (TAL 40.7, 71.9%SVL), cylindrical at base (BTAW 6.3, 11.1%SVL; BTAH 6.2, 11.0%SVL), gradually compressed posteriorly (MTAW 3.3, 5.8%SVL; MTAH 7.5, 13.3%SVL), with weak dorsal fin (MXTAH 6.9, 12.2%SVL); tip of tail slightly rounded in lateral view.

Additional measurements and counts of the holotype: IND (2.9, 5.1%SVL); IOD (3.4, 6.0%SVL); AGD (27.5, 48.6%SVL); TRL (42.7, 75.4%SVL); 2FL (2.6, 4.5%SVL); 3FL (1.7, 3.0%SVL); 3TL (4.7, 8.3%SVL); UJTN (74); LJTN (70); VTN (49).

Color

In life, dorsum light brown without markings (Fig. 8A). Underside of body lighter than dorsum, dotted with silvery white, except for throat covered with white nuptial color (Fig. 8B). Dorsal and ventral sides of tail edged with orange yellow.

Variation

Morphometric data are summarized in Table 4. The variation in the maximum 14 males is as follows. SVL 52.0±4.8 (42.5–57.9) mm. Medians and ranges are: HL 24.7 (22.6–26.6)%SVL; TRL 75.3 (73.4–77.4)%SVL; HW 17.5 (16.6–18.8)%SVL; IND 5.1 (4.7–5.9)%SVL; IOD 6.2 (5.2–7.4)%SVL; UEW 3.3 (2.6–3.6)%SVL; UEL 5.6 (5.2–6.4)%SVL; AGD 50.7 (48.6–54.4)%SVL; TAL 74.1 (70.5–81.1)%SVL; BTAW 10.6 (6.8–12.2)%SVL; MXTAH 11.0 (6.4–13.8)%SVL; FLL 26.6 (24.2–28.4)%SVL; HLL 30.3 (28.2–34.0)%SVL; MTAW 3.7

(2.4–5.8)%SVL; VTW 4.9 (4.4–5.7)%SVL; VTL 4.7 (3.7–5.6)%SVL; 2FL 4.7 (3.5–6.1)%SVL; 3FL 3.6 (3.0–4.4)%SVL; 3TL 7.8 (6.5–8.8)%SVL; 5TL 0 (0–0.98) %SVL; VTW/VTL 111.8 (87.1–139.9)%. TotL 93.7 (74.7–99.3) mm; CG 12 (12–12); LO -0.5 (-2–0.5).

A fifth toe was almost always absent, and of 134 specimens examined, 93.3% had the same number of toes between the right and left sides, and 93.7% completely lacked the fifth toe (Okawa et al., 2009).

Eggs and egg sacs

The egg sacs are string-like in shape and coiling, with a thin, wrinkled envelope lacking notable striations and any distinct whiptail structure (Fig. 8E). The clutch size ranges from 56–125, with the mean of 83.7±22.9 (n=7).

Larva

A larva at Stage 63 of Iwasawa and Yamashita (1991) had SVL of 18.2 and total length of 33.8 mm, head rounded in dorsal view and profile (Fig. 8C, D); snout short and rounded; eyes slightly protruded, inset from edge of head in dorsal view; labial fold distinct at half of upper jaw; external gills developed; caudal fin higher than head; dorsal fin higher than ventral fin; origin of dorsal fin in middle of trunk; ventral fin originating from the vent; tail tip weakly pointed; limbs slender; claws on fingers and toes absent. In life, their dorsum was light brown with dark marking; venter whitish and transparent; large black spots scattered on posterior half of tail; silver dots scattered on lower tail fin.

Range

Known from a small area in southwestern Honshu, mainly along the Japan Sea (Okawa et al., 1999; Fig. 15). Northwestern Shimane and adjacent Hiroshima Prefectures, surrounded by *H. setoi* sp. nov., *H. utsunomiyaorum* sp. nov., *H. akiensis* sp. nov., and *H. abuensis* sp. nov. Hiroshima Prefecture: Kitahiroshima-cho, Yamagata-gun (formerly Geihoku-cho). Shimane Prefecture: Masuda-shi; Gotsu-shi (including the former Sakurae-cho); Oda-shi; Hamada-shi (including the former Yasaka-mura, Asahi-machi, Kanagi-cho, and Misumi-cho).

Altitude of the known localities extends from 38–100 m, with the mean of 70.6±27.1 m (n=5).

Comparisons

Hynobius iwami sp. nov. significantly differs from *H. bakan* sp. nov. by longer head (RHL 24.7% vs. 22.5%), and shorter trunk (RTRL 75.3% vs. 77.6%) and fifth toe (R5TL 0% vs. 7.1%); from *H. nebulosus* by longer head (RHL 24.7% vs. 23.6%), greater limb overlap (LO -0.5 fold vs. -2 folds), smaller SVL (52.0 mm vs. 59.3 mm), shorter trunk (RTRL 75.3% vs. 76.4%) and fifth toe (R5TL 0% vs. 2.5%), narrower internarial (RIND 5.1% vs. 5.8%), and smaller number of costal grooves (CG 12 vs. 13).

It differs from *H. utsunomiyaorum* sp. nov. by longer vomerine teeth series (RVTL 4.7% vs. 3.7%) and narrower tail (RMTAW 3.7% vs. 5.5%); from *H. akiensis* sp. nov. by greater limb overlap (LO -0.5 fold vs. -2 folds), narrower tail (RMTAW 3.7% vs. 5.1%), and shorter fifth toe (R5TL 0% vs. 2.0%); from *H. abuensis* sp. nov. by narrower head (RHW 17.5% vs. 19.6%) and internarial (RIND 5.1% vs. 5.8%), and shorter forelimb (RFLL 26.6% vs. 27.4%), hindlimb (RHLL 33.3% vs. 34.3%), second finger (R2FL 4.7% vs. 6.3%), third finger (R3FL 3.6% vs. 5.1%), and fifth toe (R5TL 0% vs. 3.0%).

The new species is different from *H. setoi* sp. nov. by longer vomerine teeth series (RVTL 4.7% vs. 3.7%) and smaller SVL (52.0 mm vs. 58.8 mm), narrower and lower tail (RMTAW 3.7% vs. 5.3%, RMXTAH 11.0% vs. 13.4%), and shorter third finger (R3FL 3.6% vs. 4.5%) and fifth toe (R5TL 0% vs. 2.3%); from *H. setouchi* sp. nov. by shorter third finger (R3FL 3.6% vs. 4.7%) and fifth toe (R5TL 0% vs. 2.4%), and smaller number of costal grooves (CG 12 vs. 13); from *H. vandenburghi* by longer vomerine teeth series (RVTL 4.7% vs. 3.9%), greater limb overlap (LO -0.5 fold vs. -1.5 folds), shorter fifth toe (R5TL 0% vs. 2.3%, and smaller number of costal grooves (CG12 vs. 13).

The mean uncorrected pairwise sequence divergence between *H. iwami* and the other species is 10.7–15.8% (Table 2).

Natural history

Breeding occurs from January to May (Okawa et al., 1999). Twelve breeding sites recorded include marshes (41.7%), pools (25.0%), abandoned paddies (16.7%) and ditches (16.7%). Water temperature at the time of breeding varies from 5.0 to 9.6°C.

Conservation

This species is in the national Red List as VU under the name of *H. nebulosus* with all other taxa here treated (https://www.env.go.jp/nature/kisho/hozen/redlist/RL2018_5_180604.pdf). Also it has been listed as NT by Shimane Prefecture and vulnerable (VU) by Hiroshima Prefecture.

5. *Hynobius* ***bakan*** Matsui, Okawa et Nishikawa sp. nov.

[Japanese name: Yamaguchi-sansyou-uwo]
[English name: Yamaguchi salamander]
Fig. 9

Hynobius nebulosus (part, as Yamaguchi morphotype) Okawa et Utsunomiya, 1992, 12.
Hynobius nebulosus (part, as Nihonkai morphotype) Okawa et al., 2007, 58.

Holotype

KUHE OU 0391, an adult male from Warisaka, Kurumaji, Ube-shi, Yamaguchi Prefecture (34°50'36"N, 131°18'12"E, alt. 70 m), collected by H. Okawa on 5 March 2010.

Paratypes

KUHE OU 0392–0393, two males, data same as the holotype.

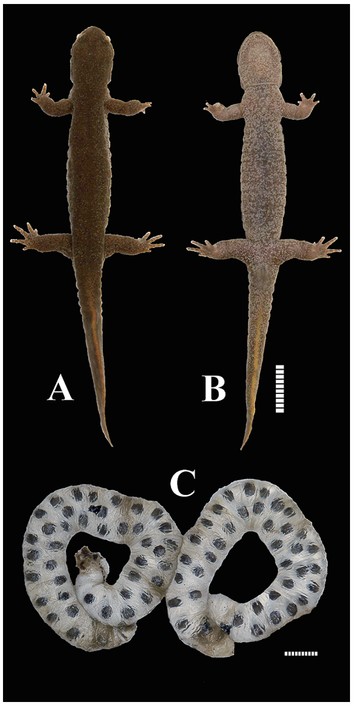

FIG. 9 *Hynobius bakan* sp. nov. Dorsal (A) and ventral (B) views of male holotype (KUHE OU 0391), scale bar=10 mm. Egg sac (C), scale bar=10 mm.

Referred specimens

Yamaguchi Prefecture: OU 0256–0257, two males, from Okoda, Isa-cho, Mine-shi. KUHE OU 0259–0260, two males, from Omine-cho, Mine-shi. KUHE OU0262, one male, from Kamiyaji, Yaji, Toyota-cho, Shimonoseki-shi. KUHE OU 0264, one male, from Oba, Tasuki, Houhoku-cho, Shimonoseki-shi. KUHE OU 0381–0382, two males, from Yamada-cho, Shimonoseki-shi. OU 0480, one male, from Ayagi, Mito-cho, Mine-shi. KUHE OU 0481–0482, two males, from Jumonji, Mana, Mito-cho, Mine-shi. Oita Prefecture: KUHE 25838, one male from Shin'ei, Bungo-takada-shi on 18 March 1999 by S. Sato. KUHE OU 0320–OU0324, five males from Yakeno, Bungo-takada-shi.

Etymology

The specific name "*bakan*" refers to the old name of Shimonoseki in Yamaguchi Prefecture, where the new species occurs.

Diagnosis

Body small (male SVL 45–63 mm, mean ±SD=51.8±5.7, n=18), forelimb medium (25.8%SVL), hindlimb medium (29.9%SVL), ratio of hindlimb to forelimb moderate (1.18). Tail medium (74.4% SVL). Always with clear yellow stripes on dorsal and ventral edges of the tail. Phylogenetically, *H. bakan* sp. nov. forms a mitochondrial DNA clade with *H. nebulosus* and *H. tsuensis*, with which *H. iwami* sp. nov. and *H. okiensis* form a larger clade. However, *H. bakan* sp. nov. differs from *H. nebulosus* by smaller SVL and wider vomerine teeth series, and from *H. iwami* sp. nov. by longer trunk and fifth toe, shorter head, and better developed fifth toe.

Description of holotype (measurements in mm)

Head-body medium for the species (SVL 52.8) and robust; head oval and not depressed, longer (HL 11.5, 21.8%SVL) than wide (HW 10.0, 18.9%SVL); snout rounded, slightly projecting beyond lower jaw; nostril close to snout tip; labial fold absent; eye moderate, protruded, slightly inset from edge of head in dorsal view; upper eyelid well developed (UEW 1.4, 2.7%SVL), shorter (UEL 3.0, 5.7%SVL) than snout (SL 4.1, 7.1%SVL); gular fold distinct, curving anteriorly; parotoid gland evident, extending from angle of jaw to gular fold; postorbital grooves distinct, branching posterior to angle of jaw, one short and running down to lower jaw, the other long and posterior to parotoid gland; vomerine teeth shallow V-shaped, series nearly touching at midline (Fig. 14E), wider (VTW 2.4, 4.6%SVL) than long (VTL 2.2, 4.1%SVL), anterior margin on line connecting choanae; tongue broad, both sides free from mouth floor; fore- and hindlimbs medium for the species and thick (FLL 13.2, 25.0%SVL; HLL 15.5, 29.4%SVL); CG 13; depressed limbs separated by two costal folds (LO –2); relative length of fingers I<IV<II<III, toes I<V<II<III<IV; fifth toe well developed (5TL 1.0, 1.9%SVL); cloaca greatly swollen with longitudinal slit; genital tubercle on anterior cloaca present; tail medium for the species (TAL 39.6, 75.0%SVL), cylindrical at base (BTAW 6.7, 11.7%SVL; BTAH 6.6, 11.4%SVL), gradually compressed posteriorly (MTAW 3.1, 3.8%SVL; MTAH 6.2, 12.7%SVL), with weak dorsal fin near tip (MXTAH 6.7, 11.7%SVL); tip of tail slightly pointed in lateral view.

Additional measurements and counts of the holotype: IND (2.6, 4.9%SVL); IOD (3.1, 5.9%SVL); AGD (27.3, 51.7%SVL); TRL (41.3, 78.2%SVL); 2FL (2.1, 3.9%SVL); 3FL (1.9, 3.6%SVL); 3TL (3.9, 7.5%SVL); UJTN (71); LJTN (71); VTN (33).

Color

In life, dorsum dark brown scattered with black markings (Fig. 9A). Underside of body lighter than dorsum covered with silvery white dots (Fig. 9B). White nuptial color on throat was not clear.

Variation

Morphometric data are summarized in Table 4. In 18 males, SVL is 51.8±5.7 (44.8–62.7) mm. Medians and ranges in males: HL 22.4 (21.1–25.3) %SVL; TRL 77.6 (74.7–78.9) %SVL; HW 18.4 (15.9–20.4) %SVL; IND 5.3 (4.5–6.5) %SVL; IOD 5.7 (4.3–7.9) %SVL; UEW 3.0 (2.4–3.5) %SVL; UEL 5.8 (5.4–6.3) %SVL; AGD 51.8 (48.9–55.2) %SVL; TAL 74.4 (66.4–82.5) %SVL; BTAW 10.9 (8.0–13.4) %SVL; MXTAH 12.4 (9.7–16.2) %SVL; FLL 25.8 (22.8–28.8) %SVL; HLL 29.9 (27.1–33.3) %SVL; MTAW 4.2 (1.0–6.5) %SVL; VTW 5.2 (4.6–6.6) %SVL; VTL 4.7 (4.1–6.2) %SVL; 2FL 5.0 (3.6–5.7) %SVL; 3FL 4.4 (3.6–5.1) %SVL; 3TL 7.1 (5.8–8.9) %SVL; 5TL 2.0 (0–3.2) %SVL. VTW/VTL 109.1 (81.7–129.9)%. TotL 88.3 (77.7–113) mm; CG 12.75 (12–13); LO –1 (-2.5–-0.5).

Yellow stripes on the tail were always seen, and clearly present on both edges in nine, but weak in two individuals seen in life. Unlike the holotype, white nuptial color was cearly seen on throat in many males. A fifth toe was almost always present, and of 24 individuals closely examined, two had rudimentary one in only on one side.

Eggs and egg sacs

The egg sacs are string-like in shape and coiling, with a thin, wrinkled envelope lacking notable striations and any distinct whiptail structure (Fig. 9C). Lengths (ESL) of 11 egg sacs before larval hatching range from 106 and 194 (mean±SD=145.9±31.0) mm and the widths (ESW) of 14.7 and 17.8 (mean ±SD=16.2±1.1) mm, with ESL/ESW being 6.9 and 11.9 (mean±SD=9.0±1.7). The clutch size ranges from 56–333 (mean ±SD=116.5±58.2, n=32). The animal pole of the egg is dark brown and vegetal pole is light brown in color.

Larva

No larvae were available.

Range

Known so far from southwestern parts of Yamaguchi Prefecture and a part of northeastern Kyushu (Fig. 15). Yamaguchi Prefecture: Ube-shi; Shimonoseki-shi (including the former Kikugawa-cho and Hohoku-cho); San'yo-Onoda-shi (the former San'yo-cho); Nagato-shi (including the former Heki-cho); Mine-shi (including the former Shuho-cho and Mito-cho). Oita Prefecture: Bungotakada-shi, Usa-shi.

The known localities extend from 15 to 280 m with the mean of 148±88 (n=15) m in altitude.

Comparisons

Hynobius bakan sp. nov. significantly differs from *H. nebulosus* by smaller SVL (51.8 mm vs. 59.3 mm), and wider vomerine teeth series (RVTW 5.2% vs. 4.7%); and from *H. iwami* sp. nov. by longer trunk (RTRL 77.6% vs. 75.3%) and fifth toe (R5TL 7.1% vs. 0%), and shorter head (RHL 22.5% vs. 24.7%).

It differs from *H. abuensis* sp. nov. by longer axilla-groin (RAGD 51.8% vs. 47.1%), greater degree of limb separation (LO -1 fold vs. 1 fold), smaller SVL (51.8 mm vs. 57.2 mm), narrower eyelid (RUEW 3.0% vs. 3.5%), and shorter tail (RTAL 74.4% vs. 86.9%) and limbs (RFLL 26.6% vs. 27.4%, RHLL 33.3% vs. 34.3%, R2FL 4.7% vs. 6.3%, R3FL 3.6% vs. 5.1%, and R5TL 0% vs. 3.0%); from *H. utsunomiyaorum* sp. nov. by deeper tail (RMXTAH 12.4% vs. 9.7%) and longer vomerine teeth series (RVTL 4.7% vs. 3.7%) and fifth toe (R5TL 2.0% vs. 0%).

The new species differs from *H. vandenburghi* by wider head (RHW 18.4% vs. 16.7%) and larger vomerine teeth series (RVTW 5.2% vs. 4.5% vs. and RVTL 4.7% vs. 3.9%); from *H. setoi* sp. nov. by longer trunk (RTRL 77.6% vs. 75.2%), axilla-groin (RAGD 51.8% vs. 48.2%), and vomerine teeth series (RVTL 4.1% vs. 3.7%), and smaller SVL (51.8 vs. 58.8 mm), and shorter head (RHL 22.5% vs. 24.8%) and hindlimb (RHLL 29.9% vs. 32.3%).

Although no significant differences were recognized in the measurements and counts, *H. bakan* sp. nov. differs from *H. setouchi* and *H. akiensis* by always having clear yellow stripes on both edges of the tail.

The mean uncorrected pairwise sequence divergence between *H. bakan* and the other species is 7.8–15.8% (Table 2).

Natural history

Breeding occurs from late January to early April. Egg sacs were found in marshes (38%), ditches (24%), pools (19%), abandoned paddies (9.5%), ponds (4.8%), and very slowly flowing streams (4.8%).

Conservation

The species has been listed as vulnerable (VU) in the Red List of Environmental Ministry (https://www.env.go.jp/nature/kisho/hozen/redlist/RL2018_5_180604.pdf), and as VU in Yamaguchi Prefecture (as *H. nebulosus* together with *H. abuensis* sp. nov.) and Oita Prefecture.

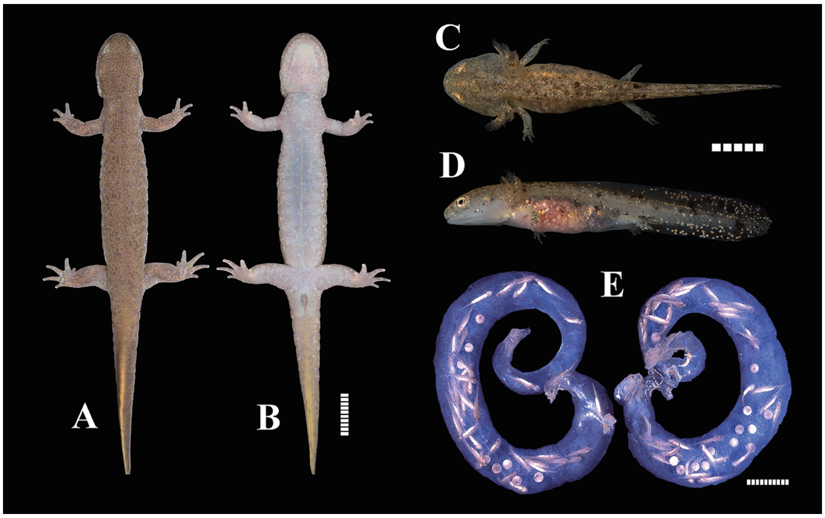

FIG. 10 *Hynobius nebulosus*. Dorsal (A) and ventral (B) views of a topotypic male (KUHE 48353), scale bar=10 mm. Dorsal (C) and lateral (D) views of a larva (Stage 63), scale bar=5 mm. Egg sac (E), scale bar=10 mm.

6. *Hynobius nebulosus* (Temminck et Schlegel, 1838)
[Japanese name: Kasumi-sansyou-uwo]
[English name: Clouded salamander]
Fig. 10

Salamandra nebulosa Temminck and Schlegel, 1838, 127, 139.
Hynobius nebulosus Tschudi, 1838, 94.
Ellipsoglossa nebulosa Duméril, Bibron, and Duméril, 1854, 100.
Hynobius ikishimae Dunn, 1923, 28.
Hynobius (Hynobius) nebulosus nebulosus Nakamura and Uéno, 1963, 6.
Hynobius nebulosus nebulosus Thorn, 1968, 43.
Hynobius (Hynobius) nebulosus Dubois and Raffaëlli, 2012, 77.
Hynobius nebulosus (part, as Kyushu morphotype) Okawa et al., 2007, 58.
Hynobius nebulosus (part, as Japan Sea morphotype) Okawa et al., 2007, 58.

Lectotype

RMNH 2309 A, 1, an adult female from “monts Mits jama (les trois monts) situés dans les environs de Nagasaki” (Japan), collected by Ph. F. von Siebold.

Paralectotypes

RMNH 2309 B–T, nine adult males, three half-grown males, five adult females, two juveniles (Hoogmoed, 1978). Same data as the lectotype; ZMB (the Museum für Naturkunde der Humboldt-Universität zu Berlin) 3620, two adults; MNHNP (the Muséum National d’Histoire Naturelle in Paris) 4691, 4691A. RMNH 2307, one larva from “Japon” by H. Bürger.

Referred specimens

Nagasaki Prefecture: KUHE 7395, 7396, two males and KUHE 7397, one female from Konpirasan, Nishiyama, Nagasaki-shi in March 1984 by M. Matsui. KUHE 8978, 8979, two males from Higashi-machi, Nagasaki-shi in January 1987 by T. Hikida. OU0218 one male from Aigawa-machi, Nagasaki-shi. KUHE 6611, one male from Nagasaki-shi, in August 1982 by K. Nishio. KUHE 7398–7417, 20 males, Tenyuji, Nishikoji-machi, Isahaya-shi on 27 March 1984 by M. Matsui and S. Tanabe, KUHE 24691–24712, 22 males, same locality on 1

February 1998 by K. Nishikawa. KUHE 24728, 24729, two males, from Iana, Tomie-cho, Goto-shi (Fukuejima Is.) on 3 February 1998 by K. Nishikawa. KUHE 24738–24748, 11 males, from Sumiyoshihigashifure, Ashibe-cho, Iki-shi on 11 February 1998 by K. Nishikawa. KUHE 24749–24758, 10 males from Kidafure Gonoura-cho, Iki-shi on 11 February 1998 by K. Nishikawa. OU 0398, one male from Nakazato, Shimomotoyama-cho, Sasebo-shi. OU 0397, one male from Shimobarumen, Yoshii-cho, Sasebo-shi on 6 March 2010 by H. Okawa. KUHE OU 0508, one male from Kaisaku, Sechibaru-cho, Sasebo-shi. OU 0505, one male from Kunimiyama, Kaisaku, Sechibaru-cho, Sasebo-shi on 10 April 2010 by H. Okawa. OU 0385, one female from Tanagata-cho, Sasebo-shi on 7 February 2010 by H. Okawa. Kagoshima Prefecture: KUHE 25773, 25774, two males from Izumi-Shi in February 1999. Kumamoto Prefecture: OU 0386, one male from Oike-Iida, Mashiki-machi, Kamimashiki-gun on 14 February 2010 by H. Okawa. KUHE OU 0476, one male from Toyono-machi, Uki-shi on 7 March 2010 by H. Okawa. Saga Prefecture: KUHE 24713–24722, 10 males from Fukuda, Imari-shi on 4 February 1998 by K. Nishikawa. KUHE OU 0384, one male from Hanamatsuritagara, Minamitaku-machi, Taku-shi on 6 February 2010 by H. Okawa. Fukuoka Prefecture: KUHE 5717–5719, three males from Ryogenji, Munakata-shi in March 1975 by M. Matsui. KUHE 7381–7394, 11 males and three females from Futajima, Wakamatsu-ku, Kitakyushu-shi on 31 March 1984 by M. Matsui and S. Tanabe. KUHE 22802–22807, five males and one female from the same locality on 30 March 1997 by S. Okada and K. Nishikawa. KUHE 24723–24726, four males from the same locality on 1 February 1998 by K. Nishikawa. KUHE 25775–25776, two females from the same locality on 17 February 1999 by K. Nishikawa. OU 0733, one male from Fujita, Araki-machi, Kurume-shi on 11 February 2013. OU 0198, one male from Idawara, Shima, Itoshima-shi on 4 February 2006. OU 0559, one male from Yamada, Munakata-shi on 13 February 2011. KUHE OU 0562, one male from Mochiyama, Munakata-shi on 13 February 2011. OU 0571, one male from Enmei-koen, Showa-machi, Omuta-shi on 5 March 2011. OU 0214, one male from Habu-jinja, Habu, Nakama-shi on 24 March 2006. OU 0332, one male from Kawasaki-Daigahara, Kawasaki-machi, Tagawa-gun on 3 March 2008 by H. Okawa.

Etymology

The specific name “*nebulosus*” is Latin signifying cloud, expressing the dorsal color, which is deep brownish yellow and clouded with deep and very fine marbling.

Diagnosis

Body large (male SVL 47–67 mm, mean ±SD=59.3±4.5, n=31), fore- and hindlimbs relatively short (25.2%SVL and 29.4%SVL, respectively), ratio of hindlimb to forelimb moderate (1.18). Tail short (69.5%SVL). Always with clear yellow stripes on dorsal and ventral edges of the tail. Phylogenetically, *H. nebulosus* forms a mitochondrial DNA clade with *H. tsuensis* and *H. bakan* sp. nov., with which *H. iwami* sp. nov. and *H. okiensis* form a larger clade. However, *H. nebulosus* differs from *H. bakan* sp. nov. by larger SVL and narrower vomerine teeth series, and from *H. iwami* sp. nov. by larger SVL, wider internarial, longer trunk and fifth toe, larger number of costal grooves, shorter head, and lesser degree of limb overlap.

Description of a topotype (KUHE 7396, measurements in mm)

Head-body large for the species (SVL 63.3) and robust; head oval and not depressed, distinctly longer (HL 14.1, 22.3%SVL) than wide (HW 11.2, 17.7%SVL); snout rounded, slightly projecting beyond lower jaw; nostril close to snout tip; labial fold absent; eye moderate, protruded, slightly inset from edge of head in dorsal view; upper eyelid well developed (UEW 1.7, 2.7%SVL), shorter (UEL 3.1, 4.9%SVL) than snout (SL 3.9, 6.2%SVL); gular fold distinct, curving anteriorly; parotoid gland evident, extending from angle of jaw to gular fold; postorbital grooves distinct, branching posterior to angle of jaw, one short and running down to lower jaw, the other long and posterior to parotoid gland; vomerine teeth V-shaped, series nearly touching at midline (Fig. 14F), slightly wider (VTW 3.0, 4.7%SVL) than long (VTL 2.9, 4.6%SVL), anterior margin on line connecting choanae; tongue broad, both sides free from mouth floor; fore- and hindlimbs short for the species and thick (FLL 14.8, 23.4%SVL; HLL 18.2, 28.8%SVL); CG 13; depressed limbs separated by three costal folds (LO -3); relative length of fingers IV<I<III<II, toes I<V<II<IV<III; fifth toe well developed (5TL 1.9, 3.0%SVL); cloaca greatly swollen with longitudinal slit; genital tubercle on anterior cloaca present; tail medium for the species (TAL 44.2, 69.8%SVL), cylindrical at base (BTAW 6.0, 9.5%SVL; BTAH 6.8, 10.7%SVL), gradually compressed posteriorly (MTAW 3.2, 5.1%SVL; MTAH 7.8, 12.3%SVL), keeled in posterior half (MXTAH 8.5, 13.4%SVL); tip of tail rounded in lateral view.

Additional measurements and counts of the topotype: IND (3.6, 5.7%SVL); IOD (3.7, 5.9%SVL); AGD (32.9, 52.0%SVL); TRL (49.2, 77.7%SVL); 2FL (3.3, 5.2%SVL); 3FL (3.1, 4.8%SVL); 3TL (5.2, 8.2%SVL); UJTN (78); LJTN (77); VTN (56).

Color

In life, dorsum dark olive scattered with darker spots (Fig. 10A). Underside of body lighter than dorsum scattered with silvery white, except for throat covered with white nuptial color (Fig. 10B). Dorsal and ventral edges of tail with yellowish stripes.

Variation

Morphometric data are summarized in Table 4. In 44 males examined, SVL was 58.4±5.1 (range=46.7–66.9) mm, and the ratios to SVL were: HL 23.1 (21.0–25.7)%SVL; TRL 76.9 (74.3–79.0)%SVL; HW 18.4 (14.3–20.4)%SVL; IND 5.8 (4.9–6.7)%SVL; IOD 6.1 (4.8–6.9)%SVL; UEW 2.9 (1.9–3.5)%SVL; UEL 5.3 (4.6–6.7)%SVL; AGD 51.1 (48.1–54.7)%SVL; TAL 69.1 (45.7–80.4)%SVL; BTAW 10.1 (8.0–13.2)%SVL; MXTAH 12.1 (8.6–14.5)%SVL; FLL 25.0 (22.3–27.9)%SVL; HLL 29.8 (26.9–34.1)%SVL; MTAW 4.8 (2.8–6.1)%SVL; VTW 4.7 (3.9–5.3)%SVL; VTL 4.6 (3.5–5.5)%SVL; 2FL 5.0 (3.6–5.7)%SVL; 3FL 4.4 (3.1–5.2)%SVL; 3TL 7.5 (5.7–9.1)%SVL; 5TL 2.5 (1.0–3.4))%SVL. VTW/VTL 103.6 (84.9–131.8)%. TotL 98.5 (78.3–119.4) mm; CG 13 (12–14); LO -2 (-3–0).

Some scattered with dark dots on dorsum. Our genetic and morphological analyses failed to split *H. ikishimae* from Iki Island from *H. nebulosus* from Nagasaki.

Eggs and egg sacs

The egg sacs are string-like in shape and coiling, with a thin, wrinkled envelope lacking notable striations and any distinct whiptail structure (Fig. 10E). In the population from Nagasaki, egg sacs are 130–164 (mean ±SD=144.8±14.8, n=6) mm in length (ESL) and 16–18 (mean±SD=17.2±1.0, n=6) mm in width (ESW), with ESL/ESW being 7.2–10.2 (mean±SD=8.5±1.3, n=6), and the clutch size is medium to large, ranging from 89–117 (mean±SD=101.7±14.2, n=3)(Sato, 1943). Clutch size of populations from all Kyushu populations is 41–333 (135.8±59.0, n=91), and the population from Kitakyushu has especially large clutch size. In color, the animal pole is blackish gray or brown, which gradually becomes lighter to yellow at vegetal pole (Sato, 1943).

Larvae

Two fully grown larvae from Nagasaki-shi at Stage 63 of Iwasawa and Yamashita (1991) had SVL ranging from 17.7–17.9 (mean=17.8) and total length of 28.9–31.5 (mean=30.2) mm, head rounded in dorsal view and profile (Fig. 10C, D); snout short and broadly rounded; eyes slightly protruded, inset from edge of head in dorsal view; labial fold distinct at half of upper jaw; external gills developed; caudal fin higher than head; dorsal fin higher than ventral fin; origin of dorsal fin in middle of trunk; ventral fin originating from the vent; tail tip weakly pointed; limbs slender; claws on fingers and toes absent. In life, their dorsum was light brown with black spots; venter whitish and transparent scattered with golden dots; large black spots scattered on tail; golden dots scattered on tail fin.

Range

Known from northern half of central and western Kyushu and adjacent islands (Fig. 15). Fukuoka Prefecture: Kurume-shi; Itoshima-shi; Munakata-shi; Omuta-shi; Nakama-shi; Kawasaki-machi, Tagawa-gun; Kitakyushu-shi. Saga Prefecture: Imari-shi; Shiroishi-cho, Kishima-gun; Ogi-shi; Taku-shi; Karatsu-shi (including the former Yobuko-cho and Ouchi-cho). Nagasaki Prefecture: Nagasaki-shi; Isahaya-shi; Sasebo-shi; Goto-shi (Fukuejima Is.); Iki-shi (Iki Is.). Kumamoto Prefecture: Uki-shi; Mashiki-machi, Kamimashiki-gun. Kagoshima Prefecture: Akune-shi; Izumi-shi.

The known localities are at the altitude of 15–670 (mean 113.5±191.6) m (n=18).

Comparisons

A part of paralectotypes of *H. nebulosus* in the collection of RMNH only slightly differ from the populations from Kyushu, including topotype of *H. ikishimae* Dunn, 1923, by relatively smaller head width (RHW 16.8% vs. 18.9%), but otherwise they are identical.

It significantly differs from *H. bakan* sp. nov. by larger SVL (59.3 mm vs. 51.8 mm) and narrower vomerine teeth series (RVTW 4.7% vs. 5.3%); from *H. iwami* sp. nov. by larger SVL (59.3 mm vs. 52.0 mm), wider internarial (RIND 5.8% vs. 5.1%), longer trunk (RTRL 76.4% vs. 75.3%) and fifth toe (R5TL 2.5% vs. 0%), larger number of costal grooves (CG 13 vs. 12), shorter head (RHL 23.6% vs. 24.7%), and lesser degree of limb overlap (LO -2 folds vs. -0.5 folds).

It differs from *H. utsunomiyaorum* sp. nov. by larger SVL (59.3 mm vs. 53.6 mm), deeper tail (RMXTAH 12.5% vs. 9.7%), longer vomerine teeth series (RVTL 4.6% vs. 3.7%) and fifth toe (R5TL 2.5% vs. 0%), and larger number of costal grooves (CG 13 vs. 12), and costal folds between adpressed limbs (LO -2 folds vs. -1 fold); from *H. akiensis* sp. nov. by larger SVL (59.3 mm vs. 49.6 mm), shorter upper eyelid (RUEL 5.3% vs. 6.1%) and narrower vomerine teeth series (RVTW 4.7% vs. 5.4%); from *H. abuensis* sp. nov. by longer trunk (RTRL 76.4% vs. 76.1%) and axilla-groin (RAGD 51.0% vs. 47.1%), greater number of costal groove (CG 13 vs. 12), smaller head (RHL 23.6% vs. 23.9% and RHW 18.9% vs. 19.6%), narrower and shorter upper eyelid (RUEW 2.8% vs. 3.8% and RUEL 5.3% vs. 7.2%), shorter and narrower tail (RTAL 69.5% vs. 86.9% and RBTAW 10.5% vs.13.3%), shorter limbs (RFLL 25.2% vs. 27.4%, RHLL 29.4% vs. 34.3%, R2FL 5.0% vs. 6.3%, and R3TL 7.5% vs. 8.9%), narrower vomerine teeth series (RVTW 4.7% vs. 6.3%), and lesser degree of limb overlap (LO -2 folds vs. 1 fold).

The species differs from *H. setoi* sp. nov. by longer trunk (RTRL 76.4% vs. 75.2%), axilla-groin (RAGD 51.0% vs. 48.2%), and vomerine teeth series (RVTL 4.6% vs. 3.7%), and larger number of costal grooves (CG 13 vs. 12), shorter head (RHL 23.6% vs. 24.8%), smaller upper eyelid (RUEW 2.8% vs. 3.1%, RUEL 5.3% vs. 5.9%), shorter hindlimb (RHLL 29.4% vs. 32.3%), and lesser degree of limb overlap (LO -2 folds vs. -0.5 folds); from *H. setouchi* sp. nov. by larger SVL (59.3 mm vs. 52.3 mm); from *H. vandenburghi* by wider head (RHW 18.9% vs. 16.7%) and internarial (RIND 5.8% vs. 4.9%), and longer vomerine teeth series (RVTL 4.6% vs. 3.9%).

The mean uncorrected pairwise sequence divergence between *H. nebulosus* and the other species is 7.8–15.8% (Table 2).

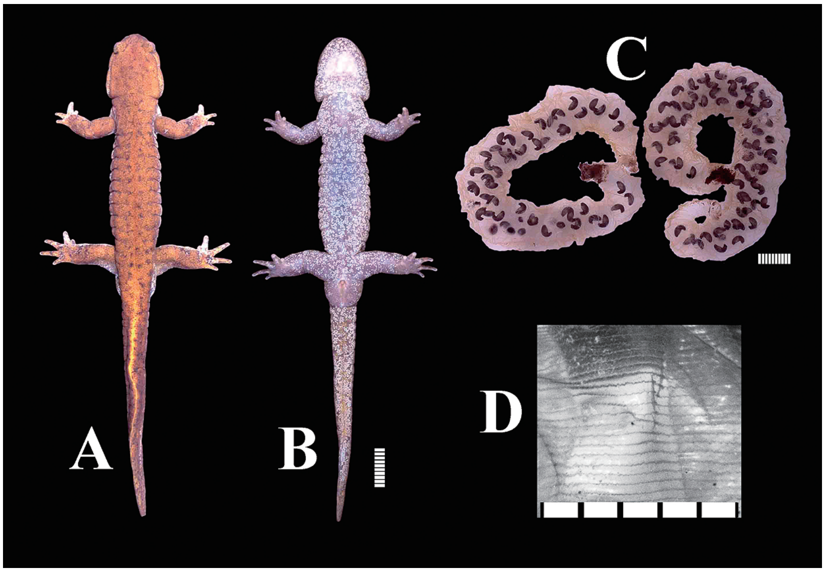

FIG. 11 *Hynobius abuensis* sp. nov. Dorsal (A) and ventral (B) views of male holotype (KUHE 35904), scale bar=10 mm. Egg sac (C), scale bar=10 mm. Striation on egg sac (D), scale bar=5 mm.

Natural history

A total of 24 breeding sites have been recorded including pools (33.3%), ditches (20.8%), ponds (16.7%), canals (12.5%), marshes (8.3%), and abandoned paddies (8.3%). Breeding occurs from late January in Iki Isl. and Nagasaki to late March in Iki Isl. Egg sacs are attached to debris or water plants in the water. Water temperature at the time of breeding varies from 7 to 18°C in Nagasaki (Kunitomo, 1910 in Sato, 1943).

Conservation

The species is in the Red List of Ministry of the Environment Government of Japan (https://www.env.go.jp/nature/kisho/hozen/redlist/RL2018_5_180604.pdf) as VU with all other taxa here treated under the name of *H. nebulosus*. The species is on the list of Fukuoka, Nagasaki, and Kagoshima Prefectures at the rank of VU and on the list of Saga and Kumamoto Prefectures at the rank of NT.

7. *Hynobius* ***abuensis*** Matsui, Okawa, Nishikawa et Tominaga sp. nov.
[Japanese name: Abu-sansyou-uwo]
[English name: Abu salamander]
Fig. 11

Hynobius nebulosus (part, as Abu morphotype) Okawa et al., 2005, 11.

Holotype

KUHE 35904, an adult male from Ato-Kaneshimo, Yamaguchi-shi, Yamaguchi Prefecture (34°28'24"N, 131°40'52"E, alt. 472 m), collected by K. Nishikawa and A. Tominaga on 9 March 2005.

Paratypes

KUHE 35905–35909, five males, collection data same as the holotype.

Referred specimens

Yamaguchi Prefecture: KUHE 35880–35890, 11 males, collected from Susa, Hagi-shi, collected by K. Nishikawa and A. Tominaga on 9 March 2005. KUHE 35912–35913, one male and one overwintered larva from

Fukudashimo, Abu-cho, Abu-gun, by K. Nishikawa and A. Tominaga on 9 March 2005. KUHE 35914, one male from the former Mutsumi-son, Hagi-shi, by K. Nishikawa and A. Tominaga on 9 March 2005. OU 0267–0270, four males from Amagoshimo, Ato-Ikumonaka, Yamaguchi-shi by H. Okawa on 11 February 2007. KUHE OU 0271–0275, five males from Higashibun, Ato-Ikumo, Yamaguchi-shi by H. Okawa on 11 February 2007. OU 0283–0284, two males from Ato-Kaneshimo, Yamaguchi-shi by H. Okawa on 10 March 2007. Shimane Prefecture: OU 0280–0282, three males from Kinogawa, Tadachi, Tsuwano-cho, Kanoashi-gun by H. Okawa on 4 March 2007. KUHE OU 0540–0542, three males from Nakagumi, Nakasono, Tsuwano-cho, Kanoashi-gun by H. Okawa on 11 February 2009. OU 0543–0544, two males from Fukutani, Nagafuku, Tsuwano-cho, Kanoashi-gun by H. Okawa on 15 February 2009.

Etymology

The specific name “*abuensis*” refers to “Abu”, the locality name of the region in the north of Yamaguchi Prefecture, around which the new species appears to be confined.

Diagnosis

The envelope of egg sacs with weak striations. Body large (male SVL 50–64 mm, mean ±SD=57.2±3.2, n=19), tail long and trunk short. Fore- and hindlimbs (30.1%SVL and 34.3%SVL, respectively), especially forelimb long, ratio of hindlimb to forelimb small (1.14). Second finger long. Tail very long (86.8%SVL). Ventral side of tail without yellow stripe. Phylogenetically, *H. abuensis* sp. nov. forms a mitochondrial DNA clade with *H. tosashimizuensis*, which is the sister clade to the clade of *H. akiensis* sp. nov. and *H. utsunomiyaorum* sp. nov. However, *H. abuensis* sp. nov. has longer, deeper, but narrower tail, longer limbs, and shorter axilla-groin than *H. utsunomiyaorum* sp. nov., and larger SVL, wider head, longer tail and limbs, greater limb overlap and shorter axilla-groin than *H. akiensis* sp. nov.

Description of holotype (measurements in mm)

Head-body medium for the species (SVL 59.3) and robust; head oval and not depressed, distinctly longer (HL 14.3, 24.1%SVL) than wide (HW 11.3, 19.1%SVL); snout rounded, slightly projecting beyond lower jaw; nostril close to snout tip; labial fold absent; eye moderate, protruded, slightly inset from edge of head in dorsal view; upper eyelid well developed (UEW 2.4, 4.0%SVL), shorter (UEL 3.4, 5.7%SVL) than snout (SL 4.3, 7.3%SVL); gular fold distinct, curving anteriorly; parotoid gland evident, extending from angle of jaw to gular fold; postorbital grooves distinct, branching posterior to angle of jaw, one short and running down to lower jaw, the other long and posterior to parotoid gland; vomerine teeth deep V-shaped, series touching at midline (Fig. 14G), slightly wider (VTW 2.9, 4.8%SVL) than long (VTL 2.6, 4.4%SVL), anterior margin on line connecting centers of choanae; tongue broad, both sides free from mouth floor; fore- and hindlimbs medium for the species and thick (FLL 18.2, 30.7%SVL; HLL 20.8, 35.1%SVL); CG 12; depressed limbs overlapped by one and half costal folds (LO 1.5); relative length of fingers IV≤I<III<II, toes I<V<II<IV<III; fifth toe well developed (5TL 1.5, 2.4%SVL); cloaca greatly swollen with longitudinal slit; genital tubercle on anterior cloaca present; tail medium for the species (TAL 53.2, 89.7%SVL), cylindrical at base (BTAW 7.2, 12.1%SVL; BTAH 6.6, 11.1%SVL), not markedly compressed (MTAW 2.9, 4.9%SVL; MTAH 7.2, 12.1%SVL), dorsal fin conspicuous (MXTAH 7.8, 13.2%SVL); tip of tail pointed in lateral view.

Additional measurements and counts of the holotype: IND (3.6, 6.1%SVL); IOD (3.4, 5.7%SVL); AGD (29.6, 49.9%SVL); TRL (45.0, 75.9%SVL); 2FL (3.3, 5.5%SVL); 3FL (2.8, 4.8%SVL); 3TL (4.4, 7.5%SVL); UJTN (82); LJTN (77); VTN (52).

Color

In life, dorsum olive scattered with brown dark and silvery-white spots (Fig. 11A). Underside of body lighter than dorsum dotted with silvery-white spots, except for throat covered with white nuptial color (Fig. 11B).

Variation

Morphometric data are summarized in Table 4. SVL of males 57.2±3.2 (49.6–64.2, n=19); HL 23.9 (22.4–25.1) %SVL; TRL 76.1 (74.9–77.6) %SVL; HW 19.4 (17.1–21.2) %SVL; IND 5.8 (5.2–6.5) %SVL; IOD 6.1 (5.3–6.9) %SVL; UEW 3.5 (2.3–3.8) %SVL; UEL 6.0 (5.4–6.8) %SVL; AGD 47.1 (43.9–52.1) %SVL; TAL 86.8 (76.5–96.2) %SVL; BTAW 11.4 (8.4–15.9) %SVL; MXTAH 12.0 (10.1–16.3) %SVL; FLL 30.1 (29.1–33.2) %SVL; HLL 34.3 (31.9–37.2) %SVL; MTAW 4.2 (3.4–6.3) %SVL; VTW 5.3 (4.4–6.6) %SVL; VTL 4.1 (3.7–5.5) %SVL; 2FL 6.3 (5.7–6.7) %SVL; 3FL 5.1 (4.0–6.0) %SVL; 3TL 8.9 (6.8–9.7) %SVL; 5TL 3.0 (1.9–4.1) %SVL. VTW/VTL 127.1 (109.1–150.2)%. TotL 105.8 (98.0–118.1) mm. CG 12 (12–12); LO 1 (-0.5–2).

Only a few specimens have been recorded with yellow stripes on either side of the tail.

Eggs and egg sacs

The egg sacs are thick, string-like in shape and coiling (Fig. 11C), with a thin, wrinkled envelope lacking any distinct whiptail structure, but having weak striations (Fig. 11D). Lengths of two pairs of egg sacs (ESL) before larval hatching ranged from 191 to 232 (mean ±SD=212.9±21.7) mm and the widths (ESW) from 20.1 to 28.0 (mean ±SD=24.4±3.7) mm, with ESL/ESW from 8.3 to 9.5 (mean±SD=8.8±0.5). The clutch size is 44–120 (mean±SD=74.6±15.0, n=49). The ova from a female ranged from 2.0–2.8 (mean±SD=2.3±0.3, n=7) mm. The animal pole is dark brown and vegetal pole is light brown in color.

Larva

An over-wintered larva collected in early March at Stage 63 of Iwasawa and Yamashita (1991) had SVL of 32.8 and total length of 59.1 mm, head rounded in dorsal view and in profile; snout short and rounded; eyes slightly protruded, inset from edge of head in dorsal view; labial fold distinct at half of upper jaw; external gills slightly degenerating; caudal fin higher than head; dorsal fin higher than ventral fin; origin of dorsal fin in middle of trunk; ventral fin originating from the vent; tail tip weakly pointed; limbs slender; claws on fingers and toes absent. In preservative, the dorsum was brown without marking; venter light brown; black spots scattered on tail.

Range

Known so far from small areas in the Chugoku region, western Honshu (Fig. 15). Shimane Prefecture: Tsuwano-cho, Kanoashi-gun. Yamaguchi Prefecture: Abu-cho, Abu-gun; Hagi-shi (including the former Susa-cho, Mutsumi-son, and Fukue-son); Yamaguchi-shi (including the former Ato-cho and Tokuji-cho).

Altitude of the localities extends from 150–970 m with the mean±SD of 305.4±155.1 m (n=25).

Comparisons

Hynobius abuensis sp. nov. significantly differs from *H. utsunomiyaorum* sp. nov. by longer, deeper, but narrower tail (RTAL 86.9% vs. 69.4%, RMXTAH 12.0% vs. 9.7%, and RMTAW 4.2% vs. 5.5%), longer limbs (RFLL 30.1% vs. 24.9%, R2FL 6.3% vs. 4.7%, R3FL 5.1% vs. 3.6%, RHLL 34.3% vs. 30.0%, R3TL 8.9% vs. 7.4%, and R5TL 3.0% vs. 0%), and shorter axilla-groin (RAGD 47.1% vs. 51.7%); from *H. akiensis* sp. nov. by larger SVL (57.2 mm vs. 49.6 mm), wider head (RHW 19.4% vs. 17.8%), longer tail (RTAL 86.9 vs. 74.3%) and limbs (RFLL 27.4% vs. 25.3%, RHLL 34.3% vs. 29.8%, R2FL 6.3% vs. 5.1%, R3FL 5.1% vs. 4.3%, R3TL 8.9% vs. 7.4%, and R5TL 3.4% vs. 2.0%), greater limb overlap (LO 1 fold vs. -2 folds), and shorter axilla-groin (RAGD 47.1% vs. 51.7%).

The new species is different from *H. nebulosus* by larger head (RHL 23.9% vs. 23.6%, RHW 19.6% vs. 18.9%), wider and longer eyelid (RUEW 3.8% vs. 2.8% and RUEL 7.2% vs. 5.3%), longer and wider tail (RTAL 86.9% vs. 69.5% and RBTAW 13.3% vs. 10.5%), longer limbs (RFLL 27.4% vs. 25.2%, RHLL 34.3% vs.29.4%, R2FL 6.3% vs. 5.0%, and R3TL 8.9% vs. 7.5%), wider vomerine teeth series (RVTW 6.3% vs. 4.7%), greater limb overlap (LO 1 fold vs. -2 folds), shorter trunk (RTRL 76.1% vs. 76.4%) and axilla-groin (RAGD 47.1% vs. 51.0%), and smaller number of costal grooves (CG 12 vs. 13); and from *H. iwami* sp. nov. by wider head and internarial (RHW 19.6% vs. 17.5% and RIND 5.8% vs. 5.1%), and longer limbs (RFLL 27.4% vs. 26.6%, RHLL 34.3% vs. 33.3%, R2FL 6.3% vs. 4.7%, R3FL 5.1% vs. 3.6%, and R5TL 3.0% vs. 0%); and from *H. bakan* sp. nov. by larger SVL (57.2 mm vs. 51.8 mm), wider eyelid (RUEW 3.5% vs. 3.0%), longer tail (RTAL 86.9% vs. 74.4%) and limbs (RFLL 27.4% vs. 25.8%, RHLL 34.3% vs. 29.9%, R2FL 6.3% vs. 5.0%, R3TL 8.9% vs. 7.1%, and R5TL 3.0% vs. 2.0%), greater limb overlap (LO 1 fold vs. -1 fold), and shorter axilla-groin (RAGD 47.1% vs. 51.8%).

The new species differs from *H. vandenburghi* by wider head (RHW 19.6% vs. 16.7%), internarial (RIND 5.8% vs. 4.9%), eyelid (RUEW 3.5% vs. 3.0%), and vomerine teeth series (RVTW 6.3% vs. 4.5%), longer and wider tail (RTAL 86.9% vs. 68.2% and RBTAW 11.4% vs. 10.3%), longer limbs (RFLL 27.4% vs. 24.8%, RHLL 34.3% vs. 29.4%, R2FL 6.3% vs. 4.9%, R3FL 5.1% vs. 4.2%, and R3TL 8.9% vs. 6.9%), greater limb overlap (LO 1 fold vs. -1.5 folds), shorter axilla-groin (RAGD 47.1% vs. 51.6%), and smaller number of costal groove (CG 12 vs. 13); from *H. setouchi* sp. nov. by wider head (RHW 19.6% vs. 17.8%), longer forelimb (RFLL 27.4% vs. 26.7%) and second finger (R2FL 6.3% vs. 4.9%), greater limb overlap (LO 1 fold vs. -1 fold), and smaller number of costal grooves (CG 12 vs. 13); and from *H. setoi* sp. nov. by wider vomerine teeth series (RVTW 5.3% vs. 4.8%) and longer second finger (R2FL 6.3% vs. 5.2%).

The mean uncorrected pairwise sequence divergence between *H. abuensis* sp. nov. and the other species is 8.8–14.6% (Table 2).

Natural history

Breeding occurs from February to March (Okawa et al., 2005). Recorded oviposition sites (n=28) are ditches (28.6%), pools (25.0%), abandoned paddies (17.9%), fountains (10.7%), marshes (10.7%), and ponds (7.1%). Water temperatures at the time of breeding varied from 8.3 to 12.0°C. Some overwintered larvae can be found the following spring.

Conservation

Together with *H. bakan* sp. nov., the new species has been listed as *H. nebulosus* and ranked as vulnerable (VU) in the Red List of Yamaguchi Prefecture (Yamaguchi Prefecture, 2018). Similarly, *H. abuensis* sp. nov. has been listed together with *H. iwami* sp. nov. and *H. setoi* sp. nov. as *H. nebulosus* and ranked as nearly threatened (NT) in the Red List of Shimane Prefecture (Shimane Prefecture, 2014). In the Red List of Japanese Government, they are listed as *H. nebulosus* and vulnerable (VU) (https://www.env.go.jp/nature/kisho/hozen/redlist/RL2018_5_180604.pdf).

8. *Hynobius* ***akiensis*** Matsui, Okawa et Nishikawa sp. nov.

[Japanese name: Aki-sansyou-uwo]
[English name: Aki salamander]
Fig. 12

Hynobius abei (part) Sato, 1943, 163.
Hynobius nebulosus (part, as Hiroshima morphotype) Okawa and Utsunomiya, 1989, 142.
Hynobius nebulosus (part, as Osakijima morphotype) Okawa and Utsunomiya, 1989, 142.
Hynobius nebulosus (part, as Transitional

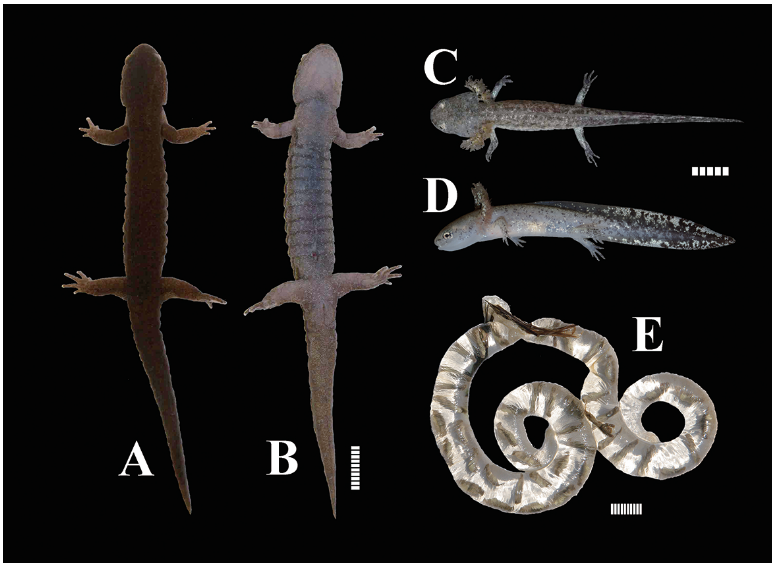

FIG. 12 *Hynobius akiensis* sp. nov. Dorsal (A) and ventral (B) views of male holotype (KUHE 35925), scale bar=10 mm. Dorsal (C) and lateral (D) views of a larva (Stage 63), scale bar=5 mm. Egg sac (E), scale bar=10 mm.

morphotype) Okawa et Utsunomiya, 1992, 12.
Hynobius nebulosus (part, as Miyoshi morphotype) Okawa et Utsunomiya, 1992, 12.
Hynobius nebulosus (part, as Island morphotype) Okawa et al., 1999, 47.
Hynobius nebulosus (part, as Aki morphotype) Okawa et al., 2005, 11.

Holotype

KUHE 35925, an adult male from Haizuka, Mirasaka-cho, Miyoshi-shi, Hiroshima Prefecture (34°46'01"N, 132°58'45"E, alt. 223 m), collected by Yasuchika Misawa and Kanto Nishikawa on 26 March 2005.

Paratypes

KUHE 35924, one male, data same as the holotype. KUHE 8682–8687, five males, two females from Nomino-o, Kurose-cho, Higashihiroshima-shi, Hiroshima Prefecture (34°20'44"N, 132°41'33"E, alt. 160 m) on 10 March 1986 by M. Matsui, T. Utsunomiya, Y. Utsunomiya and H. Okawa.

Referred specimens

Hiroshima Prefecture: KUHE OU 0297–0299, three males from Goso, Saijo-cho, Higashihiroshima-shi. OU0300, one male from Hara, Hachihonmatsu-cho, Higashihiroshima-shi. KUHE OU 0301–0302, two males from Kamiitaki, Miwa-cho, Miyoshi-shi. OU0303, one male, all on 28 March 2007 by H. Okawa. OU0309, one male from Kiyotake, Toyosaka-cho, Higashihiroshima-shi. KUHE OU 0310–0311, two males, both on 5 April 2007 by H. Okawa. KUHE OU 0319, one male, Kanashitoge, Akitsu-cho, Higashihiroshima-shi on 13 May 2007 by H. Okawa. KUHE OU 0340–0342, three males, Yoshikawa, Hachihonmatsu-cho, Higashihiroshima-shi on 28 March 2008 by H. Okawa. OU0387–0389, three males, Imodura, Miwaka-machi, Miyoshi-shi. OU0390, one male, Shimoshiwachi-machi, Miyoshi-shi on 3 March 2010 by H. Okawa. KUHE OU 0474, one male, Kurobuchi, Sera-cho, Sera-gun. OU0479, one male, Nagata, Sera-cho (former Seranishi-cho), Sera-gun on 19 March 2010 by H. Okawa. KUHE OU0510–0511, two males, Bogo, Takamiya-cho, Akitakata-shi on 2 May 2010 by H. Okawa. OU0706, one male, Nomino-o, Kurose-cho, Higashihiroshima-shi on 22 March 2012 by H. Okawa. Ehime Prefecture: KUHE OU 0343–0348, six males, Hinokuchikatakami, Namikata-cho, Imabari-shi on 5 April 2008 by H. Okawa.

Etymology

The specific name "*akiensis*" refers to the locality name "Aki" for the western part of current Hiroshima Prefecture which was used from ancient times to the present, and where the new species occurs.

Diagnosis

Body small (male SVL 44–60 mm, mean ±SD=49.6±4.3, n=21), fore- and hindlimbs relatively short (25.3%SVL and 29.8%SVL, respectivey), ratio of hindlimb to forelimb moderate (1.18). Limbs separated (LO -2), and vomerine teeth series tending to be long and wide. Tail medium (74.3%SVL). Usually lacking yellow stripes on edges of the tail. Phylogenetically, *H. akiensis* sp. nov. forms a mitochondrial DNA clade with *H. utsunomiyaorum* sp. nov., which is a sister clade to *H. abuensis* sp. nov. However, *H. akiensis* sp. nov. has longer vomerine teeth series and fifth toe, as proportions of SVL, and greater degree of limb separation than *H. utsunomiyaorum* sp. nov., and has smaller SVL and relatively longer axilla-groin, narrower head, shorter tail and limbs and lesser degree of limb overlap than *H. abuensis* sp. nov.

Description of holotype (measurements in mm)

Head-body large for the species (SVL 58.6) and robust; head oval and not depressed, distinctly longer (HL 14.8, 25.3%SVL) than wide (HW 10.0, 16.4%SVL); snout rounded, slightly projecting beyond lower jaw; nostril close to snout tip; labial fold absent; eye moderate, protruded, slightly inset from edge of head in dorsal view; upper eyelid well developed (UEW 1.9, 3.2%SVL), shorter (UEL 3.4, 5.8%SVL) than snout (SL 4.0, 6.8%SVL); gular fold distinct, curving anteriorly; parotoid gland evident, extending from angle of jaw to gular fold; postorbital grooves distinct, branching posterior to angle of jaw, one short and running down to lower jaw, the other long and posterior to parotoid gland; vomerine teeth shallow V-shaped, series nearly touching at midline (Fig. 14H), wider (VTW 3.0, 5.2%SVL) than long (VTL 2.4, 4.1%SVL), anterior margin on line connecting choanae; tongue broad, both sides free from mouth floor; fore- and hindlimbs medium for thes species and thick (FLL 14.7, 25.1%SVL; HLL 17.6, 30.0%SVL); CG 12; depressed limbs separated by a half costal fold (LO -0.5); relative length of fingers I<IV<III<II, toes I<V<II<IV<III; fifth toe well developed (5TL 1.5, 2.6%SVL); cloaca greatly swollen with longitudinal slit; genital tubercle on anterior cloaca present; tail medium for the species (TAL 42.9, 73.2%SVL), cylindrical at base (BTAW 6.6, 11.3%SVL; BTAH 5.3, 9.0%SVL), compressed posteriorly (MTAW 3.1, 5.3%SVL; MTAH 6.2, 10.6%SVL), with weak dorsal fin near tip (MXTAH 6.4, 10.9%SVL); tip of tail slightly pointed in lateral view.

Additional measurements and counts of the holotype: IND (3.0, 5.1%SVL); IOD (3.3, 5.6%SVL); AGD (28.8, 49.2%SVL); TRL (43.8, 74.7%SVL); 2FL (3.2, 5.5%SVL); 3FL (3.1, 5.3%SVL); 3TL (4.4, 7.4%SVL); UJTN (73); LJTN (63); VTN (37).

Color

In life, the dorsum is chocolate brown without markings (Fig. 12A). Underside of body grayish brown scattered with silvery white dots (Fig. 12B). White nuptial color on throat not clear.

Variation

Morphometric data are summarized in Table 4. SVL of 21 males 49.6±4.3 (44.4–59.9) mm. Medians and ranges in males: HL

23.2 (21.2–25.3) %SVL; TRL 76.8 (74.7–78.8) %SVL; HW 17.8 (16.0–19.6) %SVL; IND 5.3 (4.6–6.4) %SVL; IOD 6.3 (4.8–7.5) %SVL; UEW 3.2 (2.3–3.8) %SVL; UEL 6.1 (4.7–7.2) %SVL; AGD 51.7 (46.8–56.3) %SVL; TAL 74.3 (63.2–81.4) %SVL; BTAW 11.0 (8.3–13.2) %SVL; MXTAH 11.1 (9.9–12.8) %SVL; FLL 25.3 (22.5–27.4) %SVL; HLL 29.8 (26.0–33.8) %SVL; MTAW 5.1 (4.2–6.4) %SVL; VTW 5.4 (4.7–6.3) %SVL; VTL 4.6 (3.9–5.9) %SVL; 2FL 5.1 (3.8–6.23) %SVL; 3FL 4.3 (3.2–5.3) %SVL; 3TL 7.4 (5.5–9.0) %SVL; 5TL 2.0 (0.6–3.4) %SVL. VTW/VTL 119.0 (100.0–161.1)%. TotL 84.5 (75.2–104.6) mm; CG 12 (12–13); LO -2 (-3–-0.5).

Adpressed limbs were always separated, although the degree varied. Condition of the fifth toe is most variable among the species formerly classified as *H. nebulosus*. The proportion of the hindlimbs with four and five toes, respectively, is 37.9% and 28.7% in the Transitional morphotype, 2.7 % and 73.4% in the Hiroshima morphotype, and 2.4% and 76.8% in the Miyoshi morphotype. As a result, 10.3% and 65.3% of hindlimbs in *H. akiensis* sp. nov. have four and five toes, respectively. The remaining percentages of hindlimbs are occupied by rudimentary fifth toes (Okawa et al. 2009). Usually, white nuptial color on throat was clear unlike the holotype.

Populations from around Yatsugi (present Higashisakeya-machi), Miyoshi-shi were once identified as *H. abei* (Sato, 1943), but are now regarded as *H. nebulosus* with dark brown dorsum (Utsunomiya and Okawa, 1987). Utsunomiya et al. (1996) reported that, of 32 young *H. nebulosus* reared from a pair of egg sacs from Yatsugi, only five had dark brown color without black spots, and the remaining 27 were light yellow brown with dark spots or with white spots.

Eggs and egg sacs

The egg sacs are string-like in shape and coiling, with a thin, wrinkled envelope lacking notable striations and any distinct whiptail structure (Fig. 12E). Lengths of three egg sacs (ESL) before larval hatching ranged from 133.1–166.7 (mean=148.4) mm and the widths (ESW) from 12.4–14.3 (mean=13.4) mm with ESL/ESW from 9.3–12.4 (mean=11.2). The clutch size ranges from 20–118 with mean±SD of 51.5±16.8 (n=57). Sato (1943) reported the clutch size ranged from 36 to 78 with the average of 61.0±16.2 (n=10) for the Bingo population (as *H. abei*). For the population from Ehime Prefecture, clutch size is reported to range from 81 to 215 (Tanabe, 2014), averaging 124.7±40.1 and 127.0±22.0 in two successive years (Itano et al., 2016). The diameter of ova from a female from Kurose-cho, Higashihiroshima-shi, Hiroshima Prefecture ranges from 2.5–2.8 (mean±SD=2.63±0.13) mm. The animal pole is dark brown and vegetal pole is light brown in color.

Larvae

Fully-grown larvae at Stage 63 of Iwasawa and Yamashita (1991) from near the type locality had SVL ranging from 16.1–18.0 (mean±SD=17.0±0.8, n=4) and total length of 27.2–34.0 (mean±SD=31.4±2.6, n=4) mm (Utsunomiya et al., 1996). Head rounded in dorsal view and profile (Fig. 12C, D); snout short and broadly rounded; eyes slightly protruded, inset from edge of head in dorsal view; labial fold distinct at half of upper jaw; external gills developed; caudal fin higher than head; dorsal fin higher than ventral fin; origin of dorsal fin in middle of trunk; ventral fin originating from the vent; tail tip weakly pointed; limbs slender; claws on fingers and toes absent. In life, their dorsum was yellowish brown scattered with small dark brown marking; venter whitish and transparent; large black spots scattered on tail especially on posterior half; golden dots scattered on tail fin (Utsunomiya et al., 1996).

Range

Known so far from areas in southern central Chugoku and northwestern Shikoku (Fig. 15). The range is bordered by *H. setou-*

chi sp. nov. to the east and *H. utsunomiyaorum* sp. nov. to the north. Populations in the northern region have been called the "transition type between *H. utsunomiyaorum* sp. nov. Hiroshima Prefecture: Akitakata-shi (including the former Yoshida-cho, Mukaihara-cho, Koda-cho, Takamiya-cho, Yachiyo-cho); Higashihiroshima-shi (including the former Kurose-cho, Fukutomi-cho, Toyosaka-cho, Akitsu-cho); Mihara-shi (including the former Daiwa-cho); Kure-shi; Onomichi-shi (including the former Mitsugi-cho); Miyoshi-shi (including the former Kisa-cho, Mirasaka-cho, Miwa-cho); Kitahiroshima-cho, Yamagata-gun (including the former Chiyoda-cho, Oasa-cho); Sera-cho, Sera-gun (including the former Seranishi-cho). Ehime Prefecture: Imabari-shi.

Altitude of the known localities extends from 18 to 565 (329.4±152.1) m (n=18).

Comparisons

Hynobius akiensis sp. nov. significantly differs from *H. utsunomiyaorum* sp. nov. by longer vomerine teeth series (RVTL 4.6% vs. 3.7%) and fifth toe (R5TL 2.0% vs. 0%), and greater degree of limb separation (LO -2 folds vs. -1 fold); from *H. abuensis* sp. nov. by longer axilla-groin (RAGD 51.7% vs. 47.1%) and smaller SVL (49.6 mm vs. 57.2 mm), narrower head (RHW 17.8% vs. 19.4%), shorter tail (RTAL 74.3% vs. 86.9%) and limbs (RFLL 25.3% vs. 30.1%, RHLL 29.8% vs. 34.3%, R2FL 5.1% vs. 6.3%, R3FL 4.3% vs. 5.1%, R3TL 7.4% vs. 8.9%, and R5TL 2.0% vs. 3.0%), and lesser degree of limb overlap (LO -2 folds vs. -1 fold).

The new species differs from *H. nebulosus* by longer eyelid (RUEL 6.1% vs. 5.3%) and wider vomerine teeth series (RVTW 5.4% vs. 4.7%), and smaller SVL (49.6 mm vs. 59.3 mm); from *H. iwami* sp. nov. by wider tail (RMTAW 5.1% vs. 3.7%), longer fifth toe (R5TL 2.0% vs. 0%), and lesser degree of limb overlap (LO -2 folds vs. -0.5 fold).

It differs from *H. vandenburghi* by larger vomerine teeth series (RVTW 5.4% vs. 4.5% and RVTL 4.6% vs. 3.9%), and smaller SVL (49.6 mm vs. 56.5 mm); from *H. setoi* sp. nov. by longer trunk (RTRL 76.8% vs. 75.1%) and axilla-groin (RAGD 51.7% vs. 48.2%), larger vomerine teeth series (RVTW 5.4% vs. 4.8% and RVTL 4.6% vs. 3.7%), smaller SVL (49.6 mm vs. 58.8 mm), shorter head (RHL 23.2% vs. 24.8%) and hindlimb (RHLL 29.8% vs. 32.3%), lower tail (RMXTAH 11.1% vs. 13.4%), and lesser degree of limb overlap (LO -2 folds vs. -0.5 fold).

Hynobius akiensis sp. nov. did not show significant metric differences from *H. bakan* sp. nov. and *H. setouchi* sp. nov. However, *H. akiensis* sp. nov. differs from *H. bakan* sp. nov. by the usual lack of yellow stripes in the tail. Both *H. akiensis* sp. nov. and *H. setouchi* sp. nov. tend to lack the tail stripes, but the former is reported to be smaller in body size and more frequently lacking the fifth toe than the latter (Okawa et al., 2009).

The mean uncorrected pairwise sequence divergence between *H. akiensis* and the other species is 5.5–15.8% (Table 2).

Natural history

Breeding usually occurs from early March to late April, but from late January in Miyoshi-shi, Hiroshima (Utsunomiya and Okawa, 1987), and from late December to early May in Ehime Prefecture (Itano et al., 2016). Of 23 cases recorded, ditches around paddy fields occupied about half (48%), followed by marshes (35%). Some spawned in slowly flowing streams and small pools (each 8.7%). Egg sacs are attached to dead leaves and twigs. The minimum and maximum water temperatures at the time of breeding were 3.5–6.6°C and 8.0–13.6°C, respectively in Ehime Prefecture (Itano et al., 2016).

Conservation

Since the paddies, main breeding sites of *H. akiensis* sp. nov., are abandoned and quickly degrading, the species requires immediate conservation measures. As *H. nebulosus*, it is listed as VU in the Red List of Japan (https://www.env.go.jp/nature/kisho/hozen/redlist/

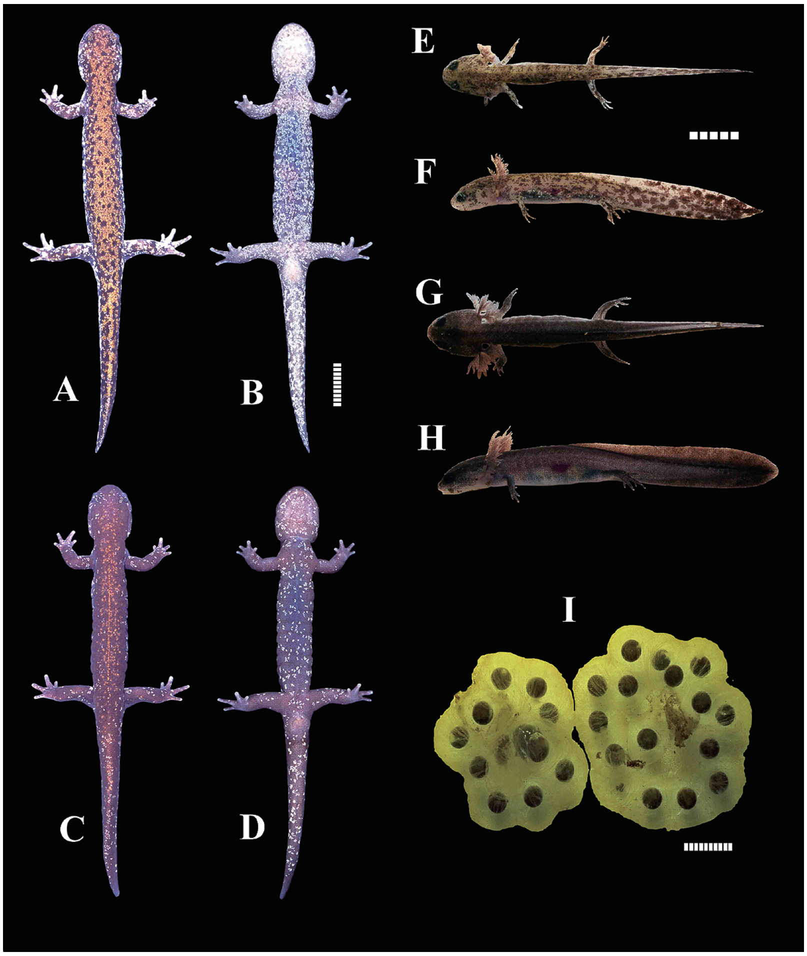

FIG. 13 *Hynobius utsunomiyaorum* sp. nov. Dorsal (A, C) and ventral (B, D) views of (KUHE 26185 and 26186, respectively), scale bar=10 mm. Dorsal (E, G) and lateral (F, H) views of two larvae (Stage 63–64), scale bar=5 mm. Egg sac (I), scale bar=10 mm.

RL2018_5_180604.pdf), together with *H. setouchi* sp. nov., *H. iwami* sp. nov., and *H. utsunomiyaorums* sp. nov. Also it has been listed as VU by Hiroshima Prefecture, and as CR+EN by Ehime Prefecture (Tanabe, 2014).

9. *Hynobius* ***utsunomiyaorum*** Matsui et Okawa sp. nov.

[Japanese name: Hiba-sansyou-uwo]
[English name: highland salamander]
Fig. 13

Hynobius nebulosus (part, as X morphotype) Okawa and Utsunomiya, 1989, 142.
Hynobius nebulosus (part, as Highland morphotype) Okawa et al., 1998, 13.

Holotype

KUHE 6491, an adult male from Rokunohara, Mt. Hibayama, Saijo-cho, Shobara-shi, Hiroshima Prefecture (35°04'20"N, 133°04'18"E, alt. 850 m) on 3 May 1982 by M. Matsui.

Paratypes

All from Hiroshima Prefecture: KUHE

6492–6494, three males from Tate-eboshiyama, Mt. Hibayama, Saijo-cho, Shobara-shi (35°03'04"N, 133°04'01"E, alt. 1216 m). KUHE 6509–6513, five males from Tatarabara, Goka, Shobara-shi (34°50'48"N, 133°08'24"E, alt. 690 m). KUHE 6514–6517, 6519–6523, nine males from Daizenbara, Mitsugaichi, Hiwa-cho, Shobara-shi (35°04'02"N, 133°02'30"E, alt. 1027 m), all on 3 May 1982 by M. Matsui, T. Utsunomiya, Y. Utsunomiya and H. Okawa.

Referred specimens

Hiroshima Prefecture: KUHE 26183–26184, two males, Tawaraharagoshi, Kamiyukawa, Takano-cho, Shobara-shi in May 1997 by K. Nishikawa. KUHE OU0513–0515, three males from Kanao-toge, Takano-cho, Shobara-shi on 25 April 2010 by H. Okawa. OU0371, one male from Kawate-machi, Shobara-shi on 29 April 2009 by H. Okawa. OU0373, one male from Shinodo, Kawakita-cho, Shobara-shi on 9 May 2009. OU 0516, one male on 3 May 2010 by H. Okawa. OU 0517–0519, three males, Tatara-bara, Goka, Shobara-shi on 5 May 2010 by H. Okawa. Shimane Prefecture: KUHE OU 0487, one male from Kamiyamasa-Shimomyo, Hirose-cho, Yasugi-shi on 7 April 2010 by H. Okawa. KUHE OU 0503, one male from Okutawara, Hirose-cho, Yasugi-shi on 7 April 2010, by H. Okawa. OU0527–OU0529, three males from Kusano, Hakuta-cho, Yasugi-shi on 12 May 2010 by H. Okawa. OU0520–OU0523, three males, Fukuharakami, Kamiai, Okuizumo-cho, Nita-gun on 9 May 2010 by H. Okawa. Tottori Prefecture: KUHE 8246–8287, 42 males, Daisen-ji, Daisen, Daisen-cho, Saihaku-gun by M. Matsui and T. Hayashi on 10 May 1985. Okayama Prefecture: KUHE 8750–8753, four males from Shinrin-koen, Kamisaibara, Kagamino-cho, Tomata-gun on 4 May 1986 by M. Matsui, T. Utsunomiya, Y. Utsunomiya and H. Okawa. KUHE 8754–8757, four males from Sanjyusichininbaka, Ningyo-toge, Kamisaibara, Kagamino-cho, Tomata-gun on 4 May 1986 by M. Matsui, T. Utsunomiya, Y. Utsunomiya and H. Okawa. U 0479–0488, six females, from Ogaya, Nishiawakura-son, Aida-gun on 3 May 1988 by T. Utsunomiya and M. Matsui. Hyogo Prefecture: KUHE11074–11081, eight males from Nishigochi, Chikusa-cho, Shiso-shi on 9 May 1990 by M. Matsui and S. Tanabe.

Etymology

The specific name "*utsunomiyaorum*" is dedicated to the late Ms. Taeko Utsunomiya and her husband Dr. Yasuaki Utsunomiya of Hiroshima University who made great contributions to the study of Japanese amphibians, and began the study of this species in the early 1980s.

Diagnosis

Body small (male SVL 43–60 mm, mean ±SD=53.6±4.0, n=16), forelimb relatively short (24.9%SVL), hindlimb medium in length (30.0%SVL), ratio of hindlimb to forelimb moderate (1.16). Phylogenetically, *H. utsunomiyaorum* sp. nov. is a member of a mitochondrial DNA clade in which it forms a clade with *H. akiensis* sp. nov., which is sister to the clade of *H. abuensis* sp. nov. and its sister species *H. tosashimizuensis*. However, *H. utsunomiyaorum* sp. nov. significantly differs from *H. akiensis* sp. nov. by greater limb overlap, and shorter vomerine teeth series and fifth finger; from *H. abuensis* sp. nov. by longer axilla-groin, wider, but shorter and lower tail, and shorter forelimb, hindlimb, third finger, third toe, and fifth toe. Except for some populations, normally lacking or at most with rudimentary fifth toe. The tail is short (69.4%SVL), low in height, and tip not pointed. Vomerine teeth series relatively short. Body color very variable. Yellow stripes absent on edges of the tail.

Description of holotype (measurements in mm)

Head-body large for the species (SVL 57.3) and thin; head oval and not depressed, distinctly longer (HL 14.6, 25.5%SVL) than wide (HW 9.4, 16.4%SVL); snout rounded, slightly projecting beyond lower jaw; nostril

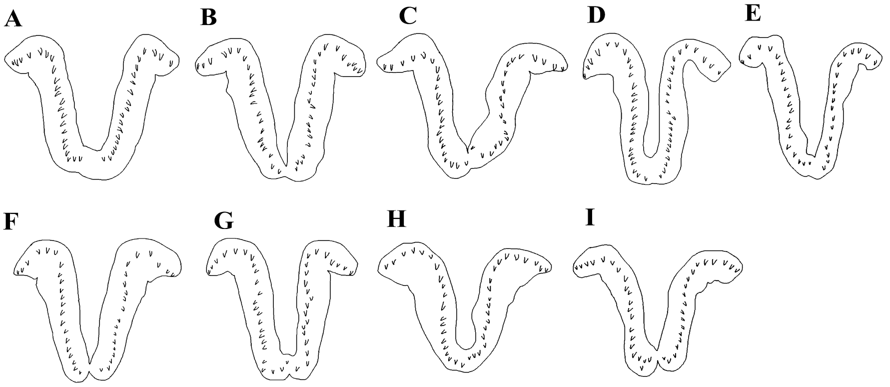

FIG. 14 Open mouths, showing the shape of vomerine teeth series in male of: (A) the holotype of *H. setoi* sp. nov. (KUHE 7313); (B) topotypic *H. vandenburghi* (KUHE 28516); (C) holotype of *H. setouchi* sp. nov. (KUHE OU 0285); (D) the holotype of *H. iwami* sp. nov. (KUHE 35891); (E) the holotype of *H. bakan* sp. nov. (KUHE OU 0391); (F) a topotypic *H. nebulosus* (KUHE 7313); (G) the holotype of *H. abuensis* sp. nov. (KUHE 35904); (H) the holotype of *H. akiensis* sp. nov. (KUHE 35925); and (I) the holotype of *H. utsunomiyaorum* sp. nov. (KUHE 6491). Not to scale.

close to snout tip; labial fold absent; eye moderate, protruded, slightly inset from edge of head in dorsal view; upper eyelid well developed (UEW 1.5, 2.6%SVL), shorter (UEL 3.3, 5.8%SVL) than snout (SL 3.9, 6.8%SVL); gular fold distinct, curving anteriorly; parotoid gland evident, extending from angle of jaw to gular fold; postorbital grooves distinct, branching posterior to angle of jaw, one short and running down to lower jaw, the other long and posterior to parotoid gland; vomerine teeth shallow U-shaped, series nearly touching at midline (Fig. 14I), wider (VTW 3.4, 5.8%SVL) than long (VTL 2.3, 4.0%SVL), anterior margin on line connecting posterior borders of choanae; tongue broad, both sides free from mouth floor; fore- and hindlimbs thin, short for the species (FLL 13.4, 23.4%SVL; HLL 15.2, 26.5%SVL); CG 12; depressed limbs separated by two and half costal folds (LO -2.5); relative length of fingers IV<I<III<II, toes I<IV<II<III; fifth toe lacking (5TL 0, 0%SVL); cloaca greatly swollen with longitudinal slit; genital tubercle on anterior cloaca present; tail long for the species (TAL 42.3, 73.8%SVL), cylindrical at base (BTAW 5.2, 9.1%SVL; BTAH 5.6, 9.8%SVL), slightly compressed posteriorly (MTAW 2.6, 4.5%SVL; MTAH 5.2, 9.1%SVL), with weak dorsal fin near tip (MXTAH 6.0, 10.5%SVL); tip of tail rounded in lateral view.

Additional measurements and counts of the holotype: IND (3.6, 6.3%SVL); IOD (3.6, 6.3%SVL); AGD (28.9, 50.4%SVL); TRL (42.7, 74.5%SVL); 2FL (2.5, 4.4%SVL); 3FL (1.9, 3.4%SVL); 3TL (4.0, 6.9%SVL); UJTN (68); LJTN (64); VTN (44).

Color

In life, gray-brown with ochre marking forming a dorsal stripe from snout to tip of tail (Fig. 13A); side of body with silvery white dots, gradually increasing toward ventrum which is lighter than dorsum in ground color (Fig. 13B). White nuptial color on throat not strong.

Variation

Morphometric data are summarized in Table 4. SVL of 16 males 53.6±4.0 (43.4–60.0) mm. Medians and ranges in males: HL 23.1 (22.2–25.5) %SVL; TRL 76.9 (74.5–77.8) %SVL; HW 17.6 (16.4–19.2) %SVL; IND 5.4 (4.3–6.3) %SVL; IOD 6.2 (5.4–6.9) %SVL; UEW 3.1 (2.5–3.7) %SVL; UEL 5.8

(5.0–7.8) %SVL; AGD 51.7 (42.1–54.3) %SVL; TAL 69.4 (60.8–79.3) %SVL; BTAW 10.9 (9.1–13.1) %SVL; MXTAH 9.7 (8.4–12.2) %SVL; FLL 24.9 (22.2–27.7) %SVL; HLL 30.0 (26.5–35.7)) %SVL MTAW 5.5 (3.9–6.4) %SVL; VTW 5.0 (4.2–5.8) %SVL; VTL 3.7 (3.1–4.7) %SVL; 2FL 4.7 (3.3–6.0) %SVL; 3FL 3.6 (2.3–5.3) %SVL; 3TL 7.4 (5.6–8.8) %SVL; 5TL 0 (0–1.7) %SVL. VTW/VTL 130.1 (110.7–152.8)%. TotL 94.5 (72.3–103.4) mm; CG 12 (12–13); LO -1 (-2.5–1.5).

Fifth toes were almost always present in the population from Daisen unlike in other populations. Body color is highly variable (Figs. 13A–D) and some individuals are very similar to *H. naevius*, having the dorsum varying from reddish- to bluish- purple in ground color, with discontinuous, grayish- to brownish-white markings.

Eggs and egg sacs

The egg sacs are string-like in shape and coiling, with a thin, wrinkled envelope lacking striations and any distinct whiptail structure (Fig. 13I). Lengths of egg sacs (ESL) range from 126–198 (mean±SD=159.5±35.0, n=4) mm and the widths (ESW) from 10.5–13 (mean±SD=11.7±1.1, n=4) mm, with ESL/ESW of 12.0–15.2 (mean±SD=13.5±1.8, n=4). The clutch size is small, ranging from 20–63 (mean±SD=39.0±10.2, n=58). The ova large, diameter from three females ranges from 3.4–3.6 (mean±SD=3.5±0.1, n=9) mm, from 3.4–3.5 (mean±SD=3.4±0.1, n=10) mm, and from 3.1–3.3 (3.2±0.1, n=8) mm. The animal pole is gray brown and vegetal pole is light gray in color.

Larvae

Larvae from Maniwa-shi at Stage 63–64 of Iwasawa and Yamashita (1991) had SVL ranging from 15.3–17.8 (mean±SD=16.4±1.3, n=3) and total length of 27.7–33.3 (mean±SD=30.7±2.8, n=3) mm, head rounded in dorsal view and profile (Fig. 13E–H); snout very short and broadly rounded; eyes slightly protruded, slightly inset from edge of head in dorsal view; labial fold distinct at half of upper jaw; external gills developed; caudal fin higher than head; dorsal fin higher than ventral fin; origin of dorsal fin posterior to middle of trunk; ventral fin originating from the vent; tail tip weakly pointed or rounded; limbs slender; claws on fingers and toes absent. In life, their dorsum was usually light brown with dark marking; venter whitish and transparent; large black spots scattered on tail; golden dots scattered on flank and tail fin (Fig. 13E, F). However, some were totally darkened without marking although they hatched with normally colored individuals (Fig. 13G, H).

Range

Known from montane areas on the backbone range of the Chugoku Mountains (Fig. 15). Hyogo Prefecture: Shiso-shi (including the former Chikusa-cho). Okayama Prefecture: Niimi-shi (including the former Tetta-cho); Kagamino-cho, Tomata-gun (including the former Nishiawakura-son, Kamisaibara-son); Takahashi-shi; Maniwa-shi. Hiroshima Prefecture: Miyoshi-shi (including the former Sakugi-son, Funo-son); Shobara-shi (including the former Soryo-cho, Takano-cho, Kuchiwa-cho, Saijo-cho, Tojyo-cho, Hiwa-cho). Tottori Prefecture: Daisen-cho, Saihaku-gun; Nichinan-cho, Hino-gun. Shimane Prefecture: Yasugi-shi; Okuizumo-cho, Nita-gun (including the former Yokota-cho, Nita-cho); Unnan-shi (including the former Yoshida-mura); Oda-shi; Iinan-cho, Iishi-gun (including the former Akagi-cho, Tonbara-cho). Misato-cho, Ochi-gun (including the former Ochi-cho).

The altitude of known localities extends from 35–1216 m, with the mean of 350±231.2 m (n=30).

Comparisons

Hynobius utsunomiyaorum sp. nov. is genetically close to *H. hidamontanus* with the mean uncorrected pairwise sequence divergence of 3.0–7.4%. Morphologically, the new species is also similar to *H. hidamontanus* in

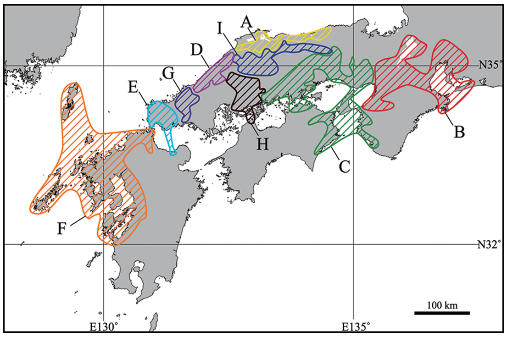

FIG. 15 Map of central and western Japan showing ranges of *Hynobius* taxa defined here. (A) *H. setoi* sp. nov.; (B) *H. vandenburghi*; (C) *H. setouchi* sp. nov.; (D) *H. iwami* sp. nov.; (E) *H. bakan* sp. nov.; (F) *H. nebulosus*; (G) *H. abuensis* sp. nov.; (H) *H. akiensis* sp. nov.; (I) *H. utsunomiyaorum* sp. nov.

usual lack of the fifth toe, the number of costal grooves (CG 12), and degree of limb overlap (LO -1). However, *H. utsunomiyaorum* sp. nov. differs from *H. hidamontanus* by larger SVL (males 43–60 mm vs. 41–54 mm), smaller head (RHL 23.1% vs. 24.1% and RHW 17.6% vs. 19.6%), shorter and lower tail (RTAL 69.4% vs. 75.2% and RMXTAH 9.7% vs. 11.9%), and longer and narrower vomerine teeth series (RVTL 3.7% vs. 2.2% and RVTW 5.0% vs. 5.3%).

Hynobius utsunomiyaorum sp. nov. significantly differs from *H. akiensis* sp. nov. by greater limb overlap (LO -1 fold vs. -2 folds), and shorter vomerine teeth series (RVTL 3.7% vs. 4.6%) and fifth toe (R5TL 0% vs. 2.0%); from *H. abuensis* sp. nov. by longer axilla-groin (RAGD 51.7% vs. 47.1%), wider (RMTAW 5.5% vs. 4.2%), but shorter and lower tail (RTAL 69.4% vs. 86.9% and RMXTAH 9.7% vs. 12.0%), and shorter forelimb (RFLL 24.9% vs. 30.1%), hindlimb (RHLL 30.0% vs. 34.3%), second finger (R2FL 4.7% vs. 6.3%), third finger (R3FL 3.6% vs. 5.1%), third toe (R3TL 7.4% vs. 8.9%), and fifth toe (R5TL 0% vs. 3.0%).

The new species differs from *H. nebulosus* by smaller SVL (53.6 mm vs. 59.3 mm), greater limb overlap (LO -1 fold vs. -2 folds), relatively lower tail (RMXTAH 9.7% vs. 12.5%), shorter vomerine teeth series (RVTL 3.7% vs. 4.6%) and fifth toe (R5TL 0% vs. 2.5%), and smaller number of costal grooves (CG 12 vs. 13); from *H. bakan* sp. nov. by lower tail (RMXTAH 9.7% vs. 12.4%), shorter vomerine teeth series (RVTL 3.7% vs. 4.7%), and shorter fifth toe (R5TL 0% vs. 2.0%); and from *H. iwami* sp. nov. by larger medial tail width (RMTAW 5.5% vs. 3.7%) and shorter vomerine teeth series (RVTL 3.7% vs. 4.7%).

It differs from *H. setouchi* sp. nov. by shorter vomerine teeth series (RVTL 3.7% vs. 4.6%), fifth toe (R5TL 0% vs. 2.4%), and third finger (R3FL 3.6% vs. 4.7%); from *H. vandenburghi* by lower tail (RMXTAH 9.7% vs. 11.7%), shorter fifth toe (R5TL 0% vs. 2.3%), and smaller number of costal grooves (CG 12 vs. 13); and from *H. setoi* sp. nov. by longer trunk (RTRL 76.9% vs. 75.2%) and axilla-groin (RAGD 51.7% vs. 48.2%), lower tail (RMXTAH 9.7% vs. 13.4%), and shorter head (RHL 23.1% vs. 24.8%), forelimb (RFLL 24.9% vs. 26.7%), third finger (R3FL 3.6% vs. 4.5%), and fifth toe (R5TL 0% vs. 2.3%).

The mean uncorrected pairwise sequence divergence between *H. utsunomiyaorum* sp. nov. and the other species formerly called *H. nebulosus* is 5.5–17.9% (Table 2).

Natural history

Breeding occurs usually from late March to late May. Of 42 cases recorded, marshes (38%) occupied the largest proportions followed by ditches (21%), pools (14.3%), and small streams (11.9%). Other than these, breeding occurred in abandoned paddies, small springs, and small ponds. Egg sacs are attached to debris or roots of water plants or directly buried in the muddy bottom of marshes or in very slowly flowing streams. Water temperature at the time of breeding was about 10°C. Overwintered larvae can be found not rarely in the following spring. Both sexes collected at the breeding season including females with eggs (KUHE 6913–6922) mostly with full stomachs including gammarids.

The species is syntopic with *H. setoi* sp. nov. at 90 m in altitude in Hirose-cho, Yasugi-shi, and at 355 m in altitude, in Daito-cho, Unnan-shi, Shimane Prefecture.

Conservation

Although the habitats of *H. utsunomiyaorum* sp. nov. are mainly in the mountain ranges, artificial modifications of the environments such as deforestation and construction of roads are steadily increasing. This species is in the national Red List as VU under the name of *H. nebulosus* with all other taxa here treated. Locally, the species, as *H. nebulosus*, is listed on the Prefectural Red Lists as EN in Okayama, as VU in Hiroshima, Tottori and Hyogo, and as NT in Shimane Prefectures.

Divergence Time

The divergence time estimations revealed relatively large degrees of overlap in the confidence intervals surrounding the estimated values between many lineages, and this hinders making a definitive conclusion regarding the timing of colonization of the present areas of distribution.

Our data suggest that *H. nebulosus* and related species began divergences from the lotic breeding species in current Japan, and spread among the current continental region, Korean Peninsula, and Japan in the early stage of the Late Miocene. Date of divergence between Clades A and B is estimated at 9.1 (CI 7.9–10.3) million years before present (MYBP) in the Late Miocene (Fig. 16, Table 5). Slightly later in the Late Miocene, divergences within Clade A (7.4 [CI 6.3–8.4] MYBP) and Clade B (7.4 [CI 6.4–8.4] MYBP) are estimated to have occurred nearly simultaneously. Clade A diverged to form (1) Clade A1 that differentiated into *H. setoi* and the clade of *H. nigrescens*, *H. takedai*, and *H. mikawaensis* at 4.1 (CI 3.2–5.0) MYBP in the Early Pliocene, and (2) Clade A2, the common ancestor of *H. tokyoensis* and *H. vandenburghi*, and *H. setouchi* at 4.8 (CI 4.0–5.6) MYBP in the Early Pliocene. In contrast, Clade B gave rise to (3) Clade leading to Korean and Chinese species at 5.9 (CI 4.4–7.2) MYBP in the Late Miocene, (4) Clade B1, ancestral to *H. nebulosus*, *H. tsuensis*, *H. dunni*, *H. bakan*, *H. iwami*, and *H. okiensis* at 6.0 (CI 5.0–6.9) MYBP in the Late Miocene, and (5) Clade B2, now differentiated into *H. abuensis*, *H. tosashimizuensis*, *H. akiensis*, and *H. utsunomiyaorum* at 5.5 (CI 4.4–6.5) MYBP in the Late Miocene.

DISCUSSION

Two hynobiid salamanders collected by von Siebold were described and named by Temminck and Schlegel (1838): *Salamandra naevia* (currently *H. naevius*) and *Salamandra nebulosa* (currently *H. nebulosus*). These represent southwestern Japanese lotic and lentic breeding species, respectively. More recently, *H. naevius* was split into two distinct species, *H. naevius* and *H. yatsui* Oyama, 1947 (Tominaga and Matsui, 2008), and the types of *H. naevius* were shown to have come from Tara-cho, Saga Prefecture (Tominaga and Matsui, 2007). Another species, *H. yatsui*, is now called *H. stejnegeri* after complicated taxonomical considerations (Matsui et al., 2017b).

In contrast, the taxonomic revision of *H. nebulosus* was delayed after early research during the 1920s to 1940s by Dunn, Oyama, and Sato. Of these, Sato published a monumental monograph of the Japanese Caudata (Sato, 1943), but seems to have studied *H. nebulosus* from Hiroshima very little, even though this is where he lived. Instead, he (Sato, 1943) mistook a population of that species from Yatsugi in Hiroshima as *H. abei*, which he had originally described from Tango Province, Kyoto Prefecture.

Shibata (1979) compiled distribution data for *H. nebulosus*, noting some interesting records in Hiroshima, Kagawa, and Nagano Prefectures. Taeko Utsunomiya energetically made a field survey (e.g., Utsunomiya and

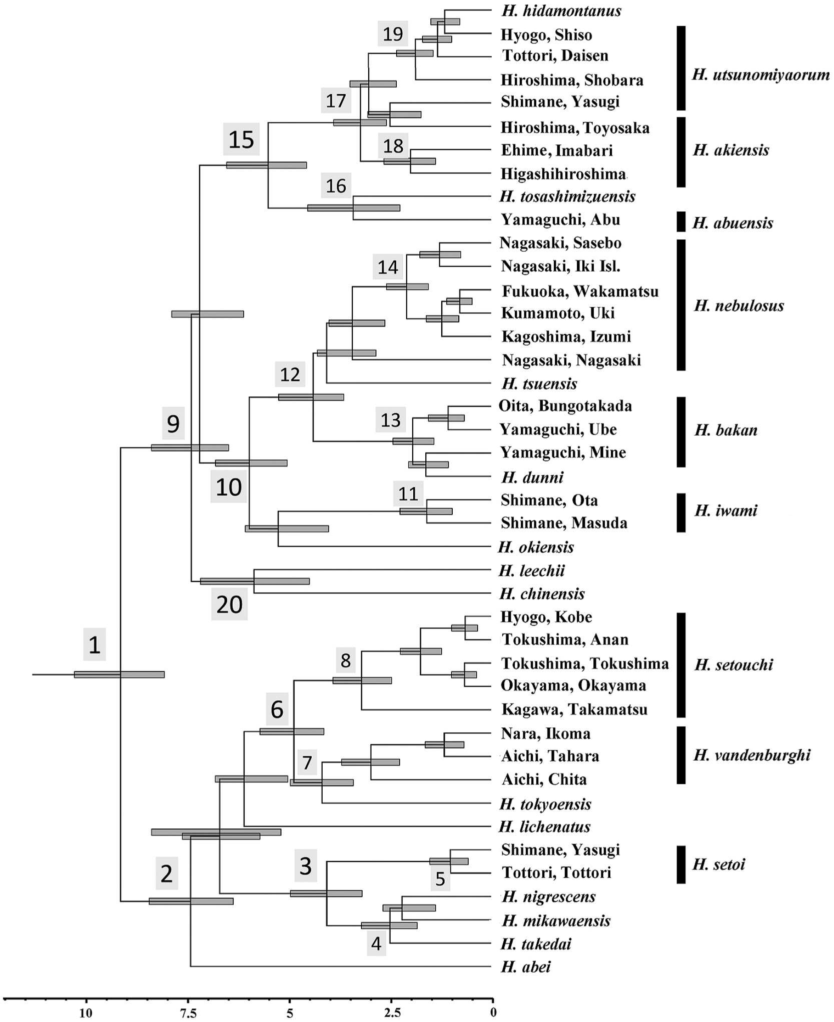

FIG. 16 Estimated times in MYBP (million years before present) for the main divergences of *Hynobius* taxa defined here. Numbers above or below branches correspond to events shown in Table 5.

Utsunomiya, 1998) with Y. Utsunomiya, H. Okawa, M. Matsui, and T. Seto, and clarified the occurrence of several distinct morphotypes including the Highland type, later discovered in wider regions covering Tottori through Hiroshima to Okayama, and eventually in western Hyogo. Because typical Highland type looks like *H. hidamontanus* that Matsui (1987) described from central Japan, he further tried to find out the distribution of the form, but has failed to find specimens further to the east. Distinct clades have been reported (e.g., Okawa and Utsunomiya, 1989; Okawa et al., 2007, 2009), chiefly on morphological grounds over these 30 years and were mostly confirmed in the present genetic investigations.

It is notable that lineages of so-called *H. nebulosus* frequently form clades in mtDNA not with other conspecific lineages but with other named species. For example, the sister species of *H. bakan* sp. nov. was *H. dunni*, and *H. abuensis* sp. nov. was the sister to *H. tosashimizuensis*. This was first proved by

TABLE 5. Estimated divergence times (MY) of main divergences of lentic breeding *Hynobius* shown in Fig. 16.

Node No.	Divergent events	Mean	CI
1	A/B	9.10	(7.90, 10.33)
9	B1/B2/China, Korea	7.39	(6.37, 8.42)
2	A1/A2/*H. lichenatus*/ *H. abei*	7.35	(6.3, 8.43)
10	B1a/B1b/*H. okiensis*	5.96	(5.04, 6.92)
20	*H. chinensis*/*H. leechi*	5.86	(4.43, 7.21)
15	B2a/B2b	5.46	(4.43, 6.50)
6	A2a/A2b	4.82	(4.01, 5.63)
12	B1b1/B1b2/B1b3/ *H. tsuensis*	4.40	(3.59, 5.18)
7	Within A2a	4.11	(3.31, 4.91)
3	A1a/A1b	4.05	(3.17, 5.00)
16	Within B2a	3.40	(2.35, 4.56)
8	Within A2b	3.17	(2.43, 3.95)
17	B2b1/B2b2/B2b3/ B2b4	3.17	(2.52, 3.75)
4	Within A1a	2.50	(1.79, 3.22)
14	Within B1b3	2.08	(1.59, 2.61)
18	Within B2b1	1.96	(1.30, 2.62)
13	Within B1b1	1.93	(1.42, 2.49)
19	Within B2b4	1.84	(1.37, 2.37)
11	Within B1a	1.58	(0.98, 2.25)
5	Within A1b	1.02	(0.58, 1.53)

mtDNA, which shows only matrilineal lineages. However, MIG-seq, including a lot of nuclear genomic SNPs, also confirmed recognition of mitochondrial lineages. Thus the molecular, as well as morphological data sets, although less clearly, provides evidence that the concept of *H. nebulosus* as a single, widespread species (as in Sato, 1943; Nakamura and Uéno, 1963) must be rejected. Instead, the present data indicate that this is a complex of as many as nine species. The sympatry of *H. setoi* sp. nov. and *H. utsunomiyaorum* sp. nov. also supports this idea.

In the phylogenetic tree based on cyt b gene, two major clades, A and B, were recognized, and in the latter, Clades B1 and B2 were recognized. Clade A included *H. lichenatus*, *H. abei*, *H. nigrescens*, *H. mikawaensis*, *H. takedai*, *H. tokyoensis*, *H. setoi* sp. nov., *H. vandenburghi*, and *H. setouchi* sp. nov., whereas Clade B1 contained *H. okiensis*, *H. tsuensis*, *H. dunni*, *H. nebulosus*, *H. iwami* sp. nov., and *H. bakan* sp. nov., and Clade B2 encompassed *H. tosashimizuensis*, *H. hidamontanus*, *H. abuensis* sp. nov., *H. akiensis* sp. nov., and *H. utsunomiyaorum* sp. nov.

These groupings fairly well agree with those obtained by Matsui et al. (2006) based on enzyme variation. Of members in Clade A, *H. setoi* sp. nov. corresponds to the Chugoku group, and *H. vandenburghi* and *H. setouchi* sp. nov. represent the Eastern group of Matsui et al. (2006) based on enzyme variation. Whereas *H. nebulosus*, *H. iwami* sp. nov., and *H. bakan* sp. nov. in Clade B1, and *H. abuensis* sp. nov. in Clade B2 are in the Western group. *Hynobius utsunomiyaorum* sp. nov. in Clade B2 corresponds to the Montane group of Matsui et al. (2006). Samples not assigned to any group in Matsui et al. (2006) are *H. akiensis* sp. nov. in Clade B2 and part of *H. iwami* sp. nov. in Clade B1.

In the present result, Clade A contained species from central and northeastern species like *H. abei* and *H. lichenatus*, and surely originated in regions northwards to central Japan. Because *H. setoi* sp. nov. formed a clade with the clade of these species it is not close to *H. nebulosus* from southwestern Japan, although the two species commonly have a large body and yellow stripes on the tail. This morphological resemblance would be a result of convergence, or some characteristics like possession of a yellow tail stripe may represent plesiomorphic states, and further studies are badly needed.

Hynobius vandenburghi proved to be phylogenetically remote from *H. nebulosus*, rejecting Sato's (1934) idea of their conspecific status, but supporting Dunn's (1923b) original view. On the other hand, it was a sister species to *H. tokyoensis*, supporting earlier taxonomy based on morphology (Sato, 1937). Intraspecific variation was great and populations from Aichi Prefecture were split in two. Of these, one is remote from more western populations like Nara and Kyoto with a large p-distance, and might be split from *H. vandenburghi* (type locality=Nara) as a distinct species.

The third species in Clade A, *H. setouchi* sp. nov., was sister to the clade including *H. vandenburghi* and *H. tokyoensis*, and occurred in regions around Seto Inland Sea, i.e., eastern Shikoku and inland regions of southwestern Kinki region. The species was found very close to *H. vandenburghi* in southern Osaka. The possibility that their sympatric occurrence is not precluded. Further detailed survey is required.

Species in Clade B1 and B2 were close to Chinese and Korean species (data not shown) and had no relatives in central to northeastern Japan except for *H. utsunomiyaorum* sp. nov., whose relative, *H. hidamontanus* occurs in highland of central Japan.

Clade B1 included *H. nebulosus*, *H. bakan* sp. nov., and *H. iwami* sp. nov. together with *H. dunni*, *H. tsuensis*, and *H. okiensis*. *Hynobius tsuensis* from Tsushima Island is actually a lotic breeder (Sato, 1943), but has been generally regarded as a close relative of *H. nebulosus* since its description (Dunn, 1923b). *Hynobius tsuensis* and *H. okiensis* have 2N=56 chromosomes, like lentic breeding *H. nebulosus* and *H. dunni*, and unlike other lotic-breeders with 58 chromosomes (Seto et al., 1987). Thus, changes in breeding habits appear to have occurred in different lineages independently of karyotypic evolution in Japanese small salamanders. Like *H. tsuensis*, *H. okiensis* is ecologically grouped with lotic-breeders such as *H. naevius*, *H. kimurae*, or *H. boulengeri* (Sato, 1943), and the degree of adaptation to the lotic environment seems greater than in *H. tsuensis* (Matsui et al., 2007b).

Hynobius okiensis did not form a clade with *H. setoi* sp. nov., even though it occurs on the opposite shore on Oki Island and thought to have diverged from eastern members (Clade A). Instead, *H. okiensis* is thought to have diverged from the western members because it was grouped in the western Clade B1.

Hynobius iwami was close to a clade of *H. nebulosus* and *H. bakan* sp. nov., although it is geographically separated from *H. nebulosus* and *H. bakan* sp. nov. by the area occupied by *H. abuensis* sp. nov. From the pattern of distribution, *H. iwami* and *H. okiensis* might have a common ancestor.

Hynobius bakan sp. nov. has isolated populations in Oita, Kyushu based on mtDNA analysis, and this confirms results from allozymes (Matsui et al., 2006). The species is also close to *H. dunni* from eastern Kyushu. Although the two species have been recorded from the same locality (Usa and Bungotakada), whether they are parapatric or sympatric is not confirmed and requires investigation (Sugawara et al., 2017).

Samples of *H. nebulosus* from Nagasaki and Isahaya, including the topotypic population, formed a clade distinct from samples from the other regions for unknown reasons. The populations from Iki Islands, once called *H. ikishimae*, were within the range of variation of other populations of *H. nebulosus* and their conspecific relationship first proposed by Oyama (1930) and Sato (1934) was confirmed.

In Clade B2, *H. abuensis* sp. nov. was the sister species to *H. tosashimizuensis* from Western Shikoku, but the species was distinct from the latter and the others with egg sacs having striations, which condition is well-known in *H. abei* and *H. lichenatus* from central to eastern Japan (Matsui and Matsui, 1980). However, they are phylogenetically not close either, and compared with these northern species, the striations of *H. abuensis* sp. nov. are much weaker. Morphology of egg-sac envelope markedly varies even in a closely related species pair like *H. mikawaensis* and *H. nigrescens*, and possession of striations on egg-sacs could be a convergence among *H. abuensis* sp. nov., *H. abei*, and *H. lichenatus*.

Hynobius akiensis sp. nov. occurs inland to coastal regions of Hiroshima and Imabari, Shikoku. Thus, salamanders hitherto called *H. nebulosus* on Shikoku seem to have two origins, one from Hiroshima (*H. akiensis* sp. nov.) and another one from western Kinki (*H. setouchi* sp. nov.). Okawa and Utsunomiya (1992) considered populations from the northern region of Hiroshima (Akitakata-shi, Miyoshi-shi, and Kitahiroshima-cho) to be transitional types from the Highland type (*H. utsunomiyaorum* sp. nov.). However, the direction of "transition" is not clear, and the populations should be better called as mixed ones. The present genetic analysis resulted in poor resolution of phylogenetic relationships in mtDNA of *H. akiensis* sp. nov. and *H. utsunomiyaorum* sp. nov. Also in MIG-seq, a few individuals in Clade B2b2 (*H. akiensis* sp. nov.) showed probable past hybridization with the neighboring clades, i.e., *H. utsunomiyaorum* sp. nov.

Interestingly, some populations of the *Hynobius* X (Okawa and Utsunomiya, 1989) or the Highland morphotype (Okawa et al., 1998) included highly variable cyt b genotypes, and some of them proved to be genetically very close to *H. hidamontanus*, suggesting possible gene flow between them, although their current geographical distribution is widely separated from each other, and no salamanders like them are known in between.

After the description of *H. hidamontanus* from Nagano Prefecture, *H. tenuis* Nambu, 1991 was described from Toyama Prefecture (Nambu, 1991), but they proved to be nearly identical in allozymes characteristics and the latter was synonymized with the former (Matsui et al., 2002). In our preliminary survey in mtDNA, populations of *H. hidamontanus* from Nagano and Toyama were very similar and both were contained in the clade of Highland morphotype (data not shown). In spite of our intensive field survey over the past 30 years, salamanders like *H. hidamontanus* or the Highland morphotype from the Chugoku regions have not been discovered from montane regions between them that are at least 260 km apart in straight-line distance. As far as we know, degree of genetic differentiation of the Highland morphotype from *H. hidamontanus* is not large, but they could be viewed as heterospecific. The mean uncorrected pairwise sequence divergences of 3.0–7.4% between them are similar to those reported for some heterospecific pairs of *Hynobius* (2.9% between *H. ikioi* and *H. amakusaensis*, 3.9% between *H. ikioi* and *H. osumiensis*, 5.7% between *H. nigrescens* and *H. takedai*, 5.9% between *H. mikawaensis* and *H. nigrescens*, and 6.7% between *H. mikawaensis* and *H. takedai*: Matsui et al., 2017a; Nishikawa and Matsui, 2014). Thus, we had at present better to treat the Highland morphotype as *H. utsunomiyaorum*, a species close to, but distinct from *H. hidamontanus*.

History of differentiation

Hynobius nebulosus and the related salamanders here revised are all endemic to Japan and mainly distributed in the area north (the Inner Zone) of MTL (Major [Median] Tectonic Line), and west of ISTL (Itoigawa-Shizuoka Tectonic Line), although a few populations have slightly invaded into the Outer Zone. MTL was formed 20–15MYBP, while ISTL was formed in the Mid Miocene (Isozaki et al., 2010).

Ages of divergences of *H. nebulosus* and related species from the lotic breeders we estimated were all later than these periods, and all events related to differentiation are thought to have occurred in the area north of MTL and west of ISTL (Kinki to Chubu regions in Honshu, coastal areas of Shikoku, and northwestern areas of Kyushu).

It has been estimated that the *Hynobius* species occurring outside of Japan arose among lineages of the Japanese species (e.g., Nishikawa et al., 2010; Weisrock et al. 2013). In our age estimation, major diversifications of *H. nebulosus* and related salamanders all occurred in the Late Miocene. These include divergence between the northeastern and southwestern clades (Clades A and B), separation of the Chinese and Korean lineages from Clade B, and divergences within the northeastern and southwestern clades in

Japan.

As far as we know, no significant vicariant events are known in western Japan around Middle to Late Miocene, while the Chugoku Mountains were formed by wide-ranging crustal uplift during the Late Miocene and the Pliocene. However, these ages experienced climatically very important changes. It is thought that large areas of the continents experienced drying, enhanced seasonality, and a restructuring of terrestrial plant and animal communities during the Late Miocene, and cooling in this epoch culminated with ocean temperatures dipping to near-modern values between about 7 and 5.4 MYBP (Herbert et al., 2016).

Unfortunately, we have no direct evidence of the effect of these climate changes upon cladogenesis of East Asian *Hynobius* salamanders, but if indeed aridity in terrestrial ecosystem increased, populations would tend to be confined to wet habitats, being isolated from each other, resulting in prevention of gene exchange and enhancement of speciation.

The Highland morphotype, *H. utsunomiyaorum* sp. nov., is particularly interesting, both biogeographically and phylogenetically. *Hynobius utsunomiyaorum* sp. nov. formed a clade with *H. akiensis* sp. nov., and their divergence was estimated to be around 3.2 MYBP in the early Pliocene. Around this epoch, about 3 to 2.5 MYBP in the late Pliocene, floral change is known to have occurred, due to cooling of climates and beginning of active mountain formation around sedimentary basins.

On the other hand, *H. utsunomiyaorum* sp. nov. is genetically very close to *H. hidamontanus* with small sequence divergences (3.0–7.4%), indicating their separation roughly 1.8 MYBP in the Early Pleistocene. Thus, in the current Chugoku area, an ancestral salamander was split to give birth to lowland *H. akiensis* and montane *H. utsunomiyaorum*. This ancestral *H. utsunomiyaorum* seems to have enlarged its range towards east, eventually to the edge of ISTL, where *H. hidamontanus* currently occurs.

Hynobius hidamontanus is now distributed from eastern and western slopes of Japan Alps (Hida Mountains) and just east of Mt. Hakusan, but the populations occur widely apart. On the other hand, *H. utsunomiyaorum* sp. nov. is now known nearly continuously from Shiso-shi, Hyogo Prefecture, westwards to Yasugi-shi. Areas between the ranges of these two species are mountainous, seemingly suitable for distribution, although there are no particularly high mountains. Thus, the past continuous distribution of their ancestor would have been split by environmental changes. It is noteworthy that so many resultant species are now genetically unique from each other and occurring allopatrically or parapatrically.

Past gene flow surely happened between ancestors of *H. utsunomiyaorum* sp. nov. and of *H. akiensis* sp. nov. as shown by MIG-seq results, but *H. utsunomiyaorum* sp. nov. and *H. hidamontanus* might never have been contacted secondarily after geographic separation, probably due to changes in the areas connecting them by crustal movements. Around 1.6 MYBP, increasing global cooling forced subalpine coniferous forests to enlarge their distribution to the areas with lower elevations (Momohara, 2017). This would have induced gene flow between *H. utsunomiyaorum* sp. nov. and *H. akiensis* sp. nov. as noted above.

Despite the high degree of morphological uniformity, the salamanders long treated as *H. nebulosus* are proving to be far more diverse than originally expected. As a result, the number of species in the genus *Hynobius* increased from current 39 species (Frost, 2018) to 46 species. At the same time, the highest diversity of the genus in Japan is confirmed by the present study. This diversity surpasses recent researches where integrative taxonomic studies have demonstrated that several other wide-ranging Japanese *Hynobius* are, in fact, species complexes (e.g., Tominaga and Matsui, 2008; Nishikawa and Matsui, 2014). Because wide areas of western Honshu, especially of the Chugoku District, have been surveyed over these 30 years, new species of *Hynobius* are not likely to be discovered. However, additional field researches in unexplored regions of Shikoku and the remaining regions of western Honshu are necessary so as to catalogue and protect this unrealized biodiversity.

Key for typical specimens

1. Dorsum usually with distinct markings...... 2
   Dorsum usually without distinct markings 3
2. Male SVL 43–60 (mean=54) mm, no yellow stripes on tail, 12 costal grooves, limb overlap -1 fold.....*H. utsunomiyaorum*
3. Yellow stripes on tail.................................4
   Usually without yellow stripes on tail....... 11
4. Hindlimb usually with four digits..............5
   Hindlimb usually with five digits................6
5. Male SVL 43–58 (mean=52) mm, 12 costal grooves, limb overlap -0.5 fold......*H. iwami*
6. 12 costal grooves, limb long, overlap -0.5 fold.. 7
   13 costal grooves, limb short, overlap -1–-2 folds...8
7. Large body with male SVL 49–69 (mean=59) mm..............................*H. setoi*
8. Body small..9
   Body large...10
9. Male SVL 45–63 (mean=52) mm, limb short, overlap -1 fold................... *H. bakan*
10. Male SVL 44–67 (mean=57) mm, head narrow (RHW 16.7%), vomerine teeth series short (RVTL 3.9%)........ *H. vandenburghi*
    Male SVL 47–67 (mean=58) mm, head wide (RHW 18.9%), vomerine teeth series long (RVTL 4.6%).............. *H. nebulosus*
11. Body large..12
    Body small... 13
12. Male SVL 50–64 (mean=57) mm, 12 costal grooves, limb very long, overlap 1 fold, egg sac with striations.....*H. abuensis*
13. Male SVL 45–58 (mean=52) mm, 13 costal grooves, limb long, overlap -1 fold ....*H. setouchi*
    Male SVL 44–60 (mean=50) mm, 12 costal grooves, limb short, overlap -2 folds .... *H. akiensis*

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