

# Morphological Discrimination of Two Genetic Groups of a Japanese Salamander, *Hynobius naevius* (Amphibia, Caudata)

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**ABSTRACT**—*Hynobius naevius*, distributed on western Honshu, Shikoku, and Kyushu Islands of Japan, includes two genetically distinct groups (Groups A and B) that have never been delimited morphologically. Using specimens from the entire species range, we investigated the possibility of distinguishing these groups morphologically. Multivariate analyses of morphometric characters resulted in recognition of two groups that corresponded well to the two genetic groups. One (Group A) was characterized by larger body, compressed tail, shallower vomerine tooth series, bluish- or reddish-purple ground color, and pale-white lateral markings. In contrast, another (Group B) was characterized by smaller body, cylindrical tail, longer vomerine tooth series, reddish-brown ground color, and white lateral markings. Group A was composed of populations from the Chugoku District of Honshu and northern Kyushu, and could not be divided into subgroups, while Group B encompassed populations from the Chubu and Kinki Districts of Honshu, Shikoku and Kyushu, and was subdivided into three local subgroups that are geographically separated by marine straits. Morphometric differentiation in Group A is presumed to have been less affected by genetic factors than by other factors, such as ecological relationships with other, coexisting species. Differentiation in Group B is assumed to have been enhanced not only by genetic but also by climatological factors.

**Key words:** morphometrics, cryptic species, sympatry, distribution, systematics

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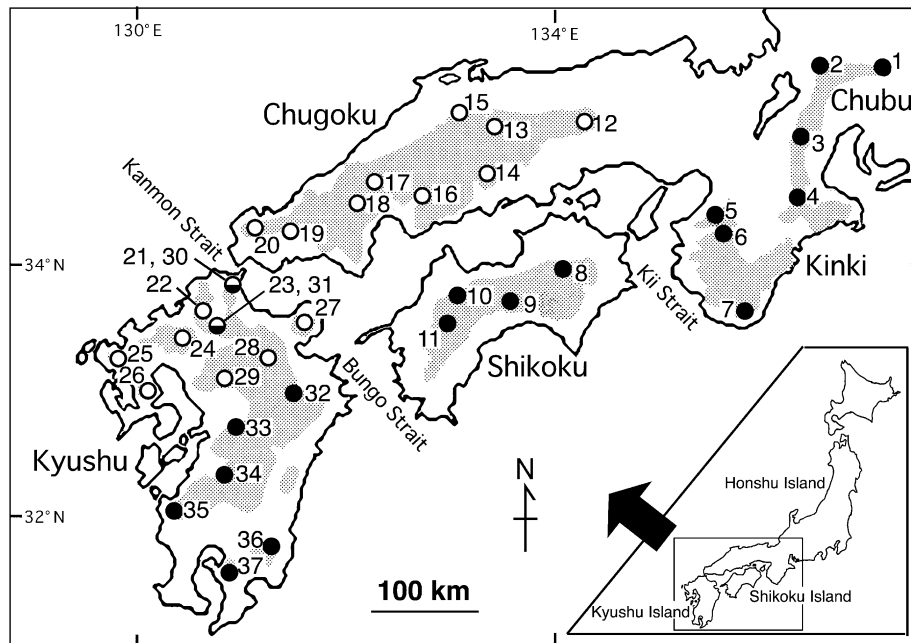
## INTRODUCTION

The family Hynobiidae, distributed in eastern Asia and considered to be a primitive group of Urodela (Duellman and Trueb, 1986), includes 44 species forming nine genera (Frost, 2004). Nearly half of these (18 species in three genera: Matsui *et al.*, 2004) occur in Japan, including 16 species of the genus *Hynobius*, all endemic to the country (Matsui, 1996a; Matsui *et al.*, 2004), and the presence of additional cryptic species has been suggested (*e.g.*, Nishikawa *et al.*, 2005). *Hynobius* is subdivided into lentic and lotic breeding types (Sato, 1943). *H. naevius* surveyed in this study is a lotic breeder, occurring widely in four discrete regions of western Japan (the Chubu-Kinki and Chugoku Districts of Honshu; Shikoku; and Kyushu: Fig. 1).

Morphological variations of this species have been studied for more than 60 years (Sato, 1943; Sato *et al.*, 1994). Sato (1943) recognized four allopatric types (the 'typical' type from Chugoku and northern Kyushu, and three local types from Kinki, Shikoku, and southern Kyushu) on the bases of the shape of the vomerine tooth series and the degree of fifth toe development. From results of a morphological study on Kyushu populations, Sato *et al.* (1994) recognized three allopatric types (northern, central, and southern Kyushu types) that differ in body size and dorsal marking of the trunk. However, the results have recently been challenged by genetic and more detailed morphological approaches (Tominaga *et al.*, 2003).

Tominaga *et al.* (2003) clarified the coexistence of two genetically and morphologically distinct types (Type A=larger type; Type B=smaller type) in four local areas of Fukuoka Prefecture in northern Kyushu. Further, these authors considered the northern and central Kyushu types

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**Fig. 1.** Map of western Japan, showing the known distributional range (hatched) and sampling localities of *H. naevius* (open circles=Group A, filled circles=Group B, half-filled circles=sympatry of two groups). For locations of the sample numbers, refer to Table 1.

recognized by Sato *et al.* (1994) to be identical, both corresponding to the larger type, and the southern Kyushu type of Sato *et al.* (1994) to correspond to the smaller type.

Recent allozyme analyses of *H. naevius* using samples from across its entire distributional range (Tominaga *et al.*, 2005) revealed that this species includes two genetically distinct groups [Group A and Group B, corresponding to Type A and Type B of Tominaga *et al.* (2003), respectively] occurring not only on northern Kyushu, but also in other regions. These groups overlap, and are possibly reproductively isolated, on northern Kyushu, as previously reported (Tominaga *et al.*, 2003).

Groups A and B of *H. naevius* should be recognized as different species from foregoing genetic studies. However, because problems remain regarding how to diagnose them for official taxonomic descriptions, studies of morphological variation and comparisons of genetic and morphological data are necessary. Actually, for reliable taxonomic identification of populations, morphological techniques usually have greater convenience in practical use than biochemical ones.

Additionally, accurate taxonomic identification of populations of *H. naevius* is pertinent to assessment of the endangered status of this species. Currently, this species is treated as geographically widespread and thus is omitted from the "Red List" within Japan (Environment Agency of Japan, 2000). However, if the taxon actually comprises more than one species, each will need appropriate reassessment by considering its more restricted range of distribution. Thus, we here elucidate the morphological characters of the two groups of *H. naevius* by comparing samples from across its entire distributional range, and discuss the

pattern of morphological differentiation in this nominal species.

## MATERIALS AND METHODS

Specimens included 457 adult males and 201 adult females collected from 1975 to 2004 from 81 localities representing the distributional range of *H. naevius* (Fig. 1; Appendix 1). Young specimens were omitted because sample size for some populations was insufficient for analyses. For convenience of comparisons among local populations, 81 collection sites were grouped into 35 sample localities. Most of the specimens used had already been investigated genetically (Tominaga *et al.*, 2003, 2005) and could be divided into two genetic groups (A and B) by several completely or nearly completely fixed allelic differences (see Tominaga *et al.*, 2005). Thus, two sympatric populations from northern Kyushu belonging to different genetic groups were treated as independent samples. In all, a total of 37 basic sample units included seven from Chubu-Kinki (all belonging to Group B genetically), four from Shikoku (Group B), nine from Chugoku (Group A), and 17 from Kyushu [nine of Group A (hereafter collectively called Kyushu-A) and eight of Group B (Kyushu-B)] (Fig. 1; Table 1).

Salamanders collected from the field were anesthetized with Chloretone saturated solution; their sex and maturity were determined by gonadal inspection; and they were fixed in 10% formalin and later preserved in 70% ethanol. We measured 25 morphometric characters and counted five meristic characters (Appendix 2) for all specimens, mainly following Tominaga *et al.* (2003). For analyses of color variations, 18 qualitative characters were selected that represented ground color and the size and color of markings on each body part, and their character states coded (Appendix 3).

For univariate analyses, snout-vent length (SVL) representing the body size, ratios (R) to SVL of the other morphometric characters, and meristic character values were employed. We chose these variables both for ease of comparison with reported taxonomic studies and for the convenience of individual identification. Preliminary investigation of sexual differentiation using Sample 31 (Amagi-B)

**Table 1.** Number, name, genotype, and size of samples used for the morphological survey, and latitude, longitude, altitude, mean annual temperature, and annual precipitation of sample localities. Sample numbers correspond to those in Fig. 1.

Sample number	Sample name	Genetic type	Sample size		Latitude (°N)	Longitude (°E)	Altitude (m)	Temperature (°C)	Precipitation (mm)
			Male	Femal					
1	Maze	B	12	8	35°48'	137°11'	440	11.1	1886
2	Fujihashi	B	20	15	35°41'	136°32'	405	10.5	2593
3	Tsuchiyama	B	8	4	34°59'	136°22'	500	10.8	1585
4	Misugi	B	6	6	34°21'	136°09'	675	10.8	1703
5	Izumi	B	7	9	34°24'	135°32'	475	11.8	1317
6	Kudoyama	B	11	–	34°15'	135°40'	405	12.4	1669
7	Kozagawa	B	7	5	33°39'	135°34'	200	14.6	2427
8	Higashiyayama	B	40	16	33°51'	134°06'	1800	3.7	2701
9	Tosayama	B	15	4	33°40'	133°31'	850	10.5	2468
10	Saijyo	B	11	10	33°47'	133°07'	1425	6.5	2303
11	Oda	B	3	6	33°30'	132°56'	800	10.3	1813
12	Katsuta	A	6	–	35°11'	134°16'	450	11.4	1617
13	Nichinan	A	14	–	34°58'	133°08'	575	10.3	1702
14	Yuki	A	9	–	34°43'	133°21'	510	11.0	1426
15	Daito	A	8	–	35°19'	133°03'	430	11.7	1757
16	Fukutomi	A	4	–	34°33'	132°43'	600	10.4	1381
17	Geihoku	A	10	–	34°41'	132°12'	820	8.3	2082
18	Yoshiwa	A	22	–	34°30'	132°05'	880	9.1	2131
19	Asahi	A	6	3	34°14'	131°27'	430	12.4	1883
20	Toyota	A	16	7	34°11'	131°02'	660	10.9	1826
21	Kitakyushu-A	A	24	6	33°49'	130°48'	345	13.6	1786
22	Wakamiya	A	7	–	33°37'	130°35'	395	12.8	1748
23	Amagi-A	A	13	8	33°29'	130°43'	630	11.4	1912
24	Fukuoka	A	16	8	33°26'	130°22'	730	11.5	1948
25	Sechiwara	A	6	4	33°14'	129°49'	680	12.6	1950
26	Tara	A	5	7	32°59'	130°06'	660	11.7	2371
27	Aki	A	10	–	33°35'	131°36'	440	12.5	1532
28	Kokonoe	A	18	4	33°09'	131°16'	930	9.6	1852
29	Kikka	A	10	4	33°05'	130°49'	460	12.6	2239
30	Kitakyushu-B	B	20	14	33°49'	130°48'	345	13.6	1786
31	Amagi-B	B	19	17	33°29'	130°43'	630	11.4	1912
32	Ume	B	11	10	32°51'	131°33'	520	12.1	2247
33	Yabe	B	21	3	32°42'	130°55'	720	11.4	2172
34	Itsuki	B	12	4	32°22'	130°50'	650	11.8	2513
35	Miyanojyo	B	10	8	31°59'	130°22'	870	10.9	2642
36	Tano	B	13	7	31°46'	131°16'	1010	10.9	2648
37	Kanoya	B	6	4	31°29'	130°49'	1100	10.6	2569

revealed sexual dimorphisms in SVL, in most ratios of the head, limbs, and tail, and in meristic characters (Student's *t* test or Mann-Whitney *U* test, see Results). Therefore, sexes were separated for subsequent comparisons among samples. However, sexes were combined for comparisons of coloration among samples, because no sexual dimorphism was detected for any of the 18 color characters when continuous character states were combined and their fre-

quencies compared by Fisher's exact test (Sokal and Rohlf, 1995).

Among samples, SVLs were compared by the Tukey-Kramer test, and character ratios by Dunn's multiple comparisons (Sokal and Rohlf, 1995). In the latter comparisons, the ratio of tail length to SVL (RTAL) was omitted for specimens with damaged or regenerated tails. Further, ratios hitherto reported to be taxonomically important [vomeric tooth series width to length (VTW/VTL), fifth to

third toe length (5TL/3TL), and width to height of the tail in its middle region (MTAW/MTAH)] were also compared among samples. All comparisons were made using  $P < 0.05$  as significant.

For multivariate analyses, log-transformed values rather than ratios were used as input variables. Because the results of simple multivariate analyses are sometimes claimed to be strongly affected by absolute body size (Carr, 1996; Adams and Beachy, 2001), we adopted multiple-group principal component analysis (MGPCA: *e.g.*

Thorpe and Leamy, 1983; Overton *et al.*, 1997). Although MGPCA is considered to require more stringent conditions than other relevant analyses (Krystufek and Macholan, 1998), it is considered to be appropriate for subsequent canonical discriminant analysis (CANDISC), because requirements for MGPCA and CANDISC are the same (Thorpe, 1988). MGPCA was conducted based on all 24 morphometric characters, except for tail length (TAL), which included missing values. "Size out" CANDISC, in which the MGPCA

**Table 2.** Mean of SVL (in mm) and medians of percentage ratios of 24 morphometric characters to SVL, and additional three ratios in males. For character abbreviations, refer to Appendix 2, and for locations of the sample numbers, refer to Table 1.

Sample	SVL	RHL	RHW	RLJL	RSL	RIND	RIOD	RUEW	RUEL	RAGD	RTRL	RTAL	RBTAW	RMTAW	RBTAH
1	56.1	24.5	18.3	15.1	6.9	5.6	5.9	3.3	5.7	51.6	75.5	69.3	10.3	7.9	9.5
2	58.3	23.7	18.2	14.9	6.9	5.5	5.7	3.1	5.7	52.1	76.3	67.0	11.4	8.0	10.3
3	58.4	23.9	18.0	15.0	6.8	5.4	5.8	3.3	5.6	52.7	76.2	63.3	10.8	8.1	9.3
4	56.9	23.6	17.8	14.5	6.7	5.5	5.7	3.2	5.8	54.3	76.4	64.8	11.1	7.8	9.2
5	57.1	24.0	18.0	15.0	6.6	5.5	6.0	3.3	5.8	53.2	76.0	64.6	10.6	7.9	10.1
6	59.8	23.1	17.3	14.2	6.6	5.3	5.6	3.1	5.5	53.6	76.9	68.4	10.6	7.5	9.4
7	56.7	23.5	17.5	14.1	6.7	5.0	5.5	3.3	5.6	53.8	76.5	66.1	10.2	7.8	9.4
8	61.8	23.3	17.1	14.2	6.9	5.5	5.5	3.3	5.7	52.9	76.7	71.4	10.5	7.5	10.1
9	59.8	23.2	17.1	14.6	6.7	5.5	5.8	3.3	5.7	52.2	76.9	67.5	10.5	7.2	9.8
10	62.1	23.6	17.1	14.4	6.8	5.4	5.5	3.3	5.6	52.0	76.4	77.1	10.8	8.6	10.9
11	59.2	23.9	16.9	14.7	6.8	5.2	5.7	3.2	5.7	52.9	76.1	70.7	11.1	7.9	9.6
12	75.3	22.9	17.5	14.4	6.8	5.7	5.7	3.1	5.4	52.2	77.2	80.7	10.5	7.9	10.2
13	76.7	22.5	18.0	14.5	6.7	5.5	5.6	3.2	5.4	54.1	77.5	74.1	11.7	7.8	10.9
14	73.0	23.5	17.5	15.0	7.1	5.8	5.8	3.2	5.6	51.6	76.5	69.9	11.2	7.3	11.1
15	75.1	22.9	17.8	14.5	6.8	5.7	5.5	3.3	5.5	53.4	77.2	73.1	10.7	7.7	9.7
16	67.8	23.8	17.8	15.0	7.0	5.5	5.8	3.2	5.5	53.2	76.2	73.5	10.6	8.0	9.7
17	66.6	24.0	18.3	14.7	7.1	6.0	5.7	3.5	5.8	51.1	76.0	78.8	12.1	8.4	10.9
18	66.7	23.7	17.5	14.6	7.1	5.8	5.5	3.4	5.9	51.6	76.3	78.0	11.6	8.2	11.2
19	63.9	23.3	18.3	15.1	7.2	5.8	5.7	3.4	5.9	51.4	76.7	74.3	11.6	7.6	10.6
20	67.4	23.4	18.2	15.0	7.1	5.7	5.5	3.6	5.8	53.4	76.6	70.9	11.1	7.9	10.1
21	73.1	23.1	18.3	14.8	6.8	5.7	5.7	3.2	5.6	53.2	76.9	73.2	11.8	8.1	10.5
22	73.9	23.8	17.7	14.4	6.6	5.4	5.7	3.0	5.6	52.5	76.2	73.4	12.3	8.8	11.2
23	73.8	23.1	17.9	14.5	6.6	5.9	5.5	3.3	5.7	53.4	76.9	72.3	11.1	6.9	9.7
24	73.5	23.0	17.5	14.2	6.6	5.3	5.7	3.1	5.4	53.1	77.0	69.9	11.4	7.8	9.6
25	69.5	23.6	18.0	14.6	6.4	5.5	5.6	3.1	5.7	53.5	76.4	72.0	10.8	7.4	9.6
26	75.1	22.9	17.4	14.0	6.8	5.8	5.5	3.2	5.4	52.7	77.1	74.5	11.3	6.3	9.9
27	76.7	22.4	17.6	14.4	6.8	5.8	5.6	3.3	5.4	53.7	77.6	76.3	11.3	8.1	10.2
28	75.3	22.9	17.7	14.4	6.7	5.9	5.5	3.2	5.6	52.8	77.1	75.8	10.9	8.1	10.0
29	73.0	22.8	18.1	14.6	6.8	5.5	5.4	3.3	5.6	53.6	77.2	76.5	11.1	7.3	10.1
30	58.4	23.3	17.8	14.6	6.5	5.4	5.8	3.4	5.8	53.2	76.7	59.8	10.7	6.9	9.8
31	60.2	23.3	17.1	14.3	6.3	5.2	5.8	3.2	5.7	53.2	76.7	62.5	10.0	7.0	9.0
32	58.1	23.9	17.2	14.1	6.6	5.2	5.7	3.2	5.9	52.4	76.1	63.7	11.1	7.5	10.0
33	59.6	23.9	17.1	14.2	6.5	5.4	5.8	3.2	5.8	52.4	76.1	63.9	11.2	7.1	10.6
34	60.2	23.7	16.9	14.4	6.6	5.3	5.8	3.2	5.5	53.0	76.3	69.3	10.1	7.3	9.0
35	60.7	23.4	17.5	14.3	6.6	5.5	5.8	3.3	5.6	53.6	76.6	65.2	10.2	7.7	9.2
36	64.5	23.0	16.8	13.9	6.6	5.4	5.5	3.3	5.7	53.1	77.0	66.3	10.1	7.8	9.5
37	59.3	23.5	17.4	14.5	6.7	5.5	5.9	3.3	5.5	54.7	76.5	68.7	10.3	7.8	9.3

To be continued.

size vector was excluded, was run. Using Mahalanobis distances thus obtained, two dimensional scaling analysis (2DS: SAS, 1990; Babik and Rafinski, 2000) was employed to visualize the overall pattern of morphometric variation among samples. To assess the relationship between genetic and morphometric differentiation, we examined correlations of Mahalanobis distances to Nei's (1978) genetic distances (Tominaga *et al.*, 2005) among samples.

Relationships between geographic (latitude, longitude, and alti-

tude) and climatological (mean annual temperature and annual precipitation) parameters of sampling sites (X: Table 1) and values of morphometric and meristic characters (Y) were surveyed by regressing Y linearly with X. Mean SVL and medians of morphometric variables in males, and averages of geographic and climatological parameters for collecting sites, were calculated for each sample. For climatological parameters, values observed by the Japan Meteorological Agency (URL: <http://www.data.kishou.go.jp>) were

**Table 2.** continued.

Sample	RMXTAH	RMTAH	RFL	RHLL	R2FL	R3FL	R3TL	R5TL	RVTW	RVTL	VTL/VTW	5TL/3TL	MTAW/MTAH
1	10.2	10.1	25.0	30.5	3.9	3.7	6.8	1.2	5.0	5.0	100.9	16.2	77.4
2	10.7	9.8	23.5	29.3	3.7	3.7	6.4	1.2	5.1	5.1	99.3	18.3	80.9
3	9.8	9.5	23.9	29.1	3.8	3.4	6.1	1.0	5.5	5.3	102.6	14.6	80.4
4	10.5	10.5	23.0	29.0	3.6	3.2	5.9	1.4	4.9	5.0	96.6	23.1	79.2
5	10.5	10.0	23.9	29.2	3.9	3.9	6.3	1.5	5.1	5.1	96.8	24.2	78.9
6	9.5	9.0	23.0	28.7	3.6	3.6	6.1	1.3	4.9	4.8	104.6	22.2	80.4
7	10.0	9.6	23.9	29.2	4.1	3.9	6.2	1.8	5.0	4.9	99.4	27.0	81.3
8	10.4	9.8	23.2	29.1	4.0	3.9	6.4	1.0	4.8	4.5	105.2	15.4	78.3
9	9.8	9.2	24.3	29.7	4.3	4.1	7.0	2.2	5.2	4.6	111.9	33.3	82.7
10	10.6	10.1	24.0	29.6	4.2	3.9	6.6	1.3	5.1	4.3	116.0	18.6	85.9
11	9.8	9.1	23.0	29.2	3.8	3.8	6.6	0.7	4.9	4.1	120.6	10.5	89.7
12	11.2	11.1	24.3	31.7	4.3	4.2	7.5	2.4	5.2	3.4	158.5	31.2	70.9
13	11.9	11.7	24.1	30.6	4.5	3.8	7.2	2.1	5.1	3.9	133.9	29.1	68.7
14	12.0	11.4	23.1	29.6	4.4	4.1	7.4	2.6	5.3	3.7	148.0	36.5	67.9
15	11.1	11.0	24.7	30.2	4.5	4.1	7.4	2.5	5.1	3.8	138.9	32.1	67.5
16	11.2	11.1	24.7	29.8	4.5	3.9	6.8	2.0	5.3	3.6	150.6	28.6	73.2
17	12.0	11.6	24.4	30.2	4.2	3.9	6.9	1.9	5.0	3.5	142.5	25.8	75.0
18	11.4	11.0	24.6	30.5	4.5	4.2	7.0	2.1	5.1	3.4	147.0	29.7	75.8
19	10.9	10.7	24.8	30.8	4.4	4.1	7.1	2.0	5.3	3.8	140.6	28.0	71.1
20	11.4	10.7	25.1	31.1	4.5	4.1	6.9	2.1	5.3	3.5	150.0	31.5	70.6
21	11.5	11.2	23.4	29.2	4.4	4.0	6.8	2.9	5.5	4.5	132.4	41.4	71.1
22	13.0	12.2	22.7	28.4	4.3	3.8	6.5	2.3	5.4	3.8	138.4	32.7	71.8
23	10.7	10.3	25.1	30.8	4.3	3.8	6.8	2.4	5.3	4.2	132.2	33.3	68.2
24	11.1	10.8	22.8	28.9	4.1	3.8	6.4	2.1	4.9	4.2	112.3	33.7	70.1
25	9.9	9.6	24.4	29.8	4.2	3.5	6.8	2.4	5.0	4.4	112.5	35.0	72.2
26	11.5	11.5	25.0	29.6	4.7	4.6	7.0	2.7	5.5	4.6	117.7	37.9	54.4
27	10.9	10.7	24.4	30.5	4.6	4.4	7.2	2.5	5.0	3.7	125.1	34.9	71.1
28	12.1	11.4	25.0	30.6	4.7	4.6	7.4	2.6	5.1	3.8	136.3	35.1	70.3
29	12.0	11.6	25.2	30.8	4.5	4.2	7.3	2.5	5.0	4.1	123.4	35.6	64.1
30	10.0	9.3	23.4	29.2	3.8	3.6	6.7	1.3	5.0	5.4	94.0	18.8	78.5
31	9.5	9.0	23.8	30.0	3.8	3.7	7.2	0.8	5.0	5.3	96.6	12.2	79.6
32	9.8	9.3	23.6	29.8	3.7	3.7	6.9	2.0	5.2	5.7	90.7	28.6	80.0
33	11.1	9.7	23.6	29.2	4.0	3.5	7.0	1.7	4.9	5.6	89.6	24.4	74.5
34	9.5	9.4	23.7	29.8	4.1	4.0	7.0	1.9	4.9	5.3	93.7	27.9	75.3
35	9.8	9.6	24.0	29.9	3.9	3.9	6.9	2.1	5.0	5.7	87.9	27.9	81.0
36	9.8	9.5	23.7	29.2	3.8	3.8	6.8	1.5	5.1	5.8	89.2	22.2	83.3
37	9.9	9.8	24.6	30.9	4.3	3.9	7.3	1.0	4.8	5.3	90.0	13.4	81.6

used. If there was no observatory at a given sampling site, averages of data from the three observatories nearest to the site were used. If the altitude differed between the sampling site and the observatory, temperature was corrected by  $-0.65^{\circ}\text{C}/100\text{m}$ , but no correction was made for precipitation.

Finally, in order to assess color variation among samples, principal component analysis (PCA) was conducted using arcsine-corrected frequencies of character states. All multivariate analyses were performed through the facilities of the Academic Center for Computing and Media Studies, Kyoto University. We used a program package designed and implemented by SAS (1990).

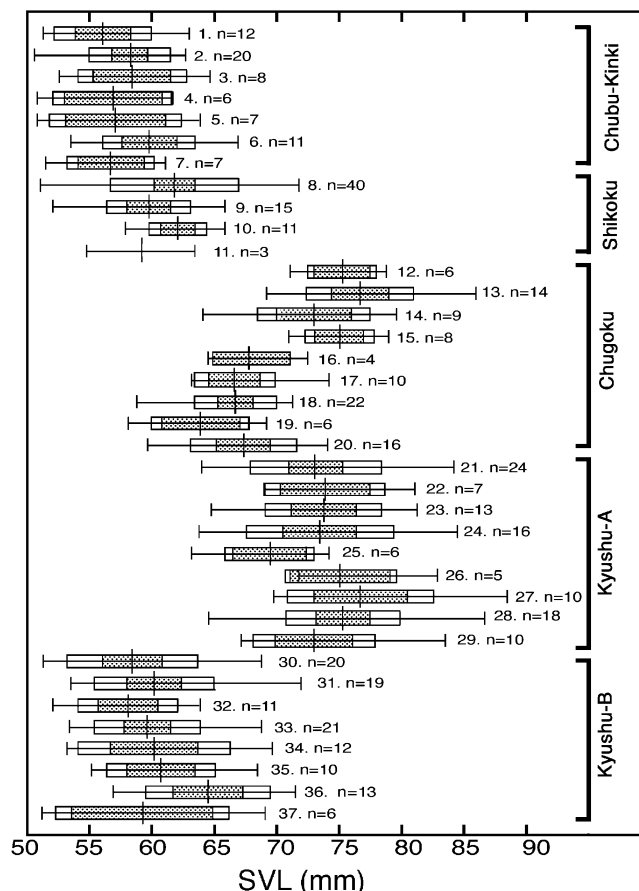
## RESULTS

### Morphometric variation

**Sexual dimorphisms:** In Sample 31, males were characterized by relatively larger features related to the head [ratios of head length to SVL (RHL), head width to SVL (RHW), lower jaw length to SVL (RLJL), snout length to SVL (RSL), internarial distance to SVL (RIND), interorbital distance to SVL (RIOD), and vomerine tooth series width to SVL (RVTW): Mann-Whitney  $U$  test,  $P < 0.05$ ], and by relatively longer limbs and tail [ratios of forelimb length to SVL (RFLL), hindlimb length to SVL (RHLL), and RTAL]. Females had larger body size (SVL: Student's  $t$  test,  $P < 0.05$ ), a relatively longer trunk [ratios of axilla-groin distance to SVL (RAGD) and trunk length to SVL (RTRL), and number of costal folds between adpressed limbs (LO)], and more numerous upper (UJTN) and lower (LJTN) jaw teeth.

**SVL:** Group A included large individuals [sample mean  $\geq 63.9$  mm in males (Fig. 2 and Table 2) and  $\geq 68.9$  mm in females (Data not shown)], whereas Group B was dominated by smaller individuals ( $\leq 64.5$  mm and  $\leq 69.2$  mm). In both sexes, Group A had significantly larger SVL (mean  $\pm 1\text{SD} = 72.1 \pm 5.7$  mm in males and  $76.7 \pm 6.9$  mm in females) than Group B ( $59.8 \pm 4.9$  mm and  $61.7 \pm 5.3$  mm). Individuals in samples 21 and 22 of Kyushu-A were larger than those in sympatric Kyushu-B samples (30 and 31). Group A from Chugoku could be divided into eastern and western samples by SVL. The eastern samples (12–15) included large individuals (male sample means  $\geq 73.0$  mm), while the western samples (16–20) were intermediate in SVL ( $\leq 67.8$  mm) between the eastern samples and Group B (Fig. 2).

**Character ratios:** The most variable character was ratio of the vomerine tooth series width to SVL (RVTL) in both sexes. Ratio of fifth toe length to SVL (R5TL) and RTAL in both sexes, ratio of second finger length to SVL (R2FL) in males, and RLJL in females also varied greatly among samples. Chubu-Kinki and Kyushu-B specimens had long vomerine tooth series (larger RVTL) compared to the others, of which Chugoku (Group A) specimens had particularly short series in both sexes. Group A specimens and Shikoku specimens (Group B) had the tail relatively longer than Chubu-Kinki (Group B) and Kyushu-B specimens in both sexes. Males of Group A had the second finger and the fifth toe relatively longer than in Group B, and this tendency



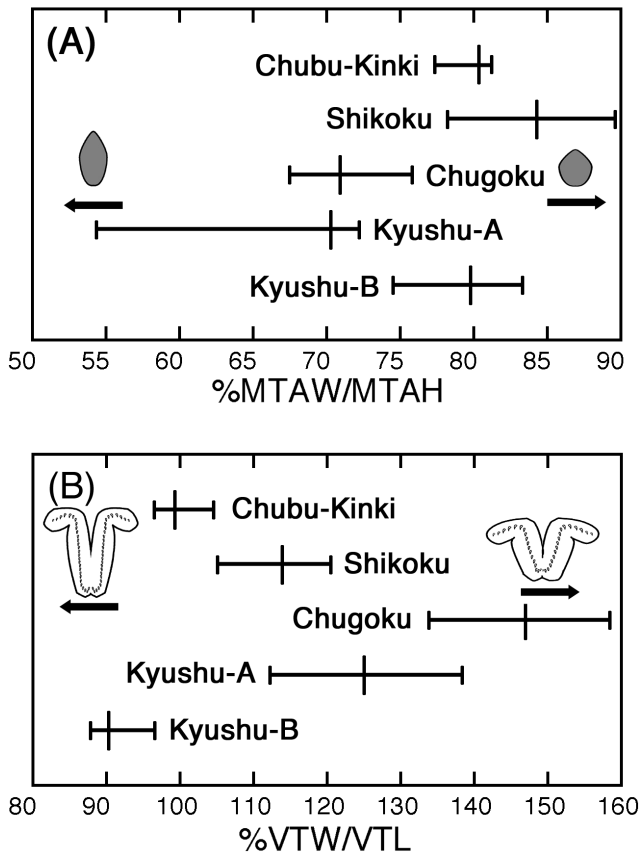
**Fig. 2.** Male SVL variation (range, mean  $\pm 1\text{SD}$  and  $2\text{SE}$ ) among 37 samples. For locations of the sample numbers, refer to Table 1.

also held for the fifth toe in females. Chubu-Kinki females had the lower jaw relatively longer than Kyushu-B females.

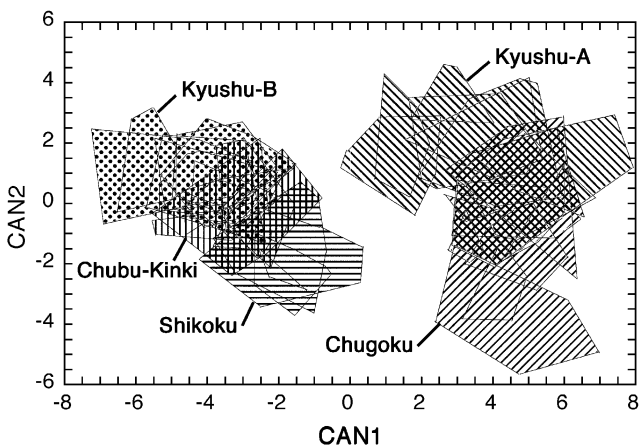
All three characters previously noted to vary geographically actually did show high geographic variation, but did the range of variation not overlap between the two groups only for MTAW/MTAH. The vomerine tooth series from Chugoku was shallow, whereas that from Chubu-Kinki and Kyushu-B was very deep, with Kyushu-A and Shikoku in-between (Fig. 3B). Group A and a part of Shikoku and Kyushu-B had well developed fifth toes (large  $5\text{TL}/3\text{TL}$ ). The tail in its middle region was more compressed in Group A than in Group B, and Sample 26 (Group A from northwestern Kyushu) had a remarkably compressed tail (*i.e.*, small MTAW/MTAH; Fig. 3A).

**MGPCA:** Because the result of MGPCA indicated the first eigenvector to represent size, where each coefficient had a similar sign and magnitude, this vector was omitted to conduct the “size out” CANDISC (Fig. 4). The eigenvalues of the first three axes of “size out” CANDISC, respectively, accounted for 14.8 (proportion: 0.63), 2.3 (0.10) and 1.15 (0.05) in males, and 18.4 (0.61), 3.6 (0.12) and 2.0 (0.07) in females [WILKS’ LAMBDA value = 0.00015 for males and 0.00003 for females ( $P < 0.0001$ )].

For males, the two groups were completely separated



**Fig. 3.** Range and median of sample medians in two diagnostic ratios (A: shape of tail section [MTAW/MTAH]; B: shape of vomerine tooth series [VTW/VTL]) in males grouped geographically and genotypically.

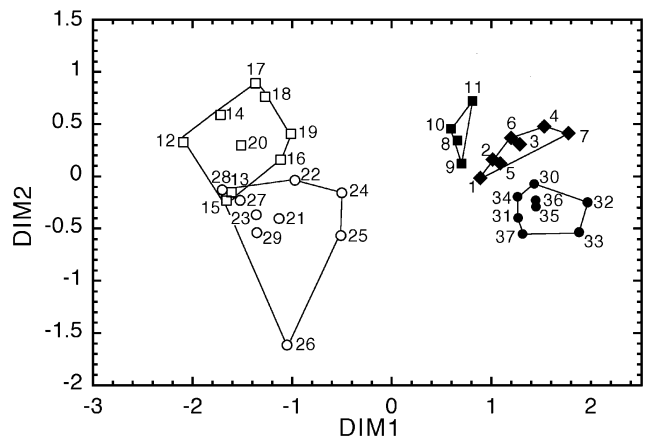


**Fig. 4.** Ranges occupied by 37 male samples on the first against second canonical axes based on scores from MGPC analysis.

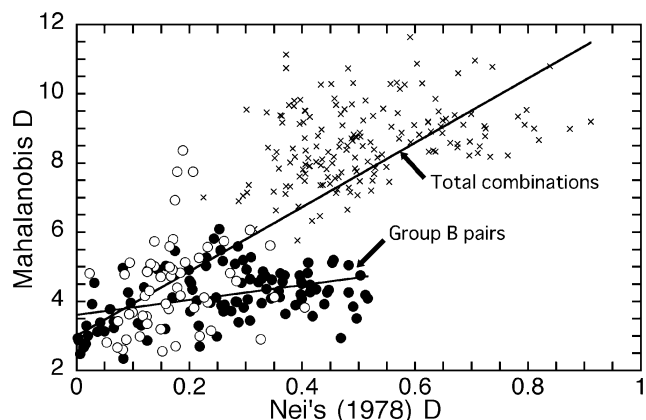
by the first and second axes (Fig. 4). Within Group A, Chugoku and Kyushu-A widely overlapped in ranges along the first two axes and could not be further divided. Similarly, within Group B, the three regional subgroups were not separated from each other along these axes. The same patterns also held for females.

**2DS:** The 2DS analysis for males reached convergences after 14 iterations with a Kruskal stress value of 0.078 and with uncorrected distance correlations of 1.00. Groups A and B were clearly split as in CANDISC (Fig. 5). Within Group A, variation ranges of Kyushu-A and Chugoku slightly overlapped, while within Group B, Chubu-Kinki, Shikoku, and Kyushu-B did not overlap and formed three regional subgroups. Of these, Shikoku and Kyushu-B were remote from each other, with Chubu-Kinki in-between. These patterns also held for females.

In summary, all but the western Chugoku samples of Group A were characterized by having the body larger than in those of Group B. Although Group A from western Chugoku could not be clearly separated from Group B in



**Fig. 5.** Clusters resolved from 2DS analysis using Mahalanobis distances in males. Open circles, open squares, filled squares, filled diamonds, and filled circles indicate scores of Kyushu-A, Chugoku, Shikoku, Chubu-Kinki, and Kyushu-B, respectively. For locations of the sample numbers, refer to Table 1.



**Fig. 6.** Correlation between Nei's (1978) unbiased distance (Gen D) and Mahalanobis distance (Morph D) observed between pairs of male samples. Regression equation in total pairs: Morph D=9.31 Gen D+3.00,  $r=0.742$ ,  $P<0.01$ ; Regression equation in Group B pairs: Morph D=2.15 Gen D+3.61,  $r=0.390$ ,  $P<0.01$ . Open squares: comparisons among Group A samples; filled circles: comparisons among Group B samples; crosses: comparisons among Group A and Group B samples combined.

body size, it differed from Group B in having a compressed tail and a shallow vomerine tooth series. These results indicated distinction of the two genetic groups (Groups A and B) by a combination of body size, tail shape, and shape of the vomerine tooth series.

**Table 3.** Medians of five meristic characters in males. For character abbreviations, refer to Appendix 2, and for locations of the sample numbers, refer to Table 1.

Sample	UJTN	LJTN	VTN	CG	LO
1	60.5	58.5	47.5	12.0	-0.5
2	61.5	59.5	48.0	13.0	-2.0
3	62.5	64.0	50.5	12.5	-1.8
4	57.5	56.0	48.0	13.0	-2.3
5	61.0	61.0	44.0	13.0	-1.5
6	62.0	60.0	48.0	13.0	-2.0
7	64.0	64.0	50.0	12.0	-2.0
8	55.0	52.0	38.0	13.0	-2.0
9	58.0	58.0	42.0	13.0	-1.5
10	58.0	60.0	44.0	13.0	-1.5
11	60.0	61.0	41.0	13.0	-2.0
12	67.0	65.0	36.5	13.0	-1.0
13	60.5	62.0	39.5	13.0	-1.5
14	71.0	72.0	44.0	13.0	-1.5
15	58.5	57.0	38.0	13.0	-1.0
16	73.5	70.5	40.0	13.0	-1.0
17	59.5	59.5	35.5	13.0	-1.0
18	65.0	65.0	36.0	13.0	-1.0
19	68.5	70.0	41.5	13.0	-1.0
20	73.0	73.0	37.5	13.0	-1.3
21	76.5	74.0	47.0	13.0	-2.0
22	68.0	69.0	43.0	13.0	-2.5
23	76.0	78.0	52.0	13.0	-1.0
24	72.0	73.0	50.0	13.0	-2.0
25	71.0	75.5	50.0	13.0	-1.8
26	74.0	76.0	55.0	13.0	-1.0
27	76.5	73.5	44.0	13.0	-1.5
28	76.0	75.5	46.0	13.0	-1.3
29	76.5	75.0	46.5	13.0	-1.5
30	61.5	62.5	52.5	13.0	-2.0
31	60.0	62.0	47.0	13.0	-2.0
32	64.0	65.0	52.0	13.0	-2.0
33	64.0	63.0	48.0	13.0	-2.5
34	62.0	61.0	49.0	13.0	-1.5
35	65.0	65.0	56.0	13.0	-2.0
36	68.0	67.0	61.0	13.0	-2.0
37	67.0	67.5	55.5	13.0	-2.0

### Relationships between morphometric and genetic variations

Mahalanobis distances positively correlated to Nei's (1978) genetic distances when Groups A and B were combined (Fig. 6;  $r=742$ ,  $P<0.0001$ ), but when each group was separately analyzed, a positive correlation was obtained

**Table 4.** Correlation between environmental parameters and SVL, ratios of morphometric characters to SVL, and meristic characters as expressed by linear regression correlation coefficients in two genetically distinct groups of *H. naevius*. Only characters with at least one significant correlation are shown.

		Latitude	Longitude	Altitude	Temperature	Precipitation
SVL	A	-0.785	0.267	-0.006	0.804	-0.002
	B	-0.956*	-0.460*	0.004**	-0.442*	0.003*
RHW	A	0.008	-0.049	-0.000	0.031	0.000
	B	0.263**	0.121**	-0.001*	0.046	-0.000
RLJL	A	0.155	0.059	-0.000	-0.013	-0.000
	B	0.197**	0.078*	-0.000	0.002	-0.000*
RSL	A	0.127	0.065	0.000	-0.065	-0.000
	B	0.063*	0.040**	0.000	-0.033*	0.000
RTAL	A	0.828	0.868	0.002	-0.703	0.002
	B	0.069	0.256	0.006**	-1.078**	0.003
RMTAW	A	0.241	0.103	-0.000	-0.080	-0.000
	B	0.082	0.080*	0.000	-0.060	-0.000
R2FL	A	-0.026	0.006	0.000	-0.013	0.000
	B	-0.066	-0.024	0.000	-0.023	0.000*
R3FL	A	-0.050	0.030	0.000	-0.027	0.000
	B	-0.067	-0.025	0.000	-0.019	0.000*
R3TL	A	0.152	0.158**	-0.000	-0.043	-0.000
	B	-0.191**	-0.123**	0.000	-0.000	0.000*
R5TL	A	-0.136	-0.038	-0.001	0.104*	0.000
	B	-0.120	-0.039	-0.000	0.054	0.000
RVTL	A	-0.325**	-0.200**	-0.000	0.142*	0.001
	B	-0.161	-0.083	-0.000	0.099*	0.000
UJTN	A	-5.933**	-2.584*	-0.002	1.914	0.000
	B	-1.525*	-0.530	-0.003	0.777*	0.001
LJTN	A	-6.693**	-3.280**	0.001	1.909	0.003
	B	-1.805**	-0.767*	-0.003	0.905*	0.001
VTN	A	-6.334**	-3.357**	-0.000	2.064*	0.008
	B	-2.271*	-0.865	-0.004	1.170*	0.003
CG	A	0.000	0.000	0.000	0.000	0.000
	B	-0.112	-0.071*	0.000	-0.041	0.000
LO	A	0.220	0.152	0.001	-0.173*	0.000
	B	0.146	0.064	-0.000	-0.012	-0.000

\*: $P<0.05$ , \*\*: $P<0.01$ .



**Table 5.** Frequency distribution of character states of each body color character analyzed. See Appendix 3 for characters and character states. For locations of the sample numbers, refer to Table 1.

Character	Sample																																							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37			
C1	0											4	5	1	3		10	1	2	17		8	10	5	8	7	9	8												
	1	3	2	1	6	5	4	4	30		6	1	2	6	2	5	2	3	2	3	12	1	1			1		1		1	7	5		2	2	5	3			
	2	16	25	8	5	11	7	7	25	15	8	8	3	6			2	7	10	4	9	12	2	13	12	5	3	3	8	6	18	28	8	9	12	16	14	7		
	3	1	8	3	1			1	1	4	7																				15	1	2	4	2	1	1			
C2	0	6	1	1				3		1		3		3			1			30	3	20	21	10	12	5	6	10												
	1	13	32	10	3	6	2	3	5	11	5	1		1	3	2	5	2	2	4		1				5	10	1	8	15	13	6	9	10	16	5				
	2	1	2		8	7	6	7	12	8	11	8	3	12	3	5	4	4	13	3	13			1			2	3	24	20	2	6	7	9	4	5				
	3			1	1	3	3	2	36		4			1	1	1	1	6	3	6											2	1		1						
C3	0							2		1		1	2							23	3	16	21	9	12	3	4	7												
	1											3	1	2	4	1	1	5	3	1	1	5	3	1	1	6	13	5												
	2	2	4	1		1				1			2	2		1	2	4	2	2		2			1			2	2											
	3	18	31	10	12	15	11	12	5	18	20	9	2	12	4	6	4	5	19	7	19							1	2	34	34	13	13	16	19	20	10			
	4			1				49																																
C4	0											4	5	6	5		2	14	4	7	29	3	20	18	10	11	8	15	14											
	1	8	8	4	9	15	8	5	53	11	10	6	2	9	3	3	3	6	6	4	16	1	1	1	1	2	1	23	25	10	3	13	14	11	4					
	2	11	26	6	3	1	3	7	3	8	11	3					1	2	2				3			2	11	11	5	10	3	4	9	6						
	3	1	1	2																																			1	
C5	0							1				2	1	2	1		1	1		6	2	5	11	6	8		4	3												
	1	18	30	9	2	5	1	3	1	5		1	2	1	2	1	1	2	1	2	1	7	1	1	1	3	4	4	4	1	2	10	4	3	1	3	3			
	2	2	5	2	7	7	7	6	14	13	11	6	2	10	5	2	2	6	13	4	14	12	12	10	1	4	6	8	6	21	23	3	5	11	13	15	5			
	3			1	3	4	3	3	40	1	10	2		2		4	1	2	7	1	8	5	3				2	1	12	11	2	4	2	5	2	2				
C6	0							1				1	2	1		1			3	1	3	11	6	8		2														
	1											4	4	3	7	4	5	7	11	26	2	15	9	3	4	10	16	14												
	2	3	4		1			1				2	1	1	1	1	1	1	4	1	2	2					5	4		3									1	
	3	17	30	11	12	15	11	12	1	18	21	9	1	8	3	3	5	15	1	8		1	1						29	32	15	12	13	19	20	9				
	4	1	1					54																															1	
C7	0											5	6	2	5		1	12	3	6	22	3	21	20	10	11	10	17	13											
	1	6	4	1	5	4	4	1	22	1	7	1	6		3	2	4	7	3	7					1			4	12	6		8	9	5	5					
	2	13	29	8	6	12	7	8	34	16	4	9		2	7		2	5	3	2	10	8	2					1	1	26	23	9	13	8	9	13	5			
	3	1	2	3	1			3		2	10																			4	1								1	2
C8	0							3				1	5	1		2	1																							
	1	18	32	11	4	9	3	7	9	8	3	3	1	2	6	1	6	1	2	4	2	1				1	3	4	2	5	7	13	2	4	5	11	1			
	2	2	3	1	8	6	8	5	43	10	18	7	2	13	2	1	3	2	17	6	18	18	1	15	14	6	11	7	11	9	25	24	2	5	11	13	8	6		
	3				1			1	1	2								3		1	10	1	6	8	4		3	3	4	5		6	1	1	1	3				
C9	0							2				1	3			1																								
	1					1	1					4	8	2	5	4	11	7	12	25	3	21	21	10	12	10	17	14		1					8	2	4			
	2	18	35	11	11	15	10	11	49	17	19	9		5	4	3	3	6	9	1	10	5		1		1			33	35	13	13	16	11	18	6				
	3	2	1	1	1			3	2	2		1	1			1	1	1											1	2										
	4							2																																

To be continued.

Table 5. continued.

Character	Sample																																					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	
C10	0											5	7	8	6		2	14	7	9	30	3	21	22	10	11	9	17	14									
	1	8	5	3	7	10	6	2	47	5	8	2	1	7		2	3	7	7	1	14						1	1		24	29	10	1	13	13	6	4	
	2	11	30	9	5	6	5	9	9	14	10	7			1	1	1	1											1	10	7	5	12	3	6	12	6	
	3	1					1				3																										2	
C11	0		1										1		1				1																			
	1	18	33	11	5	5	3	6	6	9	8		3	2	1	1	2	1		2							1	3	4	3	9	8	7	3	4	3	10	5
	2	2	2		7	9	8	6	47	10	13	8	2	13	6	6	3	7	16	8	20	15	3	19	11	10	10	7	11	8	25	26	7	9	11	14	10	4
	3				2			3				1	1	1	1	1	4		1	15		2	11		1		3	3	2	1	1	1	2		1			
C12	0														1				1	1							1											
	1	1			1	1						5	11	6	8		5	16	8	16	30	3	21	22	10	12	9	18	14									
	2	18	35	11	12	15	10	12	51	19	17	9		3	2		3	5	4		6								31	36	15	13	16	18	18	10		
	3	1	1					2		3		1			1	1													3						1	2		
	4							3		1																												
C13	0		1	1		2		5	1	2		1	10					4		2	5	2	10	11	1	4	4	5	5	4	7		4	1		2		
	1	15	3	1	1	2	7	4	27	2	3	3	3	4	6	1	4		15	7	15	15	1	8	7	4	4	3	6	6	7	11	6	7	6	11	5	5
	2	5	31	10	11	12	4	8	24	16	16	6	2		3	7		10	3	1	6	10		3	4	5	4	3	7	3	23	18	9	6	6	7	15	3
C14	0						8						1	1									1	1		1												
	1	20	34	11	10	15	10	10	36	13	9	2		1	6	4	1	9	8	4	11	9	1	1	3	4	4	8	11	5	22	12	14	7	10	16	18	8
	2		1	1	2	1	1	2	11	6	12	6	4	11	2	4	3	1	10	4	11	17	2	14	11	5	8	1	5	9	12	23	1	5	6	3	2	2
	3						1					1	1	2				4		1	4		5	8		1	1		1	1								
C15	0						5						1	1	1							1					1											
	1						1						1	5	1			4	1	1	8		7	12	2	7	4	5	11		2			4	2	3		
	2	20	34	12	12	16	11	12	50	19	21	9	4	8	8	7	4	9	18	7	22	22	2	14	10	8	5	6	12	3	34	34	15	13	16	15	18	7
	3	1															1																					
C16	0		2	1		2		1	12	4	5		2	5	1			1	3			8	1	19	13	5	6	5	6	10	4	8	6		9	13	6	6
	1	17	13	8	10	9	9	6	32	7	4	4	3	9	5	8	4	3	17	7	23	22	2	1	7	5	6	5	11	4	12	20	8	12	6	3	11	3
	2	3	20	3	2	5	2	5	12	8	12	5	1		3			6	2	1			1	2				1	18	8	1	1	1	3	3	1		
C17	0	1			1		3							1				1							1	1	1											
	1	19	33	11	9	11	10	10	26	11	9	3	4	1	5	6	1	8	7	5	11	4	1	1	1		2	7	5	3	10	7	11	6	8	9	17	8
	2	2			3	4	1	2	27	8	12	3	2	13	3	2	3	2	11	3	12	21	2	14	12	8	9	2	10	10	23	28	4	6	8	8	3	2
	3		1									3						3			5		6	9	1		3	1	1	1		1				2		
C18	0	1					2							1				1							1	1	1											
	1						1					2	7	1	3			1	3	2	3	14	1	12	13	8	11	6	10	11	1	1			4		1	
	2	19	35	11	12	16	11	12	52	19	21	9	4	7	7	5	4	9	18	6	20	16	2	9	9	1		3	8	3	33	34	15	13	16	15	20	9
	3		1				1																															1

only for Group B ( $r=0.390$ ,  $P<0.0001$ ).

#### Variations in meristic characters

Although some meristic characters were geographically variable, they were not useful in discriminating the two groups. Numbers of teeth [UJTN, LJTN, and number of

vomerine tooth (VTN)] showed great among-sample variation in both sexes. Kyushu-A and some of Chugoku had larger, and Shikoku had smaller, LJTN and UJTN than the others. Kyushu-B had larger, and Chugoku and Shikoku had smaller, VTN than the others (Table 3). No clear pattern of differentiation was found in LO or number of costal grooves (CG).

### Relationships with environmental parameters

The latitudes, longitudes, and altitudes of the sampling sites had ranges of 35°48'–31°30'N, 137°12'–129°48'E, and 200–1800 m asl, respectively; mean annual temperature and annual precipitation ranges were 3.7–14.6°C and 1317–2701 mm, respectively, among sampling sites. Sites on Shikoku were higher in altitude (mean=1219 m) and lower in temperature (mean=7.8°C) than the others (means=437 m and 11.7°C for Chubu-Kinki; 595 m and 10.6°C for Chugoku; 586 m and 12.0°C for Kyushu-A; 731 m and 11.6°C for Kyushu-B). Annual precipitation for Shikoku (mean=2321 mm) and Kyushu-B (2311 mm) was higher than for the other regions (1890 mm for Chubu-Kinki, 1756 mm for Chugoku, and 1926 mm for Kyushu-A).

More morphometric and meristic characters correlated with geographic and climatological parameters in Group B than in Group A. SVL correlated negatively with latitude, longitude, and mean annual temperature, and positively with altitude and annual precipitation in Group B, but with none of these parameters in Group A (Table 4).

Only one (RVTL) of 24 ratio variables correlated negatively with latitude in Group A, while three (RHW, RLJL, and RSL) correlated positively and one [ratio of third toe length to SVL (R3TL)] negatively in Group B. These variables correlated with longitude in the same manner; in addition, R3TL in Group A and the ratio of medial tail width to SVL (RMTAW) in Group B correlated positively with longitude. No ratios correlated with altitude in Group A, but in Group B, RHW correlated negatively and RTAL positively, with altitude. RVTL and R5TL correlated positively with mean annual temperature in Group A, while RVTL correlated pos-

itively and RSL and RTAL negatively in Group B. R2FL, ratio of third finger length to SVL (R3FL), and R3TL correlated positively with annual precipitation in Group B, but none in Group A.

Tooth numbers (UJTN, LJTN, and VTN) all correlated negatively with latitude in both groups. These three characters also correlated negatively with longitude in Group A, but only LJTN correlated negatively in Group B. Additionally, CG correlated positively with longitude in Group B. No meristic characters correlated with altitude or annual precipitation, but VTN correlated positively and LO negatively with mean annual temperature in Group A, and all measures of tooth number correlated positively in Group B.

### Color variation

Male-female comparisons using Sample 31 (Kyushu-B) failed to detect sexual dimorphism in any of 18 color characters (Fisher's exact test,  $P > 0.05$ ). Analyses of color characters among samples (Table 5) revealed that high variation in the Chugoku specimens prohibited clearly distinguishing the Groups A and B by coloration, although Kyushu-A specimens were clearly distinguishable from the rest.

Kyushu-A specimens were dorsally bluish- or reddish-purple in ground color without any markings or with pale-white markings (Fig. 7D). Chugoku specimens had the dorsum varying from bluish- to reddish-purple in ground color, with discontinuous, pale- to brownish-white markings (Fig. 7C). Group B specimens, except for Sample 8 (Fig. 7B), had the dorsum reddish-purple in ground color, with discontinuous, brownish-white markings or white dots (Fig. 7A, E); in many animals from Chubu and northern Kinki, the white dots on the

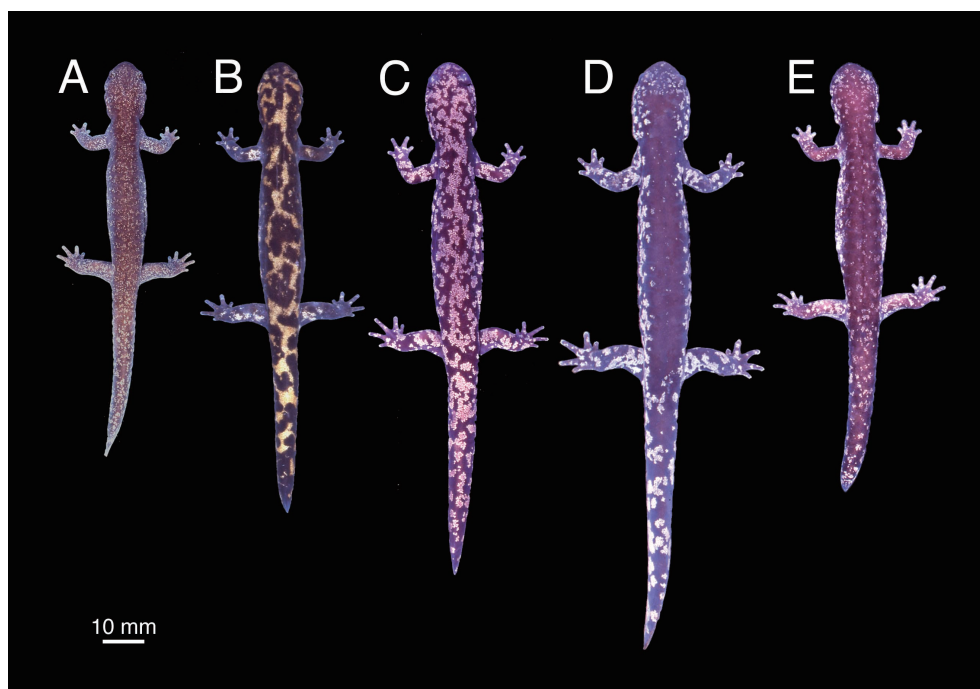


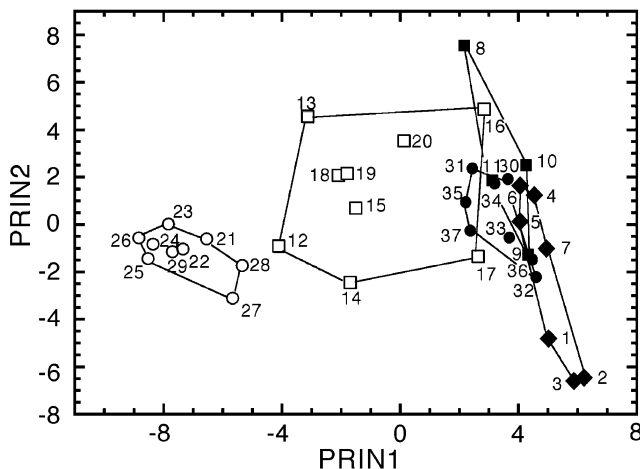
Fig. 7. Color variation in male specimens. (A): Chubu-Kinki; (B): Shikoku (Sample 8); (C): Chugoku; (D): Kyushu-A; (E): Kyushu-B.

dorsum were very small (Fig. 7A). The exceptional Sample 8 animals from Shikoku commonly had a black dorsum with continuous bright yellow markings, and differed from all the other samples (including Shikoku samples) in most color characters examined (Fisher's exact test,  $P < 0.05$ ).

Kyushu-A specimens had the flank bluish-purple in ground color, with large broken or continuous pale-white markings; the ground color of the flank in Chugoku specimens was bluish- to reddish-purple. Group B animals mostly had the flank reddish-purple in ground color, with white markings that tended to be smaller in some specimens from Chubu-Kinki and Kyushu-B than in others.

Ventrally, Group A animals were light to dark gray, or light reddish-purple, in ground color, with relatively large markings varying from pale-white to white. Ventral coloration usually differed in Group B specimens; ground color was light gray to light reddish-purple, with relatively small, white marking (Table 5). Generally, the tail was darker in ground color, with markings larger in size than on the trunk, but patterns of geographic variation were similar to those of the trunk (Table 5).

Principal component analysis based on arcsine-corrected frequencies of color character states for each sample completely separated Kyushu-A samples from others by the first axis (Fig. 8). Some Chugoku samples (12–14) were similar to Kyushu-A, but others (Samples 16–17) were similar to Group B. The three Group B subgroups overlapped along the first three axes, although Sample 8 (Shikoku) and Samples 1–3 (Chubu-Kinki) diverged from the other Group B samples.



**Fig. 8.** Plot of first against second principal component scores from PCA of color variation for 37 samples. Open circles, open squares, filled diamonds, filled squares, and filled circles indicate scores of Kyushu-A, Chugoku, Chubu-Kinki, Shikoku, and Kyushu-B, respectively. For locations of the sample numbers, refer to Table 1.

In summary, specimens of most Group A samples (83%) had pale white marking laterally on the trunk and tail, while specimens of all Group B samples (100%) had white marks. In nearly three-quarters of Group A (72%), the tail

was dorsally bluish purple in ground color and lacked marking or had pale white marks. In Group B (100%), tail ground color was black, dark blue, or reddish-purple, and the marking was brownish-white or bright yellow (in Sample 8). Finally, in half of Group A, the ground color of the flank was bluish-purple, and the marks on the ventral side of the trunk were large, whereas these characters were reddish-purple and small, respectively, in more than three-quarters of Group B (79%).

## DISCUSSION

### Two morphological groups

Two genetically distinct groups of *H. naevius* (Tominaga *et al.*, 2003, 2005) were also distinguished from each other morphologically [Group A (larger type)=Chugoku and Kyushu-A; Group B (smaller type)=Chubu-Kinki, Shikoku, and Kyushu-B]. Sato *et al.* (1994) reported specimens from northern to central Kyushu to be much larger than those from southern Kyushu. However, this result was not supported by our data, which demonstrated the presence of small specimens (Group B) in northern Kyushu; therefore, we suspect that the paucity of samples Sato *et al.* (1994) examined led them to an incorrect conclusion.

Sato (1943) reported that most of the Kinki and southern Kyushu, and some of the northern Kyushu specimens had a long vomerine tooth series. He also noted that most specimens from Kinki, Shikoku, and southern Kyushu had a short fifth toe. On the basis of these morphological variations, Sato (1943) split populations of Chugoku and northern Kyushu as the typical type from those of the Kinki, Shikoku, and southern Kyushu local types. These four types correspond well to the four groups and/or subgroups recognized in the present study (Group A, Chubu-Kinki, Shikoku, and Kyushu-B). Also the unique traits attributed to each type by Sato (1943) were confirmed to be valid, except for the degree of development of the fifth toe. This discrepancy might have resulted from the limited number of individuals surveyed by Sato (1943).

Western Chugoku samples, with a relatively small body size, were not distinct within Group A in overall morphometric characteristics. Furthermore, Group A was morphologically split not at the present geographic barrier, the Kanmon Strait between Chugoku and Kyushu, but at a region within northern Kyushu. Sample 26 from west of the boundary within Kyushu, while most divergent morphometrically in Group A, is also most divergent genetically in this group (Tominaga *et al.*, 2005), and thus might have diverged at an early stage of evolution of Group A. Correlation of Mahalanobis distances with genetic distances in Group B also suggests an association between morphometric and genetic differentiation.

In contrast, no correlation was observed between the two distances when all samples of Group A were pooled. This suggests stronger effects of environmental or ecological factors than genetic one on morphometric differentiation

within this group, as is the case in some other amphibian species (*e.g.*, Babik and Rafinski, 2000).

In Group B, geographically and genetically (Tominaga *et al.*, 2005) distant subgroups of Chubu-Kinki and Kyushu-B were similar to each other morphometrically. This situation might be a consequence of retention of ancestral character states in Shikoku, with subsequent convergence in the other two subgroups, or, alternatively, of acceleration of morphometric differentiation in Shikoku, with retention of ancestral character states in the other two subgroups.

Color differentiation seems not always to parallel genetic differentiation, as exemplified by substantial differentiation in color between Chugoku and Kyushu-A, and between Sample 8 and the remaining Shikoku samples, despite low genetic differentiation in each combination. Sample 8 from Shikoku with distinct coloration was from the sampling sites highest in altitude, and the unique color pattern might have been acquired rapidly as a yet uncharacterized adaptation to the high altitude environment.

### Effects of environmental factors on morphological variations

Morphological differentiation in amphibians is believed to be affected not only by genetic factors (Carr, 1996) but also by environmental (*e.g.*, climatological) factors (Babik and Rafinski, 2000; Lynch, 1981). Because SVL did not correlate with environmental parameters in Group A, body size variation within this group might be affected more by ecological (*e.g.*, effects of coexisting species, as mentioned below) than by physical factors. In contrast, SVL correlated with all environmental parameters examined in Group B, suggesting stronger effects of environmental factors on size variation than in Group A.

In Group B, SVL correlated positively with altitude and negatively with temperature, indicating a tendency for body size to increase in regions with lower temperatures. Similar trends have been reported for some other amphibian species (Camp and Marshall, 2000; Hairston, 1949; Kozłowska, 1971; Lynch, 1981; Ray, 1960), and have been attributed to delayed reproduction and continuous growth at lower temperatures, although counterexamples have been reported in some amphibians (Stebbins, 1949; Matsui, 1984). Whether or not the age composition in each population is related to size variation should be examined by skeletochronology (Misawa and Matsui, 1999).

Similarly to SVL, more of the other morphological characters correlated with environmental parameters in Group B than in Group A. This result suggests stronger effects of climatological and geographic factors on morphological variation in Group B than in Group A.

### Effects of coexisting species on morphological variations

Distributional ranges in Group A comprise five patterns of relationship of *H. naevius* to other, coexisting congeneric species: (1) southwestern Chugoku and northwestern Kyushu, where coexisting species are absent; (2) northeast-

ern Kyushu, where Group B coexists; (3) northwestern Chugoku, where *H. kimurae*, a relative of *H. naevius* (Nakamura and Uéno, 1963), coexists; (4) southeastern Chugoku, where *H. nebulosus* coexists; and (5) northeastern Chugoku, where *H. kimurae* and *H. nebulosus* coexist with Group A.

In eastern Chugoku, coexisting *H. nebulosus* is smaller (mean SVL=53.6 mm: Okawa and Utsunomiya, 1989) and *H. kimurae* is larger (85.3 mm: Misawa *et al.*, unpublished data) than *H. naevius* (75.2 mm), and these size differences seem to have allowed these salamanders to coexist in this region. In contrast, in western Chugoku, the presence of larger *H. kimurae* but the absence of smaller species seems to have led to smaller body size (67.0 mm) of *H. naevius*, as previously suggested by Tominaga *et al.* (2003).

Distributional ranges in Group B (mean SVL=60.5 mm) also comprise five patterns of relationship: (1) southeastern Kyushu, where coexisting species are absent; (2) Chubu and northern Kinki, where *H. kimurae* (58–71 mm in Chubu and Kinki populations: Misawa *et al.*, unpublished) coexists; (3) southern Kinki, Shikoku, and a part of central Kyushu, where *H. boulengeri* (79–107 mm: Matsui, 1996b; Nishikawa *et al.*, 2001) coexists; (4) northeastern Kyushu, where Group A of *H. naevius* (64–86 mm: this study) coexists; and (5) central to southwestern Kyushu, where *H. stejnegeri* (76–85 mm: Matsui and Misawa, 1996) coexists, with Group B. Thus, all coexisting species have larger body sizes than Group B, and similarly lay eggs in relatively wide montane streams, with their larvae living in open streams (Sato, 1943; Matsui and Misawa, 1996; Matsui, 1996b). In contrast, Group B lays eggs in very small, underground streams, where the larvae hatch and metamorphose without feeding (Tanabe, 2002). Tominaga *et al.* (2003) suggested that coexistence of the two groups of *H. naevius* in northern Kyushu may have promoted gigantism in Group A and size reduction in Group B there.

Species composition might have affected morphological differentiation especially in Group A, on which geographical and climatological factors had less effect than in Group B. Character displacement in body size between related species has been reported in various animal groups (*e.g.*, beetles: Kawano, 2002; salamanders: Jaeger *et al.*, 2002; frogs: Matsui, 1994), and is expected to have induced body size differentiation between *H. naevius* and other species in the range of sympatry. However, Group B from the ranges of allopatry and sympatry are similarly small, and thus size reduction through character displacement is not supported for this group. For further discussion of body size co-evolution, more detailed relevant information is required.

Adams and Rohlf (2000) reported an example of character displacement in head proportions between coexisting species of plethodontid salamanders. Differentiation in ratios and meristic characters, not clearly correlated with genetic or environmental factors in Group A, might be also ascribed to the effect of coexisting species in that group.

In conclusion, two groups of *H. naevius* differentiated

genetically are also divergent morphologically. Such morphological differentiation is thought to be influenced not only by genetic and abiotic environmental factors, but also by interactions with other salamander species. The present results also prompt us to consider the two groups as separate species, and surveys of type specimens of names available for the two groups are now underway.

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**Appendix 1.** Specimens used in this study. Voucher specimens are stored at the Graduate School of Human and Environmental Studies, Kyoto University (KUHE), Osaka Museum of Natural History (OMNH), Mr. Tanabe's private collection (T), Mr. Sato's private collection (ST), Mr. Okada's private collection (OK), and Ms. Sakamoto's private collection (SK).

Males: Sample 1: KUHE32250, 32252–32253, 32255–32258, 33456, 33458, 33461, 33465, 33482; Sample 2: KUHE27396–27399, 27406, 27408, 27531–27533, 27632, 27636, 28762, 28765–28766, 28775, 33361–33362, T2910, T3045–3046; Sample 3: KUHE6378, 7504, 28477, 28710, T2194, T2799, T2804, T3193; Sample 4: KUHE27007, 27011, 27732, 32509, 32511, 32527; Sample 5: KUHE5416, 33451, 34564, T2695, T2852, T2854, T2990; Sample 6: KUHE 22790–22791, 22893, 28961–28962, 29253, T3107–3108, T3112–3114; Sample 7: KUHE22793, 26096–26097, 28671, 29670–29672; Sample 8: KUHE8335, 8341, 8343–8345, 8347–8355, 8357–8360, 8362–8364, 28045, T1238–1240, T1243, T1597, T1600, T1959–1961, T1963, T2001–2002, T2093–2094, T3020, T3064, T3138–3139; Sample 9: KUHE5971–5972, 10205–10210, 18035–18040, T2709; Sample 10: T1946, T1948–1949, T1952–1954, T2170–2172, T2995, T3033; Sample 11: KUHE21712, 21785, T2689; Sample 12: KUHE30265–30267, 32639–32640, 34696; Sample 13: KUHE6495, 6952, 6954, 30268–30274, 34697–34700; Sample 14: KUHE6905–6906, 6908–6911, 6988, 6992–6993; Sample 15: KUHE18832, 32642–32645, OK1, OK3–4; Sample 16: KUHE30218–30219, 34694–34695; Sample 17: KUHE11037–11044, 11046–11047; Sample 18: KUHE11066–11067, 17812, 30276–30282, 30921–30932; Sample 19: KUHE30193, 30195–30199; Sample 20: KUHE28599–28606, 28608, 28610, 30209, 30211, 34551–34553, 34556–34557; Sample 21: KUHE12985–12987, 13607, 16746–16752, 22798, 27267, 27281, 28379–28383, 28496, 28579–28581, T2400; Sample 22: KUHE22795, 26708, 29459, OMNHAmYH131, AmYH133, AmYH149, AmYH93; Sample 23: KUHE26858, 26860, 27883, 27904, 28372, 28487–28488, 28577–28578, 28673, 28956–28957, 29364; Sample 24: KUHE26191–26194, 26196, 28958–28960, 32398–32400, 33901, 33904–33906, one male ST unnumbered; Sample 25: KUHE28583–28585, 28587, 28591–28592; Sample 26: KUHE9671–9673, 28637, one male SK unnumbered; Sample 27: KUHE14387–14390, 29986, 32578–32582; Sample 28: KUHE5705–5706, 13608–13609, 14391–14392, 26145–26147, 27561, 29983–29985, 29992, four males ST unnumbered; Sample 29: KUHE28676, 28678, 28681, 28683–28688, one male SK unnumbered; Sample 30: KUHE27129, 27162, 27268–27269, 28384, 28498–28503, 28505–28507, 28571–28573, 29958, 32585, two males ST unnumbered; Sample 31: KUHE26168, 26171, 26635–26636, 27808, 27887–27889, 27894, 27896, 27902, 27906, 28375, 28486, 28489–28493; Sample 32: KUHE14032, 22888, 25098, 27383, 28846, OMNHAm3120–3121, four males ST unnumbered; Sample 33: KUHE7502, 26072, 27302, 28007–28008, 28010–28015, OMNHAm10141, Am10143–10148, Am10150–10151, Am10155; Sample 34: KUHE34620, 34622–34623, 34626–34627, 34629, 34631, 34633, 34635, 34637–34639; Sample 35: KUHE28121–28122, 28125, 29948, 32098–32099, 32101, 32103–32104, 34640, one male KUHE unnumbered; Sample 36: KUHE 6436, 6438–6440, 28110–28112, 29955, 29957, 30214, 32079–32081; Sample 37: KUHE32088, 32091–32095.

Females: Sample 1: KUHE32251, 32254, 32260, 33457, 33459, 33462–33464; Sample 2: KUHE27407, 27571, 27637–27640, 27838, 28763–28764, 28770–28771, 28774, 28776, 28781, T3044; Sample 3: KUHE6051, 7074, 11530, T2805; Sample 4: KUHE27008–27010, 32508, 32510, T2687; Sample 5: KUHE5973, 33452, 34563, T1977, T2853, T2986–2989; Sample 7: KUHE9595, 22794, 26897, 28672, 29669; Sample 8: KUHE8336–8337, 8342, 8346, 28044, T1244, T1247, T1599, T1964, T2095, T2096, T2198, T2607, T3018–3019, T3021; Sample 9: KUHE18041, T2118, T2707–2708; Sample 10: T1436, T1947, T1950–1951, T2016, T2600–2601, T2997–2999; Sample 11: KUHE21711, 21786, T2596, T2598–2599, T2690; Sample 19: KUHE30194, 30200, OMNHAm8102; Sample 20: KUHE28600, 28607, 28609, 30207–30208, 30210, 34554; Sample 21: KUHE12984, 12988, 22799–22801, 29959; Sample 23: KUHE26857, 27282–27283, 27807, 27884, 27900, 28674, 28955; Sample 24: KUHE26136, 26195, 33898, 33900, 33907–33909, one female SK unnumbered; Sample 25: KUHE28586, 28588–28590; Sample 26: KUHE9670, 9674–9675, 28638–28639, two females SK unnumbered; Sample 28: KUHE13610, 26144, 29982, 32584; Sample 29: KUHE28677, 28679–28680, 28682; Sample 30: KUHE13605, 26706, 27127, 27161, 27911, 28201, 28378, 28385, 28497, 28501, 28504, 28508, 32586, one female ST unnumbered; Sample 31: KUHE26092, 26173, 26637, 26859, 27143, 27886, 27893, 27895, 27901, 27907, 28485, 28675, 29254–29255, 29365–29366, 34566; Sample 32: KUHE22890–22891, 24968–24969, 26093, 27381–27382, 29988, 34565, one female ST unnumbered; Sample 33: KUHE7503, 28009, OMNHAm10142; Sample 34: KUHE34568, 34621, 34628, 34632; Sample 35: KUHE27329, 28120, 28123–28124, 29949, 32100, 32102, 32105; Sample 36: KUHE6437, 18523, 19035, 28109, 29956, 30213, 30215; Sample 37: KUHE28098–28099, 32089–32090.

**Appendix 2.** Quantitative characters examined.

Morpometric characters: SVL (snout-vent length): from tip of snout to anterior tip of vent; HL (head length): from tip of snout to wrinkle of throat; HW (head width): measured at angle anterior to parotid gland; LJL (lower jaw length): from tip of lower jaw to the angle of jaw; SL (snout length): from tip of snout to anterior tip of upper eyelid; IND (internarial distance): the minimum distance between the external nares; IOD (interorbital distance): the minimum distance between the upper eyelids; UEW (upper eyelid width): the greatest width of the upper eyelid; UEL (upper eyelid length): the greatest eye diameter of the eye, including upper eyelid; AGD (axilla-groin distance): the minimum distance between axilla and groin; TRL (trunk length): from wrinkle of throat to anterior tip of vent; TAL (tail length): from anterior tip of vent to tip of tail; BTAW (basal tail width): tail width measured at root of tail; BTAH (basal tail height): tail height measured at base of tail; MTAW (medial tail width): tail width measured at middle of tail; MTAH (medial tail height): tail height measured at middle of tail; MXTAH (maximum tail height): tail height measured at highest part of tail; FLL (forelimb length): distance from axilla to tip of the longest finger; HLL (hindlimb length): distance from groin to tip of the longest toe; 2FL (second finger length): distance from base point between second and third fingers to tip of the second finger; 3FL (third finger length): distance from base point between second and third fingers to tip of the third finger; 3TL (third toe length): distance from the base point between third and fourth toes to tip of the third toe; 5TL (fifth toe length): distance from base point between fourth and fifth toes to tip of the fifth toe; VTW (vomarine tooth series width): the greatest width of vomarine tooth series; VTL (vomarine tooth series length): the greatest length of vomarine tooth series.

Meristic characters: UJTN (number of upper jaw teeth); LJTN (number of lower jaw teeth); VTN (number of vomarine teeth); CG (number of costal grooves); LO (number of costal folds between adpressed limbs).

**Appendix 3.** Color characters and their states.

C1: Dorsal ground color of trunk (0: bluish-purple; 1: black or dark blue; 2: reddish-purple; 3: reddish-brown). C2: Dorsal marking of trunk [0: absent or faintly spotted; 1: small (size=SVL/30-SVL/20); 2: large (size=SVL/20-SVL/5); 3: continuous]. C3: Color of dorsal marking of trunk (0: absent; 1: pale-white; 2: white; 3: brownish-white; 4: bright yellow). C4: Dorsal ground color of tail (0: bluish-purple; 1: black or dark blue; 2: reddish-purple; 3: reddish-brown). C5: Dorsal marking of tail [0: absent or faintly spotted; 1: small (size=SVL/30-SVL/20); 2: large (size=SVL/20-SVL/5); 3: continuous]. C6: Color of dorsal marking of tail (0: absent; 1: pale-white; 2: white; 3: brownish-white; 4: bright yellow). C7: Lateral ground color of trunk (0: bluish-purple; 1: black or dark blue; 2: reddish-purple; 3: reddish-brown). C8: Lateral marking of trunk [0: absent or faintly spotted; 1: small (size=SVL/30-SVL/20); 2: large (size=SVL/20-SVL/5); 3: continuous]. C9: Color of lateral marking of trunk (0: absent of markings 1: pale-white; 2: white; 3: brownish-white; 4: bright yellow). C10: Lateral ground color of tail (0: bluish-purple; 1: black or dark blue; 2: reddish-purple; 3: reddish-brown). C11: Lateral marking of tail [0: absent or faintly spotted; 1: small (size=SVL/30-SVL/20); 2: large (size=SVL/20-SVL/5); 3: continuous]. C12: Color of lateral marking of tail (0: absent; 1: pale-white; 2: white; 3: brownish-white; 4: bright yellow). C13: Ventral ground color of trunk (0: dark gray; 1: gray; 2: light reddish-purple). C14: Ventral marking of trunk [0: absent or faintly spotted; 1: small (size=SVL/30-SVL/20); 2: large (size=SVL/20-SVL/5); 3: continuous]. C15: Color of ventral marking of trunk (0: absent; 1: pale-white; 2: white; 3: yellowish-white). C16: Ventral ground color of tail (0: dark gray; 1: gray; 2: light reddish-purple). C17: Ventral marking of tail [0: absent or faintly spotted; 1: small (size=SVL/30-SVL/20); 2: large (size=SVL/20-SVL/5); 3: continuous]. C18: Color of ventral marking of tail (0: absent; 1: pale-white; 2: white; 3: yellowish-white).