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A New Salamander of the Genus *Onychodactylus* from Central Honshu, Japan (Amphibia, Caudata, Hynobiidae)

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Abstract: The number of species of salamanders of the genus *Onychodactylus* in Japan has increased from one to six nominal species over the past decade, by taxonomic studies based not only on morphological but also molecular phylogenetic data. However, the presence of an additional cryptic species has been suggested by a series of previous studies. Here, we describe a new species, *Onychodactylus pyrrhonotus* sp. nov., from the Kinki, Chubu and Hokuriku Districts of central Honshu, Japan, based on morphological and genetic evidence. It belongs to the *O. japonicus* species complex and is the sister species of *O. kinneburi* from Shikoku Island and westernmost part of Honshu with 5.5% sequence divergence in the mitochondrial cytochrome b gene. The range of the new species completely overlaps with that of *O. japonicus sensu stricto*, and they are syntopic in at least six known localities. As shown in a previous study, reproductive isolation between these sympatric species is substantial. The new species is morphologically most easily distinguished from congeners by coloration, a sharply defined scarlet to pinkish dorsal stripe or mottling on a black background and dusty white dots on the venter.

Key words: Central Honshu; Japan; *Onychodactylus japonicus*; *Onychodactylus pyrrhonotus* new species; Salamander; Sympatry; Taxonomy

INTRODUCTION

Genus *Onychodactylus*, referred to as clawed salamanders, is known as the basal clade of the family Hynobiidae (Zhang et al., 2006; Pyron and Wiens, 2011), and is endemic to northeast Asia. Among 10 species currently described in this genus, the *Onychodactylus*

japonicus species complex comprises six species, *O. japonicus* (Houttuyn), *O. nipponoborealis* Kuro-o, Poyarkov and Vieites, *O. tsukubaensis* Yoshikawa and Matsui, *O. kinneburi* Yoshikawa, Matsui, Tanabe and Okayama, *O. intermedius* Yoshikawa and Matsui, and *O. fuscus* Yoshikawa and Matsui, all from Honshu and Shikoku Islands of Japan. However, one cryptic species referred as Kinki group remained to be described (Yoshikawa et al., 2010b; Yoshikawa and Matsui, 2014).

The Kinki group was recognized in previous

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genetic studies based on mtDNA and allozymic variation (Yoshikawa et al., 2008, 2010a, b), and was reported to be sister group of *O. kinneburgi* from Shikoku Island and parts of the Chugoku mountains of western Honshu with substantial genetic divergence and reciprocal monophyly. The Kinki group is known to occur in the Kinki, Hokuriku and Chubu Districts of central Honshu, and is sympatric with *O. japonicus* sensu stricto (Yoshikawa et al., 2010b). Large genetic divergence from allopatric sister species, genetic distinctiveness and sympatry of a closely related congener revealed by previous studies strongly indicated distinct specific status of the Kinki group. In this paper, we describe the Kinki group from central Honshu of Japan as a new species.

MATERIALS AND METHODS

Specimens of the Kinki group examined in this study were collected in central Honshu (Fig. 1). All adult specimens used in Yoshikawa and Matsui (2014) were included and only specimens newly examined in this study are listed in section below and Appendix I. For morphological comparisons, we used adult specimens of *O. fischeri* from Russia, *O. koreanus* from South Korea, and six nominal species of *O. japonicus* species complex in Japan: *O. fuscus* (including the type series); *O. intermedius* (type series); *O. japonicus* sensu stricto (including topotypes); *O. kinneburgi* (type series); *O. nipponoborealis* (including the type series); and *O. tsukubaensis* (type series). Specimens examined in this study were fixed by 10% formalin and preserved in 70% ethanol, and are stored at the Graduate School of Human and Environmental Studies, Kyoto University (KUHE), Herpetological collection of the National Museum of Nature and Science, Tokyo (NSMT-H), Amphibian Collection of Omogo Mountain Museum (OMM-Am), Mr. Shimizu's private collection (SZ-Mie) and Mr. Tanabe's private collection (T; stored at KUHE). The 18 measurements were taken to the nearest 0.1 mm with dial calipers (Appendix II: for the definition of

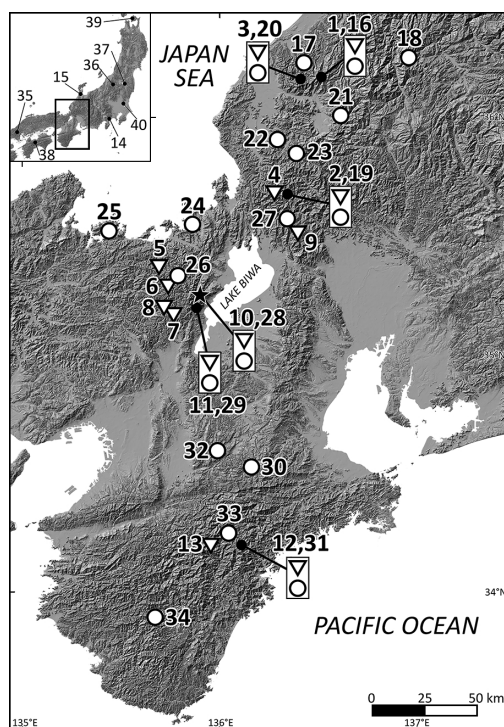


FIG. 1. Map of central Honshu showing sampling sites of *Onychodactylus pyrrhonotus* sp. nov. (inverse triangles) and *O. japonicus* (circles) and other congeners used in this study. The star indicates the type locality of the new species. For population numbers, refer to Appendix III. The map is based on the hillshade map published by the Geospatial Information Authority of Japan.

characters, refer to Yoshikawa and Matsui [2014]). We counted the number of costal grooves (CGN) separately on the right and left sides following Misawa (1989). The number of vomerine teeth (VTN) was counted separately for the right and left vomerine tooth series, and the presence or absence of a gap between the right and left series was recorded under a stereoscopic binocular microscope using a thin needle. The number of presacral vertebrae (PSVN: including the atlas) was counted from digital X-ray photographs. For larval specimens, SVL and TAL were measured. Student's t-test and Tukey-Kramer test were used for morphometric comparisons, while Mann-

Whitney's U-test and Kruskal-Wallis test were performed for ratio values. We used the significance level of $P < 0.05$ in all statistical tests. All statistical analyses were performed by R 4.1.1 (R Core Team, 2021). In the following description, average values are shown with 1 standard deviation (\pm SD).

We sequenced 1141 bp of the mitochondrial cytochrome b (cyt b) gene using primers and protocols described in Yoshikawa et al. (2008) for specimens from the Kinki, Hokuriku, and Chubu Districts of central Honshu to determine the genetic identity of specimens examined. The newly obtained cyt b sequences (LC658991–LC659003, LC663766–LC663770) and previously published sequences of *Onychodactylus* species (Zhang et al., 2006; Yoshikawa et al., 2008, 2010b, 2013; Yoshikawa and Matsui, 2014; see Appendix III) were combined, and then we conducted phylogenetic analysis to check whether each specimen belongs to Clade IV-A (Kinki group) or Clade III (*O. japonicus* sensu stricto) of Yoshikawa et al. (2008). The best substitution models for the 1st, 2nd, and 3rd codon positions were selected by ModelTest-NG (Darriba et al., 2020). Then, the maximum likelihood (ML) tree was estimated by raxmlGUI 2.0.6 (Edler et al., 2020) under the optimum model (GTR [Tavaré, 1986]+G) for each codon position. The confidence of branches in the ML tree was assessed by 1000 bootstrap pseudoreplicates (b.s.).

RESULTS

The obtained values for SVL, and ratios of the other characters relative to SVL (and partly HW), are shown in Table 1. Frequencies in three meristic characters and the presence/absence of a gap between vomerine tooth series, short sub-branch of vomerine tooth series, and marking on chest are shown in Tables 2 and 3. As the result of morphological analysis, all characters were found to be informative to discriminate candidate species from the others.

We successfully obtained sequences of the

cyt b gene. In the ML tree ($-\ln L$ 4097.876136), the *O. japonicus* species complex was monophyletic (bootstrap support=100%), comprising four major clades, Clade I (*O. nipponoborealis*), Clade II (*O. fuscus*, *O. intermedius*, and *O. tsukubaensis*), Clade III (*O. japonicus* sensu stricto), and Clade IV (*O. kinneburi* and *Onychodactylus* sp. Kinki group), although relationships among major clades were unclear (Fig. 2). *Onychodactylus* sp. Kinki group from central Honshu was most closely related to *O. kinneburi* from Shikoku Island and westernmost Honshu (mean p-distance of 5.47%), and distantly related to sympatric populations of *O. japonicus* sensu stricto (mean p-distance of 8.52%), as is reported by Yoshikawa et al. (2008, 2010b). The monophyly of each major clade was strongly supported (b.s.=99%, 98%, and 100% for Clades II, III, and IV, respectively), and the phylogenetic relationships among clades were different from those of the previous study that failed to attain statistical support (Yoshikawa et al., 2008). In topology, Clade IV diverged from the other three clades without statistical support. From the remaining three clades, Clade II, comprised of three species from northeastern Japan, diverged from Clades I and III. In the Clade IV, *O. kinneburi* and *Onychodactylus* sp. Kinki group diverged with 5.47% of mean uncorrected p-distance, and the monophyly of the latter was well supported (b.s.=100%). The observed mean p-distance between *O. kinneburi* and *Onychodactylus* sp. Kinki group was larger than the minimum values observed between the other Japanese *Onychodactylus* (e.g., 4.15% between *O. fuscus* and *O. intermedius*; Yoshikawa and Matsui, 2014). Within the *Onychodactylus* sp. Kinki group, populations from the Kii Peninsula of the southern Kinki District (Southern population; b.s.=86%) was divergent from the other populations from the northern Kinki and Hokuriku Districts (northern population; b.s.=82%) with a mean p-distance of 1.94% as reported by Yoshikawa et al. (2010b).

Based on the result of previous studies and genetic and morphological data newly

TABLE 1. Comparisons of snout-vent length (SVL: means±1 SD, followed by ranges in parenthesis, in mm), percentage ratios of each of the other character dimensions to SVL (indicated by R: medians, followed by ranges in parenthesis) and head width (indicated by HW), and mean number of vomerine teeth on right and left sides (ranges in parenthesis) in seven species of the *Onychodactylus japonicus* complex.

Species	sex	n	SVL	RTAL	RBTAL	RBTAW	RMTAH	RMTAW	RAGD	RFL	RHLL	RHL	RHW	RUEL
<i>O. pyrrhonotus</i> sp. nov.														
<i>O. japonicus</i> (central pops.)	male	33	65.7±3.2 (58.7–75.8)	120.6 (100.4–131.9)	8.4 (6.5–10.9)	9.6 (7.5–11.4)	8.3 (5.7–10.7)	6.0 (4.7–7.5)	53.3 (49.6–55.5)	27.3 (24.5–30.5)	30.4 (27.7–30.5)	22.7 (21.8–26.9)	14.5 (13.2–16.7)	6.1 (5.0–6.8)
	female	32	70.7±5.2 (58.0–79.4)	103.8 (94.6–119.7)	7.5 (4.9–9.8)	8.3 (6.4–9.7)	7.4 (4.6–9.5)	5.5 (2.6–7.0)	54.6 (51.3–58.3)	27.2 (24.9–29.9)	31.3 (28.4–33.7)	22.5 (20.2–25.4)	14.4 (12.8–16.4)	5.8 (5.3–6.7)
(Combined)	male	30	70.4±5.2 (59.0–83.3)	124.1 (100.2–151.0)	8.3 (5.0–10.9)	10.0 (7.6–11.9)	8.6 (5.0–10.0)	6.1 (3.7–8.0)	52.2 (49.4–56.6)	28.7 (24.7–30.3)	32.2 (28.7–35.0)	22.9 (20.7–24.3)	14.8 (13.3–16.7)	5.8 (5.2–6.8)
	female	34	74.6±4.7 (61.7–85.2)	110.0 (88.7–124.1)	7.8 (6.7–9.1)	8.9 (7.8–10.2)	7.8 (6.2–9.6)	5.6 (4.3–8.2)	54.3 (50.8–57.4)	27.6 (24.7–31.7)	31.0 (26.0–37.4)	22.4 (20.2–25.3)	14.6 (13.1–17.1)	5.8 (5.0–6.9)
<i>O. fuscus</i>	male	110	67.2±4.5 (58.5–83.3)	123.6 (97.4–151.0)	8.5 (5.0–11.1)	10.4 (7.6–12.9)	8.3 (5.0–10.4)	5.9 (3.7–9.2)	52.1 (48.2–56.6)	28.1 (23.6–31.8)	31.7 (27.6–37.8)	22.9 (20.7–25.4)	14.5 (12.6–16.9)	6.1 (5.2–7.2)
	female	109	70.3±5.5 (55.3–85.2)	106.8 (88.7–124.1)	7.9 (6.1–10.4)	8.9 (7.4–10.7)	7.3 (5.1–9.6)	5.3 (3.7–8.2)	54.4 (50.8–57.9)	27.9 (24.6–31.7)	31.2 (26.0–37.4)	22.6 (20.2–25.3)	14.8 (12.9–17.4)	6.1 (5.0–7.1)
<i>O. intermedius</i>	male	6	69.5±3.0 (65.0–72.7)	121.9 (111.1–131.8)	9.5 (8.8–11.3)	10.8 (9.9–12.2)	9.5 (8.6–10.2)	6.4 (3.3–6.8)	52.1 (50.3–52.8)	27.4 (26.1–28.6)	30.0 (28.2–32.6)	22.3 (21.8–23.4)	14.6 (14.2–15.2)	6.1 (5.8–6.5)
	female	5	69.0±4.8 (61.5–74.8)	99.7 (94.2–101.9)	9.3 (8.7–9.5)	9.4 (9.2–9.6)	9.2 (7.1–9.8)	6.2 (4.8–6.4)	54.7 (52.7–54.9)	27.3 (25.8–27.8)	30.9 (29.2–31.9)	22.1 (21.3–23.3)	15.5 (14.8–16.1)	6.5 (6.1–6.8)
<i>O. kinneburgi</i>	male	17	63.0±4.9 (56.0–74.3)	121.8 (102.3–136.3)	7.6 (6.4–9.5)	9.0 (8.0–10.6)	7.4 (4.9–9.7)	5.3 (4.3–7.5)	52.3 (50.5–56.3)	27.5 (24.6–29.4)	31.5 (29.3–34.4)	22.8 (20.6–24.1)	14.5 (12.9–15.4)	6.3 (5.8–7.4)
	female	10	65.5±4.1 (58.3–70.4)	101.9 (96.8–110.5)	8.0 (5.5–9.9)	9.3 (7.5–9.9)	8.5 (4.9–9.9)	5.9 (4.4–7.3)	54.5 (51.5–56.9)	28.2 (25.3–32.4)	32.1 (29.9–35.4)	22.1 (20.4–23.4)	14.8 (13.9–16.0)	6.2 (5.8–6.7)
<i>O. nipponoborealis</i>	male	36	72.2±4.3 (59.2–78.5)	117.1 (98.1–130.5)	10.1 (6.7–12.6)	10.8 (8.1–12.6)	8.6 (6.0–11.3)	6.3 (4.7–7.7)	51.9 (48.6–55.4)	27.4 (25.2–29.9)	31.2 (26.2–34.8)	22.6 (21.1–23.8)	14.7 (13.4–16.6)	6.2 (5.7–7.1)
	female	33	78.4±5.8 (64.2–88.9)	105.1 (91.1–115.1)	8.9 (6.6–10.2)	8.9 (8.1–10.9)	7.5 (6.2–9.3)	5.3 (4.5–7.2)	55.5 (52.0–57.8)	27.2 (25.2–30.1)	31.9 (29.1–35.7)	21.7 (20.4–24.0)	14.5 (13.2–16.9)	5.8 (5.2–6.5)
<i>O. tsukubaensis</i>	male	20	64.2±5.6 (56.1–76.7)	125.7 (107.3–141.2)	8.2 (6.8–10.8)	10.0 (8.5–11.5)	8.0 (5.9–9.7)	5.9 (4.4–7.2)	51.5 (47.5–53.9)	28.4 (25.3–30.5)	33.5 (29.7–36.3)	22.7 (20.0–25.3)	15.1 (12.7–16.4)	6.0 (5.1–7.0)
	female	26	64.4±5.0 (57.0–77.9)	106.7 (97.1–117.1)	8.0 (6.9–10.5)	9.0 (8.0–11.4)	7.3 (5.5–10.5)	5.4 (3.5–7.4)	53.6 (51.2–57.1)	27.2 (24.1–31.1)	32.4 (27.2–35.4)	22.4 (20.9–24.5)	15.2 (13.2–16.8)	6.0 (5.1–6.7)
<i>O. tsukubaensis</i>	male	6	69.5±3.1 (65.6–74.5)	102.3 (99.0–114.1)	8.1 (6.8–10.6)	9.1 (7.6–10.6)	7.7 (6.0–11.2)	6.0 (5.0–8.3)	51.6 (49.7–53.3)	26.6 (25.0–27.4)	31.1 (29.5–32.8)	22.9 (21.3–23.6)	15.4 (14.4–16.4)	6.2 (5.9–6.4)
	female	10	66.9±2.1 (61.9–69.3)	90.4 (82.0–95.9)	8.7 (8.2–10.1)	9.1 (8.7–10.2)	8.4 (7.2–10.9)	6.5 (4.8–7.6)	52.4 (48.6–53.6)	26.4 (24.4–28.1)	30.5 (28.2–31.9)	23.3 (20.7–24.9)	15.6 (15.1–16.3)	6.2 (5.9–6.6)

TABLE 1. (continued)

Species	sex	n	RIND	REND	RIOD	RCW	RSL	RICD	IND/HW	IOD/HW	ICD/HW	VTN (L)	VTN (R)
<i>O. pyrrhonotus</i> sp. nov.													
<i>O. japonicus</i> (central pops.)	male	33	7.7 (6.9–8.4)	3.3 (2.8–4.4)	4.8 (4.1–5.5)	12.5 (10.8–14.4)	7.5 (6.7–8.4)	9.0 (8.1–10.2)	52.2 (46.6–58.8)	32.8 (30.2–38.4)	61.9 (56.2–70.8)	15.3±4.2 (6–24)	14.6±4.6 (5–24)
	female	32	7.3 (6.5–8.8)	3.2 (2.6–3.8)	4.8 (4.0–5.5)	12.5 (10.7–13.6)	7.4 (6.6–8.8)	8.8 (7.7–9.7)	52.6 (45.6–59.1)	33.6 (27.4–40.0)	61.6 (56.0–68.2)	17.9±4.7 (8–27)	17.1±4.7 (3–26)
(Combined)	male	30	7.5 (6.1–8.9)	3.3 (2.8–4.0)	4.7 (4.0–6.2)	12.5 (11.5–14.0)	7.5 (6.6–9.3)	8.9 (7.8–10.2)	50.7 (44.5–57.1)	32.7 (29.0–38.8)	60.3 (55.1–67.7)	15.2±5.6 (5–26)	14.9±5.6 (6–25)
	female	34	7.5 (6.9–8.8)	3.4 (3.0–3.9)	4.7 (4.2–5.8)	12.8 (11.5–14.3)	7.5 (6.9–8.4)	8.7 (7.9–10.7)	50.0 (47.3–57.8)	32.4 (27.9–37.1)	59.8 (53.6–66.2)	16.3±5.2 (8–27)	16.4±4.8 (8–27)
	male	110	7.3 (6.1–9.3)	3.3 (2.6–4.0)	4.7 (3.8–6.2)	12.9 (10.8–15.0)	7.5 (6.6–9.3)	8.8 (7.7–10.2)	50.5 (44.5–57.1)	32.4 (27.2–38.8)	60.6 (51.1–68.9)	13.5±4.9 (5–26)	13.4±4.6 (6–26)
	female	109	7.2 (6.0–8.8)	3.3 (2.6–4.0)	4.8 (3.4–6.0)	13.1 (10.9–14.9)	7.5 (6.5–8.9)	8.8 (7.9–10.7)	48.9 (40.6–57.8)	32.4 (23.8–40.8)	59.6 (51.1–66.2)	14.9±4.6 (5–27)	15.3±4.4 (4–27)
<i>O. fuscus</i>													
<i>O. intermedius</i>	male	6	7.6 (7.4–7.8)	3.3 (3.2–3.4)	4.7 (4.3–5.3)	13.5 (13.1–14.3)	7.5 (7.1–8.2)	8.8 (8.3–9.6)	51.3 (50.0–53.3)	32.2 (28.7–36.2)	60.0 (55.6–65.7)	12.8±1.5 (11–15)	12.0±1.9 (9–15)
	female	5	7.5 (7.2–7.6)	3.4 (3.1–3.7)	4.6 (4.1–5.5)	13.8 (13.4–14.0)	7.8 (7.4–8.0)	8.7 (8.0–9.6)	47.5 (44.6–51.0)	30.8 (27.4–34.3)	57.1 (53.3–59.6)	14.8±3.1 (11–18)	15.2±3.3 (12–19)
<i>O. kinnebuli</i>	male	17	7.2 (6.1–7.6)	3.1 (2.7–3.9)	4.6 (3.7–5.1)	12.7 (11.7–14.6)	7.1 (6.7–8.1)	8.4 (7.9–9.3)	49.4 (45.7–52.9)	31.8 (26.6–34.7)	59.6 (57.0–62.5)	16.1±2.9 (11–22)	15.6±1.9 (12–18)
	female	10	7.2 (6.7–7.6)	3.2 (2.7–4.0)	4.9 (4.2–5.4)	13.6 (12.0–16.1)	7.3 (6.8–7.9)	8.6 (8.1–9.5)	48.3 (43.5–52.1)	32.2 (29.0–35.4)	59.3 (54.6–63.7)	16.1±4.6 (10–25)	14.9±4.1 (8–21)
<i>O. nipponoborealis</i>	male	36	7.3 (6.4–8.3)	3.1 (2.4–3.7)	4.7 (3.7–5.6)	12.3 (11.2–13.5)	7.4 (7.0–8.4)	8.8 (8.0–9.7)	49.1 (43.8–58.6)	32.4 (24.8–35.3)	60.1 (55.1–65.0)	13.6±3.3 (9–22)	13.5±3.2 (9–20)
	female	33	7.0 (6.3–7.9)	3.1 (2.5–3.6)	4.6 (3.7–5.4)	12.2 (10.6–15.2)	7.4 (6.6–8.5)	8.8 (7.6–9.6)	48.3 (43.0–54.2)	31.3 (25.0–36.2)	59.8 (51.3–64.8)	15.6±3.7 (9–23)	16.4±3.9 (8–25)
<i>O. tsukubaensis</i>	male	20	7.4 (6.7–8.1)	3.3 (2.8–3.8)	4.7 (4.0–5.9)	13.1 (11.7–14.4)	7.9 (7.0–8.6)	8.5 (7.6–10.2)	49.7 (45.3–58.3)	31.9 (27.2–37.0)	56.9 (51.9–67.0)	15.0±5.0 (4–24)	15.2±4.4 (5–23)
	female	26	7.6 (6.3–8.4)	3.1 (2.7–3.7)	4.8 (3.5–5.7)	13.0 (11.6–14.5)	7.8 (7.2–8.6)	8.6 (7.7–9.9)	50.0 (42.7–53.8)	31.9 (23.3–36.0)	56.8 (51.8–63.7)	15.4±3.8 (8–23)	15.0±3.6 (9–20)
<i>O. tsukubaensis</i>	male	6	7.3 (7.0–7.5)	3.3 (2.9–3.7)	4.4 (4.2–4.6)	13.1 (12.9–13.7)	7.7 (7.3–8.2)	9.2 (8.5–9.8)	48.3 (44.6–48.6)	28.3 (27.6–30.8)	59.0 (56.7–63.6)	16.0±3.2 (12–20)	15.5±3.0 (12–19)
	female	10	7.4 (6.9–7.8)	3.3 (3.1–3.5)	4.3 (4.0–5.0)	13.4 (13.0–14.6)	7.9 (7.4–8.3)	9.1 (8.9–9.5)	46.7 (43.5–52.5)	27.8 (24.8–30.7)	57.9 (56.0–61.5)	18.2±2.2 (14–22)	17.1±2.5 (12–20)

TABLE 2. Variation in the number of presacral vertebrae (PSVN) and costal grooves (CGN) on each side of the body in seven *Onychodactylus* species in Japan. Bold numbers indicate modal values.

Species	Sex	n	PSVN				CGN (L)				CGN (R)			
			17	18	19	20	11	12	13	14	11	12	13	14
<i>O. pyrrhonotus</i> sp. nov.														
	male	33		20	13		1	9	22	1		10	22	1
	female	32	1	19	12			5	24	3		5	24	3
<i>O. japonicus</i> (central pops.)														
	male	30		21	8	1		18	12			18	12	
	female	34	1	23	10			8	24	2		8	24	2
(combined)														
	male	110	8	75	26	1	7	68	34	1	8	68	33	1
	female	109	4	81	24		1	60	46	2	1	56	50	2
<i>O. fuscus</i>														
	male	6		3	3			1	5			1	5	
	female	5		4	1			4	1			4	1	
<i>O. intermedius</i>														
	male	17		11	6			10	7			11	6	
	female	10		8	2			4	6			5	5	
<i>O. kinneburgi</i>														
	male	36			32	4		2	32	2		1	34	1
	female	33		1	30	2			28	5		2	27	4
<i>O. nipponoborealis</i>														
	male	20	1	18	1		5	14	1		6	13	1	
	female	26	1	12	13		3	19	4		3	19	4	
<i>O. tsukubaensis</i>														
	male	6		5	1		1	5			1	5		
	female	10	1	9			1	9			2	8		

obtained, the distinct specific status of *Onychodactylus* sp. Kinki group is strongly supported, and we describe it as a new species below.

SYSTEMATICS

Onychodactylus pyrrhonotus sp. nov.

(Suggested English name: Fireback clawed salamander)

(Suggested Japanese name: Homura-hakone-sanshou-uwo)

Figs. 3 and 4

Onychodactylus japonicus (Subclade IV-A): Yoshikawa et al., 2008, p. 249.

Onychodactylus japonicus (Kinki group): Yoshikawa et al., 2010a, p. 33; Yoshikawa et al., 2010b, p. 344.

Onychodactylus sp. Kinki group: Yoshikawa and Matsui, 2013, p. 9; Yoshikawa et al., 2013, p. 441; Yoshikawa and Matsui, 2014, p. 53.

Holotype

NSMT-H 13600, an adult male from the environs of Mt. Bunagatake (35°15'N, 135°53'E, 990 m asl) in the Hira Mountains, Otsu-shi, Shiga Prefecture, Japan, collected by N. Yoshikawa on 22 June 2017.

Paratypes

A total of 29 specimens: one male (NSMT-H 13601) and two females (KUHE 47969–47970) from the type locality, collected on 22 June 2017 and 7 July 2013, respectively, by N. Yoshikawa; one male (NSMT-H 11685) and two females (NSMT-H 11759–11760) from Mt. Horai, Otsu-shi, Shiga Prefecture, col-

TABLE 3. A table showing frequencies of the presence or absence of a gap between the right and left sides of the vomerine tooth series, the short sub-branch of vomerine tooth series, and the dark marking on chest (number followed by percentage in parenthesis) in seven species of the *Onychodactylus japonicus* complex. Bold numbers indicate modal values.

	<i>O. pyrrhonotus</i> sp. nov.	<i>O. japonicus</i>		<i>O. fuscus</i>	<i>O. intermedius</i>	<i>O. kinneburi</i>	<i>O. nipponoborealis</i>	<i>O. tsukubaensis</i>
		central pops.	combined					
Gap between VTS								
present	33 (50.8%)	20 (31.2%)	72 (34.1%)	10 (90.9%)	8 (29.6%)	25 (36.2%)	15 (32.6%)	1 (6.3%)
absent	32 (49.2%)	44 (68.8%)	139 (65.9%)	1 (9.1%)	19 (70.4%)	44 (63.8%)	31 (67.4%)	15 (93.7%)
Short sub-branch VTS								
present	31 (47.7%)	36 (56.2%)	76 (36.0%)	3 (23.7%)	17 (63.0%)	27 (39.1%)	30 (65.2%)	8 (50.0%)
absent	34 (52.3%)	28 (43.8%)	135 (64.0%)	8 (72.7%)	10 (37.0%)	42 (60.9%)	16 (34.8%)	8 (50.0%)
Chest marking								
present	5 (7.6%)	51 (79.7%)	157 (71.7%)	2 (18.2%)	1 (3.7%)	—	30 (65.2%)	3 (18.8%)
indistinct	17 (26.2%)	10 (15.6%)	24 (10.9%)	1 (9.1%)	3 (11.1%)	2 (2.9%)	7 (15.2%)	2 (12.5%)
absent	43 (66.2%)	3 (4.7%)	38 (17.4%)	8 (72.7%)	23 (85.2%)	67 (97.1%)	9 (19.6%)	11 (68.8%)

lected on 22 June 2014 by N. Yoshikawa; one male (KUHE 43367) from Mt. Horai, Otsu-shi, Shiga Prefecture, collected on 31 May 2009 by N. Yoshikawa; two males (KUHE 44862, 45011) and two females (KUHE 44863, 45013) from Mt. Horai, Otsu-shi, Shiga Prefecture, collected on 12 June 2011 by N. Yoshikawa; one male (KUHE26101) from the Ashiu Research Forest of Kyoto University, Nantan-shi, Kyoto Prefecture, collected on 6 May 1999 by K. Nishikawa; one male (KUHE 37417) from Kurama-Ninose-cho, Sakyo-ku, Kyoto-shi, Kyoto Prefecture, collected on 9 April 2006 by N. Yoshikawa; one female (KUHE 38273) from Kurama-Ninose-cho, Sakyo-ku, Kyoto-shi, Kyoto Prefecture, collected on 4 November 2006 by N. Yoshikawa, K. Nishikawa, J.-P. Jiang, T. Shimada, and S. Ikeda; one female (KUHE 38600) from Kurama-Ninose-cho, Sakyo-ku, Kyoto-shi, Kyoto Prefecture, collected on 8 April 2007 by N. Yoshikawa; one male (KUHE 41268) and one female (KUHE 41269) from Kurama-Ninose-cho, Sakyo-ku, Kyoto-shi, Kyoto Prefecture, collected on 6 April 2008 by N. Yoshikawa and A. Hamidy; one female (KUHE 42955) from Kurama-Ninose-cho, Sakyo-ku, Kyoto-shi, Kyoto Prefecture, collected on 1 May 2009 by N. Yoshikawa; one female (KUHE 16046) from the environs of Mt. Odaigahara, Odai-cho, Mie Prefecture, collected on 27 May 1993 by Y. Misawa; three males (KUHE 40001–40003) and one female (KUHE 40000) from the environs of Mt. Odaigahara, Odai-cho, Mie Prefecture, collected on 23–24 June 2007 by K. Nishikawa; two females (KUHE 40204, SZ-MIE 219 [KUHE 40205]) from the environs of Mt. Odaigahara, Odai-cho, Mie Prefecture, collected on 21 July 2007 by K. Nishikawa, N. Yoshikawa, and Z. Shimizu; two males (KUHE 41523, 41525) and two females (KUHE 41524, 41526) from the environs of Mt. Misen, Tenkawa-mura, Nara Prefecture, collected on 17 May 2008 by K. Nishikawa.

Referred specimens
See Appendix I.

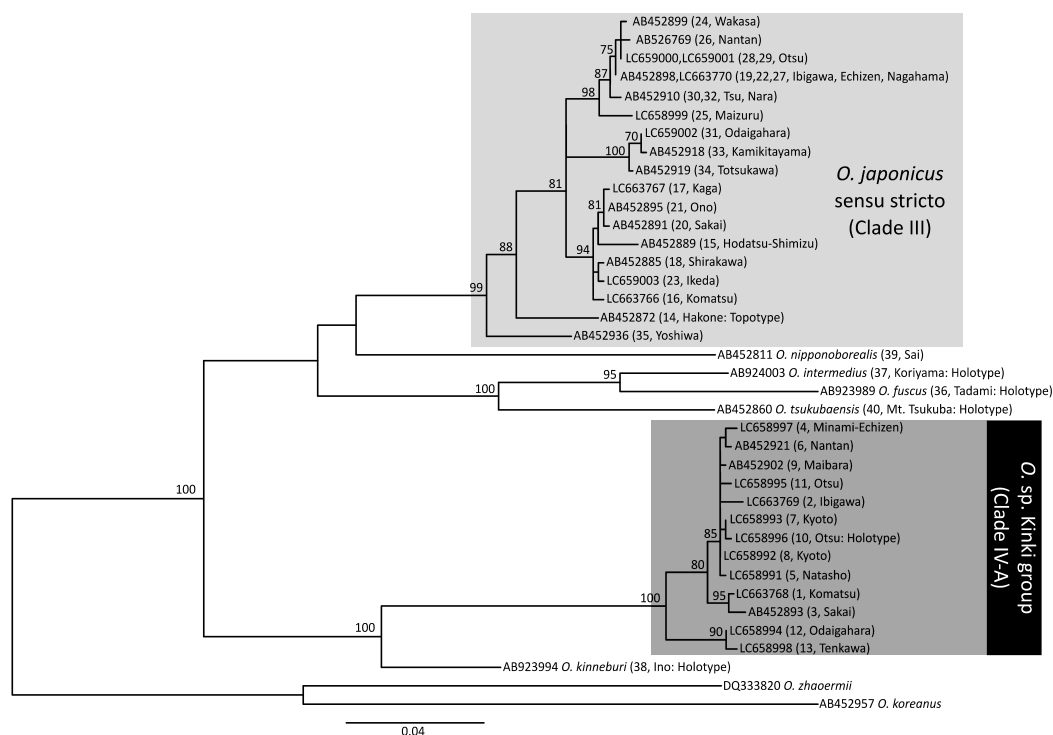


FIG. 2. The ML tree based on selected mitochondrial cytochrome b gene sequences (1141 bp). Values at nodes indicate bootstrap support based on 1000 pseudoreplicates. Numbers in parentheses indicate population numbers shown in Appendix III.

Etymology

The specific epithet, combination of ancient Greek words “pyrrho-” (fire-colored) and “-notus” (back), is derived from the beautiful scarlet dorsal coloration of the new species, and is also inspired from the coloration like the venter of the Japanese fire-bellied newt, *Cynops pyrrhogaster*. The suggested English name is after the specific epithet. The suggested Japanese name is derived from a Japanese word “Homura (flame)”.

Diagnosis

The new species is a member of the genus *Onychodactylus* and is diagnosed by the following characters: lungs absent; black horny claws present on tips of fingers and toes of larvae and breeding adults in both sexes; vomerine teeth in two short, transverse, arch-shaped series; larvae with skin folds on poste-

rior edges of limbs; nuptial males with dermal flaps on posterior edge of hindlimb; black tubercles and asperities on palm and sole in nuptial males and on sole in nuptial females; breeding in flowing water under the ground; eggs large, pigmentless, small in number. It is further assigned to a member of the *O. japonicus* species complex by the genetic evidence and presence of a distinct dorsal stripe or marking. *Onychodactylus pyrrhonotus* sp. nov. is most similar to *O. kinneburii*, but differs in morphology (smaller body, fewer presacral vertebrae, wide internarial distance relative to head width) and coloration (scarlet, orange or pink dorsal stripe or marking on purplish black background; purplish gray ventrum with dusty white dots). Body size moderate with mean SVL (± 1 SD) of 65.7 ± 3.2 mm and 70.7 ± 5.2 mm in males and females, respectively; tail longer than SVL in both sexes;

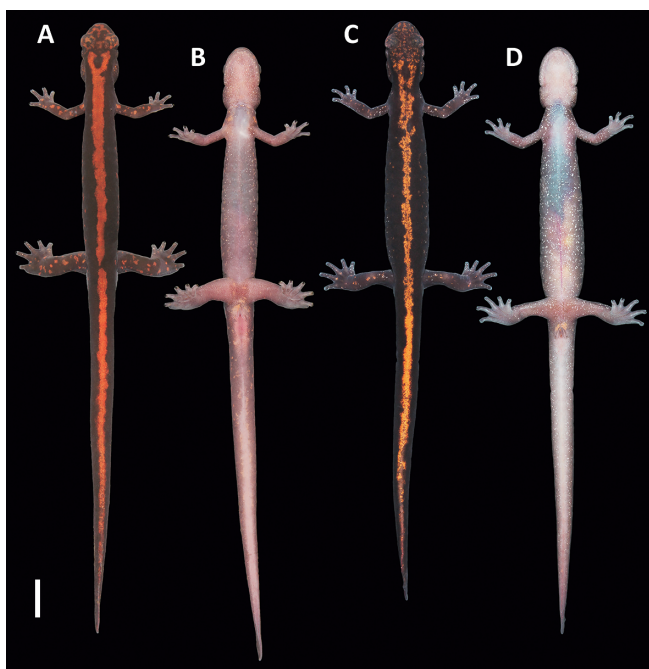


FIG. 3. Dorsal and ventral views of the male holotype (A and B; NSMT-H 13600, SVL=67.9 mm) and a female paratype (C and D; KUHE 47970, SVL=72.8 mm) of *Onychodactylus pyrrhonotus* sp. nov. Scale bar indicates 10 mm.



FIG. 4. Male holotype of *Onychodactylus pyrrhonotus* sp. nov. (NSMT-H 13600) in life.

snout relatively short; pair of dark marking on chest absent or indistinct; presacral vertebrae including atlas usually 18; costal grooves 12–13.

Description of the holotype

An adult male with SVL=67.9 mm (Figs.

3A–B and 4); body slender; skin smooth; head oval and depressed, distinctly longer than wide; snout rounded, projecting beyond lower jaw; nostril close to snout; eye large, slightly shorter than snout, prominently protruded; gular fold posteroventral to head; parotoid gland well developed, oval, ca. 1.5 times longer than wide, posterior end at level of gular fold; postorbital groove obvious; vomerine teeth in two transverse, distinctly curved arch-shaped series with slight gap in between (Fig. 5); 14 and 11 teeth on right and left series, respectively; inner end of each vomerine tooth series slightly curving to form a short sub-branch; few pigments around vomerine tooth series; forelimb thin; relative length of fingers $I < II < IV < III$; hindlimb slightly longer and distinctly more robust than forelimb; relative length of toes $I < V < II < III < IV$; adpressed limbs separated by one costal fold; nuptial characteristics obvious; dermal flap on posterior edge of hindlimb well developed; black

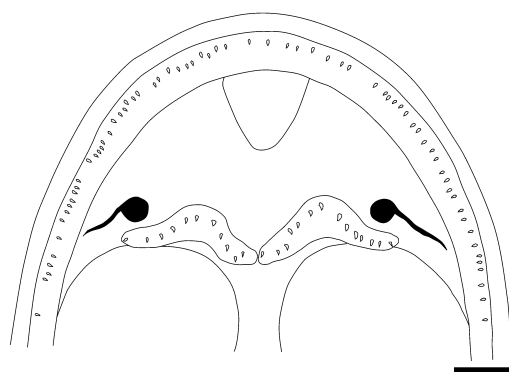


FIG. 5. Open mouth of the holotype of *Onychodactylus pyrrhonotus* sp. nov. (NSMT-H 13600) showing the shape of the vomerine tooth series. Scale bar indicates 1 mm.

horny claws on fingers and toes; slight black tubercles and asperities on palm and sole; trunk elongated and cylindrical; middorsal groove from level of parotoid gland to cloaca; well-developed 12 costal grooves on both sides of trunk; presacral vertebrae including atlas numbering 18; base of tail including around cloaca swollen; cloaca longitudinal slit, length 10.8% SVL, with anterior one-third of its edge forming inverse V-shaped precloacal skin fold; precloacal skin fold as wide as two-fifths of cloacal length; precloacal black spine series present in precloacal skin fold; tail long, 130.8% length of SVL, cylindrical at base, increasingly compressed laterally toward tip forming finny shape; tail highest (10.3% SVL) at distal one-third; tip of tail rounded in lateral view.

Coloration in life, distinct scarlet dorsal stripe from head to tip of the tail on a purplish black background (Figs. 3 and 4); dorsal stripe wavy but sharply defined from the background; on tail, dorsal stripe narrowed toward tip; sparse scarlet flecks on dorsal side of limbs; side of body purplish dark gray with sparse scarlet flecks and/or fine white dots, gradually fading toward ventrum; ventrum purplish gray with fine white dots; upper iris golden, slightly mottled with brown, lower iris dark-brown. In alcohol, color and pattern

fading generally, and dorsal scarlet coloration immediately bleached.

Measurements (in mm) of the holotype

SVL 67.9, HL 15.4, HW 9.3, TAL 88.8, AGD 36.2, FLL (L) 18.2, FLL (R) 18.2, HLL (L) 20.3, HLL (R) 20.5, UEL (L) 4.1, UEL (R) 4.2, IOD 3.0, END (L) 2.0, END (R) 2.2, ICD 6.0, IND 4.8, SL (L) 5.2, SL (R) 5.0, CW 7.9, BTAH 7.1, BTAW 7.4, MTAH 7.3, MTAW 4.5.

Variation

The following description of variation is based on the maximum number of 33 adult males and 32 adult females. Morphometric data are summarized in Tables 1–3 together with those of six congeneric species.

Males were significantly smaller in SVL and VTN (student's t-test, $P < 0.05$) than females. In values relative to SVL, males had significantly larger TAL, BTAH, BTAW, MTAH and smaller AGD than females (Mann–Whitney U test, $P < 0.05$). Males also had apparently more robust, thicker hindlimb than females.

Adult specimens collected in the breeding season showed the following characteristics: black claws on all fingers and toes, swollen parotoid glands, black precloacal spine series, and laterally compressed tail in both sexes; dermal skin fold on posterior edge of hindlimbs, black tubercles and asperities on palms and soles, swollen cloaca, and inverse V-shaped precloacal skin fold in males; inverse narrow U-shaped precloacal skin fold in females.

Color pattern in life was variable among individuals (Fig. 6). The dorsal stripe varied from straight, uneven, to broken into a continuous or discontinuous series of blotches or spots. The dorsal stripe mostly bifurcates anteriorly at the neck. The color of the dorsal stripe varied from scarlet, light orange, pink to rarely yellow (Fig. 6), while the background color was uniformly purplish black in most specimens. The border of the dorsal stripe and background color was sharply defined. The color on the lateral to ventral sides was purplish gray

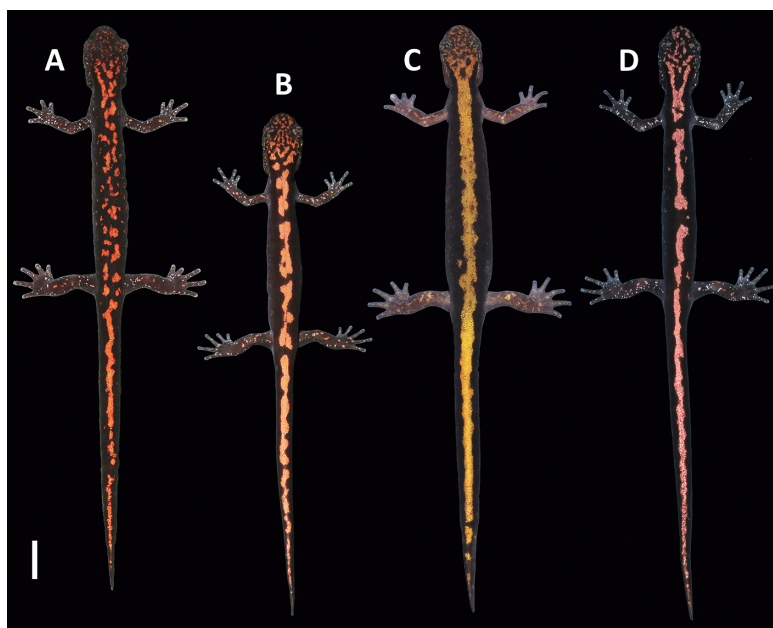


FIG. 6. Dorsal views of *Onychodactylus pyrrhonotus* sp. nov. showing variation in dorsal coloration. Discontinuous spots (A: KUHE 38273) and continuous series of blotches (B: KUHE 40002). Dorsal coloration varies the scarlet (A), light orange (B), yellow (C: KUHE 47969) and pink (KUHE 41526). Scale bar indicates 10 mm.

with a varying amount of silvery white dots.

Geographic variation in morphology is unclear, but southern populations (Kii Peninsula) tend to have a larger SVL and paler dorsal coloration than in northern populations.

Larva

Larvae found in the open streams had a SVL of 16.6–43.2 mm and TAL of 13.7–42.6 mm, resulting in total length (TL) of 30.6–85.8 mm (Fig. 7A–D). Head rectangular and blunt at snout; three pairs of short external gills; labial fold well developed at posterior half of upper jaw; caudal fin low but well-developed dorsally and ventrally; dorsal fin relatively higher than ventral fin; origin of dorsal fin at level of hindlimb to cloaca; ventral fin originating from around posterior four-fifths to two-thirds of tail; tail tip moderately rounded; skin fold on posterior edge of limb; dark asperities on surfaces of palm and sole; digits with acute and curved black claws.

The coloration of the larvae of the new species is variable, possessing uniformly dark-brown, obscured light-colored blotches/stripe on dark-brown background, or yellowish-gray with indistinct markings (Fig. 7C, D). In the premetamorphic stage (third year), they generally possess scarlet to light-orange blotches or a stripe on a black to purplish-black background, as seen in adults (Fig. 7A, B). The color of the ventrum is transparent, whitish, or purplish-gray with slight dusty white dots.

Comparisons

Morphological measurements are summarized in Tables 1–3. Some other data are cited from Poyarkov et al. (2012) and Yoshikawa and Matsui (2014).

From species of the genus *Onychodactylus* from the continental region of East Asia (*O. fischeri*, *O. koreanus*, *O. zhangyapingi*, and *O. zhaoermii*) that have indistinct markings or scattered yellow spots on dorsum, *O. pyrrho-*

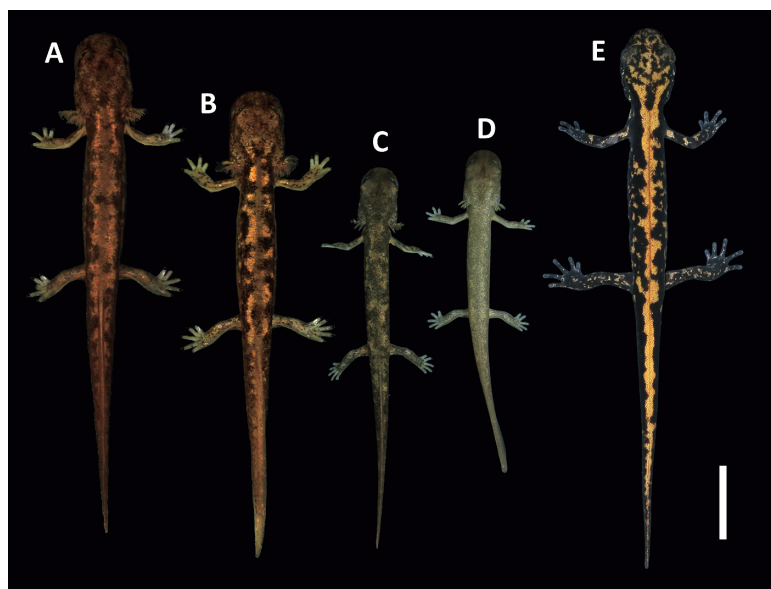


FIG. 7. Dorsal views of larvae (A–D: KUHE 38613–38614, 42369 and 42371, respectively) and a metamorphosed juvenile (E: KUHE 41328) of *Onychodactylus pyrrhonotus* sp. nov. Scale bar indicates 10 mm.

notus sp. nov. can be easily differentiated by distinct scarlet dorsal markings. In addition, *O. fischeri* has larger number of costal grooves and presacral vertebrae.

Compared with the other species of the *O. japonicus* species complex in Japan which generally possess distinct dorsal markings, *O. pyrrhonotus* sp. nov. is distinct in coloration except for *O. japonicus sensu stricto* (*O. japonicus* hereafter). The new species, with sharply defined scarlet, orange, or pinkish dorsal markings and dusty white dots on the venter, is easily differentiated from *O. fuscus* (dorsum is generally dark-brown without distinct markings). Dorsal markings of *O. intermedius*, *O. nipponoborealis*, and *O. tsukubaensis* are not always sharply defined and often obscured, and its color varies yellow, ochre, or brown to reddish-brown. *Onychodactylus kinneburi*, the sister species, has sharply defined yellow to orange dorsal markings and no white dots or spots on the venter (vs. with dusty white dots on venter in the new species).

The color pattern of *O. japonicus* is highly

variable among populations (Fig. 8), but most populations are easily differentiated from the new species in coloration. In *O. japonicus* from the northern Kinki District and eastward, which is partly sympatric with the new species, the dorsal marking is generally obscured and not always sharply defined, and its color varies from yellow to, ochre, brown (Fig. 8A and C), to orange (environs of Hakone, eastern Honshu). The color of the mottling on the venter is the same as the dorsal markings, but with fewer white spots (Fig. 8B and D) in *O. japonicus*. *Onychodactylus japonicus* from the Chugoku mountains of western Honshu (allopatric with the new species) is similar to the new species in dorsal coloration (Fig. 8E: red or reddish brown), but has a pair of dark markings on the chest (less frequent in the new species) and fewer white spots on the venter (Fig. 8F). *Onychodactylus japonicus* from the Kii Peninsula of the southern Kinki District and Mie Prefecture (sympatric with the new species) is extremely similar to the new species in coloration (Fig. 8G and H: light-orange or

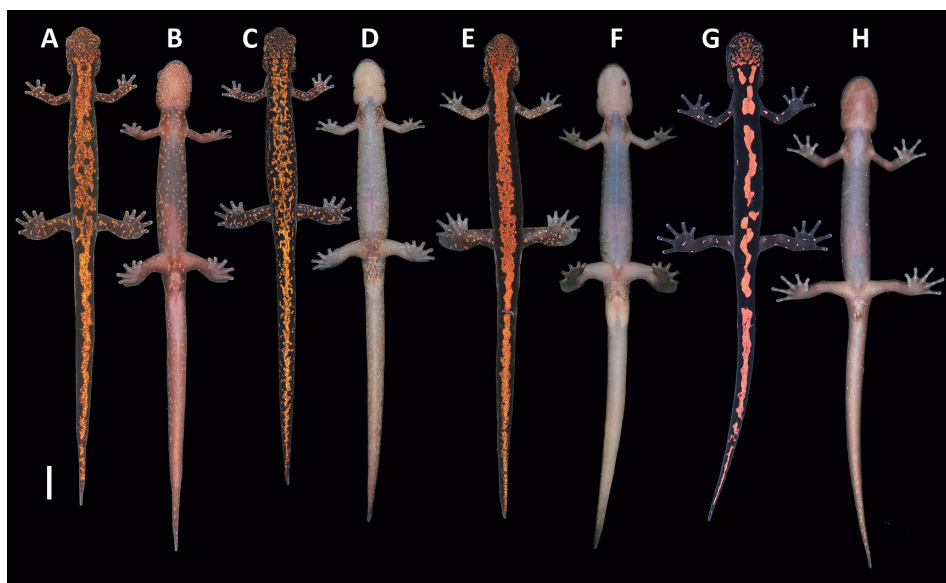


FIG. 8. Dorsal views of *Onychodactylus japonicus* showing variation in dorsal and ventral coloration. Typical morphs in the northern Kinki District of central Honshu (A–B: NSMT-H 13603; C–D: KUHE 47968), in the Chugoku mountains of western Honshu (E–F: KUHE 40195), and in the Kii Peninsula of central Honshu (G–H: KUHE 40199). Scale bar indicates 10 mm.

pink dorsal markings on a black background), and difficult to distinguish from the new species by appearance alone. However, in *O. japonicus*, a pair of dark marking on chest is more frequent and ventral white spots are often absent or scarce (Fig. 8H).

In other morphological characters, *O. pyrrhonotus* sp. nov. is significantly different from the sister species *O. kinneburi* in having a smaller SVL, REND, PSVN, and larger IND/HW in males, and a smaller SVL, PSVN and larger IND/HW in females; from *O. intermedius*, in a larger RIND and RICD in males, and in a smaller RCW in females; from *O. nipponoborealis*, a larger RAGD, ICD/HW, and CGN in males, and in a larger SVL, CGN, smaller RHW, RCW, RSL and ICD/HW in females; from *O. tsukubaensis*, in a larger RTAL and IOD/HW in males, and in a larger RTAL, RAGD, IND/HW, IOD/HW, CGN, smaller RHW, RCW, and RSL in females. From combined *O. japonicus* populations, the new species differs significantly in having a

smaller RFL and larger CGN in males and in having a smaller RCW, larger IND/HW, and CGN in females, but from central Honshu (including sympatric area) population, it differs only in having a smaller SVL in males.

Genetic characteristics

Onychodactylus pyrrhonotus sp. nov. corresponds to Subclade IV-A or Kinki group in previous studies (Yoshikawa et al., 2008, 2010a, b), and is a sister species of *O. kinneburi* from Shikoku Island and some parts of the Chugoku Mountains of western Honshu. The mean uncorrected p-distance for the cyt b gene between *O. pyrrhonotus* sp. nov. (Subclade IV-A) and *O. kinneburi* (IV-B) is 5.56% (5.08–5.87%: Yoshikawa et al., 2008). Within the new species, Kii Peninsula populations (Southern population) and other populations (Northern population), are distinctly divergent with a mean p-distance of 1.94% (Yoshikawa et al., 2010b). The new species is sympatric with *O. japonicus* throughout its entire distributional

range, but they are phylogenetically distant (mean p -distance=8.52% [7.62–10.88%]). Reproductive isolation between them is known to be substantial and only a single putative hybrid juvenile has been discovered so far (Yoshikawa et al., 2010b). As far as we examined, the genetic identifications of individuals by mtDNA and nuclear allozymic data are concordant, suggesting no mitochondrial introgression and replacement.

Fecundity and natural history

The breeding season of *O. pyrrhonotus* sp. nov. is early summer (late May to early July) based on our field observation, and winter-breeding, seen in some congeners (Akita, 1989; Yoshikawa and Matsui, 2014), has never been found. The breeding site are in underground flowing water near headstreams, as is the case in other *Onychodactylus* species. We obtained only a single pair of unfertilized egg sacs from a female (KUHE45013) from Otsu-shi, Shiga Prefecture in the laboratory on 4 August 2011 (Fig. 9). Although this female was collected on 12 June 2011, oviposition was probably greatly delayed because of the failure of spontaneous mating in captivity and non-use of gonadotropin induction. The egg sacs were attached to the stone and strongly adhered directly without gelatinous stalks. The outer gelatinous layer was transparent, elastic, and strong. The egg sacs were long and narrow, in which eggs were arranged in two rows at the basal halves and in single row at the tip. Very weak, longitudinal grooves could be seen on the surface of the egg sacs. The clutch size was 30 (15 eggs in each sac) and the diameters of 30 eggs ranged from 3.7–5.5 mm (mean=4.8±0.49). The eggs were pigmentless and entirely yellowish-white.

The larval life history of *O. pyrrhonotus* sp. nov. is poorly known, but the larvae found in open streams ranged 30.6–85.8 mm in TL. Larvae inhabit streams and may metamorphose after two or more years, as in other congeners (Yoshikawa and Matsui, 2013; Yoshikawa et al., 2013). Diets of the larvae observed in Mt. Horai, Otsu-shi, Shiga Prefecture were mayfly



FIG. 9. A pair of egg sacs of *Onychodactylus pyrrhonotus* sp. nov. deposited on 4 August 2011 in the laboratory. The ruler is graduated in millimeters.

nymphs and other small aquatic insects or invertebrates (Yoshikawa, N. personal observation). The season of metamorphosis and size at the time are unclear, but a juvenile collected on 29 April 2008 (KUHE 41328; Fig. 7E), which probably metamorphosed in the autumn of preceding year, was 36.1 mm in SVL (69.1 mm in TL). Juveniles were often found in the forest floor around the stream.

The life history of adults in the non-breeding season is not well known, but they inhabit cool and humid forest-floors near streams in well-forested mountains, like the other *Onychodactylus* species. A case of predation by a snake *Rhabdophis tigrinus* was observed in Kyoto-shi, Kyoto Prefecture on 4 November 2006 (Yoshikawa, 2008, as *O. japonicus*).

The new species is sympatric with *O. japonicus*. We observed at several breeding sites in Mt. Horai and Mt. Bunagatake in Otsu-shi, Shiga Prefecture, that breeding individuals of these two species co-occurred in June to July. We also found hatchlings of the two species coming out from the same underground streams, and larvae of the two species in a single stream. These observations suggest that the two sympatric species breed in the same place in the same season, although their reproductive isolation is evident (Yoshikawa et al., 2010b).

Range

Onychodactylus pyrrhonotus sp. nov. is currently known from Kyoto, Mie, Nara, and Shiga Prefectures of the Kinki District, Ishikawa and Fukui Prefectures of the Hokuriku District, and Gifu Prefecture of the Chubu District, all in central Honshu (Fig. 1). To date, the new species is not known from Wakayama Prefecture in the southern Kii Peninsula, but it probably occurs in the area. The geographic range of the new species overlaps entirely with the entire geographic range of *O. japonicus*, and they are sympatric in at least six localities (Fig. 1).

Conservation

Onychodactylus pyrrhonotus sp. nov. is not abundant, and its habitat seems to be fragmented and discontinuous in its known distributional range. The new species is listed as a part of *O. japonicus* on the Red List of Kyoto Prefecture as Critically endangered (Tanabe, 2015), the Red Lists of Mie and Nara Prefectures as Vulnerable (Shimizu [2015] and Sato [2017], respectively), and the Red List of Shiga Prefecture as Near Threatened (Tanabe and Matsui, 2021), but not listed in the Red List of Fukui, Gifu, and Ishikawa Prefectures. The extent of the occurrence of the new species is estimated to be less than 20,000 km² (approx. 10,000 km²), and its distributional range is fragmented in at least two areas (Northern and Southern populations). Based on the criteria used in the Japanese and IUCN Red Lists (IUCN, 2001), we propose that the new species to be designated as Vulnerable in those red lists.

DISCUSSION

Onychodactylus japonicus sensu lato was described by Houttuyn (1782) based on specimens from the Hakone mountains of eastern Honshu as the first Japanese amphibian known to science. The oldest record of *O. japonicus* sensu lato in the Kinki District of central Honshu was noted by Temminck and Schlegel (1838) (Tamba, a region spanning the middle

Kyoto and eastern Hyogo Prefectures), probably referring to “Nihon-Sankai-Meisan-Zue, vol. 2” (Shitomi, 1799). Since then, *Onychodactylus* salamanders of Japan, as well as populations in central Honshu, have long been considered a single species, *O. japonicus* sensu lato, although some researchers noted the presence of extensive geographic variation in dorsal markings (e.g., Sato, 1943; Nakamura and Uéno, 1963; Kokuryo, 1979).

Onychodactylus pyrrhonotus sp. nov. described here was first reported by Yoshikawa et al. (2008) as a distinct genetic clade (Subclade IV-A) endemic to the Kinki District of central Honshu, forming a sister clade of the geographically separated Subclade IV-B in Shikoku and parts of westernmost Honshu (currently treated as *O. kinneburi*), suggesting a close phylogeographic relationship of Kii Peninsula and Shikoku. Interestingly, Subclade IV-A was found sympatric with Clade III (*O. japonicus*) in some localities, and they were genetically divergent each other based on allozymic data (Yoshikawa et al., 2010a). Yoshikawa et al. (2010b) further studied the genetic structure and phylogeography of the two genetic groups co-occurring in central Honshu, and they proved to be reproductively isolated, separate biological species.

Phylogenetically, Yoshikawa et al. (2008) surmised that *O. pyrrhonotus* sp. nov. (as Subclade IV-A) diverged from *O. kinneburi* (as Subclade IV-B) around 2.5 MYA by the narrowing connection between Kii Peninsula and Shikoku prior to the formation of Kii Strait. A similar phylogeographic pattern is seen between montane salamanders *Hynobius guttatus* (occurring in the Kii Peninsula and surrounding area) and *H. tsurugensis* (occurring around Mt. Tsurugi of eastern Shikoku), although their divergence is estimated to have occurred more recently, around 1.7 MYA (Tominaga et al., 2019). However, this similar pattern may suggest close relationships of montane amphibians in Shikoku and the Kii Peninsula, and the importance of Kii Strait in the differentiation of fauna in each area.

Yoshikawa et al.’s (2010b) phylogeographic

reconstruction of *Onychodactylus* species in the Kinki District demonstrated that the new species (as the Kinki group) originated in the Kii Peninsula and expanded northward, and *O. japonicus* (as SW-Honshu group) entered to the Kii Peninsula vice versa, to form the current sympatric distribution. Similar secondary contact and sympatric occurrence is also found in *O. kinneburi*, the sister species, and *O. japonicus* in the Chugoku Mountains of western Honshu. It is unknown how these pairs of sympatric species avoid interspecific hybridization, but similar mechanisms of reproductive isolation may be present between them.

In this study, we described the variation in dorsal coloration in *O. pyrrhonotus* sp. nov. and the sympatric *O. japonicus*. Interestingly, dorsal pattern and coloration is generally stable across populations in the new species, showing little variation in color. In contrast, variation in *O. japonicus* is quite extensive in pattern and color. Especially in Kii Peninsula, *O. japonicus* is surprisingly similar to *O. pyrrhonotus* sp. nov. in coloration. This may be an example of convergent evolution in *O. japonicus* after colonizing into Kii Peninsula, although it is unknown what kind of selective pressure would drive such convergence in these species.

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APPENDIX I

Specimen examined

Referred specimens of the new species and newly examined specimens in this study are listed below.

Onychodactylus pyrrhonotus sp. nov.—**Adults:** Ibigawa-cho (formerly Sakauchi-mura), Gifu Pref.: KUHE 18594 (male); Natasho, Ohi-cho, Fukui Pref.: KUHE 45235 (female); Ashiu Research Forest of Kyoto University, Nantan-shi, Kyoto Pref.: KUHE 18519 (male); Kurama-Ninose-cho, Sakyo-ku, Kyoto-shi, Kyoto Pref.: KUHE 12989, 47174–47175, 48153–48154, 48492 (males), KUHE 8879, 47176, 47440, 48148–48152, 48155, 48493–48494 (females); Mt. Horai, Otsu-shi, Shiga Pref.: 42364, 42366, 43442, 44320–44321, 44729, 45012, 47964 (males), KUHE 41327, 43368 (females); Environs of Mt. Odaigahara, Odai-cho, Mie Pref.: KUHE 18667, 40203 (females); Environs of Mt. Misen, Tenkawa-mura, Nara Pref.: KUHE 41527–41529 (males). **Larvae:** Sakai-shi, Fukui Pref.: KUHE 39612–39616, 39619, 39621–39622, 39624–39628; Minami-Echizen-cho, Fukui Pref.: KUHE 39522–39532; Mt. Horai, Otsu-shi, Shiga Pref.: KUHE 36588–36589, 41312–41317, 42368–42369, 42371–42372, 43101–43116; Ashiu Research Forest of Kyoto University, Nantan-shi, Kyoto Pref.: KUHE 35946–35950, 36020–36023; Kurama-Ninose-cho, Kyoto-shi, Kyoto Pref.: 36650–36659, 38249–38253, 38352.

O. japonicus—**Adults (central populations):** Otari-mura, Nagano Pref.: KUHE 44111–44112; Hiraya-mura, Nagano Pref.: T3119; Hodatsu-Shimizu-cho, Ishikawa Pref.: KUHE 43054–43056; Toyama-shi, Toyama Pref.: KUHE 13190, 16002–16003; Takayama-shi, Gifu Pref.: KUHE 16016, 44354; Shirakawa-mura, Gifu Pref.: KUHE 14980–14996; Ibigawa-cho, Gifu Pref.: KUHE 42838; Ono-shi, Fukui Pref.: KUHE 44296; Otsu-shi, Shiga Pref.: KUHE 38824, 43364–43365, 45010, 47968, NSMT-H 13602–13603; Nantan-shi, Kyoto Pref.: KUHE 7459, 59065; Maizuru-shi, Kyoto Pref.: T3346; Environs of Mt. Odaiga-

hara, Nara (Kamikitayama-mura) and Mie (Odai-cho) Prefs.: KUHE 8323–8324, 9714, 13054, 16045, 35411–35413, 39996–39999, 40198–40200, 40202, 48012, 40207 (SZ-Mie 220), SZ-Mie 221–222 (SZ-1–2), T3013–3015; Totsukawa-mura, Nara Pref.: KUHE 27078.

Adults (eastern population): Fujinomiya-shi, Shizuoka Pref.: KUHE 16975–16976. **Larvae:** Sakai-shi, Fukui Pref.: KUHE 39611, 39617–39618, 39620, 39623, 39629; Otsu-shi, Shiga Pref.: KUHE 38450–38454, 41311, 41319, 42367, 42370, 43099–43100, 43117–43127; Kamikitayama-mura, Nara Pref.: KUHE 36888–36889.

APPENDIX II

Measurements taken in this study

1) snout-vent length (SVL); 2) head length (HL); 3) head width (HW); 4) tail length (TAL); 5) axilla-groin distance (AGD); 6) forelimb length (FLL); 7) hindlimb length (HLL); 8) upper eyelid length (UEL); 9) interorbital distance (IOD); 10) eye-nostril distance (END); 11) intercanthal distance (ICD); 12) internarial distance (IND); 13) snout length (SL); 14) chest width (CW); 15) basal tail height (BTAH); 16) basal tail width (BTAW); 17) medial tail height (MTAH); 18) medial tail width (MTAW).

APPENDIX III

Samples for phylogenetic analysis

Locality information of samples used for the phylogenetic analysis shown in Fig. 2 is listed below.

Onychodactylus pyrrhonotus sp. nov.: (1) Komatsu-shi, Ishikawa Pref. (LC663768). (2) Ibigawa-cho, Gifu Pref. (LC663769). (3) Sakai-shi, Fukui Pref. (AB452891). (4) Minami-Echizen-cho, Fukui Pref. (LC658997). (5) Natasho, Ohi-cho, Fukui Pref. (LC658991). (6) Ashiu Research Forest of Kyoto University, Nantan-shi, Kyoto Pref. (AB452921). (7) Kurama-Ninose-cho, Sakyo-ku, Kyoto-shi, Kyoto Pref. (LC658993). (8) Mt. Sajikigatake, Kita-ku, Kyoto-shi, Kyoto Pref. (LC658992).

(9) Maibara-shi, Shiga Pref. (AB452902). (10) Mt. Bunagatake, Otsu-shi, Shiga Pref. (holotype: LC658996). (11) Mt. Horai, Otsu-shi, Shiga Pref. (LC658995). (12) Environs of Mt. Odaigahara, Odai-cho, Mie Pref. (LC658994). (13) Environs of Mt. Misen, Tenkawa-mura, Nara Pref. (LC658998).

O. japonicus: (14) Hakone-machi, Kanagawa Pref. (type locality: AB452872). (15) Hodatsu-Shimizu-cho, Ishikawa Pref. (AB452889). (16) Komatsu-shi, Ishikawa Pref. (LC663766). (17) Kaga-shi, Ishikawa Pref. (LC663767). (18) Shirakawa-mura, Gifu Pref. (AB452885). (19) Ibigawa-cho, Gifu Pref. (LC663770). (20) Sakai-shi, Fukui Pref. (AB452893). (21) Ono-shi, Fukui Pref. (AB452895). (22) Echizen-shi, Fukui Pref. (AB452898). (23) Ikeda-cho, Fukui Pref. (LC659003). (24) Wakasa-cho, Fukui Pref. (AB452899). (25) Maizuru-shi, Kyoto Pref. (LC658999). (26) Ashiu Research Forest of Kyoto University, Nantan-shi, Kyoto Pref. (AB526769). (27) Nagahama-shi, Shiga Pref. (AB452898). (28) Mt. Bunagatake, Otsu-

shi, Shiga Pref. (LC659000). (29) Mt. Horai, Otsu-shi, Shiga Pref. (LC659001). (30) Tsu-shi, Mie Pref. (AB452910). (31) Environs of Mt. Odaigahara, Odai-cho, Mie Pref. (LC659002). (32) Nara-shi, Nara Pref. (AB452910). (33) Kamikitayama-mura, Nara Pref. (AB452918). (34) Totsukawa-mura, Nara Pref. (AB452919). (35) Yoshiwa, Hatsukaichi-shi, Hiroshima Pref. (AB452936).

O. fuscus: (36) Tadami-machi, Fukushima Pref. (holotype: AB923989).

O. intermedius: (37) Koriyama-shi, Fukushima Pref. (holotype: AB924003).

O. kinneburii: (38) Ino-cho, Kochi Pref. (holotype: AB923994).

O. nipponoborealis: (39) Sai-mura, Aomori Pref. (AB452811).

O. tsukubaensis: (40) Mt. Tsukuba, Sakuragawa-shi, Ibaraki Pref. (holotype: AB452860).

O. koreanus: Kangwong-do, South Korea. (AB452957)

O. zhaoermii: Jilin, China. (DQ333820)