

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

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Abstract: Recent phylogenetic studies using mtDNA and allozymes have revealed the presence of large genetic differentiation within a Japanese clawed salamander, *Onychodactylus japonicus*, suggesting the presence of several cryptic taxa in this species. Based on morphological analyses, we describe one of them from the Tsukuba Mountains of Ibaraki Prefecture, in the Kanto district of eastern Honshu, as a new species, *Onychodactylus tsukubaensis*. It is a member of the *japonicus* species complex of *Onychodactylus*, and differs from the other species of the complex by a relatively short tail, wide head, and large number of vomerine teeth.

Key words: Caudata; Eastern Japan; Morphology; *Onychodactylus tsukubaensis*; Taxonomy; Tsukuba Mountains

INTRODUCTION

The salamanders of the genus *Onychodactylus* are endemic to northeast Asia (Yoshikawa et al., 2008), and belong to the family Hynobiidae, which is a primitive lineage within the order Urodela (Zhang and Wake, 2009). Although *Onychodactylus* was once thought to be a derived lineage within the Hynobiidae (Sato, 1943; Zhao and Hu, 1988), recent molecular phylogenetic studies demonstrated that the genus represents the most basal lineage within the Hynobiidae, forming a sister taxon to the clade of all the remaining extant hynobiid genera (Zhang et al., 2006; Zheng et al., 2011). The genus *Onychodactylus* has

long been considered a small, uniform group within the Hynobiidae, comprising only two species, *O. japonicus* (Houttuyn, 1972) from the Honshu and Shikoku Islands of Japan and *O. fischeri* (Boulenger, 1886) from Russian Far East, northeast China, and Korean Peninsula (Yoshikawa et al., 2008; Frost, 2011).

Recently, Yoshikawa et al. (2008) conducted a molecular phylogenetic study and demonstrated the presence of extensive genetic divergences within *Onychodactylus*, suggesting that the two species of this genus actually include more than one cryptic species. Furthermore, Yoshikawa et al. (2010a, b, 2012) conducted large scale allozymic studies on *O. japonicus* obtained from its entire distributional range, and recognized six distinct genetic groups: N-Tohoku, S-Tohoku, Tsukuba, SW-Honshu, Kinki, and Shikoku, each corresponding to Clades or Subclades I, II-A, II-B, III, IV-A,

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and IV-B of Yoshikawa et al. (2008), respectively, found in mitochondrial phylogeny. There were clear signs of reproductive isolation between groups that are sympatric and/or parapatric in distribution, and Yoshikawa et al. (2010a, b, 2012) speculated that these six genetic groups were distinct species.

More recently, Poyarkov et al. (2012) revised the genus *Onychodactylus*, and described four new species from the continent and Japan. Of the Japanese *Onychodactylus*, they split Clade I or the N-Tohoku group of Yoshikawa et al. (2008, 2010a, 2012) from *O. japonicus* sensu stricto and described it as a new species *O. nipponoborealis* Kuro-o, Poyarkov and Vieites, 2012. However, the taxonomic status of other genetic groups of *O. japonicus* recognized by Yoshikawa et al. (2010a, b, 2012) remains undetermined. In this paper, we describe one of them as a new species on the basis of morphological data. The new species has been reported as the Tsukuba group in previous studies (Yoshikawa et al., 2010a, b, 2012), and occurs on the Tsukuba Mountains located in eastern Honshu.

MATERIALS AND METHODS

In this paper, we use the name “*Onychodactylus japonicus*” only for *O. japonicus* sensu stricto, which corresponds to Clade III or the SW-Honshu group of Yoshikawa et al. (2008, 2010a), unless otherwise specified, and “*O. japonicus* (sensu lato)” for the entire *O. japonicus* species complex. We call the other candidate cryptic species “*Onychodactylus* sp.” followed by the name of the genetic group determined by Yoshikawa et al. (2010a).

Salamanders were collected on Mts. Tsukuba and Kaba, Ibaraki Prefecture (Fig. 1, Appendix 1). For morphological comparisons, we used adult specimens of *O. japonicus* from Kanagawa (including topotypes) and Shizuoka Prefectures and *O. nipponoborealis* from Aomori, Akita, Iwate, and Miyagi Prefectures (including the type series; Fig. 1, Appendix 1). The following 18 measurements (Fig. 2) were taken for specimens fixed in 10% formalin

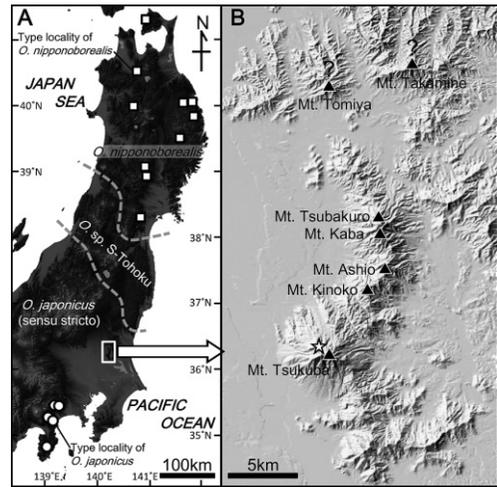


FIG. 1. (A) Map of eastern Honshu showing sampling sites of *Onychodactylus nipponoborealis* (open squares) and *O. japonicus* (open circles). (B) Map of the Tsukuba Mountains. Triangles indicate mountains where *O. tsukubaensis* sp. nov. occurs, and a star indicates the type locality. Mts. Tomiya and Takamine are shown as putative localities of the new species.

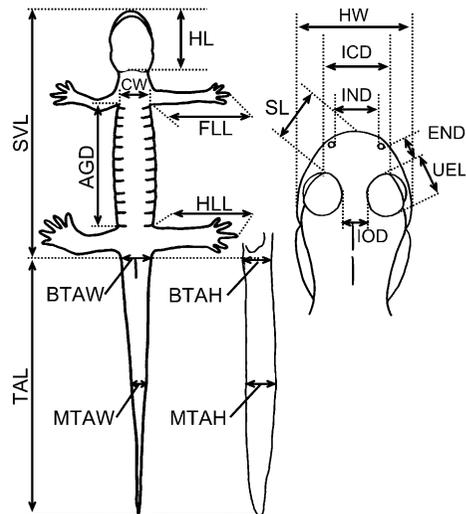


FIG. 2. Character dimensions of the *Onychodactylus* species taken in this study. For character definitions, see MATERIALS AND METHODS. Ventral view of the entire body (left), lateral view of the tail (center), and dorsal view of the head (right).

and preserved in 70% ethanol, to the nearest 0.1 mm with dial calipers: 1) snout-vent length (SVL), from snout to anterior angle of cloaca; 2) head length (HL), from tip of snout to gular fold; 3) head width (HW), measured at jaw articulation; 4) tail length (TAL), from anterior angle of cloaca to tip of tail; 5) axilla-groin distance (AGD), distance between axilla and groin (only for right side); 6) forelimb length (FLL), distance from axilla to tip of longest finger (measured separately on right and left sides); 7) hindlimb length (HLL), distance from groin to tip of longest toe (measured separately on right and left sides); 8) upper eyelid length (UEL), maximum length of upper eyelid (measured separately on right and left sides); 9) interorbital distance (IOD), minimum distance between upper eyelids; 10) eye-nostril distance (END), minimum distance between eye and nostril (measured separately on right and left sides); 11) intercanthal distance (ICD), minimum distance between anterior corners of eyes; 12) internarial distance (IND), minimum distance between external nares; 13) snout length (SL), tip of snout to anterior corner of eye (measured separately on right and left sides); 14) chest width (CW), minimum distance between right and left axillae; 15) basal tail height (BTAH), height of tail at anterior angle of cloaca; 16) basal tail width (BTAW), width of tail at anterior angle of cloaca; 17) medial tail height (MTAH), height of tail at midpoint; 18) medial tail width (MTAW), width of tail at midpoint.

The number of costal grooves (CGN) was counted separately on right and left sides following Misawa (1989). Overlap of finger and toe tips when both limbs were adpressed to the body was also recorded by the number of costal folds between the tips with ‘plus’ indicating overlap and ‘minus’ separation. 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 X-ray photographs using Fuji Medical X-ray Film.

For larvae, measurements of four characters, SVL, HL, HW, and TAL, were taken for three species, the salamander from Mts. Tsukuba and Kaba, *O. japonicus* from Kanagawa and Shizuoka, and *O. nipponoborealis* from Miyagi.

Tukey-Kramer tests were used for morphometric comparisons, while Kruskal-Wallis or Dunn’s multiple comparison tests were performed for ratio values to detect the presence or absence of differences in the frequency distributions. We separated sexes in comparing morphological variations between species. Intraspecific sexual variation in SVL and ratio values were tested by Student’s t-test and Mann-Whitney’s U-test, respectively. A significance level of 95% was used in all statistical tests.

SYSTEMATICS

Onychodactylus tsukubaensis sp. nov.

(Japanese name: Tsukuba-hakone-sanshou-uwō)

(English name: Tsukuba clawed salamander)

Figs. 3 and 4

Onychodactylus japonicus: Tago, 1907, p. 239 (part); Namiye, 1908, p. 399; Dunn, 1923, p. 506 (part); Tago, 1931, p. 200 (part); Sato, 1943, p. 288 (part).

Onychodactylus japonicus (Subclade II-B): Yoshikawa et al., 2008, p. 249.

Onychodactylus japonicus (Tsukuba group): Yoshikawa et al., 2010a, p. 33; Yoshikawa et al., 2012, p. 229.

Etymology

The specific name “*tsukubaensis*” is derived from “Mt. Tsukuba”, which is the type locality and the highest mountain of the Tsukuba Mountains where this species occurs.

Holotype

KUHE 41388, an adult male from a small stream on Mt. Tsukuba (140°6'E, 36°13'N, 600 m asl) in Sakuragawa-shi (formerly Makabe-

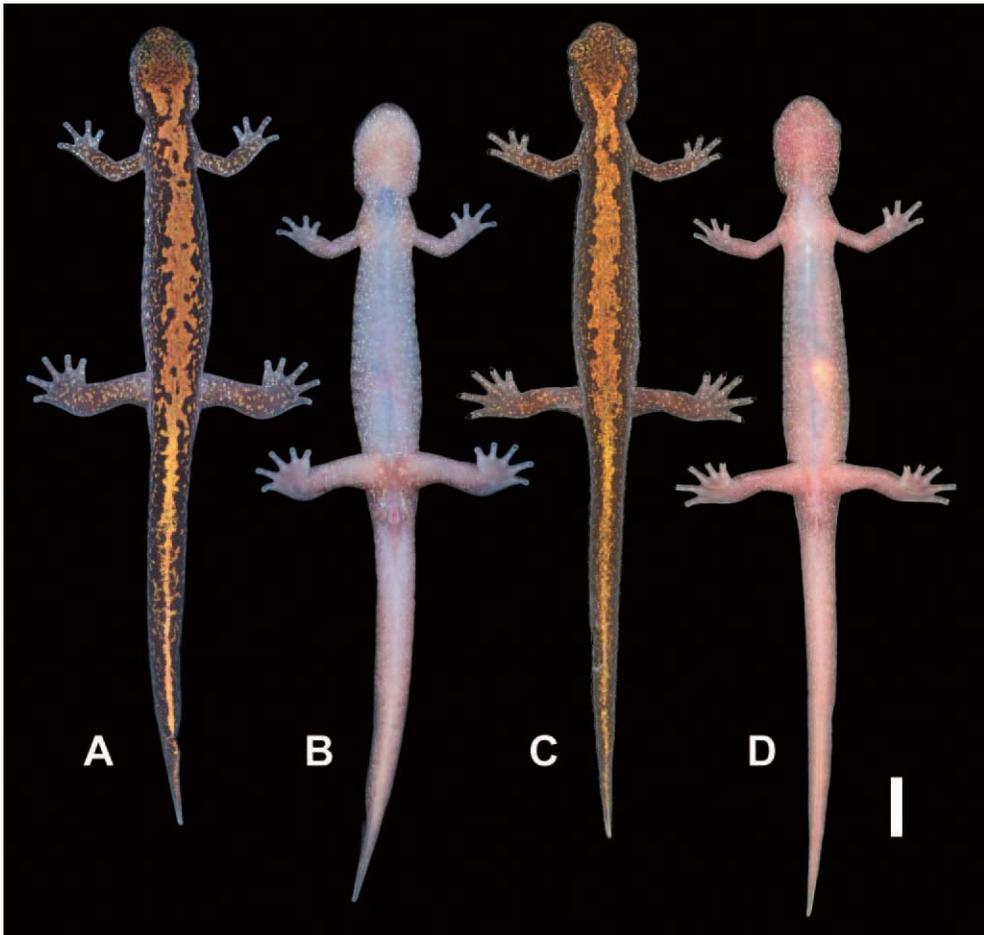


FIG. 3. Dorsal and ventral views of the male holotype (A and B; KUHE 41388, SVL=67.7 mm) and a female paratype of *O. tsukubaensis* (C and D; KUHE 44779, SVL=68.3 mm). Scale bar indicates 10 mm.



FIG. 4. Holotype of *O. tsukubaensis* in life (KUHE 41388; photo taken on 3 May 2008).

cho), Ibaraki Prefecture, Japan, collected by N. Yoshikawa on 3 May 2008.

Paratypes

A total of 15 specimens, two males (KUHE 42733–42734 collected on 27 March 2009 by N. Yoshikawa, Y. Misawa, and A. Tominaga) and seven females (KUHE 37418 collected on 16 April 2006, KUHE 41389 on 3 May 2008, KUHE 42462 on 5 December 2008, all by N. Yoshikawa; KUHE 42735–42738 on 27 March 2009 by N. Yoshikawa, Y. Misawa, and A. Tominaga) from the type locality, two males (KUHE 44930–44931 on 19 June 2011 by N. Yoshikawa and K. Eto) and three females (KUHE 44779 on 6 May 2011 by N. Yoshikawa; KUHE 44932–44933 on 19 June 2011 by N. Yoshikawa and K. Eto) from Mt. Tsukuba,

Tsukuba-shi (a stream different from the type locality), and one male (KUHE 39783 collected on 19 May 2007 by N. Yoshikawa) from Mt. Kaba, Sakuragawa-shi, Ibaraki Prefecture.

Diagnosis

A medium sized *Onychodactylus* (lung absent; black horny claws present on tips of fingers and toes of breeding adults and larvae; vomerine teeth in two short, transverse, distinctly arched series; larvae with skin folds on posterior edges of limbs; breeding males with dermal flaps on posterior edge of hindlimb; breeding in flowing water under the ground; eggs few, large, and pigmentless); body relatively thick, with clearly edged light-colored dorsal stripe; presacral vertebrae including atlas usually 18; costal grooves 12; right and left vomerine tooth series continuous, without gap, series tending to curve anteriorly at inner junction in females; vomerine teeth on one side of series usually 15–18; tail relatively short, equal to or slightly longer than SVL in males, but shorter in females; tail also short in larval stage.

Description of holotype

An adult male with SVL 67.7 mm (Figs. 3 and 4); body thick; head oval and depressed, longer than wide; neck narrower than head; snout rounded, projecting beyond lower jaw; nostril close to snout tip; eye large, shorter than snout, prominently protruding, laterally nearly reaching level of outer edge of upper jaw; gular fold posteroventral to head; parotoid gland well developed, oval, ca. 1.7 times longer than wide, extending from angle of jaw to gular fold; postorbital groove obvious, running to parotoid gland; vomerine teeth in two transverse, distinctly arched series without gap between them (Fig. 5); each series with 17 tooth; vomerine tooth series surrounded by a group of weakly dark pigments; forelimb thin, forearm slightly thicker than upper arm; relative length of fingers $I < IV < II < III$; hindlimb longer and distinctly more robust than forelimb; thigh and tibia equally thick; relative

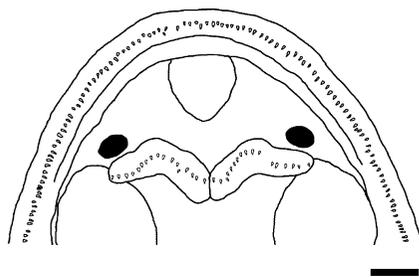


FIG. 5. Open mouth of holotype of *O. tsukubaensis* (KUHE 41388) showing the shape of the vomerine tooth series. Scale bar indicates 1 mm.

length of toes $I < V < II < III = IV$; no dermal flap on posterior edge of hindlimb; tips of fingers and toes rounded without black claws; no palmer or tarsal tubercles, or black asperities on palm or sole; trunk elongated and cylindrical; a middorsal groove from end of head to level of cloaca; 12 well developed costal grooves on both sides of trunk; presacral vertebrae including atlas numbering 18; cloaca not swollen; vent longitudinally slit, length 7.1% SVL, with anterior half of its edge slightly swollen, forming inverse V-shaped skin fold; tail short, 99% length of SVL, cylindrical at base, increasingly compressed posteriorly; posterior three-fourths of tail laterally compressed; tail highest (11.4% SVL) at distal one-fourth length.

Color

In life, color grayish brown with obvious reddish brown dorsal stripe (Figs. 3, 4); border of stripe sharply defined, but wavy and mottled; indistinctly dotted pattern on head turning into dorsal stripe, widened toward posterior end of eye but narrowed at neck, and again widened on trunk; on tail, dorsal stripe clear and brightest at base, but narrowed and obscure toward tip; side of body with dense silvery spots laterally to ventrally; upper iris uniformly golden, lower iris blackish. In alcohol, dorsal reddish brown coloration bleached, silvery dots on body somewhat indistinct, and grayish background color slightly faded. Ventral color also becomes whitish.

TABLE 1. Comparisons of snout-vent length (SVL: means \pm 1SD, followed by ranges in parenthesis, in mm) and percentage ratios of each of the other character dimensions to SVL (indicated by R: medians, followed by ranges in parenthesis) and HL (indicated by HR) in three *Onychodactylus* species.

Species	Sex	n	SVL	RTAL	RBTAH	RBTAW	RMTAH	RMTAW	RAGD	RFLL	RHLL	RHL	RHW	RUEL	RIND
<i>O. tsukubaensis</i>															
	Male	6	69.5 \pm 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)	7.3 (7.0–7.5)
	Female	10	66.9 \pm 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)	7.4 (6.9–7.8)
<i>O. japonicus</i>															
	Male	9	68.9 \pm 3.6 (63.3–72.7)	119.6 (108.7–128.1)	9.1 (7.8–11.1)	10.9 (9.4–12.3)	8.5 (7.7–9.3)	6.3 (5.6–7.4)	51.3 (48.2–54.4)	27.6 (25.9–29.0)	31.0 (27.6–37.8)	22.0 (20.8–23.4)	13.9 (13.0–14.4)	6.1 (5.5–6.5)	7.4 (7.2–7.8)
	Female	9	74.7 \pm 4.0 (68.9–78.1)	109.3 (95.1–116.8)	8.5 (7.9–10.4)	9.2 (8.2–10.7)	8.4 (6.8–9.1)	6.3 (4.3–8.0)	54.3 (53.2–56.6)	26.8 (25.5–28.6)	31.3 (28.7–33.3)	21.5 (20.7–22.6)	14.3 (13.0–15.0)	5.9 (5.4–6.4)	7.3 (6.8–8.1)
<i>O. nipponoborealis</i>															
	Male	20	64.2 \pm 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)	7.4 (6.7–8.1)
	Female	26	64.4 \pm 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)	7.6 (6.3–8.4)
Species sex n REND RIOD RCW RSL RIC RHW HRUEL HRIND HREND HRIOD HRSL HRIC															
<i>O. tsukubaensis</i>															
	Male	6	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)	67.1 (65.4–74.2)	27.8 (25.8–28.5)	32.5 (31.4–33.1)	14.8 (12.6–16.6)	19.4 (18.5–20.6)	34.3 (32.1–37.1)	41.3 (37.1–43.7)	
	Female	10	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)	67.1 (64.2–76.1)	26.5 (25.5–29.6)	31.5 (29.7–34.0)	14.1 (13.2–16.2)	19.2 (17.0–20.4)	34.9 (32.1–35.9)	39.1 (37.0–43.7)	
<i>O. japonicus</i>															
	Male	9	3.1 (3.0–3.5)	4.3 (4.0–5.1)	12.3 (10.8–13.1)	7.4 (7.2–8.3)	8.7 (8.4–9.7)	62.5 (60.0–66.7)	28.0 (24.8–29.3)	34.2 (31.5–36.9)	14.3 (13.4–15.6)	19.3 (17.8–24.1)	34.2 (32.5–36.9)	39.3 (38.1–45.4)	
	Female	9	3.2 (2.8–4.1)	4.7 (3.8–5.2)	12.6 (10.9–13.4)	7.6 (7.0–8.1)	8.8 (8.1–9.7)	65.4 (60.1–70.2)	27.4 (25.3–29.8)	33.9 (31.8–37.7)	14.9 (12.9–19.4)	21.2 (17.2–24.5)	35.3 (31.9–37.1)	41.7 (37.4–45.7)	
<i>O. nipponoborealis</i>															
	Male	20	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)	66.1 (56.8–73.6)	26.4 (23.8–29.4)	32.7 (29.4–35.5)	14.6 (12.1–16.8)	21.1 (17.7–24.4)	34.8 (30.5–39.4)	37.2 (32.9–48.6)	
	Female	26	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)	66.1 (62.5–78.7)	26.7 (24.5–31.1)	33.0 (29.3–39.3)	13.7 (11.6–17.4)	21.2 (15.8–24.2)	34.8 (31.6–38.5)	37.8 (35.3–44.4)	

Measurements (in mm) and counts of the holotype

SVL 67.7, HL 16.0, HW 11.1, TAL 67.0, AGD 34.6, FLL (L) 18.8, FLL (R) 18.5, HLL (L) 21.7, HLL (R) 21.4, UEL (L) 4.2, UEL (R) 4.3, IOD 3.1, END (L) 2.3, END (R) 2.5, ICD 6.4, IND 5.1, SL (L) 5.2, SL (R) 5.2, CW 8.7, BTAH 7.2, BTAW 7.2, MTAH 7.5, MTAW 5.6.

Number of vomerine teeth 17 in both right and left series. Costal grooves 12 on both left and right sides. Adpressed limbs overlapping by 1.0 costal fold. Number of presacral vertebrae including atlas 18.

Variation

The following description of variation is based on the maximum number of six adult males and 10 adult females of the type series. Morphometric data are summarized in Table 1 together with those of the reference species, *O. japonicus* and *O. nipponoborealis*. Although males (mean \pm 1SD = 69.5 \pm 3.1 mm, n = 6) tended to be larger in SVL than females (66.9 \pm 2.1 mm, n = 10), there was no significant sexual dimorphism (Student's t-test, $P > 0.05$). Males tended to have a longer tail relative to SVL than females, but they were not significantly different (Mann-Whitney's U test, $P > 0.05$). Males had a more robust hindlimb than females, and had a dermal skin fold on the posterior edge of the hindlimb in the breeding season. The distal end of the fibula

projected further posteriorly in males than in females. The number of costal grooves varied from 11 to 12 (Table 2), and was not significantly different between the sexes. The degree of overlap of forelimbs and hindlimbs was greater in males (median = 0.8 folds) than in females (median = 0 fold). The number of presacral vertebrae including the atlas varied from 17 to 19, although it was usually 18. The number of vomerine teeth varied from 12 to 20 (mean = 15.8 \pm 3.0) in males and 12 to 22 (17.8 \pm 2.3) in females, and the mean values tended to be larger in females, although no significant sexual variation was detected (Student's t-test, $P > 0.05$). Females usually had a vomerine tooth series in which the inner branch curved medially to form a short sub-branch directed anteriorly, but males lacked such a structure.

Color in life was highly variable among individuals. A wide dorsal stripe, straight, wavy, or mottled, was typically present and was rarely absent. The dorsal stripe was ochre or reddish-brown, while the background color varied from dark-gray or gray-brown to purplish-gray. The border between the dorsal stripe and the ground color was usually sharply defined, but was sometimes partly obscured by merging with the background. The silvery dots on the lateral and ventral sides of the body varied from sparse to dense. The upper half of the iris was either uniformly

TABLE 2. Variation in the number of presacral vertebrae (PSVN) and costal grooves (CGN) on each side of the body in three *Onychodactylus* species. R and L indicates right and left side of body, respectively.

Species	Sex	n	PSVN			CGN(L)			CGN(R)		
			17	18	19	11	12	13	11	12	13
<i>O. tsukubaensis</i>											
	Male	6		5	1	1	5		1	5	
	Female	10	1	9		1	9		2	8	
<i>O. japonicus</i>											
	Male	9		5	4	2	4	3	2	6	1
	Female	9		8	1		8	1		8	1
<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

golden or mottled with black.

Larva

SVL and TAL 18.5–45.6 mm and 11.7–39.1 mm, respectively, for a TL of 30.2–81.8 mm; head rectangular and blunt at snout (Fig. 6); 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 cloaca; ventral fin originating from around posterior one-third to one-fourth 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. Hatchling in life with blackish spots on yellowish-gray dorsum, and whitish and transparent ventrum; blackish spots expanding and joining each other dorsolaterally on trunk and tail with growth, forming narrow brownish middorsal stripe on trunk, and relatively wide light yellow dorsal stripe on upper edge of tail. Well-developed, premetamorphic larvae often with dense silvery mottling and dots, increasing with larval development on entire body; light-yellow dorsal stripe obvious on upper edge of tail; venter whitish or purplish-gray with silvery dots.

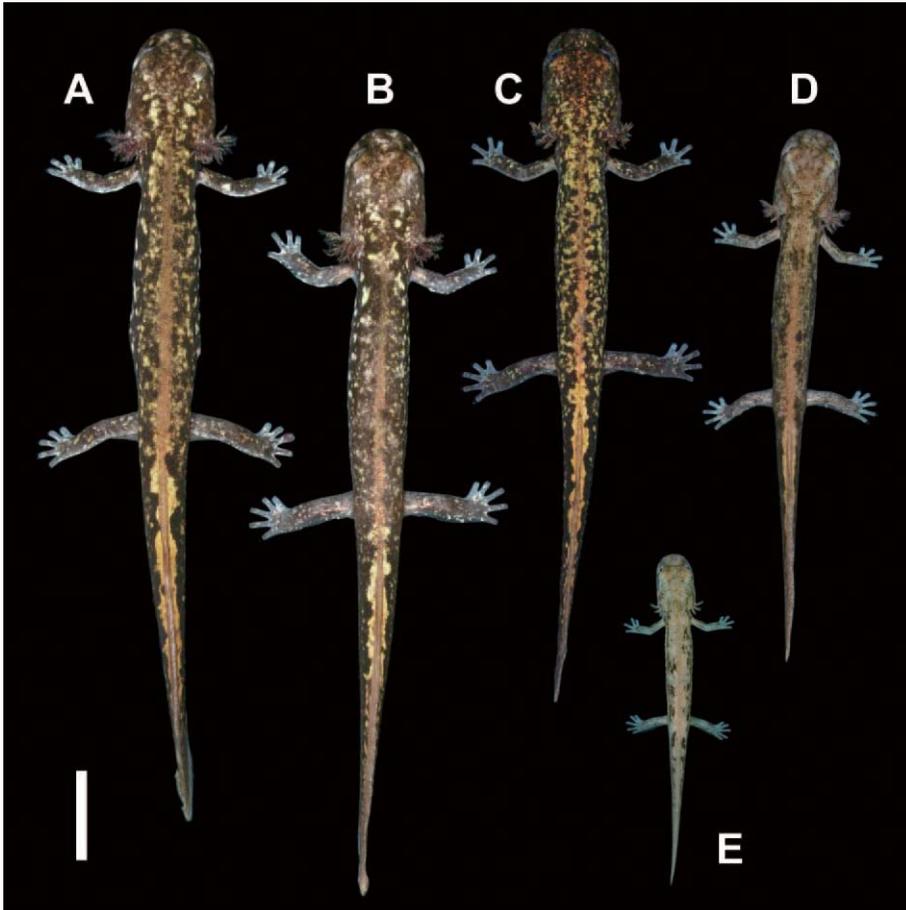


FIG. 6. Dorsal views of premetamorphic (A–C), growing (D), and hatchling (E) larvae of *Onychodactylus tsukubaensis*. Scale bar indicates 10 mm.

Comparisons

Data from Poyarkov et al. (2012) were used to compare *Onychodactylus tsukubaensis* with the other congeneric species, *O. fischeri*, *O. koreanus*, *O. zhangyapingi*, and *O. zhaoermii*.

Onychodactylus tsukubaensis differs from *O. fischeri*, *O. koreanus*, *O. zhangyapingi*, and *O. zhaoermii* in having a wide dorsal stripe (vs. absent in other species) and shorter tail relative to SVL (102.3% in males and 90.4% in females vs. 120.7–136.8% in males and 104.0–110.9% in females in other species). In addition, *O. tsukubaensis* differs from *O. fischeri* in having a shorter trunk relative to SVL (51.6% in males and 52.4% in females vs. 61.0% in males and 57.6% in

females in *O. fischeri*), a smaller number of presacral vertebrae (normally 18 vs. 20–21) and costal grooves (12 vs. 14–15), and a larger number of vomerine teeth (17.0 ± 2.7 vs. 11.5 ± 1.2). From *O. koreanus*, *O. tsukubaensis* also differs in having longer forelimbs and hindlimbs relative to SVL in females (26.4% and 30.5%, respectively, vs. 23.7% and 28.1% in *O. koreanus*), and a smaller number of presacral vertebrae and costal grooves (normally 18 and 12, respectively, vs. 18–19 and 13 in *O. koreanus*). Other than possessing (vs. lacking) a dorsal stripe, *O. tsukubaensis* differs from *O. zhangyapingi* in having a relatively shorter trunk (51.6% in males and 52.4% in females vs. 54.0% and 56.5%, respectively, in *O. zhangyapingi*) and a relatively wider head

TABLE 3. Variation in the number of vomerine teeth (means \pm 1SD, followed by ranges in parenthesis), gap between right and left sides of the vomerine tooth series, and the short sub-branch of the vomerine tooth series at the inner junction (number followed by percentage in parenthesis) in three *Onychodactylus* species. L: left; R: right.

Species	Sex	n	VTN (L)	VTN (R)	VTN (average)	Gap		Short sub-branch of VTS		
						Present	Absent	L+R	L or R	Absent
<i>O. tsukubaensis</i>										
	Male	6	16.0 \pm 3.2 (12–20)	15.5 \pm 3.0 (12–19)	15.8 \pm 3.0 (12–20)	1	5	—	1	5
						(16.7)	(83.3)	—	(16.7)	(83.3)
	Female	10	18.2 \pm 2.2 (14–22)	17.1 \pm 2.5 (12–20)	17.8 \pm 2.3 (12–22)	—	10	7	—	3
						—	(100)	(70.0)	—	(30.0)
	Combined	16	17.4 \pm 2.7 (12–22)	16.7 \pm 2.8 (12–20)	17.0 \pm 2.7 (12–22)	1	15	7	1	8
						(6.3)	(93.7)	(43.7)	(6.3)	(50.0)
<i>O. japonicus</i>										
	Male	6	12.5 \pm 2.2 (10–15)	13.8 \pm 1.3 (12–16)	13.2 \pm 1.9 (10–16)	—	6	1	1	4
						—	(100)	(16.7)	(16.7)	(66.6)
	Female	4	13.8 \pm 1.9 (11–15)	14.3 \pm 4.4 (10–19)	14.0 \pm 3.2 (10–19)	2	2	1	1	2
						(50.0)	(50.0)	(25.0)	(25.0)	(50.0)
	Combined	10	13.0 \pm 2.1 (10–15)	14.3 \pm 4.4 (10–19)	13.5 \pm 2.4 (10–19)	2	8	2	2	6
						(20.0)	(80.0)	(20.0)	(20.0)	(60.0)
<i>O. nipponoborealis</i>										
	Male	20	15.0 \pm 5.0 (4–24)	15.2 \pm 4.4 (5–23)	15.1 \pm 5.8 (4–24)	6	14	9	2	9
						(30.0)	(70.0)	(45.0)	(10.0)	(45.0)
	Female	26	15.4 \pm 3.8 (8–23)	15.0 \pm 3.6 (9–20)	15.2 \pm 3.6 (8–23)	9	17	14	5	7
						(34.6)	(65.4)	(53.8)	(19.2)	(27.0)
	Combined	46	15.2 \pm 4.3 (4–24)	15.1 \pm 3.9 (5–23)	15.2 \pm 4.2 (4–24)	15	31	23	7	16
						(32.6)	(67.4)	(50.0)	(15.2)	(34.8)

(15.4 in males and 15.6% in females vs. 14.9% and 14.5%, respectively, in *O. zhangyapingi*). Similarly, from *O. zhaoermii*, *O. tsukubaensis* differs in having a shorter trunk relative to SVL (51.6% in males and 52.4% in females vs. 52.9% and 55.9%, respectively, in *O. zhaoermii*) and a larger number of vomerine teeth (17.0 ± 2.7 vs. 13.8 ± 1.3).

Comparison of *Onychodactylus tsukubaensis* with two closely related species, *O. japonicus* and *O. nipponoborealis* produced the following results (Tables 1, 2, and 3). In SVL, males of *O. tsukubaensis* and *O. japonicus* from Kanagawa, including topotypic specimens, do not differ, but in females, *O. tsukubaensis* is smaller than *O. japonicus*. There is no significant difference in SVL between *O. tsukubaensis* and *O. nipponoborealis* from its entire range including the type series (Tukey-Kramer test, $P > 0.05$).

In SVL and HL, *O. tsukubaensis* significantly differs from *O. japonicus* in the following characters. In males, *O. tsukubaensis* has a relatively wider head than *O. japonicus* (relative to SVL and HL: 15.4% and 67.1%, respectively, vs. 13.9% and 62.5% in *O. japonicus*). Although not statistically significant, *O. tsukubaensis* tends to have a shorter tail (102.3% vs. 119.6% in *O. japonicus*), longer head (22.9% vs. 22.0%), wider chest (13.1% vs. 12.3%), larger intercanthal distance (9.2% vs. 8.7%), relative to SVL, and a smaller internarial distance (32.5% vs. 34.2%) relative to HL. In females, *O. tsukubaensis* differs from *O. japonicus* in having a significantly longer and wider head (23.3% and 15.6%, respectively, vs. 21.5% and 14.3% in *O. japonicus*) and a wider chest (13.4% vs. 12.6%), relative to SVL, and tends to have shorter tail (90.4% vs. 109.3%) relative to SVL, and smaller intercanthal (39.1% vs. 41.7%) and internarial distances (31.5% vs. 33.9%) relative to HL. *Onychodactylus tsukubaensis* also differs from *O. japonicus* in having a larger number of vomerine teeth (17.0 ± 2.7 vs. 13.5 ± 2.4 in *O. japonicus*).

In males, *O. tsukubaensis* differs from *O. nipponoborealis* in having a significantly

shorter tail relative to SVL (102.3% vs. 125.7% in *O. nipponoborealis*). Although not significant, *O. tsukubaensis* tends to have a shorter hindlimb (30.5% vs. 33.5% in *O. nipponoborealis*) relative to SVL, a larger intercanthal distance (relative to both SVL and HL: 9.2% and 41.3%, respectively, vs. 8.5% and 37.2% in *O. nipponoborealis*), and a smaller interorbital distance (19.4% vs. 21.1% in *O. nipponoborealis*) relative to HL. In females, *O. tsukubaensis* differs from *O. nipponoborealis* in having a significantly shorter tail (90.4% vs. 106.7% in *O. nipponoborealis*) relative to SVL and a smaller interorbital distance (19.2% vs. 21.2% in *O. nipponoborealis*) relative to HL. Also, *O. tsukubaensis* tends to have a shorter hindlimb (30.5% vs. 32.4% in *O. nipponoborealis*) relative to SVL, larger intercanthal distance (relative to both SVL and HL: 9.1% and 39.1%, respectively, vs. 8.6% and 37.8%), and smaller interorbital distance (19.2% vs. 21.2%) relative to HL, although these differences are not significant.

Onychodactylus tsukubaensis has a significantly smaller number of presacral vertebrae (18) than *O. nipponoborealis* (18–19) in females. The number of vomerine teeth tends to be larger in *O. tsukubaensis* (17.0 ± 2.7) than in the other two species (13.5 ± 2.4 in *O. japonicus* and 15.2 ± 4.2 in *O. nipponoborealis*), but the difference is not significant. The gap between the vomerine tooth series is rarely present in *O. tsukubaensis* (only in 6.3% of the individuals examined), whereas the gap is present in 20.0% and 32.6% of *O. japonicus* and *O. nipponoborealis*, respectively. The shape of the vomerine teeth series of *O. tsukubaensis* was most similar to that of *O. nipponoborealis*, but the occurrence of an anteriorly directed curve at the inner juncture was less frequent than in *O. nipponoborealis*.

It is not easy to distinguish *O. tsukubaensis* from *O. japonicus* or *O. nipponoborealis* by coloration, because it is highly variable and overlaps with species. However, *O. tsukubaensis* tends to differ from topotypic *O. japonicus* in having an ochre or reddish-

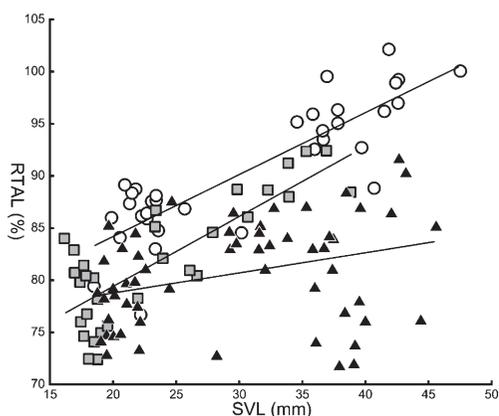


FIG. 7. Relationships between SVL and relative length of tail (RTAL) in larvae of the three species of *Onychodactylus*. Closed triangles: *O. tsukubaensis*, $RTAL = 0.19 SVL + 74.91$, $r^2 = 0.1045$; open circles: *O. japonicus*, $RTAL = 0.59 SVL + 72.36$, $r^2 = 0.7341$; shaded squares: *O. nipponoborealis*, $RTAL = 0.66 SVL + 66.04$, $r^2 = 0.6126$.

brown dorsal stripe with a distinct edge and having dense, silvery dots (vs. reddish or orangish clear dorsal stripe and few silvery dots in *O. japonicus*). *Onychodactylus tsukubaensis* is more similar to *O. nipponoborealis* in coloration, but the occurrence of a distinct broad dorsal stripe and dense silvery dots seems to be more frequent in *O. tsukubaensis*.

Larvae of the new species have a relatively shorter tail than those of the other species of the *O. japonicus* complex, due to a significantly slower growth rate of the tail relative to SVL (Fig. 7). Relative to HL, the head tends to be wider than in the two related species. Therefore, larvae of the new species look somewhat stouter than in the other species. The occurrence of silver dots and mottling on the entire body and the light yellow dorsocaudal stripe, which is comparatively more distinct and wider than in the other species, are important characteristics of *O. tsukubaensis*.

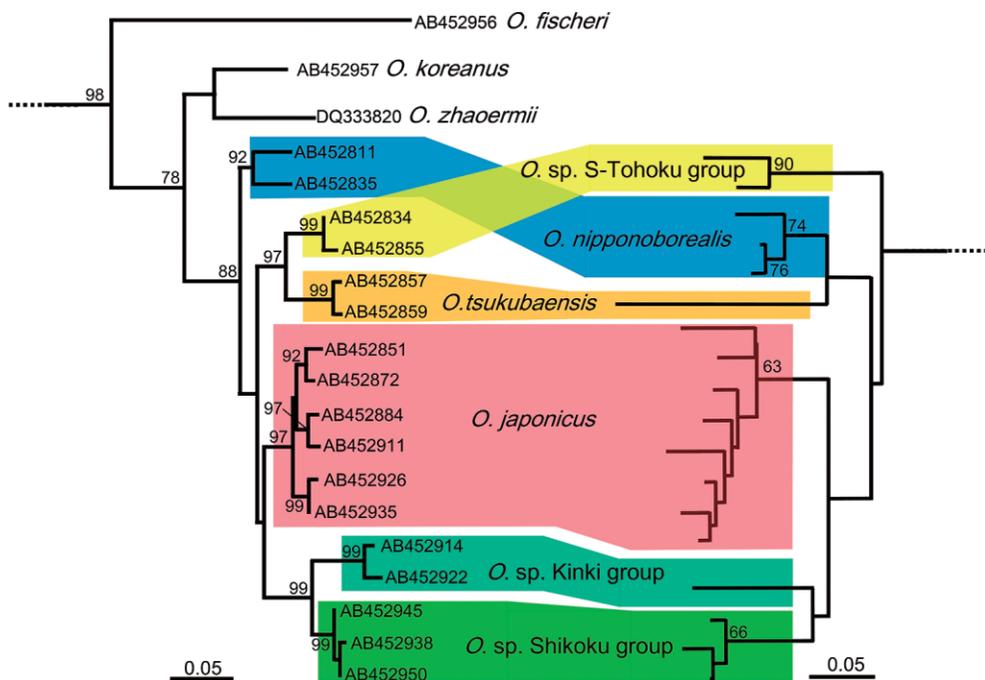


FIG. 8. A maximum likelihood tree based on 1141 bp of mitochondrial cytochrome b gene (left) and a neighbor-joining tree based on 14 allozyme loci (right), modified from Yoshikawa et al. (2008) and (2010a), respectively. Values at nodes indicate supports of 2000 and 1000 bootstrap replicates, respectively. Scale bars indicate substitutions per site and Cavalli-Sforza and Edwards' (1967) chord distance, respectively.

Karyotype

No karyotypic information is available for *O. tsukubaensis*.

Genetic characteristics

The new species is a member of the *O. japonicus* species complex, and corresponds to Clade II-B or the Tsukuba group of Yoshikawa et al. (2008, 2010a, 2012). Molecular phylogenetic analysis based on the cytochrome b gene of mtDNA has revealed that *O. tsukubaensis* is a sister taxon to Subclade II-A (*Onychodactylus* sp. S-Tohoku group; Fig. 8), with a mean uncorrected p distance of 5.23% (4.82–5.70%: Yoshikawa et al., 2008). Although the mitochondrial genetic distance between *O. tsukubaensis* and *O.* sp. S-Tohoku was relatively small, *O. tsukubaensis* is clearly distinct and divergent from the other genetic groups in allozymic analysis (Yoshikawa et al., 2012). For the degree of genetic differentiation between *O. tsukubaensis* and other described or unnamed cryptic species, refer to Yoshikawa et al. (2008, 2010a, 2012).



FIG. 9. A pair of egg sacs of *O. tsukubaensis* laid in the laboratory by a female paratype (KUHE 44779; 17 days after oviposition).

Fecundity and natural history

The fecundity of *O. tsukubaensis* in nature is unknown, and only one pair of egg sacs was obtained in the laboratory. One female (KUHE 44779) collected on 6 May 2011 and kept under completely dark conditions at a water temperature of about 11 C laid a pair of egg sacs on 24 May 2011. The egg sacs were gelatinous and spindle shaped (29.1 and 23.9 mm long immediately after oviposition; 40.4 and 31.1 mm long 17 days later) containing pigmentless, completely yellowish eggs (Fig. 9). The number of eggs in each egg sac was 10 and six, but one ripe egg remained in the ovary after oviposition, indicating that the clutch size of this female was 17. Diameters of the eggs ranged from 4.4 to 5.9 mm (mean \pm 1SD = 5.3 \pm 0.4). Eggs in the sacs were arranged in two rows immediately after oviposition, but the eggs in the larger sac moved into three rows after the egg sac swelled. The outer gelatinous layer of the egg sacs was elastic but very strong, and was difficult to tear by hand. The egg sacs adhered strongly to a stone by short, gelatinous stalks. Very weak, longitudinal grooves could be seen on the surface of the egg sac.

The ecology of adults in the non-breeding season is poorly known, but they inhabit cool, humid, well-forested mountains. From our observations on Mts. Tsukuba and Kaba, they



FIG. 10. A breeding site of *O. tsukubaensis* on Mt. Tsukuba (ca. 700 m asl). Arrow indicates opening of the underground stream.

appear to breed underground in headstreams (ca. 550–700 m asl), where the amount of water and the temperature (7.9–9.5 C) is stable throughout the year. The breeding season seems to be from late May to mid June, since we found mature adults converging on the breeding sites before this season, although Hayase and Oseki (1983) reported the arrival of breeding adults at the headstream around late April to the middle of May. Observation of actual breeding is very difficult because the known breeding sites are all deep inside underground streams, which flow out from cracks in huge rocks (Fig. 10).

According to Hayase and Yamane (1989), who studied larval life history on Mt. Tsubakuro (a peak neighboring Mt. Kaba), hatchlings (ca. 35 mm in TL) appeared in February and March, lived in the stream for three years before metamorphosis in July to October, at ca. 80 mm in TL. During our observations on Mts. Tsukuba and Kaba, hatchlings with a yolk (mean = 35.6 ± 2.0 mm in TL, $n = 15$) appeared in headstreams by late March, dispersed downstream and grew to metamorphose at TL of ca. 80 mm or more. Because metamorphosing larvae are often found near the headstream, they might return to the upper stream reaches before or during metamorphosis.

Range

Onychodactylus tsukubaensis is known so far only from eastern Japan, from Mts. Tsukuba, Kaba, Ashio, Kinoko, Tsubakuro, and the adjacent mountains of Ibaraki Prefecture (Tsukuba-shi, Sakuragawa-shi, and Ishioka-shi), in the Tsukuba Mountains (Fig. 1). The northern limit known so far is Mt. Tsubakuro, and the southern limit is the southern slope of Mt. Tsukuba. All known localities are higher than 350 m asl, and the upper limit is near the top of Mt. Tsukuba (871 m asl). Older reports suggest a much wider range of this species in the past: Sato (1943) recorded the species from Saruda, Higashinaka-mura (currently Saruda, Sakuragawa-shi), located north of Mt. Tsubakuro, and Kosuge (1979) reported a record from Mts. Tomiya and

Takamine (Fig. 1), located ca. 9 km north of Mt. Tsubakuro (all as *O. japonicus*).

Conservation

The distribution of the new species is very limited, occurring only on the upper parts of the Tsukuba Mountains, and this species may be one of the Japanese endemic salamanders with the smallest distributional ranges. At present, this species is locally abundant, and some of their habitats are protected, but this species is apparently decreasing in number and range due to recent disturbance of their habitat (Kosuge, 1979; Hayase, 2010). We are also afraid that collecting pressure for the pet trade will increase after the present description of the species, because the Tsukuba Mountains are close to urban areas and include popular spots for sightseeing. We propose to expand the current protected area to include all the habitats of this species as quickly as possible.

DISCUSSION

The salamanders inhabiting the Tsukuba Mountains have long been considered to be local populations of *O. japonicus* (sensu lato), which commonly occurs in the montane areas of Honshu and Shikoku Islands of Japan. The occurrence of *O. japonicus* (sensu lato) in the Tsukuba Mountains was first reported by Tago (1907), who listed the northern slope of Mt. Tsukuba as a locality of *O. japonicus* (sensu lato). The following year, Namiye (1908) also reported occurrence of the species on Mt. Tsukuba. Later, Sato (1943) listed Mt. Kaba and an adjacent area (Saruda, Higashi-nakamura) as well as Mt. Tsukuba, as localities of this species.

A series of genetic researches, however, have revealed the presence of extensive genetic diversity within the wide-ranging *O. japonicus* (sensu lato), suggesting that this species actually comprises several cryptic species. Yoshikawa et al. (2008) recognized four greatly differentiated major clades in *O. japonicus* (sensu lato) that were also further subdivided into several subclades. Furthermore,

they conducted allozymic studies (Yoshikawa et al., 2010a, 2010b, 2012), and found the presence of six candidate species within *O. japonicus* (sensu lato). *Onychodactylus tsukubaensis* described here was recognized as a Subclade II-B of mtDNA phylogeny and of the Tsukuba group in allozymic analysis. This group was only moderately differentiated in mtDNA, but clearly distinct in allozymes from the others (Fig. 8).

The morphological data presented in this study demonstrated that *O. tsukubaensis* is also morphologically clearly distinct, although cryptic species within the *O. japonicus* species complex share highly conservative morphology. One of the important characters of the genus *Onychodactylus* is the long tail, usually much longer than SVL, in contrast to the other genera in the family Hynobiidae, where the tail is equal to or shorter than SVL in general (Zhao and Hu, 1988; Fei et al., 2010). However, *O. tsukubaensis* described in this study has a short tail, equal to or only slightly longer in males and shorter in females than SVL. This is a unique feature in the genus *Onychodactylus*, and the most effective diagnostic character of this species. The VTN in *O. tsukubaensis* is relatively larger, and tends to differ among species in the *O. japonicus* complex in which the number is smallest in *O. japonicus*, largest in *O. tsukubaensis*, and intermediate in *O. nipponoborealis*, although the range largely overlaps among species and the validity of this character as a diagnosis seems to be limited. The shape of the vomerine tooth series of *O. tsukubaensis* is similar to that of *O. nipponoborealis*, having a distinctly curved arch and an anteriorly directed curve medially. The VTN and shape of the tooth series of *O. tsukubaensis* seem to differ between sexes, with slightly larger tooth number and more frequent occurrence of anteriorly directed curve in females. Sexual dimorphism in VTN is also reported in *O. fischeri*, with a smaller number of teeth in females (Poyarkov et al., 2012), contrary to the case in *O. tsukubaensis*. The occurrence of an anteriorly directed curve might reflect

the larger number of teeth.

Poyarkov et al. (2012) suggested that VTN and the number of trunk vertebrae (TVN; PSVN in this study) are effective characters to distinguish species of the *O. japonicus* complex. Our observation also indicated the tendency of interspecific difference in VTN. However, Poyarkov et al.'s (2012) count of VTN seems to be incorrect. We found 19 teeth on each side of vomerine tooth series in the holotype of *O. nipponoborealis* described by them, but in the original description, VTN of the holotype was reported to be 17 on each side (Poyarkov et al., 2012). In our observations, two outer teeth on each side were hidden in the gum. Poyarkov et al. (2012) noted that they counted vomerine teeth from photographs, but such a method often overlooks hidden teeth. Therefore, variation reported in the VTN among species of the genus *Onychodactylus* should be reassessed. Besides, we could not find significant differences in PSVN among three species of the *O. japonicus* complex, except for between females of *O. tsukubaensis* and *O. nipponoborealis*. Poyarkov et al. (2012) reported that the number of trunk vertebrae was greater in *O. nipponoborealis* (17–20, mode=18) than *O. japonicus* (16–18, mode=17). As shown above, we also found 18 presacral vertebrae including the atlas in the holotype of *O. nipponoborealis*, and confirmed a variation range of 17–19 vertebrae (mode=18) in *O. nipponoborealis* samples. Therefore, our counting method must be the same as that employed by Poyarkov et al. (2012). However, we could not find any *O. japonicus* samples with 16 or 17 presacral vertebrae, and all our samples had 18–19 (mode=18) vertebrae, showing no difference from *O. nipponoborealis*. Because Poyarkov et al. (2012) included samples from many localities other than the type locality of *O. japonicus* (Hakone-machi, Kanagawa Pref.), their results may have been affected by geographic variations. Thus the PSVN seems to be variable within *O. japonicus* and the validity of this character as diagnostic needs to be reassessed.

We presume that Poyarkov et al.'s (2012) morphological measurements of *O. nipponoborealis* used for interspecific comparison were based not on preserved specimens but on fresh specimens, despite the fact that measurements of all the other species were based on preserved specimens. As a result, their measurements were somewhat strange; for example, their relative head width was much larger than ours (19.1% in their study vs. 15.1% in our study, in males; Table 1), although the corresponding values for *O. japonicus* were similar (13.8% vs. 13.9%, in males; Table 1). It is commonly known that all or parts of the body are considerably shrunk by fixation and preservation (Matsui, 1984; Hayashi, 1993), and it is preferable that the condition of specimens for morphological comparisons are similar. This shrinkage may be the cause of this situation, and therefore, Poyarkov et al.'s (2012) interspecific comparisons seem to be defective, and the morphological differences between *O. nipponoborealis* and other *Onychodactylus* species need to be reexamined.

In Japan, species of *Onychodactylus* are known to occur at relatively high elevations in the southern part and the Pacific Ocean side of their range, whereas they are found in lower elevations in the northern and Japan Sea side (Sato, 1943; Nakamura and Uéno, 1963). Distributional range may be strongly related to annual temperature and winter precipitation, because this genus prefers a cold climate and requires stably abundant and cool underground streams for breeding. The range of *O. tsukubaensis* is relatively lower in elevation than the range of the other *O. japonicus* populations in adjacent areas. Adaptations to low elevation and other specific environmental factors in the Tsukuba Mountains might have enhanced morphological differentiation in this species. Yoshikawa et al. (2010a, 2012) surmised that the large genetic divergence of this species (Tsukuba group in their study) is responsible for the small population size (=limited distributional range) and long-term isolation. Morphological evolution of the new species may also have been accelerated by

such factors.

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APPENDIX 1

Voucher specimens examined are stored at the Graduate School of Human and Environmental Studies, Kyoto University (KUHE) and the Herpetological Collection of the National Museum of Nature and Science, Tokyo (NSMT-H).

Onychodactylus tsukubaensis—Adults: Mt. Tsukuba, Tsukuba-shi, Ibaraki Pref.: KUHE 37418, KUHE 42733–42738, 44779, 44930–44933; Mt. Tsukuba, Sakuragawa-shi Ibaraki Pref.: KUHE 41388–41389, 42462; Mt. Kaba, Sakuragawa-shi, Ibaraki Pref.: KUHE 39783. Larvae: Mt. Tsukuba, Tsukuba-shi, Ibaraki Pref.: KUHE 41378–41387, 42741, 42801–42804; Mt. Tsukuba, Sakuragawa-shi Ibaraki Pref.: KUHE 36556–36566, 36573–36578; Mt. Kaba, Sakuragawa-shi, Ibaraki Pref.: KUHE 39769–39778, 39782, 41375–41376; Mt. Kaba, Ishioka-shi, Ibaraki Pref.: KUHE 42743–42745, 42792–42797, 42799–42800, 43910–43915.

O. japonicus—Adults: Hakone-machi, Kanagawa Pref.: KUHE 46611–46615; Hadano-

shi, Kanagawa Pref.: KUHE 46618–46619; Yamakita-machi, Kanagawa Pref.: KUHE 46616–46617; Gotemba-shi, Shizuoka Pref.: KUHE 45989; Environs of Hakone, Kanagawa Pref.: NSMT-H 00384–00391. Larvae: Hakone-machi, Kanagawa Pref.: KUHE 46600–46610; Gotemba-shi, Shizuoka Pref.: KUHE 45990–45991; Izu-shi, Shizuoka Pref.: KUHE 36746–36749, 36763–36771, 39985–39995.

O. nipponoborealis—Adults: Sai-mura, Aomori Pref.: KUHE 46385–46386, 46388–46392; Hirakawa-shi, Aomori Pref. (Type series): NSMT-H05710–05714; Kita-akita-shi, Akita Pref.: KUHE 37707; Noda-mura, Iwate Pref.: KUHE 37022–37025; Kuji-shi (formerly Yamagata-mura), Iwate Pref.: NSMT-H01172–01174; Iwaizumi-cho, Iwate Pref.: NSMT-H01159–01160, 02063; Mt. Hayachine, Iwate Pref.: NSMT-H01165–01166; Oshu-shi, Iwate Pref.: KUHE 45644–45646; Ichinoseki-shi, Iwate Pref.: KUHE 35276; Izumi-ku, Sendai-shi, Miyagi Pref.: KUHE41348, 45868–45878, 45881, 45883–45885, 45887. Larvae: Izumi-ku, Sendai-shi, Miyagi Pref.: KUHE 45666–45698.

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