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Lefua tokaiensis, a new species of nemacheilid loach from central Japan (Teleostei: Nemacheilidae)

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Abstract

A new nemacheilid loach, *Lefua tokaiensis* sp. nov., is described from the small mountain streams in the Tokai region, central Honshu, Japan. *Lefua tokaiensis* is distinguished from all other species of *Lefua* by the following combination of characters: eyes located dorsally on the head, presence of a narrow conspicuous longitudinal mark between the base of the outer rostral barbel and the eye, absence of a rhomboid or triangular dark blotches on the middle of the caudal fin base, absence of a black longitudinal stripe on both body sides in mature males, absence of dusky cross bars on the dorsal area of the body, absence of a dusky bar beside the dorsal fin base, typical presence of small dark spots on the dorsal and caudal fins, presence of the obscure dark band in skin of the caudal fin base, caudal fin slightly squared off at the apex, and modally four hypurals.

Keywords Endangered species · Head water · Parallel evolution · Tokai region

Introduction

Species of *Lefua* Herzenstein 1888 are small, benthic freshwater fishes, distributed in Mongolia, Russia, China, Korea, and Japan (Kottelat 2012; Hosoya 2013). They are characterized by having a round caudal fin, the anterior nostril formed as long nasal barbel, and absence of postcleithrum and epural (Nichols 1943; Sawada 1982; Prokofiev 2005).

The genus presently includes six valid species: *Lefua costata* (Kessler 1876), *Lefua pleskei* (Herzenstein 1887), *Lefua nikkonis* (Jordan and Fowler 1903), *Lefua echigonia* Jordan and Richardson 1907, *Lefua sayu* (Herre and Lin 1936), and *Lefua torrentis* Hosoya, Ito and Miyazaki 2018 (Kottelat 2013; Hosoya et al. 2018). *Lefua nikkonis*, *L. echigonia*, and *L. torrentis* are endemic to Japan (Hosoya 2013; Hosoya et al. 2018). *Lefua costata* was originally distributed in the Amur drainage, Korea, and northeastern China, and is an invasive species in Yamanashi, Shizuoka, Nagano, and Toyama prefectures, central Japan (Hosoya 2013; Nakajima 2017).

Miyazaki et al. (2011) suggested the populations of the Tokai region of central Japan as an undescribed species of *Lefua* by mitochondrial and nuclear DNA sequences. This species was previously treated as a local population of *L. torrentis*, because their morphological and ecological characteristics are very similar (Miyazaki et al. 2011; Hosoya 2013). However, it is genetically and morphologically distinguishable from *L. torrentis* (Miyazaki et al. 2007; Miyazaki et al. 2011; Hosoya 2015; Nakajima 2017). Here, we describe this species as a new species.

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Materials and methods

Measurements and counts are predominately based on those of Hubbs and Lagler (2004) except for vertebral counts at each end, which followed Hosoya (1983). These were counted from radiographs and included the first four vertebrae with the Weberian Apparatus and one vertebra fused to the pleurostyle in the hypural complex. The last two rays of the dorsal and anal fins were counted as one ray, each pair being associated with a single pterygiophore. Counts and proportions are given first for the holotype, followed by those for the paratypes (if different) in parentheses. The cephalic lateral line systems were stained using Suminol Cyanine 5R. To observe the osteological features of the pectoral girdle and the caudal complex, we cleared and counterstained several specimens prepared by the method of Kawamura and Hosoya (1991). We also observed osteological features of the caudal complex using radiographs. Osteological terminology follows those of Sawada (1982) and Fujita (1990).

Abbreviations used are: CAS, California Academy of Sciences, San Francisco; CAS-SU, Stanford University, now at CAS; KUN-P, Kindai University, Nara; NSMT-P, National Museum of Nature and Science, Tsukuba; TKPM-P, Tokushima Prefectural Museum, Tokushima; TMNH-F, Toyohashi Museum of Natural History, Toyohashi; USNM, National Museum of Natural History, Smithsonian Institution, Washington; TIC, Taiki Ito private collection, Japan.

Lefua tokaiensis sp. nov.

(New English name: Tokai stream eight-barbeled loach)
(Figs. 1, 2, 3, 4, 5; Table 1)

Lefua sp.: Hosoya 2002: 277, 1467 (in part).

Lefua sp. Tokai population: Miyazaki et al. 2007: 666–675; Miyazaki et al. 2011: 416–427.

Lefua sp. 2: Hosoya 2013: 334; Nakajima 2017: 198–203, 211; Miyazaki et al. 2018: 140–148; Nakajima 2018: 116–117.

Holotype. NSMT-P 132821, 44.2 mm SL, 34° 58' 24" N, 137° 34' 52" E, Japan, Aichi, Shinshiro, Kadoya, Toyo river system, 23 Aug. 2018, collected by T. Ito and J.-I. Miyazaki.

Paratypes. 21 specimens (all from Japan). CAS 245809, 35.7 mm SL, TMNH-F 2387, 37.2 mm SL, USNM 445698, 40.8 mm SL, collected with holotype. KUN-P 44626, 36.9 mm SL, KUN-P 44628, 48.8 mm SL, KUN-P 44630, 45.1 mm SL, KUN-P 44632, 46.9 mm SL, KUN-P 44635, 53.8 mm SL, Aichi, Okazaki, Myoken, Yahagi river system, 7 Aug. 2014, collected by M. Takemoto. KUN-P 44636, 46.8 mm SL, KUN-P 44644, 49.3 mm SL, KUN-P 44645, 49.7 mm SL, Aichi, Kitashitara, Shitara, Toyo river system, 7 Aug. 2014, collected by M. Takemoto. KUN-P 44651, 43.7 mm SL, KUN-P 44654, 48.6 mm SL, KUN-P 44655, 55.0 mm SL, Aichi, Okazaki, Hosomitsu, Yahagi river system, 8 Aug. 2014, collected by M. Takemoto. KUN-P 44657, 40.6 mm SL, KUN-P 44660, 40.8 mm SL, KUN-P 44664, 52.5 mm SL, Shizuoka, Syuchi, Tenryu river system, 8 Aug. 2014, collected by M. Takemoto. KUN-P 44667, 39.3 mm SL, KUN-P 44675, 49.6 mm SL, KUN-P 44684, 41.8 mm SL, Shizuoka, Hamamatsu, Mikkabi, Tenryu river system, 8 Aug. 2014, collected by M. Takemoto. TKPM-P 7382, 37.0 mm SL, Shizuoka, Hamamatsu, Mikkabi, Tenryu river system, 22 Aug. 2018, collected by T. Ito.

Non-types. 2 specimens (all from Japan). TIC 1 (cleared and stained), 60.3 mm SL, TIC 2 (cleared and stained), 56.1 mm SL, Shinshiro, Kadoya, Toyo river system, 19 Aug. 2010, collected by T. Morishita.

Diagnosis. *Lefua tokaiensis*, *Lefua torrentis* and *Lefua echigonia* are distinguished from all other species of *Lefua* by the following combination of characters: absence of a rhomboid or triangular dark blotches on middle of caudal fin base (vs. presence in *Lefua costata*, *Lefua pleskei* and *Lefua nikkonis*), absence of a black longitudinal stripe on both body sides in mature male (vs. presence in *L. costata*, *L. pleskei* and *L. nikkonis*), absence of dusky cross bars on dorsal area of body (vs. presence in *Lefua sayu*), and absence of a dusky bar beside dorsal fin base (vs. presence in *L. sayu*).

Lefua tokaiensis and *L. torrentis* are distinguished from *L. echigonia* by eyes located dorsally on head (vs. dorso-laterally on head) and presence of a narrow conspicuous

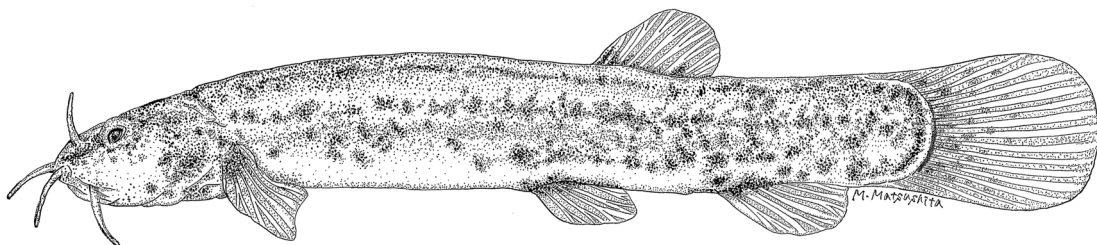


Fig. 1 *Lefua tokaiensis* sp. nov.; NSMT-P 132821, holotype, 44.2 mm SL

Fig. 2 Typical body coloration of *Lefua tokaiensis* sp. nov.; NSMT-P 132821, holotype, 44.2 mm SL



longitudinal mark between base of outer rostral barbel and eye (vs. absence, or light and/or indistinct if present).

Lefua tokaiensis is distinguished from *L. torrentis* by typical presence of small dark spots on dorsal and caudal fins (rarely absent) (vs. absence or sparse if present), presence of obscure dark band in skin of caudal fin base (vs. absence), caudal fin slightly squared off at the apex (vs. fan shaped), and modally four hypurals [vs. five (range: 4–5)].

Description. General body shape as in Figures 1–3. Morphometric data of holotype and paratypes are listed in Table 1. Body elongated, slender, and rounded anteriorly to compressed posteriorly; body depth about equal from occiput to caudal fin base. Head small (less than one-fourth of SL), slightly dorsoventrally compressed. Snout relatively long, comprising 39.7% of head length (30.9–41.5%), with tip rounded in dorsal view. Eye very small, positioned on dorsolateral surface of head (Fig. 6), not visible in ventral view of head. Mouth subterminal; small and inferior, slightly arched; upper and lower lips fleshy and smooth (Fig. 7). No median incision in upper lip. A median notch in lower lip. Three pairs of barbels;

two pairs of rostral barbels and one pair of maxillary barbels. Inner rostral barbel relatively short, reaching to or slightly past anterior border of eye when extended, outer rostral barbel longest, slightly past posterior border of eye when extended, maxillary barbel extends slightly past posterior border of eye horizontally. Nostrils separated from each other; anterior nostril formed as long nasal barbel; posterior opening larger than anterior one; anterior nostril at anterior side of a nasal barbel, close to base. Nasal barbel reaching to or slightly past posterior border of eye when extended. Dorsal and ventral keels on caudal peduncle; dorsal keel starting slightly posterior to extremity of dorsal fin base, ventral keel starting very close to posterior extremity of anal fin base. Caudal peduncle 1.2 (1.1–1.6) times longer than its depth (depth including keels). Scales embedded on body. Lateral line absent. Dorsal fin rounded. Origin of dorsal fin nearer to caudal fin base than to tip of snout. Pectoral fin horizontal. Pelvic fin origin in front of dorsal fin origin. Anal fin rounded. Anus positioned slightly anterior to anal fin. Caudal fin rounded; slightly squared-off apex.

Fig. 3 Color variant of *Lefua tokaiensis* sp. nov.; TKPM-P 7382, paratype, 37.0 mm SL



Fig. 4 Underwater photograph of typical body coloration of *Lefua tokaiensis* sp. nov. in a mountain stream of Toyo river system, Aichi, Shinshiro, Kadoya



Fig. 5 Underwater photograph of color variant of *Lefua tokaiensis* sp. nov. in a mountain stream of Tenryu river system, Shizuoka, Hamamatsu, Mikkabi

Dorsal fin with 4 simple and 6 (5–6) branched rays. Second branched ray longest. Anal fin with 4 (3–4) simple and 5 branched rays. Pectoral fin with 1 simple and 7 (7–11) branched rays. Pelvic fin with 1 simple and 5 (4–5) branched rays. No pelvic axillary lobe. Caudal fin with 1 (1–2) simple, 7 (4–7) branched, 8 (7–8) branched, and 2 (1–3) simple

principal rays. Total vertebrae 39 (38–41); abdominal vertebrae 20 (19–21); caudal vertebrae 19 (18–20).

Cephalic lateral line system composed of conspicuous superficial neuromasts. No ossified sensory canals.

Pectoral girdle (Fig. 8) consisting of cleithrum, coracoid, posttemporal, scapula, and supracleithrum; postcleithrum

Table 1 Morphometric data for holotype and paratypes (n=21) of *Lefua tokaiensis* sp. nov.

	Holotype	range (n=22)	mean	SD
Standard length (mm)	44.2	35.7–55.0		
In % of standard length				
Head length	21.3	18.8–22.1	20.3	1.0
Body depth	14.0	13.4–17.7	15.6	1.0
Body width	11.7	11.1–13.6	12.3	0.7
Depth of caudal peduncle	13.2	12.5–14.8	13.6	0.6
Length of caudal peduncle	16.1	14.9–19.5	17.2	1.1
Predorsal length	62.9	60.6–64.8	62.6	1.0
Preanal length	74.8	72.6–78.3	75.9	1.3
Prepelvic length	53.6	50.5–57.6	54.0	1.6
Height of dorsal fin	11.3	8.9–13.4	11.3	1.0
Length of dorsal fin base	8.6	8.3–9.8	8.8	0.4
Height of anal fin	10.3	9.4–14.5	12.0	1.2
Length of anal fin base	6.6	6.6–9.6	7.8	0.7
Pectral fin length	12.4	12.4–18.7	15.3	1.5
In % of head length				
Snout length	39.7	30.9–41.5	37.9	2.6
Orbit diameter	13.6	12.6–18.3	14.5	1.2
Interorbital width	36.3	33.6–49.9	40.6	4.7

SD standard deviation

Values of holotype included in range

absent. Caudal skeletal system (Fig. 9) composed of first and second preural centra, four hypurals, haemal spine and arch, opisthural cartilage, parhypural, and pleurostyle; free epural and uroneural absent; modally four hypurals (range: 4–5), 4th and 5th hypurals fused (Fig. 9).

Sexual dimorphism: no obvious sexual dimorphism.

Coloration. After fixation in formalin, body and head yellowish gray except for ventral surface, which is whitish gray (Fig. 2), sometimes dark brown blotches on side and dorsal area of body and caudal peduncle (Fig. 3). Both lips white, one pair of small brown spots inside lower lip tissue (Figs. 2, 3, 7). A narrow conspicuous longitudinal black mark between base of outer rostral barbel and eye. All fins pale to transparent with small dark spots on dorsal and caudal fins (rarely absent: Fig. 10c, d). Obscure dark band in skin of caudal fin base (Figs. 10b, d, 11). In life, body slightly translucent (Figs. 4, 5). In individuals from Tenryu river systems, typical presence of dark brown blotches on body and caudal peduncle (Fig. 3).

Habitat. *Lefua tokaiensis* inhabits small mountain streams surrounded by dense vegetation (Fig. 12). The species has a preference for sandy bottoms with gravels or pebbles, concealing itself under rocks, stones, and fallen leaves in small pools.

Distribution. *Lefua tokaiensis* is known from Aichi and Shizuoka prefectures in central Japan (Fig. 13).

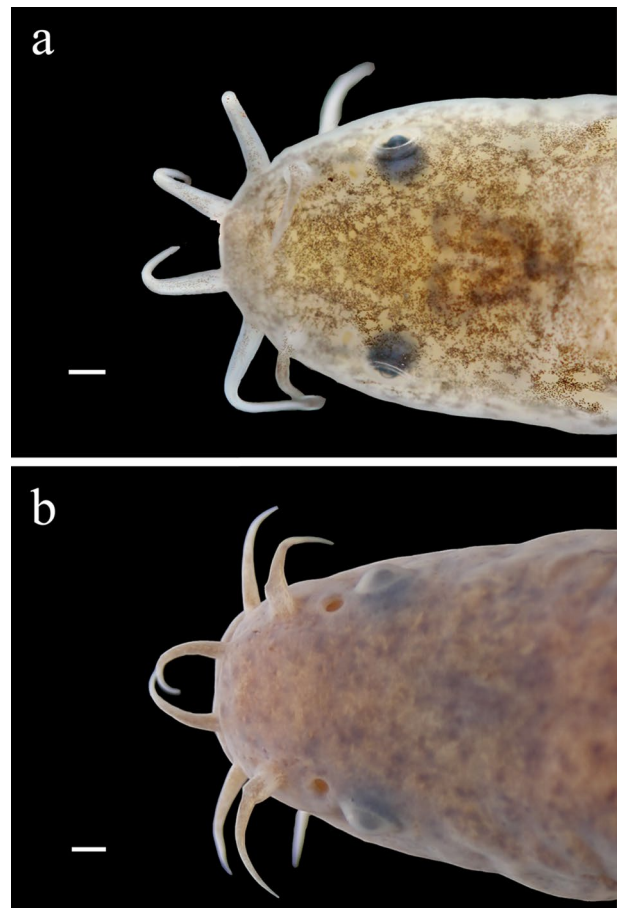


Fig. 6 Close-up of the head in dorsal view. **a** *Lefua tokaiensis* sp. nov., NSMT-P 132821, holotype, 44.2 mm SL; and **b** *L. echigonia*, FRLM 11159, 59.3 mm SL



Fig. 7 Ventral view of mouth of *Lefua tokaiensis* sp. nov., NSMT-P 132821, holotype, 44.2 mm SL. Bar 1 mm

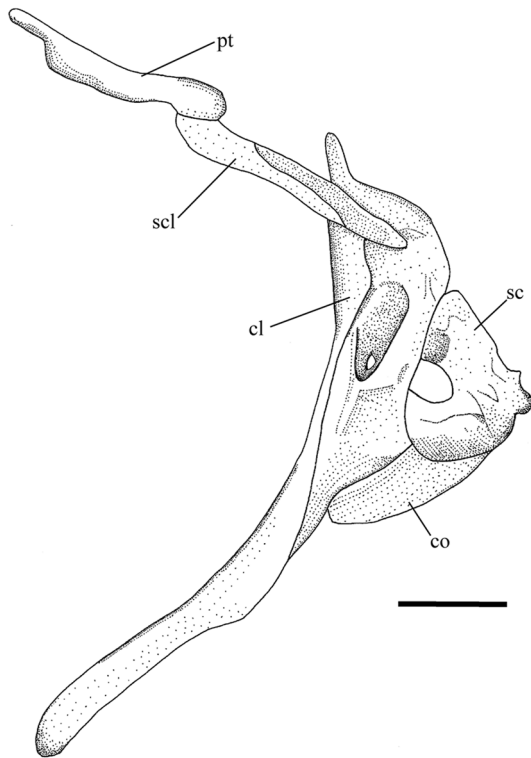


Fig. 8 Lateral view of pectoral girdle of *Lefua tokaiensis* sp. nov., TIC 1, nontype, 60.3 mm SL. *cl* cleithrum; *co* coracoid; *pt* posttemporal; *sc* scapula; *scl* supracleithrum. Bar 1 mm

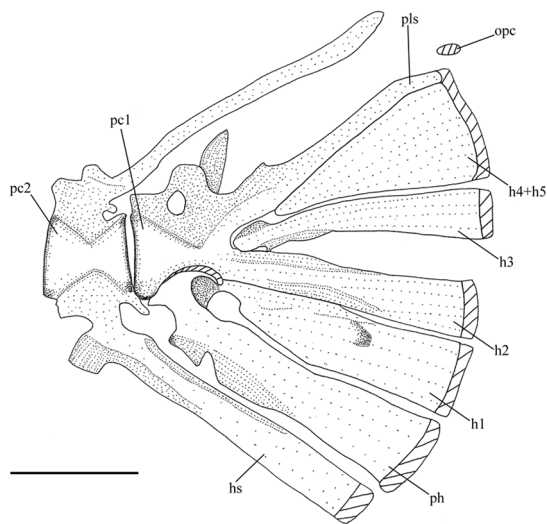


Fig. 9 Caudal skeletal system of *Lefua tokaiensis* sp. nov., TIC 2, nontype, 56.1 mm SL. Cartilage is cross-hatched. *h* hypural; *hs* haemal spine and arch; *opc* opisthural cartilage; *pc 1* first preural centrum; *pc 2* second preural centrum; *ph* parhypural; *pls* pleurostyle. Bar 1 mm

Etymology. The species name “*tokaiensis*” refers to the main distributional area of this species.

Ecology. Females lay eggs within the interstices under rocks and stones in small pools; 1–3 males pursue a female and burrow into the interstices under rocks or stones (Asaka and Uchiyama 2017). The spawning season extends from March to June (Komori et al. 2010).

Remarks. Color variant forms of *L. tokaiensis* coexist with individuals of typical body coloration form, although no supporting genetic differentiation has been found. This suggests that presence or absence of dark brown blotches on the body and caudal peduncle may be an intraspecific variation.

Nakajima (2017) noted one of the diagnostic characters between *L. tokaiensis* and *L. torrentis*: the obscure dark band at the caudal fin base (present in *L. tokaiensis*, but absent in *L. torrentis*). We reconfirmed that *L. tokaiensis* differs from *L. torrentis* by having the obscure dark band in the skin of the caudal fin base. However, this band is sometimes too light to see. To identify *L. tokaiensis* and *L. torrentis*, combining this character with another diagnostic character is needed.

Lefua tokaiensis is extremely similar to *L. torrentis*, but differs by the typical presence of small dark spots on the dorsal and caudal fins (vs. absence or sparse if present), presence of the obscure dark band in skin of the caudal fin base (vs. absence), caudal fin slightly squared off at the apex (vs. fan shaped), and modally four hypurals (vs. five).

Lefua tokaiensis and *L. torrentis* are morphologically very similar and share unique ecology in inhabiting only mountain streams, whereas other congeners have a preference for more lentic waters in swampy streams and ditches. However, *L. tokaiensis* is genetically more closely related to *L. echigonia* rather than to *L. torrentis* in mitochondrial and nuclear DNA sequences (Miyazaki et al. 2011; Miyazaki et al. 2018). Hence, Miyazaki et al. (2011) considered that the general morphological and ecological characteristics of *L. tokaiensis* evolved in parallel with *L. torrentis* from the nearest common ancestor with *L. echigonia* in adapting to the environments of the uppermost reaches of mountain streams.

In Yahagi, Toyo, and Tenryu river systems, *L. tokaiensis* and *L. echigonia* are generally distributed parapatrically. However, *L. tokaiensis* is syntopically distributed together with *L. echigonia* in the Sana River of Yahagi river system. *Lefua tokaiensis* is similar to *L. echigonia* in its genetic and morphological characters (Hosoya 2013, Miyazaki et al. 2007; Miyazaki et al. 2011; Miyazaki et al. 2018), but these two species are reproductively isolated from each other in these river systems, as shown by differences in mitochondrial and nuclear DNA sequences (see Miyazaki et al. 2007; Miyazaki et al. 2011; Miyazaki et al. 2018). In morphology, *L. tokaiensis* differs from *L. echigonia* by presence of

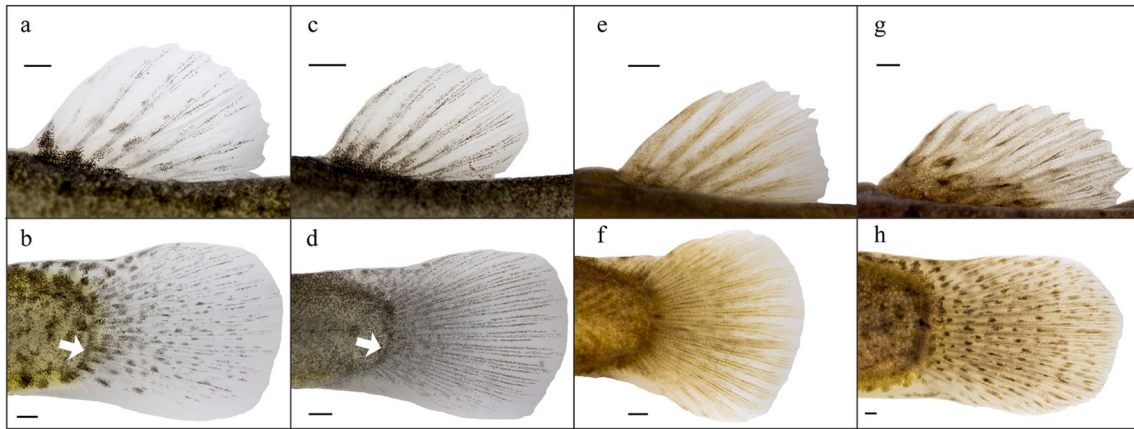


Fig. 10 Pigmentation and shapes of the dorsal (upper) and caudal (lower) fins in: **a–b** *Lefua tokaiensis* sp. nov., NSMT-P 132821, holotype, 44.2 mm SL; **c–d** *L. tokaiensis*, TMNH-F-2387, 37.2 mm SL; **e–f** *L. torrentis*, KUN-P 45408, holotype, 44.9 mm SL; and **g–h** *L.*

echigonia, KUN-P 45399, 47.6 mm SL. **a, c, e, g** dorsal fins; **b, d, f, h** caudal fins. *Arrows* indicate the obscure dark band in skin of the caudal fin base. *Bar* 1 mm

Fig. 11 Distribution of melanophores at the caudal region of *Lefua tokaiensis* sp. nov., TMNH-F-2387, 37.2 mm SL. **a** melanophores on skin and caudal fin membrane; **b** melanophore flocks in skin of the caudal fin base. *odb* obscure dark band

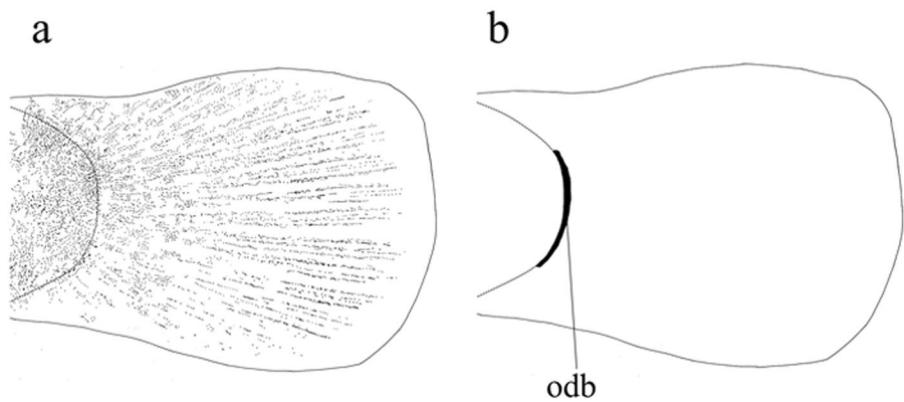
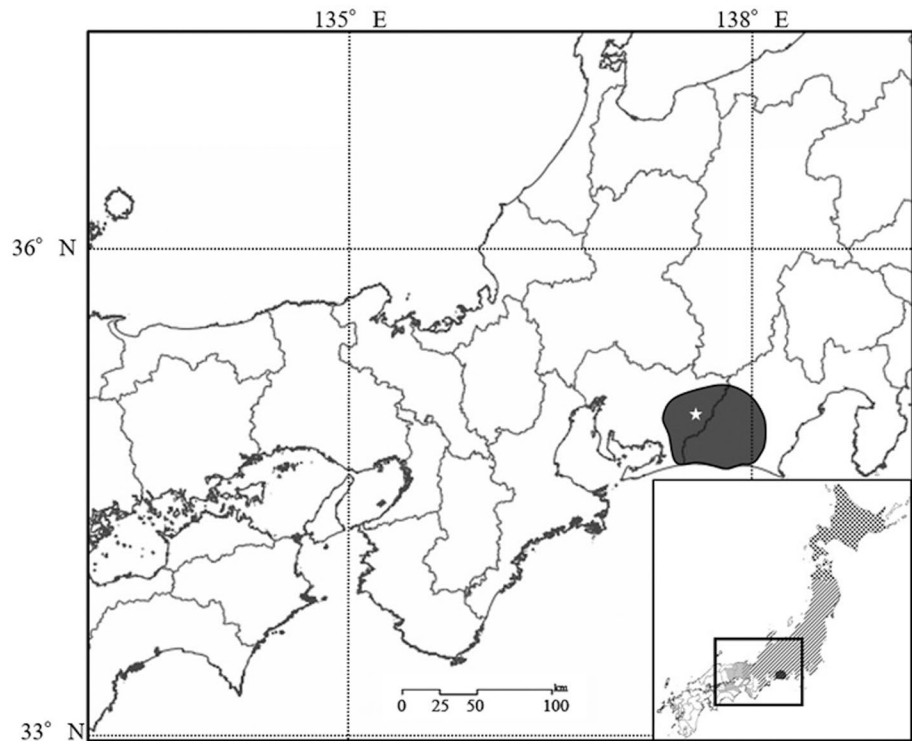


Fig. 12 Type locality of *Lefua tokaiensis* sp. nov. One of the head waters of Toyo river system, Aichi Prefecture, Japan

a narrow conspicuous longitudinal mark between the base of the outer rostral barbel and the eye (vs. absence, or light and/or indistinct if present), and eyes located dorsally on the head (vs. dorsolaterally on the head). Furthermore, *Lefua tokaiensis* inhabits only mountain streams, whereas *L. echigonia* has a preference for the lower reaches of rivers, such as swampy streams and ditches. The genetic distinctiveness of *L. echigonia* and its unique morphological and ecological features indicate that *L. tokaiensis* is a typical biological species sensu Mayr (1942; 1963).

Comparative material examined. *Lefua echigonia*, 50 specimens (27.2–71.3 mm SL), all from Japan: CAS-SU 20164, 39.3 mm SL, holotype, Niigata, Nagaoka; FAKU 115835, 115895–896, 3 specimens, 37.8–71.3 mm SL, Kyoto, Kamo River; FAKU 115849–851, 115854, 4 specimens, 30.7–58.4 mm SL, Mie, Tsu, Geino; FAKU 51051, 115871–879, 115881, 11 specimens, 32.3–52.6 mm SL, Tochigi, Otawara; FAKU 51052, 1 specimen, 47.7 mm SL, Tokyo, Akikawa; FAKU 51053, 115894, 2 specimens, 40.9–48.0 mm SL, Chiba, Yoro River; FAKU 51056,

Fig. 13 Distribution of *Lefua tokaiensis* sp. nov. (black), *L. torrentis* (gray), *L. nikkonis* (dots), *L. echigonia* (cross-hatched). The type locality of *L. tokaiensis* is indicated by a white star



115870, 2 specimens, 27.2–27.7 mm SL, Shiga, Yogo River; FAKU 51058, 1 specimen, 65.2 mm SL, Shiga, Hikone, Echigawa; FAKU 51059, 1 specimen, 53.9 mm SL, Kyoto, Uji; FRLM 11157–162, 6 specimens, 36.4–61.2 mm SL, Mie, Miya River; FRLM 11167–168, 2 specimens, 45.1–47.3 mm SL, Mie, Yokowa River; KUN-P 45398–399, 2 specimens, 47.6–62.4 mm SL, Akita, Konoura; KUN-P 45400–401, 2 specimens, 51.7–66.8 mm SL, Yamagata, Hatsume River; KUN-P 45403–407, 5 specimens, 39.3–60.9 mm SL, Hyogo, Tanba, Kasuga, Yura river system; MNHA 1014140–145, 6 specimens, 31.4–49.5 mm SL, Hyogo, Tanba: Aogaki, Kako river system. *Lefua nikkonis*, 8 specimens (40.6–59.4 mm SL), all from Hokkaido, Japan: CAS-SU 7848, 59.4 mm SL, holotype, Chitose; FAKU 51060, 115882–887, 7 specimens, 40.6–54.7 mm SL, Tomakomai, Tarumae. *Lefua torrentis*, 22 specimens (29.9–56.9 mm SL), all from Japan: KUN-P 45408, 44.9 mm SL, holotype, Hyogo, Tanba, Kasuga, Yura river system; AIFS 0001, CAS 243685, NSMT-P 127393, USNM 440343, 4 paratypes, 29.9–40.9 mm SL, Hyogo, Tanba, Kasuga, Yura river system; FAKU 50366, 1 paratype, 53.1 mm SL, Nara: Yoshino: Kumano river system; KUN-P 42271, OMNH-P 45847, 2 paratypes, 48.2–50.3 mm SL, Kagawa, Syodo-shima, Yoshida River; KUN-P 44141, 44166, 44581, 3 paratypes, 39.5–56.9 mm SL, Hyogo, Minami-Awaji, Sumoto river system, Ayuya River; KUN-P 44162, 44164, 2 paratypes, 48.9–49.3 mm SL, Hyogo, Tatsuno, Shingu, Tsunogame, Chikusa river system; KUN-P 44180, 1 paratype, 35.2 mm SL, Hyogo,

Takarazuka, Tamase, Muko river system, Sou River; KUN-P 44585, 44593, 2 paratypes, 47.9–55.7 mm SL, Okayama, Mimasaka, Ohara, Yoshino river system; KUN-P 44594, 1 paratype, 56.5 mm SL, Kagawa, Nakatado, Mannou, Doki River; KUN-P 45410, WMNH-PIS 10007, 2 paratypes, 39.6–39.9 mm SL, Wakayama, Hidaka, Hidakagawa, Hidaka river system; MNHA A-1014160–161, 2 paratypes, 44.6–48.7 mm SL, Hyogo, Kobe, Kita-ku, Muko river system, Kamagatani River; TKPM-P 7377, 1 paratype, 43.1 mm SL, Tokushima, Myozai, Kamiyama, Yoshino river system.

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