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FULL PAPER

Two new species of *Pseudogobio* pike gudgeon (Cypriniformes: Cyprinidae: Gobioninae) from Japan, and redescription of *P. esocinus* (Temminck and Schlegel 1846)

Koji Tominaga[1,2] · **Seigo Kawase**[3,4]

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Abstract

Two new species of pike gudgeon, *Pseudogobio agathonectris* from western Honshu, Japan, and *P. polysticta* from eastern Honshu, Japan, are described, and *P. esocinus* (Temminck and Schlegel 1846) is redescribed. The two new species were previously considered to be *P. esocinus*, which had been only a nominal species of the genus from Japan. The two species are distinguishable from *P. esocinus* by following a combination of characters: tip of pectoral spiny soft ray not reaching that of 6th soft fin ray, and tip of outer edge of pectoral fin strongly curved posteriorly; long barbel (15.3–36.9% of HL in *P. agathonectris*, 16.9–34.8% in *P. polysticta*, respectively), with the tip commonly extending beyond vertical through anterior edge of eye; 10–14 (modally 13) and 12–14 (13) pectoral soft fin rays; long mouth (33.2–45.3% of HL and 29.6–42.0%); short length between anus and anal fin origin (16.5–21.4% of SL and 15.9–22.4%), with 11–14 (12) and 11–16 (12) scales; and small body size (at most 150 mm SL). *Pseudogobio agathonectris* is distinguishable from *P. polysticta* by the canonical discriminant functions based on five meristic and 25 morphometric characters, and distinct dark blotches and obscure black spots on dorsal and lateral body (vs. obscure blotches and many distinct spots).

Keywords *Pseudogobio agathonectris* · *Pseudogobio polysticta* · Cryptic species · Freshwater fish · Taxonomy

Introduction

The pike gudgeon genus *Pseudogobio* (Cypriniformes: Cyprinidae) comprises benthic freshwater fishes that are widely distributed in Japan, Korea, China, and northern Vietnam (Uchida 1939; Nakamura 1969; Yue 1998; Nguyen and Ngô 2001). Species within this genus are characterized by a protruding snout that is concave in the upper region, fleshy and papillose lips, a pair of barbels, a concave edge of the dorsal fin, and two rows of pharyngeal teeth (Bănărescu and Nalbant 1965). The genus was established by Bleeker (1860) based on the type species *Gobio esocinus* Temminck and Schlegel 1846 collected in Japan. Four species are currently recognized in the genus: *Pseudogobio esocinus* (Temminck and Schlegel 1846) from Japan, Korea and northern China; *Pseudogobio vaillanti* (Sauvage 1878) from a wide area of China; *Pseudogobio guilinensis* Yao and Yang in Luo, Yue and Chen 1977 from the Pearl River system in southern China; and *Pseudogobio banggiangensis* Nguyen in Nguyen and Ngô 2001 from the Pearl River system in northern Vietnam.

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✉ Koji Tominaga
tominaga@kwansei.ac.jp

[1] Division of Biological Sciences, Graduate School of Science, Kyoto University, Kitashirakawa-oiwakecho, Sakyo, Kyoto 606-8502, Japan

[2] Present Address: Kwansei Gakuin Senior High School, 1-155 Uegahara-ichibancho, Nishinomiya, Hyogo 662-8501, Japan

[3] Graduate School of Agriculture, Kindai University, 3327-204, Nakamachi, Nara 631-8505, Japan

[4] Present Address: Osaka University of Economics and Law, 6-10, Gakuonji, Yao, Osaka 581-8511, Japan

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In Japan, *P. esocinus* has been only a nominal species of the genus. *Pseudogobio esocinus* was originally described by Temminck and Schlegel (1846) based on individuals from Japan. Although populations of *P. esocinus* have morphological variation (e.g., Nakamura 1969), few comprehensive studies of their morphology, genetics, or biogeography have been conducted. Recent phylogenetic and phylogeographic studies based on mitochondrial and nuclear DNA sequences have revealed three largely differentiated groups within *P. esocinus* in Japan: Group A from Kyushu, Shikoku, and western Honshu; Group B from the Pacific Ocean side of western Honshu; and Group C from eastern Honshu (Tominaga et al. 2009, 2016). Species trees based on nuclear DNA have indicated that these groups are independent species (Tominaga et al. 2016; Cao et al. 2017); however, detailed morphological comparisons have not yet been performed. In the present study, we demonstrated that *P. esocinus* corresponds to Group A, and that Groups B and C are undescribed species. Here, we describe Group B as *Pseudogobio agathonectris* sp. nov. and Group C as *Pseudogobio polysticta* sp. nov., redescribe *P. esocinus*, and perform detailed morphological comparisons among the three species of Japanese *Pseudogobio* with continental congeners based on specimens from a wide geographic range.

Materials and methods

Count and measurement methods followed Hubbs and Lagler (1964), and all measurements were taken to the nearest 0.1 mm using a digital caliper. The last two rays of the dorsal and anal fins were counted as one ray. We defined the length of lip (from anterior edge to posterior edge of upper lip) as mouth length (ML) because *Pseudogobio* have fleshy, fringed lips. All specimens were fixed in 10% formalin and preserved in 70% ethanol. The specimens examined in this study were deposited in the following institutions: National Museum of Nature and Science, Tsukuba (NSMT); Kyoto University Museum, Kyoto (FAKU); Kindai University, Nara (KUN); Lake Biwa Museum, Kusatsu (LBM); Osaka Museum of Natural History, Osaka (OMNH); Wakayama Prefectural Museum of Natural History, Kainan (WMNH); Mie Prefectural Museum, Tsu (MPM); Kanagawa Prefectural Museum of Natural History, Odawara (KPM); Natural History Museum and Institute, Chiba (CBM); Tokushima Prefectural Museum (TKPM); Nationaal Natuurhistorisch Museum, Leiden (RMNH); Swedish Museum of Natural History, Stockholm (NRM).

To examine whether *Pseudogobio agathonectris* sp. nov., *Pseudogobio polysticta* sp. nov., *Pseudogobio esocinus* and continental congeners could be statistically distinguished by morphological characters, we conducted canonical discriminant analysis (CDA) using all of the morphological data (except for characters with no variation) of the three Japanese species (*P. agathonectris*, $n = 52$, holotype and paratypes; *P. polysticta*, $n = 56$, holotype and paratypes; *P. esocinus*, $n = 70$), *Pseudogobio* sp. collected from Korea ($n = 26$), and *Pseudogobio vaillanti* collected from China ($n = 8$). The Japanese specimens were genetically identified in Tominaga et al. (2016): *P. esocinus* = Group A, *P. agathonectris* = Group B, and *P. polysticta* = Group C. Using the discriminant functions, we confirmed the type specimens of *P. esocinus* ($n = 7$, lectotype and paralectotypes) correspond to *P. esocinus* Group A in Tominaga et al. (2016). The analyses were performed using the "lda" function in the MASS package for R 3.5.1 (R Development Core Team 2018). We used measurements proportional to standard length (SL) or head length (HL), as data for the analyses above. The data of counts and proportional values of measurements were logarithmically transformed.

Pseudogobio agathonectris sp. nov.

(New Japanese name: Nagare-kamatsuka; Figs. 1, 2a, 3a–b, 4a, 5a–d, 6a; Tables 1, 2)

Pseudogobio esocinus (not of Temminck and Schlegel): Kawase 2015: 154–155 (in part), 155, photograph in the middle (no locality information); Mukai 2017: 80–81, photograph 38-1 (Seki, Gifu Prefecture, Japan), photograph 38-2 (Nakatsugawa, Gifu Pref., Japan) and photograph 38-3 (Gujo, Gifu Pref., Japan).

"Kamatsuka" (no designation of scientific name): Okayamatansuigyokenkyukai 1984: 53, photograph on the top (no locality information); Hibakagakukyoikushinkokai 1994: 62, photograph (Ota River, Hiroshima Pref., Japan) and 63, photograph in the middle (Ota River, Hiroshima Pref., Japan); Tominaga 2004: S-9, photograph at the bottom of left column (an irrigation ditch, Uji River., Kyoto Pref., Japan); Naito 2009: 84, photograph on the top (Ota River, Hiroshima Pref., Japan).

Holotype. NSMT-P 128089, 119.4 mm SL, Anraku River, Suzuka River system, Kameyama, Mie Pref., Japan, 24 July 2007, collected by K. Tominaga.

Paratypes. NSMT-P 128090, 112.0 mm SL, same data as holotype; FAKU 206007–206010, 4 specimens, 103.8–114.4 mm SL, same data as holotype; FAKU 206024, 84.0 mm, Nishiki River, Iwakuni, Yamaguchi Pref., Japan, 16 June 2008; FAKU 206025–206031, 7 specimens, 80.5–84.0 mm SL, Ota River, Akiota, Yamagata, Hiroshima Pref., Japan, 17 June 2008; FAKU 206036–206042, 7 specimens, 89.7–105.3 mm SL, Toyo River, Shinshiro, Aichi Pref., Japan, 1 Aug. 2008; FAKU 206043–206044, 2 specimens, 63.4–70.3 mm SL, Kushida River, Matsusaka, Mie Pref., Japan, 8 Sep. 2008, collected by T. Goto; FAKU 206045–206046, 2

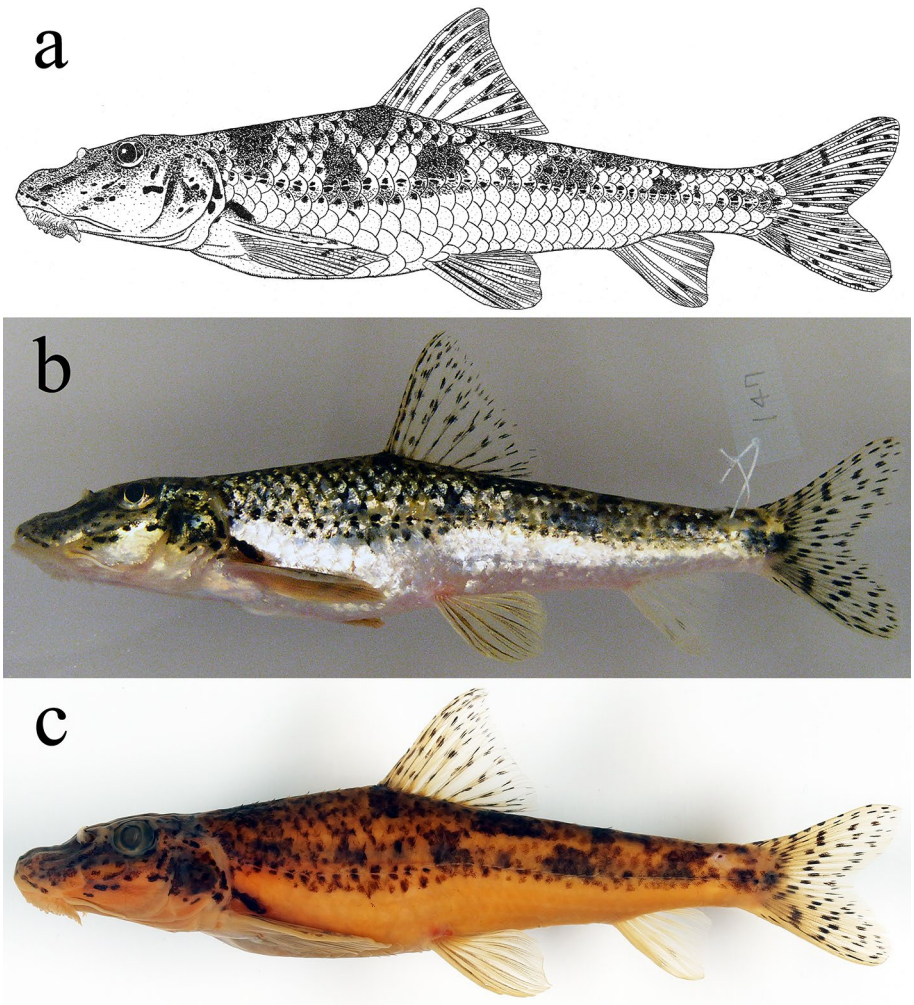

Fig. 1 *Pseudogobio agathonectris* sp. nov., NSMT-P 128089, holotype, 119.4 mm SL, Anraku River, Suzuka River system, Mie Pref., Japan. **a** original drawing; **b** immediately after fixation; **c** after preservation

specimens, 95.3–106.0 mm SL, Nabari River, Yodo River System, Nabari, Mie Pref., Japan, 16 Feb. 2009; FAKU 206047–206055, 9 specimens, 64.0–104.0 mm SL, Makuni River, Kino River system, Kinokawa, Wakayama Pref., Japan, 23 May 2009; KUN-P 47511, 91.1 mm SL, Takami River, Kino River system, Higashiyoshino, Yoshino, Nara Pref., Japan, 4 Sep. 2007; LBM 1210056397–1210056404, 8 specimens, 84.3–101.3 mm SL, Tamura River, Yasu River system, Lake Biwa, Koka, Shiga Pref., Japan, 20 Sep. 2007; OMNH-P 45709, 143.4 mm SL, same data as FAKU 206045–206046; MPM-Fi 1512–1513, 2 specimens, 117.1–120.4 mm SL, same data as FAKU 206043–206044; KPM-NI 48980–48981, 2 specimens, 112.0–113.1 mm SL, same data as holotype; CBM-ZF-0019258, 85.2 mm SL, same data as FAKU 206025–206031; WMNH-PIS.10478, 97.0 mm SL, same data as FAKU 206047–206055; RMNH.PISC. 38473, 107.1 mm SL, same data as FAKU 206024; NRM 70399, 102.2 mm SL, same data as FAKU 206036–206042. All specimens in the list without a collector's name were collected by K. Tominaga.

Non-type specimen. FAKU 207416, 105.6 mm, Shorenji River, Yodo River system, Nara Pref., Japan, 27 Sep. 2015, collected by K. Tominaga (only photographic observation).

Diagnosis. *Pseudogobio agathonectris* is distinguishable from *Pseudogobio esocinus* by the following combination of characters: tip of pectoral spiny soft ray not reaching that of 6th soft fin ray (Fig. 2a) (vs. almost reaching 6th soft ray: Fig. 2c), and tip of outer edge of pectoral fin strongly curved posteriorly (Fig. 3a) (vs. gently curved: Fig. 3e); long barbel (15.3–36.9% of HL vs. 11.9–27.9%) with the tip commonly extending beyond vertical through anterior edge of eye (Fig. 3b) (vs. not or barely reaching vertical through anterior edge of eye: Fig. 3f); 10–14 (modally 13) pectoral soft fin rays [vs. 12–16 (14)]; long mouth (33.2–45.3% of HL vs. 18.2–40.7%); short length between anus and anal fin origin (16.5–21.4% of SL vs. 18.7–26.5%), with 11–14 (12) scales [vs. 12–16 (13)]; and small body size (at most 150 mm SL vs. > 180 mm SL). This new species can be distinguished from the most similar congener *Pseudogobio polysticta* by the canonical discriminant functions based on five meristic and

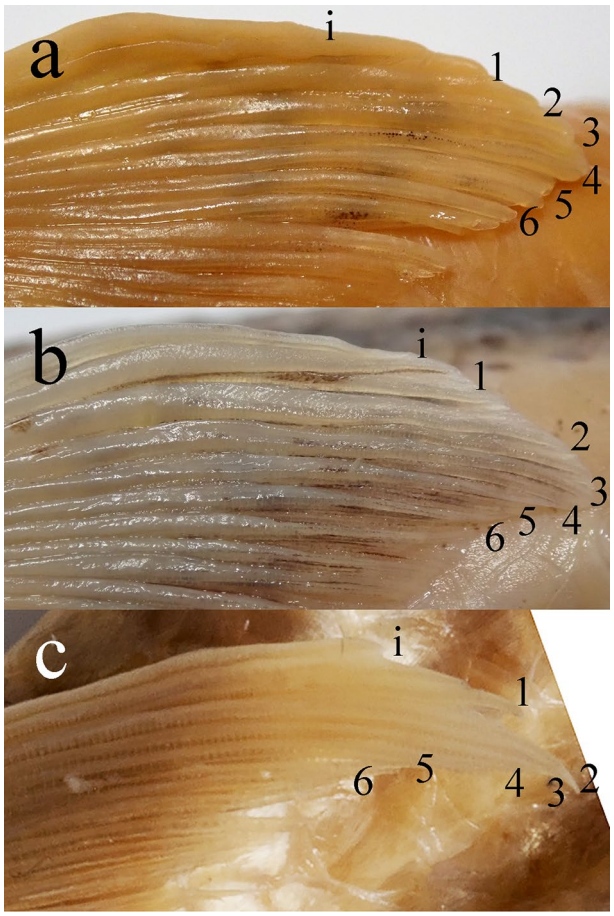

Fig. 2 Comparison of ventral view of the pectoral fin tip in three species of Japanese *Pseudogobio*. *Roman and Arabic numerals* indicate the tip of spiny soft ray and the tip of soft rays, respectively. **a** *Pseudogobio agathonectris* sp. nov., NSMT-P 128089, holotype, 119.4 mm SL; **b** *Pseudogobio polysticta* sp. nov., NSMT-P 128091, holotype, 116.9 mm SL; **c** *Pseudogobio esocinus*, RMNH 2478, lectotype, 143.2 mm SL

25 morphometric characters, and distinct dark blotches and obscure black spots on dorsal and lateral body (vs. obscure blotches and many distinct spots). *Pseudogobio agathonectris* is distinguishable from other congeners by the following combination of characters: long mouth (33.2–45.3% of HL vs. 28.8–31.5% in *Pseudogobio vaillanti*); long barbel (15.3–36.9% of HL) with the tip commonly extending beyond vertical through anterior edge of eye (vs. short barbel, 9.1–19.1% of HL and not or barely reaching vertical through anterior edge of eye in *P. vaillanti*); short length between anus and anal fin origin (16.5–21.4% of SL vs. 19.4–25.6% in *P. vaillanti*); short and high caudal peduncle (the length: height ratio < 200% vs. long and low peduncle, > 200% in *Pseudogobio guilinensis*); 10–14 (13) pectoral soft fin rays (vs. 11 in *Pseudogobio banggiangensis*); and a black stripe on lateral body absent (vs. present in *P. banggiangensis*).

Description. The meristic and morphometric measurements for type specimens are summarized in Tables 1 and 2. All of the raw data are shown in Electric Supplementary Material (ESM) Table S1. The following description is based on holotype, except for pharyngeal teeth. Variation seen in paratypes is given in parentheses.

Dorsal fin rays iii, 7; anal fin rays iii, 6 (5–6, rarely 5); pectoral fin rays i, 14 (10–14, modally 13, rarely 10–11); pelvic fin rays ii, 7. Pharyngeal teeth present in two rows with a dental formula of 1, 5–5, 1 or 2, 5–5, 2. Body elongated, cylindrical, and anteriorly tapered, with the posterior region somewhat laterally compressed. Head relatively large, 29.7 (26.7–31.4) % of SL; snout relatively long, 49.6 (44.0–52.8) % of HL, protruding and dorsally concave. Mouth long, 39.7 (33.2–45.3) % of HL, and extended downward, with fleshy lips with many well-developed papillae (Figs. 3b, 4a). Eye relatively small, 18.3 (17.0–25.5) % of HL, and positioned on upper-middle of head. A pair of long barbels, 30.4 (15.3–36.9) % of HL, with the tips commonly extending beyond vertical through anterior edge of eyes (Fig. 3b). Pectoral fin wide, positioned low on the body, and horizontally spread, with the tip of the outer edge strongly curved posteriorly because of short pectoral spiny soft ray (Figs. 2a, 3a). Dorsal fin high, 19.4 (15.8–21.6) % of SL, with a slightly concave edge. Pelvic fin origin positioned at the approximate SL center, and dorsal fin origin positioned just before it. Anus between pelvic fins, and anal fin positioned near posterior end of the body. In all, 11 (11–14, modally 12) scales between anus and anal fin origin. Caudal fin moderately forked. Trunk lateral line complete, and 38 (36–40, modally 39, rarely 36 and 40) pored lateral line scales. Cephalic lateral line complete; infraorbital canal connected with supraorbital and preoperculomandibular canals; rostral branch present; supratemporal canals of both sides connected.

Color in life. Dorsal side of head and body dark yellowish brown, mid-lateral side yellowish brown, and ventral side white. Mid-lateral body with a series of distinct bluish-black amorphous blotches, equal or larger in size than eye diameter. A series of distinct black saddle-shaped markings on mid-dorsal body, and obscure small black spots on dorsal and lateral sides of head and body (Figs. 1b, 3a, 5a–d, 6a). A bright yellow-gold stripe under the blotches on mid-lateral body. Dorsal, pectoral, and caudal fins faded yellowish brown with 3–4 rows of small black spots along the fin rays. Pelvic fin faded yellowish brown with narrow black lines along the fin rays. Anal fin almost colorless, but some individuals (> 90 mm SL) with narrow black lines along the fin rays.

Color in preservation. Dorsal and lateral side of head and body faded brown; ventral side faded yellowish brown. A series of several bluish-brown blotches on mid-lateral body, a series of dark brown saddle-shaped markings on

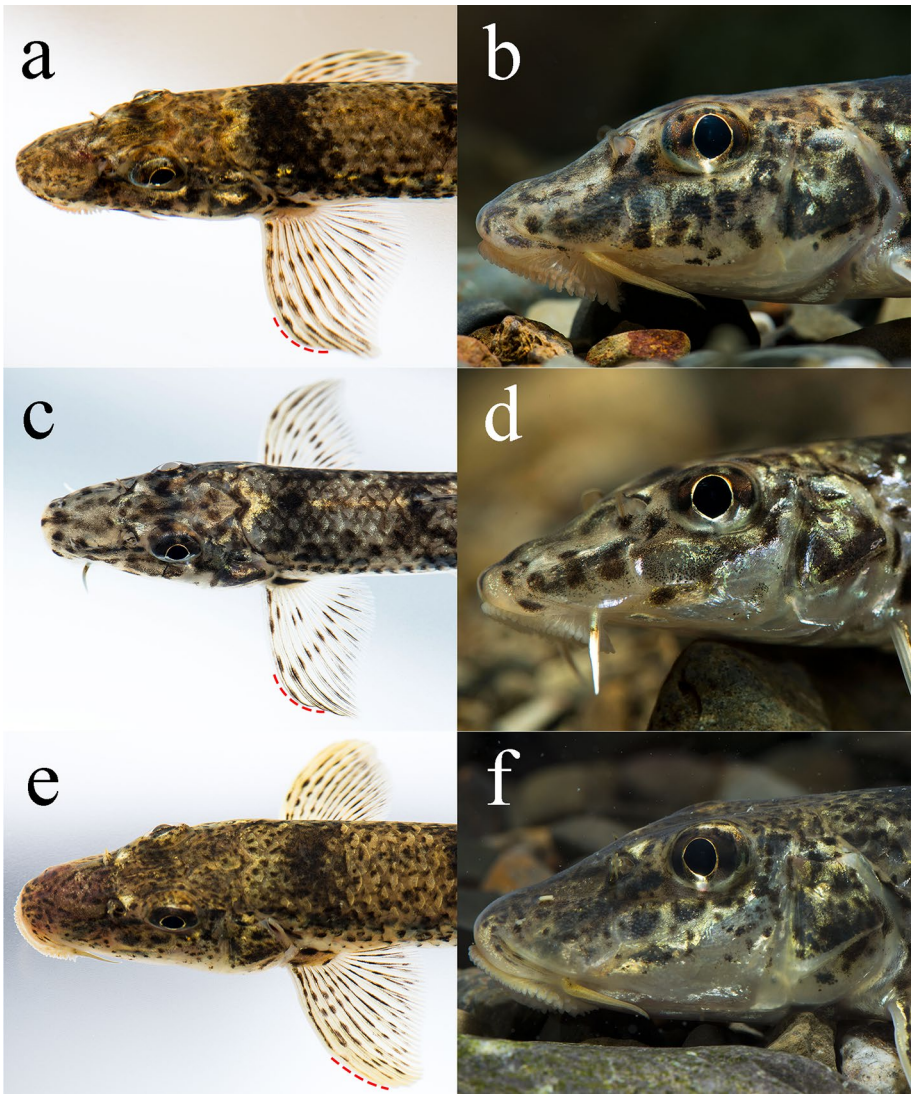

Fig. 3 Comparison of pectoral fin and head in three species of Japanese *Pseudogobio*. *Red broken lines* indicate the outer contour of the pectoral fin tip. **a**, **b** *Pseudogobio agathonectris* sp. nov., FAKU 207416, 105.6 mm SL, Shorenji River, Yodo River system, Nara Pref.; **c**, **d** *Pseudogobio polysticta* sp. nov., FAKU 207417, 93.2 mm SL, Obuta River, Ichinomiya River system, Chiba Pref.; **e**, **f** *Pseudogobio esocinus*, FAKU 207415, 122.8 mm SL, Shorenji River, Yodo River system, Nara Pref. All specimens were collected by K. Tominaga and photographed by R. Uchiyama

mid-dorsal body, and small dark brown spots on dorsal and lateral sides of head and body (Fig. 1c). Fins almost colorless with black spots or lines as same as those of living specimen.

Distribution. *Pseudogobio agathonectris* is distributed along the Pacific Ocean side of western Honshu, Japan. We have confirmed specimens from the Nishiki River system in Yamaguchi Prefecture to the Tenryu River system in Shizuoka Prefecture, but not from the southern Kii Peninsula (Fig. 7). The distribution area is enclosed in that of *P. esocinus*.

Habitat and biology. *Pseudogobio agathonectris* inhabits the sandy but somewhat rocky bottoms of the upper to middle reaches of rivers (Fig. 6b), and sometimes appears with *P. esocinus*. During the day, *P. agathonectris* often burrows in the sand accumulated behind large rocks in riffles. By contrast, *P. esocinus* is usually found in the sand accumulated in pools. Sometimes, *P. agathonectris* floats to medium depth and actively swims with pelagic fish [e.g., Ayu *Plecoglossus altivelis altivelis* (Temminck and Schlegel 1846) and *Opsariichthys platypus* (Temminck and Schlegel 1846); Fig. 6a]. Such behavior is rarely observed for *P. esocinus*. At dusk, *P. agathonectris* feeds actively in riffles. Spawning season is inferred to be May to July, when the water temperature is around 20 °C. In our aquarium observations, at night, this species scattered eggs and sperms, which drift with the current. The spawning behaviors were similar to those of *P. esocinus* (Nakajima and Onikura 2016b). However, most of the life history of *P. agathonectris* is unknown.

Ethymology. The specific name, *agathonectris*, is a compound noun of two Greek words, "agathos," meaning 'excellent, good, superior' and "nektes," meaning 'swimmer.' This species is good swimmer compared to other Japanese congeners (see the 'Habitat and biology' section), particularly *P. esocinus*, which are typical benthic fish. The Japanese word "nagare" means 'river flow'; this species inhabits upper to

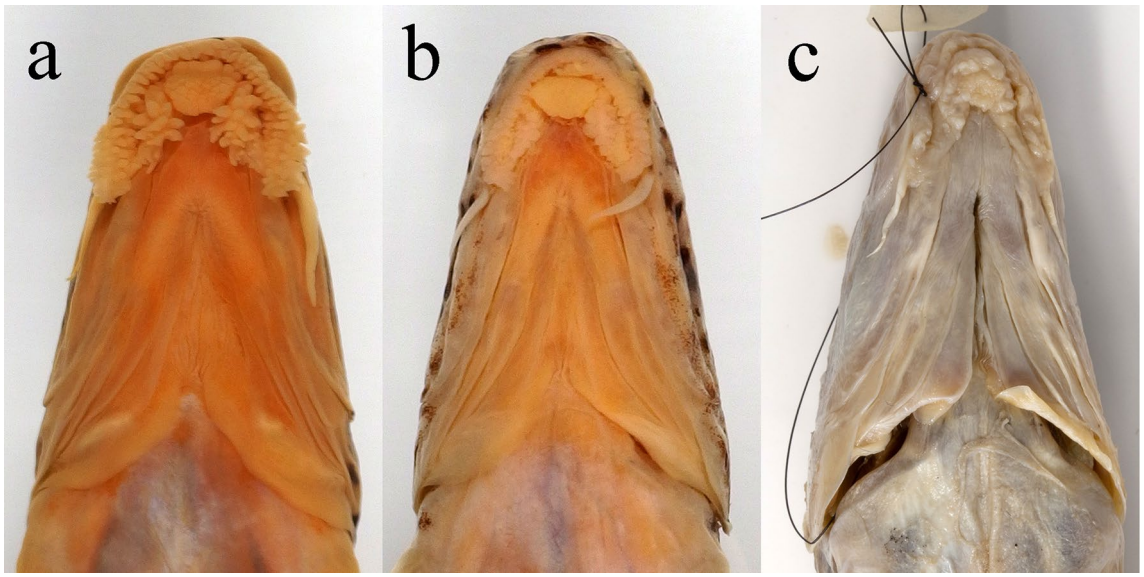

Fig. 4 Comparison of ventral view of the mouth in three species of Japanese *Pseudogobio*. **a** *Pseudogobio agathonectris* sp. nov., NSMT-P 128089, holotype, 119.4 mm SL; **b** *Pseudogobio polysticta* sp. nov., NSMT-P 128091, holotype, 116.9 mm SL; **c** *Pseudogobio. esocinus*, RMNH 2478, lectotype, 143.2 mm SL

middle reaches of clear streams and prefers riffles for their microhabitat.

Remarks. *Pseudogobio agathonectris* corresponds to *P. esocinus* Group B in Tominaga et al. (2016). See the 'Morphometric comparison of the five species of *Pseudogobio*' and 'Discussion' sections for morphological differences and phylogenetic relationships among other congeneric species.

Pseudogobio polysticta sp. nov.

(New Japanese name: Sunago-kamatsuka) (Figs. 2b, 3c–d, 4b, 5e–h, 8, 9a; Tables 1, 2)

Pseudogobio esocinus (not of Temminck and Schlegel): Aoyagi 1957: 135–137 (in part), fig. 113 (Kitakami River, Iwate or Miyagi Pref., Japan); Nakamura 1963: 123 (in part), pl. 48 (Tadami River, Agano River system, Fukushima Pref., Japan); Nakamura 1969: 143–148 (in part), pl. 45E–G (Tone River, Saitama Pref., Japan), pl. 109A–C (Tama River, Tokyo, Japan) and D–E (Tone River, Saitama Pref., Japan); Kimura 2015: 91 (in part), photograph on the top (no locality information).

Pseudogobio esocinus esocinus (not of Temminck and Schlegel): Bănărescu and Nalbant 1965: 301–308 (in part), fig. 4 (Iwai River, Kitakami River system, Ichinoseki, Iwate Pref., Japan); Watanabe 1992: 46 (in part), photograph at the bottom (juvenile, no locality information); Takahashi 2004: 32, photographs (no locality information); Nakagawasuiyuen 2016: 68, photograph at the bottom (no locality information).

"Kamatsuka" (no designation of scientific name): Niigata Nippo Jigyousha 1983: 39 (no locality information); Watanabe 1999: 76, photograph (no locality information); Watanabe and Sakadosizensikenkyukai 2000: 34 (Oppe River, Ara River system, Saitama Pref., Japan); Matsuzawa 2012: 90, photograph (no locality information).

Holotype. NSMT-P 128091, 116.9 mm SL, Hirose River, Kitakami River system, Esashi, Oshu, Iwate Pref., Japan, 2 Nov. 2008, collected by K. Tominaga.

Paratypes. NSMT-P 128092, 96.6 mm SL, same data as holotype; FAKU 206056–206061, 6 specimens, 61.2–87.3 mm SL, Funato River, Ochibori River system, Tainai, Niigata Pref., Japan, 1 June 2007 [FAKU 206057 is a marking pattern variant. See Tominaga and Kano (2019)]; FAKU 206064–206070, 7 specimens, 56.6–88.5 mm SL, Yoro River, Otaki, Isumi, Chiba Pref., Japan, 5 Mar. 2008; FAKU 206079–206084, 6 specimens, 91.6–99.0 mm SL, Arase River, Nikko River system, Sakata, Yamagata Pref., Japan, 26 Aug. 2008; FAKU 206085–206090, 6 specimens, 75.4–90.8 mm SL, Narato River, Agano River system, Tadami, Minamiaizu, Fukushima Pref., Japan, 1 Sep. 2008; FAKU 206098–206103, 6 specimens, 88.1–102.2 mm SL, Yamada River, Kuji River system, Hitachiota, Ibaraki Pref., Japan, 3 Sep. 2008; FAKU 206112–206117, 6 specimens, 58.9–85.2 mm SL, same data as holotype; FAKU 206122–206127, 6 specimens, 52.1–60.2 mm SL, Motoisago River, Natori River system, Taihaku, Sendai, Miyagi Pref.,

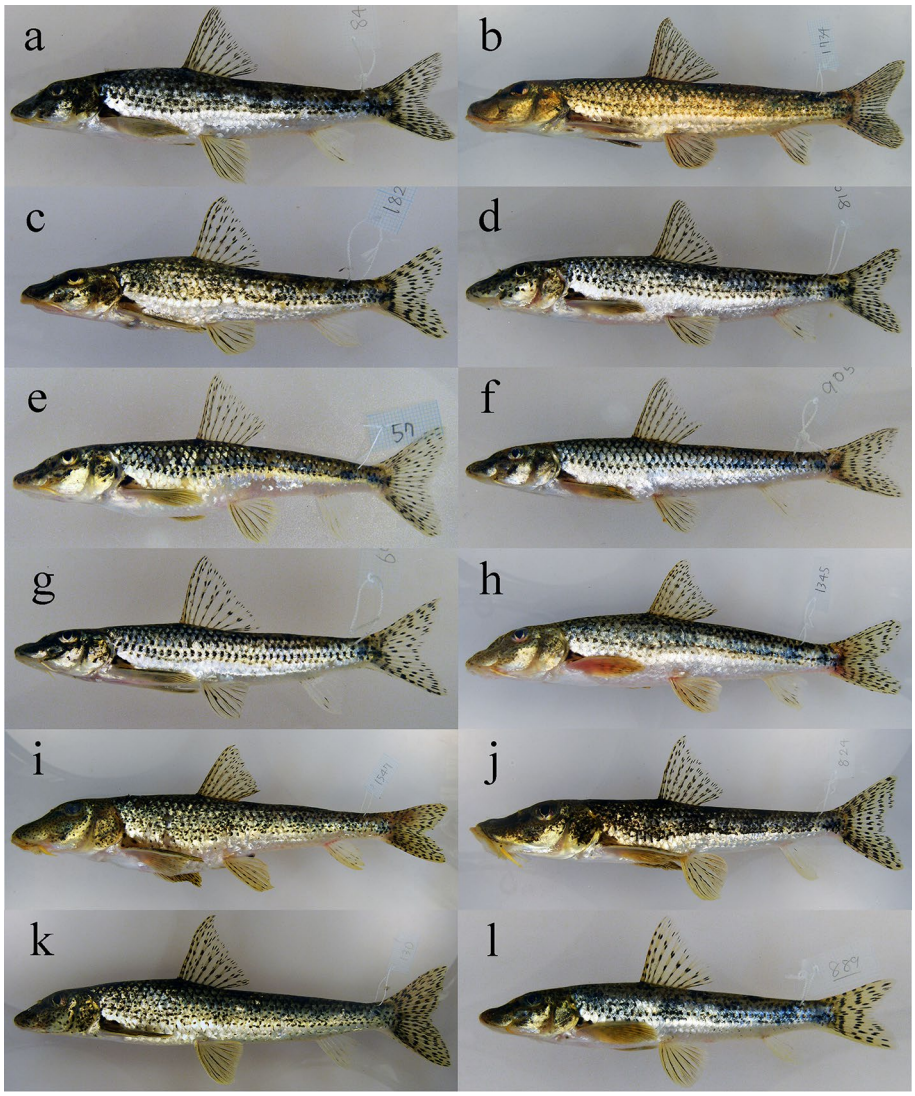

Fig. 5 Lateral views of three Japanese *Pseudogobio* immediately after fixation. **a** *Pseudogobio agathonectris* sp. nov., paratype, RMNH.PISC. 38473, 107.1 mm SL, Nishiki River, Yamaguchi Pref.; **b** *P. agathonectris*, paratype, OMNH-P 45709, 143.4 mm SL, Nabari River, Yodo River system, Mie Pref.; **c** *P. agathonectris*, paratype, WMNH-PIS.10478, 97.0 mm SL, Makuni River, Kino River system, Wakayama Pref.; **d** *P. agathonectris*, paratype, MPM-Fi 1512, 120.4 mm SL, Kushida River, Mie Pref.; **e** *Pseudogobio polysticta* sp. nov., paratype, KPM-NI 48982, 93.0 mm SL, Funato River, Ochibori River system, Niigata Pref.; **f** *P. polysticta*, paratype, WMNH-PIS.10479, 89.3 mm SL, Arase River, Nikko River system, Yamagata Pref.; **g** *P. polysticta*, paratype, CBM-ZF-0019259, 82.1 mm SL, Yoro River, Chiba Pref.; **h** *P. polysticta*, paratype, OMNH-P 45710, 137.3 mm SL, Motoisago River, Natori River system, Miyagi Pref.; **i** *Pseudogobio esocinus*, FAKU 206172, 168.4 mm SL, Hatsuki River, Sendai River system, Kagoshima Pref.; **j** *P. esocinus*, FAKU 206189, 144.9 mm SL, Yura River, Kyoto Pref.; **k** *P. esocinus*, FAKU 206147, 166.1 mm SL, Lake Biwa, Shiga Pref.; **l** *P. esocinus*, FAKU 206200, 90.0 mm SL, Fusuma River, Ota River system, Shizuoka Pref.

Japan, 3 Nov. 2008; KUN-P 47512, 128.0 mm SL, same data as FAKU 206098–206103; LBM 1210056405, 87.3 mm SL, same data as FAKU 206079–206084; OMNH-P 45710, 137.3 mm SL, same data as FAKU 206122–206127; MPM-Fi 1514–1515, 2 specimens, 86.3–88.0 mm SL, same data as FAKU 206085–206090; KPM-NI 48982–48983, 2 specimens, 85.7–93.0 mm SL, same data as FAKU 206056–206061; CBM-ZF-0019259, 82.1 mm SL, same data as FAKU 206064–206070; WMNH-PIS.10479, 89.3 mm SL, same data as FAKU 206079–206084, RMNH.PISC. 38474, 120.2 mm SL, same data as FAKU 206122–206127; NRM 70400, 99.2 mm SL, same data as FAKU 206098–206103. All of the specimens were collected by K. Tominaga.

Non-type specimen. FAKU 207417, 93.2 mm SL, Obuta River, Ichinomiya River system, Chiba Pref., Japan, 13 Oct. 2016, collected by K. Tominaga (only photographic observation).

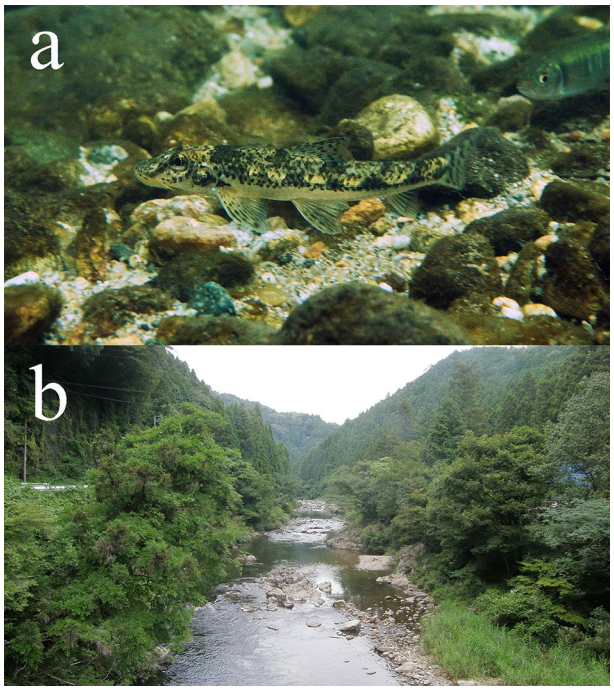

Fig. 6 **a** An underwater photograph of *Pseudogobio agathonectris* sp. nov., Shorenji River, Yodo River system, Nara Pref., 27 Sep. 2015, photograph by R. Uchiyama; **b** Habitat of *P. agathonectris* sp. nov., Toyo River, Aichi Pref. 26 Aug. 2009

Diagnosis. *Pseudogobio polysticta* is distinguishable from *Pseudogobio esocinus* by the following combination of characters: tip of pectoral spiny soft ray not reaching that of 6th soft fin ray (Fig. 2b) (vs. almost reaching 6th soft ray: Fig. 2c), and tip of outer edge of pectoral fin strongly curved posteriorly (Fig. 3c) (vs. gently curved: Fig. 3e); long barbel (16.9–34.8% of HL vs. 11.9–27.9%) with the tip commonly extending beyond vertical through anterior edge of eye (Fig. 3d) (vs. not or barely reaching vertical through anterior edge of eye: Fig. 3f); 12–14 (modally 13) pectoral soft fin rays [vs. 12–16 (14)]; long mouth (29.6–42.0% of HL vs. 18.2–40.7%); short length between anus and anal fin origin (15.9–22.4% of SL vs. 18.7–26.5%), with 11–16 (12) scales [vs. 12–16 (13)]; and small body size (at most 150 mm SL vs. > 180 mm SL). This new species can be distinguished from the most similar congener *Pseudogobio agathonectris* by the canonical discriminant functions based on five meristic and 25 morphometric characters, and obscure dark blotches and many distinct black spots on dorsal and lateral body (vs. distinct blotches and obscure spots). *Pseudogobio polysticta* is distinguishable from other congeners by the following combination of characters: long mouth (29.6–42.0% of HL vs. 28.8–31.5% in *Pseudogobio vaillanti*); long barbel (16.9–34.8% of HL) with the tip commonly extending beyond vertical through anterior edge of eye (vs. short barbel, 9.1–19.1% of HL and not or barely reaching vertical through anterior edge of eye in *P. vaillanti*); short length between anus and anal fin origin (15.9–22.4% of SL vs. 19.4–25.6% in *P. vaillanti*); short and high caudal peduncle (the length: height ratio < 200% vs. long and low peduncle > 200% in *Pseudogobio guilinensis*); 12–14 (13) pectoral soft fin rays (vs. 11 in *Pseudogobio banggiangensis*); and a black stripe on lateral body absent (vs. present in *P. banggiangensis*).

Description. The meristic and morphometric measurements for type specimens are summarized in Tables 1 and 2. All of the raw data are shown in ESM Table S1. The following description is based on holotype, except for pharyngeal teeth. Variation seen in paratypes is given in parentheses.

Dorsal fin rays iii, 7; anal fin rays iii, 6; pectoral fin rays i, 13 (12–14, modally 13, rarely 14); pelvic fin rays ii, 7. Pharyngeal teeth in two rows, with a dental formula of commonly 1, 5–5, 1 or rarely 2, 5–5, 2. Body elongated, cylindrical, and anteriorly tapered, with the posterior region laterally compressed. Head relatively large, 27.5 (26.7–31.8) % of SL; snout relatively long, 48.3 (44.4–53.0) % of HL, protruding and dorsally concave. Mouth relatively long, 30.2 (29.6–42.0) of HL, and extended downward, with fleshy lips with many well-developed papillae (Figs. 3d, 4b). Eye relatively small, 20.6 (16.2–24.7) % of HL, and positioned on upper-middle of head. A pair of long barbels, 20.2 (16.9–34.8) % of HL, with the tips commonly extending beyond vertical through anterior edge of eyes (Fig. 3d). Pectoral fin wide, positioned low on the body, and spread horizontally, with the tip of the outer edge strongly curved posteriorly because of short pectoral spiny soft ray (Figs. 2b, 3c). Dorsal fin high, 18.7 (18.0–24.1) % of SL, with a slightly concave edge. Pelvic fin origin positioned at the approximate SL center, and dorsal fin origin positioned just before it. Anus between pelvic fins, and anal fin positioned near posterior end of the body. In all, 14 (11–16, modally 12, rarely 11 and 16) scales between anus and anal fin origin. Caudal fin moderately forked. Trunk lateral line complete, and 38 (36–39, modally 38, rarely 36) pored lateral line scales. Cephalic lateral line complete; infraorbital canal connected with supraorbital and preoperculomandibular canals; rostral branch present; supratemporal canals of both sides connected.

Color in life. Dorsal side of head and body dark yellowish brown, mid-lateral side yellowish brown and ventral side white. Mid-lateral body with a series of obscure bluish-black, amorphous blotches, equal or slightly larger in size than eye diameter. A series of obscure, black saddle-shaped markings on mid-dorsal body, and many small, obvious black spots on dorsal and lateral sides of head and body (Figs. 3c, 5e–h, 8b, 9a). A narrow yellowish-gold stripe under the blotches on lateral body. Dorsal, pectoral, and caudal fins faded yellowish brown with a row of small black spots along the fin rays. Pelvic fin faded yellowish brown

Table 1 Counts and morphometric measurements of *Pseudogobio agathonectris* sp. nov., *Pseudogobio polysticta* sp. nov., *Pseudogobio esocinus*, *Pseudogobio* sp., and *Pseudogobio vaillanti*

	P. agathonectris sp. nov.		*P. polysticta* sp. nov.		*P. esocinus*		*Pseudogobio* sp.	*P. vaillanti*
	Holotype	Paratypes ($n=51$) Mean (range)	Holotype	Paratypes ($n=55$) Mean (range)	Lectotype	Paralectotypes ($n=6$) + Other specimens ($n=70$) Mean (range)	($n=26$) Mean (range)	($n=8$) Mean (range)
Standard length (mm)	119.4	94.7 (63.4-143.4)	116.9	83.3 (52.1-137.3)	143.2	117.2 (66.7-185.0)	75.8 (44.4-107.4)	116.0 (92.7-143.3)
Counts								
Branched dorsal soft fin rays	7	7.0 (7)	7	7.0 (7)	7	7.0 (7)	7.0 (7)	7.0 (7)
Branched anal soft fin rays	6	6.0 (5-6)	6	6.0 (6)	6	6.0 (5-6)	6.0 (6)	6.0 (6)
Pectoral soft fin rays	14	12.9 (10-14)	13	12.7 (12-14)	15	14.2 (12-16)	14.1 (13-15)	14.0 (13-16)
Pelvic soft fin rays	7	7.0 (7)	7	7.0 (7)	7	7.0 (7)	7.0 (7)	7.0 (7)
Lateral line scales	38	38.5 (36-40)	38	37.8 (36-39)	37	38.2 (36-41)	38.9 (37-40)	40.0 (40)
Scales above lateral line	5	5.0 (5)	5	5.0 (5)	5	5.1 (5-6)	5.0 (5-6)	5.0 (5)
Scales below lateral line	4	4.0 (4)	4	4.0 (4)	4	4.0 (4)	4.0 (4)	4.0 (4)
Scales between anus and anal fin origin	11	12.1 (11-14)	14	12.8 (11-16)	12	13.6 (12-16)	14.8 (14-17)	13.4 (13-14)
In % standard length								
Head length	29.7	29.0 (26.7-31.4)	27.5	28.9 (26.7-31.8)	30.0	29.8 (25.8-35.5)	29.3 (27.7-31.2)	27.9 (25.8-29.1)
Body depth	21.8	18.5 (16.3-21.1)	20.3	18.0 (16.4-20.6)	20.1	18.6 (15.5-21.9)	17.8 (14.8-22.5)	18.2 (17.1-19.9)
Body width	17.8	16.1 (12.6-18.8)	16.6	15.7 (13.4-18.3)	15.7	15.8 (12.4-18.3)	14.6 (12.3-17.3)	14.1 (12.7-16.8)
Caudal peduncle depth	8.5	8.4 (7.4-9.2)	8.6	8.1 (7.0-8.8)	8.9	8.0 (6.6-9.2)	7.3 (6.9-7.8)	7.3 (6.3-7.8)
Caudal peduncle length	11.3	11.9 (9.1-14.6)	12.6	12.3 (10.3-14.7)	13.0	12.0 (8.8-15.9)	11.7 (9.0-13.7)	13.0 (12.2-14.5)
Predorsal length	49.5	46.8 (41.9-50.4)	45.0	46.3 (43.4-51.0)	50.6	46.8 (44.0-50.3)	47.1(44.7-50.2)	45.7 (44.0-47.2)
Prepelvic length	55.0	50.2 (46.4-56.4)	49.1	50.0 (47.1-56.5)	52.6	50.3 (47.1-54.6)	49.2 (45.6-58.7)	50.4 (48.7-52.5)
Preanal length	79.0	77.7 (74.8-81.7)	75.8	77.2 (74.0-80.2)	81.2	79.2 (75.8-82.1)	79.8 (75.9-83.3)	79.6 (78.4-80.9)
Length between end of gill cover and dorsal fin origin	23.5	22.5 (20.9-24.2)	22.0	22.0 (20.5-23.9)	22.7	21.3 (17.9-23.9)	21.2 (18.6-24.2)	22.2 (20.3-24.0)
Length between end of gill cover and pelvic fin origin	27.5	24.8 (22.5-26.8)	24.4	24.8 (22.8-29.0)	22.4	24.0 (19.6-26.4)	22.8 (20.9-24.6)	24.4 (23.2-25.6)
Length between end of gill cover and anal fin origin	52.0	52.5 (49.5-54.7)	51.8	52.5 (50.2-55.5)	53.2	53.4 (49.4-57.3)	53.7 (51.0-56.5)	53.8 (49.7-56.9)

Table 1 (continued)

	P. agathonectris sp. nov.		*P. polysticta* sp. nov.		*P. esocinus*		*Pseudogobio* sp.	*P. vaillanti*
	Holotype	Paratypes ($n=51$) Mean (range)	Holotype	Paratypes ($n=55$) Mean (range)	Lectotype	Paralectotypes ($n=6$) + Other specimens ($n=70$) Mean (range)	($n=26$) Mean (range)	($n=8$) Mean (range)
Length between pectoral fin origin and pelvic fin origin	26.7	23.5 (20.9-26.6)	22.9	23.1 (20.5-27.4)	24.1	22.7 (20.1-26.8)	21.4 (19.3-23.9)	23.5 (22.0-25.9)
Length between pelvic fin origin and anal fin origin	25.5	28.6 (25.8-31.3)	28.1	28.5 (25.8-31.2)	28.7	30.4 (27.3-34.8)	31.5 (28.6-33.5)	30.8 (27.9-33.6)
Length between anus and anal fin origin	16.1	19.0 (16.5-21.4)	19.8	19.2 (15.9-22.4)	23.6	22.1 (18.7-26.5)	23.7 (20.8-26.5)	22.6 (19.4-25.6)
Dorsal fin height	19.4	19.8 (15.8-21.6)	18.7	20.4 (18.0-24.1)	21.2	19.9 (16.3-24.0)	21.7 (19.7-23.9)	21.0 (18.2-22.7)
Dorsal fin base length	12.8	12.8 (11.2-14.3)	13.8	12.9 (11.5-14.8)	13.9	12.6 (10.8-14.7)	12.6 (10.8-13.8)	13.5 (11.9-14.7)
Anal fin height	13.7	15.1 (13.3-17.3)	15.6	15.3 (13.3-16.9)	15.2	14.7 (11.7-17.6)	15.2 (13.5-17.7)	14.5 (11.0-16.4)
Anal fin base length	6.9	7.7 (6.6-8.6)	7.5	7.8 (6.5-9.1)	7.3	7.2 (6.0-8.7)	7.2 (6.3-8.8)	7.3 (5.6-8.2)
Pectoral fin length	21.8	23.9 (21.7-27.6)	22.3	23.5 (21.1-25.8)	23.2	23.5 (19.0-27.9)	22.2 (20.1-24.3)	21.8 (19.1-23.1)
Pelvic fin length	16.5	17.4 (15.4-19.8)	17.5	16.9 (14.5-19.6)	20.3	16.7 (14.5-21.2)	17.1 (15.8-20.4)	16.8 (15.3-18.0)
In % head length								
Snout length	49.6	47.9 (44.0-52.8)	48.3	48.5 (44.4-53.0)	55.7	50.7 (44.7-55.6)	52.5 (45.1-56.8)	51.0 (48.6-53.7)
Mouth length	39.7	38.7 (33.2-45.3)	30.2	35.1 (29.6-42.0)	33.0	30.6 (18.2-40.7)	30.5 (26.1-35.0)	30.3 (28.8-31.5)
Barbel length	30.4	24.8 (15.3-36.9)	20.2	25.0 (16.9-34.8)	22.4	19.3 (11.9-27.9)	19.4 (15.9-25.1)	14.0 (9.1-19.1)
Eye diameter	18.3	20.8 (17.0-25.5)	20.6	19.8 (16.2-24.7)	18.7	20.0 (15.1-28.7)	21.8 (19.2-24.5)	19.5 (17.7-21.3)
Interorbital width	23.7	26.1 (22.4-30.7)	28.3	25.6 (21.3-30.3)	26.6	25.6 (22.2-30.7)	24.0 (22.1-26.7)	24.2 (23.3-26.0)

Table 2 Frequency distributions of counts in *Pseudogobio agathonectris* sp. nov., *Pseudogobio polysticta* sp. nov., *Pseudogobio esocinus*, *Pseudogobio* sp., and *Pseudogobio vaillanti*

	Pectoral soft fin rays							Lateral line scales						Scales between anus and anal fin origin						
	10	11	12	13	14	15	16	36	37	38	39	40	41	11	12	13	14	15	16	17
P. agathonectris sp. nov.	1		7	40	4*			1	3	20*	27	1		10*	32	6	4			
P. polysticta sp. nov.			16	38*	2			1	18	29*	8			2	24	19	4*	5	2	
P. esocinus			1	9	46	18*	3	4	19*	23	19	11	1		5*	33	26	12	1	
Pseudogobio sp.				2	19	5			1	6	13	6					10	11	4	1
P. vaillanti				3	3	1	1					8				5	3			

*including holotype or lectotype

Branched anal soft fin rays and scales above lateral line were excluded because of rare variation (see Table 1)

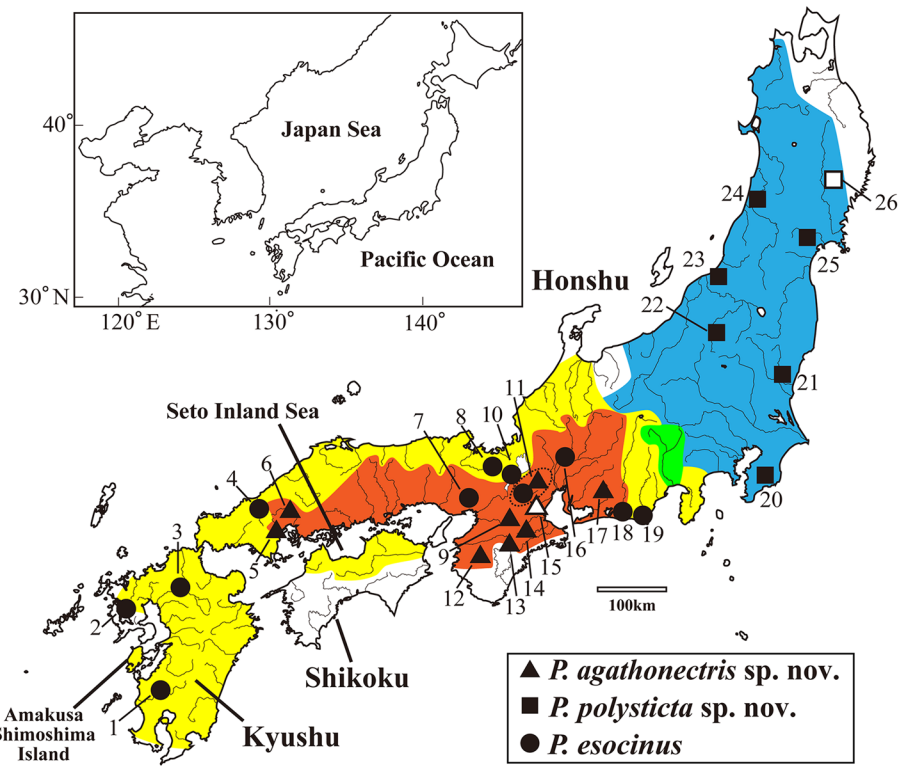

Fig. 7 Sampling localities and distribution of *Pseudogobio agathonectris* sp. nov. (*triangles*), *Pseudogobio polysticta* sp. nov. (*squares*), and *Pseudogobio esocinus* (*circles*). Open symbols indicate the type locality of the species (type locality of *P. esocinus* is Japan but precise location unknown). Colored areas indicate putative natural distribution of each species (*yellow*: *P. esocinus*; *orange*: *P. agathonectris* and *P. esocinus*; *blue*: *P. polysticta*; *green*: putative hybrid zone of *P. polysticta* and *P. esocinus*). 1, Hatsuki River (Sendai River system); 2, Kawatana R.; 3, Koishiwara R. (Chikugo R. s.); 4, Hodogan R. (Takatsu R. s.); 5, Nishiki R.; 6, Ota R. 7, Muko R.; 8, Yura R.; 9, Nabari R. (Yodo R. s.); 10, Otsu (Lake Biwa); 11, Tamura R. (Yasu R. s., Lake Biwa); 12, Makuni R. (Kino R. s.); 13, Takami R. (Kino R. s.); 14, Kushida R.; 15, Anraku R. (Suzuka R. s.); 16, Kawaura R. (Nagara R. s.); 17, Toyo R.; 18, Fusuma R. (Ota R. s.); 19, Kiku R.; 20, Yoro R.; 21, Yamada R. (Kuji R. s.); 22, Narato R. (Agano R. s.); 23, Funato R. (Ochibori R. s.); 24, Arase R. (Nikko R. s.); 25, Motoisago R. (Natori R. s.); 26, Hirose R. (Kitakami R. s.)

with narrow black lines along the fin rays. Anal fin almost colorless, but some individuals (>90 mm SL) with narrow black lines along the fin rays.

Color in preservation. Dorsal and lateral sides of head and body faded brown; ventral side faded yellowish brown. A series of several bluish-brown blotches on mid-lateral body, a series of obscure, dark brown saddle-shaped markings on mid-dorsal body, and many small dark brown spots on dorsal and lateral sides of head and body (Fig. 8c). Fins almost colorless with black spots or lines as same as those of living specimen.

Distribution. *Pseudogobio polysticta* is distributed across eastern Honshu, Japan. We have confirmed specimens from the Fuji River system in Yamanashi and Shizuoka Prefectures to the Kitakami River system in Iwate Prefecture on the Pacific Ocean side (but not from the Izu Peninsula, and the Hamadori region of Fukushima Prefecture, except for the Natsui River system), and from the Seki River system in Niigata Prefecture to the Iwaki River system in Aomori Prefecture on the Japan Sea side (Fig. 7). However, it is not known whether its natural distribution extends north of Akita Prefecture (Sugiyama 1985; Sawara 2000). The population in the Fuji River system, which includes the western edge of the Pacific Ocean side distribution of *P. polysticta* and eastern edge of the *P. esocinus* distribution, is a putative natural hybrid of *P. polysticta* and *P. esocinus* (Tominaga et al. 2016).

Habitat and biology. *Pseudogobio polysticta* inhabits the sandy bottoms of the upper to lower reaches of rivers (Fig. 9b). Although the habitat of *P. polysticta* is similar to that of *P. agathonectris*, the former species' range extends downstream. During the day, *P. polysticta* often burrows in the sand accumulated in pools or behind rocks in riffles. At dusk, this species feeds actively around the sandy bottom. The peak period of spawning season is inferred to be mid-June in the Tone River system in Gunma Prefecture and this species is mature at approximately 70 mm SL (Sato et al. 1996). In aquarium observations, at night, this species scatters eggs and sperms near the surface of the water (Sato et al. 1997). The spawning behaviors seemed to be similar to those

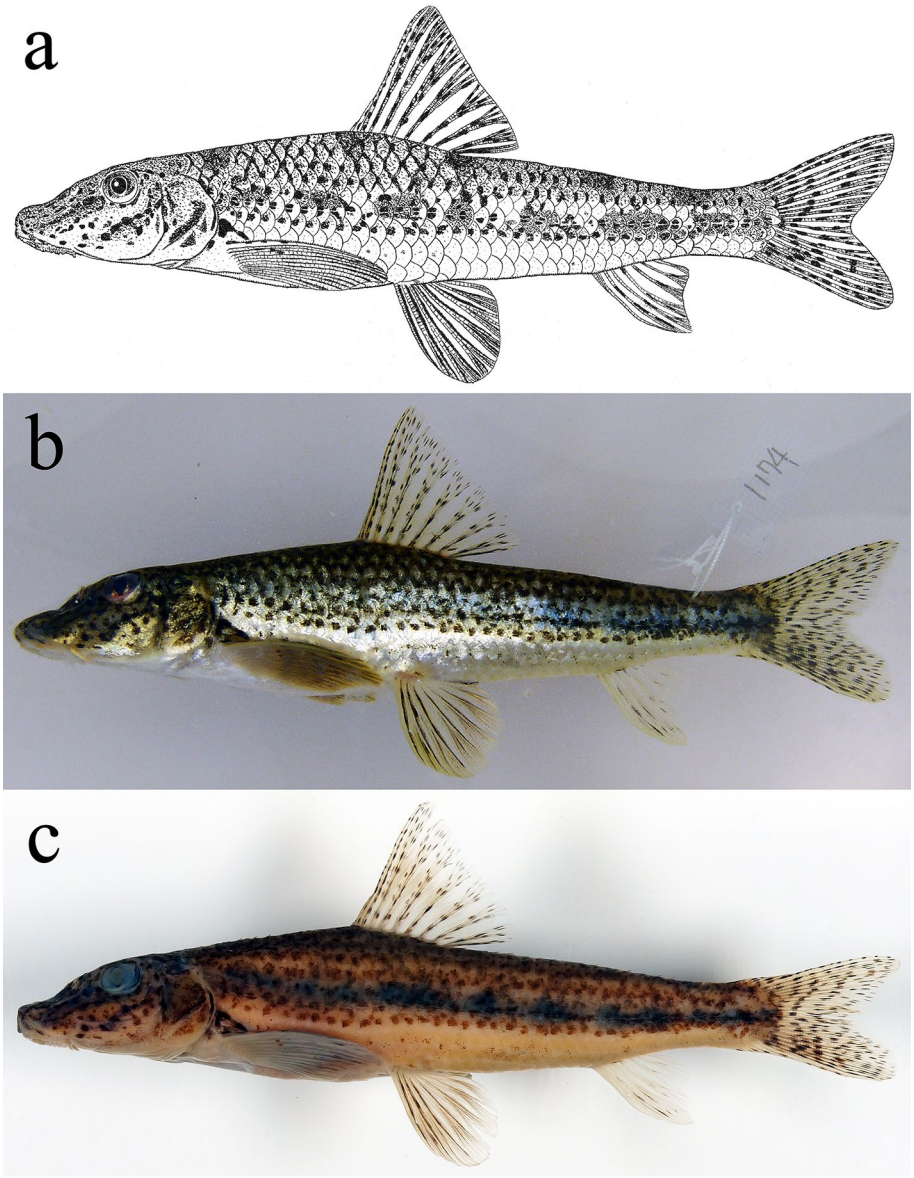

Fig. 8 *Pseudogobio polysticta* sp. nov., NSMT-P 128091, holotype, 116.9 mm SL, Hirose River, Kitakami River system, Iwate Pref., Japan. **a** original drawing; **b** immediately after fixation; **c** after preservation

of *P. esocinus* (Nakajima and Onikura 2016b). Nakamura (1969) described the morphological features of juvenile. However, most of the life history of *P. polysticta* is unknown.

Ethymology. The specific name, *polysticta*, is a compound of two Greek words, "polys," meaning 'many' and "stiktos," meaning 'spotted.' This new species is characterized by many distinct black spots on the dorsal and lateral body. The Japanese name "sunago" comes from the lyrics of a Japanese nursery rhyme "Kin gin sunago," which means 'dust of gold and silver.' The edges of scales on the dorsal and lateral body of this new species are glittery, like gold dust.

Conservation. Recently, *P. esocinus* has been introduced artificially into the range of *P. polysticta*, concomitant with the stocking of Ayu from Lake Biwa. It is hypothesized that this introduction may have resulted in hybridization between the two species, or the replacement of *P. polysticta* by *P. esocinus* (see Tominaga et al. 2016). To promote species conservation, any hybridization and replacement by *P. esocinus* should be comprehensively investigated in the distribution area of *P. polysticta*, and basic biological investigations, including mechanisms of hybridization and replacement, are required.

Remarks. *Pseudogobio polysticta* corresponds to *P. esocinus* Group C in Tominaga et al. (2016). See the 'Morphometric comparison of the five species of *Pseudogobio*' and 'Discussion' sections for morphological differences and phylogenetic relationships among other congeneric species.

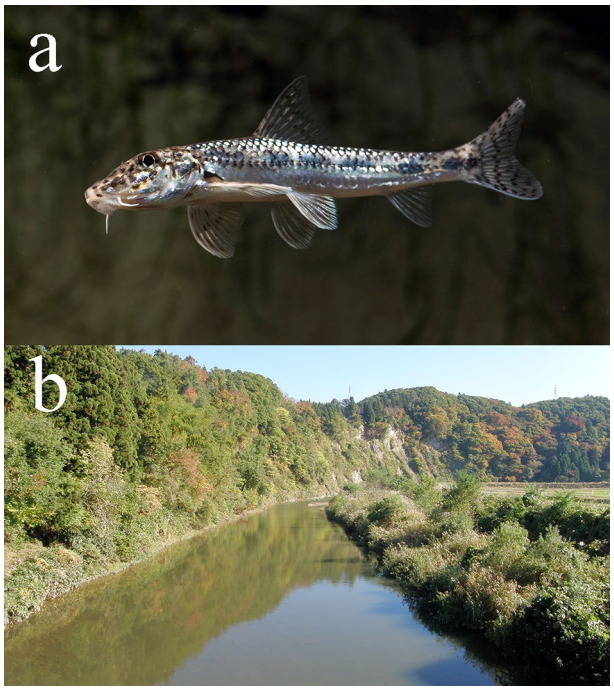

Fig. 9 **a** A photograph of *Pseudogobio polysticta* sp. nov. in an aquarium, collected from Obuta River, Ichinomiya River system, Chiba Pref., photograph by R. Uchiyama; **b** habitat of *P. polysticta* sp. nov., Kariyata River, Shinano River system, Niigata Pref., 5 Nov. 2008

Pseudogobio esocinus (Temminck and Schlegel 1846)

(Japanese name: Kamatsuka; Figs. 2c, 3e–f, 4c, 5i–l, 10; Tables 1, 2)

Gobio esocinus Temminck and Schlegel 1846: 196–198, pl. 99(2) [Japan (precise locality unknown)]

Pseudogobio esocinus (Temminck and Schlegel 1846): Boeseman 1947: 155; Nakamura 1969: 143–148 (in part), pl. 45A–C and A′–B′ (Lake Biwa, Shiga Pref., Japan), pl. 108A–B (Lake Biwa, Shiga Pref., Japan): 400–401, table (Lake Biwa, Shiga Pref., Japan): 402–403, table (Maruyama River, Hyogo Pref., Japan).

Pseudogobio esocinus esocinus: Bănărescu and Nalbant 1965: 301–308 (in part); Bănărescu and Nalbant 1973: 231–235 (in part); Hosoya (1986): 484–501; Hosoya 2001: 296, photograph A (Banjo River, Oita Pref., Japan), 314–315 (in part), photographs (Banjo River, Oita Pref., Japan; Nishina River, Shizuoka Pref., Japan); Hosoya 2013: 325 (in part).

Specimens examined. RMNH 2478 [lectotype designated by Boeseman (1947)], 143.2 mm SL, Japan (precise locality unknown), collected by P. F. von Siebold; RMNH 36376 (paralectotypes), 6 specimens, 72.1–94.4 mm SL, same data as RMNH 2478; FAKU 206137–206144, 8 specimens, 80.2–103.9 mm SL, Muko River, Sanda, Hyogo Pref., Japan, 22 Apr. 2007, collected by K. Tominaga; FAKU 206146–206153, 8 specimens, 124.4–176.2 mm, Lake Biwa, Otsu, Shiga Pref., Japan, 12 July 2007, collected

Fig. 10 *Pseudogobio esocinus*. **a** RMNH 2478, lectotype, 143.2 mm SL, Japan; **b** an underwater photograph, Ai River, Tenryu River system, Shizuoka Pref., 27 Aug. 2009

by K. Tominaga; FAKU 206156–206158, 3 specimens, 129.6–143.9 mm SL, Kawaura River, Nagara River system, Minokamo, Gifu Pref., Japan, 31 July 2007, collected by K. Watanabe; FAKU 206159–206160, 2 specimens, 75.3–118.6 mm SL, Tamura River, Yasu River system, Lake Biwa, Koka, Shiga Pref., Japan, 26 Sep. 2007, collected by K. Tominaga; FAKU 206161–206168, 8 specimens, 81.2–130.2 mm SL, Hodogan River, Takatsu River system, Tsuwano, Kanoashi, Shimane Pref., Japan, 15 June 2008, collected by K. Tominaga; FAKU 206172–206179, 8 specimens, 108.8–168.4 mm SL, Hatsuki River, Sendai River system, Okuchi, Kagoshima Pref., Japan, 5 Aug. 2008, collected by J. Nakajima and K. Tominaga; FAKU 206188–206195, 8 specimens, 129.0–152.0 mm SL, Yura River, Nantan, Kyoto Pref., Japan, 28 Sep. 2008, collected by H. Yamane and K. Tominaga; FAKU 206198–206199, 2 specimens, 81.1–112.2 mm SL, Kiku River, Shimada, Shizuoka Pref., Japan, 5 Sep. 2008, collected by K. Tominaga; FAKU 206200–206203, 4 specimens, 66.7–90.0 mm SL, Fusuma River, Ota River system, Mori, Shuchi, Shizuoka Pref., Japan, 5 Sep. 2008, collected by K. Tominaga; FAKU 206204–206211, 8 specimens, 118.2–185.0 mm SL, Koishiwara River, Chikugo River system, Asakura, Fukuoka Pref., Japan, 10 Aug. 2008 and 4 Oct. 2008, collected by Y. Nakatani; FAKU 206212–206222, 11 specimens, 88.0–126.5 mm SL, Kawatana River, Hasami, Higashisonogi, Nagasaki Pref., Japan, 6 Nov. 2008, collected by J. Nakajima; FAKU 207415, 122.8 mm SL, Shorenji River, Yodo River system, Nara Pref., Japan, 27 Sep. 2015, collected by K. Tominaga (only photographic observation).

Diagnosis. *Pseudogobio esocinus* is distinguishable from *Pseudogobio agathonectris* and *Pseudogobio polysticta* by the combination of characters mentioned above. This species is distinguishable from other congeners by the following combination of characters: short and high caudal peduncle (the length: height ratio < 200%, commonly < 170% vs. long and low peduncle > 200% in *Pseudogobio guilinensis*, commonly > 170% in *Pseudogobio vaillanti*); relatively long barbel (11.9–27.9% of HL, commonly > 15% vs. 9.1–19.1%, commonly < 15% in *P. vaillanti*); 12–16 (modally 14) pectoral soft fin rays (vs. 11 in *Pseudogobio banggiangensis*); and a black stripe on lateral body absent (vs. present in *P. banggiangensis*).

Description. The meristic and morphometric measurements for type specimens and other examined specimens are summarized in Tables 1 and 2. All of the raw data are shown in ESM Table S1. The following description is based on lectotype, except for pharyngeal teeth. Variation seen in paralectotypes and other examined specimens is given in parentheses.

Dorsal fin rays iii, 7; anal fin rays iii, 6 (5–6, rarely 5); pectoral fin rays i, 15 (12–16, modally 14, rarely 12, 16); pelvic fin rays ii, 7. Pharyngeal teeth present in two rows with a dental formula of 2, 5–5, 2 or 1, 5–5, 1. Body elongated, cylindrical, and anteriorly tapered, with the posterior region somewhat laterally compressed. Head large, 30.0 (25.8–35.5) % of SL; snout long, 55.7 (44.7–55.6) % of HL, protruding and dorsally concave. Mouth relatively short, 33.0 (18.2–40.7) % of HL, and extended downward, with fleshy lips with many well-developed papillae (Figs. 3f, 4c). Eye relatively small, 18.7 (15.1–28.7) % of HL, and positioned on upper-middle of head. A pair of barbels, 22.4 (11.9–27.9) % of HL, with the tips not or barely reaching vertical through anterior edge of eyes (Fig. 3f). Pectoral fin wide, positioned low on the body, and horizontally spread, with the tip of the outer edge gently curved posteriorly (Figs. 2c, 3e). Dorsal fin high, 21.2 (16.3–24.0) % of SL, with a slightly concave edge. Pelvic fin origin positioned at the approximate SL center, and dorsal fin origin positioned just before it. Anus between pelvic fins, and anal fin positioned near posterior end of the body. In all, 12 (12–16, modally 14) scales between anus and anal fin origin. Caudal fin moderately forked. Trunk lateral line complete, and 37 (36–41, modally 39, rarely 36 and 41) pored lateral line scales. Cephalic lateral line complete; infraorbital canal connected with supraorbital and preoperculomandibular canals; rostral branch present; supratemporal canals of both sides connected.

Color in life. Dorsal side of head and body brown, mid-lateral side light brown, and ventral side white. Mid-lateral body with a series of slightly obscure bluish-black amorphous blotches, equal or larger in size than eye diameter. Slightly obscure black saddle-shaped markings on mid-dorsal body, and small black spots on dorsal and lateral sides of head and body (Figs. 3e, 5i–l, 10b). An obscure, narrow yellow-gold stripe under the blotches on mid-lateral body. Dorsal, pectoral, and caudal fins faded yellowish brown with 3–4 rows of small black spots along the fin rays. Pelvic fin faded yellowish brown with narrow black lines along the fin rays. Anal fin almost colorless, but some individuals (> 150 mm SL) with narrow black lines along the fin rays.

Color in preservation. Dorsal and lateral sides of head and body faded brown; ventral side faded yellowish brown. A series of several bluish-brown blotches on mid-lateral body, a series of dark brown saddle-shaped markings on mid-dorsal body, and small dark brown spots on dorsal and lateral sides of head and body. Fins almost colorless with black spots or lines as same as those of living specimen.

Distribution. The native range of *Pseudogobio esocinus* is Kyushu, Shikoku and western Honshu, Japan (Fig. 7). Although we have not examined the specimens, morphological characters of the specimen collected from Amakusa Shimoshima Island, which is located near Kyushu, shown in Ikematsu (1980) correspond to those of *P. esocinus*. There are records of fish considered to be *P. esocinus* from other two islands near Kyushu. Bănărescu and Nalbant (1965)

examined the specimens of *Pseudogobio* collected from "Goto Island" (no specific names of island of the Goto Islands). However, we cannot identify the specimens as *P. esocinus* because we have not examined them, the data shown in the study were insufficient to identify species, and there are no reliable records from the islands since that study. Kobayashi and Kihira (1977) recorded some species of *Pseudogobio* from Iki Island. However, the specimens or photographs have not been left, and there are no recent records of *Pseudogobio* in surveys in the island (e.g., Yoshigou 2003).

In Kyushu, this species is widely distributed in north of the Manose River system in the Satsuma Peninsula in Kagoshima Prefecture and the Kamino River system in the Osumi Peninsula in Kagoshima Prefecture. In Shikoku, the native range is considered to be east of the Shigenobu River system in Ehime Prefecture and north of the Yoshino River system in Tokushima and Kochi Prefectures (Ito 1960; Nakamura 1969). In Honshu, the native range is considered to be west of the Jinzu River system in Toyama Prefecture on the Japan Sea side. The eastern limit of the native range on the Pacific Ocean side seems to be Shizuoka Prefecture. Although Itai (1982) indicated that the limit was around the Niino River system in western Shizuoka Prefecture, the results of phylogeographic analyses supported that eastern Shizuoka Prefecture was included in the native range (Tominaga et al. 2016). Recently, *P. esocinus* has been introduced in non-native range, including eastern Honshu, concomitant with the stocking of Ayu mainly from Lake Biwa.

Habitat and ecology. *Pseudogobio esocinus* inhabits the sandy bottoms of middle and lower reaches of rivers, and lakes. During the day, this species often burrows in the sand accumulated at pools. We refer to previous studies below which we have been able to identify the research materials as *P. esocinus* by observation of the specimens, photographs or movies. Hirano et al. (1984) observed the burrowing behavior and revealed that this species preferred to burrow in the sand bottom of the diameter of 0.50 to 2.00 mm. Although some individuals of this species move upstream in the early spawning season, most individuals stay at the same area (about 300 m long) through the year (Ueno 2001). This species is mature at 1–3 years when it reaches approximately 70–80 mm SL, the spawning season is March to August (the peak period is April to June), and the life span was estimated to be 4–6 years (Ueno et al. 2000; Ueno 2002; Nakajima and Onikura 2016a). However, the age at maturity, the spawning season and the life span are variable according to thermal characteristics of the habitat (Nakajima and Onikura 2016a). At night, this species scatters eggs and sperms, which drift with the current, and the eggs adhere to substrates, such as bottom sand and algae (Sata 2014; Nakajima 2015; Nakajima and Onikura 2016b). Several studies have investigated about the early life ecology: effect of temperature on egg hatching (Nakajima et al. 2006), larval and juvenile morphology (Nakamura 1969; Nakajima and Onikura 2015) and larval phototaxis (Nakajima 2006). The larvae and juveniles inhabit shallow and slow-flowing water near the shore (Nakajima et al. 2008).

Remarks. *Pseudogobio esocinus* corresponds to *P. esocinus* Group A in Tominaga et al. (2016). The distribution area of *P. esocinus* has been regarded as Japan, Korea and the Liao River system in northern China (Uchida 1939; Nakamura 1969; Hosoya 2013). However, we demonstrated that Japanese and Korean populations could be distinguished by the canonical discriminant functions based on five meristic and 25 morphometric characters (see the 'Morphometric comparison of the five species of *Pseudogobio*' and 'Discussion' sections) and the body coloration (slightly obscure dark blotches and relatively distinct black spots on the dorsal and lateral body in Japanese population vs. relatively distinct blotches and obscure spots in Korean population). Phylogenetic analyses revealed that there was substantial genetic differentiation between Japanese population and Korean population + *P. vaillanti* from the Liao River (Xia et al. 2005; Tominaga et al. 2016). Therefore, here we treat *P. esocinus* as an endemic species of Japan, and populations in Korea and northern China as an independent species, *Pseudogobio* sp.

Populations of *P. esocinus* have morphological variations. For example, the individuals from Lake Biwa in Shiga Prefecture have slender body, shorter head, mouth and barbels, and obscure blotches on the dorsal and lateral body (Fig. 5k), whereas the individuals from the Yura River system in Kyoto Prefecture have stubby body, longer head, mouth and barbels, and distinct blotches on the dorsal and lateral body (Fig. 5j), as referred in Nakamura (1969). These variations are considered to be a result of local adaptation and reflect wide geographic and longitudinal distribution, and high environmental adaptability of this species.

Morphometric comparison of the five species of *Pseudogobio*

The three species of Japanese *Pseudogobio*, *Pseudogobio* sp., and *Pseudogobio vaillanti* were morphologically discriminated with 96.7% accuracy by CDA based on five meristic and 25 morphometric characters (Tables 3, 4, Fig. 11). The accuracy in *Pseudogobio agathonectris* was 96.0%, *Pseudogobio polysticta* was 94.6%, *Pseudogobio esocinus* was 99.0%, *Pseudogobio* sp. was 96.1%, and *P. vaillanti* was 100%. The contribution of canonical variate (CAN) 1 was 73.2% (canonical correlate = 0.954, eigenvalue = 10.118, Wilks' lambda = 0.00866, approximate $F = 13.628$, $df = 120$, $P < 0.001$), CAN 2 was 13.4% (canonical correlate = 0.805, eigenvalue = 1.845,

Table 3 Results of classification using discriminant functions induced by canonical discriminant analyses of the five species of *Pseudogobio*

Species	Result of classification				
	P. agathonectris	*P. polysticta*	*P. esocinus*	*Pseudogobio* sp.	*P. vaillanti*
P. agathonectris sp. nov.	*50	2	0	0	0
P. polysticta sp. nov.	3	*53	0	0	0
P. esocinus	0	0	69	0	1
Pseudogobio sp.	0	0	1	25	0
P. vaillanti	0	0	0	0	8
Type specimens of *P. esocinus*	0	0	*5	1	0

*including holotype or lectotype

Table 4 Canonical variate coefficients of the five species of *Pseudogobio* for canonical variate (CAN) 1 to CAN 4

	Coefficients			
	CAN 1	CAN 2	CAN 3	CAN 4
Counts				
Anal soft fin rays	0.310 (0.003)	10.229 (0.094)	-11.88 (-0.109)	24.644 (0.227)
Pectoral soft fin rays	-23.386 (-0.698)	0.829 (0.025)	13.739 (0.410)	0.120 (0.004)
Lateral line scales	30.196 (0.346)	23.388 (0.268)	59.745 (0.685)	36.777 (0.422)
Scales above lateral line	-10.384 (-0.187)	-5.726 (-0.103)	8.689 (0.157)	-1.187 (-0.021)
Scales between anus and anal fin origin	-8.720 (-0.354)	-6.813 (-0.276)	-10.123 (-0.411)	-11.012 (-0.447)
Proportion to SL				
Head length	-35.571 (-0.782)	-20.703 (-0.455)	18.169 (0.399)	-18.791 (-0.413)
Body depth	-9.727 (-0.300)	-1.534 (-0.047)	4.094 (0.126)	-8.062 (-0.249)
Body width	1.960 (0.075)	0.840 (0.032)	-1.482 (-0.056)	-1.122 (-0.043)
Caudal peduncle depth	10.163 (0.323)	-26.830 (-0.853)	7.345 (0.233)	-11.742 (-0.373)
Caudal peduncle length	-3.351 (-0.154)	-4.140 (-0.190)	-0.584 (-0.027)	-0.335 (-0.015)
Predorsal length	5.492 (0.084)	2.681 (0.041)	13.173 (0.202)	-35.133 (-0.540)
Prepelvic length	-17.907 (-0.300)	-16.754 (-0.281)	-39.302 (-0.658)	34.766 (0.582)
Preanal length	19.160 (0.198)	20.954 (0.217)	59.940 (0.619)	-29.505 (-0.305)
Length between end of gill cover and dorsal fin origin	10.628 (0.263)	16.837 (0.417)	12.870 (0.319)	19.690 (0.488)
Length between end of gill cover and pelvic fin origin	2.315 (0.053)	-22.851 (-0.527)	-24.159 (-0.557)	8.245 (0.190)
Length between end of gill cover and anal fin origin	-8.119 (-0.107)	3.567 (0.047)	-36.625 (-0.482)	-13.602 (-0.179)
Length between pectoral fin origin and pelvic fin origin	0.801 (0.021)	-3.762 (-0.099)	40.718 (1.067)	1.127 (0.030)
Length between pelvic fin origin and anal fin origin	-13.267 (-0.350)	-7.389 (-0.195)	3.947 (0.104)	5.808 (0.153)
Length between anus and anal fin origin	-20.337 (-0.975)	-7.109 (-0.341)	6.397 (0.307)	0.838 (0.040)
Dorsal fin height	-9.375 (-0.304)	22.637 (0.735)	-11.301 (-0.367)	12.549 (0.407)
Dorsal fin base length	-3.024 (-0.076)	8.855 (0.222)	3.302 (0.083)	8.399 (0.211)
Anal fin height	-7.856 (-0.240)	5.082 (0.156)	-5.167 (-0.158)	-2.500 (-0.077)
Anal fin base length	1.303 (0.045)	6.038 (0.210)	-3.800 (-0.132)	0.485 (0.017)
Pectoral fin length	8.238 (0.234)	-19.75 (-0.562)	11.495 (0.327)	11.554 (0.329)
Pelvic fin length	12.332 (0.352)	10.434 (0.298)	4.808 (0.137)	-5.507 (-0.157)
Proportion to HL				
Snout length	-29.524 (-0.685)	15.787 (0.366)	-11.495 (-0.267)	-1.821 (-0.042)
Mouth length	11.341 (0.685)	11.069 (0.669)	11.018 (0.665)	-6.635 (-0.401)
Barbel length	2.693 (0.278)	0.900 (0.093)	-5.682 (-0.587)	-5.740 (-0.593)
Eye diameter	-7.655 (-0.320)	8.194 (0.343)	17.607 (0.737)	-19.632 (-0.822)
Interorbital width	1.554 (0.049)	-13.842 (-0.432)	-0.073 (-0.002)	0.112 (0.003)

Values in parentheses indicate standardized coefficients

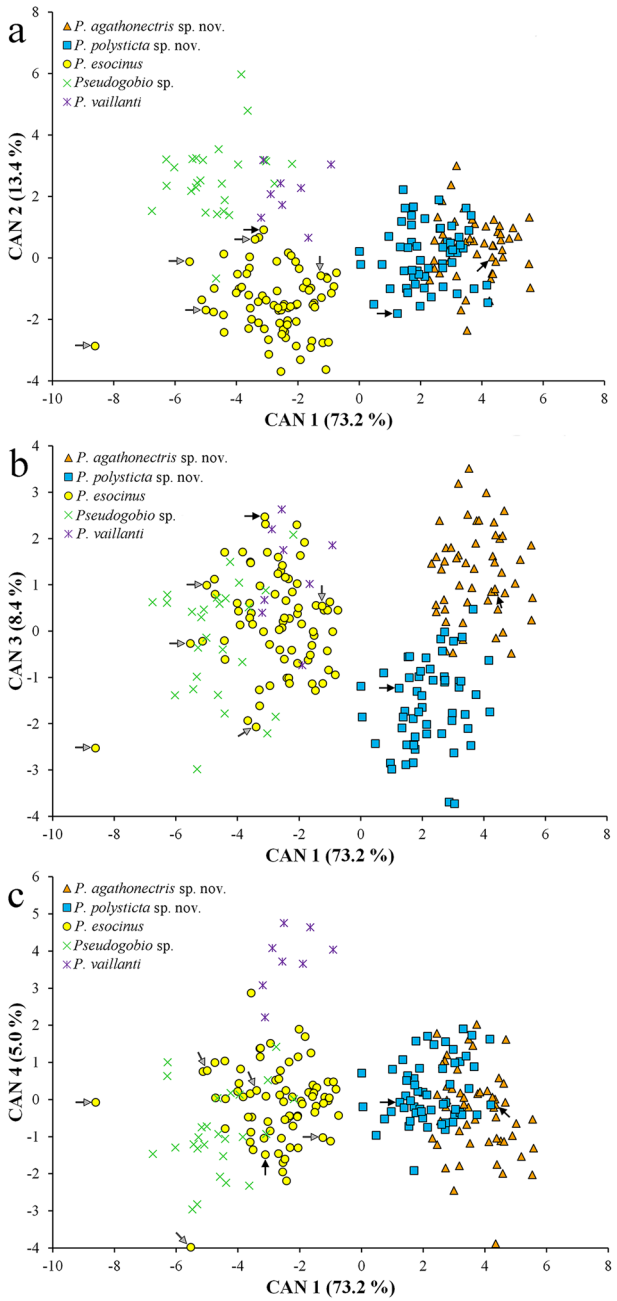

Fig. 11 The plots of canonical discriminant scores based on five meristic and 25 morphometric characters of the five species of *Pseudogobio* with canonical variate (CAN) 1 and CAN 2 (**a**), CAN 3(**b**), and CAN4 (**c**). *Triangles*: *P. agathonectris*; *squares*: *P. polysticta; circles*: *P. esocinus*: *crosses*: *Pseudogobio* sp.; *asterisks*: *P. vaillanti*. *Black arrows* indicate the holotype or lectotype and *gray arrows* indicate the paralectotypes of *P. esocinus*

Wilks' lambda = 0.0963, approximate $F = 7.316$, $df = 87$, $P < 0.001$), CAN 3 was 8.4% (canonical correlate = 0.733, eigenvalue = 1.163, Wilks' lambda = 0.274, approximate $F = 5.856$, $df = 56$, $P < 0.001$) and CAN 4 was 5.0% (canonical correlate = 0.638, eigenvalue = 0.688, Wilks' lambda = 0.592, approximate $F = 4.611$, $df = 27$, $P < 0.001$). Between *P. agathonectris* + *P. polysticta* and *P. esocinus* + *Pseudogobio* sp. + *P. vaillanti*, discriminant scores showed a different trend in CAN 1 (Fig. 11a–c). For CAN 1, the larger standardized canonical variate coefficient was ML, and smaller ones included length between anus and anal fin origin, head length, pectoral soft fin rays, and snout length (absolute value > 0.5, Table 4). Between *P. esocinus* and *Pseudogobio* sp. + *P. vaillanti*, discriminant scores showed a different trend in CAN 2 (Fig. 11a). For CAN 2, the larger standardized canonical variate coefficients were dorsal fin height, and ML and smaller ones were caudal peduncle depth, pectoral fin length, and length between end of the gill cover and pelvic fin origin (GP_2L) (absolute value > 0.5, Table 4). Between *P. agathonectris* and *P. polysticta*, discriminant scores showed a different trend in CAN 3 (Fig. 11b). For CAN 3, the larger standardized canonical variate coefficients included length between pectoral fin origin and pelvic fin origin, eye diameter (ED), lateral line scales (LLS), ML, and preanal length; and smaller ones were prepelvic length (PrP_2L), barbel length (BL), and GP_2L (absolute value > 0.5, Table 4). Between *P. esocinus* + *Pseudogobio* sp. and *P. vaillanti*, discriminant scores showed a different trend in CAN 4 (Fig. 11c). For CAN 4, the larger standardized canonical variate coefficient was PrP_2L and smaller ones were ED, BL, and predorsal length (absolute value > 0.5, Table 4).

Lectotype and all but one paralectotypes of *Pseudogobio esocinus* were discriminated as *P. esocinus* Group A in Tominaga et al. (2016) using the discriminant functions (Table 3, Fig. 11). The one paralectotype with 72.7 mm SL was discriminated as *Pseudogobio* sp. The one paralectotype with 89.8 mm SL included missing data of dorsal fin height because of damage. Although the data of the specimen were excluded in CDA, we confirmed that the specimen was classified into Group A using the discriminant functions derived without data of dorsal fin height (data not shown).

CDA results divided the five species of *Pseudogobio* broadly into two groups: *P. agathonectris* + *P. polysticta* and *P. esocinus* + *Pseudogobio* sp. + *P. vaillanti* (Fig. 11: CAN 1). The former two species have longer lips, shorter distance between anus and anal fin origin, a relatively small head, and a smaller number of pectoral soft fin rays than the latter three species (Tables 1, 2, 4). This indicates that *P. agathonectris* and *P. polysticta* share morphological characters. However, there are also different trends between the two species: the pelvic fins of *P. polysticta* are located more posteriorly and the anal fin is located more anteriorly in *P. polysticta*, which also has relatively smaller eyes, fewer lateral line scales, shorter lips, and longer barbels (Tables 1, 2, 4). There are different trends of morphological characters between *P. esocinus* and *P. vaillanti*: the former species has

shorter dorsal fin and lips, a higher caudal peduncle, longer barbels and pectoral fins, and larger eyes (Tables 1, 2, 4). *Pseudogobio* sp. combines the features of both *P. esocinus* and *P. vaillanti*.

Discussion

The results of morphometric comparisons were concordant with the phylogenetic relationships: *Pseudogobio agathonectris* and *Pseudogobio polysticta* formed a sister group, but *Pseudogobio esocinus* and the former two species were not monophyletic (Fig. 12: modified from Tominaga et al. 2016). However, *P. agathonectris* and *P. polysticta* can be distinguished by the discriminant functions based on morphological characters, and body coloration. The nuclear DNA species tree (Fig. 12a) and the depth of divergence time of the two species (ca. five million years; Fig. 12b) support the decision that they are independent species.

Pseudogobio agathonectris and *P. esocinus* occur sympatrically in parts of western Japan, and are apparently different in morphological characters. In addition, the two species show different trends of longitudinal distributions. Although *P. polysticta*, separately distributed in eastern Japan, has morphological features similar to those of *P. agathonectris*, the former species has some features in common with *P. esocinus* (e.g., shorter mouth and less developed papillose lips). Moreover, *P. polysticta* shows wider longitudinal distribution than *P. agathonectris*. These phenomena are important to consider regarding speciation and evolutionary processes in Japanese *Pseudogobio*.

Although the three species of Japanese *Pseudogobio* can be distinguished from other congeners based on morphological characters, the taxonomy of the remaining species is controversial. Bănărescu and Nalbant (1965) examined specimens and descriptions of *P. esocinus* from Japan and Korea, *Pseudogobio longirostris* Mori 1934 (now considered *Pseudogobio vaillanti*) from northern China, and *P. vaillanti* from Zhejiang and Fujian in China; they considered the three species conspecific because they could find no characters delimitated them sharply while recognizing variation in morphological characters among populations. They also concluded that the two subspecies were recognized in *P. esocinus*: *P. esocinus esocinus* in Japan, Korea, and northern China (including *P. longirostris*), and *P. esocinus vaillanti* in southern China based on differences in number of pharyngeal teeth [*P. e. esocinus*: 2, 5(6)–5, 2, *P. e. vaillanti*: mostly 1, 5–5, 1]. However, Luo et al. (1977) upgraded *P. e. vaillanti* to an independent species without clear basis, including

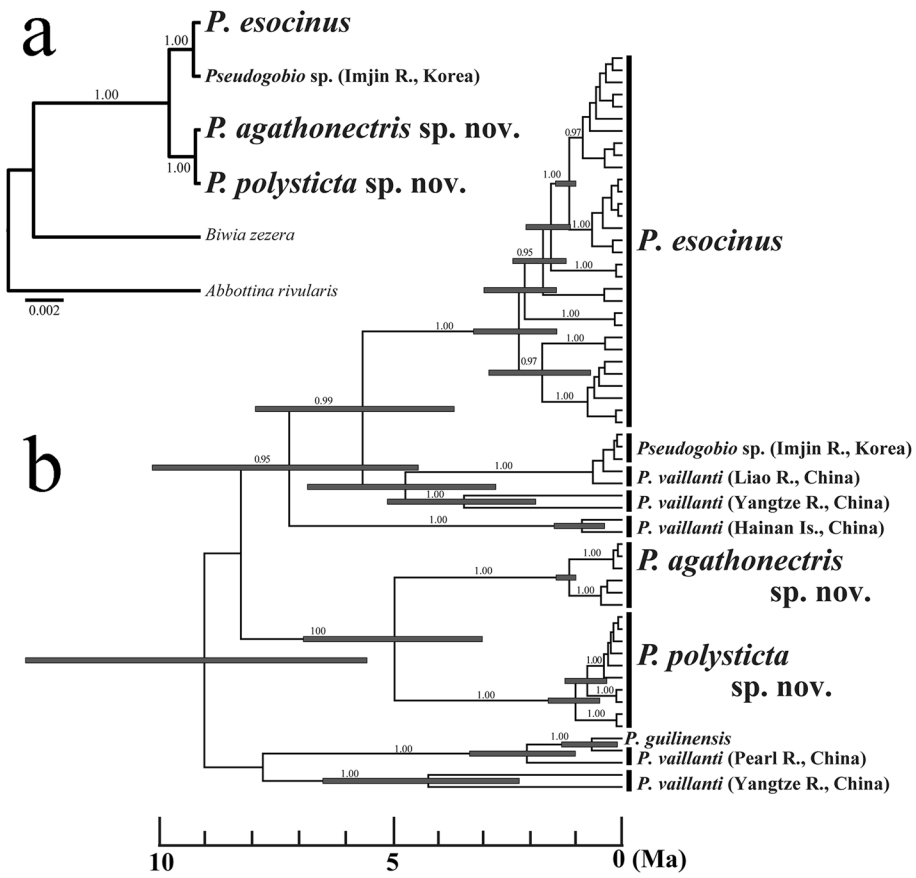

Fig. 12 Molecular phylogenies of *Pseudogobio* (modified from Tominaga et al. 2016). **a** The species tree based on the three nuclear loci (myh6, RYR3, and Glyt; total 2,370 bp) shown with posterior probability (> 0.9); **b** Time calibrated Bayesian phylogenetic tree based on the entire mitochondrial cyt*b* sequence (1,140 bp), shown with posterior probability (> 0.9) and node bars with a 95% credibility interval for major nodes

three subspecies: *P. vaillanti vaillanti*, new subspecies *P. vaillanti guilinensis*, revived *P. longirostris* as *P. vaillanti longirostris*. Moreover, they reported all three subspecies had 2, 5–5, 2 pharyngeal teeth. Now, several studies treat *P. v. guilinensis* as an independent species and *P. v. longirostris* as a synonym of *P. vaillanti* (e.g., Yue 1998; Zhang and Zhao 2016). Furthermore, considering the descriptions and a photograph of the *P. banggiangensis* holotype, the specimen may not belong to the genus *Pseudogobio* because it has a shorter, rounded snout that is not concave in the upper region.

Recent phylogenetic and morphological studies have indicated that *P. vaillanti* includes several cryptic species and that *P. esocinus* is most closely related to some continental lineages from Korea and the Liao River in northern China (Xia et al. 2005; Tominaga et al. 2016; Cao et al. 2017). The lineages from Korea and the Liao River in northern China are monophyletic and there is little genetic differentiation between them. We demonstrated that *P. esocinus* and the Korean population (*Pseudogobio* sp.) could be distinguished in morphological characters. These data support that the populations in Korea and northern China together comprise an independent species. Cao et al. (2017) found that the population of *P. vaillanti* in northern China was genetically and morphologically different from that in other regions. They proposed to revive *P. longirostris* and assigned it to the population of *P. vaillanti* in northern China. However, there is not enough morphological evidence to show that the population corresponds to *P. longirostris*. Further taxonomic reexaminations are needed for the continental species of *Pseudogobio*.

Key to six valid species of *Pseudogobio*

1a. Pectoral soft fin rays 11; a black stripe on lateral body……*P. banggiangensis*
1b. Pectoral soft fin rays commonly equal to or more than 12; no black stripe on lateral body……2
2a. Long and low caudal peduncle (the length: height ratio >200%)……*P. guilinensis*
2b. Short and high caudal peduncle (the length: height ratio <200%)……3
3a. Tip of barbel commonly not or barely reaching vertical through anterior edge of eye; tip of pectoral spiny soft ray almost reaching that of 6th soft fin ray; tip of outer edge of pectoral fin gently curved posteriorly……4
3b. Tip of barbel commonly extending beyond vertical through anterior edge of eye; tip of pectoral spiny soft ray not reaching that of 6th soft fin ray; tip of outer edge of pectoral fin strongly curved posteriorly……5
4a. Long and low caudal peduncle (commonly the length: height ratio >170%); short barbel (commonly <15% of HL)……*P. vaillanti*
4b. Short and high caudal peduncle (commonly the length: height ratio <170%); long barbel (commonly >15% of HL)……*P. esocinus*
5a. Distinct dark blotches and obscure black spots on dorsal and lateral body……*P. agathonectris* sp. nov.
5b. Obscure dark blotches and many distinct black spots on dorsal and lateral body……*P. polysticta* sp. nov.

Comparative materials *Pseudogobio* sp.: TKPM-P 19389, 3 specimens, 44.4–65.4 mm SL (one specimen with 65.4 mm SL was excluded from the analyses because of congenital malformation), Han River system, Heungeop-myeon, Wonju-si, Gangwon-do, Korea, 28 June 1973; TKPM-P 19390, 6 specimens, 55.2–96.2 mm SL, Muhan River, Yesan-eup, Yesan-gun, Chungcheongnam-do, Korea, 29 June 1973; TKPM-P 19391,16 specimens, 50.9–107.4 mm SL, Gosan River, Mangyeong River system, Gosan-myeon, Wanju-gun, Jeollabuk-do, Korea, 30 June 1973; TKPM-P 19392, 97.4 mm SL, Seomjin River, Inwol-myeon, Namwon-si, Jeollabuk-do, Korea, 30 June 1973; TKPM-P 19393, 102.0 mm SL, Nakdong River system, Ton-myeon, Namwon-si, Jeollabuk-do, Korea, 1 July 1973. All specimens were collected by N. Mizuno and S. R. Jeon. *Pseudogobio anderssoni* Rendahl 1928 (synonym of *P. vaillanti*): NRM 9977, syntypes, 6 specimens, 99.3–143.3 mm SL, Minjiang River, Min Jiang drainage, Changting, Hsiu-chiao Bridge, Fujian Province, China, 4 Apr. 1921. *Pseudogobio vaillanti*: KUN-P 45106–45107, 2 specimens, 93.1–98.7 mm SL, Xinxiang, Henan Province, China, Mar. 1981. All of the raw data of meristic and morphometric measurements are shown in ESM Table S1.

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References

Aoyagi H (1957) General notes on the freshwater fishes of the Japanese Archipelago. Taishukan, Tokyo

Bănărescu P, Nalbant TT (1965) Revision of *Pseudogobio* (Pisces, Cyprinidae) with notes on related genera. Rev Roum Biol, Sér Biol 10:301–308

Bănărescu P, Nalbant TT (1973) Pisces, Teleostei: Cyprinidae (Gobioninae). In: Mertens R, Hennig W, Wermuth H (eds) Das Tierreich: eine Zusammenstellung und Kennzeichnung der rezenten Tierformen. Walter de Gruyter, Berlin, Lieferung 93:1–304

Bleeker P (1860) Conspectus systematis Cyprinorum. Natuurkund Tijdschr Ned Indië 20:421–441

Boeseman M (1947) Revision of the fishes collected by Bürger and von Siebold in Japan. Zool Meded 28: 1–242

Cao K, Li YH, He H, Li MY, Fu CZ (2017) Species delimitation and phylogenetic relationships of *Pseudogobio* fishes in the subfamily Gobioninae. Acta Hydrobiol Sin 41:1–12

Hibakagakukyoikushinkokai (1994) Freshwater fishes of Hiroshima Prefecture ("Hiroshimaken no tansuigyo"), enlarged and revised edition. Chugoku Shimbun, Hiroshima

Hirano O, Yamamoto K, Konno H (1984) Burrowing behavior and preference of the bottom condition in the *Pseudogobio esocinus*. J Shimonoseki Univ Fish 32:75–81

Hosoya K (1986) Interrelationships of the Gobioninae (Cyprinidae). In: Uyeno T, Arai R, Taniuchi T, Matsuura K (eds) Indo-Pacific fish biology: proceedings of the second international conference on Indo-Pacific fishes. The Ichthyological Society of Japan, Tokyo, pp 484–501

Hosoya K (2001) *Pseudogobio esocinus esocinus*. In: Kawanabe H, Mizuno N, Hosoya K (eds) Freshwater fishes of Japan, third edition. Yama-kei Publishers, Tokyo, pp 314–315

Hosoya K (2013) Cyprinidae. In: Nakabo T (ed) Fishes of Japan with pictorial keys to the species, third edition. Tokai University Press, Hadano, pp 308–327

Hubbs CJ, Lagler KF (1964) Fishes of the Great Lakes region; with a new preface. University of Michigan Press, Ann Arbor

Ikematsu N (1980) Freshwater fishes of Amakusa Shimoshima Island ("Amakusa Shimoshima no tansuigyo"). Kumamotoseibutsu 21:43–54

Itai T (1982) Freshwater fishes of Shizuoka Prefecture ("Shizuokaken no Tansuigyorui"). Daiichihoki, Tokyo

Ito T (1960) Occurrence of the pike gudgeon in the Hiji River, Ehime Prefecture ("Hijikawa ni arawareta Kamatsuka"). Ehime no shizen 2:21

Kawase S (2015) Pike gudgeon. In Hosoya K (ed) Freshwater fishes of Japan. Yama-kei Publishers, Tokyo, pp 154–155

Kimura Y (2015) Freshwater fishes of Japan ("Nihon no tansuigyo"), enlarged and revised edition. Gakken Kyoiku Shuppan, Tokyo

Kobayashi T, Kihira H (1977) Freshwater fishes, mussels and snails in Iki and Tsushima Islands ("Iki, Tsushima no Tansuigyokairui"). Tansuigyo 4:23–27

Luo YL, Yue PQ, Chen YY (1977) Gobioninae. In: Wu HW (ed) The cyprinid fishes in China. vol 2. Shanghai People's Press, Shanghai, pp 439–549

Matsuzawa Y (2012) A guidebook to catch and keep freshwater fish ("Kawazakana no shiiku to saishu wo tanoshimu hon"). Gakken Kyoiku Shuppan, Tokyo

Mori T (1934) The fresh water fishes of Jehol. Rep First Sci Exped Manchoukuo 5:1–61

Mukai T (2017) Clade B of *Pseudogobio esocinus* (Temminck Schlegel, 1846). In: Mukai T (ed), Fishes of Gifu, pp 80–81. Gifu Shimbun, Gifu

Naito J (2009) The Ota River Aqualium ("Otagawa suizokukan"), Shinsei Art, Hiroshima

Nakagawasuiyuen (2016) Freshwater fishes of Tochigi, enlarged and revised edition. Shimotsuke Shimbun, Tochigi

Nakajima J (2006) Larval phototaxis of the pike gudgeon, *Pseudogobio esocinus esocinus* (Cyprinidae). Bull Hoshizaki Green Found 9:244

Nakajima J (2015) A note on the spawning and early life ecology of the pike gudgeon *Pseudogobio esocinus* (Cypriniformes, Cyprinidae). Aquacult Sci 63:65–70

Nakajima J, Onikura N (2015) Larval and juvenile development of pike gudgeon, *Pseudogobio esocinus* (Cyprinidae: Gobioninae). Ichthyol Res 62:268–273

Nakajima J, Onikura N (2016a) Life history of pike gudgeon, *Pseudogobio esocinus* (Cypriniformes, Cyprinidae): differences between the upper and lower reaches in a single river. Ichthyol Res 63:46–52

Nakajima J, Onikura N (2016b) Spawning behaviour and male mating success of pike gudgeon, *Pseudogobio esocinus* (Cypriniformes, Cyprinidae), in an experimental tank. Ichthyol Res 63:39–45

Nakajima J, Onikura N, Oikawa S, Matsui S (2006) Effect of temperature on hatching of eggs of the pike gudgeon, *Pseudogobio esocinus esocinus*. Aquacult Sci 54:515–519

Nakajima J, Onikura N, Oikawa S (2008) Habitat of pike gudgeon *Pseudogobio esocinus esocinus* in Nakagawa River, northern Kyushu, Japan. Fish Sci 74:842–845

Nakamura M (1963) Key to the freshwater fishes of Japan fully illustrated in colors. Hokuryukan, Tokyo

Nakamura M (1969) Cyprinid fishes of Japan. Research Institute of Natural Resources, Tokyo

Nguyen VH, Ngô SV (2001) Freshwater fishes of Vietnam. Volume I. Family Cyprinidae. Agricultural Publisher, Ho Chi Minh

Niigata Nippo Jigyousha (1983) A picture book of inland water animals in Niigata Prefecture ("Niigataken rikusuidoubutsu zukan"). Niigata Nippo Jigyousha, Niigata

Okayamatansuigyokenkyukai (1984) Freshwater fishes of Okayama ("Okayama no tansuigyo"). Sanyo Shimbun, Okayama

R Development Core Team (2018) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/. Accessed 26 July 2018

Rendahl H (1928) Beiträge zur Kenntnis der chinesischen Süsswasserfische. I. Systematischer Teil. Arkiv för Zool 20A:1–194

Sata T (2014) Eggs of the pike gudgeon fluttering like snow storm ("Fubuki no youni mau Kamatsuka no tamago"). In: Nagata Y (ed) Introduction to freshwater fish study. Tokai University Press, Hadano, pp 211–222

Sato A, Arai H, Teshima C (1996) Research for protection and multiplication of fishes in Gunma Prefecture: seed production of *Pseudogobio esocinus*–I ("Gunmakensan gyorui no hogo zoshoku kenkyu: Kamatsuka no shubyo seisan–I"). Rep Gunma Fish Exp Stn 2:43–46

Sato A, Tanaka H, Teshima C (1997) Research for protection and multiplication of fishes in Gunma Prefecture: seed production of *Pseudogobio esocinus*–II ("Gunmakensan gyorui no hogo zoshoku kenkyu (Kamatsuka no shubyo seisan–II"). Rep Gunma Fish Exp Stn 3:28–31

Sauvage HE (1878) Note sur quelques Cyprinidae et Cobitidae d'espèes inédites, provenant des eaux douces de la Chine. Bull de la Soc philomath Paris 7:86–90

Sawara Y (2000) On the cyprinid fish, *Pseudogobio esocinus*, "recorded" from the Tsugaru District in Edo Era. J Nat Hist Aomori 5:33–35

Sugiyama H (1985) Freshwater fishes of Akita ("Akita no tansuigyo"). Akita Sakigake Shinpo, Akita

Takahashi K (2004) *Pseudogobio esocinus esocinus*. In: Miyagi Prefecture Freshwater Fisheries Experimental Station (ed) Freshwater

fishes in Miyagi Prefecture. https://www.pref.miyagi.jp/uploaded/attachment/633495.pdf. Accessed 6 January 2019
Temminck CJ, Schlegel H (1846) Les Goujons. In: Siebold PF, Temminck CJ, Schlegel H, Haan W (eds) Fauna Japonica (Pisces). Lugduni Batavorum, pp 196–200
Tominaga K (2004) Meeting Japanese freshwater fishes–think about the rivers and the fish ("Nihon no tansuigyo wo tazunete–kawa to sakana wo yomu"). Kwansei Gakuin University Press, Nishinomiya
Tominaga K, Kano Y (2019) Marking pattern variants of the pike gudgeon *Pseudogobio esocinus* from streams in Niigata and Kyoto Prefectures, Japan. Jpn J Ichthyol. https://doi.org/10.11369/jji.19-002
Tominaga K, Nakajima J, Watanabe K (2016) Cryptic divergence and phylogeography of the pike gudgeon *Pseudogobio esocinus* (Teleostei: Cyprinidae): a comprehensive case of freshwater phylogeography in Japan. Ichthyol Res 63:79–93
Tominaga K, Watanabe K, Kakioka R, Mori S, Jeon SR (2009) Two highly divergent mitochondrial DNA lineages within *Pseudogobio esocinus* populations in central Honshu, Japan. Ichthyol Res 56:195–199
Uchida K (1939) The fishes of Tyōsen (Korea). Part 1. Nematognathi, Eventognath. Bull Fish Exp Sta Gov Gen Tyōsen 6:1–458
Ueno S (2001) Movement of pike gudgeon (*Pseudogobio esocinus esocinus*) in a river. Biol InlandWater 16:21–26
Ueno S (2002) Mature age and size of the pike gudeon, *Pseudogobio esocinus esocinus*. Biol Inland Water 17:33–41
Ueno S, Nio M, Nagata Y (2000) Growth and reproductive ecology of the pike gudgeon, *Pseudogobio esocinus esocinus*. Memoir Osaka Kyoiku Univ Ser III Natl Sci Appl Sci 48:97–106
Watanabe M (1992) Field Selection 12, Freshwater fishes ("Tansuigyo"). Hokuryukan, Tokyo
Watanabe M (1999) Natural history of the rrivers and the fish ("Kawa to sakana no hakubutsushi"). Kawade Shobo Shinsha, Tokyo
Watanabe M, Sakadosizensikenkyukai (2000) The Oppe River as seen from the eyes of fish ("Sakana no me kara mita Oppegawa"). Matsuyama Shobo, Higashimatsuyama
Xia X, Zhang X, Liu H (2005) A molecular biogeographic analysis of *Pseudogobio vaillanti*. Prog Nat Sci 15:1064–1069
Yoshigou H (2003) Inland water fishes of the Tsushima and Iki Islands, Nagasaki Prefecture, Japan. Misc Rep Hiwa Mus Nat Hist 42:1–29
Yue PQ (1998) Gobioninae. In: Chen YY (ed) Fauna Sinica, Osteichthys, Cypriniforms II. Science Press, Beijing, pp 232–389
Zhang CG, Zhao YH (2016) Species diversity and distribution of inland fishes in China. Science Press, Beijing