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A new species of nine-spined stickleback, *Pungitius modestus* (Gasterosteiformes, Gasterosteidae), from northern Honshu, Japan

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Abstract

A new species of nine-spined stickleback, *Pungitius modestus*, is described based on the holotype and 17 paratypes (38.7–51.7 mm standard length) collected from the inland area of Yamagata Prefecture, northern Honshu, Japan. The new species is distinguished from the other species of *Pungitius* by the following combination of characters: 30–32 small unconnected lateral plates; dorsal-fin rays VIII–X (usually IX)+9–11; anal-fin rays 7–10 (usually 8); pectoral-fin rays 10; a short spiny dorsal fin base (26.5%–29.8% SL); the first spiny dorsal fin spine behind the pectoral-fin base; a long pre-anal fin (59.9%–67.4% SL); the anal-fin spine below the 1st–3rd dorsal-fin rays; a short pelvic-fin spine (6.3%–9.1% SL); a short anal-fin spine (4.1%–6.0% SL); a long caudal peduncle (14.3%–19.7% SL); no body markings; membranes of the dorsal-fin spines dark brown with black pigments; the entire male body, and soft dorsal and anal fins, becoming black in the breeding season; the anteroventral process of the ectocoracoid present; and the dorsal extension of the ascending process of the pelvis level with the dorsal-most actinost.

Key words: Gasterosteidae, nine-spined stickleback, new species, Yamagata Prefecture, northern Japan

Introduction

Fishes of the genus *Pungitius* Coste, 1848 (Gasterosteiformes, Gasterosteidae), generally known as nine-spined sticklebacks, are widely distributed in fresh and brackish waters in Eurasia and North America (Keivany & Nelson, 2000). Similar in general appearance to three-spined sticklebacks of the genus *Gasterosteus* Linnaeus, 1758, the nine-spined sticklebacks differ in having more dorsal-fin spines (V–XIII) and a more slender body (Wootton 1976; Keivany & Nelson 2000). Nine-spined sticklebacks also differ from three-spined sticklebacks in nest-building behavior. Nests of nine-spined sticklebacks consist of a bundle of fine fragments of vegetation, forming a mass about 4 cm in diameter and positioned among the branches and leaves of aquatic vegetation (Wootton 1976, Chae and Yang 1993). Those of three-spined sticklebacks, however, are built on the substrate of rivers and lakes (Wootton 1976). The genus *Pungitius* is currently represented by 11 species (Fricke *et al.* 2020), as follows: *P. pungitius* (Linnaeus, 1758), nearly circumboreal; *P. laevis* (Cuvier, 1829), in the Seine, Scheldt and Loire basins (France); *P. vulgaris* (Mauduyt, 1848), in the Loire, Gironde and Dordogne basins (France); *P. platygaster* (Kessler, 1859), in the Black, Caspian and Aral Sea basins; *P. sinensis* (Guichenot,1869), in the Kuril Islands, Kamchatka, and southward to the Tumannaya River (China); *P. stenurus* (Kessler, 1876), in northern China and Mongolia; *P. bussei* (Warpachowski in Warpachowski & Herzenstein, 1888), in the Amur River basin (Russia); *P. tymensis* (Nikolskii, 1889), in Russian Far East and Japan; *P. kaibarae* (Tanaka, 1915), in Japan, Korea, and Russian Far East; *P. hellenicus* (Stephanidis, 1971), in Greece; and *P. polyakovi* (Shedko *et al*. 2005), in Sakhalin Island (Russia). However, the taxonomy of

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species found in Europe and East Asia has not yet to be clarified (Keivany & Nelson 2000; Takahashi *et al*. 2001, 2016; Denys *et al*. 2018; Wang *et al.* 2015).

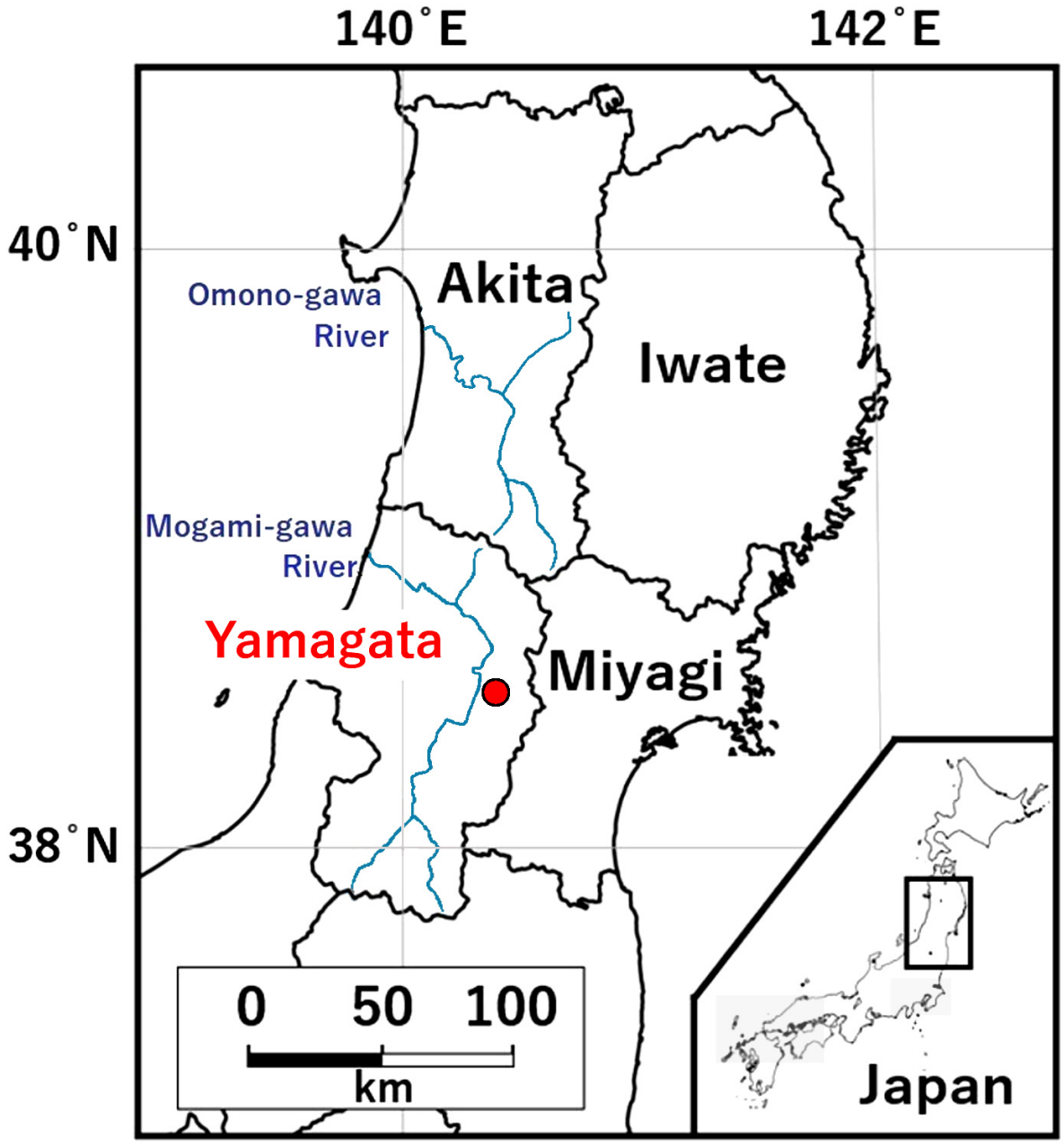

FIGURE 1. Map showing the type locality (solid circle) of *Pungitius modestus,* **sp. nov.**

Over the past few decades, the nine-spined sticklebacks of Japan have been extensively studied using morphological, ecological and/or molecular approaches (e.g., Ikeda 1933; Takata *et al.* 1987a, b; Meguro *et al*. 2016; Takahashi *et al*. 2016). These studies resulted in the recognition of several *Pungitius* "types" such as freshwater and brackish water types (Takata *et al.* 1987a, b, Takahashi *et al.* 2001, and Hosoya 2013), and Omono type (Takata *et al.* 1987b, Takahashi *et al*. 2001, Hosoya 2013). Although most authors recognized these "types" as biological species, they have not yet been given scientific names. Takahashi *et al*. (2016) showed that the fish referred to in the Japanese literature as "the Omono type" was composed of two separate clades, one found in the Mogami-gawa River system in Yamagata Prefecture (Fig. 1), and the other in the Omono-gawa River system in Akita Prefecture. They also clarified that the two lineages of the Omono type were genetically closely related to *P. kaibarae* in Korea and Russia, though clearly differentiated from the latter. Before Takahashi *et al.* (2016) published a molecular

phylogenetic analysis of the *Pungitius* found in East Asia, Wang *et al.* (2015) included the Omono type from Akita Prefecture in their molecular phylogenetic analysis of *Pungitius*. Although they did not study the other population in Yamagata Prefecture, they showed that the clade of the Omono type in Akita Prefecture was clearly differentiated from the other species of *Pungitius*. The lineage of nine-spined stickleback distributed in Yamagata Prefecture is restricted to only a few inland locations (Hosoya 2013; Hanzawa *et al.* 2019).

The main aim of this paper is to describe the nine-spined stickleback found only in the Mogami-gawa River system as a new species under the name *Pungitius modestus*, with a description of its distinguishing characteristics. We also compare the new species with all known species of *Pungitius* based on examination of available type specimens and the original descriptions of the previously described species. Because populations of *P. modestus*, **sp. nov.** are found in only a few places in the inland area of Yamagata Prefecture, it has been listed as an endangered species (Hanzawa *et al*. 2019). In terms of conservation policy, it is urgently necessary to give scientific names to threatened and undescribed species. This is why we focus on describing and naming the nine-spined stickleback of Yamagata Prefecture. Although other authors have suggested the presence of other undescribed nine-spined sticklebacks in Japan, we do not address these because they are being studied by other researchers.

Materials and methods

The specimens used in this study were deposited in the following institutions: Natural History Museum, London (BMNH); Muséum National d'Histoire Naturelle, Paris (MNHN); Department of Zoology, National Museum of Nature and Science, Tsukuba (NSMT); Yamagata Prefectural Museum, Yamagata (YAMA); Yamagata University Museum, Yamagata (YUMB); Zoological Institute, Russian Academy of Sciences, St. Petersburg (ZIN); and University Museum, The University of Tokyo, Tokyo (ZUMT). Counts and measurements generally followed the methods of Hubbs & Lagler (1964), with the following exceptions: body depth, measured as the largest distance between the dorsal and ventral body contours just in front of the pelvic-fin origin, and oblique body depth, measured as the distance between the origins of the soft dorsal and anal fins. Standard, total, and head lengths are abbreviated as SL, TL, and HL, respectively. Considering changes in body shape with growth, specimens longer than 35 mm SL were selected for analysis, although Denys *et al*. (2018) used those longer than 30 mm SL. Osteological descriptions and illustrations were based on a paratype (NSMT-P 140554, 40.5 mm SL) cleared and double-stained according to the protocol of Kelly and Bryden (1983). Measurements were made with calipers to the nearest 0.1 mm. Radiographs were used to count the number of fin rays. Counts and measurements of the holotype are given first, followed by those of the paratypes in parentheses. Characteristics described in the Diagnosis are not repeated in the Description. For ease of assimilation in Japan, the word "type", such as in "Omono type", refers to a morph, especially as used in the Japanese literature, and not as generally understood in zoological nomenclature.

Pungitius modestus, sp. nov.

New Japanese name: Kakure-tomiyo (Figure 2a).

Pungitius sp. 3 (in part) Hosoya, 2013: 607.

Holotype. NSMT-P 133674, 45.4 mm SL, tributary of Mogami-gawa River, Tendo, Yamagata Prefecture, Japan, 30 October 2014, collected by R. Sato and N. Hanzawa.

Paratypes. 17 specimens in total. 8 specimens, 39.7–48.4mm SL, tributary of Mogami-gawa River, Tendo, Yamagata Prefecture, Japan, NSMT-P 133670, NSMT-P 133671, NSMT-P 133672, NSMT-P 133673, 30 October 2014; NSMT-P 133676, 2 November 2018; NSMT-P 136595, NSMT-P 136596, NSMT-P 136597, 26 March 2020. 7 specimens, 43.6–51.7mm SL, small pond connected to Mogami-gawa River, Tendo, Yamagata Prefecture, Japan, NSMT-P 133667, NSMT-P 133668, NSMT-P 133669, 22 October 2014; NSMT-P 133675, NSMT-P 133677, 7 July 2018; NSMT-P 133678, 22 October 2014; NSMT-P 133679, 22 October 2014. 2 specimens, 38.7–40.5mm SL, small pond connected to Mogami-gawa River, Higashine, Yamagata Prefecture, Japan, NSMT-P 140553, NSMT-P 140554, 29 September 2020.

Non-type material. YAMA 3Pi000564, 3 specimens, 43.9–49.9 mm SL, small river connected to Mogami-gawa River, Higashine, Yamagata Prefecture, Japan, 21 February 1973; YAMA 3Pi000566, 3 of 4 specimens, 38.4–49.9 mm SL, locality same as the holotype, 18 September 1985; YAMA 3Pi000567, 1 of 2 specimens, 42.9 mm SL, tributary connected to Mogami-gawa River, Higashine, Yamagata Prefecture, Japan, 20 June 1973; YUMB I-1-9, 7 specimens, 39.8–57.0 mm SL, locality same as the preceding, collection date unknown.

Comparative material. *Gasterosteus platygaster* Kessler, 1859, syntypes, BMNH 1897.7.5.2, 2 specimens, 39.3–44.4 mm SL; *Gasterosteus sinensis* Guichenot, 1869, syntypes, MNHN-IC-0000-5228, 7 specimens, 21–26 mm SL; *Gasterosteus stenurus* Kessler, 1876, syntypes, ZIN 2471, 5 specimens, 46–55 mm SL; *Gasterosteus bussei* Warpachowski, 1888, syntypes, ZIN 7100, 4 specimens 42–53 mm SL; *Gasterosteus tymensis* Nikolskii, 1889, syntype, BMNH 1892.4.28.1, 58.5 mm SL; *Pygosteus kaibarae* Tanaka, 1915, ZUMT 8197, holotype, 45.0 mm SL, Kyoto, Japan; 7 paratypes, 29.4–45.0 mm SL, ZUMT 59848–59854, collection data same as the holotype; NSMT-P 29878, 10 non-type specimens, 35.0–40.7 mm SL, Kyoto, Japan; YAMA 3Pi000592, 3 non-type specimens, 38.1–41.7 mm SL, Kyoto, Japan; NSMT-P 140555, non-type specimens, 30.8 mm SL, Gangneung, Gangwon-do, Korea; NSMT-P 140556, 31.8 mm SL, collection data same as the preceding; NSMT-P 140557, 31.4 mm SL, collection data same as the preceding.

Diagnosis. *Pungitius modestus* is distinguished from other species of *Pungitius* by the following combination of characters (see also Remarks): dorsal-fin rays IX (VIII–IX)+9 (9–11); pectoral-fin rays 10 (10); 30 (30–32) small unconnected lateral plates running from just behind the dorsal end of the gill opening to the caudal peduncle (Fig. 2b); keel on the caudal peduncle present; dorsal-fin spine inclining alternately to left and right of the mid-line; dorsal, pelvic and anal-fin spines short, 3.6% (2.7%–4.9%) SL, 7.4% (6.3%–9.1%) SL and 5.0% (4.1%–6.0%) SL, respectively; base of the spiny dorsal fin short, 28.7% (26.5%–29.8%) SL; first dorsal-fin spine located above or slightly behind the pectoral-fin base; anal-fin spine located below the 1st (1st–3rd) dorsal-fin ray; ascending process of the pelvis relatively developed and extending dorsally to the level of the dorsal-most actinost (Fig. 3a); antero-ventral process of the ectocoracoid present, right and left ectocoracoids articulating with each other at the anterior end (Fig. 3b); and membranes of the dorsal-fin spines dark brown with many black pigments.

FIGURE 2. a) Freshly dead holotype of *Pungitius modestus,* **sp. nov.**, NSMT-P 133674, female, 45,4 mm SL, Tendo, Yamagata Prefecture, Honshu, Japan. b) Holotype preserved in 70% ethanol.

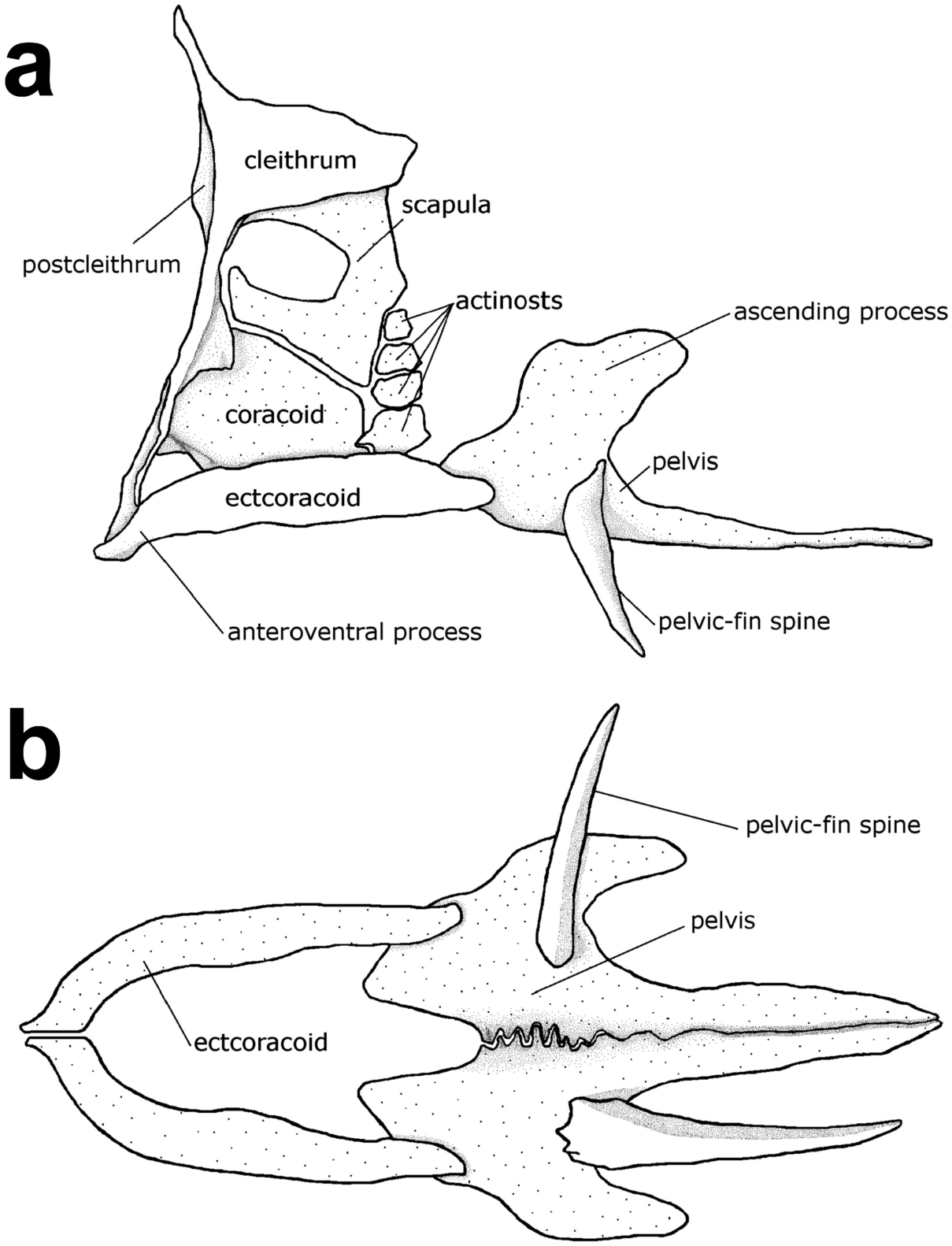

FIGURE 3. Pectoral and pelvic girdles of *Pungitius modestus,* **sp. nov.**, paratype, NSMT-P 140554, male, 38.7 mm SL, Higashine, Yamagata Prefecture, Honshu, Japan. a) Lateral view. b) Ventral view.

TABLE 1. Frequency distributions of dorsal-fin spines and dorsal-fin soft rays in 12 species of *Pungitius*. Counts are based on voucher specimens in BMNH, MNHN, NSMT, YAMA, YUMB, ZIN and ZUMT, and data provided by Keivany *et al*. (1997)[1], Denys *et al*. (2018)[2] and Shedko *et al.* (2005)[3]. Counts of *P. modestus*, **sp. nov.** and *P. kaibarae* come from non-type as well as type specimens.

	Dorsal-fin spines											
	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
P. modestus, **sp. nov.**							7	21*	2			
P. bussei							1*	3*				
P. hellenicus[1]	1	4	9	5	1							
P. kaibarae							17*	4				
P. laevis[2]							14*	9	1			
P. platygaster[1]							7	16*	3			
P. polyakovi[3]							1	20	115	26		
P. pungitius[2]							4	8	11	1		
P. sinensis[3]							17	141*	27			
P. stenurus							4*	1*				
P. tymensis[3]									13	97	86*	14
P. vulgaris[2]							11	11*	2			

*Name bearing types

TABLE 1. (Continued)

	Dorsal-fin rays				
	8	9	10	11	12
P. modestus, **sp. nov.**		7*	19	6	
P. bussei			Not available		
P. hellenicus[1]			Not available		
P. kaibarae	1	6*	11	3	
P. laevis[2]	4	8	8	4*	
P. platygaster[1]		2*			
P. polyakovi[3]			Not available		
P. pungitius[2]	1	3	11	7	2
P. sinensis[3]			5*		
P. stenurus		2*	1*	2*	
P. tymensis[3]				1*	
P. vulgaris[2]	1	11	11*	1	

*Name bearing types

Description. Counts and morphometric measurements are shown in Tables 1–4. Body elongate and laterally compressed, tapering posteriorly to the caudal peduncle, body depth greatest just in front of the pelvic-fin origin; lateral plates on the side of the body small, oval in shape, unconnected; dorsal profile of the head almost straight, gently ascending from the mouth to dorsal-fin origin, ventral profile slightly convex; mouth small, slightly subterminal, posterior end of the upper jaw not reaching the level of the anterior edge of the eye; eye large and rounded, diameter 6.2% SL (5.7%–7.4%), larger than the caudal peduncle depth; snout conical in lateral view, slightly shorter than the eye diameter; dorsal-fin spines strong and sharply pointed, all fin spines almost equal in length; soft dorsal-fin opposite to the anal-fin, all fin rays of soft dorsal and anal-fins branched; pectoral fin fan-shaped, all rays simple; pelvic-fin composed of 1 short spine and 1 (1–2) soft ray; anal-fin composed of 1 short spine and 8 (7–9) rays; caudal-fin slightly rounded with 12 (11–13) rays composed of 10 (9–11) branched rays, and simple dorsal- and ventral-most rays; ectocoracoid located on the ventral side of the pectoral girdle, curving antero-mesially to articulate with its opposite element; anterior side of the pelvic girdle articulating with the posterior end of the ectocoracoid.

Color of fresh specimens. Dorsal and lateral sides of the head and body dark brown with a yellowish tinge, ventral side silvery white (Figs. 2a, 4a); membranes of dorsal-fin spines dark brown with many black pigments (Fig. 2a); pectoral-fin hyaline; soft dorsal, anal, and caudal fins with yellowish brown rays and commonly hyaline membranes; pelvic-fin spine and membrane white; in the breeding season, male body and dorsal, anal and pelvic fins black (Fig. 4b), female body light brown with numerous dark brown blotches and spots (Fig. 4c).

Color in preservation. Dorsal and lateral surfaces of the head and body dark brown, ventral side light brown (Fig. 2b).

Distribution. *Pungitius modestus* is distributed in the inland area of Yamagata Prefecture in northern Honshu, Japan. This species was recorded from the northeastern area of Yamagata Prefecture in the 1930s by Hashimoto (1938). However, the northeastern population is now thought to have been extirpated because it has not been observed in the area for several decades.

TABLE 2. Frequency distributions of anal-fin and pectoral-fin rays in 12 species of *Pungitius*. Counts are based on voucher specimens in NSMT, YAMA, YUMB and ZUMT, and data provided by Denys *et al.* (2018)[1]. Counts of *P. modestus*, **sp. nov.** and *P. kaibarae* come from non-type and type specimens.

	Anal-fin rays							
	6	7	8	9	10	11	12	13
P. modestus, **sp. nov.**		3	23*	5	1			
P. bussei				Not available				
P. hellenicus				Not available				
P. kaibarae			6*	15				
P. laevis[1]		2	9	9	4*			
P. platygaster		2*						
P. polyakovi				Not available				
P. pungitius[1]		1	4	9	7	1	2	
P. sinensis					5*			
P. stenurus				2*	3*			
P. tymensis					1*			
P. vulgaris[1]		1	12	9*	2			
	Pectoral-fin rays							
P. modestus, **sp. nov.**				3	29*			
P. bussei				Not available				
P. hellenicus				Not available				
P. kaibarae					21*			
P. laevis[1]					10	12*	1	1
P. platygaster					2*			
P. polyakovi				Not available				
P. pungitius[1]				1	18	5		
P. sinensis				Not available				
P. stenurus					5*			
P. tymensis				Not available				
P. vulgaris[1]					1	20*	2	1

*Name-bearing types

Habitat and biology. *Pungitius modestus* inhabits small streams and ponds refreshed by abundant cold spring water from the bottom, with temperatures of around 16°C throughout the year. Adults feed mainly on amphipods such as *Jesogammarus jesoensis* (identified by K. Tomikawa), which are extremely abundant in the type locality of *P. modestus*. In contrast, larvae of *P. modestus* feed on tubificids, and 30-day-old juveniles feed mainly on chironomids and copepods (Kumada *et al*. in preparation). *Pungitius modestus* tends to hide in water grasses, and also

frequently remains still, suspended just above the bottom. The peak of the breeding season is May, with courtship behavior tending to occur at night, although also observed in the daytime. Females usually spawn at night, between 9 pm and dawn (T. Takeda, personal communication).

Etymology. The species name, *modestus*, refers to its moderate behavior: adults do not fight each other except during the reproductive season. As stated above, *Pungitius modestus* tends to hide in water grasses, and also frequently remains still just above the bottom. However, other species of *Pungitius* frequently swim in the water column (Hart 2003, personal observation). The new Japanese name "Kakure-tomiyo" is proposed for the new species. "Kakure" implies its hiding behavior, and "tomiyo" refers to nine-spined sticklebacks.

Remarks. As stated in Introduction, *Pungitius* is currently represented by 11 species. Below, we describe the differences between these known species and *P. modestus*.

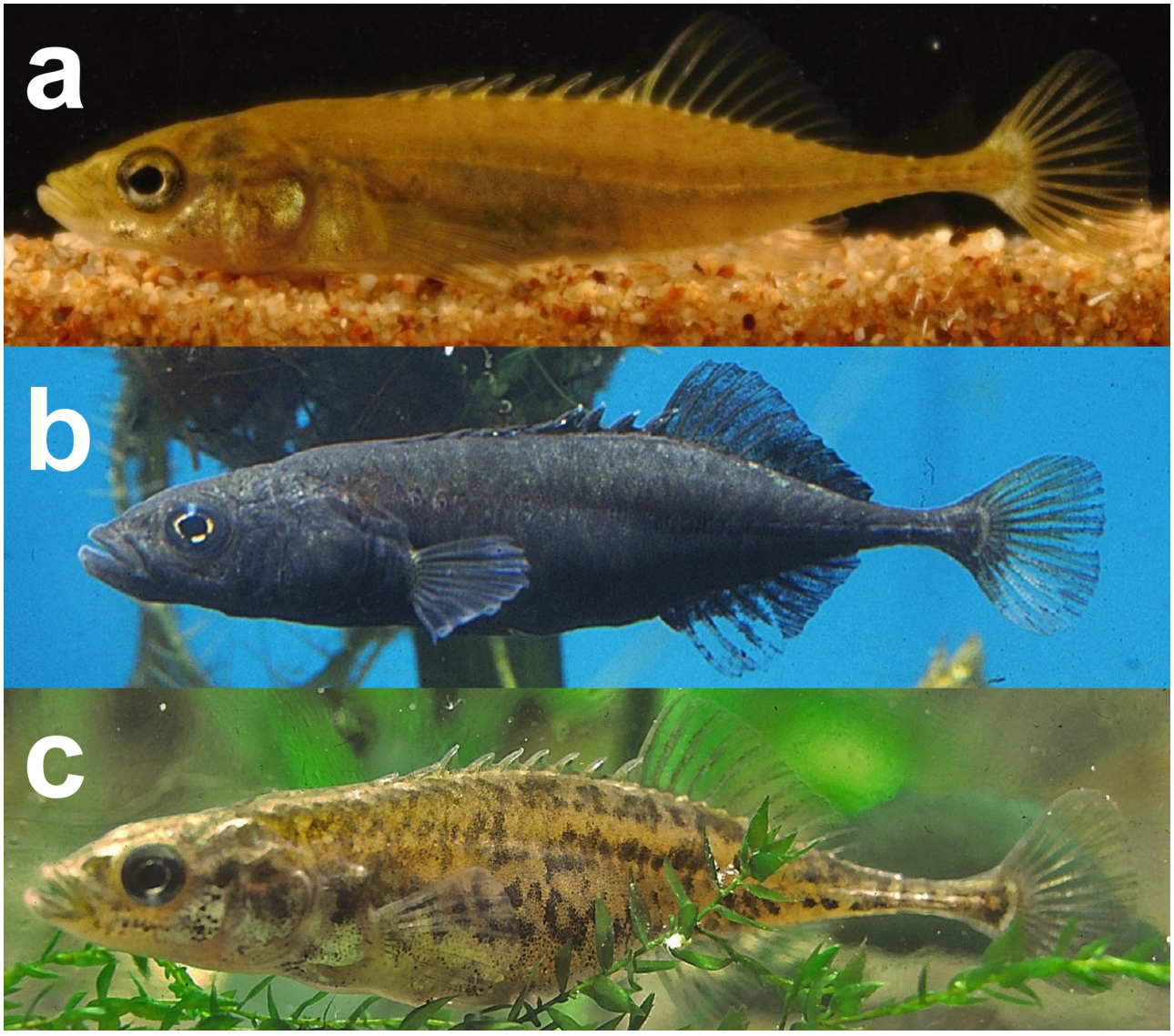

FIGURE 4. a) Living female *Pungitius modestus,* **sp. nov.** out of the breeding season. NSMT-P 136597, 41.3 mm SL, Tendo, Yamagata Prefecture, Honshu, Japan. b) Living male in the breeding season (photographed by T. Takeda, May 1992). c) Living female in the breeding season (photographed by T. Takeda, May 1992).

Denys *et al*. (2018) studied the morphological and genetic characteristics of *Pungitius* in France, recognizing three valid species: *P. pungitius*, *P. laevis*, and *P. vulgaris*. They synonymized *P. breviceps* (Blanchard, 1866) and *P. lotharingus* (Blanchard, 1866) with *P. laevis*, and *P. burgundianus* (Blanchard, 1866) with *P. pungitius*. Based on the descriptions and data provided by Keivany & Nelson (2000), Shedko *et al.* (2005) and Denys *et al*. (2018), *P. modestus* is distinguished from these three valid species as follows: it is distinguished from *P. pungitius* by the greater number of lateral plates (30–32, vs. 0–12), smaller number of dorsal-fin spines (VIII–X [usually IX], vs.

VIII–XI [usually X]), and smaller number of anal-fin rays (7–10 [usually 8], vs. 7–12 [usually 9]), the smaller diameter of the eye (21.1%–25.6%, vs. 27.3%–33.1% HL), the lack of markings on the body (vs. brownish gray blotches arranged irregularly), dark brown membranes of spiny dorsal fin (vs. hyaline), and in the breeding season, blackening of the soft dorsal and anal fins of the male (vs. hyaline).

Pungitius modestus differs from *P. laevis* by the larger number of lateral plates (30–32, vs. 0–4, and only on the caudal peduncle), the lack of markings on the body (vs. blackish blotches), dark brown membranes of spiny dorsal fin (vs. hyaline); and the ascending process of the pelvis, which is relatively well developed and extends dorsally to the level of the dorsal-most actinost (vs. less developed and smaller). The new species differs from *P. vulgaris* by the larger number of lateral plates (30–32, vs. no lateral plates), a keel on the caudal peduncle (vs. no keel), the smaller number of pectoral-fin rays (9–10 [usually 10], vs. 10–13 [usually 11]), and the smaller diameter of the eye (21.1%–25.6%, vs. 25.3%–33.9% HL).

TABLE 3. Frequency distributions of lateral plates including those on the caudal peduncle of five species of *Pungitius*. Counts are based on voucher specimens in NSMT, YAMA, YUMB and ZUMT, and data provided by Denys *et al.* (2018)[1]. Counts of *P. modestus*, **sp. nov.** and *P. kaibarae* come from non-type and type specimens. Frequency distributions of other species are not available.

	Lateral plates												
	0	1	2	3	4	5	6	7	8	9	10	11	12
P. modestus, **sp. nov.**													
P. kaibarae													
P. laevis [1]	16*	4	1	1	2								
P. pungitius [1]	6	1			2		8		3	2		1	1
P. vulgaris [1]	22*												

TABLE 3. (Continued)

	Lateral plates					
	29	30	31	32	33	34
P. modestus, **sp. nov.**		10*	16	6		
P. kaibarae	1	1		11*	5	3
P. laevis [1]						
P. pungitius [1]						
P. vulgaris [1]						

*Name-bearing types

Upon our request, James Maclaine of the BMNH kindly provided us with photographs and morphological data of syntypes of *Gasterosteus platygaster* (Fig. 5). In addition, we included the counts and morphometric data documented in Keivany & Nelson (2000) and Shedko *et al.* (2005) for comparisons of *Pungitius modestus* and *P. platygaster*. Available characteristics differentiate *P. modestus* from *P. platygaster* by its lateral plates (small and unconnected, and running from just behind the dorsal end of the gill opening to the posterior side of the caudal peduncle, vs. large connected lateral plates), a keel on the caudal peduncle (vs. no keel), the larger number of dorsal-fin rays (9–11, vs. 6–10), the first dorsal-fin spine above or behind the pectoral-fin base (vs. in front of the pectoral-fin base), and dark brown membranes of spiny dorsal fin (vs. hyaline).

Guichenot (1869) did not provide counts of fin rays in his original description of *Gasterosteus sinensis*; however, Jonathan Pfliger of MNHN kindly provided us with photographs and counts of *G. sinensis* syntypes (Fig. 6). The morphological data of the syntypes and those given by Shedko *et al*. (2005) clearly differentiate *Pungitius modestus* from *P. sinensis* by its lateral plates (small and unconnected, and running from just behind the dorsal end of the gill opening to the posterior side of the caudal peduncle, vs. large connected lateral plates), dark brown membranes of spiny dorsal fin (vs. hyaline), and in the breeding season, blackening of the male soft dorsal and anal fins (vs. hyaline).

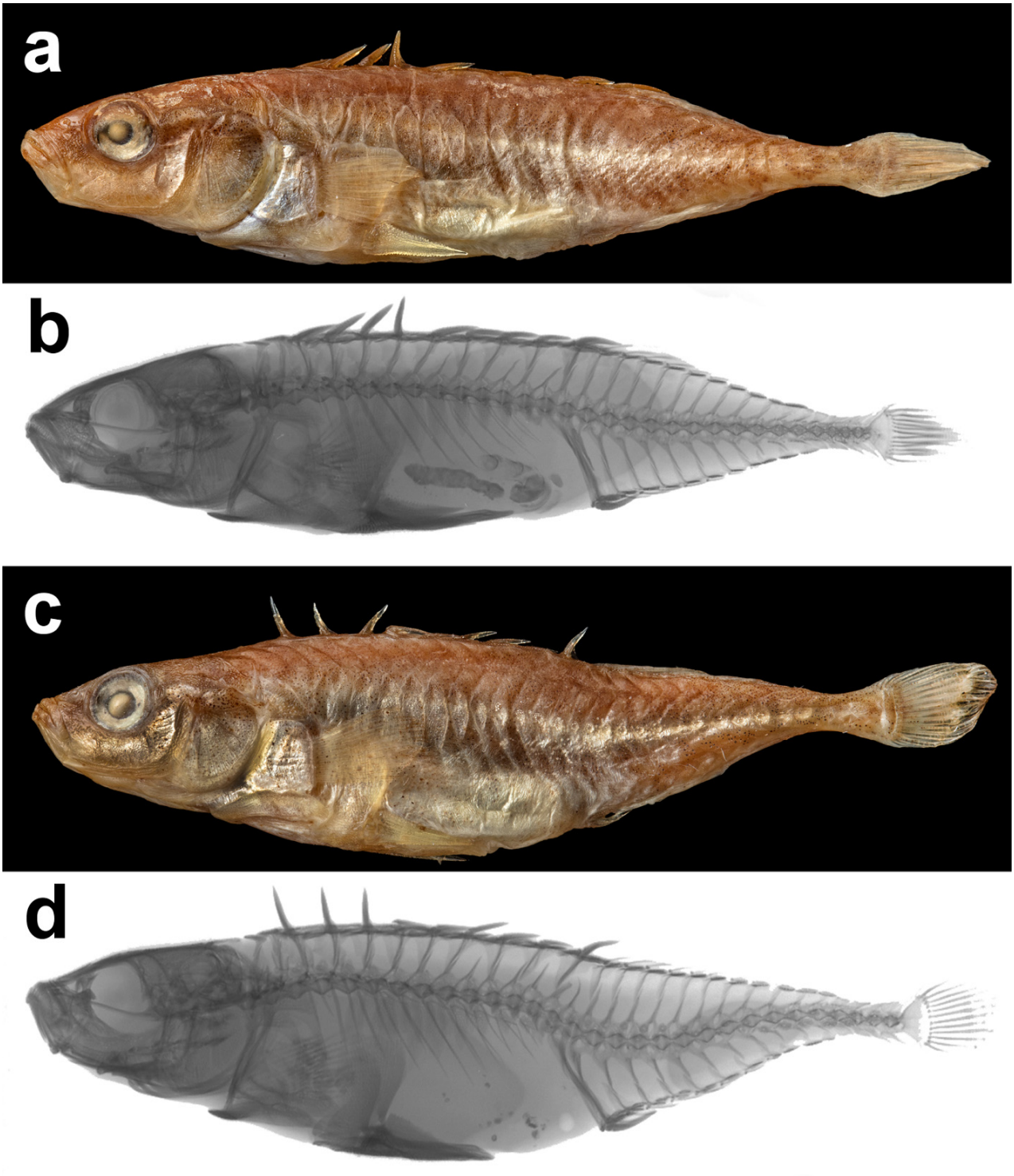

FIGURE 5. a) Syntypes of *Gasterosteus platygaster* Kessler, 1859, BMNH 1897.7.5.2, 44.4 mm SL. b) X-ray photograph of syntypes of *G. platygaster*, 44.4 mm SL. c) Syntypes of *G. platygaster* Kessler, 1859, BMNH 1897.7.5.2, 39.3 mm SL. d) X-ray photograph of syntypes of *G. platygaster*, 39.3 mm SL.

We obtained photographs and counts of syntypes of *Gasterosteus stenurus* through help of Anastasia Yurtseva of ZIN (Fig. 7). The morphological data clearly differentiate *Pungitius modestus* from *P. stenurus* by its lateral plates (small and unconnected, and running from just behind the dorsal end of the gill opening to the posterior side of the caudal peduncle, vs. large connected lateral plates), the smaller number of anal-fin rays (7–10 [usually 8], vs. 9–10), and the first dorsal-fin spine above or behind the pectoral-fin base (vs. in front of the pectoral-fin base).

FIGURE 6. Syntypes of *Gasterosteus sinensis* Guichenot, 1869, MNHN 0000-5228, 21–26 mm SL.

FIGURE 7. Syntypes of *Gasterosteus stenurus* Kessler, 1876, ZIN 2471, 46–55 mm SL.

FIGURE 8. a) Syntypes of *Gasterosteus bussei* Warpachowski, 1888, ZIN 7100, 42–53 mm SL. b) X-ray photograph of syntypes of *G. bussei*.

TABLE 4. Proportional measurements of type specimens of *Pungitius modestus*, **sp. nov.** expressed as percentages of standard length and head length.

	Holotype	17 paratypes	
	NSMT-P 133674	range	mean±standard deviation
Standard length (mm)	45.4	38.7–51.7	45.3±3.7
As % SL			
Body depth	19.1	16.9–19.8	18.6±0.9
Oblique body depth	16.8	15.5–20.0	17.6±1.0
Body width	10.6	9.3–13.2	11.6±1.0
Head length	27.4	26.3–30.0	28.5±1.2
Head width	11.7	10.0–12.9	11.7±0.9
Snout length	7.0	6.4–8.0	7.4±0.4
Eye diameter	6.2	5.7–7.4	6.6±0.4
Interorbital width	4.9	4.5–6.5	5.1±0.5
Post-orbital length	13.3	12.8–16.0	14.6±0.9
Pre-dorsal-fin length	33.2	33.0–35.8	34.3±0.8
Dorsal-fin spine base length	28.7	26.5–29.8	27.5±1.0
Soft dorsal-fin base length	22.4	21.5–24.8	23.1±1.1
Pre-pectoral-fin length	31.9	31.5–35.4	33.1±1.2
Pre-pelvic-fin length	37.8	36.6–41.2	38.8±1.3
Pre-anal-fin length	59.9	60.5–67.4	62.9±1.6
Anal-fin base length	20.7	19.8–24.4	21.4±1.2
Caudal peduncle length	18.8	14.3–19.7	16.6±1.6
Caudal peduncle depth	3.3	2.9–4.1	3.4±0.3
Longest dorsal-fin spine length	3.6	2.7–4.9	3.8±0.7
Dorsal-fin ray length	11.4	9.4–11.9	10.5±0.8
Pectoral-fin length	13.7	12.0–15.9	14.0±1.1
Pelvic-fin spine length	7.4	6.3–9.1	7.8±0.9
Anal-fin ray length	11.7	10.1–12.9	11.0±0.7
Anal-fin spine length	5.0	4.1–6.0	5.0±0.5
Caudal-fin length	14.6	12.1–15.5	14.0±1.0
As % HL			
Snout length	25.7	23.1–28.5	26.1±1.6
Eye diameter	22.5	21.1–25.6	23.2±1.3
Interorbital width	18.0	16.0–24.6	18.0±2.1
Post-orbital length	48.6	48.5–54.3	51.3±1.9
Counts			
Dorsal-fin rays	IX+9	VIII–IX+9–11	
Pectoral-fin rays	10	9–10	
Anal-fin rays	8	7–9	
Caudal-fin rays	i, 10, i	i, 10, i	
Lateral plates	30	30–32	

Staff members of ZIN attempted to locate four syntypes (ZIN 7100) of *Gasterosteus bussei*, but unfortunately, they were missing. However, Hiroshi Takahashi of the National Fisheries University in Japan was able to provide a photograph and X-ray of the syntypes (Fig. 8) sent to him from Valentina G. Sideleva of ZIN in 2017. The available morphological data differentiate *Pungitius modestus* from *P. bussei* by the first dorsal-fin spine located above

or behind the pectoral-fin base (vs. in front of the pectoral-fin base), the lateral plates (small and unconnected, and running from just behind the dorsal end of the gill opening to the posterior side of the caudal peduncle, vs. large connected lateral plates), the longer pre-dorsal (33.0%–35.8%, vs. 27.0%–29.4% SL), the shorter base of the spiny dorsal fin (26.5%–29.8%, vs. 32.1%–33.5% SL), and the shorter pelvic-fin spine (6.3%–9.1%, vs. 10.0%–11.6% SL) and the longer caudal-fin (12.1%–15.5%, vs. 10.7%–10.9% SL).

TABLE 5. Proportional measurements and counts of type specimens of *Pungitius kaibarae* expressed as percentages of standard length and head length. Although all paratypes were examined, the proportional measurements of ZUMT 59852 are excluded from the table because it is shorter than 35 mm SL.

	Holotype	7 paratypes	
	ZUMT 8197	range	mean±standard deviation
Standard length (mm)	45.0	39.4–45.0	41.2±2.0
As % SL			
Body depth	20.9	20.9–24.2	22.8±1.4
Oblique body depth	18.7	16.9–23.0	19.3±2.5
Head length	28.4	27.8–32.0	29.7±1.5
Snout length	6.7	5.8–8.7	7.3±1.0
Eye diameter	6.2	6.2–9.3	7.9±0.7
Post-orbital length	14.7	13.8–16.9	15.4±1.2
Pre-dorsal-fin length	30.0	25.9–30.8	28.7±2.1
Dorsal-fin spine base length	32.0	28.0–36.3	32.7±3.6
Soft dorsal-fin base length	23.3	21.5–28.3	25.3±2.5
Pre-pectoral-fin length	31.1	29.5–33.8	31.4±1.9
Pre-pelvic-fin length	35.3	36.3–40.7	38.9±1.7
Pre-anal-fin length	54.7	60.0–61.9	60.7±0.8
Anal-fin base length	23.8	24.0–26.8	25.2±1.1
Caudal peduncle length	21.3	14.5–16.8	15.6±0.6
Caudal peduncle depth	2.9	2.7–3.6	3.2±0.3
Pectoral-fin length	14.7	14.2–16.0	14.9±0.6
Pelvic-fin spine length	10.9	8.2–13.3	11.0±1.7
As % HL			
Snout length	23.4	18.5–30.0	24.7±4.1
Eye diameter	21.9	23.2–29.8	26.4±2.3
Post-orbital length	51.6	47.5–56.7	51.9±3.1
Counts			
Dorsal-fin rays	VIII+9	VIII–IX+9–11	
Pectoral-fin rays	10	10	
Anal-fin rays	I, 8	I, 8–9	
Caudal-fin rays	i, 10, i	i, 10, i	
Lateral plates	32	32–34	

James Maclaine of BMNH kindly provided us with photographs and morphological data of syntypes of *Gasterosteus tymensis* (Fig. 9). This plus the characteristics of *Pungitius tymensis* provided in Keivany & Nelson (2000) and Shedko *et al.* (2005) differentiate *P. modestus* from *P. tymensis* by its lateral plates (small and unconnected, and running from just behind the dorsal end of gill opening to the posterior side of the caudal peduncle, vs. several lateral plates in the area just behind the end of the gill opening and the caudal peduncle only), the smaller number of dorsal-fin spines (VIII–X, vs. VIII–XIII), the smaller number of anal-fin rays (7–10, vs. 8–12), the ascending process of the pelvis (relatively developed and extending dorsally to the level of the dorsal-most actinost, vs. less developed and smaller).

FIGURE 9. a) Syntype of *Gasterosteus tymensis* Nikolski, 1889, BMNH 1892.4.28, 58.5 mm SL. b) X-ray photograph of syntype of *G. tymensis*.

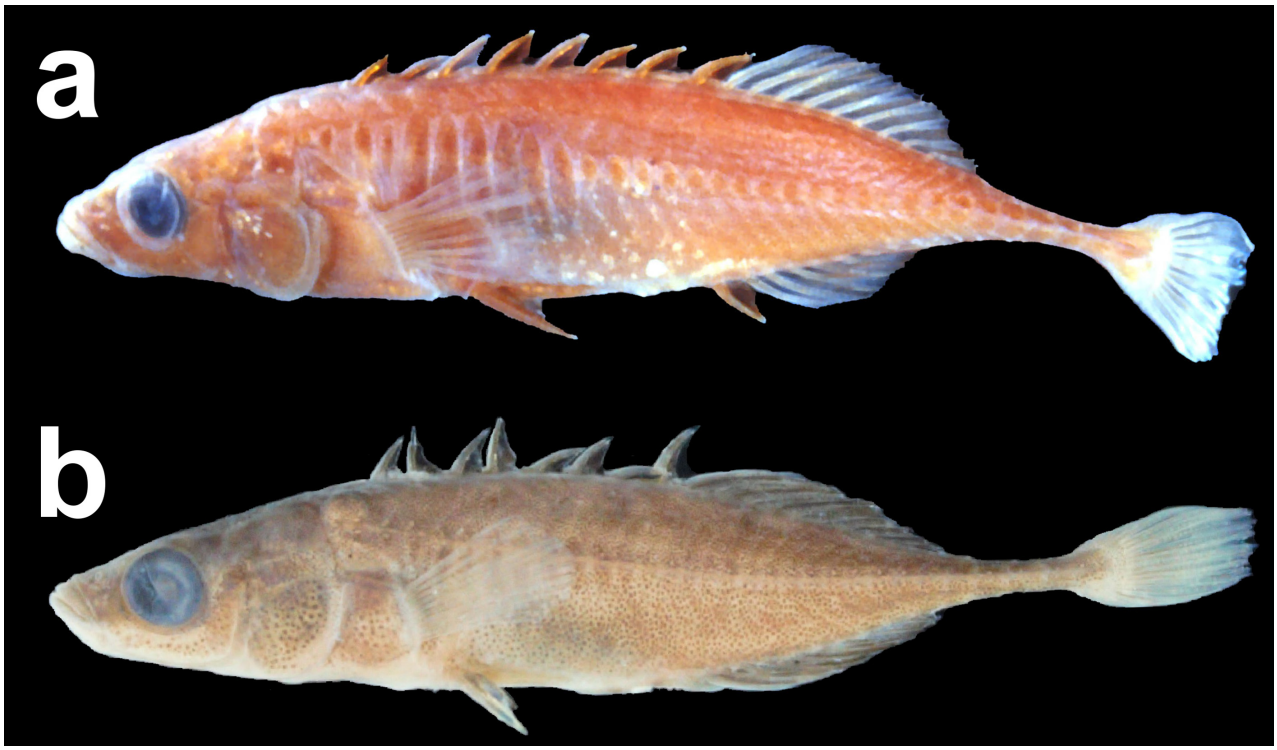

FIGURE 10. a) Holotype of *Pygosteus kaibarae* Tanaka, 1915, ZUMT 8197, 45.0 mm SL (photographed by K. Hosoya). b) *Pungitius kaibarae* from Korea, NSMT-P 140556, 31.8 mm SL, Gangneung, Gangwon-do, Korea.

Within the genus *Pungitius, P. modestus* is most similar to *P. kaibarae*, which was described by Tanaka (1915) based on the type specimens collected from Kyoto Prefecture (Fig. 10a). Since this original description, this species has also been collected from Hyogo, Kyoto, and Osaka prefectures in Japan as well as from Korea (Tanaka *et al.* 1982, Chae and Yang 1993, Hosoya 2013, Bae and Suk 2015). Although all Japanese populations were extirpated in the period from the 1930s to 1950s (Hosoya 2018), the species is still found along the eastern side of the Korean Peninsula (Bae & Suk 2015) (Fig. 10b). However, Hosoya (2013) suggested that the Kyoto and Korean populations of *P. kaibarae* differ at the species level. Our examinations differentiate *P. modestus* from the type specimens of *P.*

kaibarae (Table 5) and specimens from Korea by its lateral plates (small and unconnected, and running from just behind the dorsal end of gill opening to the posterior side of the caudal peduncle, vs. large connected lateral plates) (Fig. 11a–c), the location of the first dorsal-fin spine (behind, vs. in front of the pectoral-fin base) (Fig. 12a–c), the larger number of dorsal-fin spines (VIII–X [usually IX], vs. VIII–IX [usually VIII]) and the larger number of anal-fin rays (7–10 [usually 8], vs. 8–9 [usually 9]), the shorter base of the spiny dorsal fin (26.5%–29.8%, vs. 28.0%–36.3% SL), the longer pre-anal fin (59.9%–67.4%, vs. 54.7%–61.9% SL) and the anal-fin spine below the 1st–3rd dorsal-fin rays (vs. below or in front of the 1st dorsal-fin ray), the shorter pelvic-fin spine (6.3%–9.1%, vs. 8.2%–13.3% SL), and dark brown membranes of spiny dorsal fin (vs. black).

FIGURE 11. Comparisons of lateral plates of *Pungitius modestus* and *P. kaibarae*. a) *Pungitius modestus,* **sp. nov.**, paratype, NSMT-P 140554, 40.5 mm SL. b) *P. kaibarae*, holotype, ZUMT 8197, 45.0 mm SL. c) *P. kaibarae* from Korea, non-type, NSMT-P 140556, 31.8 mm SL. Edges of the lateral plates are shown in red.

Keivany *et al*. (1997) re-described in detail *Pungitius hellenicus*, which is restricted to central Greece. In addition, its color was detailed in Keivany & Nelson (2000). *Pungitius modestus* differs from *P. hellenicus* by VIII–X dorsal-fin spines inclining alternately left and right along the mid-line (vs. II–VI dorsal-fin spines vertically arranged), a keel on the caudal peduncle (vs. no keel), a lack of markings on the body (vs. dark bars or blotches on the body), dark brown membranes of spiny dorsal fin (vs. hyaline), the presence of the anteroventral process of the ectocoracoid (vs. absence of the anteroventral process of the ectocoracoid), and the presence of a pelvic girdle (vs. absence of a pelvic girdle).

Shedko *et al*. (2005) described *Pungitius polyakovi* based on the holotype and 25 paratypes collected from southeastern Sakhalin Island, Russia. Their detailed description clearly differentiates *P. modestus* from *P. polyakovi* by the larger number of lateral plates (30–32, vs. 4–6), the smaller number of dorsal-fin spines (VIII–X [usually IX], vs. VIII–XI [usually X]), smaller dorsal-fin rays (9–11, vs. 10–12), the smaller number of anal-fin rays (7–10, vs. 9–11); the longer caudal peduncle (14.3%–19.7%, vs. 7.8%–11.6% SL); the lack of markings on the body (vs. 4–8 transverse brown bars on the body); in the breeding season, blackening of the entire male body (vs. blackening of only the ventral side of the head and body); the presence of the anteroventral process of the ectocoracoid (vs. absence of the anteroventral process of the ectocoracoid); articulation of the right and left ectocoracoids at the anterior end (vs. no articulation of the right and left ectocoracoids); and the ascending process of the pelvis (extending dorsally to the level of the dorsal-most actinost, vs. less developed and smaller).

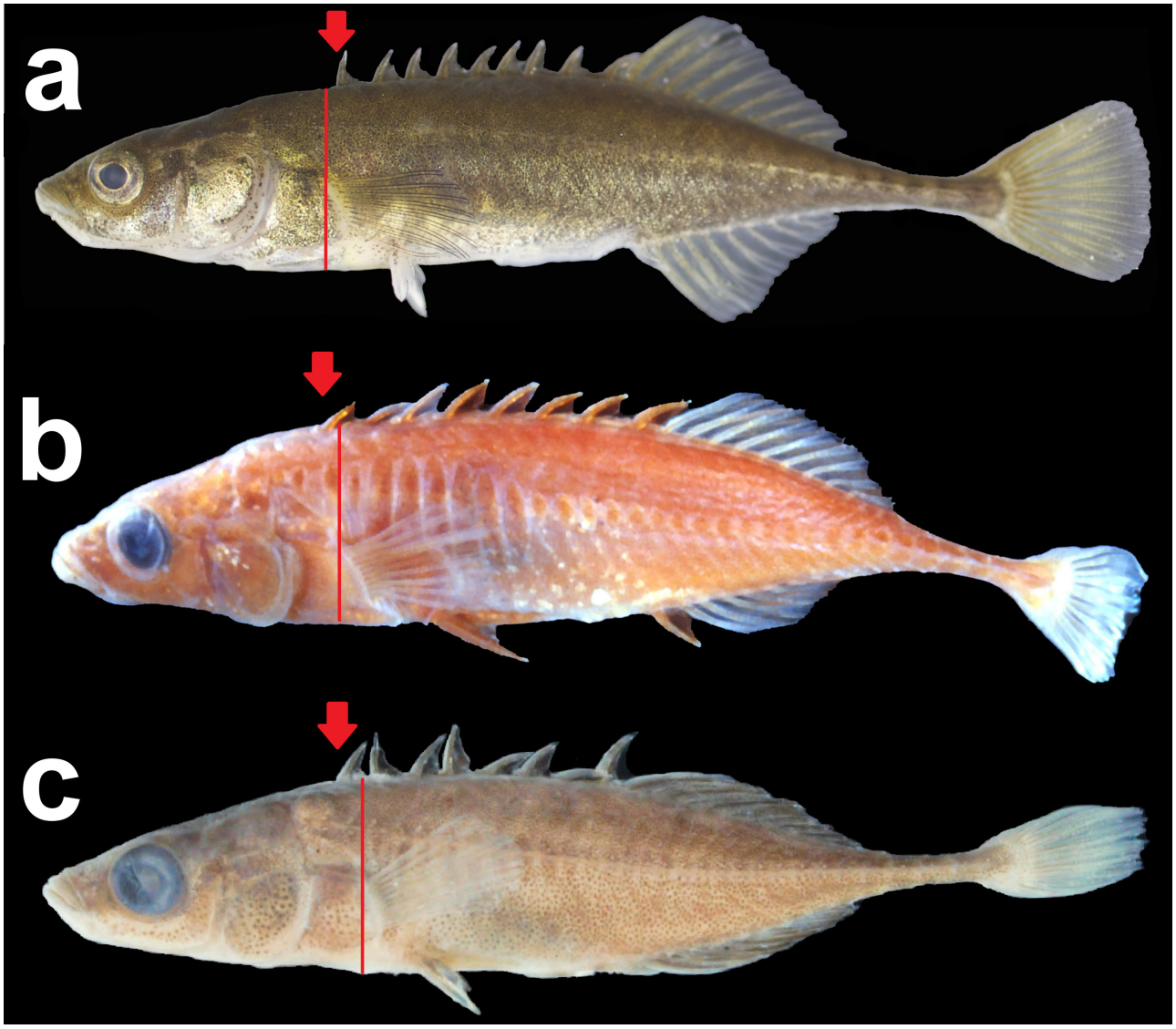

FIGURE 12. Comparisons of the origin of the spiny dorsal fin in *Pungitius modestus* and *P. kaibarae*. a) *Pungitius modestus,* **sp. nov.**, holotype, NSMT-P 133674, 45.4 mm SL. b) *P. kaibarae*, holotype, ZUMT 8197, 45.0 mm SL. c) *P. kaibarae* from Korea, non-type, NSMT-P 140556, 31.8 mm SL. The first dorsal-fin spine is shown by a red arrow, and the origin of the pectoral-fin base is shown by a red vertical line.

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