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Morphological Characteristics of Three Rare Freshwater Populations of the Stickleback, *Gasterosteus aculeatus aculeatus* (Teleostei, Gasterosteidae)

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Abstract Morphological characteristics of three rare freshwater populations of *Gasterosteus aculeatus aculeatus* (Paramushir population from the Kuril Islands, Ohtsuchi population from Iwate Prefecture and Okanjichi population from Tochigi Prefecture) are demonstrated for the first time, from museum specimens. The Paramushir population, with partially armored lateral plates, is thought to represent an ancient freshwater resident lineage, whereas the Ohtsuchi population, with fully armored lateral plates, numerous vertebrae and a relatively larger body, has possibly become a freshwater population comparatively recently. The Okanjichi population, also with fully armored lateral plates, differed from the closest Chikazono population in the same river system, the latter exhibiting a considerable lateral plate variation, from partially to fully armored. These populations, representing very important morphological diversity, should be accorded high conservation status in the future.
Key words: Threespine stickleback, Freshwater resident, Morphology, Lateral plate, Conservation, Natural monument.

Introduction

The threespine stickleback genus *Gasterosteus*, including *G. aculeatus* Linnaeus, 1758 (type species), *G. wheatlandi* Putnam, 1867, and *G. nipponicus* Higuchi *et al.*, 2014, is an intensively studied model for evolutionary biology, exhibiting wide morphological and ecological variations (e.g. Tinbergen, 1951; van den Assem, 1967; Hagen, 1967; McPhail, 1969; Wootten, 1976; Bell and Foster, 1994; McKinnon and Rundle, 2002; Goto and Mori, 2003). *Gasterosteus wheatlandi* is primarily distinguished from *G. aculeatus* by having pelvic fins with one spine with two well-developed pointed cusps at the base, and two soft rays (vs. one cusp and one soft ray in the latter) (Scott and Crossman, 1973). *Gasterosteus nipponicus* differs from *G. aculeatus* in having the lateral plates abruptly reducing in size above the anus, and its caudal keels thin and membranous (vs. gradually reducing in size with tough bony keels, or the lateral plates partially degenerate in some freshwater populations, see below) (Higuchi *et al.*, 2014).

Gasterosteus aculeatus is known to exhibit wide variations in plate morphology, from fully armored to non-armored (e.g. Cuvier, 1829; Cuvier and Valenciennes, 1829; Regan, 1909), and life mode, from anadromous to fully freshwater (e.g. Hagen, 1967; Moodie, 1972; Bell, 1976; McPhail, 1984; Ziuganov *et al.*, 1987). Whilst all anadromous individuals are fully

Table 1. Freshwater resident populations of *Gasterosteus acculeatus aculeatus* and *G. a. microcephalus* examined (museum catalogued specimens measured during present study; remaining populations cited from literature)

Species and population	Abbreviation	*n*	Catalogue Number or Literature	Collection date	No. in Fig. 1
Freshwater resident of *G. a. aculeatus*					
Kamchatka Peninsula					
Azabachije Lake	Azabachije	332	Ziuganov *et al.* (1987)		1
Kuril Islands					
Shumshu Is. small lake	Shumshu	1	Ikeda (1935)		2
Paramushir Is. small lake	Paramushir	9	HUMZ 152759–152768 (exp. 152761)	August, 1997	3
Itrup Is. Motori L.	Motori	1	Ikeda (1935)		4
Itrup Is. Kamuikotan L.	Kamuikotan	1	Ikeda (1935)		5
Kunashir Is. Higashibiroku L.	Higashibiroku	1	Ikeda (1935)		6
Honshu Is., Japan					
Ohtsuchi, Gensui R., Iwate Prefecture	Ohtsuchi	11	NSMT-P 114420–114430	April, 2001	7
Tahara, Agano R. system, Fukushima Pref.	Tahara	17	Ikeda (1933)		8
Ichinozawa, Okanjichi, Naka R. s., Tochigi Pref.	Okanjichi	12	NSMT-P SK8948	August, 1964	9
Chikazono, Naka R. s., Tochigi Pref.	Chikazono 1	26	Ikeda (1933)		10
Chikazono, Naka R. s., Tochigi Pref.	Chikazono 2	71	Igarashi (1970)		10
Ohno, Kuzuryu R. s., Fukui Pref.	Ohno	32	Ikeda (1933)		11
G. a. microcephalus (*G. aculeatus* subsp. 2 sensu Hosoya, 2013)					
Kanou, Nagara R. s., Gifu Pref.	Kanou	33	Ikeda (1933)		12
Yahata, Ibi R. s., Gifu Pref.	Yahata	6	Ikeda (1933)		13
Nanatori, Ibi R. s., Mie Pref.	Nanatori	27	Ikeda (1933)		14
Toyosato, Uso R. s., Shiga Pref.	Toyosato	17	Ikeda (1933)		15
Imashuku, Yasu R. s., Shiga Pref.	Imashuku	14	Ikeda (1933)		16

armored, semi- (lateral plates separated into front series and caudal keels), less- (lacking caudal keels) and non-armored (without plates, sometimes with degenerate dorsal spines) populations are prevalent in freshwater environments. These varieties have been traditionally classified into three subspecies (Miller and Hubbs, 1969; Eschmeyer, 1998); fully armored *G. a. aculeatus* (Linnaeus, 1758), partially (semi- or less-) armored *G. a. microcephalus* (Girard, 1854), and non-armored *G. a. williamsoni* (Girard, 1854). However, less than fully armored populations are thought to have been derived separately, following adaptation to local freshwater environments (Bell, 1976; MacPhail, 1984; Bell and Foster, 1994; McKinnon and Mori, 2003; Colosimo *et al.*, 2005). Accordingly, it has been proposed that the application of these subspecies names to any local populations (including the type locality populations) should be carefully re-examined (Sakai and Yabe, 2003; Watanabe *et al.*, 2003; Higuchi *et al.*, 2014).

Many freshwater stocks of *G. aculeatus* are known from the mainland Asian coast, Japan, the Kuril Islands, and Kamtchatka Peninsula (e.g. Ikeda, 1933, 1935; Igarashi, 1964, 1965, 1970; Ziuganov *et al.*, 1987; Higuchi and Goto, 1996; Watanabe *et al.*, 2003), being represented by

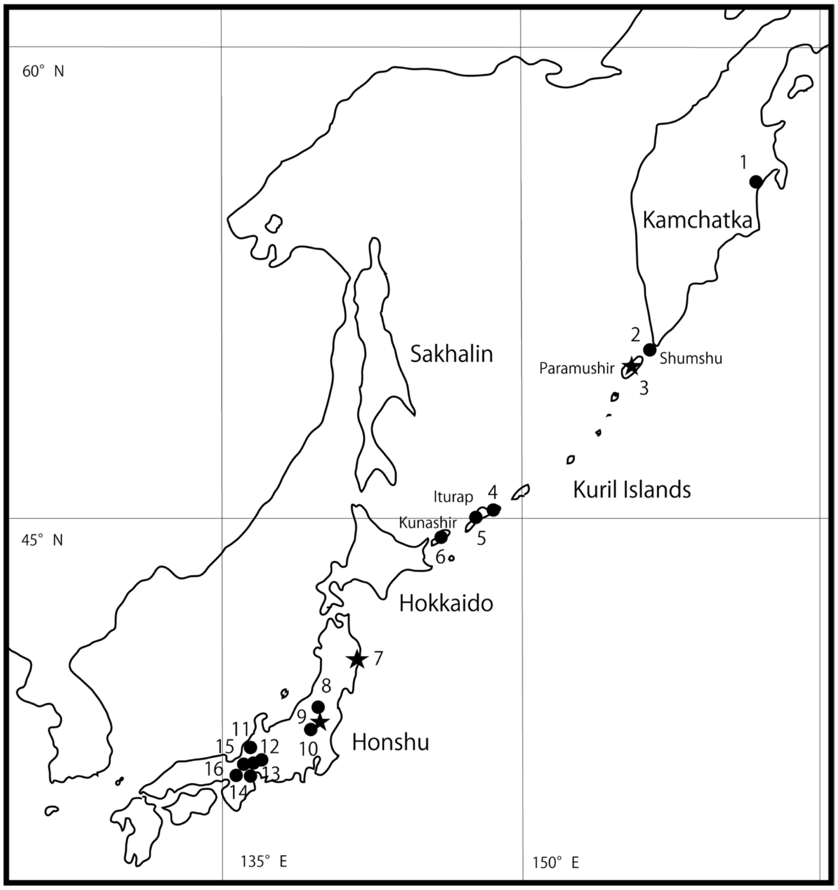

Fig. 1. Map from Kamchatka Peninsula to the Japanese Archipelago, showing localities of the three freshwater *Gasterosteus aculeatus aculeatus* populations examined in the present study (stars) and populations described in the literature (circles). 1 Azabachije, 2 Shumushu, 3 Paramushir, 4 Motori, 5 Kamuikotan, 6 Higashibiroku, 7 Ohtsuchi, 8 Tahara, 9 Okanjichi, 10 Chikazono, 11 Ohno, 12 Kanou, 13 Yahata, 14 Nanatori, 15 Toyosato, and 16 Imashuku populations. Population abbreviations shown in Table 1.

fully or partially (semi- or less-) armored forms. In particular, semi-armored populations in springs in Shiga Prefecture, and less-armored populations in Gifu and Mie Prefectures, Japan (Ikeda, 1933; Watanabe and Mori, 2003; Watanabe *et al.*, 2003), have traditionally been classified as *G. a. microcephalus* (called 'Hariyo' in Japanese), but Hosoya (2013) has tentatively called 'Hariyo' as *G. aculeatus* subsp. 2 and the other freshwater populations of threespine sticklebacks as subsp. 1.

During an examination of museum-held *Gasterosteus* specimens prior to a morphological comparison of *G. aculeatus* and *G. nipponicus* (Sakai *et al.*, 2013; Higuchi *et al.*, 2014), one of the authors (HS) became aware of three rare freshwater populations of *G. a. aculeatus* from Japan and Kuril Islands, with as yet unreported morphological characteristics. The morphology of these populations, especially lateral plate characteristics, are documented and compared with literature descriptions of neighboring populations.

Materials and Methods

In the present study, *G. a. microcephalus* is applied to the so-called 'Hariyo' and *G. a. acu-*

Table 2. Morphological comparisons of freshwater resident of *Gasterosteus aculeatus aculeatus* from Paramushir, Ohtsuchi and Okanjichi populations

	Paramushir (n = 9)	Ohtsuchi (n = 11)	Okanjichi (n = 12)
Standard length (SL mm)	48.4 ± 2.2 (45.3–51.8)*	69.8 ± 18.5 (47.2–99.5)**	29.9 ± 8.0 (20.2–41.0)***
In % of SL			
Head length (HL)	32.1 ± 1.2 (30.9–33.3)	30.2 ± 1.6 (27.5–32.6)	35.0 ± 1.2 (33.2–37.6)
Body depth	24.8 ± 1.1 (23.7–25.8)	24.3 ± 0.9 (22.9–25.7)	29.3 ± 1.0 (27.3–31.1)
Caudal peduncle length	14.5 ± 0.6 (13.9–15.1)	12.2 ± 1.3 (10.6–14.9)	9.6 ± 0.9 (8.2–11.2)
Caudal peduncle depth	4.3 ± 0.4 (3.9–4.7)	3.9 ± 0.4 (3.3–4.2)	6.0 ± 0.7 (5.2–7.2)
Predorsal length	65.2 ± 1.0 (64.2–66.2)	64.9 ± 1.2 (62.5–66.6)	67.1 ± 1.4 (64.0–68.9)
Preanal length	73.8 ± 1.8 (72.0–75.6)	73.5 ± 1.7 (70.5–76.6)	76.1 ± 2.3 (72.4–80.6)
Prepelvic length	49.2 ± 1.7 (47.5–50.9)	46.4 ± 1.3 (44.1–48.7)	47.0 ± 1.9 (42.0–49.3)
Dorsal fin base length	20.7 ± 1.4 (19.3–22.1)	24.3 ± 1.3 (16.0–26.8)	25.2 ± 1.5 (22.7–27.2)
Anal fin base length	14.0 ± 1.2 (12.8–15.2)	15.1 ± 2.0 (10.1–17.4)	16.6 ± 1.2 (15.1–19.5)
First dorsal spine length	7.9 ± 1.3 (6.6–9.2)	8.8 ± 1.4 (7.3–11.7)	9.4 ± 1.5 (7.3–11.3)
Second dorsal spine length	9.1 ± 1.6 (7.5–10.7)	10.2 ± 0.9 (8.7–11.9)	9.7 ± 1.2 (7.8–11.3)
Third dorsal spine length	3.2 ± 0.4 (2.8–3.6)	3.2 ± 0.9 (2.0–4.5)	3.8 ± 0.8 (2.7–5.0)
Pelvic spine length	14.0 ± 1.1 (12.9–15.1)	14.3 ± 1.5 (12.2–16.5)	16.8 ± 2.9 (13.1–22.6)
Pectoral fin length	15.9 ± 0.8 (15.1–16.7)	15.4 ± 0.8 (14.6–17.2)	17.0 ± 1.6 (14.1–19.5)
Length of longest dorsal ray	10.2 ± 0.9 (9.3–11.1)	8.9 ± 1.4 (6.9–11.7)	11.1 ± 1.0 (9.8–12.5)
Length of longest anal ray	9.5 ± 1.0 (8.5–10.5)	8.8 ± 1.2 (6.5–10.7)	10.1 ± 1.4 (8.0–11.9)
In % of HL			
Snout length	28.8 ± 1.2 (27.6–30.0)	30.9 ± 2.2 (23.2–37.0)	24.1 ± 1.6 (22.2–27.0)
Upper jaw length	22.4 ± 1.6 (19.9–24.1)	22.2 ± 1.7 (19.9–25.5)	24.7 ± 2.2 (20.3–28.0)
Eye diameter	27.0 ± 1.5 (24.5–29.5)	24.5 ± 3.0 (20.9–32.8)	30.1 ± 2.7 (26.5–34.8)
Interorbital width	22.3 ± 1.1 (21.2–24.7)	22.2 ± 2.0 (20.2–24.8)	21.7 ± 1.8 (18.2–24.3)
Number of isolated dorsal spines	3	3	3
Number of pectoral fin rays	10	10	10
Number of dorsal soft rays	10.3 ± 0.5 (10–11)	11.4 ± 0.9 (10–13)	11.2 ± 0.6 (10–12)
Number of anal soft rays	8.7 ± 0.9 (8–10)	8.4 ± 0.8 (7–9)	7.8 ± 0.4 (7–8)
Number of lateral plates	6.0 ± 1.4 (5–9)	32.2 ± 1.0 (31–34)	30.6 ± 0.5 (30–31)
Number of vertebrae	31.6 ± 0.5 (31–32)	32.4 ± 1.1 (31–34)	30.0 ± 0.0 (30–30)

*: mean ± SD (min–max)
**: bimodal size distribution including four smaller individuals than 56 mm SL and seven larger individuals than 67 mm SL.
***: bimodal size distribution including six smaller individuals than 26 mm SL and six larger individuals than 34 mm SL.

leatus to the other freshwater resident populations tentatively. The three populations examined in the present study, together with 13 freshwater populations of *G. a. aculeatus* and *G. a. microcephalus* discussed in the literature (Ikeda, 1933, 1935; Igarashi, 1970; Ziuganov *et al.*, 1987), are outlined in Table 1 and Fig. 1. Of the three populations, the Paramushir population (Kuril Islands), deposited in HUMZ (Marine Zoology, Hokkaido University), is a new record, the Ohtsuchi population (Iwate Prefecture), deposited in NSMT-P (National Museum of Nature and Science, Tokyo), was collected before the 2011 Great East Japan Earthquake, and the Okanjichi population, deposited in NSMT-P, is now designated as a Natural Monument by Ohtawara City, Tochigi Prefecture (Yoshida *et al.*, 2011). The last population specimens represent those before the disappearance around 1974 in the upper reaches of the Okanjichi River, and nowadays population has re-established naturally around 2007 in the lower reaches (Yoshida *et al.*, 2011). Both specimens of the Ohtsuchi and Okanjichi populations consist of two size groups, smaller and larger individuals, mean 81.1 mm ± 12.3 standard deviation (67.5–99.5 mm) and 50.0 ± 3.9 (47.2–55.6) in the Ohtsuchi population, and 37.3 ± 2.2 (34.4–41.0) and 22.4 ± 1.8 (20.2–25.6) in the Okanjichi population (Table 2), origin or background of the size groups unknown but not excluding a possibility of age classes. Arai *et al.* (2003) documented that the two size

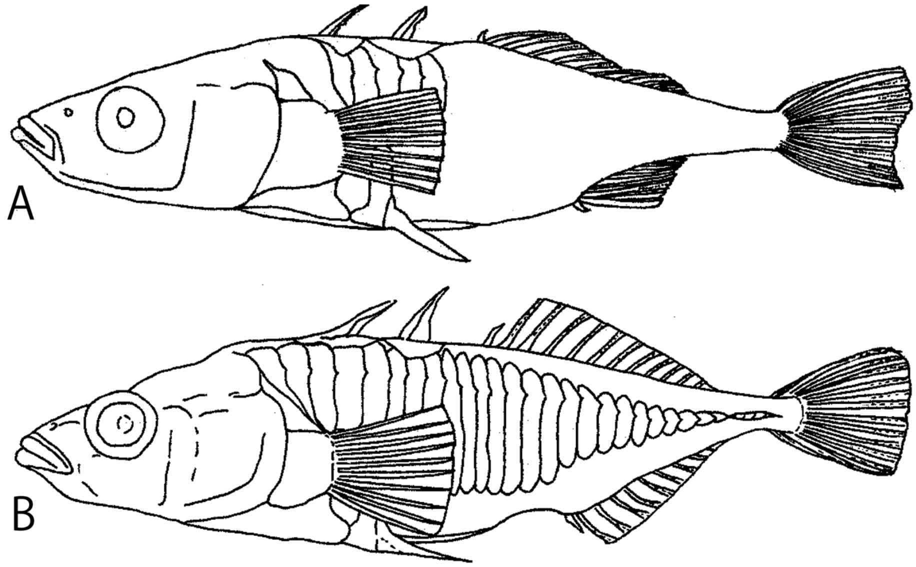

Fig. 2. Diagrammatic illustrations of *Gasterosteus aculeatus aculeatus*. A, HUMZ 152768 (50.9 mm SL), collected from the Paramushir population in August 1997; B, NSMT-P SK 8948 (41.0 mm SL), collected from the Okanjichi population in August 1964.

groups from the same locality of the Ohtsuchi River had no trait of sea migration in their otoliths, indicating their freshwater resident life.

Counts and measurements generally followed Hubbs and Lagler (1958). Measurements were made with dial calipers to the nearest 0.1 mm. All relative measurements, except for standard length (SL), are given as percentages of SL or head length (HL). Vertebral counts and dorsal and anal fin ray counts were made from soft *X*-ray photos. Differences in lateral plate numbers among five less-armored populations of *G. a. aculeatus* and *G. a. microcephalus* and among four fully armored populations of *G. a. aculeatus* with more than five individuals were tested with Mann-Whitney *U*-test (with sequential Bonferroni correction by BH method, Benjamini and Hochberg, 1995). Differences in standard length (SL) and number of vertebrae were also tested by the same manner.

Results and Discussion

Measurements and counts of the three populations are given in Table 2, with frequency distributions of lateral plates in Table 3, together with known freshwater populations of *G. a. aculeatus* and *G. a. microcephalus*.

The Paramushir and Okanjichi (larger size group) populations consisted of smaller individuals than the Ohtsuchi population (larger size group) in standard length (Paramushir–Ohtsuchi: $U = 0.0$, $P < 0.01$; Okanjichi–Ohtsuchi: $U = 0.0$, $P < 0.01$) (Table 2). It has been determined that freshwater resident sticklebacks generally have smaller size, and fewer vertebrae and lateral plates (e.g. Ikeda, 1933, 1934; McKinnon and Rundle, 2002; McKinnon and Mori, 2003). The adult size of the recent Okanjichi population has been reported as around 40 mm SL (Yoshida *et al.*, 2011). Therefore, the specimens of the Paramushir population and larger specimens of the Okanjichi population (Table 2) are considered as adult. The Ohtsuchi population (eight individuals countable) had more vertebrae than the Okanjichi population ($U = 0.0$, $P < 0.01$) and had a tendency to have a little more vertebrae than the Paramushir population although the difference was marginally significant ($U = 19.0$, $0.05 < P < 0.1$) (Table 2), the Ohtsuchi poplation being

Table 3. Variations in number of lateral plates in *Gasterosteus aculeatus*

Abbreviation	Number of																	
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Freshwater resident of *G. a. aculeatus*																		
Kamchatka Peninsula																		
Azabachije							6	77	203	37	6	3						
Kuril Islands																		
Shumshu						1												
Paramushir						5	1	2		1								
Motori								1										
Kamuikotan																		
Higashibiroku																		
Honshu Is., Japan																		
Ohtsuchi																		
Tahara																		
Okanjichi																		
Chikazono 1																		
Chikazono 2									1									
Ohno																		
G. a. microcephalus (*G. aculeatus* subsp. 2 sensu Hosoya, 2013)																		
Kanou				1		7	19	6										
Yahata						1	5											
Nanatori						6	13	7						1				
Toyosato						6	2	6	1	1				1				
Imashuku							3	2	2	2		2	3					

similar to anadromous *G. nipponicus* (mean 32.1) and *G. a. aculeatus* (mean 32.3) in this regard (Higuchi *et al.*, 2014).

It has also been well established that *G. a. aculeatus* has become freshwater resident at various times (Ikeda, 1935; Bell and Foster, 1994; McKinnon and Rundle, 2002; McKinnon and Mori, 2003), as recently as several decades ago in dammed lakes (Higuchi and Goto, 1994; Higuchi *et al.*, 1996; Higuchi, 2003). Thus, the Ohtsuchi population is likely to have become freshwater resident far more recently than the Paramushir and Okanjichi populations. In reality, the Ohtsuchi population is reported as being less derived genetically from the anadromous population than the Ohno and Aizu populations, the latter being a freshwater population like as the Okanjichi population (Watanabe *et al.*, 2003). The Ohtsuchi population has undergone hybridization with anadromous *G. nipponicus* since the 2011 Great East Japan Earthquake, and some measures to protect the genetic structure of the original population are necessary (Mori, 2013).

The Paramushir population was partially armored (Fig. 2A, Table 3), and the Ohtsuchi and Okanjichi populations fully armored (Fig. 2B, Table 3). Other partially armored populations have been recorded from the Kuril Islands (Ikeda, 1935) and Kamchatka Peninsula (the Azabachije population, Ziuganov *et al.*, 1987), although the population from Paramushir Island, adjacent to Shumshu Island (Ikeda, 1935), is a new record. Ikeda (1935) who had also collected fully armored individuals from populations in lakes near the seashore of Itrup and Kunashir Islands, discussed the possibility of such populations having become freshwater resident more recently than the partially armored populations. A similar argument might be made for the Ohtsuchi population, having fully armored lateral plates.

Comparisons of number of lateral plates among five less-armored populations of *G. a. aculeatus* and *G. a. microcephalus* and among four fully armored populations of *G. a. aculeatus* are shown in Table 4. Only the Azabachije population, geographically nearest to the Paramushir population, was significantly different from the other less-armored populations, indicating the Paramushir population is rather similar to the less-armored *G. a. microcephalus*. As being well known (Ikeda, 1933; Watanabe and Mori, 2003;

aculeatus and *G. a. microcephalus* populations (abbreviations as in Table 1)

lateral plates																			Mean	SD
18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36		
																			7.9	0.8
																			6.0	1.4
															1					
															1					
													2	4	4	1			32.4	0.9
														2	15				32.9	0.3
													5	7					31.6	0.5
1	1			1	4	2	4	1	4						7				26.6	4.6
		2	2	3	5	4	6	6	5	8	7	3	4	9	5		1		27.3	4.4
												4	26	1	1				31.0	0.5
																			5.9	0.8
																			5.8	0.4
																			6.3	1.5
																			6.7	2.0
																			8.7	2.3

Table 4. Mann–Whitney *U*-test with sequential Bonferroni correction by BH method (Benjamini and Hochberg, 1995) for differences of number of lateral plates among five less-armored populations of *Gasterosteus aculeatus aculeatus* and *G. a. microcephalus* (left diagonal) and four fully armored populations of *G. a. aculeatus* (right diagonal) with more than five individuals (abbreviations as in Table 1). *U* values are shown. *: $P < 0.05$, **: $P < 0.001$.

	Five less-armored populations of *G. a. aculeatus* and *G. a. microcephalus*					Four fully armored populations of *G. a. aculeatus*		
	Azabachije	Paramushir	Kanou	Yahata		Ohtsuchi	Tahara	Okanjichi
Paramushir	440.5**				Tahara	59.0*		
Kanou	330.0**	133.0			Okanjichi	33.0*	7.0**	
Yahata	15.0**	24.0	92.0		Ohno	38.0**	10.5**	85.5**
Nanatori	691.5**	92.0	365.0	60.0				

Watanabe *et al.*, 2003), the Toyosato and Imashuku populations, being semi-armored, are different from the less-armored *G. a. microcephalus*. On the other hand, all fully armored populations are different significantly. The Chikazono population collected in 1933 and 1970 (Ikeda, 1933; Igarashi, 1970) is different from all the other populations by having a wide variation of lateral plate morphology as discussed bellow. The result would suggest most of these freshwater populations have evolved separately, adapting to the local environments as discussed by many authors (e.g. Bell, 1976; MacPhail, 1984; Bell and Foster, 1994; McKinnon and Mori, 2003; Colosimo *et al.*, 2005).

The Okanjichi population with smaller-sized body and fewer vertebrae than the Ohtsuchi population (Table 2) is thought to have become freshwater resident in a more ancient time than the latter. Interestingly, the Chikazono population (Ikeda, 1933; Igarashi, 1970), just a few kilometers distant from the Okanjichi population in the same Naka River system, had widely variable lateral plates, including partially to fully armored individuals (Ikeda, 1933; Igarashi, 1970: Table 3). Whatever their provenance, the two populations are now discrete, without any genetic exchange. The Okanjichi population is designated as a Natural Monument by Ohtawara City, and the Chikazono population designated similarly by Tochigi Prefecture (Yoshida *et al.*, 2011). Both populations are deserving of high conservation status in the future.

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