Ichthyol Res (2014) 61:341–351
DOI 10.1007/s10228-014-0403-1

FULL PAPER

A new threespine stickleback, *Gasterosteus nipponicus* sp. nov. (Teleostei: Gasterosteidae), from the Japan Sea region

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Received: 8 September 2013 / Revised: 24 February 2014 / Accepted: 3 March 2014 / Published online: 17 May 2014

Abstract *Gasterosteus nipponicus* sp. nov. is described from the holotype, 35 paratypes, and 60 additional specimens. The species differs from congeners in the following combination of characters: lateral plates complete, abruptly reducing in size above the anus, depth of lateral plate above the anus < 60 % that of the deepest plate; caudal keels thin, membranous. The new species is distributed in coastal Japan facing the Sea of Japan from Kyushu to Hokkaido Islands, along the Pacific coast of northern Japan from the Chiba Prefecture to Hokkaido, along the Sea of Okhotsk of Hokkaido, west to the southern and eastern coasts of Korean Peninsula, the Maritime Territory and north to Sakhalin Island, Russia.

This article was registered in the *Official Register of Zoological Nomenclature* (ZooBank) as FF559A95-456C-48B3-A9D5-473EFEDBF4A2.
This article was published as an Online First article on the online publication date shown on this page. The article should be cited by using the doi number.

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Keywords New species · Taxonomy · Morphology · Lateral plate · Anadromy

Introduction

The threespine stickleback species complex, *Gasterosteus aculeatus* Linnaeus 1758, is a well-known and intensively studied model for evolutionary biology, exhibiting an array of morphological, physiological, ethological, reproductive, and genetic variations (Tinbergen 1951; Assem 1967; Hagen 1967; Wootton 1976; Bell and Foster 1994; McKinnon and Rundle 2002; Goto and Mori 2003). Due to such variations, more than 50 nominal species have been described under the genus (Eschmeyer 1998; Sakai and Yabe 2003).

According to Eschmeyer's (1998) list, the *Gasterosteus* species are rearranged into two species with three subspecies, namely *Gasterosteus wheatlandi* Putnam 1867, *G. aculeatus aculeatus*, *Gasterosteus aculeatus microcephalus* (Girard 1854) (known as "Hariyo" in Japanese), and *Gasterosteus aculeatus williamsoni* (Girard 1854), synonymizing most of the nominal species with *G. a. aculeatus* [see table 1 of Sakai and Yabe (2003)]. *Gasterosteus wheatlandi* is distinguished from *G. aculeatus* in having pelvic fins with one spine with two well-developed pointed cusps at the base (vs. one cusp in *G. aculeatus*) and two soft rays (vs. one soft ray), and many black spots along the sides (vs. without spots) (Scott and Crossman 1973).

Gasterosteus aculeatus aculeatus includes semi-armored (previously known as *Gasterosteus gymnurus* Cuvier 1829 or *Gasterosteus leiurus* Cuvier in Cuvier and Valenciennes 1829) and unarmored (previously known as *Gasterosteus hologymnus* Regan 1909) forms that occurred as freshwater populations [Kottelat (1997) synonymized *G.*

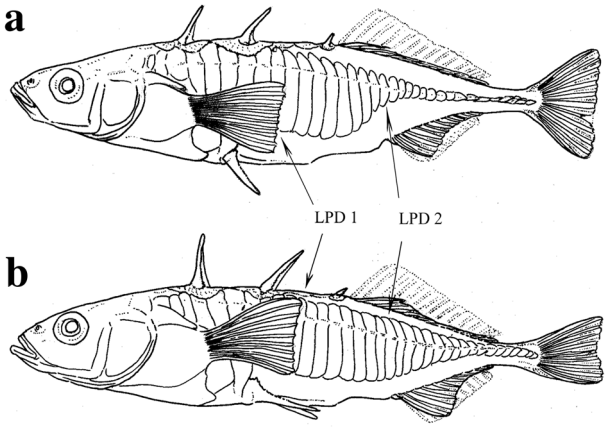

Fig. 1 Line drawings of *Gasterosteus nipponicus*, paratype, LBM 1210054324, 65.5 mm SL, female, Fukawa River, Nagato, Yamaguchi, Japan, 19 March 2007 (**a**), and *G. aculeatus aculeatus*, HUMZ 20790, 74.8 mm SL, female, Kodiak Island, Alaska, USA, 1965 (**b**). Dorsal and anal fins illustrated by dotted lines indicate the situation when fanned out. *LPD1* and *LPD2* indicate the deepest lateral plate and the lateral plate above the anus, respectively (see text)

leiurus and *G. hologymnus* with *G. gymnurus;* Berg (1949) synonymized all the three with *G. aculeatus*], in addition to the more common full-armored form (previously known as *Gasterosteus trachurus* Cuvier in Cuvier and Valenciennes 1829) that occurs as both anadromous and freshwater populations. As the lateral plates of *G. a. microcephalus* and *G. a. williamsoni* are semi-armored and unarmored, respectively (Girard 1854), their classification should be restudied, following detailed comparisons with semi-armored and unarmored populations of *G. a. aculeatus* (see Sakai and Yabe 2003).

Within the *G. aculeatus* complex, many "species" pairs have been recognized (McPhail 1994; McKinnon and Rundle 2002), including anadromous vs. freshwater (full-armored vs. full-, semi- or non-armored, as mentioned above, Hagen 1967; Bell 1976; Ziuganov et al. 1987), black vs. red (both semi-armored, McPhail 1969), lake resident vs. stream resident (both semi-armored, Moodie 1972), limnetic vs. benthic (both semi-armored, McPhail 1984), white vs. typical color (both full-armored, Blouw and Hagen 1990), and Japan Sea vs. Pacific Ocean (differently full-armored with each other, Higuchi and Goto 1996) forms.

The last-mentioned Japan Sea and Pacific Ocean forms, the former being first identified by Ikeda (1933) as differing in lateral plate morphology (see Figs. 1, 2) are more distantly related to each other than the components of other species pairs (Haglund et al. 1992; Higuchi and Goto 1996). In contrast to the Pacific Ocean form whose depth of lateral plates became smaller gradually along the sides, those of the Japan Sea form reduced abruptly from above the anus (Ikeda 1933; Sakai et al. 2013). According to the

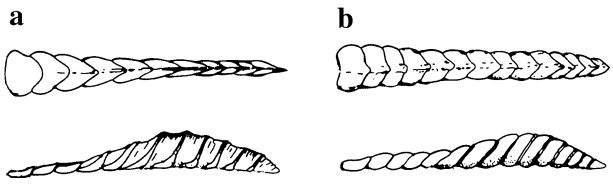

Fig. 2 Line drawings of caudal keels from lateral view (top) and upper view (bottom) of *Gasterosteus nipponicus*, paratype, LBM 1210054324, 65.5 mm SL, female, Fukawa River, Nagato, Yamaguchi, Japan, 19 March 2007 (**a**), and of *G. a. aculeatus*, HUMZ 20790, 74.8 mm SL, female, Kodiak Island, Alaska, USA, 1965 (**b**)

photos of the syntypes of *G. aculeatus* (Fig. 3, Linnean Society of London 2007), their lateral plates gradually reduce in depth, corresponding to the states of the full-armored form of the Pacific Ocean form (as group) described by Higuchi and Goto (1996). Higuchi and Goto (1996) included the semi-armored form ("Hariyo" in Japanese, *G. a. microcephalus*) also in the Pacific Ocean form (normally full-armored) because the genetic distance between the semi- and full-armored forms from both the Pacific and Atlantic Oceans is much closer than that between the two forms and the Japan Sea form (Haglund et al. 1992; Higuchi and Goto 1996). Although further study concerning the classification of the Pacific Ocean form is necessary as mentioned above, the full-armored form of the Pacific Ocean form ("Itoyo" in Japanese) is thought to represent *G. aculeatus* by Linnaeus (1758), namely *G. a. aculeatus* in the present study.

Studies of courtship behavior (Ishikawa and Mori 2000; Ishikawa et al. 2006; Kitano et al. 2007a, 2008), distribution and migration patterns (Kume et al. 2005, 2010), chromosome numbers (Kitano et al. 2009), egg and clutch sizes (Kume 2011), and lateral plate morphology (Higuchi and Goto 1996; Sakai et al. 2013) of the two forms have resulted in a significant level of understanding. In addition, hybrids of female Japan Sea and male Pacific Ocean forms are sterile (Yamada et al. 2001; Kitano et al. 2007a). Although all the information to hand suggests that the two forms are specifically distinct, the Japan Sea form has at no time been formally described. Because the plate morphology of the latter is unique and stable throughout its distribution range (Sakai et al. 2013), the Japan Sea form is described herein as a new species of *Gasterosteus* and compared in detail with the Pacific Ocean form, common full-armored *G. a. aculeatus*.

Methods

Counts and measurements generally followed Hubbs and Lagler (1958), except for depths of the deepest lateral plate (LPD1) and the lateral plate above the anus (LPD 2), the

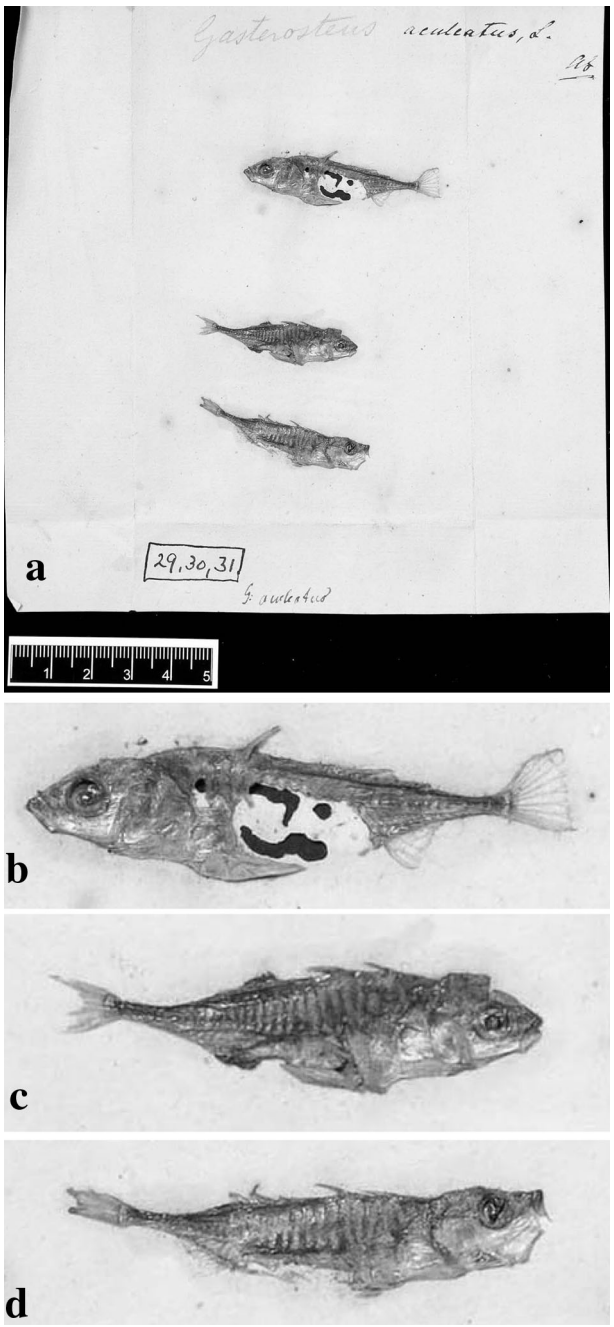

Fig. 3 Syntype specimens of *Gasterosteus aculeatus* Linnaeus 1758 appeared in the online collections of the Linnean Society of London (**a**), magnified figures of LINN 29 (**b**), LINN 30 (**c**), and LINN 31 (**d**), the image being available by permission of the society

plates being shown by arrows in Fig. 1. Measurements were made with dial calipers to the nearest 0.1 mm. All the relative measurements except for standard length (SL) are given as percentage of SL or head length (HL), and LPD1 is given as percentage of LPD2 as well. Vertebral counts and dorsal and anal fin ray counts were made from soft X-ray photos. Differences in relative measurements and counts are tested by analysis of co-variance (ANCOVA) and Mann–Whitney's *u* test (M–W *u* test), respectively. Institutional codes are as follows: HUMZ, Hokkaido University Museum, Hakodate; LBM, Lake Biwa Museum, Kusatsu; NSMT, National Museum of Nature and Science, Tsukuba (formerly National Science Museum, Tokyo).

Gasterosteus nipponicus sp. nov.

(New Japanese name: Nihon-itoyo) (Figs. 1a, 2a, 4)

Gasterosteus aculeatus (not of Linnaeus 1758): Mori 1956: 10 (Maruyama and Yura Rivers, Kyoto Prefecture, Japan); Choi et al. 1983: 28 (east coast of Korea, Mangyeong River, west coast of Korea); Haglund et al. 1992: 432 (in part, Ishikawa and Aomori Prefectures, Japan); Tajima 1995: 144, photos (Saga Prefecture, Japan); Kim 1997: 386, pl. 34 photos 131a–131e (east coast of Korean Peninsular, Mangyeong River, west coast of Korea); Katayama et al. 2000: 209 (Lake Ogawara, Aomori Prefecture, Japan)

Gasterosteus aculeatus, anadromous form: Choi et al. 2002: 163, photo (east coast of Korea, Mangyeong River, west coast of Korea)

Gasterosteus aculeatus, Japan marine: McKinnon and Rundle 2002: 481, fig. 1f (coast of Hokkaido Island, Japan)

Gasterosteus aculeatus, Japan Sea form: Yamada et al. 2001: 269 (Setabetsu and Kunebetsu Rivers, Akkeshi Bay, Hokkaido Prefecture, Koyoshi River, Akita Prefecture, Japan); Arai et al. 2003a: 9 (Tokachi River, Hokkaido Prefecture, Japan); Arai et al. 2003b: 223 (Lake Akkeshi, Hokkaido Prefecture, Japan); Kume et al. 2005: 189 (Akkeshi Bay, Hokkaido Prefecture, Japan): Ishikawa et al. 2006: 938 (Biwase River, Hokkaido Prefecture, Shinano River, Niigata Prefecture, Lake Shinji, Shimane Prefecture, Japan); Kitano et al. 2007b: 671, fig. 2A (Akkeshi, Hokkaido Prefecture, Japan); Kume 2008: 21 (Isumi and Kuriyama Rivers, Chiba Prefecture, Japan); Kume and Mori 2009: 2845 (Biwase Bay, Hokkaido Prefecture, Japan); Kitano et al. 2009: 1079 (Akkeshi Bay, Hokkaido Prefecture, Japan); Kume et al. 2010: 1436 (Bekanbeushi River, Hokkaido Prefecture, Japan); Kume 2011: 309 (Bekanbeushi River, Hokkaido Prefecture, Japan); Kitano et al. 2012: 294, fig. 1 upper panel (Chiba, Akita, Niigata, Ishikawa, Fukui Prefectures, Japan); Sakai et al. 2013: 57, fig. 1A, fig. 1C (Japan Sea region from Japan, Russia to Korea, around Sakhalin and Hokkaido Islands, Pacific coast of Honshu Island from Aomori to Chiba Prefectures, Japan)

Gasterosteus aculeatus, Japan Sea group: Higuchi and Goto 1996: 1, fig. 4 (Hokkaido, Aomori, Niigata Prefectures, Japan, east coast of Korea); Ishikawa and Mori 2000: 1065 (Niigata Prefecture, Japan); Nakajima and Onikura 2009: 285, fig. 2 (Oita, Fukuoka, Saga, Nagasaki, Kumamoto Prefectures, Japan)

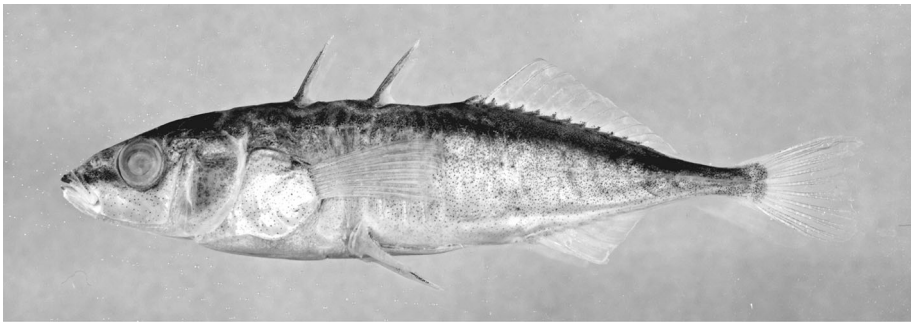

Fig. 4 Holotype of *Gasterosteus nipponicus*, HUMZ 97486, 72.3 mm SL, male, Usujiri, Minami-kayabe, Hokkaido, Japan, 19 May 1983

Gasterosteus aculeatus, Japan Sea lineage: Kitano et al. 2007a: 337, fig. 3A (Akkeshi Bay, Hokkaido Prefecture, Japan, Lake Krestonosthka, Sakhalin Island, Russia); Kitano et al. 2008: 443 (Akkeshi Bay, Hokkaido Prefecture, Japan)

Gasterosteus aculeatus, trachurus form: Mori 1987: 165 (Kahoku-gata Lagoon, Ishikawa Prefecture, Japan)

Gasterosteus aculeatus aculeatus: Ikeda 1933: 142, figs. 1B, 8A, 9A, 10B (in part, the sea of Japan coast of Hokkaido and Honshu Islands, Pacific coast of Honshu Island from Miyagi, Fukushima and Ibaragi Prefectures, Japan, Sakhalin Island, Russia); Ikeda 1935: 213, fig. 1B (in part, Itrup Island); Ikeda 1936: 15 (Miomote River, Niigata Prefecture, Japan); Ikeda 1937: 1, fig. 2 (Oita and Nagasaki Prefectures, Japan); Honma 1956: 82 (Sado Island, Niigata Prefecture, Japan); Katayama and Fujioka 1971: 82, pl. III-14 (Hikari, Yamaguchi Prefecture, Japan); Amaoka and Haruta 1972: 129, fig. 2 (Nagata River, Yamaguchi Prefecture, Japan); Nakamura 1976: 29, 130, photo (Natori River, Miyagi Prefecture, Japan); Hirai 1989: 433, photos of fish from Tedori and Chitose Rivers (in part, Honshu Island from Chiba through Aomori to Yamaguchi Prefectures, around Hokkaido Island, Japan)

Gasterosteus aculeatus aculeatus, anadromous form: Sugiyama 1985: 90, photo of anadromous form (in part, Akita Prefecture, Japan); Taniguchi et al. 1990: 231 (Abashiri and Hakodate, Hokkaido Prefecture, Niigata, Niigata Prefecture, Japan); Mori and Uchiyama 1997: 78, 224, photos of fish from the Shibetsu and Kuzuryu Rivers (in part, from Hokkaido Island west to Yamaguchi Prefecture and south to the Tone River, Japan)

Gasterosteus aculeatus aculeatus, sea-run type: Takeuchi 1994: 80, photos (Kuji River, Iwate Prefecture, Japan)

Gasterosteus cataphractus (not of Pallas 1814): Mori 1930: 9 (Tumen River, North Korea)

Gasterosteus sp., Japan Sea lineage: Hosoya 2013: 606, key, fig. (from Hokkaido Island west to Shimane Prefecture and south to the Tone River, Japan, Sakhalin and Kuril Islands, Russia, east coast of Korea)

Gasterosteus sp., Japan Sea type: Hatama and Ohashi 2009: 26 (Fukawa River, Yamaguchi Prefecture, Japan)

"Itoyo" (no designation of scientific name): Inoue 1983: 48, photos (in part, Niigata Prefecture, Japan); Okabe 2000: 122, photos (Shonai area, Yamagata Prefecture, Japan); Aoyama 2002: 118, photos (Lake Shinji, Shimane Prefecture, Japan)

Holotype. HUMZ 97486: 72.3 mm SL, male, Usujiri, Minami-kayabe, Hokkaido, Japan, 19 May 1983.

Paratypes. Thirty females and 5 males, 52.7−78.8 mm SL. HUMZ 92318 (54.6 mm SL): 1 female, same locality as holotype, 17 May 1982. HUMZ 95325 (59.6 mm SL): 1 female, same locality as holotype, 24 May 1982. HUMZ 97692 (66.4 mm SL): 1 female, same locality as holotype, 23 June 1982. HUMZ 102011 (52.7 mm SL): 1 female, same locality as holotype, 20 June 1984. HUMZ 104755 (75.2 mm SL): 1 female, same locality as holotype, 10 May 1985. HUMZ 140390−140395, 140397, 140399 (61.5−67.1 mm SL): 8 females, Saverny Bay Lagoon, North Sakhalin, Russia, 23 July 1995. HUMZ 140396 (66.0 mm SL), 140398 (56.4 mm SL): 2 males, Saverny Bay Lagoon, North Sakhalin, Russia, 23 July 1995. LBM 1210054324 (65.5 mm SL): 1 female, Fukawa River, Nagato, Yamaguchi, Japan, 19 March 2007, coll. by T Hatama. LBM 1210054325−1210054327, 12154329−1210054334 (62.4−78.8 mm SL): 9 females, Monbetsu coast, Monbetsu, Hokkaido, Japan, 19 March 2007, coll. by Y Suda. LBM 1210054328 (69.1 mm SL): 1 male, Monbetsu coast, Monbetsu, Hokkaido, Japan, 19 March 2007, coll. by Y Suda. NSMT-P SK 1305-1 (72.9 mm SL), 1305-3 (69.8 mm SL): 2 females, Yoroi-gata pond, Nishi-kanbara, Niigata, Japan, 21 April 1958, coll. by M Nakamura. NSMT-P SK 1305-2 (61.2 mm SL): 1 male, Yoroi-gata pond, Nishi-kanbara, Niigata, Japan, 21 April 1958, coll. by M Nakamura. NSMT-P SK 10302 (69.4 mm SL): 1 female, Lake Kitaura, Itako, Ibaraki, Japan, 22 April 1965, coll. by M Nakamura. NSMT-P SK 10595 (65.1 mm SL): 1 male, Hitachi River, Tone River system, Kashima, Ibaraki, Japan, 29 April 1965, coll. by M Nakamura. NSMT-P SK 21571 (64.9 mm SL): 1 female, Hitachi River, Tone River system, Kashima, Ibaraki, Japan, 24 March 1967, coll. by M Nakamura. NSMT-P SK 21695-1 (72.4 mm SL), 21695-2 (71.0 mm SL): 2 females, Tone River, Kashima, Ibaraki, Japan, 19 April 1966, coll. by M Nakamura. NSMT-P SK 18223 (75.6 mm SL): 1 female,

Table 1 Morphometrics and counts of the holotype and paratypes of *Gasterosteus nipponicus*

	Holotype (male)	Paratypes	
		Females (N = 30)	Males (N = 5)
Standard length (SL mm)	72.3	52.7–78.8	56.4–69.1
In % of SL			
Head length (HL)	28.4	28.3±1.0 (26.9–31.2)	30.1±1.2 (28.0–30.9)
Body depth	22.1	22.8±1.5 (19.8–27.2)	22.6±0.9 (21.4–23.8)
Caudal peduncle length	9.41	0.0±1.0 (8.0–13.2)	9.8±0.4 (9.6–10.5)
Caudal peduncle depth	4.1	3.6±0.3 (3.2–4.0)	3.9±0.3 (3.5–4.1)
Predorsal length	62.4	64.1±1.5 (61.8–67.9)	63.5±1.2 (61.5–64.7)
Preanal length	69.8	71.7±1.6 (68.7–74.9)	70.1±1.4 (68.5–72.0)
Prepelvic length	44.8	44.5±1.9 (41.3–51.5)	44.4±1.1 (43.3–45.4)
Dorsal fin base length	33.6	29.0±1.6 (23.9–31.1)	29.6±0.6 (28.9–30.3)
Anal fin base length	23.8	19.3±1.4 (16.1–22.7)	21.9±1.7 (18.9–23.2)
First dorsal spine length	11.2	10.2±1.2 (8.1–12.8)	11.2±1.1 (10.3–12.9)
Second dorsal spine length	11.9	11.8±1.3 (9.6–14.8)	12.8±1.0 (11.4–14.0)
Third dorsal spine length	3.9	3.7±1.0 (2.5–8.5)	3.5±0.7 (2.8–4.5)
Pelvic spine length	14.2	15.5±1.5 (12.4–21.6)	16.3±1.1 (14.9–17.7)
Pectoral fin length	19.4	19.4±1.1 (17.5–21.6)	20.4±1.3 (18.6–22.2)
Length of longest dorsal ray	10.4	11.3±2.1 (7.1–17.6)	11.1±2.0 (8.1–13.8)
Length of longest anal ray	9.8	9.5±1.2 (6.5–12.2)	10.3±1.6 (8.8–12.2)
LPD1	16.6	16.8±1.2 (14.0–18.6)	16.7±1.4 (14.7–18.4)
LPD2	7.5	6.7±0.9 (4.6–8.6)	8.2±0.8 (7.0–9.2)
LPD1 in % of LPD2	45.0	40.1±5.9 (27.0–54.1)	49.2±6.6 (41.8–56.2)
In % of HL			
Snout length	33.7	31.1±1.6 (26.9–35.5)	32.2±1.2 (30.6–33.3)
Upper jaw length	26.3	24.1±2.6 (17.8–31.5)	24.4±1.6 (21.7–25.4)
Eye diameter	25.9	26.5±1.5 (24.5–30.7)	26.7±1.2 (25.3–28.2)
Interorbital width	23.9	24.0±0.9 (22.3–25.8)	23.7±0.7 (22.7–24.7)
Isolated dorsal spines	3	3	3
Pectoral rays	10	10	10
Dorsal soft rays	14	13.0±0.8 (11–14)	13.2±1.1 (12–14)
Anal soft rays	11	9.7±0.7 (7–11)	10.2±0.8 (9–11)
Lateral plates	31	32.6±1.3 (29–34)	33.0±1.0 (32–34)
Vertebrae	32	32.0±0.8 (30–33)	32.0±0.7 (31–33)

LPD1 deepest lateral plate depth; *LPD2* depth of lateral plate above the anus

Tone River, Inba, Chiba, Japan, 16 May 1966, coll. by M Nakamura.

Non-type specimens. Forty-five females and 15 males, 59.5−76.4 mm SL. HUMZ 183229−183238 (60.2−71.4 mm SL): 6 females and 4 males, Aynskaya River estuary, southwestern Sakhalin, Russia, 22 July 1994. NSMT-P 114370−114379 (65.5−76.4 mm SL): 6 females and 4 males, Sacheon River, Gangneung, Korea, 1987, coll. by SR Jeon. NSMT-P 114380−114389 (65.8−75.3 mm SL): 10 females, Lake Ogawara, Misawa, Aomori, Japan, 19 May 1990, coll. by M Higuchi. NSMT-P 114390−114399 (64.1−73.7 mm SL): 8 females and 2 males, Sarufutsu River, Souya, Hokkaido, Japan, 22 June 1992, coll. by M Higuchi. NSMT-P 114400−114409 (61.2−72.9 mm SL): 5 females and 5 males, Biwase River, Akkeshi, Hokkaido, Japan, 1 October 1990, coll. by M Higuchi. NSMT-P 114410−114419 (59.5−76.4 mm SL): 10 females, Ryukei River, Hokuto, Hokkaido, Japan, 22 August 1976, coll. by A Goto.

Diagnosis. *Gasterosteus nipponicus* differs from congeners in the combination of the following characters: lateral plates complete, abruptly reducing in size above the anus, depth of lateral plate above the anus < 60 % that of the deepest plate; caudal keels thin and membranous.

Description. Measurements and counts of the holotype and paratypes are given in Table 1, and together with those of non-type specimens in Table 2. Frequency distributions of counts are also shown in Table 3. Body compressed, elongated, tapering to a slender caudal peduncle. Head small, pointed. Mouth oblique, maxillary not reaching to level with eye. Gill membrane joined to isthmus. Three separate serrated dorsal spines, each with a triangular

Table 2 Morphological comparisons between *Gasterosteus nipponicus* and Pacific Ocean form of *G. aculeatus aculeatus*

	G. nipponicus (N=96)				*G. a. aculeatus* (N=83)				ANCOVA between species			
			ANCOVA between sex				ANCOVA between sex		Females		Males	
	Females (N=75)	Males (N=21)	*f*-value	*p*	Females (N=59)	Males (N=24)	*f*-value	*p*	*f*-value	*p*	*f*-value	*p*
Standard length (SL mm)	52.7–78.8	56.4–72.3			46.6–89.0	43.0–99.5						
In % of SL												
Head length (HL)	28.3±1.2 (25.7–32.0)	30.0±1.9 (24.2–33.0)	16.986	0.000***	29.7±1.6 (27.3–33.4)	31.2±1.8 (26.8–35.0)	13.385	0.000***	31.049	0.000***	5.348	0.026*
Body depth	23.1±1.6 (19.8–27.9)	22.4±0.8 (21.1–24.2)	2.666	0.106	23.2±1.5 (16.8–25.5)	22.9±1.0 (20.5–24.7)	0.901	0.345	0.065	0.798	4.121	0.049*
Caudal peduncle length	10.6±1.5 (8.0–18.1)	10.2±0.9 (8.9–12.5)	1.521	0.221	11.5±1.2 (8.4–13.7)	12.4±1.5 (8.8–15.0)	6.329	0.014*	15.198	0.000***	31.428	0.000***
Caudal peduncle depth	3.5±0.3 (2.9–4.1)	3.7±0.3 (2.9–4.1)	5.675	0.019*	3.8±0.4 (3.0–4.8)	4.0±0.3 (3.2–4.4)	3.154	0.080	34.297	0.000***	8.459	0.006**
Predorsal length	63.5±2.0 (58.8–71.9)	63.3±1.2 (61.1–65.4)	0.518	0.474	64.3±1.5 (61.3–67.6)	64.4±1.7 (61.3–67.9)	0.001	0.973	8.035	0.005**	6.005	0.019*
Preanal length	72.3±1.8 (68.7–79.4)	70.3±1.3 (67.9–73.0)	16.481	0.000***	73.3±1.8 (69.7–78.6)	71.8±1.1 (70.2–74.4)	12.127	0.001**	7.629	0.007**	18.129	0.000***
Prepelvic length	44.1±2.2 (39.2–51.5)	45.6±1.8 (42.4–50.1)	3.252	0.075	46.6±2.4 (42.4–52.6)	48.3±2.4 (44.1–51.6)	6.884	0.010*	48.679	0.000***	22.976	0.000***
Dorsal fin base length	28.3±2.2 (20.5–31.7)	28.9±2.2 (24.3–33.6)	3.461	0.066	24.9±2.0 (16.0–28.5)	25.3±1.8 (22.6–29.5)	2.261	0.137	104.699	0.000***	40.009	0.000***
Anal fin base length	18.3±1.8 (13.9–22.7)	21.9±2.2 (18.9–29.5)	46.492	0.000***	15.6±1.5 (10.1–19.0)	16.7±1.1 (14.8–18.8)	13.917	0.000***	87.853	0.000***	103.683	0.000***
First dorsal spine length	9.9±1.2 (6.1–12.8)	11.4±1.3 (8.8–13.6)	18.898	0.000***	9.9±1.1 (7.3–12.2)	10.1±1.0 (8.5–12.1)	0.798	0.374	0.008	0.931	12.554	0.001**
Second dorsal spine length	11.2±1.5 (6.4–14.8)	12.6±0.9 (11.1–14.0)	9.773	0.002**	10.7±1.2 (6.9–12.8)	10.9±1.0 (9.0–12.9)	1.165	0.284	4.764	0.031*	27.054	0.000***
Third dorsal spine length	3.5±0.9 (2.2–8.5)	3.7±0.6 (2.7–5.0)	0.594	0.443	3.7±0.9 (1.3–5.8)	3.8±0.8 (2.5–5.9)	0.623	0.433	0.863	0.355	0.316	0.577
Pelvic spine length	15.1±1.6 (11.1–18.6)	15.8±1.0 (14.2–17.9)	2.768	0.100	15.1±1.5 (10.9–18.6)	14.4±1.2 (12.2–17.5)	3.816	0.054	0.087	0.768	12.940	0.001**
Pectoral fin length	19.0±1.4 (14.0–21.6)	19.5±1.1 (17.5–22.2)	1.485	0.226	18.3±2.7 (13.5–23.0)	18.6±1.8 (14.6–21.5)	0.638	0.427	4.633	0.033*	3.295	0.077
Length of longest dorsal ray	10.6±1.7 (7.1–17.6)	10.9±1.7 (8.2–15.3)	0.072	0.788	10.0±1.5 (6.9–14.0)	10.9±1.5 (7.5–13.7)	8.116	0.006**	5.103	0.026*	0.156	0.695
Length of longest anal ray	9.3±1.1 (6.5–12.2)	10.0±1.3 (8.3–12.9)	3.114	0.081	9.4±1.0 (7.1–11.7)	9.8±1.1 (7.9–12.0)	5.847	0.018*	0.181	0.671	0.769	0.385

Table 2 continued

	G. nipponicus (N=96)				*G. a. aculeatus* (N=83)				ANCOVA between species			
			ANCOVA between sex				ANCOVA between sex		Females		Males	
	Females (N=75)	Males (N=21)	*f*-value	*p*	Females (N=59)	Males (N=24)	*f*-value	*p*	*f*-value	*p*	*f*-value	*p*
LPD1	16.2±1.2 (11.9–18.8)	16.3±0.8 (14.7–18.4)	0.051	0.822	15.2±1.3 (12.5–18.1)	15.5±1.5 (11.4–18.5)	3.430	0.068	30.385	0.000***	8.989	0.005**
LPD2	6.4±0.9 (4.5–8.7)	7.5±1.0 (5.8–9.2)	16.374	0.000***	10.4±1.3 (8.3–12.8)	11.3±1.4 (7.8–13.9)	14.192	0.000***	443.510	0.000***	112.590	0.000***
LPD1 in % of LPD2	39.7±5.7 (27.0–54.9)	46.3±5.7 (36.8–56.3)	23.240	0.000***	68.6±4.9 (61.0–80.6)	72.8±6.4 (61.4–88.1)	9.641	0.003**	814.290	0.000***	182.705	0.000***
In % of HL												
Snout length	31.4±1.8 (26.3–36.7)	33.5±2.7 (30.6–42.6)	16.638	0.000***	31.1±2.5 (23.8–36.3)	32.0±2.2 (25.3–36.5)	3.270	0.074	4.553	0.035*	3.995	0.052
Upper jaw length	22.7±2.5 (17.2–31.5)	24.8±2.1 (21.5–29.7)	11.973	0.001**	22.6±2.6 (12.2–28.6)	23.6±2.5 (17.6–28.4)	2.869	0.094	0.137	0.712	2.856	0.098
Eye diameter	25.3±2.1 (20.7–30.7)	26.0±2.0 (23.0–31.1)	2.476	0.119	24.2±3.0 (20.1–36.0)	24.2±2.4 (19.1–21.8)	0.005	0.942	0.072	0.789	4.208	0.047*
Interorbital width	23.5±1.3 (19.6–25.8)	23.5±1.5 (21.0–28.4)	0.005	0.942	22.2±1.9 (18.5–26.3)	22.3±1.4 (19.5–24.9)	0.010	0.920	14.320	0.000***	4.802	0.034*

			M-W *u*-test between sex				M-W *u*-test between sex		M-W *u*-test between species			
			u-value	*p*			*u*-value	*p*	*u*-value	*p*	*u*-value	*p*
Isolated dorsal spines	3	3	–	–	3	3.0±0.2 (3–4)	737.500	0.117	–	–	262.500	0.350
Pectoral rays	10	10	–	–	10	10	–	–	–	–	–	–
Dorsal soft rays	12.9±0.8 (10–14)	13.1±0.8 (12–15)	842.500	0.591	11.8±0.8 (10–13)	11.9±0.7 (11–13)	717.500	0.918	787.500	0.000***	77.500	0.000***
Anal soft rays	9.7±0.7 (7011)	10.2±0.7 (9–11)	1310.000	0.003**	8.7±0.8 (7–10)	8.9±0.7 (7–11)	812.000	0.238	800.000	0.000***	46.000	0.000***
Lateral plates	32.7±1.1 (29–34)	32.8±1.1 (31–35)	775.500	0.912	32.8±1.2 (30–35)	33.3±1.0 (31–35)	848.500	0.143	2343.000	0.544	317.000	0.122
Vertebrae	Vertebrae	32.2±0.6 (31–33)	921.500	0.193	32.3±0.8 (30–34)	32.2±0.8 (30–34)	664.500	0.625	2640.000	0.038*	241.000	0.772

ANCOVA analysis of co-variance: *M–W u test* Mann–Whitney's u test: *, significantly different at 5 % level: **, significantly different at 1 % level: ***: significantly different at 0.1 % level: *LPD1* deepest lateral plate

Table 3 Frequency distribution of counts in *Gasterosteus nipponicus* and Pacific Ocean form of *G. aculeatus aculeatus*

	Dorsal spines		Dorsal soft rays						Anal soft rays				
	3	4	10	11	12	13	14	15	7	8	9	10	11
G. nipponicus													
Female	75		1	3	13	42	16		1	4	18	49	3
Male	*21				5	10	*5	1			3	11	*7
G. a. aculeatus													
Female	59		1	19	27	12			3	20	29	7	
Male	23	1		8	11	5			1	3	19		1

	Lateral plates							Vertebrae				
	29	30	31	32	33	34	35	30	31	32	33	34
G. nipponicus												
Female	1	8	18	26	21	1		2	16	39	15	3
Male			3	*4	9	4	1			2	*12	7
G. a. aculeatus												
Female		2	6	14	17	17	3	1	6	31	17	4
Male			2	3	7	11	1	1	1	16	4	2

*: including holotype

membrane and lockable in erect position. Bases of first and second dorsal spines broad, joined with each other and with lateral plates. Third dorsal and anal spines short, hooked, not attached to soft ray fins. Dorsal and anal soft rays forming a straight distal margin. Lower processes of right and left shoulder girdles extended, contacting anteroventrally, extending posteriorly to pelvic girdle. Upper process of shoulder girdle joined with lateral plates. Pectoral fin large, fan-shaped with truncated straight margin. Posterior processes of pelvic girdle extended, forming a pair of sheaths of ventral spines. Pelvic fin with a single long serrated spine, lockable when erect, and triangular membrane with a single soft ray. Lateral plates complete, reducing abruptly in size above anus (Fig. 1a), forming thin membranous lateral keels on caudal peduncle (Fig. 2a). Caudal fin fan-shaped with truncated straight end.

Sexual dimorphism. Relative preanal length was significantly greater in females than in males, although the latter had significantly greater head length, caudal peduncle depth, anal fin base length, first and second spine lengths, depth of lateral plate above anus, snout length, upper jaw length and LPD1 in LPD2 (Table 2). Anal fin soft ray numbers were also greater in males.

Color in life (*based on literature records*). Sides silver with silvery-greenish dorsum in marine environment [color photos are presented by Inoue (1983), Sugiyama (1985), Hirai (1989), Kim (1997), and Aoyama (2002)]. In the spawning season in brackish to freshwater, belly tinted red and eyes, dorsum and sides blue in males [color photos are presented by Inoue (1983), Hirai (1989), Takeuchi (1994), Tajima (1995), Kim (1997), Mori and Uchiyama (1997), Okabe (2000), Aoyama (2002), Choi et al. (2002), and Kitano et al. (2012)], belly silvery and dorsum and sides mottled brown in females [color photos are presented by Takeuchi (1994), Tajima (1995), Mori and Uchiyama (1997), and Okabe (2000)].

Color in alcohol. Dorsum brown, belly lighter brown.

Distribution. Coastal Japan facing the Sea of Japan from Kyushu to Hokkaido Islands, along the Pacific coast of northern Japan from Chiba Prefecture to Hokkaido, along the Sea of Okhotsk of Hokkaido, Japan, west to the southern and eastern coasts of Korean Peninsula, the Maritime Territory and north to Sakhalin Island, Russia (Fig. 5).

Etymology. The species name "*nipponicus*" refers to "Nippon (Japan)".

Comparative note. All populations of *G. nipponicus* are anadromous (Higuchi and Goto 1996), although otolith microchemistry analyses suggested that they include estuary-resident forms as well (Arai et al. 2003a, b). As their lateral plates are complete, being of the full-armored form, the following comparison was restricted to *G. nipponicus* and the full-armored form (Pacific Ocean form) of *G. a. aculeatus*.

The lateral plates become abruptly smaller from just above the anus in *G. nipponicus* (Fig. 1a), gradually smaller from the trunk to the caudal peduncle in *G. a. aculeatus* (Fig. 1b); depth of the plate just above the anus (LPD2) is < 60 % of that of the deepest plate (LPD1) in the

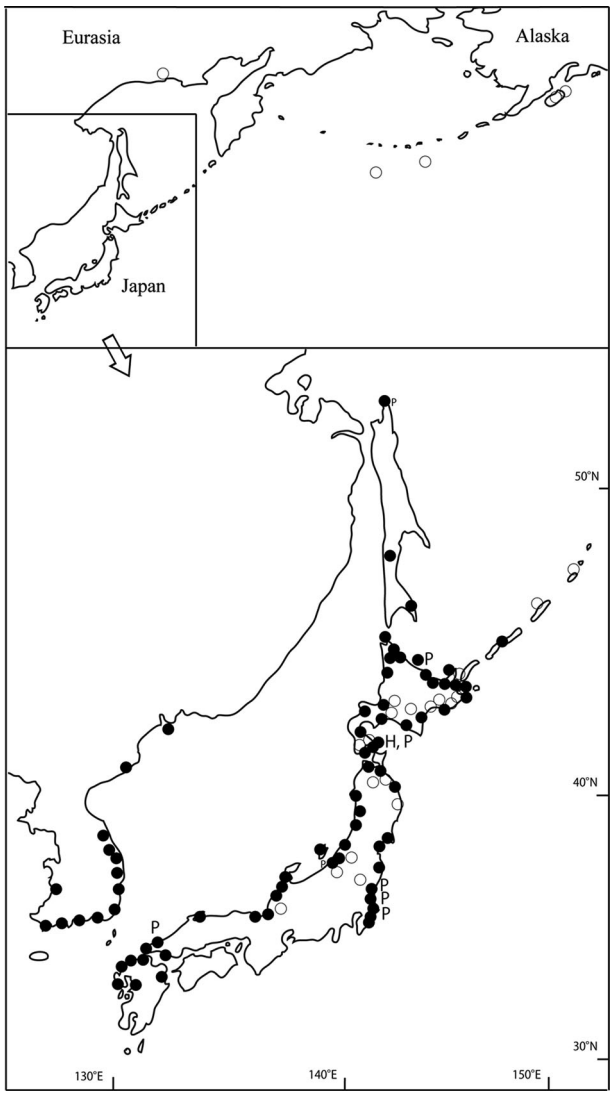

Fig. 5 Map showing the distribution of *Gasterosteus nipponicus* sp. nov. (closed circles) and *G. aculeatus aculeatus* (open circles) modified from the figure shown by Sakai et al. (2013), adding localities listed in the synonym list of the present study. Localities of the holotype and paratypes of *G. nipponicus* sp. nov. are indicated by letters "*H*" and "*P*", respectively

former, > 60 % in both sexes of the latter (Table 2). The caudal keels are thin and membranous in *G. nipponicus* (Fig. 2a), and bony and thick in *G. a. aculeatus* (Fig. 2b).

Sexual dimorphism in *G. nipponicus* was more extensive than in *G. a. aculeatus* (Table 2), with 10 morphometric and 1 meristic character differing significantly between sexes in the former and only 9 morphometric characters in the latter. In both species, males had larger heads, shorter preanal length, and greater anal fin base length than females, as shown by Kitano et al. (2007b) and Kume at al. (2010).

Both sexes of *G. nipponicus* had significantly greater relative lengths of dorsal fin base, anal fin base, second dorsal spine and LPD1, and greater interorbital width than *G. a. aculeatus* (Table 2). In addition, *G. nipponicus* has significantly more dorsal and anal soft rays than *G. a. aculeatus*. Both sexes of *G. a. aculeatus*, on the other hand, had significantly greater head length, caudal peduncle length and depth, predorsal length, preanal length, prepelvic length, LPD2, and LPD1 in LPD2. Female *G. nipponicus* had significantly greater pectoral fin, longest dorsal ray and snout lengths, and less vertebral count, and males had greater first dorsal and pelvic spine lengths and shorter body depth and smaller eye diameter.

Although the genetic distance between the species, based on allozyme data, is quite large (Haglund et al. 1992; Higuchi and Goto 1996), *G. nipponicus* has the same mitochondrial DNA haplotypes with some of those found in *G. a. aculeatus*, probably because of mitochondrial introgression between them through past hybridization events (Yamada et al. 2001). However, the two species rarely hybridize now (Higuchi and Goto 1996), with mating of female *G. nipponicus* and male *G. a. aculeatus* resulting in sterile offspring (Yamada et al. 2001; Kitano et al. 2007a). Differences in male courtship behavior, a rolling approach in *G. nipponicus* vs. zigzag approach in *G. a. aculeatus* (Ishikawa and Mori 2000; Ishikawa et al. 2006; Kitano et al. 2007a, 2008), as well as in chromosome number (n = 42 in females, 41 in males of *G. nipponicus* vs. n = 42 in both sexes of *G. a. aculeatus*) (Kitano et al. 2009) may be responsible for their reproductive isolation.

The spawning migration patterns also differ (Kume et al. 2005, 2010). Though *G. nipponicus* adults migrate upward to freshwater in mono-specific condition such as in the Shinano or Mogami Rivers (Ikeda 1936; Okabe 2000), they tend to remain in brackish water in the co-existing populations such as of the Bekanbeushi River, whereas *G. a. aculeatus* adults move upstream to freshwater (Kume et al. 2005, 2010). *Gasterosteus nipponicus* spawns a larger clutch of smaller eggs than *G. a. aculeatus*, a reproductive strategy that may be related to the differences in spawning migration or the spawning and nursery grounds (Kume 2011).

In contrast to the relatively narrow distribution range of *G. nipponicus*, primarily around Japan Sea region, the range of *G. a. aculeatus* is circum-North Pole (Haglund et al. 1992; Sakai et al. 2013).

On the basis of distribution and genetic independency of *G. nipponicus*, Higuchi and Goto (1996) argued that the species (as "*G. aculeatus*, Japan Sea group") may have originated from an ancestral stock of threespine stickleback locked into the ancient Sea of Japan, which was separated from the Pacific Ocean by geological changes and a significant lowering of sea levels at least ca. two million years before the present (Lindberg 1972; Nishimura 1974).

Comparative materials. *Gasterosteus aculeatus. aculeatus*. Fifty-nine females and 24 males (all are full-armored form), 43.0−99.5 mm SL. HUMZ 20795, 20787−20793 (65.5−75.5 mm SL): 7 females and 1 male, Kodiak Island, Alaska, USA, 1965. HUMZ 44156−44165 (64.4−79.2 mm SL): 5 females and 5 males, Little River, Kodiak Island, Alaska, USA, 3 July 1965. HUMZ 94828−94832 (43.0−70.9 mm SL): 2 females and 3 males, North Pacific Ocean, 49°00.2′N, 179°59.8′E, 31 July 1980. HUMZ 97804−97807 (46.6−74.0 mm SL): 3 females and 1 male, North Pacific Ocean, 49°33.3′N, 175°36.5′E, 7 August 1982. HUMZ 144337 (72.5 mm SL), 144380 (74.3 mm SL): 2 females, Shinshiru Island, Kuril Islands, Russia, 1995. HUMZ 144500 (66.1 mm SL): 1 male, Shinshiru Island, Kuril Islands, Russia, 1995. HUMZ 159998−160007 (69.1−84.5 mm SL): 6 females and 4 males, Gertner Bay, Magadan, Russia, 28 July 1997, coll. by A. Goto. NSMT-P 114420−114427 (49.4−99.5 mm SL): 5 females and 3 males, Otsuchi River, Kamihei, Iwate, Japan, 29 April 2001, coll. by A Goto. NSMT-P 114431−114438 (56.8−77.6 mm SL): 6 females and 2 males, Horonai River, Tomakomai, Hokkaido, Japan, 21 July 1989, coll. by M Higuchi. NSMT-P 114441−114449 (75.6−84.6 mm SL): 8 females and 1 male, Charo River, Shiranuka, Hokkaido, Japan, 5 June 1990, coll. by M Higuchi. NSMT-P 114451−114458 (74.0−83.7 mm SL): 7 females and 1 male, Biwase River, Akkeshi, Hokkaido, Japan, 1 October 1990, coll. by M Higuchi. NSMT-P 114460−114469 (53.8−75.5 mm SL): 8 females and 2 males, Ryukei River, Hokuto, Hokkaido, Japan, 22 August 1976, coll. by A Goto.

Acknowledgments We are indebted to H. Imamura (HUMZ), G. Shinohara (NSMT) and M. Kuwahara (LBM) for their cooperation and support in the deposition and loan of specimens, and to the Linnean Society of London for permitting the use of the digital image of the syntypes of *Gasterosteus aculeatus*. We express our gratitude to T. Hatama, S.-R. Jeon, T. Kitamura, M. Kume, Y. Suda, K. Takata, H. Takahashi and M. Yamada for their help in collecting specimens. Thanks also go to R. Miyauchi and D. Takeda for their technical help. We appreciate the English correction made by G. S. Hardy.

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