Zootaxa 3575: 49–62 (2012)
www.mapress.com/zootaxa/

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

Article

urn:lsid:zoobank.org:pub:34842C82-0E34-4D59-A014-18BB3483DF6B

A new frog species of the genus *Rugosa* from Sado Island, Japan (Anura, Ranidae)

KUNIO SEKIYA[1], IKUO MIURA[2] & MITSUAKI OGATA[3,4]

[1]*Department of Environmental Science, Faculty of Science, Niigata University, Niigata 950-2181, JAPAN.*
E-mail: ksekiya@mbe.nifty.com
[2]*Institute for Amphibian Biology, Graduate School of Science, Hiroshima University, Higashi-Hiroshima 739-8526, JAPAN.*
E-mail: imiura@hiroshima-u.ac.jp
[3]*Laboratory of Zoo Biology, Preservation and Research Center, Yokohama 241-0804, JAPAN. E-mail: zvp06246@nifty.ne.jp*
[4]*Corresponding author*

Abstract

We describe a new frog species in the genus *Rugosa* from Sado Island, Japan. This new species, *Rugosa susurra*, is genetically close to *R. rugosa* Temminck and Schlegel, but is morphologically distinguishable and postzygotically isolated from the latter species. The skin over the entire body is much smoother, and the abdomen and ventral surface of hindlimb are deep yellow whereas these are pale yellow or grayish yellow in *R. rugosa*. In addition, duration of the advertisement call of *Rugosa susurra* is definitely longer than that of *R. rugosa*. *Rugosa susurra* is the first endemic amphibian species to Sado Island in Japan.

Key words: *Rugosa susurra* **sp. nov.**, advertisement call, postzygotic isolation, smooth skin

Introduction

The wrinkled frog, *Rugosa rugosa* is a species of a convoluted taxonomic history. Fei *et al*. (1991 "1990") proposed a new genus name of *Rugosa* to the three frog species of *Rana rugosa* Temminck and Schlegel, *R. emeljanovi* Nikolsky and *R. tientaiensis* Chang, and the name of *Glandirana* to the species *Rana minima* Ting and Ts'ai. Dubois (1992) relegated *Rugosa* and *Glandirana* as subgenera of *Rana* and set them into the different sections (*Rugosa* into the section *Pelophylax* and *Glandirana* into the section *Hylarana*). However, the two sections were considered polyphyletic by Che *et al*. (2007). More recently, Frost *et al*. (2006) combined the two genera, *Rugosa* and *Glandirana,* into one genus *Glandirana* based on the molecular phylogenetic data. However, *Glandirana* (sensu Frost *et al*. 2006) was sometimes considered as non-monophyletic (Wiens *et al*. 2009). In addition, several morphological characters, particularly toe webbing, of *Rana minima* were largely different from the remaining three species (Frost *et al*. 2006). Thus, we consider that it is premature to recognize *Glandirana* (sensu Frost *et al*. 2006) as a good genus for the four species. Also, we consider the Dubois's classification invalid, because the two subgenera *Rana* (*Glandirana*) and *Rana* (*Rugosa*) were placed into polyphyletic sections as mentioned above. In contrast, monophyletic relationship of the three species *Rana rugosa*, *R. emeljanovi* and *R. tientaiensis* seems stable although the name of *R. rugosa* was not used in several studies (Frost *et al*. 2006; Che *et al*. 2007; Wiens *et al*. 2009). Therefore, in this study, we follow Fei's classification.

The wrinkled frog, *Rugosa rugosa* was thought to be distributed in the northeastern parts of Asia, including Japan archipelago (Maeda & Matsui 1999). However, the Chinese populations of *R. rugosa* was given a distinct species name, *Rugosa emeljanovi* (Fei *et al*. 1991"1990"). Recently, the populations in Korean peninsula were also treated as *R. emeljanovi* (Kuzumin *et al*. 2004). Following these treatments, we use the species name *Rugosa rugosa* for only the Japanese populations in this study. The type locality of the species was restricted to Nagasaki of Kyusyu district in the Western part of Japan (Stejneger 1907). The unique biological characteristic of *R. rugosa* is the diversity in sex determining mechanisms. A total of five geographic races are currently recognized on the basis

Accepted by M. Vences: 24 Oct. 2012; published: 7 Dec. 2012

of molecular phylogenetic and cytogenetic studies (Miura 2007). In three (XY, ZW and Neo-ZW) of the five races, chromosomes 7 out of 13 haploid complements are the heteromorphic sex chromosomes, while they are homomorphic in the remaining two races (West- and East-Japan). The type locality of the species is on the area of West-Japan race (Fig.1). Very interestingly, two (ZW and Neo-ZW) of the five races have a female heterogametic sex determining mechanism whereas the remaining three have a male heterogametic one (Nishioka *et al*. 1994; summary in Fig. 1). Since co-existence of the two different kinds of sex determining mechanisms within a single species is very rare, this frog is reputed to be an excellent and valuable research material for the study on evolutionary mechanisms of sex determination (Graves 2008). In contrast to the high diversity of sex chromosomes and sex determining mechanisms, the species is poor in variation of the external morphology (Maeda & Matsui 1999), and thus evoked little taxonomical interest. The dorsum of this species is largely muddy brown and bears many short ridges, and the abdomen is pale yellow or grayish yellow. Recently, we have found a unique morphotype of this species on Sado Island, Japan, whose abdomen and ventral surface of hindlimb are deep yellow and the skin is much smoother (Sekiya *et al*. 2010). Also, *R. rugosa* (ZW race) whose color of abdomen is pale yellow inhabits the southern hilly area (Kosado Hills) and the north part of the same island, while most of the unique morphotype occupies flat lowland (Kuninaka Plain) in the central region of the island (Fig.1). The two forms are genetically differentiated and are postzygotically isolated from each other (Ohtani *et al*. 2012). Almost all of the reciprocal hybrids produced from artificial crossings become males with low abundance of sperm in the testes, and showing an extremely low fertility when backcrossed to the females of both types (Ohtani *et al*. 2012). We describe this unique morphotype occurring in Sado Island as a new species of the genus *Rugosa*.

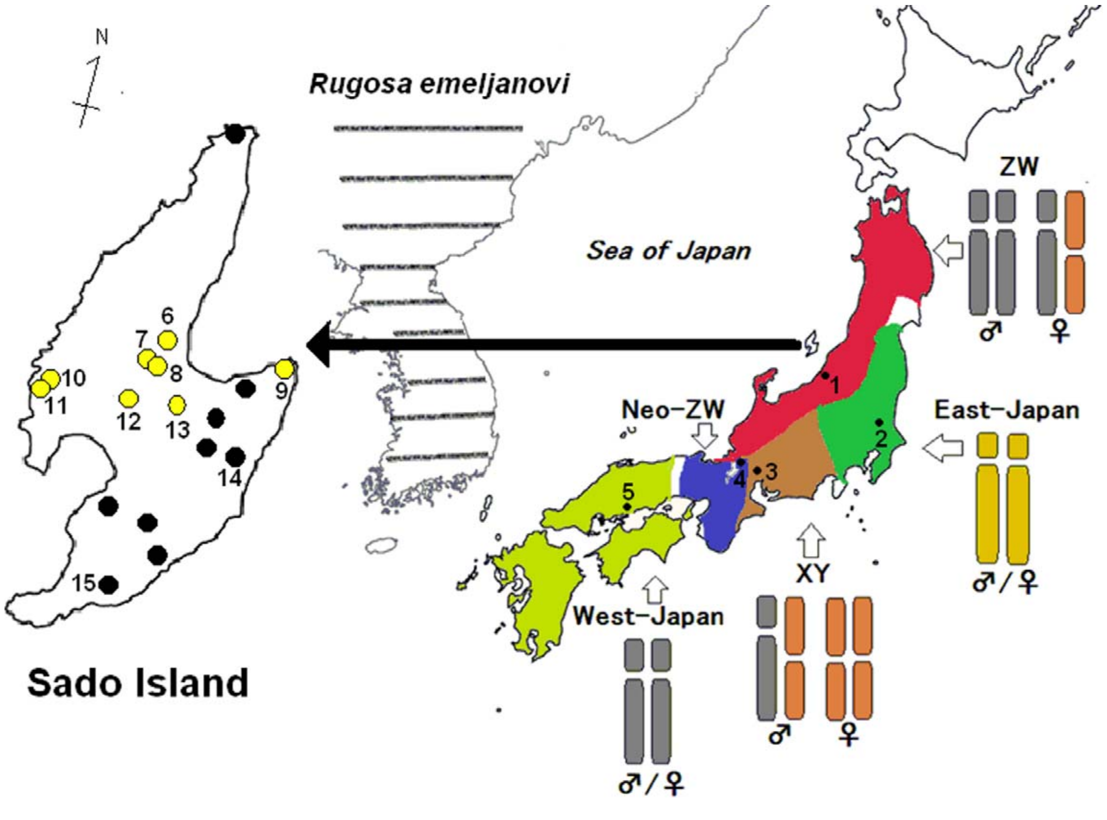

FIGURE 1. Distribution map of the five geographic races of *Rugosa rugosa* and the localities of specimens (closed circle) used in this study including those (yellow circle) of *R. susurra* in Sado Island. Circles without number are the localities of *R. rugosa* on Sado Island referred from Sekiya *et al*. (2010). Chromosomes 7 are given for the groups except Neo-ZW. Names of localities are as follows: 1, Nagaoka; 2, Hitachi-Oomiya; 3, Motosu; 4, Kinomoto; 5, Higashi-Hiroshima; 6, Umetsu; 7, Nagae; 8, Akitsu; 9, Suitsu; 10, Tassya; 11, Ogawa; 12, Nakaoku; 13, Niibo-Iuchi; 14, Akadama; 15, Hamochi-Ohashi.

Materials and methods

Morphology. Adult specimens of the new species and *Rugosa rugosa* were collected in the period from June 2008 to July 2011. The collected specimens were fixed with 10% formalin and were finally stored in 80% ethanol after being rinsed with water. Tadpoles of the new species were fixed in 10% formalin. A set of 13 characters of preserved adult specimens and head-body length of preserved tadpole specimens were measured to the nearest 0.05mm with dial calipers by mainly following the study of Ueda *et al*. (1998). Ten of the earlier mentioned characters are snout-vent length (SVL), head width (HW), head length (HL, in parallel with the line connecting angle of jaw), eye distance (ED), tympanum diameter (TD), distance between tympanum and eye (T-ED), interorbital distance (IOD), snout length (SL, in parallel with the line connecting angle of jaw), tibia length (TIBIA) and internarial distance (IND). Upper eyelid width (UEW) and distance between nostril and eye (N-ED) were measured following the method of Maeda and Matsui (1999). Arm length (AL) was defined as a distance from the proximal end of inner metacarpal tubercle to elbow in the present study. Webbing formula is based on that of Myers and Duellman (1982). Furthermore, we examined the presence of skin granules on the ventral side of jaw in all specimens. For tadpole specimens, dental formula and developmental stages were examined according to Gosner’s staging table (1960). Size of ova was measured to the nearest 0.05mm under a binocular dissecting microscope with dial calipers. All statistical analyses were conducted using R 2.11.1 software of R development core team (2010); males and females were separated for each analysis, while the data of specimens less than five in total number were excluded from analysis. Also, the data of granule distribution on the ventral surface of jaw from plural populations of each species were put together and used for statistical analysis. Specimens used in this study were deposited in Kanagawa Prefectural Museum of Natural History, Japan (KPM) and Institute for Amphibian Biology, Graduate School of Science, Hiroshima University, Japan (IABHU). Specimens of *R. rugosa* for morphological comparison were shown in Appendix

Call. Advertisement call was recorded in the field from males of the new species belonging to one population on 19 June 2011. In order to compare the calls of new species and *Rugosa rugosa*, we injected pituitary suspension of *R. rugosa* or *Pelophylax porosus brevipoda* Ito into the abdomen of three males each of the new species and *R. rugosa* both collected from Sado Island, and recorded their calls at laboratory following the method of Hasegawa *et al*. (1999). For recording, we used a microphone (NTG2, Rode) and a digital recorder (44.1kHz, 16bit, ICR-PS004M, Sanyo). Call duration and number of pulse in a single call were measured using Praat ver 5.1.44 (Paul 2001) and Raven lite version 1.0 (Cornel lab of ornithology). Mean value of each parameter for three males was calculated.

Molecular phylogeny. A mitochondrial gene tree was constructed based on the sequences of 12SrRNA (357bp) and 16SrRNA (510bp) genes from *Rugosa rugosa* (6 populations), the new species, *R. emeljanovi* (Korean and Chinese populations), *R. tientaiensis* Chang and *Glandirana minima* Ting and Ts’ai from Fujian Province, China obtained from NCBI database (*R. rugosa*, AB430340-430345 and AB430352; the new species, AB430349; *R. emeljanovi*, Korean population AB430346; Chinese population, AF205561 and AF315155; *R. tientaiensis*, DQ359954 and DQ359985; *G. minima*, DQ359968 and DQ359998). DNA sequences of the new species, *R. rugosa* and *R. emeljanovi* (Korean population) were determined in our previous study (Sekiya *et al*. 2010). The sequence of *Pelophylax nigromaculatus* Hallowell was used as an outgroup (DQ283137). Phylogenetic analysis was conducted by the methods of neighbor-joining, maximum parsimony and maximum likelihood with MEGA ver.5 software (Tamura *et al*. 2011). DNA distances among the sequences were estimated by Kimura’s two-parameter methods with the same software.

Systematics

Rugosa susurra sp. nov.

(Japanese name: Sado gaeru)
(Figs. 2–4)

Rana rugosa (yellow type): Sekiya *et al*. 2010, p. 71, figs. 2A–B.

Diagnosis. Genetically close to *Rugosa rugosa* than other species of the genus *Rugosa*. A medium sized species of *Rugosa*, with males 33.0–44.2 mm, females 38.1–49.6 mm in SVL; web rather well developed; dorsum khaki in color with many dermal ridges of varying size, dorso-lateral fold absent, supratympanic fold strong; venter posteriorly deep yellow; anterior ventral less granulated than posterior ventral, especially around jaw granule almost absent. This new species is differentiated from *R. rugosa* by its yellow ventral coloration and jaw almost without granules and no vocal sac in male.

Holotype: KPM-NFA000078, an adult male from Akitsu, Sado city, Sado Island, Niigata Prefecture, Japan (38°4'N, 138°24'E, 11m a.s.l), Collected on 14 July 2009 by M. Ogata.

Paratypes: Two adult females and an adult male (KPM-NFA000075-77) from the same locality and collected on the same day by M. Ogata and Y. Ogata.

Referred specimens: Is. Sado, Akitsu (10 males, 7 females, collected on 22 June 2008 by M. Ogata IABHU F2462-F2471,F2475-F2481, 3 males collected on 30 May 2011 by M. Ogata IABHU F2472-F2474, one female collected on 11 July 2011 by M. Ogata IABHU F2482) and Suitsu (38°4'N, 138°33'E, 33m a.s.l, 10 males, 3 females, collected on 22 June 2008 by M. Ogata, IABHU F2483-F2495), Nakaoku (38°3'N 138°21'E, 187m a.s.l, 9 tadpoles, collected on 1 July 2010 by K. Sekiya, IABHU F2513-F2521).

Description of holotype (measurements in millimeters). Snout-vent length 37.6; head about as long (14.0) as broad (13.8); snout (5.5) longer than eye (5.0), tip of snout rounded; distance between nostril and eye (3.4) about as same as upper eyelid width (3.3) and internarial distance (3.5), but narrower than interorbital width (4.4), snout dorsally concave and slightly pointed; lore concave; supratympanic fold strong; tympanum conspicuous, diameter (3.8), separated from eye by about one-fifth of tympanum diameter (0.7).

FIGURE 2. Holotype of *Rugosa susurra* (KPM-NFA000078) in life. Dorsal side (A) and ventral side (B).

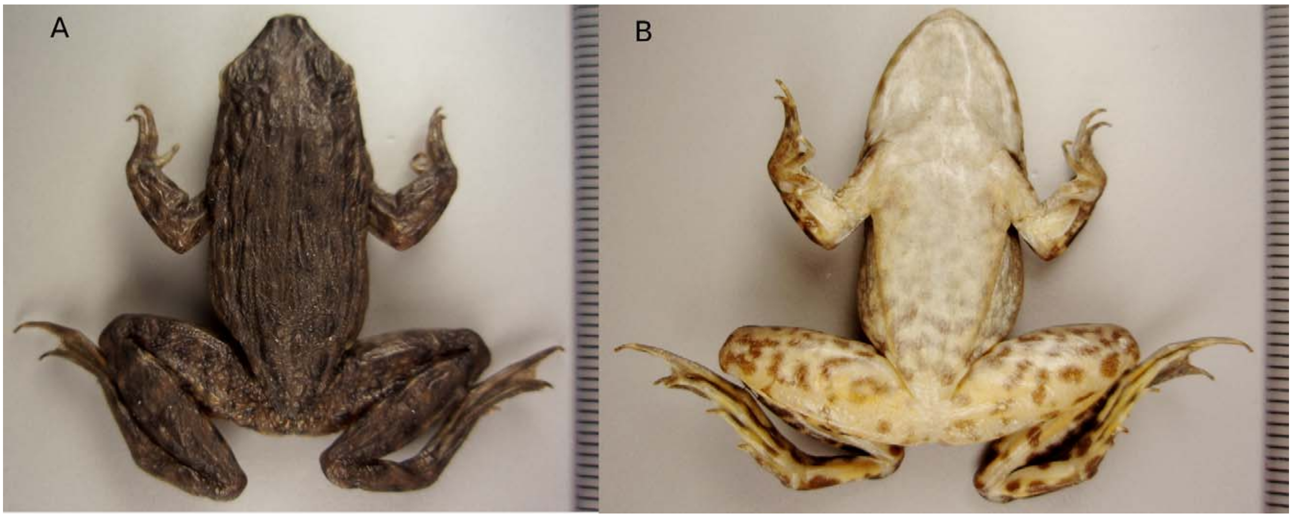

FIGURE 3. Holotype of *Rugosa susurra* (KPM-NFA000078) in alcohol. Dorsal side (A) and ventral side (B). Scale bars represent 1mm.

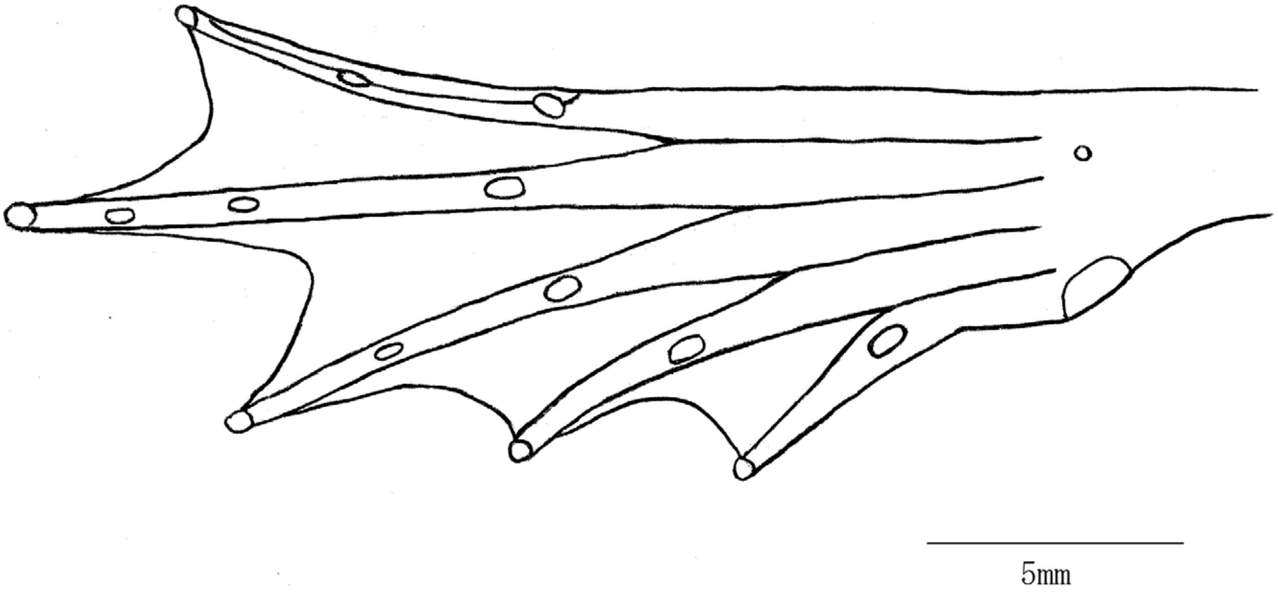

FIGURE 4. Ventral view of left foot of holotype of *Rugosa susurra* in alcohol.

Fingers slender, no webbing, tips of fingers slightly depressed, third finger longer than fourth; subarticular tubercle strong; inner metacarpal tubercle circular; outer metacarpal tubercle elliptical.

Tibia (19.2) about 2.9 times length of arm (6.6); toes slender, tips of toes slightly depressed, web rather well developed, webbing formula: I 1 - 2 II 1 - 2 III 1 - 2 IV 2 – 1 V; excision of membrane between two outer toes reaching near middle subarticular tubercle of fourth toe when toes in contact; inner metatarsal tubercle elliptical, outer metatarsal tubercle weak, tarsal ridge rather strong, skin fold slightly developed on outer margin of fifth toe from near proximal subarticular tubercle to near distal articulation.

Dorsal surface with slightly large dermal ridges and minute tubercles, dorso-lateral fold absent, granules on ventral surface of forelimb, hindlimb and abdomen, but absent on jaw.

Nuptial pad developed on dorso-medial area on first finger from base to subarticular tubercle but weakly elongated to tip.

Dorsum grayish brown, mid-dorsal stripe weak and slender, black transect line on hindlimb.

Colors in life. Dorsum almost khaki. Abdomen almost whitish anteriorly, deep yellow posteriorly (Fig. 2A and 2B). Similar yellow color is observed on ventral side of arm and ventral side of hindlimb but excluding fifth toe. Some dark brown spots on ventral side of hindlimb.

Variation. Females are larger than males in Akitsu population (Table 1A and 1B, t-test, p<0.001). Male has longer tibia, relative to SVL, than female. Tympanum distance, interorbital width, head width, snout length, distance between nostril and eye, internarial distance and head length of male are larger, all relative to SVL, than female (Mann-Whitney U-test, p<0.05). Males of Akitsu population are smaller than males of Suitsu population (t-test, p<0.001). Males of Akitsu have larger head width, relative to SVL, than males of Suitsu (Mann-Whitney U-test, p<0.01). Some individuals of Akitsu have much less weakly skin granule but others no granule (Table 2). Ventral colors of some individuals are clear yellow but others less yellow. Mid-dorsal stripe is absent in some individuals (no stripe: Akitsu population=59.1%, Suitsu population=100%).

Comparison. Morphological characters of *Rugosa susurra* are similar to *R. rugosa* (Table 1A and 1B). When *R. susurra* is morphologically compared with *R. rugosa* from five geographic races, the following differences are detected. In females, head width of *R. susurra*, relative to SVL, are shorter than those of *R. rugosa* from the ZW race on Sado Island population (Kruskal-wallis test with nonparametric multiple comparison, p<0.05). In males, *R. susurra* have longer tibia than *R. rugosa* from West-Japan race, shorter snout length than the XY race, all relative to SVL (Kruskal-wallis test with nonparametric multiple comparison, p<0.05). Most of *R. rugosa* (95.6%) have tiny granules especially around ventral surface of jaw, whereas most of *R. susurra* (94.1%) do not have any there (Table 2, Chi-square test, p<0.001). A deep yellow coloration of abdomen and ventral surface of hindlimb is observed in *R. susurra* but not in *R. rugosa* (Maeda & Matsui 1999). Also, *R. susurra* differs from other species of the genus

TABLE 1A. Measurements of 13 morphological characters in males of *Rugosa susurra* (two populations) and *R. rugosa* (six populations).

Species	*Rugosa susurra*		*Rugosa rugosa*					
Locality (Geographic race)	Akitsu	Suitsu	Akadama (ZW)	Nagaoka (ZW)	Hitachi-Oomiya (East-Japan)	Motosu, (XY)	Kinomoto (Neo-ZW)	Higashi-Hiroshima (West-Japan)
No. of specimens	n=12	n=10	n=8	n=6	n=15	n=14	n=8	n=9
SVL(mm)	35.5±1.70	39.3±2.09	41.5±2.79	41.5±4.29	39.5±1.21	38.4±2.40	41.7±1.53	37.6±2.72
	(33.0–37.6)	(37.3–44.2)	(37.1–45.3)	(36.5–47.2)	(37.2–41.5)	(34.4–41.9)	(39.5–44.0)	(33.5–42.6)
AL(%)	19.7	19.0	19.8	17.1	18.4	18.8	18.0	17.9
	(16.4–21.2)	(17.6–19.8)	(18.4–21.5)	(16.7–19.0)	(16.8–19.4)	(16.0–20.3)	(15.5–21.2)	(14.0–19.5)
TIBIA(%)	51.4	50.7	53.6	54.3	48.9	50.0	49.9	46.5
	(49.2–55.4)	(49.9–52.0)	(48.4–55.3)	(50.6–57.1)	(46.4–51.3)	(47.4–57.0)	(45.7–52.8)	(43.4–49.2)
ED(%)	14.1	14.3	14.0	15.3	12.3	13.8	13.1	12.4
	(12.7–15.5)	(12.2–16.4)	(13.3–16.2)	(12.3–16.4)	(10.5–14.0)	(12.0–15.7)	(12.6–14.9)	(11.2–13.8)
TD(%)	10.7	11.0	11.4	12.5	11.2	10.9	11.0	10.0
	(9.3–11.7)	(8.8–11.5)	(9.4–12.6)	(10.7–12.9)	(9.3–12.3)	(9.4–13.0)	(9.6–12.0)	(8.5–11.3)
T–ED(%)	2.8	2.9	3.4	3.1	3.5	2.5	2.9	2.8
	(1.9–3.5)	(2.3–3.5)	(2.4–4.2)	(2.5–3.3)	(2.2–3.9)	(1.4–3.3)	(1.8–3.8)	(2.3–3.9)
IOD(%)	10.3	9.7	9.4	9.3	9.8	10.4	9.2	10.2
	(8.2–12.0)	(8.5–11.3)	(8.1–10.9)	(7.6–9.6)	(8.6–10.8)	(8.9–12.5)	(8.8–11.1)	(8.6–10.8)
HW(%)	38.5	37.6	42.0	40.5	37.4	40.4	40.8	39.2
	(36.7–42.5)	(35.4–38.3)	(39.3–43.7)	(39.5–43.4)	(35.3–39.3)	(37.7–43.4)	(38.5–42.2)	(37.3–41.2)
SL(%)	14.8	14.1	15.7	17.5	14.8	16.1	16.3	15.0
	(13.3–15.5)	(13.3–15.0)	(15.4–17.5)	(15.3–18.8)	(13.5–15.8)	(15.1–18.2)	(14.9–17.5)	(13.6–15.5)
IND(%)	9.4	9.7	10.5	11.0	9.3	10.1	9.7	9.6
	(8.8–10.4)	(8.5–10.3)	(10.0–11.6)	(9.0–11.8)	(8.1–12.0)	(9.1–12.0)	(8.9–11.4)	(8.5–11.0)
HL(%)	38.2	38.3	40.2	43.3	37.9	39.9	41.1	38.9
	(37.2–39.3)	(37.6–39.9)	(38.2–41.8)	(41.0–44.9)	(35.8–39.2)	(36.7–43.4)	(39.8–44.6)	(34.0–42.1)
UEW(%)	8.8	8.5	10.2	10.2	8.6	9.8	9.2	9.3
	(7.9–10.9)	(7.7–9.4)	(8.4–11.7)	(8.3–10.4)	(7.4–9.8)	(8.4–15.7)	(8.0–10.6)	(8.0–10.7)
N–ED(%)	8.5	8.1	9.0	9.1	8.7	9.2	9.5	8.6
	(7.4–10.4)	(7.5–9.6)	(7.9–9.7)	(9.0–10.4)	(7.5–10.8)	(7.7–10.1)	(8.0–11.4)	(8.0–10.7)

SVL: mean±1*standard deviation (range); Other characters: median of ratio to SVL (range); See the text for abbreviations of the other characters.

TABLE 1B. Measurements of 13 morphological characters in females of *Rugosa susurra* (two populations) and *R. rugosa* (six populations).

Species	*Rugosa susurra*		*Rugosa rugosa*					
Locality (Geographic race)	Akitsu	Suitsu	Akadama (ZW)	Nagaoka (ZW)	Hitachi–Oomiya (East–Japan)	Motosu (XY)	Kinomoto (Neo–ZW)	Higashi–Hiroshima (West–Japan)
No. of specimens	n=9	n=3	n=6	n=5	n=8	n=2	n=3	n=6
SVL(mm)	45.6±3.40 (38.1–49.6)	44.6±0.78 (44.1–45.5)	46.9±5.41 (40.2–54.0)	50.3±3.58 (44.1–53.0)	50.3±2.97 (46.5–55.2)	50.1±2.05 (48.6–51.5)	48.9±3.52 (44.9–51.4)	41.2±2.35 (39.0–43.9)
AL(%)	19.0 (17.8–20.5)	19.2 (18.7–20.0)	18.6 (17.2–21.3)	18.8 (16.3–21.2)	18.4 (16.8–19.1)	18.9 (16.3–21.4)	19.8 (18.9–19.8)	17.4 (15.7–19.5)
TIBIA(%)	48.2 (47.1–52.5)	50.5 (49.9–50.6)	50.8 (46.6–53.8)	53.8 (52.3–55.8)	46.7 (44.8–49.9)	48.7 (48.4–48.9)	49.3 (49.2–51.7)	45.3 (44.6–50.5)
ED(%)	13.7 (12.0–14.8)	12.7 (12.2–14.9)	14.3 (13.3–15.2)	13.8 (11.9–14.6)	11.7 (9.7–13.2)	14.2	12.2 (11.9–12.3)	11.5 (9.4–12.3)
TD(%)	8.9 (8.4–10.0)	9.7 (8.8–9.8)	9.9 (8.9–10.3)	9.2 (8.9–10.2)	9.3 (8.1–9.9)	9.1 (8.8–9.3)	9.8 (8.1–9.9)	8.9 (7.3–10.1)
T–ED(%)	2.9 (2.0–3.2)	2.9 (2.3–3.7)	3.3 (2.6–4.3)	3.5 (2.8–3.6)	2.8 (2.4–3.8)	2.9	4.0 (3.1–5.0)	3.1 (2.3–4.4)
IOD(%)	9.4 (8.2–10.6)	9.5 (9.5–9.7)	9.2 (7.8–11.4)	8.5 (8.3–9.8)	9.2 (8.9–10.7)	9.6 (8.8–10.3)	8.9 (8.7–10.0)	9.6 (8.5–10.3)
HW(%)	36.4 (33.1–37.5)	36.3 (35.8–36.4)	40.9 (38.5–47.2)	39.9 (38.1–43.6)	35.5 (35.2–37.3)	39.8 (38.7–40.8)	41.2 (40.7–43.0)	37.3 (36.2–39.8)
SL(%)	13.0 (12.4–15.0)	14.3 (14.3–15.0)	14.9 (13.1–16.7)	15.8 (14.0–16.2)	13.7 (13.3–14.5)	14.1 (13.4–14.8)	15.8 (14.7–15.8)	14.0 (13.7–14.5)
IND(%)	8.3 (7.9–9.4)	9.7 (8.8–9.8)	10.1 (9.8–11.4)	9.1 (8.3–10.2)	8.8 (8–9.4)	9.3 (8.8–9.7)	9.5 (8.7–9.9)	8.6 (7.9–9.9)
HL(%)	35.3 (33.1–35.8)	36.1 (35.8–37.8)	38.5 (35.6–39.8)	41.4 (38.8–43.4)	35.1 (34.4–36.3)	36.1 (35.6–36.5)	39.7 (37.0–41.2)	36.6 (34.9–37.7)
UEW(%)	7.9 (6.9–9.2)	7.7 (7.7–8.4)	9.2 (8.7–10.1)	9.3 (8.3–10.2)	8.4 (8–9.2)	9.8 (9.1–10.5)	9.9 (9.1–9.9)	9.0 (8.2–9.5)
N–ED(%)	7.4 (6.6–8.9)	8.1 (7.5–8.4)	8.7 (7.7–9.5)	9.1 (8.8–10.0)	7.8 (7.3–8.6)	9.3	8.5 (8.2–9.7)	8.0 (7.1–8.2)

SVL: mean±1*standard deviation (range); Other characters: median of ratio to SVL (range); See the text for abbreviations of the other characters.

Rugosa in the following characters (Chang 1933; Nikolsky 1918; Fei *et al*.1991“1990”; 2005): dorsal surface with slightly large dermal ridges and minute tubercles (*R. tientaiensis* has oval or elongated narrow warts on dorsal surface, Chang 1933) and smooth ventral skin without granule especially around jaw (*R. emeljanovi* and *R. tientaiensis* have rough skin both on dorsum and ventral side, Chang 1933; Fei *et al*. 1991“1990”; 2005). Ventral surface is deep yellow (dirty-yellow in *R. emeljanovi*, Nikolsky 1918). Since the genus *Glandirana* was considered as related with *Rugosa* (Che *et al*. 2007), we compared *R. susurra* and *G. minima* to clarify their morphological difference. *R. susurra* differs from *G. minima* in the following characters: webbing on toes is well developed (half-webbed on toes in *G. minima*, Fei *et al*. 1991“1990”), and ventral surface is deep yellow (gray in *G. minima* Ting & Ts’ai 1979).

TABLE 2. Distribution of granules on the ventral side of jaw in *Rugosa susurra* and *R. rugosa*.

Species	Locality (Geographic race)	Number of frogs with granules		Number of frogs without granules	
		Male	Female	Male	Female
Rugosa susurra	Akitsu	1	1	11	8
	Suitsu	0	0	10	3
Rugosa rugosa	Akadama (ZW)	8	6	0	0
	Nagaoka (ZW)	6	5	0	0
	Hitachi-Oomiya (East-Japan)	11	8	4	0
	Motosu (XY)	14	2	0	0
	Kinomoto (Neo-ZW)	8	3	0	0
	Higashi-Hiroshima (West-Japan)	9	6	0	0

Eggs. A female (IABHU F2482 collected from Akitsu on 11 July 2011) contained a total of 710 matured ova in a pair of ovaries. The diameter of twenty ova ranged from 1.2 to 1.5mm (mean±2SE=1.38±0.04). The animal pole is dark brown while the vegetable pole is cream in color.

Tadpole. Developmental stages of the nine tadpoles (IABHU F2513-F2521 collected from Nakaoku) varied from St.29 to St.42. In preserved specimens, dorsum almost grayish brown, abdomen white (Fig. 5). Tail and tail fin fine and grayish brown network. A lot of small white spots were distributed on entire body including tail fin.
Head and body oval shaped, body slightly rounded below. Dermal ridges strongly on the dorsum of tadpoles at St.41 and St.42. Head-body length of two tadpoles at St.29 was 16.5mm and 18.0mm and that of tadpoles at St. 31 and St. 32 was 19.1 mm and 19.9 mm. Head-body length of four tadpoles at St.41 and St.42 varied from 22.6 mm to 27.0 mm whereas that of tadpole at St. 39 is 25.0 mm. Tail fin slightly develop around the center of tail.

Oral slightly below. Dental formula of six tadpoles (St.29-St.32 and St.39-St.42) was 1:1+1/1+1:2 having relatively large interrupted portion on upper dentition, while that of the remaining three tadpoles was 1:1+1/3 (St.41) or 1/1+1:2 (St.29).

Range. *Rugosa susurra* is mainly distributed in the Kuninaka Plain of Sado Island including the type locality (Fig. 1), not in any areas outside Sado Island, Japan. Five localities are known other than the three as mentioned above, Umetsu (38°6’N, 138°24’E, 75m a.s.l.), Nagae (38°5’N, 138°23’E, 54m a.s.l), Tassya (38°4’N, 138°15’E, 77m a.s.l.), Ogawa (38°3’N, 138°15’E, 29m a.s.l.), Niibo-Iuchi (38°1’N, 138°27’E, 102m a.s.l.). The elevations of the eight localities range from 11m to 187m (in a.s.l.). Five of the eight localities are on the Kuninaka Plain (Fig.1, No.6–No.8 and No.12–No.13)

Calls. A total of five calls from one male of *Rugosa susurra* were recorded in Akitsu at air and water temperatures of 18.5°C and 21.5°C, respectively. Call duration ranged from 1112 to 1646 ms (mean±SD = 1341.7±226.9 ms), whereas number of pulse in a single call ranged from 67 to 93 (mean±SD = 78.8±11.6) (Fig. 6). When the male frog was calling, we could not observe any apparent expansion of vocal sac as *R. rugosa* is doing. Recording at laboratory was conducted at air temperatures of 24.6–26.8°C for *R. susurra* collected from Akitsu on 30 May 2011 (IABHU F2472-2474) and *R. rugosa* from Akadama on Sado Island on 30 May 2011 (ZW race, IABHU F2504-2506). A total of 16 calls (*R. susurra*) and 19 calls (*R. rugosa*) were recorded. Call duration of *R. susurra* is much longer than that of *R. rugosa* (*R. susurra*, mean±SD = 1333.5±382.4ms; *R. rugosa*, mean±SD = 111.7±9.1ms, t-test $p<0.05$). Also, number of pulse in a single call is completely different between them (*R.*

susurra, mean±SD =63.0±6.4; *R. rugosa*, 6.8±0.7, $p<0.005$, t-test). In the three male specimens of *R. susurra*, any vocal sac and vocal opening could not be identified inside the mouth by dissection. Therefore, *R. susurra* has no vocal sac.

FIGURE 5. Tadpole of *Rugosa susurra* (IABHU F2521). Dorsal (A), ventral (B) and lateral (C) views. Developmental stage is 41 (Gosner 1961). Scale bars represent 1mm.

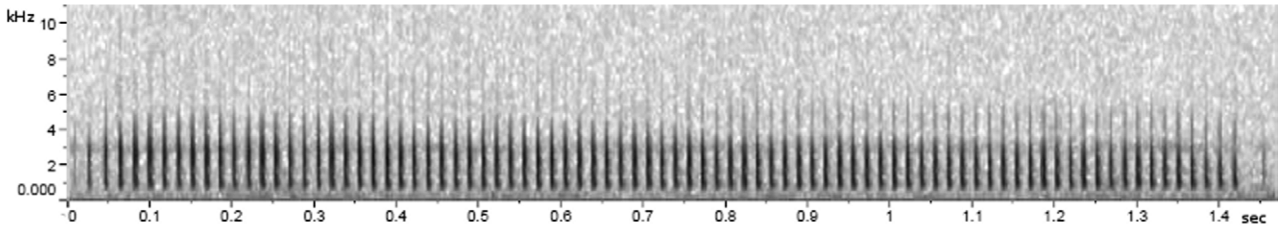

FIGURE 6. Spectrogram of an advertisement call of *Rugosa susurra*. Air temperature and water temperature are 18.5°C and 21.5°C, respectively

Natural history. Adult frogs of *Rugosa susurra* are always observed near the water, such as in rims of rice field, small streams and ponds. Egg masses laid are attached to short withered branches or water grass (Fig. 7). Breeding seems to occur from middle May to early August, because egg masses are observed in ponds during the season. In winter, both adult frogs and tadpoles are observed in the mud under water. Thus, the tadpoles of *R. susurra* can hibernate successfully like those of *R. rugosa* (Maeda & Matsui 1999), although we have no information whether all tadpoles that are produced in the season hibernate or not.

FIGURE 7. Egg masses of *Rugosa susurra* from Tassya on Sado Island.

Karyotype. 2n= 26 (males and females) with no heteromorphic pair of sex chromosomes, and the chromosome 7 is very similar in morphology and late replicating banding pattern to that of East-Japan (Kanto) race of *R. rugosa*, suggesting their genetically close relationship (cf. Fig. 3 in Sekiya *et al*. 2010).

Molecular phylogeny. The bootstrap consensus tree of mitochondrial genes was constructed by maximum likelihood method (Fig. 8). *Rugosa rugosa* comprises two main lineages: one contains East-Japan (Kanto), XY and Neo-ZW races, while the other contains West-Japan and ZW races. *R. susurra* was always clustered with the lineage comprising East-Japan (Kanto), XY and Neo-ZW races of *R. rugosa* in the three topologies with high bootstrap probability. Topologies of the other two methods were almost the same as that of Fig. 6, although two sequences of *R. emeljanovi* were clustered with the linage (East-Japan +XY+Neo-ZW + *R. susurra*) in maximum parsimony methods with low bootstrap probability (<50%). DNA distances between *R. susurra* and *R. rugosa* are 0.041 to 0.062 whereas those of *R. susurra* and the remaining three species are 0.068–0.075 (*R. emeljanovi*), 0.071 (*R. tientaiensis*) and 0.096 (*Glandirana minima*).

Etymology. The specific epithet is derived from "susurrus" in Latin meaning “whispering”. The advertisement call of *Rugosa susurra* is much quieter than those of other anuran species in the same locality (*Hyla japonica* Günther, *Rhacophorus arboreus* Okada and Kawano), and sounds like whispering among them.

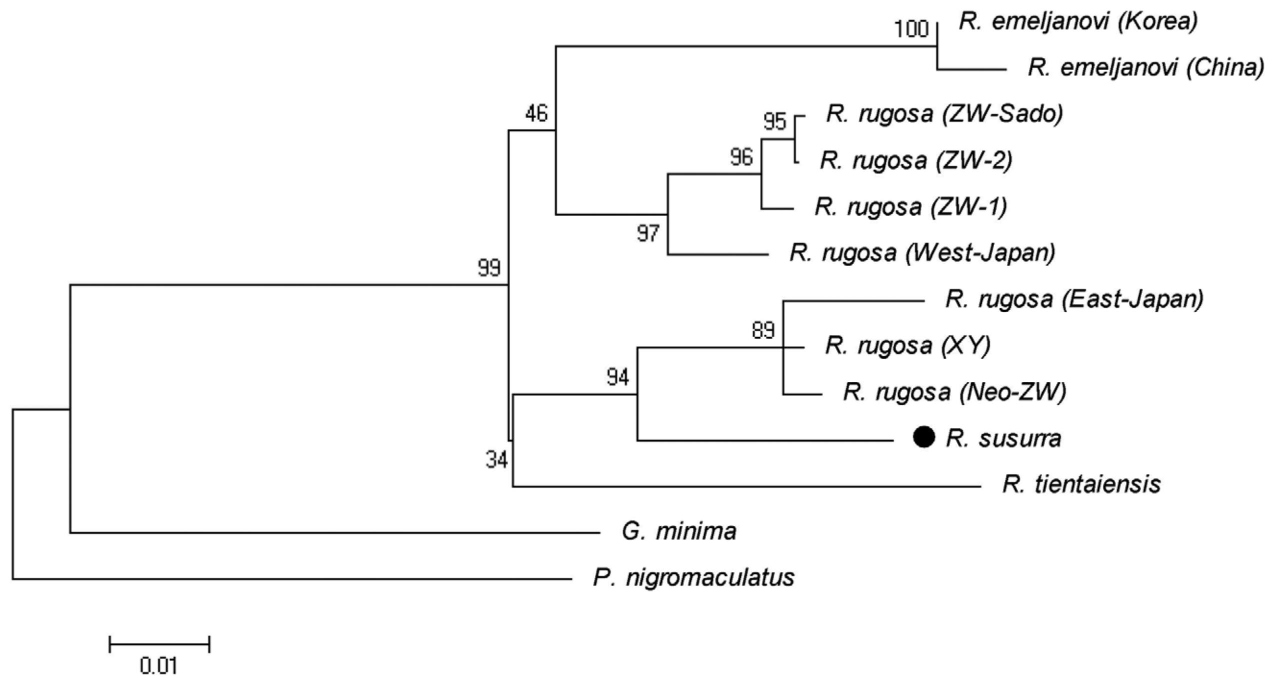

FIGURE 8. Maximum-likelihood tree based on the 867bp sequences of the mitochondrial 12S rRNA and 16S rRNA genes of genus *Rugosa*. Numeral at each node denotes the bootstrap support (% in 1000 replicates). *R. susurra* indicates a specimen from Akitsu in Sado Island (AB430349). East-Japan (AB430340), West-Japan (AB430341), XY (AB430342), Neo-ZW (AB430343), ZW1 (AB430345) and ZW2 (AB430344) indicate specimens from Isehara City in Kanagawa Prefecture, Miyoshi City in Hiroshima Prefecture, Ohtsu City in Shiga Prefecture, Kanazawa City in Ishikawa Prefecture and Niigata City in Niigata Prefecture, respectively. ZW-Sado (AB430352) indicates a specimen from Hamochi-Ohashi in Sado Island. See other abbreviations in the text.

Discussion

Though genetically close to *Rugosa rugosa* (Fig. 8), *R. susurra* is definitely distinguished from *R. rugosa* in morphology such as yellow color of the ventral surface, much smoother skin without granules around ventral surface of the jaw, and advertisement call with a longer call duration, as mentioned in this article. Also, *R. susurra* has no internal vocal sac whereas *R. rugosa* has it (Maeda & Matsui 1999). In addition, when *R. susurra* is artificially crossed with two ancestral races (West- and East-Japan) of *R. rugosa*, sex ratios of the reciprocal hybrids extremely skewed to males (more than 90%) with some abnormal hermaphrodites (Ohtani *et al*. unpublished). Particularly, *R. susurra* shows a severe postzygotic isolation from ZW race of *R. rugosa* on the same island by all hybrid maleness with extremely low fertility when they are artificially hybridized (*R. susurra* of Akitsu x *R. rugosa* of Hamochi-Ohasi on Sado Island; Ohtani *et al*. 2012). Therefore, *R. susurra* is recognized as a distinct species from *R. rugosa*.

Rugosa rugosa comprises five geographic races in Japanese islands and they are ZW, XY, East-Japan (Kanto), Neo-ZW and West-Japan (Miura 2007). Two of the five races, ZW and XY, share the evolutionary origin at hybridization between the West-Japan and East-Japan (Kanto) races (Miura *et al*. 1997; Ogata *et al*. 2003), and the Neo-ZW has another more recent origin at re-hybridization between West-Japan and XY races (Ogata *et al*. 2008). Based on the phylogenetic analysis of mitochondrial DNA sequences, these five races are divided into two major lineages. One lineage comprises East-Japan (Kanto), XY and Neo-ZW, while the other, West-Japan (including the type locality of the frog) and ZW (Sekiya *et al*. 2010). The ZW race also occurs in Sado Island (Sekiya *et al*. 2010). Interestingly, *R. susurra* is much closer in the mtDNA tree to the former lineage of *R. rugosa* than the latter including the ZW race that is geographically closest to (Fig. 6). Sekiya *et al*. (2010) hypothesized that *R. susurra* evolutionarily originated from the relic East-Japan (Kanto) population of *R. rugosa*, which had been distributed in the whole eastern region of Honshu. After *R. susurra* appeared, the ZW population of *R. rugosa* emerged from hybridization between the primary West-Japan and East-Japan (Kanto) races and subsequently expanded its distribution to the northwestern mainland of Japan and finally reached Sado Island (Sekiya *et al*. 2010). Although there is no information about the time when *R. rugosa* (ZW race) invaded Sado Island, the small value of DNA distance (0.001) between the two populations of the ZW race (Niigata vs Sado) indicate that the invasion of the ZW race was much more recent than speciation of *R. susurra*. Thus, it is evident that *R. susurra* has been isolated from *R. rugosa* for a long time on the island. And such a long time isolation would have made several differences in morphology and call (advertisement call duration, color of abdomen, and smooth skin), and evolved a postzygotic isolation between *R. susurra* and *R. rugosa* (ZW race). In sharp contrast, there are no postzygotic isolations among the five races of *R. rugosa* itself on the basis of artificial crossings: reciprocal hybrids among the races are all viable and fertile (Nishioka *et al*. 1993a; Nishioka & Hanada 1994; Ogata *et al*. 2002; 2003; Ohtani *et al.* unpublished data). As mentioned above, three of the five races, XY, ZW and Neo-ZW, share the hybrid origins between the West- and East-Japan races of *R. rugosa* (Miura *et al*. 1997; Ogata *et al*. 2003; Ogata *et al*. 2008), suggesting that natural hybridization had actually occurred in the field. In addition, based on the allozyme analysis (Nishioka *et al*. 1993b), the ZW race belongs to the lineage of East-Japan race (an opposite topology to that based on sequences of mitochondrial genes), and one western population of the Neo-ZW belongs to that of the West-Japan race while another eastern populations, to that of XY race. These results indicate that the nuclear genomes of the five races are varying continuously. Also, it is impossible to identify each of the frogs belonging to the five races in external morphology at least based on our data (Table1 and Table2). Thus, we consider that the five races should be now treated as one same species.

Sado Island is located in the Sea of Japan, ca 35 km off the coast of the Echigo Plain, the closest shore of the mainland Japan (Honshu). This island was geologically formed at least in middle Pliocene (Machida *et al*. 2006), and had already been separated from the mainland at middle Pleistocene (Ohshima 1990). On the other hand, a biogeographical study suggests that movement of animals between Sado Island and the mainland of Japan has continued until more recently (Motokawa 2008). Thus, it is unclear when emigration of animals between the mainland of Japan and the island ceased. The amphibian fauna of Sado Island is rendered to a mere subset of the mainland Japanese amphibian fauna (Sato 1940; Maeda & Matsui 1999). So far, although just one endemic urodele *Hynobius sadoensis* Sato has ever been reported (Sato 1940), it is currently treated as a synonym of *H. nigrescens* Stejneger in the northern mainland of Japan on the basis of genetic studies (Matsui *et al*. 1992). Thus, *Rugosa susurra* is a true, and the only endemic amphibian species in Sado Island. In other vertebrates, an endemic mole *Mogera tokudae* Kuroda is described. This mole is most closely related to *Mogera etigo* Yoshiyuki and Imaizumi (DNA distance of mitochondrial cytochrome *b* gene = 0.025), whose distribution is restricted to the Echigo plain, located in the opposite shore of Sado Island across the Sea of Japan (Shinohara *et al*. 2005). In contrast, *R. rugosa* inhabiting the Echigo Plain is the ZW race having a lower genetic affinity to *R. susurra* (DNA distance of mitochondrial 12SrRNA and 16SrRNA genes = 0.062). The most closely related group to *R. susurra* is the lineage comprising East-Japan (Kanto) and XY (DNA distance of 12SrRNA and 16SrRNA genes = 0.041–0.055), whose geographic distributions are far distant from the island (Fig. 1). Compared to the endemic mole, the evolutionary origin of *R. susurra* is unique, and raises a question why only the amphibian has completed speciation in Sado Island, while most other amphibians and animals have not. This is a new issue on *R. susurra* to be solved.

Lastly, we mention a possible reason of allopatry of *Rugosa susurra* and *R. rugosa* (ZW race) on Sado Island. As mentioned earlier and shown in Fig. 1, distributional ranges of the two species are mutually exclusive on the island, *R. susurra* on the central region while *R. rugosa* on the southern region and northern top. The fact that the two forms locate distantly within the molecular phylogeny of *R. rugosa* + *R. susurra* (Fig. 8) attests that the separation of the distributional ranges of the two forms does not mean initial geographical speciation of the two forms on the Sado Island but later invasion of *R. rugosa*. Ohtani *et al.* (2012) showed that there is virtually perfect postzygotic isolation between *R. susurra* and Is. Sado populations of *R. rugosa*. Thus, the present allopatry of *R. susurra* from *R. rugosa* in Sado Island may have been maintained after invasion of the latter by existence of such a reproductive isolating mechanism. Further genetic and ecological investigations focused on the populations located at the boundary region between the two species are necessary.

Acknowledgements

We gratefully thank N. Tsurusaki for critical comments; S. Horie for help in the recording of advertisement calls; Y. Hasegawa for help in the analysis of advertisement calls; J. Marunouchi for assistance in the deposition of holotype at Kanagawa museum, and Y. Ogata for help in the collection of specimens of *R. susurra*. This work was partially supported by a grant-in-aid for scientific research from Ministry of Education, Culture, Sports, Science and Technology of Japan to M.O. (no. 20918010) and by a grant for scientific research from Niigata University to K.S.

Literature cited

Chang, T.K. (1933) Two new amphibian records from Chekiang. *Peking Natural History Bulletin*, 8, 75–80.

Che, J., Pang, J., Zhao, H., Wu, G., Zhao, E. & Zhang, Y. (2007) Phylogeny of Raninae (Anura: Ranidae) inferred from mitochondrial and nuclear sequences. *Molecular Phylogenetics and Evolution*, 43, 1–13.

Dubois, A. (1992) Notes sur la classification des Ranidae (Amphibiens, Anoures). *Bulletin de la Société d'anthropologie de Lyon*, 61, 305–352.

Fei, L., Ye, C. & Huang, Y. (1991 "1990") *Key to Chinese amphibians*. Publishing house for scientific and technological literature, Chongqing, 364pp. (in Chinese)

Fei, L., Ye, C., Jiang, J., Feng, X. & Huang, Y. (2005) *An illustrated key to Chinese amphibians*. Sichuan publishing house of science and technology, Chongqing, 340pp. (in Chinese)

Frost, D.R., Grant, T., Faivovich, J., Bazin, R.H., Haas, A., Haddad, C.F.B., de Sa´ , R.O., Channing, A., Wilkinson, M., Donnellan, S.C., Raxworthy, C.J., Campbell, J.A., Blotto, B.L., Moler, P., Drewes, R.C., Nussbaum, R.A., Lynch, J.D., Green, D.M. & Wheeler, W.C. (2006) The amphibian tree of life. *Bulletin of the American museum of natural history*, 297, 1–370.

Gosner, K.L. (1960) A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica*, 16, 183–190.

Graves, J.A.M. (2008) Weird animal genomes and the evolution of vertebrate sex and sex chromosomes. *Annual Review of Genetics*, 42, 565–586.

Hasegawa, Y., Ueda, H. & Sumida, M. (1999) Clinal geographic variation in the advertisement call of the wrinkled frog, *Rana rugosa*. *Herpetologica*, 55, 318–324.

Kuzmin, S., Matsui, M., Wenge, Z. & Kaneko, Y. (2004) *Glandirana emeljanovi*. In: IUCN 2011. IUCN Red List of Threatened Species. Version 2011.2. Available from http://www.iucnredlist.org (accessed 30 November 2011)

Machida, H., Matsuda, T., Umetsu, M. & Koizumi, T. (2006) *Landscape of Japan (5)* Tokyo University Press, Tokyo, 392pp. (in Japanese)

Maeda, N. & Matsui, M. (1999) *Frogs and toads of Japan. Revised edition.* Bun-Ichi Sogo Shuppan, Tokyo, 223pp. (in Japanese with English abstract)

Matsui, M., Iwasawa, H., Takahashi, H., Hayashi, T. & Kumakura, M. (1992) Invalid specific status of *Hynobius sadoensis* Sato: Electrophoretic evidence (Amphibia: Caudata). *Journal of Herpetology*, 26, 308–315.

Miura, I., Ohtani, H., Hanada, H., Ichikawa, Y., Kashiwagi, A. & Nakamura, M. (1997) Evidence for two successive pericentric inversions in sex lampbrush chromosomes of *Rana rugosa* (Anura: Ranidae). *Chromosoma*, 106, 178–182.

Miura, I. (2007) An evolutionary witness: the frog *Rana rugosa* underwent change of heterogametic sex from XY male to ZW female. *Sexual Development*, 1, 323–331.

Motokawa, M. (2008) *Mammalogy in Japan (1).* Tokyo University Press, Tokyo, 311pp. (in Japanese)

Myers, C. W. & Duellman, W. E. (1982) A new species of *Hyla* from Cerro Colorado, and other tree frog records and geographical notes from western Panama. *American Museum Novitates*, 2752, 1–32.

Nikolsky, A. M. (1918) *Fauna of Russia and adjacent countries. Amphibia*. Israel program for Scientific Translation, Jerusalem, 225pp. (Original in Russian)

Nishioka, M., Miura, I., & Saitou, K. (1993a) Sex chromosomes of *Rana rugosa* with special reference to local differences in sex determining mechanism. *Scientific Report of the Laboratory for Amphibian Biology, Hiroshima University*, 12, 55–81.

Nishioka, M., Kodama, Y., Sumida, M. & Ryuzaki, M. (1993b) Systematic evolution of 40 populations of *Rana rugosa* distributed in Japan elucidated by electrophoresis. *Scientific Report of the Laboratory for Amphibian Biology, Hiroshima University*, 12, 83–131.

Nishioka, M., Hanada, H., Miura, I. & Ryuzaki, M. (1994) Four kinds of sex chromosomes in *Rana rugosa*. *Scientific Report of the Laboratory for Amphibian Biology, Hiroshima University*, 13, 1–34.

Nishioka, M. & Hanada, H. (1994) Sex of reciprocal hybrids between the Hamakita (XX-XY type) population and the Murakami (ZW-ZZ type) population of *Rana rugosa. Scientific Report of the Laboratory for Amphibian Biology, Hiroshima University*, 13, 35–50.

Ogata, M., Lee, J.Y., Kim, S., Ohtani, H., Sekiya, K., Igarashi, T., Hasegawa, Y., Ichikawa, Y. & Miura, I. (2002) The prototype of sex chromosomes found in Korean populations of *Rana rugosa*. *Cytogenetic and Genome research*, 99, 185–193.

Ogata, M., Ohtani, H., Igarashi, T., Hasegawa, Y., Ichikawa, Y. & Miura, I. (2003) Change of the heterogametic sex from male to female in the frog. *Genetics*, 164, 613–620.

Ogata, M., Hasegawa, Y., Ohtani, H., Mineyama, M. & Miura, I. (2008) The ZZ/ZW sex-determining mechanism originated twice and independently during evolution of the frog, *Rana rugosa*. *Heredity*, 100, 92–99.

Ohshima, K. (1990) The history of straits around the Japanese Islands in the late-Quaternary. *The Quaternary Research (Daiyonki Kenkyu)*, 29, 193–208. (in Japanese with English abstract)

Ohtani, H., Sekiya, K., Ogata, M. & Miura, I. (2012) The postzygotic isolation of a unique morphotype of frog *Rana rugosa* (Ranidae) found on Sado Island, Japan. *Journal of Herpetology*, 46, 325–330.

Paul, B. (2001) Praat, a system for doing phonetics by computer. *Glot International* 5:9/10, 341–345.

R Development Core Team (2010) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available from http://www.R-project.org.. (accessed 20 September 2010)

Sato, I. (1940) On a *Hynobius* from Sado Island. *Bulletin of the Biogeographical Society of Japan*, 10, 163–170.

Sekiya, K., Ohtani, H., Ogata, M. & Miura, I. (2010) Phyletic diversity in the frog *Rana rugosa* (Anura: Ranidae) with special reference to a unique morphotype found from Sado Island, Japan. *Current Herpetology*, 29, 69–78.

Shinohara, A., Campbell K.L. & Suzuki, H. (2005) An evolutionary view on the Japanese talpids based on nucleotide sequences. *Mammal Study*, 30, S19–S24.

Stejneger, L. (1907) Herpetology of Japan and adjacent territory. *Bulletin of United States National Museum*, 58, 1–577.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., & Kumar, S. (2011) MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, 28, 2731–2739.

Ting, H.P. & T'sai, M.C. (1979) A new species of frog (*Rana minimus*) from Fujian Province. *Acta Zootaxonomica Sinica*, 4, 297–300. (in Chinese with English abstract)

Ueda, H., Hasegawa Y., & Marunouchi, J. (1998) Geographical differentiation in a Japanese stream-breeding frog, *Buergeria buergeri*, elucidated by morphometric analyses and crossing experiments. *Zoological Science*, 15, 615–622.

Wiens, J.J., Sukumaran, J., Pyron, R.A., & Brown, R.M. (2009) Evolutionary and biogeographic origins of high tropical diversity in old world frogs (Ranidae). *Evolution*, 63, 1217–1231.

APPENDIX. Specimens used for comparison and measurements.

Rugosa susurra: KPM-NFA 000075-000078, IABHU F2462-F2471, F2475-F2481, F2483-F2495 and F2513-F2521

Rugosa rugosa: Adult frogs were collected from Akadama on Sado Island (38°0'N, 138°30'E, IABHU F2496-F2503 and F2507-F2512), Nagaoka in Niigata Prefecture (37°24'N, 138°55'E, IABHU F2374-F2384), Hitachi-Oomiya in Ibaraki Prefecture (36°36'N, 140°16'E, IABHU F2385-F2407), Motosu in Gifu Prefecture (35°28'N 136°40'E, IABHU F2408-F2423), Kinomoto in Shiga Prefecture (35°31'N 136°14'E, IABHU F2424-F2434), Higashi-Hiroshima in Hiroshima Prefecture (34°27'N 132°46'E IABHU F2435-F2449). These six populations belong to the following geographic races of *R. rugosa* (Miura 2007), and they are ZW (Nagaoka and Akadama), East-Japan (Kanto) (Hitachi-Oomiya), XY (Motosu), Neo-ZW (Kinomoto), West-Japan (Higashi-Hiroshima)