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Speciation of two sympatric coastal fish species, *Girella punctata* and *Girella leonina* (Perciformes, Kyphosidae)

Shiro Itoi, Takashi Saito, Sayaka Washio, Mai Shimojo, Noriyuki Takai, Kiyoshi Yoshihara, Haruo Sugita*

Department of Marine Science and Resources, Nihon University, Fujisawa, Kanagawa 252-8510, Japan

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Abstract

Girella punctata and *Girella leonina* are sympatric sister species showing extensive distributional overlap in shallow rocky reefs in the Pacific Ocean south of the Japanese Islands. Differences between the two species in external morphological characters, such as number of pored lateral line scales, colour of opercular flap and shape of caudal fin, are congruent with genetic divergence. Nucleotide identity between the two species in the 3.3 kbp region of partial mitochondrial DNA containing the D-loop region, in 12S and 16S ribosomal RNA (rRNA) and transfer RNA genes is 95%. To estimate divergence time, Bayesian analysis was conducted using a dataset comprising concatenated nucleotide sequences from the two rRNA genes of three girellid and nine other fish species. Using the Elopomorpha – Clupeocephala split (265 million years ago (mya)) as a calibration point, divergence between *G. punctata* and *G. leonina* is estimated as having occurred 6.0 ± 1.4 mya. Speciation is suggested to have been caused by geographical isolation associated with formation of the Japanese Islands, which resulted in disjunction of *Girella* habitat.

Keywords: *Girella mezina*; Biogeography; Molecular clock; mtDNA; 12S rRNA; 16S rRNA

Introduction

In East Asia, the genus *Girella* is represented by three species, *Girella punctata* Gray, 1835, *Girella leonina* (Richardson, 1846) and *Girella mezina* Jordan and Starks, 1907; the known distribution area extends from Hong Kong to Japan. Despite slight differences in habitat preference, the three species are closely related (Yagishita and Nakabo 2000, 2003). Kanda and Yamaoka (1995) suggested that *G. mezina* is herbivorous, *G. leonina* omnivorous, and *G. punctata* intermediate. *G. punctata* and *G. leonina*, which are regarded as sister species, occur sympatrically in an area of overlap between their distributional ranges: the shallow rocky reefs in the Pacific Ocean south of the Japanese Islands (Fig. 1) (Kanda and Yamaoka 1995; Yagishita and Nakabo 2000, 2003). There has been some taxonomic confusion about *Girella* in East Asia: specimens were classified variously as *Crenidens melanichthys* Richardson, 1846 or as *Girella melanichthys*; this name was placed in varying junior synonymy with *G. punctata* or *Crenidens leoninus* (the original combination for *G. leonina*; Richardson 1846; Yagishita and Nakabo 2000), and *G. melanichthys* was incorrectly used as the valid name for *G. leonina* (Yagishita and Nakabo 2000). Yagishita and Nakabo (2000) recently revised the classification of *Girella* in East Asia. However,

*Corresponding author. Tel./fax: +81 466 843679.
E-mail address: sugita@brs.nihon-u.ac.jp (H. Sugita).

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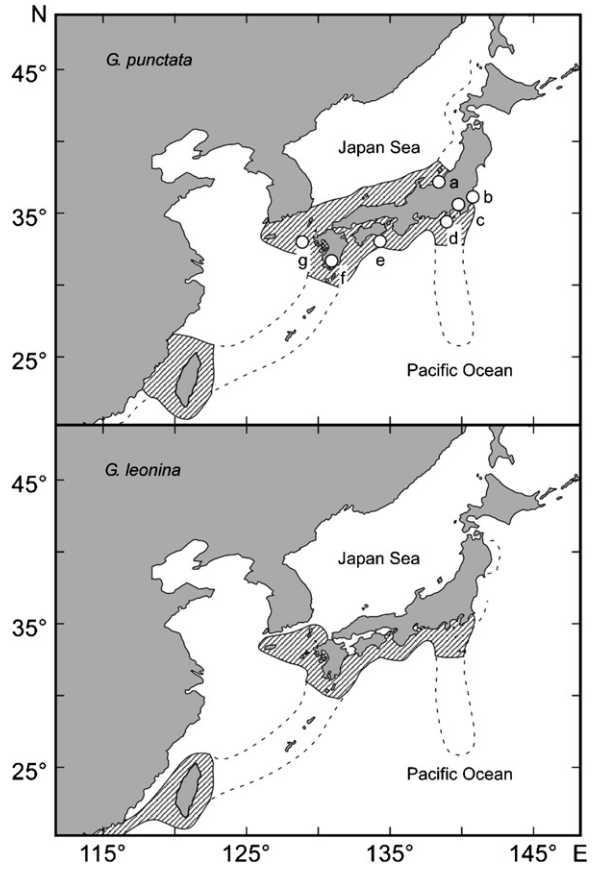

Fig. 1. Geographic distribution and sampling localities of *G. punctata* and *G. leonina*; modified from Yagishita and Nakabo (2001). Principal distribution areas enclosed by solid lines and obliquely shaded, additional areas enclosed by broken lines. Open circles on map for *G. punctata* represent localities of *Girella* samples: a = Joetsu; b = Kashima; c = Kawasaki; d = Shimoda; e = Muroto; f = Izumi; g = Goto.

the genetic relationships and behaviour of *G. punctata* and *G. leonina* remain interesting, particularly within a phylogenetic context.

In the present study, we use a combination of external characters and mitochondrial DNA (mtDNA) sequences that included part of D-loop region, 12S and 16S ribosomal RNA (rRNA) genes, and transfer RNA (tRNA) genes, in order to differentiate between the two species. In addition, we estimate the time of divergence between *G. punctata* and *G. leonina*, and discuss their speciation in lights of their respective environments, with a view to understanding organismal diversity.

Material and methods

Fish specimens

A total of 113 specimens belonging to the genus *Girella* were collected at Joetsu, Niigata ($n = 28$, 168 – 478 g); Kashima, Ibaraki ($n = 5$, 77 – 291 g); Kawasaki, Kanagawa ($n = 5$, 58 – 139 g); Shimoda, Shizuoka ($n = 21$, 24 – 168 g); Muroto, Kochi ($n = 29$, 550 – 1100 g); Izumi, Kagoshima ($n = 10$, 269 – 448 g); and Goto, Nagasaki ($n = 11$, 388 – 900 g) in Japan (Fig. 1). These specimens were stored at below −20 °C until use. Four *G. mezina* specimens (26 – 52 g) were collected at Shimoda, Shizuoka (Fig. 1).

Morphological analysis

With the exception of four *G. mezina* specimens, the morphological character states of 109 specimens were evaluated. Characters included numbers of pored lateral line scales, of spines and soft rays in the dorsal fin and soft rays in the anal fin, depth of emargination of the caudal fin, and colour of the opercular flap (Fig. 2A), based on the report by Yagishita and Nakabo (2000).

DNA extraction and PCR amplification

A small portion of skeletal muscle, liver or caudal fin was excised from each specimen. Total genomic DNA was extracted from skeletal muscle, liver or caudal fin of all 113 specimens, using the method of Sezaki et al. (1999). The mtDNA fragment including part of D-loop region, $tRNA^{Phe}$, 12S rRNA, $tRNA^{Val}$, 16S rRNA and

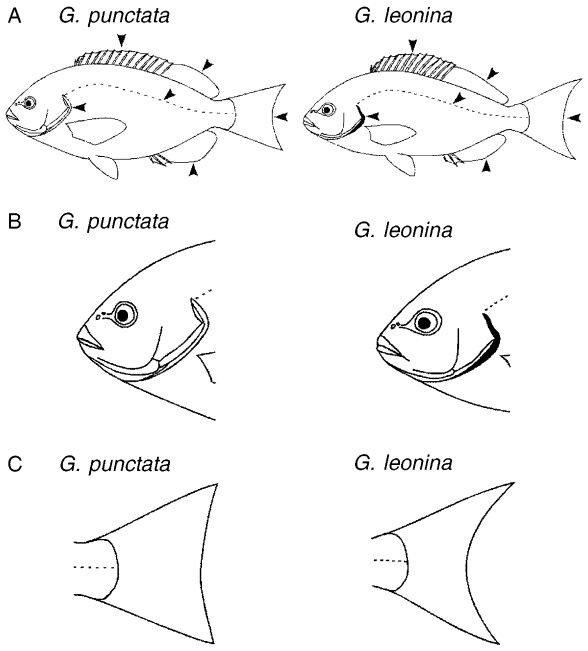

Fig. 2. Differences in external characters between *G. punctata* and *G. leonina:* (A) body overviews; arrowheads indicate locations of characters evaluated in this study (see text); (B) pigmentation of opercular flap: blackish in *G. leonina*, "not black" in *G. punctata* (C) shape of caudal fin: shallowly emarginate in *G. punctata*, deeply emarginate in *G. leonina*. For additional characters see Tables 1, 2.

part of $tRNA^{Leu}$ genes were amplified by PCR. Primers fDloop_F (5′-TTCCTGGCATTTGGTTCCTACTT-CAG-3′) and ftRLeu_R (5′-CTGTTBRAAGGGCT-TAGGBCTTTTGC-3′) were designed by referring to the corresponding regions of *Pagrus major* (GenBank accession number NC_003196), *Takifugu rubripes* (AJ421455), and *Tranchurus tranchurus* (AB108498). PCR amplification was performed using a reaction mixture containing genomic DNA as a template, 2 μl of 10 × *Ex Taq* DNA polymerase buffer, 0.8 μl of 10 μM primers, 2 μl of 2 mM dNTP, and one unit of *Takara Ex Taq* DNA polymerase (Takara, Otsu, Japan), brought to a total volume of 20 μl with sterile water. The PCR profile consisted of initial denaturation at 95 °C for 1 min, followed by 40 cycles of denaturation at 95 °C for 10 s, annealing at 55 °C for 10 s, and extension at 72 °C for 90 s, with a final extension step at 72 °C for 2 min. PCR products were cloned into the TA site of pGEM-T Easy vector (Promega, Madison, WI, USA) according to Marchuk et al. (1991), using *Escherichia coli* strain DH5α as a host bacterium.

Sequencing of PCR products

Sequencing of PCR products was performed for both strands with an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA), using a BigDye Terminator v3.1 Cycle Sequencing Ready Reaction Kit (Applied Biosystems). Alignment of partial mtDNA sequences for *G. punctata*, *G. leonina* and *G. mezina* obtained in this study with several elopocephalan taxa from the DDBJ/EMBL/GenBank databases was carried out using the program CLUSTAL W (Thompson et al. 1994, 1997).

Phylogenetic analysis

The nucleotide sequences of the 16S rRNA gene for various elopocephalan taxa were collected and aligned with those of the girellid species examined using CLUSTAL W (Thompson et al. 1994, 1997). Maximum-parsimony (MP) and neighbor-joining (NJ) methods were used to construct phylogenetic trees using PAUP* 4.0b 10 (Swofford 2003) and heuristic searches with TBR branch swapping. Additional trees were constructed under the Bayesian approach, using the MrBAYES ver 3.1.1 program (Huelsenbeck and Ronquist 2001) with a best-fit model (GTR + G) and parameters optimized by the program MrModeltest ver. 2.2 (see http://www.ebc.uu.se/systzoo/staff/nylander.html). A set of 12S and 16S rRNA gene sequences from *Anguilla japonica* was used as an outgroup.

Bayesian analysis of divergence time (Thorne and Kishino 2002) was conducted using the multidistribution package (see http://statgen.ncsu.edu/thorne/multidivtime.html) with a set of 12S and 16S rRNA genes. Branch lengths were estimated using the test-branches_dna program in conjunction with tree topology. As recommended by the authors of the multidistribution package, we used the baseml program in the PAML ver. 3.14b package (Yang 1997) to optimize parameters for the F84 (Felsenstein 1984) model and gamma distribution.

Divergence time was estimated using the program multidivtime. Markov chain Monte Carlo (MCMC) approximations were obtained with a burn-in period of 100,000 cycles. Subsequently, samples of the Markov chain were taken every 100 cycles until a total of 10,000 samples were obtained. To diagnose the possibility of failure arising from an inability of the Markov chains to converge and acquire a stationary distribution, we performed at least three replicate MCMC runs with different initial starting points for each analysis. Application of the multidivtime program requires a mean value for the prior distribution for the time separating the ingroup root from the present (rttm). As a reference point for dating, the divergence time of the Elopomorpha – Clupeocephala split (265 million years ago (mya)) was used for the age of the root node following previous analyses based on fossil records and molecular data (Inoue et al. 2005). Data used for the analysis were 12S and 16S rRNA genes in the mitochondrial DNA of *A. japonica* (NC_002707), *Arctoscopus japonicus* (NC_002812), *Coregonus lavaretus* (AB034824), *Cyprinus carpio* (X61010), *Pagrus major* (NC_003196), *Polymixia japonica* (AB034826), *Pterocaesio tile* (NC_004408), *Salmo salar* (NC_001960), and *Sardinops melanostictus* (NC_002616).

Results

Differences in morphological characters

Analysis of morphological characters using 109 specimens belonging to the genus *Girella* yielded the following results. The numbers of pored lateral line scales divided the specimens into two discrete groups: one ranging between 50 and 56, which corresponds to *G. punctata*; the other group, *G. leonina*, with 60 – 65 (Table 1). Pigmentation of the opercular flap was blackish in specimens corresponding to *G. leonina*, whereas in *G. punctata* it was not black (Fig. 2B). Furthermore, the shape of the caudal fin in *G. punctata* was shallowly emarginate, whereas in *G. leonina* it was deeply emarginate with acute upper and lower lobes (Fig. 2C). No difference between the two species was observed in the other morphological characters examined. The numbers of spines and soft rays in the dorsal fin ranged from 14 to 15 and from 12 to 16, respectively,

Table 1. Number and distribution of pored lateral line scales in *Girella punctata* and *G. leonina*; numbers in parentheses after Yagishita and Nakabo (2000)

	Pored lateral line scales																
	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	Total
G. punctata	11 (1)	17 (2)	29 (13)	17 (31)	16 (11)	4 (12)	1 (5)										95 (75)
G. leonina								(6)	(6)	(11)	1 (27)	4 (25)	2 (52)	3 (46)	3 (12)	1 (6)	14 (191)

Table 2. Dorsal and anal fin ray counts in *G. punctata* and *G. leonina*; numbers in parentheses after Yagishita and Nakabo (2000)

	Dorsal fin									Anal fin				
	Spines			Soft rays						Soft rays				
	14	15	16	8	12	13	14	15	16	11	12	13	14	Total
G. punctata	20 (8)	75 (69)		(1)	3 (7)	50 (53)	38 (15)	4 (1)		2 (3)	79 (67)	14 (6)	(1)	95 (77)
G. leonina	2 (48)	12 (152)	(1)		1 (1)	(16)	5 (99)	8 (82)	1 (3)		1 (24)	12 (175)	1 (2)	14 (201)

and those of the soft rays in the anal fin ranged between 11 and 14 (Table 2). The number of tooth rows was not measured in this study, as in *G. punctata* they develop gradually with age, increasing from a single row in juveniles to two or three rows in adults (Yagishita and Nakabo 2000).

The resulting classification according to external characters corroborates that derived for *G. punctata* and *G. leonina* in a previous report on the revision of the genus *Girella*, including *G. mezina*, *G. punctata* and *G. leonina*, by Yagishita and Nakabo (2000). These results strongly suggest that the 109 specimens examined in the present study comprised 95 specimens of *G. punctata* and 14 specimens of *G. leonina*. No *G. leonina* specimens were detected in the samples from Kashima, Kawasaki and Muroto, although they have been reported from these areas (Yagishita and Nakabo 2000).

Sequence analysis

Sequence analysis was performed for the partial mtDNA sequence amplified with the primers fDloop_F and ftRLeu_R. Nucleotide sequences of the amplified DNA fragments were approximately 3.3 kbp in size and included the D-loop region, as well as the $tRNA^{Phe}$, 12S rRNA, $tRNA^{Val}$, 16S rRNA and $tRNA^{Leu}$ genes from 14 specimens of *G. punctata*, five specimens of *G. leonina*, and four specimens of *G. mezina*. Given that several haplotypes were obtained for both partial mtDNA sequences of *G. punctata and G. leonina* from various areas (data not shown), the majority consensus sequences of 12S and 16S rRNA genes were used for the phylogenetic analysis of *G. punctata*, *G. leonina* and *G. mezina*. The partial sequences for the D-loop region revealed 86% identity between *G. punctata* and *G. leonina*, whereas those of the tRNAs and rRNAs were highly conserved, with identities of 96 – 100%. In addition, comparison of the partial mtDNA sequences from *G. punctata* and *G. leonina* revealed that it may be possible to distinguish between these two *Girella* taxa using RFLP haplotypes of the D-loop region generated with *Dde*I and *Xba*I, and for the 16S rRNA gene with *Hin*fI. These partial mtDNA sequences that included part of the D-loop region, together with the $tRNA^{Phe}$, 12S rRNA, $tRNA^{Val}$, 16S rRNA and $tRNA^{Leu}$ genes from *G. punctata*, *G. leonina* and *G. mezina* have been submitted to the DDBJ/EMBL/GenBank databases under the accession numbers AB208648, AB208649 and AB214535, respectively. Additional haplotypes of partial mtDNA sequences from *G. punctata* and *G. leonina* have been registered under the accession numbers AB233479 – AB233491 and AB233475 – AB233478, respectively, whereas those from *G. mezina* have been registered under the accession numbers AB236128 – AB236130.

Phylogenetic analysis and estimation of divergence time

A combined analysis was performed using a non-clupeocephalan species of the Elopocephala, *A. japonica*, as an outgroup. The phylogenetic trees were constructed from the topology retrieved by Bayesian analysis with Bayesian posterior probabilities support

(Fig. 3), and by maximum-parsimony and neighbor-joining analysis with bootstrap probabilities support (data not shown). These phylogenetic trees support the monophyly of *Girella* (100% support; node I in Fig. 3 and Table 3), with the tree showing *G. punctata* and *G. leonina* as sister species (64 – 95% support; node J in Fig. 3 and Table 3).

The Bayesian approach using the Elopomorpha – Clupeocephala split (265 mya) as a calibration point estimated the divergence between *G. punctata* and *G. leonina* at 6.0 ± 1.4 mya with a standard deviation interval of 4.6 – 7.4 my (node J in Table 3 and Fig. 4). Divergence between *G. punctata* + *G. leonina* and *G. mezina* was estimated at 7.3 ± 1.6 mya (node I in Table 3 and Fig. 4). Divergence times of other splits (nodes A – F in Table 3 and Fig. 4) were consistent with those estimated by Inoue et al. (2005).

Discussion

In the present study, *G. punctata* and *G. leonina* have been found to differ in the number of pored lateral line scales, colour of the opercular flap, and shape of the caudal fin (see Table 1). While these findings corroborate those of Yagishita and Nakabo (2000), no differences were observed in the other morphological characters examined, including the numbers of spines and soft rays in the dorsal and anal fins (see Table 2). The phylogeny derived using mtDNA was congruent with the grouping obtained using the morphological characters of *G. punctata* and *G. leonina*.

Although the absolute divergence time inferred using molecular data cannot be calculated due to the absence of a fossil record in *Girella*, it can be evaluated by the molecular clock approach with calibration against the fossil record for other fish species. Recently, Inoue et al. (2005) estimated divergence times for various fish species to infer the age of the two coelacanths, *Latimeria*

Table 3. Support values and estimated divergence times for nodes in Fig. 5

Node	Tree support value (%)			Divergence time (million years ago)
	MP	NJ	BA	
A	—	—	—	226.2 ± 1.1
B	59	54	100	203.7 ± 10.5
C	88	100	100	195.4 ± 9.2
D	100	100	100	49.6 ± 12.5
E	99	100	100	137.6 ± 12.5
F	—	—	100	89.6 ± 10.8
G	98	100	100	80.0 ± 10.4
H	52	85	98	72.3 ± 9.9
I	100	100	100	7.3 ± 1.6
J	64	66	95	6.0 ± 1.4

Tree support values are bootstrap scores for maximum parsimony (MP) and neighbor joining (NJ) analyses, and Bayesian posterior probabilities for Bayesian analysis (BA). Only values exceeding 50% are shown. Divergence time estimated using the Elopomorpha-Clupeocephala split (265 mya; Inoue et al. 2005) as calibration point; shown as mean ± standard deviation.

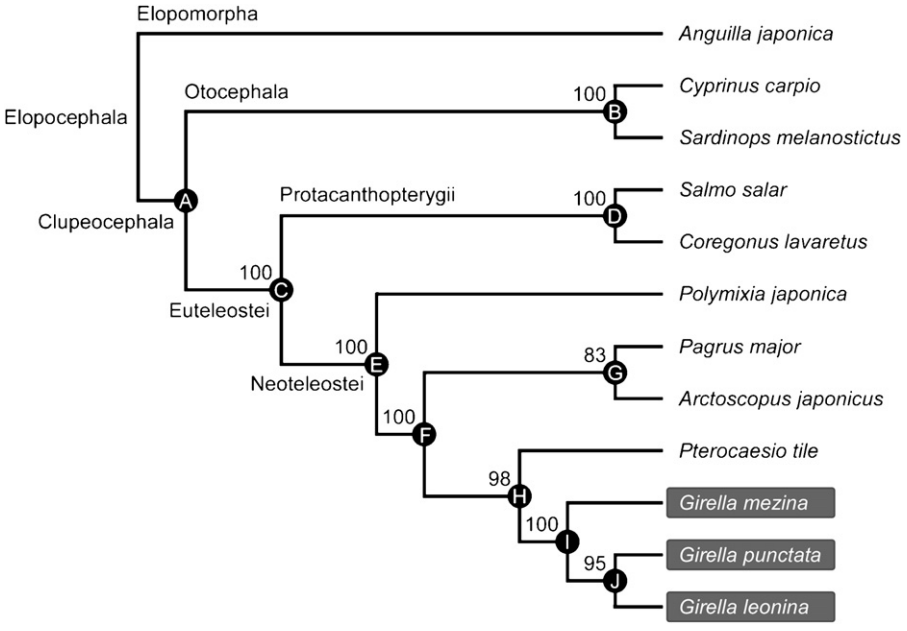

Fig. 3. Phylogenetic tree resulting from Bayesian analysis of complete 16S rRNA gene sequences; *A. japonica* used as outgroup. *Girella* species indicated by white letters in black boxes. Numbers at nodes are Bayesian posterior probabilities (%); only values > 50% are shown.

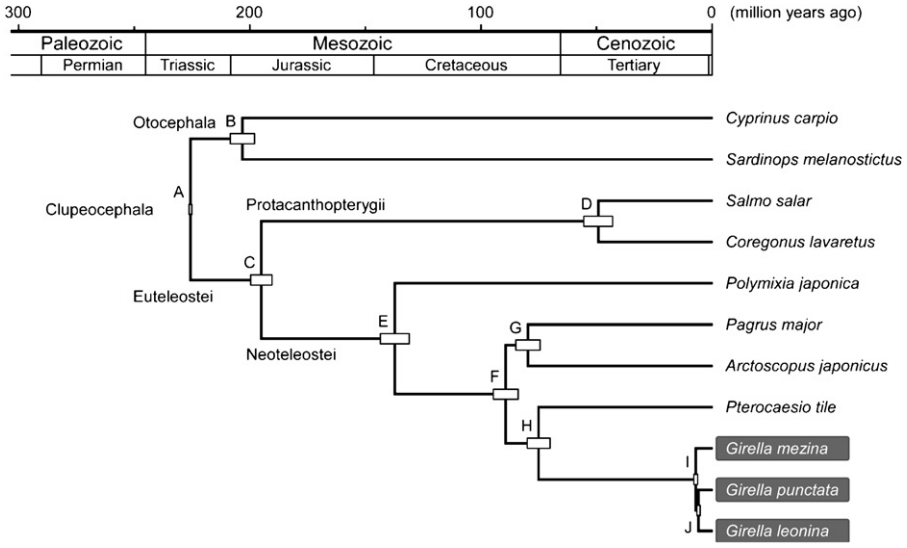

Fig. 4. Posterior distribution of divergence times among Clupeocephala, based on Bayesian approach described under Material and methods. Tree nodes positioned according to estimated divergence times derived from Bayesian analysis (Table 3); divergence times estimated using the Elopomorpha – Clupeocephala split (265 mya; Inoue et al. 2005) as calibration point. White horizontal rectangles represent estimated standard deviation intervals of divergence times; *Girella* species indicated by white letters in black boxes.

menadoensis and *Latimeria chalumnae*. Using their calculated times for calibration purposes, we estimated divergence between *G. punctata* and *G. leonina* at approximately 6.0 mya, and that between *G. mezina* and *G. punctata* + *G. leonina* at approximately 7.3 mya (see Table 3 and Fig. 4). Divergence times of other nodes were similar to those reported by Inoue et al. (2005) using whole mtDNA sequences. Our results show that 12S and 16S rRNA gene sequences are as useful as whole mtDNA sequences for accurate estimation of divergence time. However, we used only a single and fairly distant calibration point (the Elopomorpha – Clupeocephala split); more accurate divergence time estimation would require the use of multiple and close calibration points.

The distribution pattern of *Girella* species in East Asia has prompted important suggestions about their dispersal and biogeography. Okuno (1971) suggested that the original areas previously inhabited by *G. punctata* and *G. leonina* were in the Japan Sea and the Pacific Ocean, respectively. Yagishita and Nakabo (2000, 2002) also suggested that *G. punctata* and *G. leonina* might have diverged from a common ancestral species. Some of its members may have become isolated in the Japan Sea while those remaining in the Pacific Ocean might have differentiated into *G. punctata* and *G. leonina*. Assuming that the common ancestor of the three *Girella* species was distributed in paleo-East/South Asia, alternative models have been proposed to explain the evolutionary history of the *Girella* species in East Asia. One scenario involves changes of vegetation in the habitat region. The period of 6 – 7 mya was characterized by important climatic variations leading to drastic vegetation changes all over the world according to isotope studies (Cerling et al. 1997). According to the second model, geographic isolation of the habitat region of *Girella* in paleo-East/South Asia had been caused by tectonic processes and/or by the ice ages, leading to changes in sea surface level. Considering these alternatives, we believe that the estimated times of divergence between the three *Girella* species make the hypothesis of geographic isolation caused by tectonic processes the most likely scenario. Thus we describe the latter as follows.

As shown in Fig. 5, the divergence time between *G. mezina* and *G. punctata* + *G. leonina* corresponds to the period when the East/South China Sea was isolated from the Pacific Ocean by the subduction of the Philippine Sea plate leading to uplift of the Ryukyu Arc (Taira 2001). Following that event, the divergence time between *G. punctata* and *G. leonina* corresponds to the period when the Japanese Islands, during their geographical formation, were connected to the Korean Peninsula for extended periods of time (Taira et al. 1989; Taira 2001). Moreover, the analysis of the hydrocarbon potential showed that approximately 6.0 mya the paleo-Japan Sea was isolated from the Pacific Ocean, except in the north, and that this period was followed by a period of cooling with glaciation of the Japan Sea approximately 4.0 mya (Tada 1994, 1995). Therefore, we propose that the tectonic activity surrounding the paleo-Japanese Islands, followed by climate changes in the Japan Sea, was responsible for the divergence of *G. punctata* and *G. leonina* (see Fig. 5).

In summary, we identified *G. punctata* and *G. leonina* based on differences in their external characters and

nucleotide sequences of partial mtDNA. We estimated divergence between *G. punctata* and *G. leonina* to have taken place approximately 6.0 mya; the divergence event may have been caused by geographical isolation associated with the formation of the Japanese Islands. Further investigations are required in order to reveal the physiological and ecological differences between *G. punctata* and *G. leonina*. At present, the differences in species distribution appear to be due to the cold temperature of their habitat in winter.

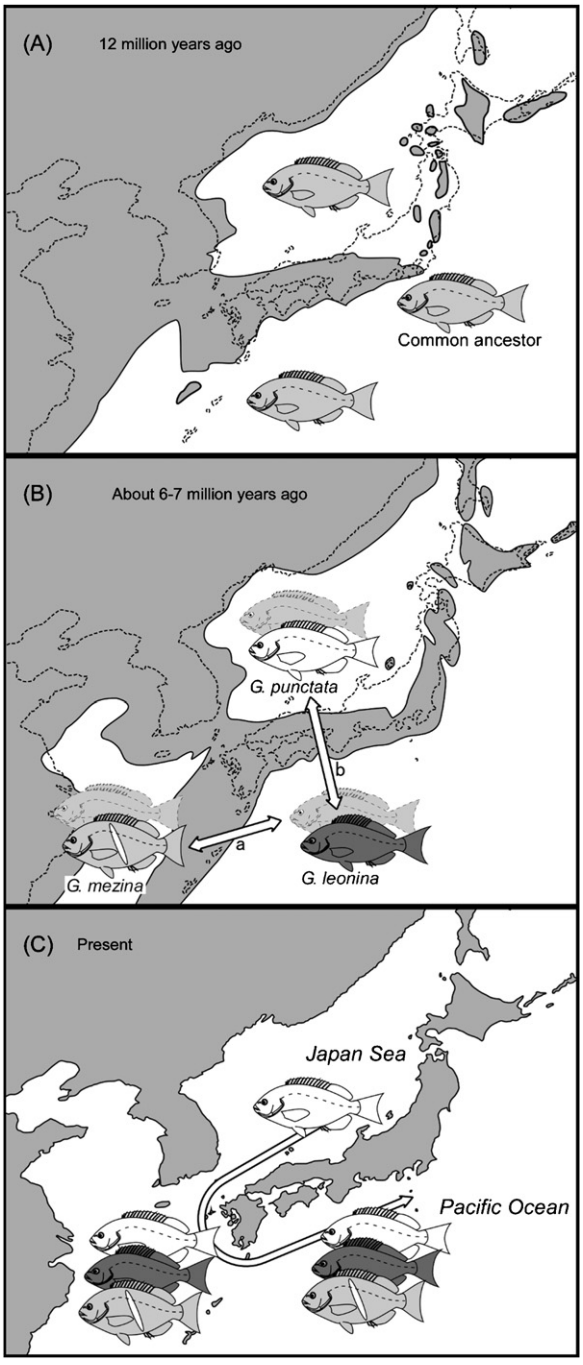

Fig. 5. Hypothesized process of speciation between *G. punctata* and *G. leonina*. Maps based on present geography (after Taira 2001); dotted lines indicate present coastlines: (A) the common ancestor of *G. punctata* and *G. leonina* was widely distributed in the paleo-Japan Sea which was connected to the Pacific Ocean approximately 12 mya; (B) about 7 mya, uplift of the Ryukyu Arc induced by subduction of the Philippine Sea plate captured a population of the common ancestor in the paleo-East/South China Sea (double-headed arrow a); about 6 mya, a landbridge between the Japanese Islands and the Korean Peninsula captured a population of the common ancestor in the Japan Sea (double-headed arrow b). (C) When geographical features of the present Japanese Islands formed, *G. punctata* spread along the coast of the Pacific Ocean (white arrow). *G. leonina* and *G. mezina* could not disperse along the coast of the Japan Sea, resulting in sympatric occurrence of the three species in the Pacific Ocean only.

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References

Cerling, T.E., Harris, J.M., MacFadden, B.J., Leakeys, M.G., Quade, J., Eisenmann, V., Ehleringer, J.R., 1997. Global vegetation change through the Miocene/Pliocene boundary. Nature 389, 153–157.

Felsenstein, J., 1984. Distance methods for inferring phylogenies: a justification. Evolution 38, 16–24.

Huelsenbeck, J.P., Ronquist, F., 2001. MrBAYES: Bayesian inference of phylogeny. Bioinformatics 17, 754–755.

Inoue, J.G., Miya, M., Venkatesh, B., Nishida, M., 2005. The mitochondrial genome of Indonesian coelacanth *Latimeria menadoensis* (Sarcopterygii: Coelacanthiformes) and divergence time estimation between the two coelacanths. Gene 349, 227–235.

Kanda, M., Yamaoka, K., 1995. Tooth and gut morphology in relation to feeding in three girellid species (Perciformes: Girellidae) from southern Japan. Neth. J. Zool. 45, 495–512.

Marchuk, D., Drumm, M., Saulono, A., Collins, F.S., 1991. Construction of T-vectors, a rapid and general system for direct cloning of unmodified PCR products. Nucleic Acids Res. 19, 1154.

Okuno, R., 1971. In: [Ecology of rock fish]. Sogensha, Osaka, pp. 151–176 [in Japanese].

Richardson, J., 1846. Report on the ichthyology of the seas of China and Japan. Rep. Brit. Assoc. Adv. Sci. 1845, 187–320.

Sezaki, K., Begum, R.A., Wongrat, P., Srivastava, M.P., SriKantha, S., Kikuchi, K., Ishihara, H., Tanaka, S., Taniuchi, T., Watabe, S., 1999. Molecular phylogeny of Asian freshwater and marine stingrays based on the DNA

nucleotide and deduced amino acid sequences of the cytochrome *b* gene. Fish. Sci. 65, 563–570.

Swofford, D.L., 2003. PAUP*: Phylogenetic Analysis Using Parsimony (* and other Methods), Version 4.0b 10. Sinauer Associates, Sunderland, MA.

Tada, R., 1994. Paleooceanographic evolution of the Japan Sea. Palaeogeogr. Palaeoclimatol. Palaeoecol. 108, 487–508.

Tada, R., 1995. Paleooceanographic evolution of the Japan Sea and its implication to the hydrocarbon potential evaluation. Chishitsu News 495, 59–71.

Taira, A., 2001. Tectonic evolution of the Japanese Island arc system. Annu. Rev. Earth Planet. Sci. 29, 109–134.

Taira, A., Tokuyama, H., Soh, W., 1989. Accretion tectonics and evolution of Japan. In: Ben-Avraham, Z. (Ed.), The Evolution of Pacific Ocean Margins. Oxford University Press, New York, pp. 100–123.

Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22, 4673–4680.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The Clustal_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 24, 4876–4882.

Thorne, J.L., Kishino, H., 2002. Divergence time estimation and rate evolution with multilocus data sets. System Biol. 51, 689–702.

Yagishita, N., Nakabo, T., 2000. Revision of the genus *Girella* (Girellidae) from East Asia. Ichthyol. Res. 47, 119–135.

Yagishita, N., Nakabo, T., 2003. Evolutionary trend in feeding habits of *Girella* (Perciformes: Girellidae). Ichthyol. Res. 50, 358–366.

Yang, Z., 1997. PAML: a program package for phylogenetic analysis by maximum likelihood. CABIOS 15, 555–556.