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Evolution of Pacific Ocean and the Sea of Japan populations of the gobiid species, *Pterogobius elapoides* and *Pterogobius zonoleucus*, based on molecular and morphological analyses

Akihito [a], Akishinonomiya Fumihito [b,c], Yuji Ikeda [d], Masahiro Aizawa [d], Takashi Makino [e,1], Yumi Umehara [e], Yoshiaki Kai [f], Yuriko Nishimoto [b,g], Masami Hasegawa [b,e,h], Tetsuji Nakabo [i], Takashi Gojobori [b,e,*]

[a] *The Imperial Residence, 1-1 Chiyoda, Chiyoda-ku, Tokyo 100-0001, Japan*
[b] *The Graduate University for Advanced Studies (SOKENDAI), Shonan Village, Hayama, Kanagawa 240-0193, Japan*
[c] *The University Museum, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan*
[d] *Imperial Household Agency, 1-1 Chiyoda, Chiyoda-ku, Tokyo 100-8111, Japan*
[e] *Center for Information Biology and DDBJ, National Institute of Genetics, Mishima, Shizuoka 411-8540, Japan*
[f] *Maizuru Fisheries Research Station, Field Science Education and Research Center, Kyoto University, Maizuru 625-0086, Japan*
[g] *The Research Institute of Evolutionary Biology, 2-4-28 Kamiyoga, Setagaya-ku, Tokyo, 158-0098, Japan*
[h] *The Institute of Statistical Mathematics, 4-6-7 Minami-Azabu, Minato-ku, Tokyo, 106-8569, Japan*
[i] *The Kyoto University Museum, Kyoto University, Kyoto, 606-8501, Japan*

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ABSTRACT

Pterogobius elapoides and *Pterogobius zonoleucus* are common free-swimming gobies found in rocky and weedy shores along the temperate coast of Japan. We collected individuals of both species from 23 locations around the coast of Japan and compared the mitochondrial nucleotide sequences of two gene regions, CytB and ND2. Phylogenetic trees constructed using the neighbor-joining, maximum parsimony, and maximum likelihood methods consistently indicated that all 125 samples of the two species, which are collected from a variety of locations in Japan, can be clearly divided into the following four clades: "Pacific *P. elapoides*" (Pa-*ela*), "Sea of Japan *P. elapoides*" (SJ-*ela*), "Pacific *P. zonoleucus*" (Pa-*zon*), and "Sea of Japan *P. zonoleucus*" (SJ-*zon*). These four monophyletic clades were supported with very high bootstrap values. Although Pa-*ela* and SJ-*ela* composed a monophyletic clade, it is noteworthy that the two clades of *P. elapoides* also formed a monophyletic group together with SJ-*zon* with a bootstrap value of 95% and 97% by the maximum likelihood and neighbor-joining methods, respectively. We observed several morphological differences between Pa-*ela* and SJ-*ela*, including; 1) six dark bands on the body in the former versus seven dark bands in the latter and 2) more pectoral-fin rays numbering 21–24 (mode 22) in the latter compared to the former (19–22, mode 21). Furthermore, the scatter plots of scores on principal components 1 and 2 based on the morphometric characters roughly separated the populations from each other. Moreover, we documented the following morphological differences between Pa-*zon* and SJ-*zon* for the first time; 1) six light bands on the body in the former versus five light bands in the latter and 2) the light bands from both eyes forming a complete U-shaped marking on the occipital region occurred in 55% of the specimens in the former versus 16% in the latter. However, no significant differences were found in the morphometric characters between the two populations of *P. zonoleucus*. The estimated divergence time of the two *P. zonoleucus* populations was 15.06±2.72 (mean±1 S.E.) times earlier than that of the two *P. elapoides* populations. However, the morphological differences between the two populations of the former were much smaller than those of the latter. An explanation for this obvious discrepancy between morphological and molecular features is proposed from an evolutionary point of view.

Abbreviations: AIC, Akaike Information Criterion; ANCOVA, Analysis of covariance; BP, Bootstrap Probability; CytB, cytochrome *b*; LCA, Last Common Ancestor; mtDNA, mitochondrial DNA; MEGA, Molecular Evolutionary Genetics Analysis; ML, Maximum Likelihood; MP, Maximum Parsimony; ND2, NADH dehydrogenase 2; NJ, neighbor-joining; Pa, Pacific Ocean; Pa-*ela*, Pacific *P. elapoides*; PAML, Program package for phylogenetic Analysis by Maximum Likelihood; PAUP*, Phylogenetic Analysis Using Parsimony and other methods; Pa-*zon*, Pacific *P. zonoleucus*; PCA, Principal Component Analysis; SJ, Sea of Japan; SJ-*ela*, Sea of Japan *P. elapoides*; SJ-*zon*, Sea of Japan *P. zonoleucus*; SL, Standard Length; tRNA, transfer RNA.

* Corresponding author. Center for Information Biology and DDBJ, National Institute of Genetics, Mishima, Shizuoka 411-8540, Japan.
[1] The present address: Trinity College, Dublin 2, Ireland.

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1. Introduction

Four species of the goby genus *Pterogobius* are found in Japan and along the southern coast of Korea: *P. virgo* (Temminck and Schlegel, 1845), *P. elapoides* (Günther, 1871), *P. zacalles* (Jordan and Snyder, 1901), and *P. zonoleucus* (Jordan and Snyder, 1901). Among these four species, *P. elapoides* (Fig. 1) and *P. zonoleucus* (Fig. 2) resemble each other in having more similarly positioned bands on both the head and body than the other two congeneric species. In addition, these two species are almost the same in their geographical distribution and habitats in Japan and along the southern coast of Korea, although there is an ecological difference in adults between *P. elapoides* and *P. zonoleucus*: the former swims solitary near rocky bottoms (Fig. 3A), whereas the latter swims in mid-water in schools (Fig. 3B).

For both *P. elapoides* and *P. zonoleucus*, there have been unsolved problems about their evolutionary intra- and inter-species relationships. For example, *P. elapoides* was originally described by Günther (1871) based on a single specimen, probably from Japan, and his illustration shows seven narrow, dark bands on the body. Then, Jordan and Snyder (1901) described a specimen of *P. elapoides* as a new species called *P. daimio*, owing to it having just six wider, dark bands on its body. Snyder (1912) later recognized that *P. daimio* should have been described as a male of *P. elapoides*.

Almost twenty years later, Tanaka (1931) examined many specimens collected from various localities in Japan, and identified them as *P. elapoides* or *P. daimio* respectively by the presence or absence of a seventh dark band on the body. It was revealed that the specimens with the seventh band were distributed mainly along the Sea of Japan coast, whereas the specimens without the seventh band were distributed along the Pacific coast of central-to-southern Honshu. Specimens from the Seto Inland Sea mostly have an interrupted band or just a spot at the position of the seventh band. Moreover, specimens which showed intermediate color patterns were sometimes found along the Sea of Japan coast. Because of the presence of intermediate color patterns between *P. elapoides* and *P. daimio*, Tanaka regarded them as a local variation, and recognized the latter as a junior synonym of the former.

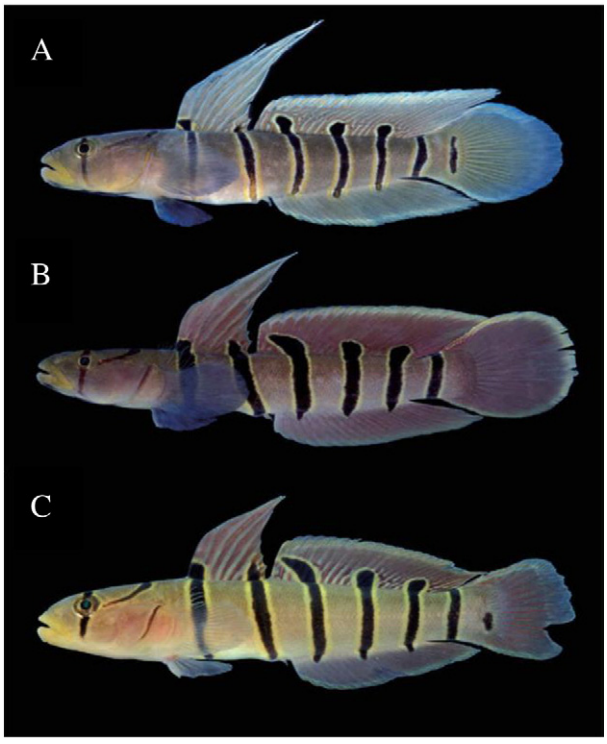

Fig. 1. *Pterogobius elapoides*: (A) The Sea of Japan, Ishikawa Prefecture (BLIH20020439); (B) The Pacific Ocean, Chiba Prefecture (BLIH20030467); (C) The Inland Sea, Ehime Prefecture (BLIH20070130).

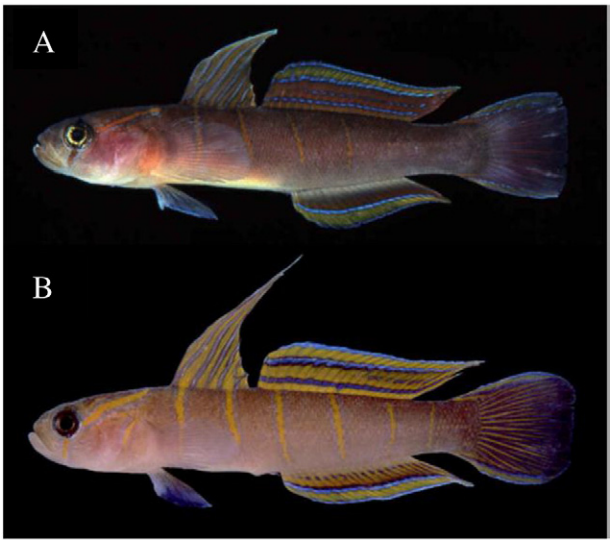

Fig. 2. *Pterogobius zonoleucus*: (A) The Sea of Japan, Ishikawa Prefecture (BLIH20050502); (B) The Pacific Ocean, Chiba Prefecture (BLIH20060883).

Akihito (1984) noted that there are two types of *P. elapoides* which have different geographic distributions: the first type, which has six bands, is found along the Pacific coast of central-to-southern Honshu; the second type, which has seven bands, is found along the coast of Kyushu, the Sea of Japan coast of Honshu and the Sanriku District; in addition, an intermediate type, which has an imperfect seventh band, is found in the Seto Inland Sea. Since then, no detailed morphological and geographical studies of this species have been published. Further, no geographical variation in *P. zonoleucus* has been described in the literature in terms of morphological characters. However, we found some morphological differences between the Sea of Japan and the Pacific Ocean populations.

As no molecular evolutionary studies of *P. elapoides* and *P. zonoleucus* have been published to date, we conducted a molecular phylogenetic analysis on these two species, in addition to morphological studies. From each of 23 sampling localities in the Sea of Japan, Pacific Ocean and the Seto Inland Sea, we collected 5 individuals and sequenced their mtDNA for two genes.

2. Materials and methods

2.1. Samples used (Table 1, Fig. 4)

For *P. elapoides*, 5 individuals were collected at each of 14 different locations in Japan divided equally between the coasts of the Pacific Ocean and the Sea of Japan. For *P. zonoleucus*, 5 individuals were also collected at each of 9 different locations in Japan: 4 locations were along the coast of the Pacific Ocean and 5 locations were along the Sea of Japan (Fig. 4).

For *P. zacalles* and *P. virgo*, which were used as the outgroup in phylogenetic tree construction, 5 individuals for each of the two species were collected from Aomori and Ishikawa Prefectures, respectively. Sampling of these populations was conducted at 2 single locations only. Thus, a total of 125 individuals were sampled from 25 locations along the Pacific Ocean and the Sea of Japan (Table 1).

Abbreviations used in specimen identification numbers are: BLIH — Biological Laboratory, Imperial Household; and NMCI — Noto Marine Center, Ishikawa.