Morphological differences among snipefishes (Macroramphosidae: Macroramphosus) from the Pacific and Atlantic oceans

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Abstract: A non-metric multi-dimensional scaling (nMDS) analysis based on 10 quantitative morphological characteristics showed significant morphological differences among snipefishes of the genus Macroramphosus from the northwestern Pacific including the East China Sea and the southern Pacific, northern Atlantic, and southern Atlantic oceans. Individuals of these four sea areas were significantly different from each other for at least one characteristic. Considering these results along with those of previous molecular analyses, Macroramphosus is suggested to have speciated or genetically diverged among sea areas.

Key words: Macroramphosus, snipefishes, morphology, Pacific, Atlantic, nMDS

Snipefishes of the genus Macroramphosus (Macroramphosidae: Gasterosteiformes: Syngnathoidei) are compressed and streamlined fish with a long second spine on a dorsal fin, distributed at ocean depths shallower than 600 m in tropical and subtropical areas around the world (Ehrich 1976; Bilecenoglu 2006; Nelson et al. 2016). Two morphotypes in Macroramphosus are recognized. The first is a plankton feeding morphotype (M. scolopax-type), which has a red-orange deep body and longer second dorsal fin spines with roughly serrated posterior margins. The second is a benthos feeding morphotype (M. gracilis-type), which has a dark or brownish slender body and shorter second dorsal fin spines with smooth posterior margins. These morphotypes are frequently distributed sympatrically in the Atlantic and northwestern Pacific oceans (Ehrich & John 1973; Matthiessen et al. 2003; Miyazaki et al. 2004). Although their feeding habitats are unknown, the two morphotypes have been reported from the Mediterranean Sea (Bilecenoglu 2006). The taxonomic status of these two morphotypes has been debated for a long time (Okada & Suzuki 1951; Ehrich & John 1973; Ehrich 1976; Miyazaki et al. 2004; Bilecenoglu 2006; Robalo et al. 2009). Clarke (1984) also reported that both plankton- and benthos-feeding individuals in southeast Australian waters showed the M. scolopax-type morphology, although other significant morphological differences were shown between them. In the eastern Pacific, only M. gracilis-type individuals were reported (Miller & Lea 1972).

The taxonomic status of Macroramphosus inhabiting other oceans has also been debated. While Mohr (1937) revised 17 nominal species in Macroramphosus into two cosmopolitan species, namely, M. scolopax and M. gracilis, Kuranaga & Sasaki (2000) compared development of the larvae of M. scolopax from Japan and the Mediterranean and concluded they were not conspecific.

In our previous study (Noguchi et al. 2015), based on morphological and molecular analyses we showed that Macroramphosus from Japanese waters should be classified into a single species in spite of large morphological differences between the M. scolopax-type and the M. gracilis-type. Further, comparing nucleotide sequences determined in this study with those of Robalo et al. (2009), we had reported that significant genetic deviation, probably at the specific level, exists between snipefishes in Japanese waters (the northwestern Pacific including the East China Sea) and those in the northeastern Atlantic (off the coast of Portugal). Although it is necessary to examine the possibility of speciation between sea areas to determine taxonomic status of Macroramphosus (Clarke 1984; Robalo et al. 2009), statistical examination of inter-oceanic differences in morphological characteristics has
not been reported. In this study, we compared the morphology of individuals of *Macroramphosus* from the northwestern Pacific including the East China Sea, the southern Pacific, the northern Atlantic, and the southern Atlantic seas.

We used 133 adult individuals of *Macroramphosus* that we collected in Japanese waters and measured in the previous study (standard length ranging between 7.10–16.68 cm) (Noguchi et al. 2015). In addition, we used formalin-fixed adult specimens, which had been deposited at the National Museum of Nature and Science, Tsukuba (NSMT-P). These were from the Pacific coast of Japan (N=5; standard length ranging between 10.33–15.80 cm), the Emperor Seamount Chain (N=2; standard length ranging between 11.83–16.30 cm), Southeast Australia (N=15; standard length ranging between 10.18–11.77 cm), New Zealand (N=1; standard length of 12.09 cm), and Morocco (N=5; standard length ranging between 13.02–15.87 cm). An additional three formalin-fixed specimens (standard length ranging between 10.93–13.13 cm) from the southern Atlantic that had been deposited at Hokkaido University (HUMZ) were also used (Table 1).

As in a previous study (Noguchi et al. 2015), snout length, eye diameter, postorbital head length, head length, postocular body length, length of the second dorsal fin spine, distance between the two dorsal fins, maximum body depth, length before the second dorsal fin spine, length after the second dorsal fin spine, and standard length of additional specimens were measured using a vernier caliper (0.05 mm accuracy). A non-metric multi-dimensional scaling (nMDS) analysis was performed using the Bray-Curtis similarity index (Clarke & Warwick 2001) based on values of the the first 10 characteristics divided by the standard length. Permutational multivariate analysis of variance (PERMANOVA) was used to examine the statistical significance of the differences (Anderson

### Table 1. List of specimens used in the present study.

| Sea area                  | Sampling site            | Position              | Depth (m) | Date          | N  | Specimen # |
|---------------------------|--------------------------|-----------------------|-----------|---------------|----|------------|
| Northwestern Pacific      | East China Sea           | 31°34.5′N, 127°30.8′E | 131       | 16 Sept 2010  | 22 | NSMT-P 49857 |
|                           |                          | 31°31.9′N, 127°32.1′E | 134       | 16 Sept 2010  | 2  | NSMT-P 72803 |
|                           |                          | 31°28.3′N, 127°32.1′E | 134       | 31 Aug 2010   | 7  | NSMT-P 101011 |
|                           |                          | 31°45.8′N, 127°44.6′E | 143       | 24 Apr 2012   | 10 | NSMT-P 72795 |
|                           |                          | 31°45.5′N, 127°45.8′E | 140       | 30 Oct 2011   | 8  | NSMT-P 72803 |
|                           |                          | 31°43.3′N, 127°50.0′E | 143       | 22 Aug 2011   | 19 | NSMT-P 75088 |
|                           |                          | 31°34.8′N, 127°50.8′E | 149       | 22 Apr 2011   | 20 | NSMT-P 43421 |
|                           |                          | 31°45.3′N, 127°57.3′E | 153       | 25 Apr 2005   | 22 | NSMT-P 49857 |
|                           |                          | 31°09.9′N, 128°11.5′E | 300       | 6 Nov 2004    | 2  | NSMT-P 99517 |
|                           |                          | 31°31.8′N, 128°23.5′E | 333       | 9 Nov 2005    | 3  | NSMT-P 99519 |
|                           |                          | 32°29.8′N, 129°09.6′E | 231       | 8 Mar 2010    | 1  | NSMT-P 99519 |
| off Tosa Bay              |                          | No data               | No data   | 30 Oct 2011–  | 15 | No data    |
|                           |                          |                       |           | 24 Apr 2012   |    |            |
| off Pacific coast of      |                          | 36°29.9′N, 140°57.1′E | 150       | 17 Nov 2010   | 1  | NSMT-P 79667 |
| NE Japan                 |                          |                       |           |               |    |            |
|                           |                          | 37°36.9′N, 141°35.8′E | 210       | 31 Oct 2011   | 1  | NSMT-P 78926 |
|                           |                          | No data               | No data   | 9 Sep 2007    | 1  | NSMT-P 78926 |
| off Pacific coast of      |                          | 34°45.5′N, 138°44.2′E | 113       | 30 Oct 1980   | 2  | NSMT-P 110047 |
| Kochi, Japan              |                          |                       |           |               |    |            |
| off Pacific coast of      |                          |                       |           |               |    |            |
| Shizuoka, Japan           |                          |                       |           |               |    |            |
| off Pacific coast of      |                          |                       |           |               |    |            |
| Chiba, Japan              |                          |                       |           |               |    |            |
| off Pacific coast of      |                          |                       |           |               |    |            |
| Ibaraki, Japan            |                          |                       |           |               |    |            |
| Emperor Seamount Chain    |                          |                       |           |               |    |            |
| off Taiwan                |                          |                       |           |               |    |            |
| Southern Pacific          |                          | 35°40′N, 171°03′E     | 528       | 7 Oct 2005    | 1  | NSMT-P 110047 |
|                           |                          | 31°02′N, 175°53′E     | 291       | 29 Sept 2005  | 1  | NSMT-P 110047 |
|                           |                          | No data               | No data   | 13 July 2005  | 1  | NSMT-P 110047 |
| Southern Pacific          | off Southeast Australia  | 34°47′S, 151°05′E     | 166       | 30 Dec 1975   | 15 | NSMT-P 110047 |
|                           | off New Zealand          | 32°42′S, 167°30′E     | 180       | 2 Mar 1983    | 1  | NSMT-P 43421 |
| Northern Atlantic         | off Morocco              | 30°21.7′N, 9°58.4′E   | 200       | 12 Dec 1993   | 5  | NSMT-P 49857 |
| Southern Atlantic         |                          | 11°35′S, 5°16′W       | 210       | 25 Jan 1982   | 3  | HUMZ 99517–99519 |

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\(^{a}\) Referred from Noguchi et al. (2015)

\(^{b}\) Formalin-fixed
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When the overall test was significant, Tukey’s HSD (honestly significant difference) test was used for pairwise comparisons. Analysis of variance (ANOVA) was performed to compare each of the 10 morphological characters among the four sea areas. All analyses were undertaken in R (R Development Core Team 2008), using the package vegan (Oksanen et al. 2008).

In the nMDS ordination space based on the 10 quantitative morphological characters (Fig. 1), significant differences were detected among Macroramphosus snipefishes from the northwestern Pacific including the East China Sea, the southern Pacific, the northern Atlantic, and the southern Atlantic (PERMANOVA, \( P<0.001 \)). Of the six pairs of sea areas, all but the northwestern Pacific and the southern Atlantic pair showed significant differences (PERMANOVA, \( P<0.05 \) for the southern Pacific and the southern Atlantic pair and \( P<0.001 \) for the other pairs). The interoceanic differences in the 10 quantitative morphological characters are shown in Fig. 2. Individuals of the four sea areas were significantly different from each other for at least one characteristic. Individuals of the southern Pacific (off Southeast Australia and New Zealand) showed deeper bodies with longer second dorsal fin spines than those of other sea areas, which corresponds to the report by Clarke (1984). In this study, only a limited number of Atlantic specimens were available and examination of additional specimens is necessary to confirm morphological differences, especially that between the northwestern Pacific and the southern Atlantic.

The previous molecular phylogenetic studies (Robalo et al. 2009; Noguchi et al. 2015) showed no genetic deviation between morphotypes inhabiting single sea areas, namely, Portuguese and Japanese waters, while individuals of these two sea areas were completely deviated from each other. The pres-

Fig. 1. The non-metric multi-dimensional scaling (nMDS) ordination of Macroramphosus snipefish from the northwestern Pacific including the East China Sea (closed circles), the southern Pacific (closed triangles), the northern Atlantic (crosses), and the southern Atlantic (open circles) based on 10 quantitative morphological characters.

Fig. 2. The ratios of snout length (a), eye diameter (b), postocular head length (c), head length (d), postopercular body length (e), length of the second dorsal fin spine (f), distance between the two dorsal fins (g), maximum body depth (h), length before the second dorsal fin spine (i), and length after the second dorsal fin spine (j) to the standard length of Macroramphosus snipefish from the northwestern Pacific including the East China Sea (NP), the southern Pacific (SP), the northern Atlantic (NA), and the southern Atlantic (SA). Bars denote standard deviations. ***, **, and * denote significant differences at the 0.1%, 1%, and 5% levels, respectively.
ent results also showed the morphological difference between the northwestern Pacific and the northern Atlantic (off the coast of Morocco). It seems reasonable to think that *Macroramphosus* snipefish have deviated in different sea areas and they can be discriminated from each other by morphological and/or molecular characteristics. As morphological deviation corresponding to feeding habitats were reported in both the Atlantic and Pacific oceans (Ehrich & John 1973; Clarke 1984; Matthiessen et al. 2003; Miyazaki et al. 2004), trophic polymorphism might have evolved in a common ancestor of *Macroramphosus* snipefish and been maintained in deviated lineages. If so, such long-term persistence of trophic polymorphism, which is thought to be an intermediate step in speciation (Smith & Skúlason 1996), is a very interesting phenomenon.

*Macroramphosus* is a valuable example of a taxon that has experienced a unique evolutionary history, and they can be used as a model group for studying adaptive evolution in the open ocean. For this purpose, comprehensive studies of their morphology, molecular phylogeny, and ecology using additional specimens from the remaining sea areas including the Indian and eastern Pacific oceans, is imperative.

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