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Short Note

Genetic identification of two types of *Ariosoma* leptocephali

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Abstract—Comparisons of genetic sequences of the mitochondrial DNA 16S rRNA gene were used to match two species of congrid eels of the genus *Ariosoma* to two established *Ariosoma*-types of leptocephali. 18 *Ariosoma* sp. 7 leptocephali (40–151 mm TL) were matched with an *A. major* adult (333 mm TL) collected from Tosa Bay of eastern Japan with 0.2–0.7% sequence differences, and 1 *Ariosoma* sp. 8 leptocephalus (330 mm TL) was matched with two *A. shiroanago* adults (317 and 320 mm TL) also from Tosa Bay with no sequence difference. *Ariosoma* sp. 7 leptocephali have been collected in many regions of the western North Pacific (WNP), and the leptocephali from both the East China Sea and the WNP were matched with *A. major*, suggesting that the leptocephali of this species are widely distributed in the region. The leptocephali of *Ariosoma* sp. 8 appear to be much less common in most areas that have been sampled recently.

Key words: Congridae, leptocephali, *Ariosoma*, larval species identification

Introduction

Many species of marine eels have been collected and identified in the waters around Japan (Hatooka 2000) and in the East Asia region (Froese and Pauly 2006), but the leptocephalus larvae of only a few of these species have been matched with the adults. Tabeta and Mochioka (1988) described the morphological characteristics of more than 55 taxa of leptocephali of eels in the far western region of the North Pacific in the waters around Japan, but due to a lack of knowledge about the species identity of these larvae, only 1/3 of them were identified to species, which included the leptocephali of important commercial species such as *Anguilla japonica*, *Conger myriaster*, *Muraenesox cinereus*, *M. bagio*, and various other leptocephali of genera that include a few species.

The big problem with identifying leptocephali to the species level is that these unusual fish larvae generally show no morphological resemblance to the juveniles or adults of each species, because the body forms of the two different stages are completely different (Böhlke 1989a, b, Miller and Tsukamoto 2004). Leptocephali have almost totally transparent, laterally compressed bodies filled with gelatinous material, but after the full-grown larvae undergo metamorphosis their bodies become round and eel-like in shape and totally pigmented (Smith 1989a, Miller and Tsukamoto 2004). The only character consistently carried through metamorphosis is the total number of myomeres (TM), which is equivalent to the total number of vertebrae (TV) in the adults (Smith 1989a). But it is difficult to match the larvae to the juveniles and adults using ranges of TM and TV unless there is little overlap in the ranges of similar species. In parts of the world such as the western North Atlantic (WNA), the eel fauna has been well studied (Böhlke 1989a, b), but in the Indo-Pacific, there are many more species of most tropical eel families, so the process of matching leptocephalus types with their adult species is only just beginning. In the larger families of eels that have many species, such as the Congridae, Muraenidae, and Ophichthidae, most species overlap in their TV, making it difficult to match them with their larval forms only using the TM of the leptocephali.

The Congridae is an important family of marine eels that live from shallow water to depths of 2,000 m or more on the continental slope worldwide. There appear to be more than 85 species of congrids in the Indo-Pacific (Froese and Pauly 2006, Ma 2006) compared to about 32–42 in the WNA (Smith 1989b), where most congrid species have been matched with their leptocephali (Smith 1989c). The genus *Ariosoma* and other members of the subfamily Bathymyrinae are a good example of this. In the WNA there are 4–5 species of *Ariosoma*, 1 of *Parabathymyrus*, and 2–4 of *Paraconger* (Smith 1989b). In the Indo-Pacific, there are at least 11 species of *Ariosoma*, 3 of *Parabathymyrus*, 2 *Bathymyrus*, 2 *Poeciloconger*, and 1 *Chiloconger* (Froese and Pauly 2006, Ma 2006). This greater diversity and a lack of many taxono-

mists actively studying these eels in recent years has made the process of identifying leptocephali of the Congridae difficult despite the detailed descriptions of many different types of congrid larvae from the Indo-Pacific region (Castle 1964, 1997, Mochioka et al 1982, 1991, Tabeta and Mochioka 1988, Castle and Smith 1999, Ma 2006).

As an effective alternative identification method that was not available to early taxonomists, genetic techniques have been successfully used recently to identify the eggs, leptocephali, or glass eels of the genus *Anguilla* (e.g. Aoyama et al. 1999, 2000, 2001a, 2003). These techniques have also been used to characterize the species or types of some other families of leptocephali in early life history studies (Ma et al. 2005). More recently 66 different taxa were distinguished using sequence data of the mitochondrial DNA (mtDNA) 16S rRNA gene from 395 congrid leptocephali from the western Pacific and eastern Indian Ocean regions (Ma 2006). In the present study, we describe the species identification of two established types of *Ariosoma* leptocephali by matching their genetic sequences with the homologous sequences of two adult species of *Ariosoma* from eastern Japan.

Materials and Methods

As part of the larger study on many taxa of congrid leptocephali in the Indo-Pacific region (Ma 2006), 18 specimens (40–151 mm TL) of *Ariosoma* sp. 7 leptocephali (Type III in Mochioka et al. 1991) collected in the East China Sea (ECS) and WNP regions and 1 specimen (330 mm TL) of *Ariosoma* sp. 8 leptocephalus (Type IV in Mochioka et al. 1991) collected in the WNP (Fig. 1) were used in the sequence analyses of their mtDNA 16S rRNA gene in this study. The leptocephali were collected during 2 research cruises (KT-00-16 and KH-04-2) in the WNP region (Table 1) using the large Isaacs Kidd Midwater Trawl (IKMT) that was fished primarily within the upper 300 m (Miller et al. 2002). The leptocephali were sorted fresh from the plankton, examined and identified according to Tabeta and Mochioka (1988), and then preserved in 99% ethanol. The adult specimens were collected during trawling surveys for benthic fishes carried out by the Fisheries Research Agency (FRA) in Tosa Bay (Fig. 1) and were frozen after collection. In the laboratory, the adult specimens were identified to species according to the key in Hatooka (2000). Juveniles and adults of *A. shiroanago* were common at the 100–250 m depths trawled from 2003–2005 (N=452, 129–375 mm TL, Minagawa 2006), and one individual of *A. major* was also collected. The same mtDNA region of 1 specimen (333 mm TL) of *A. major* and 2 specimens (317 and 320 mm TL) of *A. shiroanago* was also sequenced. Three voucher specimens of adult species were deposited in the National Science Museum, Tokyo, Japan as *A. major* (NSMT-P71846) and *A. shiroanago* (NSMT-P71847 and NSMT-P71848).

In the laboratory, the total genomic DNA was extracted

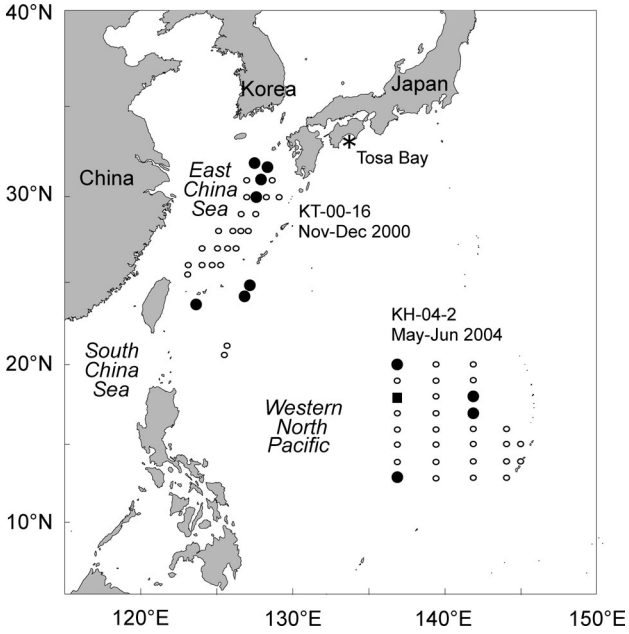

Figure 1. Map of the collection locations of the *Ariosoma* leptocephali (black circles: *Ariosoma* sp. 7; black square: *Ariosoma* sp. 8; white circles: negative locations) and adults (asterisk), whose genetic sequences were found to match.

Table 1. The collection data and size of the specimens of leptocephali and adult eels that were matched using sequence data of the mtDNA 16S rRNA gene in the present study. Leptocephali were collected during cruises of the R/V Hakuho Maru (KH) and Tansei Maru (KT), and the adult specimens were collected by the Fisheries Research Agency (FRA).

Species	Source	Date	Location	TL (mm)	Species match
Leptocephali					
Ariosoma sp. 7	KT-00-16	27 Nov–7 Dec 2000	ECS	40–148	*A. major*
Ariosoma sp. 7	KH-04-2	16–22 May 2004	WNP	105–151	*A. major*
Ariosoma sp. 8	KH-04-2	21 Jun 2004	WNP	330	*A. shiroanago*
Adults					
A. major	FRA	22 Jul 2004	Tosa Bay	333	*Ariosoma* sp. 7
A. shiroanago	FRA	6 Oct 2003	Tosa Bay	320	*Ariosoma* sp. 8
A. shiroanago	FRA	27 Aug 2003	Tosa Bay	317	*Ariosoma* sp. 8

from tissue samples of each leptocephalus and adult specimen by incubation in 500 μl of a 5% chelex solution (Bio Rad) at 98°C for 15 min. Two contiguous, overlapping fragments of the mtDNA 16S rRNA gene were amplified by polymerase chain reaction (PCR) using 2 sets of universal primers: L1803 and H2590, L2510 and H3058 (Miya and Nishida 1999, Inoue et al. 2001). The PCR amplifications were carried out in a GeneAmp PCR system 9700 (Applied Biosystems) with 25 cycles of denaturation at 94°C for 15 s, annealing at 50°C for 15 s, and extension at 72°C for 20 s. The PCR products were electrophoresed in 1% agarose gel to verify the amplified fragment length. Then double-stranded PCR products were purified using a Pre-Sequencing Kit (USB), and were subsequently used for direct sequencing with dye-labeled terminators (Applied Biosystems). The primers used were the same as those for PCR. All sequencing reactions were performed according to the manufacturer's instructions. Labeled fragments were analyzed on a Model 3130 genetic analyzer (Applied Biosystems). Sequences were obtained from the light and heavy strands of each fragment, and the final sequences combined with the two fragments were deposited in the DDBJ/EMBL/GenBank nucleotide sequence database under accession numbers: AB299447–AB299468.

The DNA sequences were edited and analyzed with EditView ver. 1.0.1, AutoAssembler ver. 2.1 (Applied Biosystems), and DNASIS, ver. 3.2 (Hitachi Software Engineering Co. Ltd.). All sequences from the 19 leptocephali and 3 adults examined were initially aligned with the software package Clustal X 1.83 (Thompson et al. 1997) and subsequently adjusted by eye with MacClade ver. 4.05 (Maddison and Maddison 2002). The pairwise sequence differences among samples were obtained by PAUP ver. 4.0s (Swofford 2002).

Results and Discussion

A total of 1,243 sites of aligned sequence data of the mtDNA 16S rRNA gene were used to compare the differences between specimens. The 18 *Ariosoma* sp. 7 leptocephali were found to have sequence differences of only 3–9 sites (0.2–0.7%) compared to the *A. major* adult, but had sequence differences of 49–55 sites (3.9–4.4%) compared to both of the *A. shiroanago* adults (Table 2). The sequence differences within the *Ariosoma* sp. 7 leptocephali were 0–10 sites (0–0.8%). The *Ariosoma* sp. 8 leptocephalus was completely matched with two *A. shiroanago* adults with no genetic difference between them, while it had sequence differences of 54 sites (4.3%) compared to the *A. major* adult (Table 2). The genetic differences between the *Ariosoma* sp. 7 leptocephali and *A. major* (0.2–0.7%) can be regarded as intra-species variations, since they are similar to the sequence differences within the *Ariosoma* sp. 7 leptocephali (0–0.8%), which are much less than that between the adult species of *A. major* and *A. shiroanago* (4.3%). These values

Table 2. Pairwise sequence differences among the specimens of *Ariosoma* leptocephali and adults examined in this study. The numbers of nucleotide substitutions are below the diagonal, and the percent sequence differences are above the diagonal.

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)	(18)	(19)	(20)	(21)	(22)
(1) *Ariosoma* sp. 7	—	0.4	0.2	0.3	0.3	0.3	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.7	0.5	0.6	0.4	0.4	0.5	4.3	4.3	4.3
(2) *Ariosoma* sp. 7	5	—	0.2	0.2	0.1	0.2	0.3	0.2	0.2	0.2	0.2	0.2	0.2	0.5	0.4	0.5	0.2	0.3	0.4	3.9	3.9	3.9
(3) *Ariosoma* sp. 7	3	2	—	0.1	0.1	0.1	0.2	0	0.1	0	0	0	0.1	0.5	0.2	0.3	0.2	0.2	0.2	4.1	4.1	4.1
(4) *Ariosoma* sp. 7	4	3	1	—	0.2	0.2	0.2	0.1	0.2	0.1	0.1	0.1	0.2	0.6	0.3	0.4	0.2	0.2	0.3	4.0	4.0	4.0
(5) *Ariosoma* sp. 7	4	1	1	2	—	0.2	0.2	0.1	0.2	0.1	0.1	0.1	0.2	0.4	0.3	0.4	0.1	0.2	0.3	4.0	4.0	4.0
(6) *Ariosoma* sp. 7	4	3	1	2	2	—	0.2	0.1	0.2	0.1	0.1	0.1	0.2	0.6	0.3	0.4	0.2	0.2	0.3	4.2	4.2	4.2
(7) *Ariosoma* sp. 7	3	4	2	3	3	3	—	0.2	0.1	0.2	0.2	0.2	0.2	0.6	0.4	0.5	0.3	0.3	0.4	4.3	4.3	4.3
(8) *Ariosoma* sp. 7	3	2	0	1	1	1	2	—	0.1	0	0	0	0.1	0.5	0.2	0.3	0.2	0.2	0.2	4.1	4.1	4.1
(9) *Ariosoma* sp. 7	2	3	1	2	2	2	1	1	—	0.1	0.1	0.1	0.2	0.6	0.3	0.4	0.2	0.2	0.3	4.2	4.2	4.2
(10) *Ariosoma* sp. 7	3	2	0	1	1	1	2	0	1	—	0	0	0.1	0.5	0.2	0.3	0.2	0.2	0.2	4.1	4.1	4.1
(11) *Ariosoma* sp. 7	3	2	0	1	1	1	2	0	1	0	—	0	0.1	0.5	0.2	0.3	0.2	0.2	0.2	4.1	4.1	4.1
(12) *Ariosoma* sp. 7	3	2	0	1	1	1	2	0	1	0	0	—	0.1	0.5	0.2	0.3	0.2	0.2	0.2	4.1	4.1	4.1
(13) *Ariosoma* sp. 7	4	3	1	2	2	2	3	1	2	1	1	1	—	0.6	0.3	0.4	0.2	0.2	0.3	4.2	4.2	4.2
(14) *Ariosoma* sp. 7	9	6	6	7	5	7	8	6	7	6	6	6	7	—	0.7	0.8	0.5	0.6	0.7	4.4	4.4	4.4
(15) *Ariosoma* sp. 7	6	5	3	4	4	4	5	3	4	3	3	3	4	9	—	0.2	0.4	0.4	0.5	4.3	4.3	4.3
(16) *Ariosoma* sp. 7	7	6	4	5	5	5	6	4	5	4	4	4	5	10	3	—	0.5	0.5	0.6	4.4	4.4	4.4
(17) *Ariosoma* sp. 7	5	2	2	3	1	3	4	2	3	2	2	2	3	6	5	6	—	0.3	0.4	4.1	4.1	4.1
(18) *Ariosoma* sp. 7	5	4	2	3	3	3	4	2	3	2	2	2	3	8	5	6	4	—	0.4	4.3	4.3	4.3
(19) *A. major*	6	5	3	4	4	4	5	3	4	3	3	3	4	9	6	7	5	5	—	4.3	4.3	4.3
(20) *Ariosoma* sp. 8	54	49	51	50	50	52	53	51	52	51	51	51	52	55	54	55	51	53	54	—	0	0
(21) *A. shiroanago*	54	49	51	50	50	52	53	51	52	51	51	51	52	55	54	55	51	53	54	0	—	0
(22) *A. shiroanago*	54	49	51	50	50	52	53	51	52	51	51	51	52	55	54	55	51	53	54	0	0	—

Table 3. Comparisons of the total myomeres (TM) of *Ariosoma* sp. 7 and sp. 8 leptocephali and the total vertebrae (TV) of the *Ariosoma* adult species referred to in the text.

Species	Leptocephali/adult	N	TM/TV	Source
Ariosoma sp. 7	Leptocephali	18	143–150	This study
		788	136–150	Tabeta and Mochioka, 1988
Ariosoma sp. 8	Leptocephali	1	163	This study
		7	158–162	Tabeta and Mochioka, 1988
Ariosoma major	Adult	1	144	This study
		—	144–147	Hatooka, 2000
Ariosoma shiroanago	Adult	6	158–161	This study
		—	156–161	Hatooka, 2000
Ariosoma anago	Adult	—	143	Hatooka, 2000
Ariosoma sazonovi	Adult	5	146–148	Karmovskya, 2004
Ariosoma meeki	Adult	—	149–159	Hatooka, 2000

(0.2–0.7%) are much smaller than the mean intra-species differences of the same mtDNA gene in the conger eel, *C. myriaster* (1.66–1.87%, Kimura et al. 2004) and the minimum inter-species differences in the genus *Anguilla* (1.15%, Watanabe 2003), and are similar to the differences within other species of eels such as *A. celebesensis* and *A. interioris* (0–0.4%, Aoyama et al. 2001b). These results indicate a clear match between the *Ariosoma* sp. 7 leptocephali and *A. major*, and between the *Ariosoma* sp. 8 leptocephalus and *A. shiroanago*.

The TM ranges of the *Ariosoma* sp. 7 (143–150 in this study; 136–150 in Tabeta and Mochioka 1988) and *Ariosoma* sp. 8 (163 in this study; 158–162 in Tabeta and Mochioka 1988) leptocephali also matched the known ranges of the TV in *A. major* (144 in this study; 144–147 in Hatooka 2000) and *A. shiroanago* (158–161 in this study; 156–161 in Hatooka 2000) adults (Table 3). The correspondences between these TM and TV data support the identifications of these two species of *Ariosoma* leptocephali.

This detection of clear genetic matches between larval and adult specimens using their 16S rRNA sequence data provides the first likely species identification of *Ariosoma* leptocephali from the WNP. The possible match between the *Ariosoma* sp. 8 leptocephalus type and *A. shiroanago* adult species had been suggested by Tabeta and Mochioka (1988), and the present study now confirms the match. However, the *Ariosoma* sp. 7 leptocephalus type likely consists of several different species or populations within the larger Indo-Pacific region (Ma 2006). In contrast to *Ariosoma* sp. 8 leptocephali, the leptocephali of *Ariosoma* sp. 7 are widespread in the far western region of the WNP (Mochioka et al. 1991). *Ariosoma* sp. 7 leptocephali are also consistently present in the North Equatorial Current region of the WNP and are abundant in the Kuroshio Extension region (Miller, M. J. and Tsukamoto, K. unpublished data). These observations and the presence of genetically identified specimens matching with *A. major* in both the ECS and WNP regions (Fig. 1), suggest that most of the individuals of this common leptocephalus type in the WNP may be those of *A. major*.

A. major may be a common species in sandy areas from Taiwan to Japan (Froese and Pauly 2006) and especially over the shelf of the ECS in the waters less than 100 m as suggested by trawling surveys (Yamada 1986), although the two similar species of *A. major* and *A. shiroanago* may not be typically separated in fisheries data sets. Based on the abundance of *A. shiroanago* at greater depths of 100–250 m in Tosa Bay however, and the rarity of *A. major* at those depths (Minagawa 2006), it appears likely that *A. major* is the shallow water species that may be more abundant in the region than the deeper living species, *A. shiroanago*, and this could contribute to its larvae being much more abundant in the WNP region.

There are other species of *Ariosoma* adults known from the WNP region that have overlapping ranges of TV with *A. major* however, so not all *Ariosoma* sp. 7 leptocephali in the region can be assumed to be *A. major*. The adult species of *A. anago* known from Japan and further south in the Indo-Pacific (143 TV in Hatooka 2000) and *A. sazonovi* from the Philippines (146–148 TV in Karmovskya 2004) have overlapping ranges of TV with the TM of *Ariosoma* sp. 7 leptocephali (136–150, Table 3), so the presence of other species among the *Ariosoma* sp. 7 leptocephali is possible, especially in regions further away from the ECS and the Kuroshio gyre system (Ma 2006). Another apparently rare species in the region, *A. meeki* (149–159 TV in Hatooka 2000), overlaps slightly with the TM range of *Ariosoma* sp. 7 leptocephali, but this range is more similar to those of several species of the exterilium-*Ariosoma* type leptocephali (Mochioka et al. 1982). Due to the genetic matches and lack of TM or TV overlap with other species of *Ariosoma*, the identification of the leptocephalus of *A. shiroanago* appears certain. However, only one *Ariosoma* sp. 8 leptocephalus and one *A. major* adult were collected in this study, so additional specimens need to be examined to confirm these identifications.

The finding of a match between an apparently abundant shallow water species and a common leptocephalus type, and a deeper water eel species with a leptocephalus type rarely collected in offshore areas, is important both taxonomically and ecologically. The rarity of the leptocephali of *A. shiroanago* offshore and their apparent abundance at deeper depths in Tosa Bay, suggest the possibility that this species may have a different spawning and larval dispersal and recruitment strategy than that of *A. major*. Congrid eels have a variety of fascinating and difficult to understand life histories (McCleave and Miller 1994, Miller 2002, Miller et al. 2002, Kimura et al. 2004, 2006), so further studies are needed on the leptocephali and adults of these two species. Additional efforts should be made to match other eel species with their leptocephalus larvae in the region to enable new ecological discoveries about poorly known groups of eels such as those of the genus *Ariosoma*.

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