First collection of amphidromous goby post-larvae of *Sicyopterus japonicus* in the ocean off Shikoku, Japan

by

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ABSTRACT. - The oceanic early life of Sicydiinae gobies has remained mysterious although considerable knowledge has been accumulated about their freshwater life. Two marine post-larvae of *Sicyopterus japonicus*, were collected by otter trawl in the ocean off Shikoku, Japan on 18 July and 22 August 2007. These specimens were identified as *S. japonicus* using meristic characters and the 16S ribosomal RNA mitochondrial gene, which were compared to *S. japonicus* adults and two other species of Sicydiinae gobies. Morphological observations documented that *S. japonicus* post-larval characters were the presence of a red pigment at the bases of the second dorsal and caudal fins in fresh samples, as well as the presence of melanophores on the upper jaw, caudal fin base and the lateral body posterior to the anal fin base. Otolith analysis showed that their post-larval durations were 278 and 286 days, and their birth dates were 13 October and 9 November 2006. It is likely that these two post-larvae may have been transported by the Kuroshio Current from the southern part of their geographic species range such as from Okinawa or Taiwan and may have had recruited to a river around Kochi.

RÉSUMÉ. - Première capture de post-larves océaniques du gobie amphidrome, *Sicyopterus japonicus*, au large du Shikoku, Japon.

La phase de vie océanique des gobies Sicydiinae reste mystérieuse alors qu'une quantité non négligeable de connaissance a été accumulée sur leur phase de vie en eau douce. Deux post-larves de *Sicyopterus japonicus* ont été collectées dans l'océan, au large des côtes japonaises du Shikoku, à l'aide d'un chalut à panneaux, les 18 juillet et 22 août 2007. Ces spécimens ont été identifiés à l'aide de l'étude de caractères méristiques et génétiques (ADN mitochondrial codant pour le gène d'ARN ribosomal 16S) par comparaison à des adultes de *S. japonicus* et de deux autres espèces de Sicydiinae. L'observation morphologique des post-larves de *S. japonicus* montre la présence d'un pigment rouge à la base de la seconde nageoire dorsale et de la caudale sur des individus frais, ainsi que la présence de mélanophores sur la mâchoire supérieure, la base de la nageoire caudale et sur les flancs en arrière de la base de la nageoire anale. L'analyse des otolithes montre que leur durée de vie larvaire en mer est de 278 et 286 jours, et que leur date de naissance est le 13 octobre et le 9 novembre 2006. Il est probable que ces deux post-larves aient été transportées par le courant du Kuroshio depuis des zones situées au sud de leur distribution géographique comme Okinawa ou Taiwan, et qu'elles auraient pu recruter dans une rivière autour de Kochi.

Key words. - Sicydiinae - Post-larvae - Larval morphology - Oceanic larval duration.

All species of the Sicydiinae (Gobiidae), except for *Sicyopterus japonicus* (Tanaka, 1909), are found mainly on tropical and subtropical oceanic islands from the western Indian Ocean through Indonesia, the Eastern Pacific Ocean, and from the Caribbean Sea to western Africa (Keith, 2003; Keith and Lord, 2011). *Sicyopterus japonicus* has the northernmost distribution among the subfamily (Iida *et al*., 2009) and the geographic distribution of this species ranges from Taiwan to Fukushima Prefecture, Japan (Fig. 1, Akihito *et al*., 2000).

Like all Sicydiinae, *S. japonicus* is an amphidromous fish that spawns in freshwater, and its larvae drift downstream to the sea where they undergo larval development before migrating back to rivers to grow and reproduce in freshwater (McDowall, 1988; Keith and Lord, 2011). Recently, there have been several studies on the larval development of Sicydiinae gobies, especially morphological transformations of hatching larvae in rivers (Iida *et al*., 2010b; Valade *et al.,* 2010), post-larvae returning to rivers (Keith *et al*., 2008), and larval duration (Radtke *et al*., 2001; Hoareau *et al*.,

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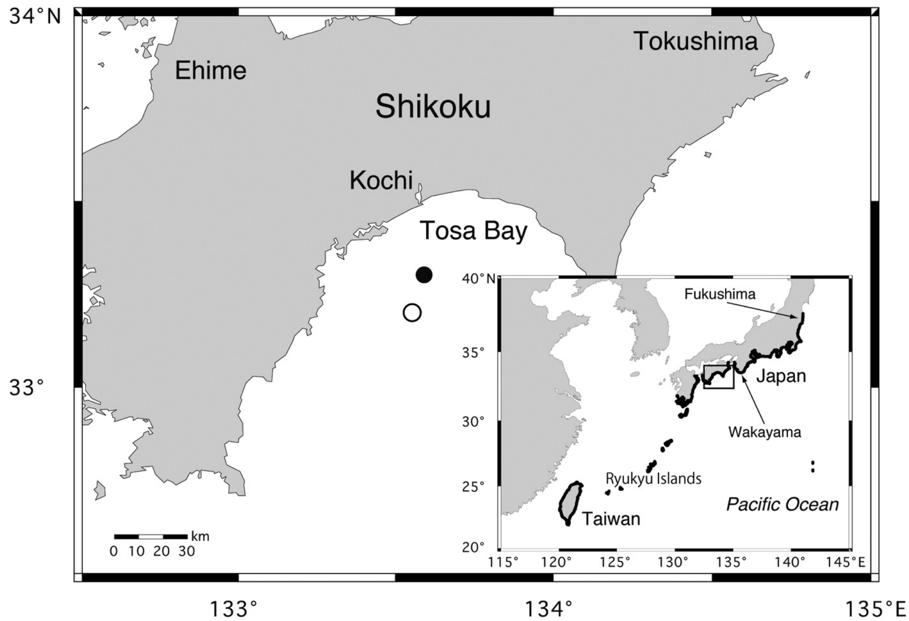

Figure 1. - The collection locations of the two oceanic post-larvae and the geographic distribution of *Sicyopterus japonicus* (areas covered by thick lines in the inset). Open and solid circles show the localities respectively for BSKU 103632 collected on 18 July 2007 and BSKU 103633 collected on 22 August 2007.

Table I. - Five meristic characters in comparison with two post-larvae and three Sicydiinae species predominant in Japan.

Meristic characters \ Stage	BSKU 103632 and 103633	*Sicyopterus japonicus*		*Sicyopterus lagocephalus*	*Stiphodon percnopterygionus*
		Post-larvae	Adult	Adult	Adult
Dorsal fin	VI-I, 10	VI-I, 10	VI-I, 10	VI-I, 11	VI-I, 10
Anal fin	I, 10	I, 10	I, 10	I, 10	I, 10
Pectoral fin	19	17-19	19	20	13-15
Pelvic fin	I, 5	I, 5	I, 5	I, 5	I, 5
Total number of vertebrae	26	26	26	26	26

2007; Lord *et al*., 2010). After the marine life, post-larvae recruit back to rivers while undergoing an important metamorphosis, which starts at sea (Keith *et al*., 2008). However, the oceanic early life of Sicydiinae gobies has still remained mysterious.

In *S. japonicus*, there have been several studies on the population structure (Ju, 2001; Watanabe *et al*., 2006), larval duration (Yamasaki *et al*., 2007; Iida *et al*., 2008; Shen and Tzeng, 2008) and otolith microchemistry (Shen *et al*., 1998; Shen and Tzeng, 2002). These studies have helped to begin to understand the possible magnitude of the oceanic dispersal and larval duration of *S. japonicus* and have suggested that this species has no genetic population structure, with their larvae possibly being frequently dispersed by the powerful Kuroshio Current during their oceanic stage. There are a few studies that have attempted to reveal the oceanic larval phase in Sicydiinae with several hypotheses concerning larval movements in the water column (Murphy and Cowan, 2007; Sorensen and Hobson, 2005; Iida *et al*., 2010c), but not a single Sicydiinae larva has ever been reported to have been collected in the ocean away from estuaries.

This paper reports the first catch of two post-larval specimens of *S. japonicus* at sea far from the estuary near Japan, which were identified by morphological and molecular genetic analyses. The purpose of this study was to describe the morphological characteristics of the oceanic post-larval stage of this amphidromous species to assist future identification of these post-larvae. Furthermore, we determine their oceanic larval duration using otolith daily rings and discuss the oceanic larval dispersal of *S. japonicus*.

MATERIAL AND METHODS

Collection of marine larvae

Two specimens were collected in the ocean within Tosa Bay of Shikoku, Japan (Fig. 1) (BSKU 103632 and 103633, BSKU: Laboratory of Marine Biology, Faculty of Science, Kochi University), by the trawl survey made by the R/V

Table II. - Genetic distance of the mitochondrial 16SrRNA gene sequences (544 basepair) in comparison with two marine larvae and 3 Sicydiinae species predominant in Japan.

	Larvae		Adults					
			Sicyopterus japonicus				*Sicyopterus lagocephalus*	*Stiphodon percnopterygionus*
	Kouchi	Kouchi	Kouchi	Kouchi	Taiwan	Taiwan	Okinawa	Okinawa
	BSKU 103632	BSKU 103633	NSMT-P90035	NSMT-P90036	NSMT-P90029	NSMT-P90030	NSMT-P90033	NSMT-P90034
BSKU 103632	0	0.006	0.004	0.006	0.004	0.002	0.019	0.073
BSKU 103633		0	0.006	0.004	0.002	0.004	0.015	0.073
NSMT-P90035			0	0.006	0.004	0.002	0.019	0.077
NSMT-P90036				0	0.002	0.004	0.019	0.073
NSMT-P90029					0	0.002	0.017	0.075
NSMT-P90030						0	0.017	0.075
NSMT-P90033							0	0.067
NSMT-P90034								0

Kotaka Maru (30 m long, 59 tons, 1,000 ps) of the Fisheries Research Agency on 18 July (33°13.86'N, 133°34.32'E to 33°12.31'N, 133°32.86'E) and 22 August 2007 (33°18.33'N, 133°36.15'E to 33°17.25'N, 133°34.04'E). These localities where two specimens were caught at sea were about 26 and 32 km (BSKU 103633 and 103632) from the nearest shoreline. The trawl depths were 150 to 163 m on 18 July 2007 and 120 to 119 m on 22 August 2007. The specimens were preserved in 95% ethanol. One specimen (BSKU 103632) was photographed before preservation on board (Fig. 2).

The gear used was a small otter trawl (cod-end mesh size 2.0 mm). The trawling stations were chosen to research the abundances of 0-age deep-sea smelt *Glossanodon semifasciatus* (Kishinouye, 1904), which is one of the most important fish for the demersal fishery in the Pacific coastal waters of central and southern Japan. All trawls were conducted during the daytime because this trawl was dragged on the bottom. Trawl courses were set bathymetrically as much as possible. The duration of the each trawl survey was 10 or 15 minutes (mostly 15 min) from the set of the towing line (8 mm diameter stainless steel) until the start of the retrieval of the net. The towing speed was approximately 2 knots. The fish samples, except for the two specimens of oceanic post-larvae of *S. japonicus*, were divided into taxonomical groups and preserved in 5% seawater neutralized formalin, and in the laboratory they were washed with tap water and then preserved in 80% ethanol within 24 h after sampling.

Morphological analysis

The standard length (SL) of the post-larvae was measured, the existence of scales was observed and pectoral fin rays in left side were counted using stereomicroscope (SMZ 800) and a micrometer after preservation in 95% ethanol. We had compared their pigmentation pattern between these specimens caught at sea and a developmental series of post-larvae (Fig. 3) collected in the estuary of the Nagano River in Wakayama to the east of Shikoku. The two specimens were x-rayed by Soft-X (Softex Co., Ltd.) to count their meristic characters of total number of vertebrae and dorsal and anal fin rays for comparison with 3 Sicydiinae species, *S. japonicus*, *S. lagocephalus* (Pallas, 1770) and *Stiphodon percnopterygionus* Watson and Chen, 1998 that are predominant in Japan.

Molecular genetic identification

We sequenced the mitochondrial (mtDNA)16S ribosomal RNA gene (16SrRNA) for species identification of the post-larvae. The 16SrRNA is a relatively conservative gene (Meyer, 1993) and is frequently used for identification studies for *Anguilla* species (Aoyama *et al*., 2000; Watanabe *et al*., 2005). In addition, we used six adult specimens of 3 Sicydiinae species predominant in Japan, which were *S. japonicus* from Kochi (N = 2; NSMT-P90035 and P90036,

Figure 2. - Oceanic post-larva of *Sicyopterus japonicus* (BSKU 103632) at the time of collection by an otter trawl deployed by the Kotaka Maru on 18 July 2007.

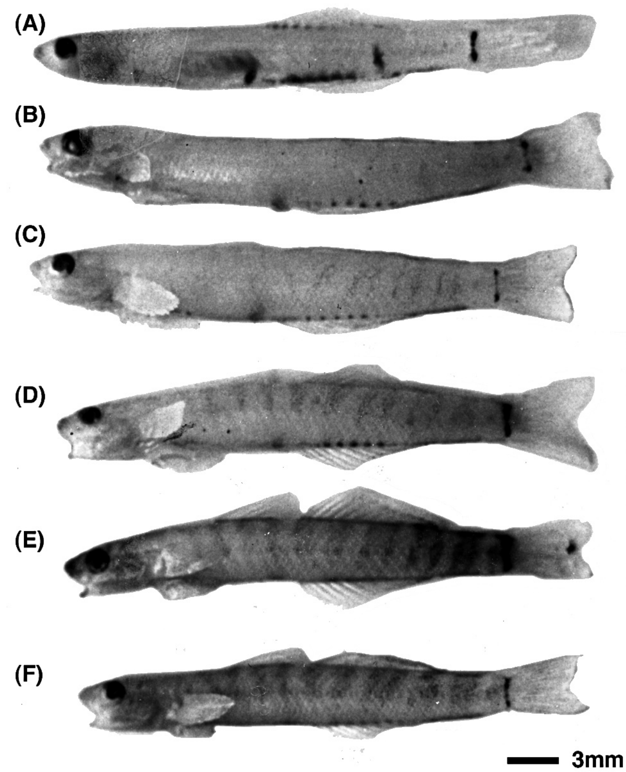

Figure 3. - A developmental series of post-larvae of *Sicyopterus japonicus* collected in the Nagano River in Wakayama, Japan by a square lift net (Photograph provided by S. Fukui). The top specimen (**A**) is post-larval stage 1 (PL1) and other specimens (**B** to **F**) are post-larval stage 2 (PL2) according to Keith *et al*. (2008).

NSMT: National Science Museum, Tokyo, collection locality and date: Monobe River, 5 November 2003) and Taiwan (N = 2; NSMT-P90029 and P90030, Sekitei River, 17 May 2005), *S. lagocephalus* from Okinawa (N = 1; NSMT-P90033, Genka River, 26 May 2005) and *Stiphodon percnopterygionus* from Okinawa (N = 1; NSMT-P90034, Aha River, 4 March 2002).

The oceanic larvae were genetically identified by sequencing their 16SrRNA in the laboratory following the standard protocol of Aoyama *et al*. (1999), which was a method used for identifying anguillid leptocephali. A pair of oligonucleotide primers, L2510 (5'-CGC CTG TTT ATC AAA AAC AT-3') and H3080 (5'- CCG GTC TGA ACT CAG ATC ACG T -3') (Palumbi *et al*., 1991) was used for polymerase chain reaction and the direct cycle sequencing reaction.

All sequences were edited with the multiple sequence editor DNASYS (Hitachi Software Engineering Co. Ltd, Tokyo, Japan) and preliminary alignment was achieved using CLUSTAL W (Thompson *et al*., 1994), the output of which was later confirmed by eye. The genetic distances (Kimura, 1980) of 16SrRNA were examined to compare the amount of sequence differences among the two oceanic larvae and six adults. The final haplotype sequences were deposited in GenBank/EMBL/DDBJ (Accession numbers / Museum specimen number / species: AB602474 / BSKU 103632 / *S. japonicus*, AB602475 / BSKU 103633 / *S. japonicus*, AB602476 / NSMT-P90035 / *S. japonicus*, AB602477 / NSMT-P90036 / *S. japonicus*, AB602478 / NSMT-P90034 / *Stiphodon percnopterygionus*, AB540216 / NSMT-P90029 / *S. japonicus*, AB540217 / NSMT-P90030 / *S. japonicus*, and AB540220 / NSMT-P90033 / *S. lagocephalus*).

Otolith analysis

Sagittal otoliths were extracted from each specimen for age determination. Otoliths were mounted on glass slides with euparal (Chroma-Gesellschaft), and examined at 50-500x under transmitted light to take photographs. The otolith increments were counted on the photographs. This species appears to have daily periodicity in the deposition of otolith growth increments based on an otolith deposition validation study on *S. japonicus* larvae at Wakayama, Japan (Iida *et al*., 2010a). The total age of the two post-larvae was estimated by counting the otolith increments from the core to the otolith edge. The hatching date of each specimen was obtained by back-calculation from the sampling date using its total age.

RESULTS

Morphological description

The SL of the two post-larvae was 26.8 mm (BSKU 103632) and 27.5 mm (BSKU 103633) after preservation in 95% ethanol. These post-larvae had semi-transluscent bodies, conspicuous swim bladders immediately anterior to the anus and emarginate caudal fins (Fig. 2). Their gut is straight and extends to about midbody. The head is of moderate length, slightly rounded and gently sloping. Eyes are round in shape. The sucker which corresponds the highly modified pelvic fins in Sicydiinae was already formed. These morphological characters typical of the Gobioidei are fairly distinctive from the larvae of other teleosts (Puple, 1984).

The most important characteristic of these post-larvae was their pigmentation pattern that appeared to consist of two types of chromatophores that included red coloured

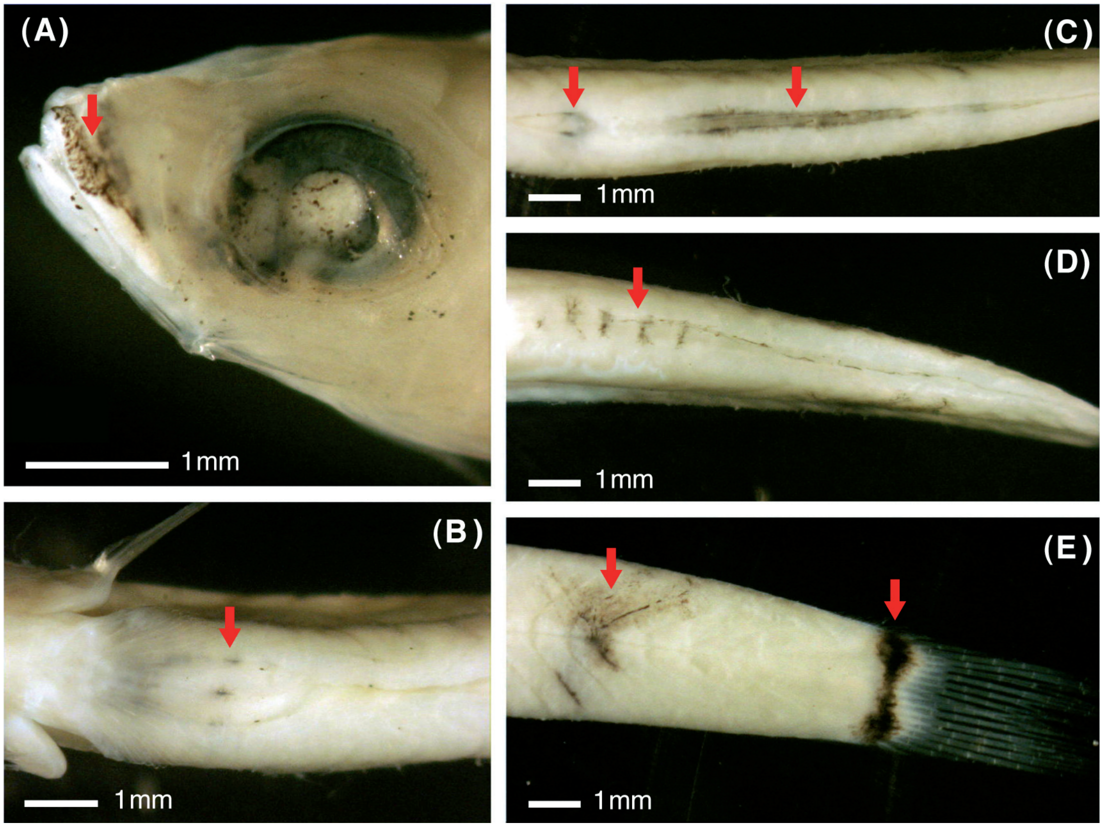

Figure 4. - The external and/or internal chromatophores at the upper margin of the upper jaws (**A**), the pelvic fin base and the ventral chest (**B**), around the anus and the anal fin base (**C**), the base of the second dorsal fin (**D**), and the posterior lateral body side posterior to the anal fin base and the caudal fin base (**E**). The arrows show each chromatophore placement.

erythrophores and dark coloured melanophores (Fujii, 2000). The red erythrophores were located at the second dorsal and caudal fin bases, but were visible only in the fresh specimens (Fig. 2 and 4-D, E), because they lost their red pigment colour and became black after ethanol preservation. They also had conspicuous external and/or internal melanophores at the upper margin of the upper jaws (Fig. 4-A), anal (Fig. 4-C), second dorsal (Fig. 4-D) and caudal fin bases (Fig. 4-E), the ventral chest region under the pelvic fin (Fig. 4-B) and around the anus (Fig. 4-C). Furthermore, there was conspicuous dark pigmentation including some highly diffuse melanophores on the lateral body side posterior to the anal fin base (Fig. 4-E). A larger melanophore extended over several myomeres from the mid to dorsal side of the body in this area, with a smaller one being on the ventral side between myomeres (Fig. 4-E). These pigmentation patterns in the two specimens were similar to those of post-larva just recruited to the estuary (Fig. 3). However, the red erythrophores in the second dorsal and caudal fin bases and the melanophores on the lateral body side posterior to the anal fin base appear to disappear during metamorphosis from post-larvae to juveniles in the estuary (Fig. 3).

The meristic characters of number of fin rays and vertebrae of the two oceanic post-larvae were as follows: dorsal fin, VI-I, 10; anal fin, I, 10; pectoral fin, 19; pelvic fin, I, 5; total number of vertebrae, 26 (Tab. I). Number of pectoral fin rays in these specimens was the same as in the post-larvae and adults of *S. japonicus* and was different from those in the two other species (Tab. I).

Molecular genetic identification and post-larvae stage

Genetic distances of the sequences of 544 sites of 16SrRNA mtDNA between all pairings between the two larvae and the six adults ranged from 0.002 to 0.057 (Tab. II). The genetic distance between the two post-larvae from Kochi was 0.006. The genetic distances between adult pairs of *S. japonicus* (0.002-0.006) were the same values as the genetic distances between two larvae and four adults of *S. japonicus* from Kochi and Taiwan (0.002-0.006). However, these values were lower than those among adult pairs of *S. japonicus*, *S. lagocephalus* and *Stiphodon percnopterygionus* (0.017-0.057). Based on these results of the genetic distances, the two post-larvae from Kochi were confirmed to be *S. japonicus*.

Table III. - Fish list collected by otter trawl net. Asterisks show larva or juvenile.

Date	Species	Family	Order	Number of individuals	Weight (g)
18 Jul. 2007	*Ariosoma shiroanago shiroanago*	Congridae	Anguilliformes	4	237.2
	Glossanodon semifasciatus	Argentinidae	Argentiniformes	3	97.2
	Glossanodon semifasciatus *	Argentinidae	Argentiniformes	5	30.3
	Synodus kaianus	Synodontidae	Aulopiformes	1	38.4
	Paraulopus oblongus	Paraulopidae	Aulopiformes	19	50.9
	Parazen pacificus	Parazenidae	Zeiformes	1	16.6
	Chelidonichthys spinosus	Triglidae	Scorpaeniformes	1	301.7
	Lepidotrigla abyssalis	Triglidae	Scorpaeniformes	5	165.5
	Lepidotrigla hime	Triglidae	Scorpaeniformes	14	509.3
	Lepidotrigla guentheri	Triglidae	Scorpaeniformes	2	147.8
	Hoplichthys gilberti	Hoplichthyidae	Scorpaeniformes	11	271.4
	Syngrops japonicus	Acropomatidae	Perciformes	3	8.9
	Syngrops philippinensis	Acropomatidae	Perciformes	18	50.7
	Malakichthys elegans	Acropomatidae	Perciformes	138	370.0
	Dentex hypselosomus	Sparidae	Perciformes	1	148.2
	Parapercis mimaseana	Pinguipedidae	Perciformes	3	130.2
	Parapercis aurantiaca	Pinguipedidae	Perciformes	1	0.6
	Osopsaron sp.	Percophidae	Perciformes	1	0.5
	Acanthaphritis unoorum	Percophidae	Perciformes	1	0.5
	Champsodon snyderi	Champsodontidae	Perciformes	1	2.5
	Bathycallionymus kaianus	Callionymidae	Perciformes	7	28.5
	Repomucenus virgis	Callionymidae	Perciformes	20	21.3
	Sicyopterus japonicus *	Gobiidae	Perciformes	1	0.2
	Crossorhombus kobensis	Bothiade	Pleuronectiformes	3	21.0
	Arnoglossus polyspilus	Bothiade	Pleuronectiformes	18	738.9
	Pleuronichthys japonicus	Pleuronectidae	Pleuronectiformes	3	21.0
22 Aug. 2007	*Mustelus griseus*	Triakididae	Carcharhiniformes	1	167.7
	Ophisurus macrorhynchus	Ophichthidae	Anguilliformes	1	361.0
	Glossanodon semifasciatus *	Argentinidae	Argentiniformes	17	36.5
	Saurida macrolepis	Synodontidae	Aulopiformes	9	869.9
	Synodus macrops	Synodontidae	Aulopiformes	2	59.7
	Zeus faber	Zeidae	Zeiformes	4	1907.1
	Antigonia rubicunda	Caproidae	Zeiformes	1	1.3
	Chelidonichthys spinosus	Triglidae	Scorpaeniformes	1	354.7
	Lepidotrigla abyssalis	Triglidae	Scorpaeniformes	2	27.7
	Lepidotrigla guentheri	Triglidae	Scorpaeniformes	1	0.7
	Lepidotrigla kishinouyei	Triglidae	Scorpaeniformes	20	537
	Bembras japonicus	Bembridae	Scorpaeniformes	4	183.6
	Syngrops japonicus	Acropomatidae	Perciformes	5	5.1
	Syngrops philippinensis	Acropomatidae	Perciformes	2	5.0
	Chelidoperca pleurospila	Serranidae	Perciformes	8	292.5
	Dentex hypselosomus	Sparidae	Perciformes	18	1599.9
	Evynnis tumifrons	Sparidae	Perciformes	3	432.1
	Upeneus japonicus	Mullidae	Perciformes	19	646.8
	Parapercis sexfasciatus	Pinguipedidae	Perciformes	1	29.9
	Parapercis sexfasciatus *	Pinguipedidae	Perciformes	1	0.1
	Bathycallionymus sp.	Callionymidae	Perciformes	1	0.4
	Repomucenus virgis	Callionymidae	Perciformes	76	53.8

Table III. - Continued.

Date	Species	Family	Order	Number of individuals	Weight (g)
	Calliurichthys japonicus	Callionymidae	Perciformes	1	104.7
	Sicyopterus japonicus *	Gobiidae	Perciformes	1	0.2
	Cryptocentrus sp.	Gobiidae	Perciformes	1	0.3
	Tarphos elegans	Paralichthyidae	Pleuronectiformes	1	1.1
	Crossorhombus kobensis	Bothidae	Pleuronectiformes	9	63.9
	Psettina tosana	Bothidae	Pleuronectiformes	1	11.7
	Plagiopsetta glossa	Samaridae	Pleuronectiformes	1	38.2
	Triacanthodes anomalus	Triacanthodidae	Tetraodontiformes	1	21.2

Keith *et al*. (2008) reported that it was possible to define three post-larval stages (PL0 to PL2) and two juvenile stages (J1 and J2) for *S. lagocephalus*. Both specimens in the present study were post-larval stage 0 (PL0) as defined by Keith *et al*. (2008) because of the appearance of the sucker.

The fish collected with post-larvae of *S. japonicus*

There were a total of 44 species including post-larvae *S. japonicus* collected in two trawl deployments. 25 and 29 species were collected on 18 July 2007 and 24 August 2007, respectively (Tab. III). 10 species (*Chelidonichthys spinosus, Crossorhombus kobensis, Dentex hypselosomus,* juveniles *Glossanodon semifasciatus, Lepidotrigla abyssalis, Lepidotrigla guentheri, Parapercis sexfasciatus, Repomucenus virgis, Syngrops japonicus* and *Syngrops philippinensis*) were collected in both trawl deployments. Most of the fish collected were benthic and epibenthic and inhabitants of the continental shelf that mostly consists of sand and/or mud bottoms.

Otolith analysis

Total age of these post-larvae was estimated as 278 and 286 days for the BSKU 103632 and BSKU 103633 specimens, respectively. Their estimated hatching dates were 13 October and 9 November 2006, for BSKU 103632 and BSKU 103633, respectively. There was no obvious metamorphosis check (Shen and Tzeng, 2002) in the otoliths of the two post-larvae.

DISCUSSION

Identification of *S. japonicus* post-larvae caught at sea

This appears to be the first report of post-larvae of *S. japonicus* caught at sea far from the estuary and the surf zone, or even of any Sicydiinae species. The external morphology of the two post-larvae examined in this study had all of the general characteristics of gobioid larvae, and the meristic characters of these post-larvae were also in accordance with other Sicydiinae species. The use of both morphological and molecular genetic tools enabled these two post-larvae to be identified as *S. japonicus*. Molecular genetic analysis was required to identify them with certainty to the species level, due to the general similarity of Gobioidei larvae. But several morphological characters may be useful for identifying them. The meristic characters such as pectoral fin rays vary greatly among the gobioids and are particularly useful in distinguishing them at the family, genus and species levels (Hoese, 1984; Birdsong *et al*., 1988). There are several reports about the arrangement of melanophores in the post-larval stage of Sicydiinae species (Erdman, 1961; Nishimoto and Kuamo'o, 1997; Benbow *et al*., 2004; Keith *et al*., 2006; Yamasaki *et al*., 2007). There have been descriptions mentioning the pigment located on the lateral side posterior to the anal fin base in post-larvae of *Sicydium plumieri* (Erdman, 1961) and *Stiphodon percnopterygionus* (Yamasaki *et al*., 2007), so it may be present in various different species of Sicydiinae. The melanophores of the pelvic fin base and the ventral chest (Nishimoto and Kuamo'o, 1997) and the red pigment on the back and the caudal fin base in the fresh specimens were also a characteristic of post-larvae in Sicydiinae. These morphological characteristics such as red and black chromatophores may be useful characters to help distinguish these post-larvae from other goby species in future studies on the early life history of Sicydiinae larvae in the ocean.

Oceanic dispersal and recruitment

The finding of two post-larvae of *S. japonicus* is a first step to learn about the oceanic early life in Sicydiinae gobies, which has still remained mysterious due to a lack of information. There were several reports about the oceanic larval duration (OLD) in Sicydiinae species (Radtke *et al*., 1988, 2001; Hoareau *et al*., 2007; Yamasaki *et al*., 2007; Iida *et al*., 2008; Shen and Tzeng, 2008; Lord *et al*., 2010). *Sicyopterus japonicus* and *S. lagocephalus* have the longest OLD (*S. japonicus* from Wakayama in eastern Japan, range: 173 to 253 days, mean ± SD: 208 ± 22 days; Iida *et al*., 2008, *S. lagocephalus* in Reunion Island, range: 133 to 266 days, 199 ± 33 days, Hoareau *et al*., 2007, *S. lagocephalus* in New

Caledonia and Vanuatu, mean ± SD: 131 ± 3.4 days, Lord *et al.*, 2010) compared to any other species of the Sicydiinae. However, the OLD of the two marine post-larvae in this study (278 and 286 days) were the longest OLD values reported for Sicydiinae species, including *S. japonicus*. There were no obvious metamorphosis check mark (Shen and Tzeng, 2002) in the otoliths of the two oceanic post-larvae, which are assumed to be deposited when the fish metamorphosed from the larval to the juvenile stage. This would be expected since these larvae were collected in the ocean and were still at the post-larval stage PL0.

It is possible that the longer OLD in *S. japonicus* in Japan is due to lower water temperature. Indeed, *S. japonicus* in Japan may avoid the lower temperature of the river in the winter of temperate zone as the recruitment to the river is observed in spring. PL0 stage takes place in salt water and individuals acquire the physiological and morphological abilities for the start of metamorphosis and colonization of the adult environment (Murphy and Cowan, 2007; Keith *et al.*, 2008). Their SL (26.8 and 27.5 mm) were within the same size range of post-larvae recruited to a river mouth in Wakayama, Japan (range: 23.5 to 30.0 mm, mean ± SD: 26.3 ± 1.1 mm, N = 761, Iida *et al.*, 2008), suggesting that these were large enough to recruit to a river soon.

The hatching dates of the two post-larvae (13 October and 9 November 2006) is also later than the spawning season of July and August in Wakayama Japan (Iida *et al.,* 2009). However, the spawning periods backcalculated from the otolith increments of recruiting larvae in Taiwan suggested that the spawning of *S. japonicus* was almost year-round, except during winter, with more apparent spawning in autumn, September and October (Shen and Tzeng, 2008). Therefore, it is likely that the two post-larvae in this study may be from more southern latitudes such as the Ryukyu Islands (including Okinawa) or Taiwan and that they probably had a longer OLD due to dispersion in the Kuroshio Current. Iida *et al.* (2010c) showed, using a Lagrangian modelling approach, a numerical simulation, that the Kuroshio could transport larvae of *S. japonicus* northward from Taiwan to northern Japan in about 180 days.

The Kuroshio and long OLD of *S. japonicus* would play an important role to maintain the apparent lack of population structure of this species from Taiwan to northern Japan (Ju, 2001).

It is difficult however, to know where the 2 larvae collected in the present study were residing within the water column when they were collected. The trawls collected many fishes presumably on the bottom, but the *S. japonicus* larvae could have been collected when the trawl was passing through the shallower depths or while they were on the bottom. The larvae could have left the Kuroshio and were swimming across continental shelf to coastal areas or they could have originated more locally. Discrete depth sampling for oceanic goby larvae will be needed to learn about their depth distributions and larval behaviours.

Further studies are also needed on how the oceanic larvae of these gobies can be identified morphologically, where they are distributed, and how much they are either dispersed or retained in various areas to be able to better understand the early life history of these interesting amphidromous gobies.

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