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Northernmost record of the bicolor eel *Anguilla bicolor* from mainland Kyushu, and notes on previous occurrences in Japan

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Abstract: A single individual of the bicolor eel *Anguilla bicolor* that is distributed mainly in the tropics of the Indo-Pacific was collected in the Shode River, Miyazaki Prefecture, mainland Kyushu, Japan and is reported as the northernmost record of the distribution of this species. This individual was 466 mm in total length and was at the yellow eel stage, which is the continental growth phase of their catadromous life history. In addition, the previous occurrences of this species in Japan, which were mainly written in Japanese, were reviewed in order to share the information worldwide.

Keywords : *Anguilla bicolor*, distribution, Kuroshio, northernmost record

1. Introduction

The catadromous freshwater eels, genus *Anguilla*, have a unique life history of open ocean spawning, transport of their leptocephalus larvae by ocean currents towards their continental glass eel recruitment habitats and then spending most of their lives as yellow eels before transforming into silver eels (silvering) to begin their migration to offshore spawning areas (TESCH, 2003). The leptocephalus period is relatively longer than the larval periods of most other fish species, and their larval morphology is uniquely well suited for transportation and dispersal by ocean currents (MILLER, 2009; KUROKI *et al*., 2014). The geographic distributions of anguillid eels during their continental growth phase are greatly affected not only by the location of species-specific spawning areas and their larval durations, but also by physical oceanographic conditions such as ocean currents (KIMURA and TSUKAMOTO, 2006; ZENIMOTO *et al*., 2009). This suggests that eel populations and distributions are sensitive to changes in the marine environments.

The bicolor eel *Anguilla bicolor* is widely distributed mainly in the tropics of the western Indo-Pacific and has been classified further into two populations or subspecies, which are the Indian Ocean population (or subspecies, Indian bicolor eel *A. bicolor bicolor*) and the northwestern Pacific population (or subspecies, Pacific bicolor eel *A. bicolor pacifica*) (EGE, 1939; MINEGISHI *et al*., 2012; WATANABE *et al*., 2014). It was reported that glass eels of *A. bicolor* recruited to a small island south of mainland Japan by being transported to that area by the Kuroshio Cur-

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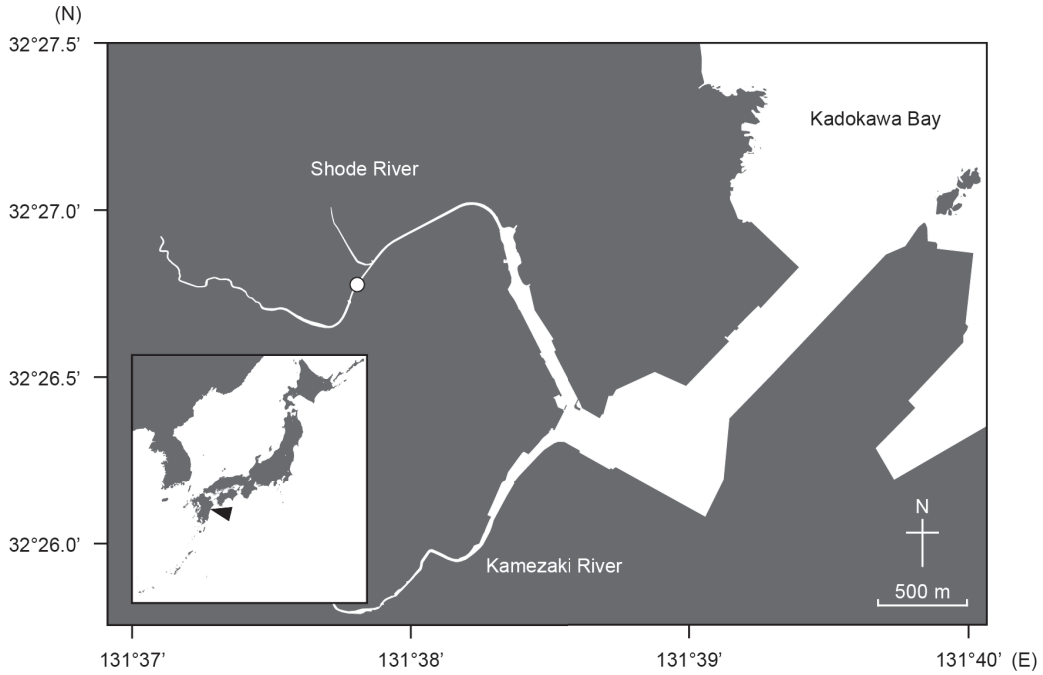

Fig. 1 Map of the study area and its location in Japan (inset) showing the sampling site (open circle) in the Shode River where the *Anguilla bicolor* yellow eel was collected.

rent, and that location had been considered to be the northern limit of the geographical distribution of this species (YAMAMOTO *et al*., 2000). Recently, occurrences of *A. bicolor* glass eels in mainland Kyushu have been reported (SEKIYA *et al*. 2017, 2018), but it is unknown if those eels can survive and grow in the inland waters of the temperate zone. *Anguilla bicolor* likely faces a variety of threats (*e.g*., barriers to migration, habitat loss and unsustainable glass eel capture for aquaculture) and they have been listed as Nearly Threatened in the IUCN Red List based on an expected reduction in population size of close to 30% over the next 21 years (PIKE *et al*., 2019). Therefore, basic information on the geographic distribution and migration ecology of this species is important, especially because our knowledge about this species is much less than for temperate eels such as the Japanese eel *A. japonica* and the European eel *A. anguilla*.

This study reports the new northernmost record of the distribution of *A. bicolor* based on a yellow eel that that was living in eastern Kyushu, the southernmost landmass of mainland Japan. In addition, there are several previous reports of occurrences of this species in southern Japan, but the information was not sufficiently shared worldwide because they were mostly written in Japanese, so this study also reviews the previous reports of the species in Japan.

2. Materials and methods

A single individual of *A. bicolor* was collected by an electric shocker on 22 October 2019 in the middle reach (freshwater area) of the Shode River in Miyazaki Prefecture, eastern mainland Kyushu, Japan, which is a temperate area (32° 26'44" N, 131° 37'47" E; Fig. 1).

The total length (TL, mm), predorsal length (PDL, mm), preanal length (PAL, mm), head length (HL, mm), body weight (BW, g), horizontal (hED, mm) and vertical (vED, mm) left eye diameters, and left pectoral fin length (FL, mm) were measured. Then, condition factor (K), eye index (EI), pectoral-fin index (FI) and anodorsal index (AD%) were calculated according to EGE (1939), PANKHURST (1982) and HAGIHARA *et al.* (2012), as follows: $K = 10^6\ BW\ TL^{-3}$, $IE = 100\ \pi\ TL^{-1}\ [0.25\ (hED + vED)]^2$, $FI = 100\ FL\ TL^{-1}$, $AD\% = 100\ (PAL - PDL)\ TL^{-1}$. Silvering stage of the eel was determined by the examination of the color of the pectoral fins and the body coloration according to the silvering index stages made for *A. japonica* (OKAMURA *et al.*, 2007). This silvering index has some validity for other anguillid species such as *A. bicolor*, the Celebes eel *A. celebesensis* and the Indo-Pacific eel (giant mottled eel) *A. marmorata* (HAGIHARA *et al.*, 2012, 2020).

A fin clip was collected from the dorsal fin and genetic identification was carried out based on partial sequences of the mitochondrial 16S ribosomal RNA gene following the standard protocol of AOYAMA *et al.* (1999). The obtained sequence of the eel was compared to the RefSeq (NCBI Reference Sequence Database) sequences of all 19 species/subspecies of the genus *Anguilla* (*A. anguilla*, INSD accession number NC_006531.1; *A. australis australis*, NC_006532.1; *A. australis schmidtii*, NC_006533.1; *A. bengalensis bengalensis*, NC_006544.1; *A. bengalensis labiata*, NC_006543.1; *A. bicolor bicolor*, NC_006534.1; *A. bicolor pacifica*, NC_006535.3; *A. borneensis*, NC_006536.1; *A. celebesensis*, NC_006537.1; *A. dieffenbachii*, NC_006538.1; *A. interioris*, NC_006539.1; *A. japonica*, NC_002707.2; *A. luzonensis*, NC_011575.1; *A. marmorata*, NC_006540.1; *A. megastoma*, NC_006541.1; *A. mossambica*, NC_006542.1; *A. obscura*, NC_006545.1; *A. reinhardtii*, NC_006546.1; *A. rostrata*, NC_006547.2).

The measurements and fin-clipping were carried out under anesthesia using 0.2‰ eugenol, and after recovery from anesthesia, the eel has been displayed alive in an exhibition tank at the International Eel Laboratory (Misato-town, Miyazaki Prefecture, Japan) for the education and awareness of the public.

3. Results

Both morphological and genetic characteristics confirmed that the 466 mm TL eel collected in this study was *A. bicolor* that was at the yellow eel stage (Fig. 2; Table 1). The AD% of the eel was a low value (−0.43), which was within the AD% range of *A. bicolor* (−6.0–3.9; EGE, 1939) and outside of the AD% ranges of other anguillid species distributed in East Asia (*A. japonica*: 5.0–12.9; *A. luzonensis*: 9.3–13.9; *A. marmorata*: 12.0–18.9; EGE, 1939; WATANABE *et al.*, 2009).

The partial sequence of the mitochondrial 16S ribosomal RNA gene of the eel in this study (619 bp) was highly similar to the RefSeq sequence of *A. bicolor pacifica* (99.8% similarity), followed by *A. bicolor bicolor* (99.4%), *A. obscura* (99.2%) and *A. interioris* (98.9%).

Other morphological characteristics and indices are described in Table 1, which indicate that the eye diameter was too low to be a silver eel and the pectoral fin was not black, so the eel was a yellow eel as seen in Fig. 2.

4. Discussion

According to the previous reports described below, the single eel collected in this study is presently the northernmost record of *A. bicolor*. One factor to consider though, because some species of glass eels are presently transported between different regions for aquaculture, is that stocking captured eels into natural water bodies

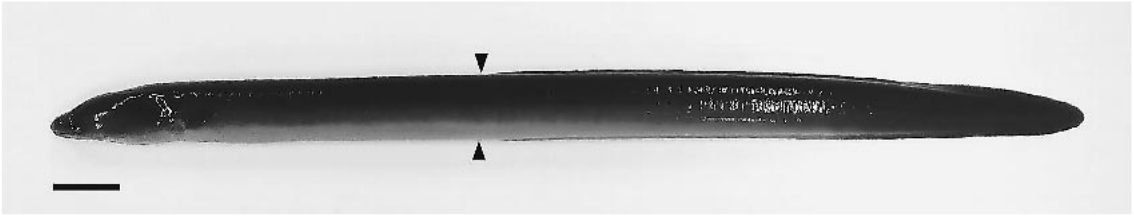

Fig. 2 Lateral view of the 466 mm *Anguilla bicolor* yellow eel collected in this study. Arrowheads show the anus (anal fin just posterior of that) and origin of the dorsal fin. Scale bar is 30 mm.

Table 1. Morphometric characteristics of the *Anguilla bicolor* yellow eel collected in mainland Kyushu, Japan.

Morphometric character and index	Value
Total length (mm)	466
Body weight (g)	201
Head length (mm)	62.4
Snout length (mm)	10.4
Predorsal length (mm)	193
Preanal length (mm)	191
Horizontal eye diameter (mm)	4.81
Vertical eye diameter (mm)	4.7
Pectoral-fin length (mm)	17.6
Ano-dorsal index (AD%)	−0.43
Eye index	3.81
Pectoral-fin index	3.78
Condition factor	1.99
Silvering index	Y1

is often conducted globally for the purposes of conservation and/or enhancement of fisheries catches. In Japan, the stocked eels can sometimes unintentionally include non-native eel species such as the European eel, which have been collected in some rivers (Arai *et al.*, 2017). A single individual of *A. bicolor* was reported from the Geum River in South Korea, but it was suggested that it was an escaped eel from culture ponds near the river (Hong *et al.*, 2017). However, eels have not been stocked into the Shode River where the *A. bicolor* was collected in this study. In addition, there are no eel farms or eel restaurants in the Shode River area. Therefore, the *A. bicolor* collected in this study is considered to be a naturally recruited individual rather than an artificially introduced one. The previous northernmost record of *A. bicolor* was of glass eels collected at the mouth of the Hitotsuse River (32° 03′ N) (Sekiya *et al.* 2017, 2018), whereas the Shode River (32° 26′44″ N), the collection site of this study, is further north. Furthermore, the previous northernmost record of the juvenile river life stage (yellow eel stage) of *A. bicolor*, after recruiting to rivers, was at Iriomote Island (24° 23′ N) near the southern edge of Japan (closer to Taiwan than to mainland Japan) (Inoue *et al.*, 2021), so the yellow-phase *A. bicolor* collected in this study substantially exceeded the previous record.

The northern limit of geographic distribution of *A. bicolor* had been previously considered to be Luzon Island, Philippines (Ege, 1939; Tabeta *et al.*, 1976), but the presences of *A. bicolor* were reported in Taiwan (Tzeng and Tabeta, 1983; Leander *et al.*, 2012) and southern part of mainland China (Chen *et al.*, 2010; Zhan *et al.*, 2018), and glass eels have been sporadically reported in the Kuroshio region of southern Japan in recent years (Table 2; Fig. 3). Six *A. bicolor* glass eels were found at the mouth of the Ibaro River on Tanegashima Island during 1999–2003 (Shinoda, 2004) and 19 glass eels were collected the Miyanoura port of Yakushima Island in 1997 during 4 different months, with the highest catch being in October (Yamamoto *et al.*, 2000, 2001). Twenty

Table 2. Information on the occurrences of *Anguilla bicolor* in Japan in previous studies and in this study.

Latitude	Longitude	Area	Location	Year	N	TL (mm)	Stage	Reference (Language)
32° 26′ 44″ N	131° 37′ 47″ E	Mainland Kyushu	Shode River	2019	1	466	Yellow eel	This study (English)
32° 03′ N	131° 30′ E	Mainland Kyushu	Hitotsuse River	2014–2015	5	~50	Glass eel	Sekiya et al. 2017 (Japanese)
32° 03′ N	131° 30′ E	Mainland Kyushu	Hitotsuse River	2016	4	~50	Glass eel	Sekiya et al. 2017 (Japanese)
32° 03′, 31° 54′ N	131° 30′, 131° 27′ E	Mainland Kyushu	Hitotsuse & Oyodo River	2016–2017	7	~50	Glass eel	Sekiya et al. 2018 (Japanese)
31° 54′ N	131° 27′ E	Mainland Kyushu	Oyodo River	2013–2014	2	~50	Glass eel	Sekiya et al. 2017 (Japanese)
31° 54′ N	131° 27′ E	Mainland Kyushu	Oyodo River	2016	2	~50	Glass eel	Sekiya et al. 2017 (Japanese)
30° 32′ N	130° 55′ E	Tanegashima Island	Ibaro River	1991–2003	6	~50	Glass eel	Shinoda 2004 (Japanese)
30° 25′ N	130° 34′ E	Yakushima Island	Miyanoura port	1997	19	~50	Glass eel	Yamamoto et al. 2000 (English)
24° 23′ N	123° 45′ E	Iriomote Island	Irrigation channel	2019	2	652, 713	Yellow eel	Inoue et al. 2021 (Japanese)
24° 23′ N	123° 45′ E	Iriomote Island	Irrigation channel	2019	1	879	Silver eel	Inoue et al. 2021 (Japanese)

N, number; TL, total length.

A. bicolor glass eels were also collected at the mouths of the Oyodo and Hitotsuse rivers in mainland Kyushu during 2013–2017 (Sekiya *et al.*, 2017, 2018). In addition, two *A. bicolor* yellow eels and one silver eel were found in an irrigation channel on Iriomote Island (Inoue *et al.*, 2021). These occurrence reports are important information on the geographical distribution of this species, but all of them except Yamamoto *et al.* (2000), were written in Japanese, so the review in this study should be useful for sharing the information worldwide.

Global environmental changes, such as global warming, have a significant impact on the physical environment of the ocean, including water temperature, currents, dissolved oxygen concentration, pH and sea level, which in turn affects marine organisms (Kumagai *et al.*, 2018; Mckenzie *et al.*, 2021). Future global warming is expected to increase the velocity of the Kuroshio Current (Sakamoto *et al.*, 2005), and this is likely to have a significant impact on the transportation, survival and geographic distribution of many marine organisms. In fact, the northward extension of the distributions of several marine fish and diadromous fish species in the Kuroshio region has already been reported (Yamakawa *et al.*, 2018, 2020). Although it is difficult to conclude now whether the recent set of reports of the occurrence of *A. bicolor* in southern Japan are a result of changes in the marine environment, this study provides important information for monitoring future changes in the distribution of eels of this species that typically lives in tropical areas.

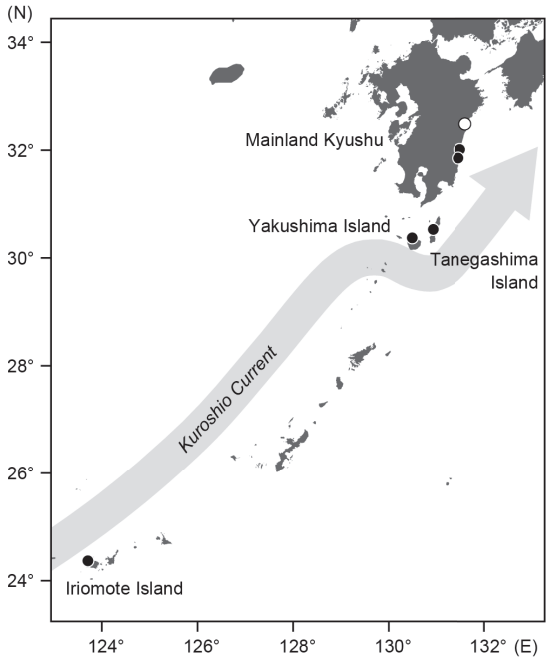

Fig. 3 Map of the southern part of Japan showing sites where *Anguilla bicolor* were recorded in previous studies (closed circles) and in this study (open circle). All sites were glass eels except for the present study and Iriomote Island.

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