First Japanese records of *Anguilla luzonensis* (Osteichthyes: Anguilliformes: Anguillidae) glass eels from Okinawa-jima Island, Ryukyu Archipelago, Japan

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Six specimens of *Anguilla luzonensis* Watanabe, Aoyama, and Tsukamoto, 2009 were collected from Okinawa-jima Island, Okinawa Prefecture, southern Japan. This species was previously known from Luzon Island, Mindanao Island and Taiwan. Therefore, the present specimens represent the first records of *A. luzonensis* from Japan and extend the northern distribution limit of the species. A new standard Japanese name, “Uguma-unagi”, is proposed for the species.

**Key Words:** Ichthyology, catadromous fish, northernmost distribution, upstream migration, 16S rRNA.

**Introduction**

The freshwater eel genus *Anguilla* Garsault 1764, comprises 19 species and subspecies worldwide (Ege 1939; Castle and Williamson 1974; Watanabe et al. 2009). Only three species of *Anguilla* have been reported in Japan (Hattooka 2013), including Japanese Eel, *Anguilla japonica* Temminck and Schlegel, 1847, Giant Mottled Eel, *A. marmorata* Quoy and Gaimard, 1824, and Indian Shortfin Eel, *A. bicolor pacifica* Schmidt, 1928.

Recently, the 16th anguillid eel species, Luzon Mottled Eel, *A. luzonensis* Watanabe, Aoyama, and Tsukamoto, 2009, was discovered on Luzon Island in the northern Philippines (Watanabe et al. 2009). The northernmost record of the species has been from Taiwan (Leander et al. 2012; Han et al. 2016); however, six specimens of *A. luzonensis* glass eels were collected on Okinawa-jima Island, Okinawa Prefecture, southern Japan. These specimens are described here as the first records of *A. luzonensis* from Japan, and the northernmost records of this species. Here, we describe the morphological characteristics of the specimens of *A. luzonensis* glass eels collected in Japan, and proposes a new standard Japanese name for this species.

**Materials and Methods**

Glass eels were collected using a hand net and underwater lamp (Hapyson, YF-500) at the estuary of the Hiji River, Okinawa-jima Island, on 9 October 2018, 30 August 2019, 31 August 2019, and 29 October 2019. The collection of glass eels took place at night during the high tide of the new moon for two days. The underwater lamp was deployed for 2 hours from 90 minutes before high tide to 30 minutes after high tide. Sampling of the glass eels was carried out with the permission from the Fishery Division of the Okinawa Prefecture Government.

Methods for measures of the collected glass eels followed Tzeng and Tabeta (1983). Ontogenetic stages in the glass eels were categorized according to Tesch (2003) and represented the following characteristics: VA stage was completely metamorphosed, eel-like in form, no external pigment (glass eel) except for the caudal spot; V B stage was no pigment on the back, body or tail region, except for the skull, caudal spot and some rostral pigment; VI AI stage was development of pigmentation along the whole dorsum, post-anal dorso-lateral pigment develops, post-anal, no clear mediolateral pigment; VI AII stage was no pre-anal ventrolateral pigment, clear pre-anal development of mediolateral pigment, postanally over almost entire dorsum, pigment rows along the myosepta, and in places doubling of the mediolateral melanophores; VI AIV stage was clear development of pre-anal ventrolateral pigmentation, initially with a doubling of the mediolateral melanophores in the pre-anal region in some places, a post-anal pigment between the myosepta in the ventral region, and finally a...
similar changes in the pre-anal region; VI₈ stage was pigment rows along the myosepta becoming indistinct, and in addition, Lateral line still recognisable, as are the individual melanophores on the head, cheek, behind and below the eyes and on the lower jaw. All the specimens were put under anesthesia with 2-phenoxyethanol before being measured and observed. The vertebral count was made from soft X-ray photo, and the mean vertebral formula followed Böhlke (1982). The specimens were identified using morphological characteristics and molecular analysis according to Leander et al. (2012). Based on the measured total length (TL), preanal length (PAL), and predorsal length (PDL) of the specimens, we then calculated the proportion of the vertical distance between the origin of the dorsal fin and the anus to TL using the following equation: ADL% TL= (PAL−PDL)/ TL×100. We confirmed that the specimens had caudal pigmentations and ADL% TL values of <13%, which were subsequently identified by molecular methods.

Tissue samples of the glass eels were dissected from the right side of the body. DNA extraction, polymerase chain reaction (PCR) amplification of the 16S rRNA, and sequencing were conducted according to Tawa et al. (2012). The primers for PCR amplification and sequencing were 5'-GGT CCW RCC TGC CCA GTG A-3' and 5'-CCG GTC TGR ACY AGA TCA CGT-3'. The generated sequences were compared to the 16S rRNA sequences of known species and subspecies in the genus Anguilla that occur in the Indo-Pacific waters retrieved from the National Center for Biotechnology Information GenBank database (accession nos.: AB021755, AB097702, AB188425, AB188426, AB188442, LC218809, AB303369, AB490285, AJ244824.2, KT728351, LC218744, LC222580, LC433764, and MH289501). These sequences were used to construct a phylogenetic tree, and then species were subsequently identified by molecular methods.

Materials examined. Six specimens: the Hiji River, Okinawa-jima Island, Okinawa Prefecture, 26°43′48″N, 128°10′10″E, KYUM-PI-05434, 49.8 mm in total length, collected from Okinawa-jima island, Japan.

Anguilla luzonensis Watanabe, Aoyama, and Tsukamoto, 2009
[New standard Japanese name: Uguma-unagi] (Figs 1, 2; Tables 1, 2)

Fig. 1. Fresh specimen of Anguilla luzonensis glass eel, KYUM-PI-05434, 49.8 mm in total length, collected from Okinawa-jima island, Japan.

Habitat and biology. This species was firstly collected from a small stream in the upper reaches of the Pinacanauan River system, a tributary of the Cagayan River on northern Luzon Island, the Philippines (holotype; Watanabe et al. 2009).

Knowledge on this species is mainly pertains to in its early
First records of *Anguilla luzonensis* from Japan

Life history, and we refer to previous studies on the presumed spawning area and transport routes of *A. luzonensis*. Comparison of otolith increment counts between *A. marmorata* and *A. luzonensis* suggested that they may have at least partially overlapping spawning areas (Han et al. 2016). Previous studies indicate that the estimated spawning area of *A. marmorata* (12–17°N, 131–143°E; Kuroki et al. 2009) overlaps that of *A. japonica* (12–16°N, 137–143°E; Tsukamoto 1992, 2006; Kuroki et al. 2006, 2009). The spawning area of *A. luzonensis* may also be in these ranges (Han et al. 2016). In fact, Kuroki et al. (2012) reported that a 29.2 mm leptocephalus of *A. luzonensis* was collected at 13°N, 140°E in 2009. A simulated tracer experiment indicated that leptocephali of *A. luzonensis* may reach the North Equatorial Current bifurcation site when the Kuroshio Current is intensified, and leptocephali of *A. luzonensis* are likely to be transported by the northward-flowing Kuroshio Current in the direction of Luzon Island (Han et al. 2016). Therefore, *A. luzonensis* are likely to be transported westward from their spawning areas by the North Equatorial Current, and then transported to Okinawa-jima Island by the Kuroshio Current.

**Remarks.** The specimens collected as a part of our monthly sampling for ecological studies of glass eels on Okinawa-jima Island, Okinawa Prefecture, the specimens were identified as *A. luzonensis* by the combination of morphological and molecular methods. Morphometric and caudal pigmentation characteristics of all known species and subspecies of *Anguilla* in Japan are shown in Table 2 (Tzeng 1982). The glass eel of *A. luzonensis* is easily distinguished from *A. b. pacifica* by ADL% TL value of >5% (vs. <5%), and from *A. japonica* by the presence of caudal pigmentation (vs. absence). However, the glass eels of *A. luzonensis* and *A. marmorata* are not completely distinguished because they have the same caudal pigmentation pattern and some

Table 1. Counts and measurements of *Anguilla luzonensis* glass eels.

| Museum reg. no. of *Anguilla luzonensis* | KYUM-PI-05433 | KYUM-PI-05434 | KYUM-PI-05435 | KYUM-PI-05436 | KYUM-PI-05437 | KYUM-PI-05438 |
|----------------------------------------|---------------|---------------|---------------|---------------|---------------|---------------|
| Total length (mm)                      | 48.4          | 49.8          | 55.1          | 50.4          | 51.8          | 52.4          |
| Counts                                 |               |               |               |               |               |               |
| Predorsal vertebrae                    | 24            | 24            | 22            | 26            | 23            | 21            |
| Preanal vertebrae                      | 35            | 36            | 33            | 36            | 37            | 33            |
| Total vertebrae                        | —             | 104           | 101           | 103           | —             | 101–104       |
| Mean                                   |               |               |               |               |               | 102.7         |
| Measurements                           |               |               |               |               |               |               |
| As % of total length                   |               |               |               |               |               |               |
| Head length                            | 13.2          | 12.3          | 10.3          | 12.7          | 11.3          | 10.3          |
| Predorsal length                       | 29.2          | 27.8          | 26.5          | 28.2          | 26.1          | 26.8          |
| Preanal length                         | 37.7          | 38.8          | 35.3          | 39.2          | 36.5          | 36.5          |
| Ano-dosal length                       | 8.5           | 11.0          | 8.8           | 11.1          | 10.4          | 9.8           |
| Body height at dorsal fin origin       | 4.2           | 4.4           | 4.1           | 4.1           | 3.9           | 4.6           |
| As % of head length                    |               |               |               |               |               |               |
| Snout length                           | 16.2          | 15.4          | 17.7          | 14.0          | 16.7          | 18.3          |
| Eye diameter                           | 13.0          | 11.5          | 10.7          | 10.7          | 11.8          | 14.4          |
| Upper-jaw length                       | 20.2          | 18.3          | 21.3          | 20.0          | 18.3          | 21.9          |
| Lower-jaw length                       | 21.5          | 20.7          | 23.5          | 23.2          | 20.6          | 26.1          |
| Pectral fin length                     | 28.1          | 23.1          | 27.2          | 22.3          | 16.0          | 19.8          |

Fig. 2. Caudal fin and caudal pigmentation pattern of *Anguilla luzonensis* glass eel (fresh specimens), KYUM-PI-05434.
According to Leander et al. (2012), among specimens with caudal pigmentation, those with ADL% TL values $>13\%$ are $A.\ marmorata$ and those with values of $<13\%$ are classified as $A.\ marmorata$ or $A.\ luzonensis$. Therefore, specimens with an ADL% TL $<13\%$ are distinguished between $A.\ marmorata$ and $A.\ luzonensis$ using molecular methods. A neighbor-joining tree based on the genetic sequences is shown in Fig. 3. As a result of comparison with other species of $Anguilla$ in Indo-Pacific waters based on 16S sequences, the sequences of the six specimens were found to be contained in the clade of $A.\ luzonensis$. Accordingly, the six specimens

### Table 2. Comparison of morphometric and caudal pigmentation characters among the four species/subspecies of $Anguilla$ (glass eels). All values are in millimeters except for ADL% TL and indicate range (mean ± SD). Present study was measured after anesthesia with 2-phenoxyethanol, while Tzeng (1982) was measured after fixation with 10% formalin solution.

| Characteristic of caudal pigmentation | Present study | Tzeng (1982) |
|--------------------------------------|--------------|--------------|
| **A. luzonensis** (n=6)               |              |              |
| Total length                         | 48.4–55.1 (51.3±2.1) | 45.3–57.8 (53.0±4.5) |
| Predorsal length                     | 13.5–14.6 (14.1±0.3) | 12.0–15.1 (13.2±1.6) |
| Preanal length                       | 18.2–19.8 (19.1±0.5) | 15.9–19.5 (18.5±1.6) |
| Ano-dosal length                     | 4.1–5.6 (5.1±0.5)   | 3.8–7.0 (5.3±1.4)   |
| ADL% TL                              | 8.5–11.1 (9.9±1.0)  | 8.2–13.0 (10.0±2.4) |
| Caudal pigmentation                  | Present       | Absent       |
| Coefficient                          | A dense belt of melanophores on the caudal peduncle | — |
| Characteristic of caudal pigmentation |              |              |
| **A. japonica** (n=5)                |              |              |
| Total length                         | 45.3–57.8 (53.0±4.5) | 46.5–58.0 (53.0±4.6) |
| Predorsal length                     | 12.0–15.1 (13.2±1.6) | 9.0–14.4 (11.4±0.9) |
| Preanal length                       | 15.9–19.5 (18.5±1.6) | 17.0–23.9 (19.1±1.3) |
| Ano-dosal length                     | 3.8–7.0 (5.3±1.4)   | 6.2–9.9 (7.7±1.0)   |
| ADL% TL                              | 8.2–13.0 (10.0±2.4) | 13.3–20.4 (15.6±1.8) |
| Caudal pigmentation                  | Present       | Present      |
| Coefficient                          | A dense belt of melanophores on the caudal peduncle | Fan-shaped of melanophores on the tip of caudal fin |
| Characteristic of caudal pigmentation |              |              |
| **A. marmorata** (n=30)              |              |              |
| Total length                         | 46.5–58.0 (53.0±4.6) | 45.9–49.2 (47.3±1.1) |
| Predorsal length                     | 9.0–14.4 (11.4±0.9) | 16.5–18.6 (17.8±0.6) |
| Preanal length                       | 17.0–23.9 (19.1±1.3) | 16.8–18.7 (18.0±0.6) |
| Ano-dosal length                     | 6.2–9.9 (7.7±1.0)   | 0.2–0.1 (0.1±0.4)   |
| ADL% TL                              | 13.3–20.4 (15.6±1.8) | 0.2–0.8 (0.4±0.2)   |
| Caudal pigmentation                  | Present       | Present      |
| Coefficient                          | A dense belt of melanophores on the caudal peduncle | Present |
| Characteristic of caudal pigmentation |              |              |
| **A. bicolor pacifica** (n=13)       |              |              |
| Total length                         | 45.9–49.2 (47.3±1.1) | 45.9–49.2 (47.3±1.1) |
| Predorsal length                     | 16.5–18.6 (17.8±0.6) | 16.5–18.6 (17.8±0.6) |
| Preanal length                       | 16.8–18.7 (18.0±0.6) | 16.8–18.7 (18.0±0.6) |
| Ano-dosal length                     | 0.2–0.1 (0.1±0.4)   | 0.2–0.1 (0.1±0.4)   |
| ADL% TL                              | 0.2–0.8 (0.4±0.2)   | 0.2–0.8 (0.4±0.2)   |
| Caudal pigmentation                  | Present       | Present      |
| Coefficient                          | A dense belt of melanophores on the caudal peduncle | Fan-shaped of melanophores on the tip of caudal fin |
| Characteristic of caudal pigmentation |              |              |

Fig. 3. Neighbor-joining phylogenetic tree of species of $Anguilla$ in the Indo-Pacific waters based on partial 16S rRNA sequences. The numbers beside internal branches indicate bootstrap probabilities for 1,000 replicates.
were identified as *A. luzonensis* based on their morphometric characteristics and DNA sequences. In present study, we followed Leander et al. (2012) to identify *A. luzonensis* glass eels using ADL% TL value of <13%. However, Shiritori et al. (2016) reported that the range of ADL% TL for *A. luzonensis* glass eels was 9.6–14.7 (n = 4), and included specimen with ADL% TL value of >13%. On the basis of the above report, key to the species of *Anguilla* (glass eel) in Japan is presented.

In Japan, there have been reports of exotic eel species imported for aquaculture from Europe and Southeast Asia escaping or being released from eel culture ponds into the rivers (Tabeta et al. 1976, 1977; Arai et al. 2017). Eels are currently cultured in a pond in Kin Town, Okinawa-jima Island. However, there are no eel culture ponds around the Hiji River Basin, where the specimens were collected. Thus, our specimens are not escapes from culture ponds.

In Watanabe (2019), a Japanese name of “Ruson-unagi” was used. However, because this Japanese name is based on unpublished data, it is not possible to determine retrospectively which species is *A. luzonensis*, making “Ruson-unagi” used by Watanabe (2019) ineligible as a standard Japanese name. Therefore, we herein propose a new standard Japanese name, “Uguma-unagi” for this species based on the specimens (KYUM-PI-05434). The proposed species name is based on the body color of adult of this species [see photo in Watabata et al. (2009)] and is a combination of “uguma” meaning “sesame” in the Okinawan dialect to express the irregular mottled coloration pattern on the body of the species, and “unagi” meaning “eel” in Japanese.

### Key to the species of *Anguilla* (glass eel) in Japan

The following characteristics of *Anguilla biclor pacifica*, *A. japonica* and *A. marmorata* were from Leander et al. (2012).

1. **ADL% TL value of less than 5%; fan-shaped of mottled coloration pattern on the tip of caudal fin** ...... *A. biclor pacifica*
   — **ADL% TL value of more than 5%** ......... ............................. 2
2. **Absence of caudal pigmentation** .......... *A. japonica* ...... ............................. 3
   — **Presence of dense belt of caudal pigmentation** ................. 3
3. **ADL% TL value of more than 14.7%** .... *A. marmorata* .... ............................. 4
   — **ADL% TL value of more than 14.7% or lower** ...... ............................. 4
4. **Identification by molecular methods is required** ...... ............................. *A. marmorata* or *A. luzonensis*

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