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The European eel, *Anguilla anguilla* (L.), in Japanese waters

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Abstract
To gain information about the relative proportion of introduced anguillid eel species inhabiting Japanese natural waters, 98 eels were collected and genetically identified – 46 from the Uono River, Niigata, Japan, during their downstream spawning migration, and 52 silver eels from the East China Sea. Species identification using RFLP analysis of the mitochondrial 16SrRNA domain suggested that the European eel, *Anguilla anguilla*, was more abundant (93.5%) than the Japanese eel, *A. japonica*, (4.3%) in the Uono River. One specimen was identified as American eel, *A. rostrata*, which is the first record of genetic identification of this species in Japanese waters. In addition, a migrating European silver eel was identified among the sample of silver eels in the East China Sea. The genetic species identification of these specimens was in agreement with the morphological data. The present study suggests that imported Atlantic eels develop normally to metamorphosis into the silver phase and then begin the oceanic spawning migration.

Keywords: anguillid eel, species identification, mitochondrial DNA, introduced species.

Introduction
The importance of anguillid eels as a fishery resource in Japan has led to large-scale imports of a variety of species from around the world. While only two species, *A. japonica* and *A. marmorata*, naturally inhabit Japanese inland waters (Tabeta *et al.* 1977), glass eels of a total of ten species of *Anguilla* have been commercially imported to Japan from other regions, such as Indonesia, the Philippines, New Zealand, USA and Europe. These introduced eels have dispersed into natural river systems by artificial releases, which were carried out for the purpose of eel stocking, or as a result of eels accidentally escaping from culture ponds (Tabeta *et al.* 1977). Zhang *et al.* (1999) recently found the European eel, *A. anguilla*, in two of six sampling areas in southern Japan, identifying 31.4% of the eels collected from Shinjiko Lake and 12.4% from Mikawa Bay as European eels, the remainder being Japanese eels. This relatively high proportion of European eels in some areas necessitates an assessment of the impacts of introduced eels on the conservation biology of the Japanese native eel species and the local ecosystems. However, the characteristics of introduced eels in Japanese natural waters, such as their growth, maturation and migration, have never been studied.

This paper presents our findings on the extraordinary dominance of European eel in a Japanese river, and on a reproductively maturing European silver eel in the East China Sea, based on a species identification method using mitochondrial 16SrRNA domains.

These findings, and especially the capture of a sexually maturing European silver eel off Japan, suggest that studies on the ecology of introduced eel species in Japanese waters are urgently needed to assess possible risks to native eel stocks in Japan.

Materials and methods

Specimens collected in Japanese waters

Liver tissue samples were taken from a total of 46 eels collected by commercial weir in the Uono River, Niigata, Japan, during the downstream migration season, July to November, in 1997. In addition, 52 eel specimens were collected by dip-netting from a fishing boat in the East China Sea about 70 km off Goto Island during 1997-98 (Figure 1).

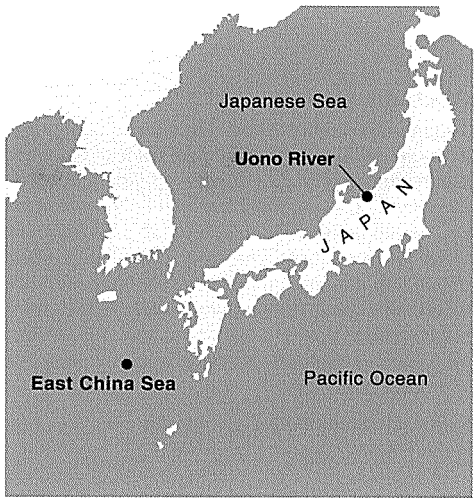

Figure 1. Sampling localities of the anguillid eels analyzed in the present study.

The liver tissue extracted from each eel was minced in 95% Ethanol or in a buffer containing 8M urea, 10 mM Tris-HCl pH 8.5, 125 mM NaCl, 50 mM EDTA and 1% v/w SDS (Aoyama & Tsukamoto 1997). After tissue samples were taken, eel specimens were preserved in 20% formalin. The eels collected in the Uono River could be classified as either sexually advanced yellow eels or silver eels based on their body coloration observed at the time of sampling. The specimens from the East China Sea were obviously silver-phase eels (GSI 1.94-3.53 in females, a large eye diameter and silver coloration), likely to be in their spawning migration.

Species identification using PCR-RFLP analysis

Species identification using RFLP analysis of the mitochondrial 16SrRNA domain was carried out according to Aoyama *et al.* (2000). Briefly, DNA purification was carried out with a phenol/chloroform standard protocol and PCR amplification for the mitochondrial 16S ribosomal RNA gene using the primers L1854 and H 3058. The PCR products were cleaved by six restriction enzymes: Alu I, Bsp1286 I, EcoT14 I, Hha I, Mva I (Takara Shuzo Co., Ltd), and BbrPI (Toyobo Co., Ltd), and finally compared with previously described species-specific restriction fragment patterns (Aoyama *et al.* 2000).

The mitochondrial 16SrRNA domain of one specimen collected in the East China Sea was sequenced and compared with those deposited in EMBL/GenBank/ DDBJ, to confirm the species identification by RFLP analysis.

Observations of the morphological key characters described in Ege (1939) were made when possible.

Results

The genetic analyses indicated that 43 of the 46 specimens (93.5%) from the Uono River were European eels and one was an American eel, *A. rostrata* (Table 1). Remarkably, only two specimens were identified as Japanese eels. Although detailed morphological observations could not be conducted because of absence of complete specimens, their external morphological characteristics (long-finned with plain skin, see Ege 1939) were consistent with the genetic species identification.

Table 1. Results of the species identification using PCR-RFLP method.

Sampling locality	Number of specimens analyzed	*A. japonica*	*A. anguilla*	*A. rostrata*	Others
Uono River, Niigata	46	2(4.3%)	43(93.5%)	1(2.1%)	0
East China Sea	52	51(98.0%)	1(2%)	0	0

All but one of the silver eels collected in the East China Sea were genetically identified as Japanese eels, the outlying specimen being unexpectedly found to be a European eel (Table 1). This specimen was collected around 32'00"N, 132'19"E in March 1998, and had a total length of 838 mm, a body weight of 961.6 g, a GSI of 2.41 and an eye index of 10.1 (Pankhurst & Lythgoe 1982) (Figure 2).

The sequence comparison between this specimen and the previously examined homologous region of the anguillid eels (Aoyama *et al.* 1999) clearly suggested that it was a European eel with only one site difference in 516 base-pair sequences of the

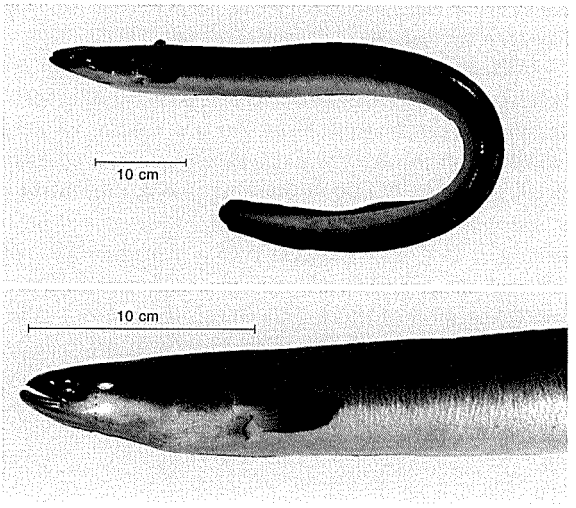

Figure 2. The European silver eel collected in the East China Sea, around 32'00"N, 132'19"E, in March 1998. The observed morphological characters were as follows: total length 838 mm, body weight 961.6 g, GSI 2.41, eye index 10.1 (Pankhurst & Lythgoe 1982), pre-anal length 347 mm, pre-dorsal length 263 mm, head length 103 mm, length of gape 26.4 mm, number of vertebrae 117, number of abdominal vertebrae 44, groove of maxillary band absent.

16SrRNA domain, whereas it had 18 site differences from Japanese eels. A homology search using FASTA3, EMBL database also indicated >99.5% homology between the present sequence and the mitochondrial 16SrRNA portion of deposited European eel specimens (accession numbers AJ244828, AJ244826, AJ244831 and AJ244827 (Bastrop *et al.* 2000)). A detailed morphological examination of this specimen completely agreed with the key characters of European eels (Ege 1939) (Figure 2).

Discussion

The presence of introduced European eels in Japanese waters has been previously reported (Tabeta *et al.* 1977, Zhang *et al.* 1999), but the findings of this study suggest that this species may be much more abundant than native Japanese eels in some areas. The present study suggests that there is a considerably higher proportion of European eels in the Uono River (more than 90% of the eels examined) than in the two other areas where this species has been genetically identified (12.1 and 31.4% of the eels examined) by Zhang *et al.* (1999). Assuming that the eel stock in the Uono River has been primarily sustained by artificial stocking, with only minimal native recruitment (Miyai 2000), it gives rise to serious questions about this type of management policy. In addition, the originally low population abundance of the native eel stock in the Uono River (Miyai 2000) suggests the possibility that artificial stocking beyond local biological carrying capacity may be occurring. Because anguillid eels are one of the top predators in freshwater ecosystems, the biological impacts of stocked European eels would not only affect native Japanese eel species, but also other aquatic animals. Accordingly, the policy of stocking non-native eel species to enhance freshwater fishery resources in Japan must now be carefully re-evaluated.

The downstream spawning migration of the North Atlantic anguillid species occurs mainly from September to October, which coincides with that of the Japanese eel (Tesch 1977). Miyai (2000) found that all eels collected by weir in the Uono River during this season were sexually matured, with an eye index (Pankhurst & Lythgoe 1982) exceeding 6.5. This suggests that most of the eels examined in the present study in the Uono River were European silver eels captured at the beginning of their spawning migration towards the ocean.

Although the downstream migration of silver eels in fresh water is well studied, the behaviour and reproductive ecology of anguillid eels during their oceanic migration and spawning is unknown. However, the finding of a matured European eel at least 70 km from the nearest freshwater habitat provides additional evidence that European eels can develop normally until metamorphosis in the silver phase and probably undergo oceanic spawning migration, even in Japanese waters far from their native habitats. Although Zhang *et al.* (1999) suggested that introduced European eels should not affect the gene pool of the Japanese eel due to the impossibility of European eel migrating into the spawning area of the Japanese eel, the present study is the first to find a European eel inhabiting sympatrically with American eel in Japanese waters. Consideration should be given to the potential for genetic contamination among anguillid eels, because the two Atlantic eel species which have been found in Japanese waters were suggested to be capable of interbreeding (Avise *et al.* 1990).

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