

Discovery of mature freshwater eels in the open ocean

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All freshwater eels of the genus *Anguilla* are catadromous, in that they perform long-distance migrations to offshore spawning area after years of growth in freshwater and near-shore habitats [1]. Finding younger and smaller leptocephali indicated the spawning area to be located in the Sargasso Sea for European and American eels (*Anguilla anguilla* and *A. rostrata*, respectively) [2, 3] and in the western North Equatorial Current (NEC) area of the western North Pacific to the west of Mariana Islands for Japanese and giant mottled eels (*A. japonica* and *A. marmorata*, respectively) [4–6]. Recent investigation could further narrow down the area and timing for spawning of the Japanese eel (*A. japonica*), where a large number of

small pre-leptocephali ($n = 130$, 4.2–6.5 mm in total length) were collected during the new moon in June 2005 around 14°N, 142°E to the west of the Suruga Seamount of the West Mariana Ridge [7]. No matured adult eels have been captured or observed to date in the spawning area, not only for the four species mentioned above, but also for any species of freshwater eel. The Fisheries Agency of Japan concerned recent declines in catches of glass eel (small juveniles migrating to freshwater area), and dispatched R/V Kaiyo Maru to capture adult Japanese eel for collecting biological information. Here, we report the first discovery of fully matured anguillid eels in the open ocean.

We used a large mid-water trawl net (maximum mouth opening 65 m wide and 55 m height with 7 mm mesh cod end) (Nichimo Co., Ltd., Tokyo), which could be towed at 4–5 knots. A total of 21 nighttime tows were performed for 0.5–4 h at a depth mainly of 170–300 m in the area adjacent to the southern part of the West Mariana Ridge (Fig. 1a) during 25 May to 9 June 2008 (Fig. 1, closed circles). Eventually, three anguillid eels were caught in two tows on 3 and 4 June operated at 13°00'N to 13°05'N, 142°08'E to 142°25'E (Fig. 1c, red circles), approximately 130 km south of the Suruga Seamount. Two individuals (48.5 and 62.3 cm total length, 112.0 and 313.5 g body weight) were caught in a single set on 3 June (Fig. 1c, larger red circle), where stepwise towing was performed at a depth of 230–280 m for the first 2 h followed by a shallower depth from 175 to 225 m for next 2 h. The water temperature at 170–300 m ranged from 13.6°C to 25.4°C. Since the largest individual was alive, drifted or vigorously swam around in the holding tank for 4 h without staying on the bottom until sacrificed for dissection (Fig. 2a, d); we assumed that the eels were caught in the shallower layer. However, a subsequent set towed in the opposite direction following the same course at a depth of 160–210 m for 2 h

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failed to catch any eels. On 4 June (new moon), another individual (51.3 cm, 151.5 g) (Fig. 2b) was caught in a similar stepwise towing at a depth of 220–270 m and 170–220 m for 2 h each (Fig. 1c, smaller red circle). Again, we failed to catch any eels in a subsequent set following the same course at a depth of 170–220 m for 2 h, suggesting the eels may be captured at depth deeper than 230 m where the water temperature was lower than 20°C.

Molecular genetic identification in the laboratory using mitochondrial 16S rDNA sequence analysis revealed the largest individual to be a giant mottled eel (*A. marmorata*) and the other two to be Japanese eel (*A. japonica*) (GenBank accession numbers AB445230–AB445232). The body color was dark brown or dark blackish gray throughout (Fig. 2a–d) entirely different from those of the yellow eels in freshwater and early silver eels in coastal waters [8]. In the process of silvering (metamorphosis from resident yellow eel to migrating silver eel), the eye diameter increases in size [9]. The eye index ($\{[(A + B)/4]^2 \times \pi/TL\} \times 100$, where *A*, *B*, and *TL* are horizontal and vertical eye diameters and total length in mm, respectively) [8] of *A. marmorata* (Fig. 2c) was 14.91, and that of two *A. japonica* was 7.51 and 8.76, considerably larger than that of male yellow eels (2.4–4.8) in freshwater area and relatively larger than that of silver eels (3.1–7.9) in coastal water [8]. All individuals had well-matured testes (Fig. 2e). The milt was not obtained by squeezing the body but was found when dissecting the testes, and spermatozoa were confirmed in the milt of all individuals. The gonad-somatic

index (GSI: relative weight of gonad to body weight) was 18.8% in one of the *A. japonica* and 13.4% in the *A. marmorata*, much higher than those of wild male silver-stage *A. japonica* from coastal areas of Japan (GSI < 1.0%) [10, 11] and comparable or even higher than those of fully matured male eels induced by hormonal injection in the laboratory [12–14]. These indicate that the three individuals were in breeding condition ready to participate in a spawning event. According to the otolith daily growth rings of leptocephali and pre-leptocephali, spawning of *A. japonica* was estimated to begin several days before the new moon [5, 7], and our eel specimens were caught 1 day before and during the new moon. Simultaneous plankton net surveys by R/V Hakuho Maru, The University of Tokyo, collected more than 200 pre-leptocephali of *A. japonica* with a size range of 3.8–6.1 mm in an adjacent area (around 12°30'N, 141°45'E) several days after the new moon during 6–9 June 2008, spatiotemporally corresponding to the catch of matured eels. These findings indicate that the area in which we found the fully matured males was considerably close to where spawning was occurring.

Unlike the Sargasso Sea for the European and American eels, there are several seamounts at the southern part of the West Mariana Ridge. These seamounts (Pathfinder, Arakane, and Suruga, from north to south) (Fig. 1a) have been proposed to be spawning area of the Japanese eels or landmarks for the eels to aggregate prior to spawning [15, 16]. The collection of small leptocephali of the Japanese eel around the Pathfinder and Arakane seamounts in 1998 [4] and many pre-leptocephali 2–5 days old in waters approximately 70 km west of the Suruga Seamount [7] support this hypothesis. However, our trawl and plankton net survey by R/V Kaiyo Maru around the Suruga Seamount during 29 May–1 June (Fig. 1b) and a more intensive plankton survey by R/V Hakuho Maru during 27 May to 4 June at the same seamount failed to catch any eels, eggs or early larvae. The area in which the adult eels were caught was deep (1,200–3,000 m) and far (130 km) from the closest seamount (the Suruga Seamount), suggesting that adult Japanese eels in offshore environment are pelagic. Thus, spawning area of the Japanese eels must be located in the southern part of the West Mariana Ridge as indicated previously [4, 5, 7], but the intimate association with shallow marine substrate such as seamounts is left unknown.

The present discovery puts an end to the century-old mystery that no mature eel has ever been found in the open ocean, and may serve as the first step for further investigating the enigmatic biological characteristics and behavior of their marine migration phase.

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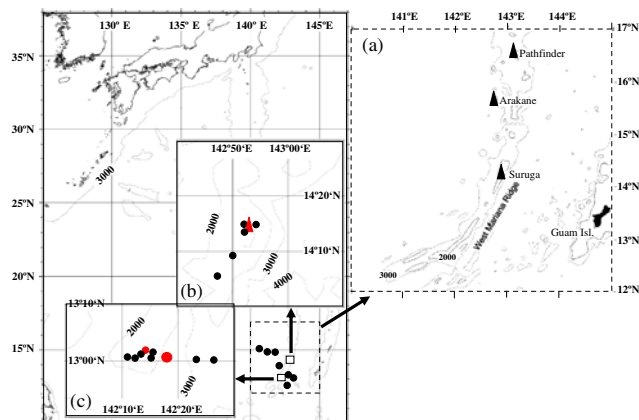


Fig. 1 Search area and collection sites of adult male eels of *Anguilla japonica* and *A. marmorata*. Closed circles indicate the middle position on the course where the mid-water trawl net was towed. **a** Southern part of the West Mariana Ridge and three seamounts (triangles) (Pathfinder, Arakane, and Suruga from north to south). **b** Sites of trawl net towing performed close to the Suruga Seamount. **c** Sites of trawl net towing at approximately 130 km south of the Suruga Seamount. Larger and smaller red circles indicate sites where two and one eels were caught, respectively. The red triangle marks the Suruga Seamount



Fig. 2 The Japanese eel (*Anguilla japonica*) and giant mottled eel (*A. marmorata*) caught in the southern part of the West Mariana Ridge. **a** Lateral view of *A. marmorata* (62.3 cm total length, 313.5 g weight). **b** Lateral view of one of *A. japonica* (51.3 cm total length, 151.5 g weight). **c** Head of *A. marmorata*. Note the enlarged eye.

d The giant mottled eel in the holding tank kept drifting and swimming for 4 h and never stayed on the bottom. **e** Ventral dissection showing fully matured testis of *A. japonica* in **(b)**. Testis weight of this individual was 28.5 g

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