



# HOKKAIDO UNIVERSITY

Title	II. MOLECULAR PHYLOGENETIC APPROACH TO THE EVOLUTION OF THE FRESHWATER EELS, ANGUILLA
Author(s)	AOYAMA, Jun; NISHIDA, Mutsumi; TSUKAMOTO, Katsumi
Citation	MEMOIRS OF THE FACULTY OF FISHERIES HOKKAIDO UNIVERSITY, 44(1), 2-10
Issue Date	1997-05
Doc URL	<a href="https://hdl.handle.net/2115/21896">https://hdl.handle.net/2115/21896</a>
Type	departmental bulletin paper
File Information	44(1)_P2-10.pdf



## I. Preface

Hiroshi UEDA and Howard A. BERN

The review papers comprising this collation were presented at an International Symposium on Fish Migration, held May 31-June 2, 1996, at Lake Toya, Hokkaido, Japan. The symposium addressed the most recent advances in all aspects of fish migration including physiology, ecology, evolution, behavior, population dynamics, genetics, and bioengineering. The rapidly accumulating new data are proving valuable in our better understanding of fish migration, particularly anadromous salmon migration, catadromous eel migration, and long distance migration of various marine fishes.

We are especially grateful to Professor Kohei Yamauchi, Dean of the Faculty of Fisheries of Hokkaido University, for his encouragement to hold this symposium following the Third International Symposium on Fish Endocrinology in Hakodate, and to the students of the Toya Lake Station (Masahiro Fukaya, Kiyoshi Orito, Hiroyuki Sakano, Ayako Sato, Koji Sato, Munetaka Shimizu and Toshiaki Yamamoto) for their help in organizing the meeting. Finally, we would like to thank the participants whose contributions made the symposium a success.

## II. MOLECULAR PHYLOGENETIC APPROACH TO THE EVOLUTION OF THE FRESHWATER EELS, *ANGUILLA*

Jun AOYAMA<sup>4)</sup>, Mutsumi NISHIDA<sup>5)</sup> and Katsumi TSUKAMOTO<sup>4)</sup>

<sup>4)</sup>Ocean Research Institute, University of Tokyo, Nakano-ku, Tokyo 164, Japan,

<sup>5)</sup>Department of Marine Bioscience, Fukui Prefectural University, Obama, Fukui 917, Japan

### Abstract

The phylogeny of 12 *Anguilla* spp. collected from various areas of the world was constructed from mitochondrial cytochrome b sequences. Considering paleogeography and global paleocirculation, a hypothesis on the evolutionary process of freshwater eels is also presented. The phylogenetic tree obtained by neighbor-joining analysis placing *A. celebesensis* as an out-group suggests that *A. mossambica*, *A. australis australis*, *A. rostrata* and *A. anguilla* form a clade; *A. japonica* is placed in another clade. Most of the speciation in the lineage occurred mainly in the Eocene (57-36 million years ago); however, the separation of two Atlantic species, *A. anguilla* and *A. rostrata*, is estimated to have occurred much later, approximately 10 million years ago. The hypothetical evolutionary process of eels is as follows: in the Eocene, the ancestor of eels originated from the western Pacific

ocean, near present-day Indonesia. One group was dispersed westward by global circumequatorial current along the north ridge of the Tethys sea. This group branched into several lineages. One of these was the ancestor of two Atlantic species which were carried by continuous currents into the Atlantic ocean.

### Introduction

The freshwater eels of the genus *Anguilla* Shaw (family Anguillidae) share a common life history and are widely distributed throughout the world (Fig. 1). Freshwater eels are catadromous and have a relatively long larval development, a leptocephalus phase which is larval form peculiar to elopomorph fishes and highly adapted to a planktonic life in the open ocean. The continental distribution of freshwater eels appears to be related to the subtropical circulation of the oceans, with most species being located on the west side of the Atlantic, Pacific and Indian oceans (Ege, 1939; Tesch, 1977; Fig. 1). Warm westward flowing equatorial currents reach and flow along the east coast of each continent, whereas the west coasts are swept by cold currents which originate in high latitudes. Their association with specific hydrographic conditions may explain why eels inhabit the east coasts of Eurasia, Australia, Africa and North America. No eels inhabit the west coasts of North and South America, Australia and Africa. Although the European eel, *A.anguilla*, is able to inhabit Europe and the Mediterranean because of the warm eastward flow of the Gulf Stream and the North Atlantic drift, it is not known why there are no freshwater eels along the east coast of South America despite the existence of the warm Brazil Current.

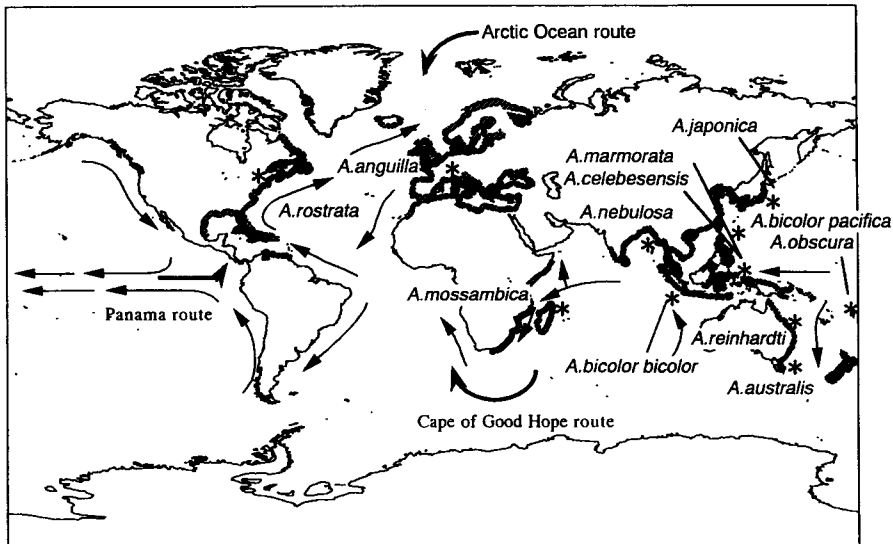


Fig. 1. Distribution of freshwater eels throughout the world (Shaded line) and the sampling location of 12 species studied (asterisks). Thick arrows show possible route for ancestral eel to invade to the Atlantic Ocean, from place of origin. Thin arrows indicate global circulation.

The geographic distribution and unique life history traits of anguillid eels give rise to many questions, such as when these eels evolved to form the 18 extant species including 3 subspecies (Castle, 1974), and how they achieved their curious geographical distribution. In particular, how did the two Atlantic species become isolated from the majority of other eel species in the Indo-Pacific, and why are there no eels in the South Atlantic? To answer these questions, the phylogenetic relationships of these eels needed to be clarified. Some informations have emerged in the last 80 years about the unusual life history of the freshwater anguillid eels, but little has been learned about most of the 15 species. Questions about the phylogenetic relationships of this group and the implications of their long spawning migration and larval phase on their global distributions have not yet been addressed.

In this report, we present the molecular phylogeny of twelve species of *Anguilla* distributed in seven different regions of the world, and by considering information about global paleogeography and paleocirculation we develop a hypothetical evolutionary scenario for speciation and dispersal to account for the unusual geographic distribution of anguillid eels.

## Materials and Methods

### 1. Sample sources

Twelve species and subspecies of freshwater eel, *A. celebesensis*, *A. marmorata*, *A. bicolor bicolor*, *A. bicolor pacifica*, *A. obscura*, *A. nebulosa nebulosa*, *A. mossambica*, *A. japonica*, *A. anguilla*, *A. reinhardi*, *A. australis australis*, and *A. rostrata*, were collected from various localities during March 1979 and August 1995 (Fig. 1). Liver tissue of individual fish was extracted and preserved in 99% ethanol or in the following buffer: 8M Urea, 10mM Tris-HCl PH8.5, 125mM NaCl, 50mM EDTA, 1%v/w SDS, at the time of capture (Aoyama et al., 1996). The rest of specimens were preserved in 20% formalin to being identified. These samples were transported to laboratory at ambient temperatures, and species identification was performed using the morphological key of Ege (1939).

### 2. DNA preparation and sequencing

DNA extraction was carried out on the solution described above, using phenol/chloroform (Kocher et al., 1989). Mitochondrial cytochrome b gene was amplified by polymerase chain reaction using two oligonucleotide primers, GLU-L (Palumbi et al., 1991) and H15149 (Kocher et al., 1989), and the following conditions: 30 cycles-30sec 94Åé, 30sec 55Åé, 90sec 72Åé. The sequencing was performed by Uniplex Cycle DNA Sequence kit (Millipore MBBLUNP02, Bedford, MA, USA).

### 3. Phylogenetic analysis

Phylogenetic analysis was performed with the neighbor-joining method (Saitou and

Nei, 1987) based on the Kimura's 2-parameter distance (Kimura, 1980) by means of the software package PHYLIP Ver.3.5 (Felsenstein, 1993). To examine the effect of saturation of transitional substitution to topological stability, analysis with different weighting schemes, (TV/TS);1.0-10.0, was carried out. According to previous morphological studies by Ege (1939), we selected *A.celebesensis* as an out-group, which in Ege's view has some ancestral characters of the genus.

## Results and Discussion

### 1. Phylogenetic relationships

Phylogenetic analysis with the neighbor-joining method suggested that two basal groupings exists: one including *A.nebulosa*, *A.marmorata*, *A.obscura*, *A.bicolor bicolor* and *A.bicolor pacifica*, the other including *A.japonica*, *A.reinhardtii*, *A.mossambica*, *A.australis*, *A.rostrata* and *A.anguilla* (Fig. 2). This same grouping appeared when the weighting of transversion was increased from 1:1 to 10:1 over transitions (data not shown). This phylogenetic tree suggests that Atlantic and African species are members of the same clade with one of the two Australian species, *A.australis*. And in this group, interestingly, *A.reinhardtii*, the other Australian eel, is a member of a different clade with *A.australis*

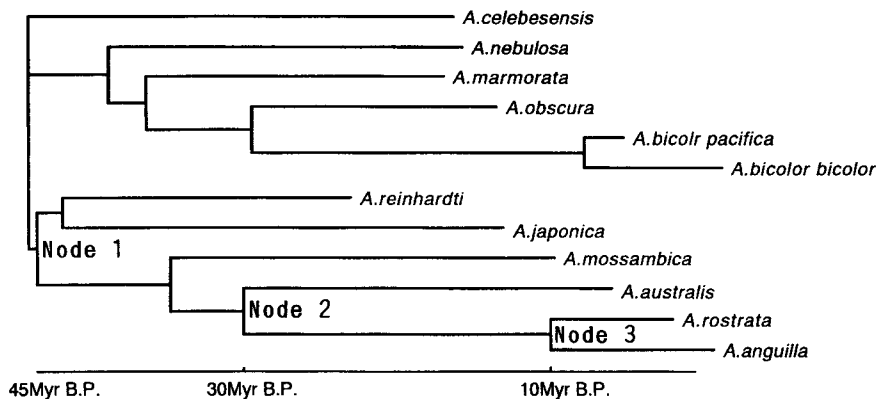


Fig. 2. Phylogenetic tree of 12 *Anguilla* species constructed from 410bp of mitochondrial cytochrome b sequence by Neighbor-joining analysis. The node indicated as "Node 2" was used to estimate divergence time (see text).

despite their sympatry. The speciation of the two Atlantic species occurred much later than most of the others. And as expected, two subspecies, *A.bicolor bicolor* and *A.bicolor pacifica*, which show no marked morphological distinction (Ege, 1939), also separated later.

### 2. Comparison with previous morphological study

The one previous study of the phylogeny of freshwater eels recognized 19 species

(revised to 18 species by Castle, 1974) including three subspecies, divided into four groups (Ege, 1939). If the scheme of species relationships proposed by Ege (1939) is transformed as a phylogeny with the node species to be the most likely sister species, then the scheme can be interpreted as follows: the group including the ancestral species *A.celebesensis* originated first, next the second group containing *A.nebulosa*, *A.marmorata* and *A.reinhardti*

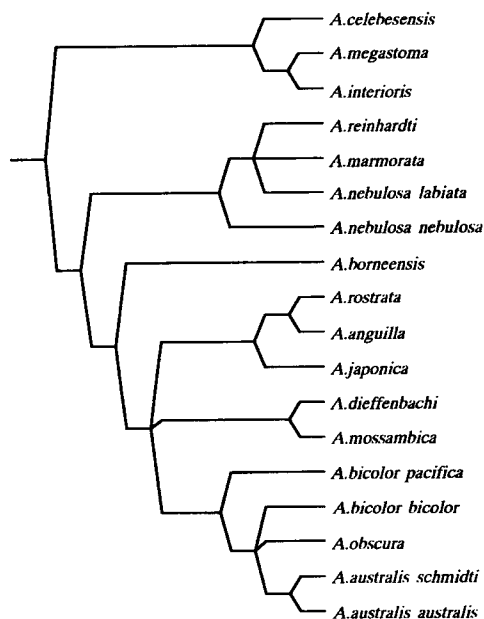


Fig. 3. Phylogenetic tree of genus *Anguilla*, constructed from Ege (1939) assuming the nodal species to be plesiomorphic with respect to terminal species.

appeared, then the third group consisting of *A.japonica*, *A.mossambica*, *A.anguilla* and *A.rostrata*, and finally the fourth group including *A.australis*, *A.bicolor* and *A.obscura* (Fig. 3). The phylogenetic tree obtained in this study roughly agrees with that of Ege (1939), except for the following points: 1) the position of *A.japonica* and *A.reinhardti*, 2) the monophyletic short-finned group proposed by Ege was subdivided, although 2 species, *A.bicolor* and *A.obscura*, formed a clade.

Ege (1939) concluded that the two Atlantic species, *A.anguilla* and *A.rostrata*, were closely related to *A.japonica* of the North Pacific. However, despite morphological similarities, their present geographical distributions (separated into different oceans) and the apparent lack of close genetic similarity suggest that the Atlantic and Pacific eel species do not have a particularly close phylogenetic relationship. The morphological similarity between the Atlantic species and *A.japonica* may be parallel or convergent evolution as a response to their temperate habitats and long oceanic spawning migrations for which

common morphological characteristics and functions may be required.

According to our phylogenetic analysis, it is possible that the “short-finned” morphological characteristics are not monophyletic. In the light of molecular phylogeny, polyphyletic relationships have emerged for morphological or ecological characteristics in some animals (Bradley et al., 1991; Foighil and Smith, 1995; Hadfield et al., 1995). However, the existence of polyphyletic relationships was not highly supported statistically in our study.

### 3. Origin of eels

Freshwater eels might have originated in the tropics, because two-thirds of the recognized 18 *Anguilla* species inhabit the tropics while only five species have their growth habitat in temperate regions. Seven of the 18 species occur in the western Pacific around Indonesia, and *A. celebesensis*, which mainly inhabits Sulawesi Island (Celebes) in Indonesia, has been considered the ancestral species of the Anguillidae (Ege, 1939; Castle, 1974). All these considerations strongly suggest that the western Pacific in and around Indonesia could be the place of origin of freshwater eels.

### 4. Invasion to Atlantic ocean

Present knowledge of zoogeography, paleogeography and ocean circulation suggests four potential routes of dispersal into the Atlantic Ocean (Fig. 1). One possibility is that ancestral eels moved across the wide paleo-Pacific Ocean from the western end to the eastern end. However, freshwater eels do not occur on Easter Island and Galapagos Islands, nor on the west coast of the American continents. Another possibility is via the Cape of Good Hope (southern tip of Africa), but there are no freshwater eels along either the east coast of South America nor the west coast of Africa (except in the far north). The low temperature and great distance would act as a barrier to prevent dispersal of ancestral eels from tropical regions through the Arctic Ocean. Moreover, the migratory distance for one life cycle would be too long for these eels which are likely to have dispersed as larvae from their breeding place in low latitudes.

The most likely route would seem to have been the Tethys Sea that separated Laurasia (North American and Eurasian Continents) from Gondwana (Africa, South America, and India) thereby providing a circum-global oceanic connection at low paleo-latitudes. The Tethys Sea existed during the Mesozoic to the beginning of the Tertiary period (approximately over 200–30 million years ago; Haq, 1984). During this period of time, continents were separated from each other and there was a global circumequatorial current which flowed from east to west encircling the earth along the equator (Obata, 1993; Haq, 1984). It is likely that the ancestral eels would disperse westwards in this current, from the eastern end of the Tethys Sea to the Atlantic. The ancestors of Atlantic eels might therefore have dispersed through the Tethys Sea to enter the paleo-Atlantic Ocean from the

western Pacific, via the present-day Indian Ocean.

### 5. Age estimation

The Tethys Sea was closed near the mid-late Oligocene (about 30 Myr ago; Haq, 1984; Andel, 1985). This suggests that separation of the node between the ancestor of the two Atlantic species and the other species in the neighbor-joining tree (Node 2 in Fig. 2) should have occurred about 30 Myr ago, namely before the closure of the Tethys Sea. On the basis of this dating, the split of *A. celebesensis* and the ancestor of the other seven species (Node 1 in Fig. 2) was approximately 45 Myr ago, *i.e.*, in the Eocene (57-36 Myr ago). Similarly, the split of *A. anguilla* and *A. rostrata* (Node 3 in Fig. 2) is estimated at approximately 10 Myr ago. This aging is possibly underestimated because the speciation between the Atlantic species and *A. australis* (Node 2 in Fig. 2) may have already proceeded by reproductive isolation of adults in different oceans, even if the Tethys Sea were opened and there were some supply of leptocephali from the Indian Ocean. This means that the timing of the closure of the Tethys Sea could possibly be located much later in the phylogenetic tree, suggesting much earlier speciation of anguillid eels, in the Cretaceous (145-65 Myr ago), for instance.

### 6. Estimated rate of DNA evolution of the eel

The sequence divergence between *A. anguilla* and *A. rostrata* was 0.02, which is approximately congruent with the 0.03 value calculated by mitochondrial DNA restriction-site polymorphism (Avise et al., 1986, 1990). These authors estimated the separation time between *A. anguilla* and *A. rostrata* as 1.5 million years ago, using a conventional mitochondrial DNA clock calibration of 2% sequence divergence per million years. Applying this value of separation time to the phylogenetic tree obtained in this study, the oldest branch (Node 1 in Fig. 2) was calculated to be only 6.4 million years ago. This would indicate that the speciation events of the *Anguilla* occurred mainly during late Miocene to early Pliocene (approximately 7-3 Myr ago). However, this period was characterized by the accentuation of a cooling climate associated with the development of the Antarctic ice-sheet (Haq, 1984). If this time estimation is adopted, the westward invasion of eels into the Atlantic would be impossible since the "Tethys Corridor" would already have closed much earlier and as an alternative the Arctic region would have been too cold. Although the Isthmus of Panama was still absent in that period, the current direction was wrong for the transport of larvae into the Atlantic, even if some larvae had drifted across the Pacific Ocean. Therefore, it is possible to conclude that the molecular evolution of eels may be much slower than for other animals, since the evolution of cytochrome b amino acid replacement may have occurred five times more slowly in cold-blooded than in warm-blooded animals (Kocher et al., 1989; Meyer, 1992). However, the rate of DNA evolution in fishes remains uncertain, and will require more data and some other calibration point such as

the existence of fossils.

### 7. Tethys corridor hypothesis

Based on our estimate of the timing of divergence for *Anguilla* species and molecular phylogenetic trees (Fig. 2), we propose the following evolutionary scenario for the speciation and dispersal of freshwater eels.

In the Eocene (57-36 Myr ago), the ancestral eel originated in the western Pacific, around present-day Indonesia, and then split into several species. One of them dispersed widely and gave rise to three groups (Fig. 4). First was the ancestor of the two Atlantic species, which dispersed westward by the global circumequatorial current along the northern margin of the Tethys Sea into the Atlantic Ocean. The second group was the ancestor of *A. mossambica* which dispersed southward in the current that flows along the east coast of the African continent. The last group was the lineage leading to *A. australis* which dispersed south-eastward. The ancestor of *A. anguilla* and *A. rostrata* that entered the Atlantic Ocean may have populated the coastal regions of the North Atlantic, as a result of larval dispersal in the paleo-North Atlantic gyre, since the exit of the Tethys Sea opened directly in the North

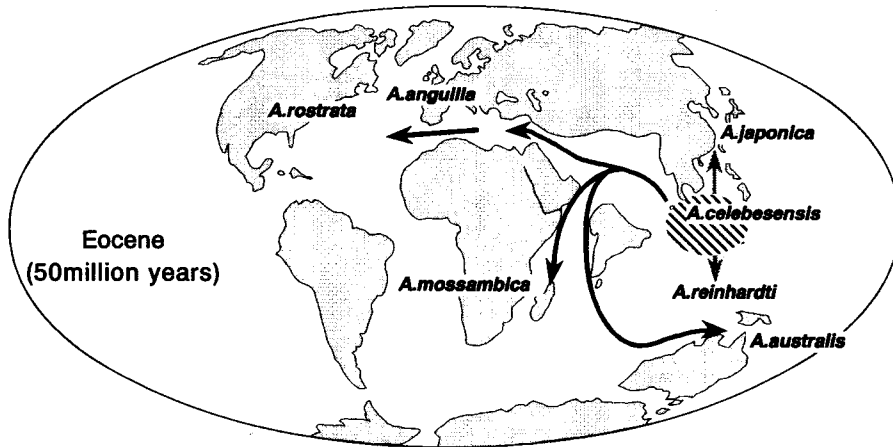


Fig. 4. Diagrammatic representation of proposed scenario for the dispersal and speciation of anuillid eels. The map shows an Eocene global paleogeographic reconstruction.

Atlantic. However, this species may not have been able to spread into the South Atlantic because the paleo-South Atlantic had not opened sufficiently to have a warm subtropical gyre suitable for larval development. Even after a gyre was formed, the circulation of the northern and southern gyres was probably removed. That is may be why no eels now occur in the South Atlantic.

In the latest part of the Eocene and early Oligocene (40-35 Myr ago), the

Australian continent had moved north after its separation from Antarctica, and was populated by the lineage of *A.reinhardti* from nearby Indonesia. Eels that inhabit the Australian continent thus now consist of two groups that may have arrived there by way of different routes at different times (Fig. 4).

The Japanese eel, *A.japonica*, is apparently not closely related to the Atlantic eels, as has previously been supposed, even though recent findings have shown them to have similar migratory and recruitment strategies (Tsukamoto, 1992). Instead, the Japanese eel is only distantly related to the North Atlantic eels, together with all eels which appear to have diverged from a common Indonesian ancestor. Keys to this scenario are the availability of the Tethys corridor and larval dispersal westwards and southwards on the paleo-circumglobal current. Our findings provide a new basis for further evaluation of the evolutionary implications of the remarkable life history strategy of freshwater eels of the genus, *Anguilla*.

#### **Acknowledgments**

We thank Drs. P.H.J. Castle and M. Miller for constructive comments and for improving the manuscript at an early stage. We also thank Drs. M Gross and JC Avise for critical reading of the manuscript and comments.

### **III. PHYSIOLOGICAL ECOLOGY OF PACIFIC SALMON MIGRATION**

W. Craig CLARKE

Department of Fisheries and Oceans, Pacific Biological Station, Nanaimo,  
B.C. V9R 5K6, Canada

#### **Abstract**

Anadromous Pacific salmon (genus *Oncorhynchus*) undergo a series of habitat shifts during their elaborate life cycle, characterized by various migrations, most notably between ocean feeding areas and freshwater spawning sites. The physiological preparation for and conduct of these migrations are adapted to annual cycles of temperature and photoperiod.

#### **Introduction**

The dramatic and accurate homing migration of adult salmon from the open ocean back to their natal streams has inspired observers for centuries. The complex life cycle of anadromous Pacific salmon involves a series of habitat changes, including outmigration of juveniles from rivers to the sea, a feeding migration in the North Pacific Ocean, and finally a purposeful migration of adults back to their native rivers, lakes and streams to reproduce and