Biology and Ecology of Anguillid Eels

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Preface

Freshwater eels of the genus *Anguilla* are exotic animals, and despite a large number of scientific studies conducted on eels, crucial aspects of their biology still remain a mystery. No one has yet observed eels spawning in the natural environment, as spawning areas are located in the open ocean. Since these eels have a unique catadromous life history and are used as food resources, they are one of the most important eel families from a conservation standpoint. Recently, however, the juvenile population has declined dramatically, in all probability due to the use of wild juveniles in cultivation. These eels are captured in estuaries and almost all of the world's total eel supply comes from aquaculture. Therefore, the supply of eel resources for human consumption is completely dependent on wild catch.

The causes of decline in stock and recruitment are not well understood. Overfishing, habitat loss, migration barriers, increased natural predation, parasitism, ocean climate variation, and pollution might all have an impact. Ever since the European eel was listed by CITES under Appendix II and came under protection in March 2009, and since the export/import ban was issued by the EU in 2010, the international trade of juvenile eels has changed drastically. Most recently, the Japanese and American eels were added to the IUCN's list with an endangered classification, suggesting that they too face a high risk of extinction. In the future, even more eel species could be classified as endangered unless there is comprehensive conservation and protection. Therefore, it is highly possible that we may not be able to see such a unique animal on the earth in the near future.

In this book, the latest information regarding the biology and ecology of the freshwater eel genus *Anguilla*, has been compiled. It will be useful not only for scientists and researchers but also for students and members of the public in order to better understand the eel itself. I would like to express sincere thanks to all authors for this book. I hope the book will be a milestone for fish biology and ecology.

May 2015   **Takaomi Arai**

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Takaomi Arai

Introduction

Freshwater eels of the genus *Anguilla* Schrank (1798) consist of 16 species, three of which are further divided into two subspecies (Ege 1939; Castle and Williamson 1974; Watanabe 2003; Watanabe et al. 2004, 2005, 2009a, 2013, 2014a,b). All of these species make oceanic migrations at various scales from a few hundred to thousands of kilometers (Arai 2014). They are globally distributed in temperate, tropical, and subtropical areas and are considered to be prevalent nearly worldwide, except for the land masses adjacent to the South Atlantic and the eastern Pacific oceans (Ege 1939). They all have a catadromous life-history strategy, spawning in remote tropical seas with larvae that are transported back by currents to their nursery grounds in freshwater or coastal areas. Tesch (2003) divided the freshwater eels into tropical and temperate species according to their geographic distributions. The tropical and temperate eels consist of 11 and 5 species, respectively. The basic biology of temperate eels is generally known, but information about the tropical eels that comprise two-thirds of all anguillid eel species is much more limited. A recent molecular genetic study indicated that the genus *Anguilla* originated in the deep ocean (Inoue et al. 2010). Furthermore, studies have revealed that tropical eels are the most basal species originating in the Indonesian region and that freshwater eels radiated out from the tropics to colonise the temperate regions (Minegishi et al. 2005).

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The only comprehensive revisions of the genus *Anguilla* were conducted by Kaup (1856), Günther (1870), and Ege (1939) using morphological analyses. In the last revision by Ege (1939), this genus was classified into 16 species, three of which were divided into two subspecies, i.e., *A. celebesensis* Kaup (1856); *A. interioris* Whitley (1938); *A. ancestralis* Ege (1939); *A. megastoma* Kaup (1856); *A. nebulosa nebulosa* McClelland (1844); *A. nebulosa labiata* Peters (1852); *A. marmorata* Quoy and Gaimard (1824); *A. reinhardtii* Steindachner (1867); *A. borneensis* Popta (1924); *A. japonica* Temminck and Schlegel (1846); *A. rostrata* Lesueur (1817); *A. anguilla* Linnaeus (1758); *A. dieffenbachii* Gray (1842); *A. mossambica* Peters (1852); *A. bicolor pacifica* Schmidt (1928); *A. bicolor bicolor* McClelland (1844); *A. obscura* Günther (1872); *A. australis australis* Richardson (1841) and *A. australis schmidtii* Phillips (1925) (Table 1). However, 35 years later, Castle and Williamson (1974) reported that based on morphological

Table 1. Comparison of anguillid eel taxonomies by Ege (1939) and the Latest.

Ege (1939)	Latest*
A. celebesensis	*A. celebesensis*
A. interioris	*A. interioris*
A. ancestralis	*A. megastoma*
A. megastoma	*A. bengalensis bengalensis*
A. nebulosa nebulosa	*A. bengalensis labiata*
A. nebulosa labiata	*A. marmorata*
A. marmorata	*A. reinhardtii*
A. reinhardtii	*A. borneensis*
A. borneensis	*A. japonica*
A. japonica	*A. rostrata*
A. rostrata	*A. anguilla*
A. anguilla	*A. dieffenbachii*
A. dieffenbachii	*A. mossambica*
A. mossambica	*A. luzonensis*
A. bicolor pacifica	*A. bicolor pacifica*
A. bicolor bicolor	*A. bicolor bicolor*
A. obscura	*A. obscura*
A. australis australis	*A. australis australis*
A. australis schmidtii	*A. australis schmidtii*

*: Referred from Castle and Williamson (1974), Watanabe (2003) and Watanabe et al. (2004, 2005, 2009a, 2013, 2014a,b).

analysis, *A. ancestralis*, which was described by Ege (1939) using only glass eels, was a synonym of *A. celebesensis*. Furthermore, *A. nebulosa nebulosa* and *A. nebulosa labiata* are synonyms of *A. bengalensis bengalensis* (Gray 1831) and *A. bengalensis labiata* (Peters 1852) respectively. Although *A. borneensis* was thought to be a synonym of *A. malgumora* (Kaup 1856), it was determined that *A. borneensis* is the valid species after re-examination of morphological characters, and *A. malgumora* is instead suggested to be a junior synonym of *A. borneensis* (Watanabe et al. 2014a).

Ege's (1939) systematics have long been widely accepted by biologists. However, Watanabe et al. (2004) suggested that the morphological characters described by Ege (1939) were not sufficient to classify all species of this genus and that geographic distribution was an indispensable character for Ege's taxonomy of freshwater eels. When only morphological characters were used, the freshwater eels could be classified into only four groups (Watanabe et al. 2004). The geographic distribution of each species is not a suitable taxonomic character of the freshwater eels because it overlaps among species and is highly variable depending on the environment (Shapovalov et al. 1959; Jellyman et al. 1996). In this regard, Ege's (1939) taxonomy is not robust. Furthermore, the only complete morphological key for species identification of the genus *Anguilla* was provided by Ege (1939), but this has been suggested to be unsatisfactory due to critical underestimates of the intraspecific variation among the characters (Aoyama et al. 2000a). Watanabe et al. (2005) examined molecular genetic data in combination with morphological information in order to evaluate Ege's (1939) taxonomy of the genus *Anguilla* from a different perspective. The results suggested that the present morphologically based taxonomy of the freshwater eels proposed by Ege (1939) is basically sound, and 15 taxa were confirmed within the genus *Anguilla* (Watanabe et al. 2005).

Recently, two new species, *Anguilla luzonensis* (Watanabe et al. 2009a) and *A. huangi* (Teng et al. 2009) were discovered almost simultaneously in the Cagayan River system on the northern Luzon Island of the Philippines and from glass eels collected from the Cagayan River estuary that were reared in a culture pond in Taiwan, respectively. However, further study suggested that *A. huangi* is a junior synonym of *A. luzonensis* (Watanabe et al. 2013). Thus, the genus *Anguilla* is currently recognised as being comprised of 16 species (Ege 1939; Castle and Williamson 1974; Watanabe et al. 2004, 2005, 2009a, 2014b), although three of these are further divided into two subspecies (Ege 1939).

This chapter summarizes the latest information about the taxonomy and distribution of the genus *Anguilla*. The information should contribute towards further biological and ecological studies in the genus. Most of the content in this chapter is cited and referenced from the latest taxonomical studies by Watanabe (2003), Watanabe et al. (2004, 2005, 2009a, 2013, 2014b).

Morphological identification

Ege (1939) examined a total of 25,265 specimens, which included 12,793 adults and 12,472 juveniles (glass eels and elvers). Although a great number of specimens were examined, Ege's raw data have never been located (Silfvergrip 2009), and many statistical analyses have therefore not been possible to perform, as they require data sets in a matrix shape. However, recent taxonomic studies by Watanabe et al. (2004, 2005, 2009a, 2013, 2014b) have provided useful morphological characters in combination with molecular analysis.

There are some technical requirements for identification of freshwater eels of unknown origin (Silfvergrip 2009). Morphological identification of glass eels requires a binocular stereoscope ("stereomicroscope") and a sharp-jawed vernier caliper graded to 0.1 mm. Specimens larger than 200 mm in total length also require low-voltage radiograph equipment (Silfvergrip 2009).

There is no comprehensive information available for the identification and comparison of eel eggs using morphology (Castle 1984), but in general, the eggs of *Anguilla* resemble large clupeid eggs, e.g., like those of herring (McGowan and Berry 1984). Identification of anguillid eggs and leptocephali requires molecular analysis (Aoyama et al. 2007).

Identification of eel samples using morphology is often the fastest means of eel identification. Based on the ano-dorsal length (ADL) as a percentage of total length (TL), the glass eels and elvers can first classify as either long-finned or short-finned (Ege 1939; Castle and Williamson 1974; Tabeta et al. 1976a; Arai et al. 1999) without counting the vertebrae. However, further identification for unknown origin samples is difficult, especially in case of the tropical anguillid species that occur sympatrically in a single habitat. Even in known origin samples, it is hard to identify the species using morphological characters only due to the overlap of such characters among different species (Ege 1939; Watanabe et al. 2004). To determine the species composition of the tropical eels, Arai et al. (1999) examined a total of 21,633 glass eels collected in the North Sulawesi of Indonesia. According to Ege (1939) and Castle and Williamson (1974), 1 short-finned eel, *A. bicolor pacifica*, and 3 long-finned eels, *A. celebesensis*, *A. marmorata* and *A. borneensis*, are known in the area. However, *A. celebesensis* and *A. borneensis* are difficult to distinguish by their vertebral characteristics alone (Tabeta et al. 1976a). Thus, Arai et al. (1999) examined the species composition using molecular analysis. Because juvenile eels such as glass eels and elvers develop less colour markings on their skin, and because the representative morphological characters are scarce and less developed when compared to those of adult specimens (Tabeta et al. 1976a; Arai et al. 1999; Silfvergrip 2009), molecular analysis in combination with morphological analysis should be conducted for precise species identification. Silfvergrip (2009) suggested that intact specimens smaller than 200 mm TL require a sharp-legged vernier caliper graded to 0.1 mm and a binocular stereomicroscope for identification.

Regarding species identification of adult specimens, Ege's (1939) systematics have long been widely accepted. However, Watanabe et al. (2004) found that the morphological characters described by Ege (1939) were not sufficient to classify all species of this genus and that geographic distribution was an indispensable character for his taxonomy of these eels. When only morphological characters were used, the freshwater eels could be classified into only four groups (Watanabe et al. 2004). The geographic distribution of each species alone is not a suitable taxonomic character of the freshwater eels because it overlaps among species and is highly variable depending on the environment (Shapovalov et al. 1959; Jellyman et al. 1996). Recently, freshwater eels have been transported around the world for aquaculture. The prevalent trade in glass and young eels has resulted in many reports of accidental or incidental introduction of several exotic species of eels into natural river systems or the sea in some areas (Skinner 1971; Tabeta et al. 1976b, 1977; McCosker 1989; Sasai et al. 2001; Okamura et al. 2008; Arai et al. 2009). In this regard, Ege's (1939) taxonomy is not robust. Furthermore, the only complete morphological key for species identification of the genus *Anguilla* was provided by Ege (1939), but this has been suggested to be unsatisfactory due to critical underestimates of the intraspecific variation among the characters (Aoyama et al. 2000a; Watanabe et al. 2004). Based on the morphological characters such as colour markings, maxillary bands, position of the dorsal fin origin and vertebral counts in combination with genetic clustering, the genus *Anguilla* is recognised as being comprised of 16 species (Ege 1939; Castle and Williamson 1974; Watanabe 2003; Watanabe et al. 2004, 2005, 2009a, 2013).

Molecular identification

Although investigations of morphological characteristics in freshwater eels as well as other taxa are fundamental and important as conventional identification methods, the characteristics are not necessarily useful in identifying the eels. Consequently, this situation requires a critical evaluation of the present classification of the genus *Anguilla* by Ege (1939). It is important to find new defining characteristics for the taxonomy of freshwater eels that are valid regardless of the geographical distribution from which an eel is collected or obtained. Watanabe et al. (2005) found that molecular genetic characteristics are useful in understanding the taxonomy of the genus *Anguilla*. They studied the mitochondrial 16S ribosomal RNA domain (16S rRNA) as a new characteristic for the taxonomy of the freshwater eels. This relatively conservative gene is frequently used for evolutionary studies at the species or genus level in fishes (Meyer 1993). Aoyama et al. (2000b) also suggested that the 16S rRNA was appropriate for identifying eels at the species level.

Recently, the anguillid eels found in the Peninsular Malaysia were identified, using a morphological analysis, as *Anguilla bengalensis bengalensis* and *A. bicolor bicolor* and that identification was further validated by an

analysis of the eels' mitochondrial cytochrome oxidase subunit I (COI) and 16S ribosomal RNA (16S rRNA) sequences (Arai and Wong 2016). Previous studies had reported the occurrence of the tropical eel species *A. marmorata* in Peninsular Malaysia without validating the identification genetically. After re-examination of a number of key morphological characteristics of a preserved sample of *A. marmorata*, the sample from Peninsular Malaysia was identified as *A. bengalensis bengalensis*. This was also the first recorded occurrence of *A. bengalensis bengalensis* in Malaysian waters that was confirmed by both morphological and molecular genetic analyses. Although one other sample was identified as *A. borneensis* on the basis of key morphological characteristics, molecular genetic analyses showed that the sample was actually *A. bicolor bicolor*. These results indicate the difficulty of accurately identifying tropical eels solely on the basis of morphological analyses, due to the sympatric distribution of a number of closely related eels.

When using molecular identification of freshwater eels, Watanabe et al. (2005) suggested that because of potentially high levels of variation in population structure, large sample sizes should be used when developing genetic techniques for species identification. They analysed the 16S rRNA gene from 8–66 specimens for each species and found a considerable amount of genetic variation both within and among species (Watanabe et al. 2005). These results suggest that exact species identification may be difficult due to greater sequence variations if a small sample size is utilized. However, once these techniques are refined using larger sample sizes, it should be possible for any eel specimen to be identified using genetic characters, regardless of the stage of growth (Watanabe et al. 2005).

Recent progress in taxonomy studies

Before Watanabe (2003) and Watanabe et al. (2004, 2005, 2009a, 2013, 2014b) examined Ege's work on the taxonomy of the genus *Anguilla*, the comprehensive revisions of the genus *Anguilla* were conducted by Kaup (1856), Günther (1870), and Ege (1939) using morphological analyses only. In the last revision by Ege (1939), the genus was classified into 16 species, three of which were divided into two subspecies. Thereafter, a total of 1736 specimens comprising of 1501 specimens collected worldwide and 235 specimens obtained from museums around the world were examined for both morphological and molecular analyses (Watanabe 2003; Watanabe et al. 2004, 2005, 2009a, 2013, 2014b). Specimen collection and research is ongoing at present.

Watanabe (2003) and Watanabe et al. (2004) have summarized the history of the taxonomy of the genus *Anguilla*, problems with Ege's taxonomy revision and have introduced a new taxonomy. The results suggested that there is not a major difference between the morphologically based taxonomy of the freshwater eels proposed by Ege (1939) and that by Watanabe (2003) and Watanabe et al. (2004). The results of morphological and meristic

measurements, counts, and observations suggested that skin with or without variegated marking, wide or narrow maxillary bands of teeth, and short or long dorsal fin were important as valid characteristics for the taxonomy of the genus *Anguilla* (Watanabe 2003; Watanabe et al. 2004). Using those three characteristics, Watanabe (2003) and Watanabe et al. (2004) suggested that the freshwater eels could be classified into four groups as follows (Fig. 1):

Group 1. Variegated body marking, with broad maxillary bands of teeth
Group 2. Variegated body marking, with narrow maxillary bands of teeth
Group 3. Without variegated body markings and with a long dorsal fin
Group 4. Without variegated body markings and with a short dorsal fin

Group 1. Variegated body marking, with broad maxillary bands of teeth
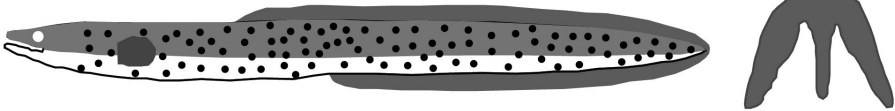
Group 2. Variegated body marking, with narrow maxillary bands of teeth

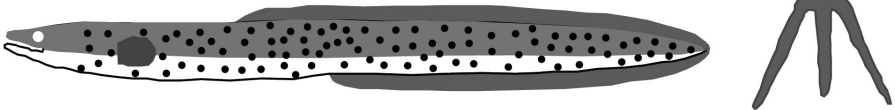Group 3. Without variegated body markings and with a long dorsal fin

Group 4. Without variegated body markings and with a short dorsal fin

Fig. 1. Classification of the genus *Anguilla* with no consideration of the geographic distribution by Watanabe (2003) and Watanabe et al. (2004). The eels could be classified into four groups.

In groups 3 and 4, the classifications were same as those of Ege's groups of III and IV as follows. Groups 1 and 2 were partly different from those of Ege's groups of I and II as follows.

  I. Variegated species with broad, undivided maxillary and mandibular bands of teeth.
 II. Variegated species with a toothless longitudinal groove in the maxillary and mandibular bands of teeth.
III. Species without variegated markings and with a long dorsal fin.
IV. Species without variegated markings and with a short dorsal fin.

The main difference between Ege (1939) and Watanabe (2003) and Watanabe et al. (2004) in their classifications were the characteristics of the teeth. Watanabe (2003) and Watanabe et al. (2004) used the width of the midpart of the maxillary band, divided by the length of the maxillary band and the number of teeth of the midpart of the maxillary band, as the dentition characteristic, instead of groove, as Ege did (1939). Watanabe (2003) suggested that his classification was more accurate than that of Ege (1939).

If we do not take into account the geographic distribution of the genus *Anguilla*, each specimen can be classified into four groups by means of their key morphological characters. Watanabe et al. (2005) studied those four groups using a molecular genetic analysis for further classification. The 16S rRNA region was processed with 10 restriction enzymes; *Alu*I, *Apa*I, *Bsp*1286I, *Eco*OI109I, *Eco*T14I, *Hha*I, *Msp*I, *Mva*I and *Van*91I (Takara Shuzo Co., Ltd.) and *Bbr*PI (Toyobo Co., Ltd.) (Watanabe 2003; Watanabe et al. 2005). Aoyama et al. (2000b) had examined the species identification in the genus using six of these restriction enzymes and suggested that the 16S rRNA was appropriate for identifying eels at the species level. There were 14 clades in the dendrogram, which were distinguished using both molecular genetic markers and the four groups classified by morphological characteristics (Watanabe et al. 2004, 2005). Furthermore, 1 of the 14 clades was divided into 2 clades based on the number of vertebrae (Watanabe 2003). A total of 15 taxa were found in the genus *Anguilla* using their morphological and genetic characteristics (Watanabe et al. 2004, 2005).

Problems of subspecies

Ege (1939) was able to name 16 species (and 3 subspecies) by systematic analyses. Castle and Williamson (1974) suggested that *A. ancestralis* was a synonym of *A. celebesensis*, and therefore reduced the genus to 15 species and 3 subspecies. Thereafter, Watanabe et al. (2004, 2005) identified 15 species, mostly consistent with Ege's (1939) milestone study. However, "18 species, including subspecies" until 2008 or "19 species including subspecies with a recently discovered new species in 2009" is still referred to and cited. In other words, systematic analyses by Ege (1939) have been widely accepted until now.

In a molecular genetic study on the genus *Anguilla*, one specimen of each of the 15 taxa was used to sequence a 1485 base pair segment of the 16S rRNA region (Watanabe et al. 2005). The genetic distance between each species ranged from 0.0115 to 0.0571 (Watanabe 2003). The genetic distance between *A. anguilla* and *A. rostrata* was 0.0115, which was the lowest value between taxa (Watanabe 2003). Watanabe (2003) also examined the differences between specimens of the subspecies of *A. bengalensis*, *A. bicolor* and *A. australis* classified by Ege (1939) and *A. australis* classified by Dijkstra and Jellyman (1999). The values between the subspecies within *A. bengalensis*, *A. bicolor* and *A. australis* were 0.0061, 0.0068 and 0.0034, respectively, and these values were much lower than those between the each of the 15 species (Watanabe 2003). Watanabe (2003) suggested that there were 15 definite taxa that presented 15 species, but that the subspecies designations were questionable.

More recently, Watanabe et al. (2014b) argued the subspecies designation details as follows. Recent information about the population structures of anguillid eels also raises questions about how to approach the use of the subspecies or population taxonomic categories for these unique catadromous fishes. There is now clear evidence of both morphological and/or molecular genetic differences between not only each of the subspecies pairs of *A. bicolor*, *A. bengalensis* and *A. australis* (Dijkstra and Jellyman 1999; Watanabe et al. 2006, 2008a; Shen and Tzeng 2007; Minegishi et al. 2009, 2012) but also between the multiple populations of *A. marmorata* (Minegishi et al. 2008; Watanabe et al. 2008b, 2009b). The genetic distances between these subspecies or between the populations of *A. marmorata* are slightly less than those between the most recently diverged species of *A. rostrata* and *A. anguilla* that are present in the North Atlantic, which have the smallest genetic divergence of all species pair comparisons of anguillid eels (Watanabe et al. 2008b; Minegishi et al. 2009). Considering the morphological and molecular genetic differences of these taxonomic groups of eels and the concept of reproductively isolated biological units (populations), there are three possible taxonomic approaches to the subspecies/populations issue of the genus *Anguilla*: (i) because recent molecular genetic (Ishikawa et al. 2004; Minegishi et al. 2008; Gagnaire et al. 2011) and morphological (Watanabe et al. 2008a, 2009b) studies recognised several populations in *A. marmorata*, the two subspecies of *A. bicolor*, *A. bengalensis* and *A. australis* could also be regarded merely as population variations within species. However, the levels of divergences among or between the populations of *A. marmorata*, *A. bicolor*, *A. bengalensis* and *A. australis* appear to differ slightly in terms of both morphological characteristics and the degree of genetic divergence (Watanabe et al. 2008b; Minegishi et al. 2009), possibly due to when the divergences occurred or due to how much subsequent gene exchange has occurred. (ii) If the validity of the two subspecies of *A. bicolor*, *A. bengalensis* and *A. australis* is to be accepted, the populations or regional metapopulations of *A. marmorata* with similar differences could also be regarded as subspecies. (iii) If the focus is on the reproductive isolation of populations of the genus *Anguilla*, without

considering the degree of differences in morphological and molecular genetic characters, then each population should be regarded as a species, and the subspecies taxonomic concept would not be used with the genus *Anguilla*.

The morphological and molecular genetic differences that have been found and the allopatric distributions of each of the subspecies pairs of *A. bicolor*, *A. bengalensis* and *A. australis* agree with the subspecies concept defined by Mayr and Ashlock (1991). However, in order to use and expand the use of the subspecies concept to *A. marmorata* as in case (ii), the use of two taxonomic (species and subspecies) and one ecological (population) units are required. Case (i) or (iii) would be simpler concepts than case (ii) because these cases use just species and population designations without any subspecies. Furthermore, some biologists have suggested that the subspecies as a category rank should be abolished (Wilson and Brown 1953; Burt 1954; Hagmeier 1958).

Most recently, a molecular genetic study accurately identified the two subspecies of *A. bicolor* (Tanaka et al. 2014). A single DNA nucleotide substitution in the mitochondrial DNA 16S rRNA gene was employed to identify the two subspecies where *A. bicolor bicolor* and *A. bicolor pacifica* possessed adenine and guanine, respectively (Tanaka et al. 2014). This substitution was highly conserved at 100% in *A. bicolor bicolor* (108/108) and 99.9% in *A. bicolor pacifica* (181/182), and the misidentification rate was estimated to be 0.34% (Tanaka et al. 2014). These results suggest that *A. bicolor* is further divided taxonomically into the two subspecies of *A. bicolor bicolor* and *A. bicolor pacifica*, although they are almost identical in their morphological characteristics except for the mode of their number of vertebrate (Watanabe et al. 2014b).

Based on the discussion, concept and idea proposed by Watanabe et al. (2014b) and recent genetic molecular analysis by Tanaka et al. (2014), the subspecies of *Anguilla bicolor*, *A. bengalensis* and *A. australis* are valid at the moment. However, further molecular genetic identification study is required for each species, in combination with population structure and life history studies.

New species

The genus *Anguilla* has long been recognised as being comprised of 15 species (Ege 1939; Castle and Williamson 1974; Watanabe 2003; Watanabe et al. 2004, 2005). After the first comprehensive taxonomic revision by Ege (1939), three new species, *A. breviceps* Chu and Jin (1984), *A. foochowensis* Chu and Jin (1984), and *A. nigricans* Chu and Wu (1984) were described from China (Chu 1984). However, their identifications were based only on the external morphology and body proportions of single individuals, so a re-examination of these doubtful species was suggested (Tabeta 1994). Watanabe et al. (2009) and Teng et al. (2009) discovered new species of the genus *Anguilla* almost simultaneously, *A. luzonensis* and *A. huangi*, respectively, from the Cagayan River system on the northern Luzon Island of the Philippines and from the glass eels collected from the Cagayan River estuary that were reared in a culture pond in Taiwan,

respectively. These discoveries are the first in seventy years since Ege's (1939) revision. Comparisons using morphological and molecular genetic characteristics clearly showed that *A. luzonensis* and *A. huangi* are the same species (Watanabe et al. 2013). Thus, *A. luzonensis* became the valid species name and *A. huangi* became a junior synonym of *A. luzonensis* (Watanabe et al. 2013). The results all lead to the conclusion that the genus *Anguilla* has been recognised as being comprised of 16 species (Ege 1939; Castle and Williamson 1974; Watanabe 2003; Watanabe et al. 2004, 2005, 2009a, 2013, 2014b).

Latest taxonomy

Based on the systematic analyses by Ege (1939) and further analyses by morphological and molecular genetic characters by Castle and Williamson (1974), Watanabe (2003) and Watanabe et al. (2004, 2005, 2009a, 2013, 2014a,b), the genus *Anguilla* is classified as 16 species, three of which were divided into two subspecies (Table 1); *A. celebesensis*, *A. interioris*, *A. megastoma*, *A. luzonensis*, *A. bengalensis bengalensis*, *A. bengalensis labiata*, *A. marmorata*, *A. reinhardtii*, *A. borneensis*, *A. japonica*, *A. rostrata*, *A. anguilla*, *A. dieffenbachii*, *A. mossambica*, *A. bicolor bicolor*, *A. bicolor pacifica*, *A. obscura*, *A. australis australis*, and *A. australis schmidtii* (Table 1).

Worldwide distribution

The genus *Anguilla* is currently recognised as being comprised of 16 species. They are widely distributed in most of the tropical and subtropical areas of the world, except for South America and West Africa (Fig. 2). Tesch (2003) divided the freshwater eels into tropical and temperate species according to their geographic distributions. The tropical and temperate eels consist of 11 and 5 species, respectively. The continental distributions of the temperate species appear to be related to the subtropical circulation of the oceans, with most species located along the west sides of the Atlantic, Indian, and Pacific Oceans, except for the European eel, *A. anguilla* (Ege 1939; Watanabe 2003; Fig. 2). However, anguillid eels are absent along the east coast of South America, despite the existence of the warm Brazil Current. Based on this geographic pattern, the Atlantic species (*A. anguilla* and *A. rostrata*) are geographically separated from their other congeners in the Pacific and Indian Oceans (Minegishi et al. 2005). Such a unique geographic distribution has recently attracted the attention of biologists, and numerous molecular phylogenetic studies have been conducted (e.g., Aoyama and Tsukamoto 1997, 2001; Bastrop et al. 2000; Lehmann et al. 2000; Lin et al. 2001; Minegishi et al. 2005; Teng et al. 2009).

    Of the eleven species found in tropical areas, six species occur in the western Pacific around Indonesia, i.e., *A. celebesensis*, *A. interioris*, *A. bengalensis*, *A. marmorata*, *A. borneensis*, and *A. bicolor* (Fig. 3) (Ege 1939; Castle and Williamson 1974; Arai et al. 1999). Molecular phylogenetic researches on
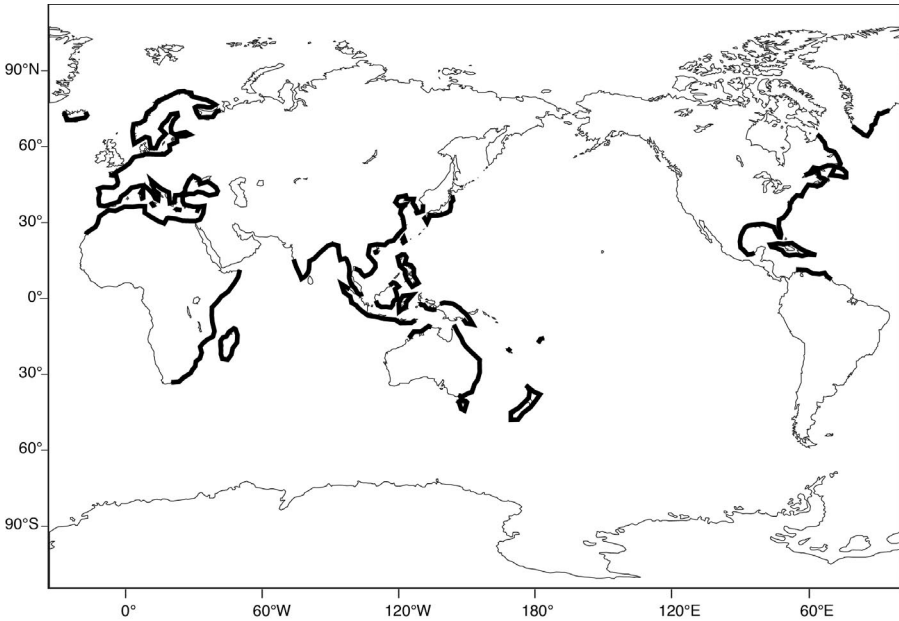
Fig. 2. Global distribution of genus *Anguilla* (thick lines).

freshwater eels have recently revealed that tropical eels are the most basal species originating in the Indonesian region and that freshwater eels radiated out from the tropics to colonise the temperate regions (Minegishi et al. 2005).

Knowledge of the geographic distributions of the genus *Anguilla* has increased dramatically since Ege's (1939) study. Passive transport of leptocephali for long periods of 3 months or more can easily change the range of recruitment of glass eels, thus the geographic distribution of the species can change from one year to another. In fact, there have been many new findings regarding the geographic distributions of the freshwater eels since Ege's (1939) comprehensive studies, e.g., *A. rostrata* in Iceland (Boëtius 1980; Williams and Koehn 1984; Avise et al. 1990), *A. reinhardtii* in New Zealand (Jellyman et al. 1996), *A. marmorata* in the Galápagos Islands (McCosker et al. 2003), and *A. celebesensis's* absence in New Guinea (Aoyama et al. 2000a). Furthermore, there have been several reports which introduced non-native species of freshwater eels that have been caught in several areas of the world (e.g., Shapovalov et al. 1959; Skinner 1971; Tabeta et al. 1976b, 1977; McCosker 1989; Zhang et al. 1999; Sasai et al. 2001; Han et al. 2002; Okamura et al. 2008; Arai et al. 2009) as a result of the international trade in glass eels and young eels for aquaculture.

Representative geographical distribution range for each anguillid species by distinct morphological characteristics classified into four groups by Ege (1939) Watanabe (2003) and Watanabe et al. (2004, 2005, 2009, 2013, 2014a,b) is summarized in Table 2.

Fig. 3. Geographical distribution of genus *Anguilla* shown by Ege (1939) with the latest taxonomy. Distribution of each species from "a" to "d" is followed by the classification of morphological characters from group 1 to group 4 as shown in Fig. 1.
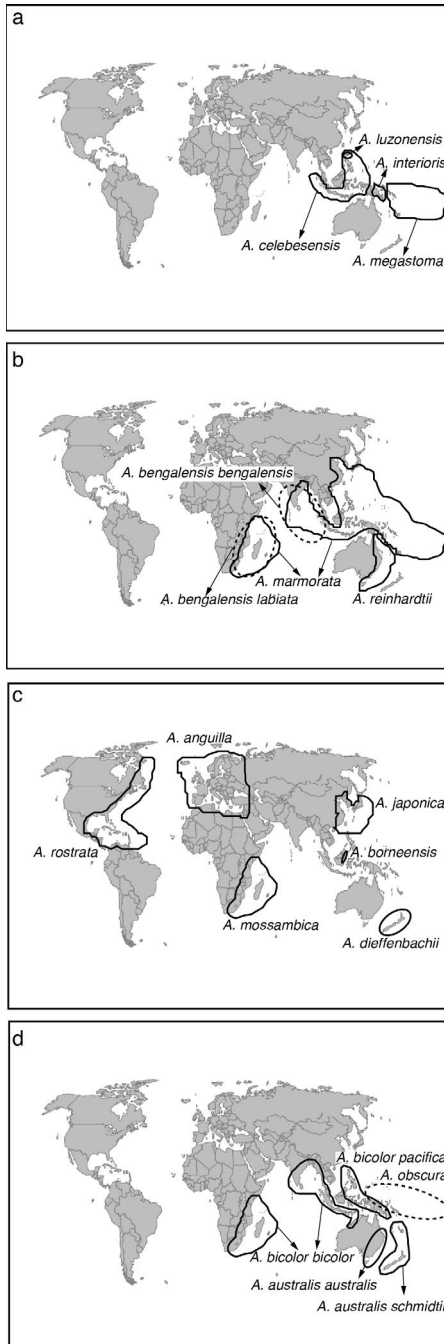
Table 2. Distinct morphological characters classified into four groups with representative geographical distribution range for each anguillid species.

Group	Species	Distinct morphological characters	Geographical distribution range
1	A. celebesensis	variegated skin	Jawa, Sumatra, Sulawesi Island in Indonesia
		broad maxillary bands of teeth	Philippines, New Guinea
	A. interioris	long dorsal fin	New Guinea
	A. megastoma		Solomon Islands, New Caledonia, Fiji Islands, Cook Islands
	A. luzonensis		Northern Philippines
2	A. bengalensis bengalensis	variegated skin	Sri Lanka, Bangladesh, India, Myanmar, Malaysia
		narrow maxillary bands of teeth	Sumatra Island in Indonesia and Andaman Islands
	A. bengalensis labiata	long dorsal fin	mid-southeastern part of Africa
	A. marmorata		longitudinally from the east coast of Africa to the Marquesas Islands in the southeast Pacific Ocean and as far north as southern Japan
	A. reinhardtii		Eastern Australia, Northern New Zealand
3	A. borneensis	nonvariegated skin	Borneo Island
	A. japonica	long dorsal fin	Japan, China, Korea, Taiwan, Northern Philippines
	A. rostrata		North and South America
	A. anguilla		Europe, North Africa
	A. dieffenbachii		New Zealand
	A. mossambica		mid-southeastern part of Africa, Madagascar
4	A. bicolor pacifica	nonvariegated skin	Philippines, Sulawesi Island in Indonesia, New Guinea
	A. bicolor bicolor	short dorsal fin	Africa, India, Sri Lanka, Bangladesh, Myanmar, Malaysia northwestern Australia, Greater Sunda Islands
	A. obscura		northeastern Australia, New Caledonia, Fiji Islands, Samoa, Tahiti, Cook Islands, Maluku Islands
	A. australis australis		Southeastern Australia, Tasmania
	A. australis schmidtii		New Zealand, New Caledonia, North Norfolk Island

References: Ege (1939), Watanabe (2003), Watanabe et al. (2004, 2005, 2009, 2013, 2014a,b) and Arai and Wong (2016).

Group 1: *A. celebesensis*, *A. interioris*, *A. megastoma* and *A. luzonensis* (Fig. 3a). Ege (1939) described that the geographical range of *A. celebesensis* is southward from Luzon Island (Philippines) to Rotti Island (Indonesia) and eastward from Noas Island (Indonesia) to Humboldt Bay on the coast of New Guinea. However, *A. celebesensis* was not found in New Guinea based on a molecular genetic study (Aoyama et al. 2000a). *A. interioris* is an endemic species in New Guinea only (Ege 1939). *A. megastoma* is distributed along a belt of islands south of the equator in the western South Pacific Ocean (WSP) (Ege 1939; Beumer et al. 1981; Allen 1991; Marquet and Galzin 1991). *A. luzonensis* is a recently discovered species just found in the Cagayan River at the northern tip of the Luzon Island, Philippines (Teng et al. 2009; Watanabe et al. 2009, 2013).

Group 2: *A. bengalensis*, *A. marmorata* and *A. reinhardtii* (Fig. 3b). The distribution range of *A. bengalensis* includes India, from Bombay in the northwest to Calcutta in the northeast, Ceylon, Sandoway, the coast of Burma, Andamans, and Serdang, the west coast of North Sumatra and Peninsular Malaysia, and the east coast of southern Africa from Kenya to South Africa (Fig. 3b, Ege 1939). *A. marmorata* is a unique tropical anguillid that reaches large sizes of almost 2 m in length with a maximum weight of 21 kg (Castle 1984). This species has the widest geographic distribution of the 16 species of the genus *Anguilla* (Ege 1939; Castle and Williamson 1974; Fig. 1) and is found longitudinally from the east coast of Africa to the Marquesas Islands in the southeast Pacific Ocean and as far north as southern Japan (Ege 1939). Recently this species was found at the Palmyra Atoll in the central Pacific (Handler and James 2006) and even farther to the east in the Galápagos Islands (McCosker et al. 2003), which indicates that it has an even wider geographic range than previously thought. *A. reinhardtii* is found mostly in New Caledonia, eastern Australia and New Zealand (Ege 1939; Beumer 1996; Jellyman et al. 1996).

Group 3: *A. borneensis*, *A. japonica*, *A. rostrata*, *A. anguilla*, *A. dieffenbachii* and *A. mossambica* (Fig. 3c). The freshwater growth habitat of *A. borneensis* is limited to the east-central part of Borneo (Ege 1939). The freshwater distribution of *A. japonica* ranges from Taiwan, through China, Korea, and north to Japan (Tesch 2003). Tabeta et al. (1976c) investigated the anguillid glass eels in the Cagayan River at the northern tip of Luzon Island of the Philippines, which is the southern-limit of the distribution range of the species. The habitat range of *A. rostrata* spans more than 50 degrees of latitude along the Atlantic from the southern tip of Greenland to north-eastern South America (Boëtius 1985; Nilo and Fortin 2001; Tesch 2003). *Anguilla anguilla* is distributed from North Cape in Northern Norway, southwards along the coast of Europe, all coasts of the Mediterranean and along the North African Coast (Schmidt 1909; Dekker 2003). *A. dieffenbachii* is endemic in New Zealand (Ege 1939; Jellyman 2003). *A. mossambica* inhabits rivers and lakes in south-eastern Africa and Madagascar (Jubb 1964; Tesch 2003).

Group 4: *A. bicolor*, *A. obscura* and *A. australis* (Fig. 3d). *A. bicolor* has the second widest geographic distribution in the genus *Anguilla* (Ege 1939), and is distributed from the eastern coast of Africa through the Indonesian Seas to New Guinea adjacent to the Pacific Ocean (Ege 1939). The species was found in Taiwan (Tzeng and Tabeta 1983) and Yagushima Island in southern Japan (Yamamoto et al. 2001). *A. obscura* is distributed along a belt of islands south of the equator in the WSP ranging from western New Guinea to Tahiti (Ege 1939; Jellyman 1991). *A. australis* is widely distributed in southeast Australia, Tasmania, New Caledonia, Norfolk Island, Lord Howe Island and New Zealand (Ege 1939; Beumer 1996; Jellyman et al. 1996).

Conclusion

The recent comprehensive revisions of the genus *Anguilla* have been performed by Watanabe (2003) and Watanabe et al. (2004, 2005, 2014b) with reports on a new species in the genus (Teng et al. 2009; Watanabe et al. 2009a, 2013) using morphological and molecular genetic analyses after the Ege's (1939) revision. In the last revision, this genus is definitively classified into 16 species, three of which are divided into two subspecies, although subspecies problems are still in discussion. Regarding juvenile eels such as glass eels and elvers, specimens can be classified as either long-finned or short-finned without vertebrae counting. Further identification, however, is difficult for samples of unknown origin. Thus, molecular genetic analyses are indispensable in order to precisely identify species, especially in case of tropical species, as a number of key morphological characteristics are overlapping among these species. Regarding adult specimens, Ege's (1939) systematics have long been widely accepted. However, Watanabe et al. (2004) found that the morphological characters described by Ege (1939) were not sufficient to classify all species of this genus and that geographic distribution was an indispensable characteristic for his taxonomy of these eels. The geographic distribution of each species is not a suitable taxonomic characteristic of the freshwater eels by itself because it overlaps among species and is highly variable depending on the environment. Furthermore, the new species discovered recently suggests the possibility of finding other new species in the future. When only morphological characters were used, the freshwater eels could be classified into only four groups (Watanabe et al. 2004). Thus, analyses of morphological characters in combination with molecular genetic characters are indispensable for precise species identification in the genus.

Keywords: *Anguilla*, taxonomy, classification, distribution, morphology, molecular genetics, subspecies

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Overview and Current Trends in Studies on the Evolution and Phylogeny of *Anguilla*

Mei-Chen Tseng

Introduction

Anguilliforms are a distinctive group of teleosts that comprise of 820 species which are further classified into 20 families and 147 genera and share a unique leptocephalus larva (Nelson 2006; Inoue et al. 2010; Johnson et al. 2011). Anguilliforms first appeared as fossils in the Aptian age, about 113–119 million years ago (Mya) (Patterson 1993; Belouze et al. 2003). Johnson et al. (2011) discovered the living fossil eel *Protoanguilla palau* at Palau. Phylogenetic analysis and divergence time estimation based on whole mitogenome sequences from various actinopterygians indicated that *P. palau* is one of the most primitive and independent lineages of true eels. Its evolutionary history dates back to the Triassic Age (251–199.6 Mya) and suggests that the origin of Anguilliformes should be about 200–250 Mya (Fig. 1).

Fossil records of the genus *Anguilla* have been found in Europe from the Eocene epoch (Ypresian, about 50–55 Mya) (Patterson 1993). Nevertheless, according to mitochondrial analyses using the molecular clock for bony fishes calibrated by Kumazawa et al. (1999), the estimated divergence time between *Anguilla* and *Serrivomer* is 52 Mya and the speciation of extant anguillid

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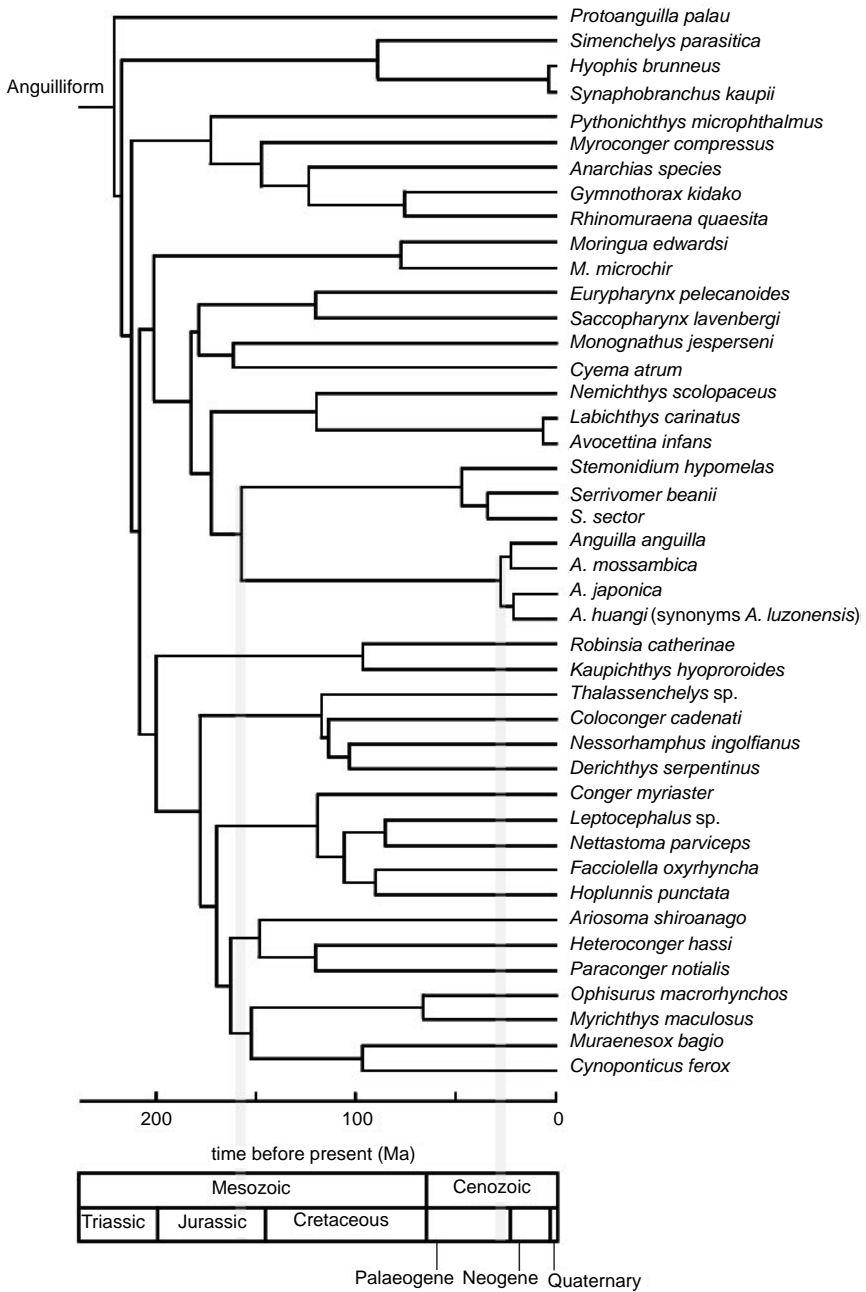 E-mail: mctseng@mail.npust.edu.tw

Fig. 1. Partial phylogenetic tree and divergence time estimation using mitogenome sequences from Anguilliform fishes. The figure was modified from Johnson et al. (2011).

species began approximately 20 Mya (Minegishi et al. 2005; Johnson et al. 2011). However, the fossil evidence is slightly inconsistent with molecular evidence, as the latter infers a more recent speciation pattern in *Anguilla* eels. Minegishi et al. (2005) indicated that the divergence time deduced from molecular data could be underestimated due to the group's peculiar ecological characteristics associated with slow metabolism. On the other hand, Teng et al. (2009) described the existence of *Anguilla* species 55 Mya based on fossil records, and several radiation events that probably occurred around 20–55 Mya. The last common ancestor of all living *Anguilla* began speciation about 20 Mya. However, these inferences need to be explicitly proven by more substantial evidence.

In all relative studies of *Anguilla* eel evolution, several questions have always mystified us. How many species of *Anguilla* are there in the world? Where did the *Anguilla* genus originate? Are all temperate freshwater eels closely related sister species? Which species of *Anguilla* is the most ancestral? How did *A. anguilla* and *A. rostrata* come to occupy the Atlantic Ocean? Fortunately, there have been major advances in molecular technologies during the past two decades that have enabled researchers to solve some of the foregoing problems using molecular genetic markers.

How many *Anguilla* species have occurred over time?

There is no information on how many *Anguilla* species have existed on earth during the last 50 million years. More than 30 species of *Anguilla* eels have been recorded, but some of them are synonyms (http://www.fishbase.org/ Nomenclature/Scientific NameSearchList.php?). Nineteen species/subspecies are unequivocally identified via morphological characters and DNA barcoding, including 2 Atlantic eels, 3 Oceanian eels, 1 Western Pacific eel, and 13 Indo-Pacific eels (Minegishi et al. 2005; Teng et al. 2009). Even so, it is possible that some cryptic species or undetected species of *Anguilla* eels exist. More precisely, the names of uncommon species still need to be verified, e.g., the species *A. breviceps* Chu and Jin 1984, seems to have never been examined. Cryptic species are worth examining more thoroughly because there are instances that prove their existence; for example, a new species was recently discovered from the Cagayan River estuary, northern Luzon, Philippines, by Lin et al. (2002). In order to identify this species, they spent five years rearing elvers into adulthood and measured their morphological characters, naming the species *A. huangi* (Teng et al. 2009). This species was coincidentally described in the same year under another name, *A. luzonensis*, by Watanabe et al. (2009). Cryptic *Anguilla* species must be found and identified if the genus' phylogeny is to be accurately determined.

Where is the original center of *Anguilla* eel evolution?

Almost all eel researchers believe that the Indo-Pacific waters are the original location of *Anguilla* eels (Table 1). The distributions of inshore marine fishes can be divided into four regions based on the order of decreasing biodiversity: Indo-West Pacific, western Atlantic, eastern Pacific, and eastern Atlantic. The Indo-West Pacific region ranges from South Africa and the Red Sea, east through Indonesia and Australia to Hawaii and through the South Pacific islands to Easter Island, harboring approximately one-third of all shallow marine fishes (*ca*. 3000 species). This region is also a significant reservoir of biodiversity for other marine taxa (Briggs 1974; Helfman et al. 1997), and is considered a "center of origin" from where many species evolved and then dispersed to wherever they are found today. In addition, Carpenter and Springer (2005) indicate that the global maxima of marine biodiversity is found in the Indo-Malay-Philippines Archipelago (IMPA), which is part of the Indo-Pacific Ocean. This has long been considered to be the area of highest marine biodiversity, with decreasing latitudinal and longitudinal gradients in species richness radiating from this center (Mora et al. 2003). Apparently, almost half of the *Anguilla* eels also reside in the IMPA. This high level of biodiversity is inferred to have possibly resulted from numerous vicariant and island integration events (Carpenter and Springer 2005). Present distributions can be compared to phylogenetic relationships in order to assess the relative movements of biota. In all pertinent phylogenetic studies of *Anguilla* eels, *A. borneensis* and *A. mossambica* are deduced as possible ancestors (Aoyama et al. 2001; Minegishi et al. 2005) of the other species distributed around the Indo-Pacific Ocean. It is firmly believed that this ocean is the original center of *Anguilla* speciation.

Are all temperate freshwater eels close sister species?

All temperate eels are like their sister group except *A. japonica*. Ege (1939) subdivided *Anguilla* eels into four distinct groups based on morphological characters. Four temperate *Anguilla* eels (*A. rostrata*, *A. anguilla*, *A. japonica*, and *A. dieffenbachii*) and two tropical species (*A. mossambica* and *A. malgumora*) having similar long dorsal fins and uniform coloration are in the same group. In contrast, temperate *A. australis* and tropical *A. bicolor* and *A. obscura*, which have shorter dorsal fins and uniform coloration, were placed in the same group (Fig. 2a). Although *A. anguilla*, *A. rostrata* and *A. japonica* have similar adult-phase morphological characteristics and rest in temperate water, their mitochondrial DNA (Minegishi et al. 2005) (Fig. 2b) and microsatellite flanking regions (Tseng 2012) suggest that *A. japonica* is not clustered with other temperate eels in the phylogenetic tree. It is interesting that inconsistent morphological and molecular phylogenetic trees cover *A. australis* and *A. japonica*. In brief, *A. rostrata*, *A. anguilla*, *A. australis*, and *A. dieffenbachii*

Table 1. Hypotheses of *Anguilla* eels dispersion patterns have been proposed below.

Published	Species number	Molecular Marker	Description
Tethys Sea Route			
Aoyama and Tsukamoto (1997) Tsukamoto and Aoyama (1998)	8 species	Cytochrome b (*Cyt b*) (410 bp)	*A. celebesensis* was the possible ancestral species of the Anguillidae. In the Eocene epoch (57–36 Mya) the ancestral eel originated in the western Pacific in and around Indonesia. The ancestors of Atlantic eels may thus have dispersed through the Tethys Sea to enter the paleo-Atlantic Ocean from the western Pacific occurred over 25–30 Mya. The split between two Atlantic eels is estimated at approximately 10 Mya.
Aoyama et al. (2001)	18 species/ subspecies	16S rRNA+ *Cyt b* (1,427 bp + 1,140 bp)	*Anguilla* was a monophyletic group. *A. bornensis* was the earliest derived species. Species disperse from Indonesia and move to Atlantic through the Tethys Corridor. The ancestral species of Atlantic eels moved into the Atlantic Ocean at least before the closure of the Tethys Sea at around 30 Mya. *Anguilla* originated about 50–60 Mya.
Bastrop et al. (2000)	7 species	16S rRNA (558 bp)	*A. marmorata* has the most ancestral lineage. *Anguilla* eels had originated in the Indo-Malaysian region. Atlantic eels were separated from congeners by circumglobal Tethys Seaway about 18 Mya.
Multiple radiation events + Central American Isthmus route Isthmus			
Lin et al. (2001)	12 species/ subspecies	12S rRNA + *Cyt b* (950 bp + 1140 bp)	No ancient species was inferred. *Anguilla* first radiated about 20 million years ago. The ancestors of Atlantic eels trekked across the Central American Isthmus to the Sargasso Sea for spawning. Multiple radial speciation events have occurred in the Indo-West Pacific region during *Anguilla* evolution.
Teng et al. (2009)	19 species/ subspecies	13 mt protein coding sequences (11,394 bp)	A new species *Anguilla huangi* was discovered, description and nomenclature. The phylogeny of freshwater eels is polytomies which are likely derived from multiple radiation events. Atlantic eels dispersed by the Central American Isthmus.
Multidirectional dispersion			
Minegishi et al. (2005)	18 species/ subspecies	protein-coding genes and RNA genes of Whole mitochondrial (mt) genome (15,187 bp)	*A. mossambica* was the most basal species of *Anguilla* eels. The unique geographic distribution of *Anguilla* eels has resulted from multiple dispersal events. The beginning of speciation was estimated as 20 Mya but it may be underestimated.