

Distribution and dispersal of anguillid leptocephali in the western Pacific Ocean revealed by molecular analysis

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ABSTRACT: To determine the distribution and dispersal of leptocephalus larvae of freshwater eels *Anguilla* spp. in the western Pacific, mitochondrial DNA sequences of 63 larvae were compared with those of adults. All larvae were definitively identified as 1 of the following 6 species: *A. marmorata*, *A. bicolor pacifica*, *A. reinhardtii*, *A. australis*, *A. megastoma* or *A. japonica*. Species-specific patchiness in the distribution of these leptocephali in the western Pacific was apparent. *A. marmorata* showed 2 different areas of patchiness in both the North and South Pacific (around 15° N and 10° S). Horizontal distributions of anguillid leptocephali are apparently closely related to the freshwater habitat of adults by way of ocean currents. Accordingly, specific dispersal routes of leptocephali from likely spawning areas to their freshwater habitats are suggested.

KEY WORDS: Leptocephali · *Anguilla* · Western Pacific · DNA identification · Dispersal · Spawning areas

INTRODUCTION

Based on extensive earlier research it has become well known that freshwater eels *Anguilla* spp. are catadromous and have a long period of larval oceanic dispersal. The collection of great numbers of anguillid leptocephali in the open ocean suggests that the North Atlantic species *A. anguilla* and *A. rostrata* spawn in the Sargasso Sea (Schmidt 1923, Kleckner & McCleave 1985), and the Japanese eel *A. japonica* to the west of the Mariana Islands (Tsukamoto 1992). However, in the South Pacific, where 6 species occur (Ege 1939,

Jespersen 1942), the spawning areas and larval dispersal routes of these species are still unknown. Only 16 anguillid leptocephali have been reported so far from this part of the Pacific: 2 *A. megastoma*, 4 *A. australis*, 3 *A. obscura* and 7 *A. marmorata* / *A. reinhardtii* (Jespersen 1942, Castle 1963).

The leptocephalus larva, which is the unique larval form of elopomorph fishes, is so distinct from the adults that their study almost constitutes a separate science (Smith 1989). Even for leptocephali of *Anguilla*, which have been well described from the morphological characteristics of their adults, identification to species level is often difficult, especially for small specimens because their morphological keys are not fully developed (Castle 1963, Mochioka 1996).

Molecular techniques, which have recently seen great advancements, are able to identify characters which continue on from the larva through the metamorphosis to the adult. These molecular characters, determined from fully developed adults, are likely to

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be a powerful tool for the identification of small leptocephali which are not well developed morphologically.

In this paper we identified western Pacific anguillid leptocephali from mitochondrial DNA sequences and revealed their horizontal distributions and oceanic movements. Based on these results it might now be possible to explore further the spawning area(s) for the Australian and New Zealand short-finned eel *Anguilla australis* in particular.

MATERIALS AND METHODS

Materials. A total of 372 anguillid leptocephali were collected during the research cruise KH95-2 of RV 'Hakuho-Maru' of the Ocean Research Institute, University of Tokyo in the western Pacific (Fig. 1). The leptocephali were sampled by Isaacs-Kidd Midwater Trawl (IKMT) with a 8.7 m² mouth opening and 0.5 mm mesh. Net sampling involved a 60 min oblique tow from the surface to 300 m depth. The following morphological characters of fresh specimen were recorded using a binocular microscope: total length (to the nearest 0.1 mm), total number of myomeres, positions of the origin of dorsal and anal fins, anterior margin of gall bladder, and vertical blood vessels. The identification of species was made based on these morphological characters, following Jespersen (1942), Castle (1963) and Tabeta & Mochioka (1988). A total of 128 leptocephali were definitively identified as *Anguilla japonica* by morphological characters, and separated from the total of 372. Of the remaining 244, 63 specimens, comprising all morphological types, were subsampled and preserved in 95% ethanol or in a deep freezer at -80°C for subsequent genetic analysis (Table 1). The specimens which could not be examined genetically were considered as unidentified specimens.

DNA analysis. Total genomic DNA extraction from each leptocephalus was carried out following a standard protocol (Aoyama & Tsukamoto 1997). A portion of mitochondrial 16S ribosomal RNA gene was amplified via polymerase chain reaction (PCR) using the oligonucleotide primers H2510 and H3058 (Miya & Nishida 1996). Amplification parameters were 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 60 s. Double-stranded DNA products from PCR were sequenced following the manufacturer's protocol (Applied Biosystems, Inc.) on a 373A DNA sequencer (Applied Biosystems, Inc.). Sequences were obtained from light strand only.

Species identification by mitochondrial DNA sequences. Using MacClade version 3.0 (Maddison & Maddison 1992), we first compared sequences previously determined from identified adult specimens of the Pacific species *Anguilla japonica*, *A. marmorata*,

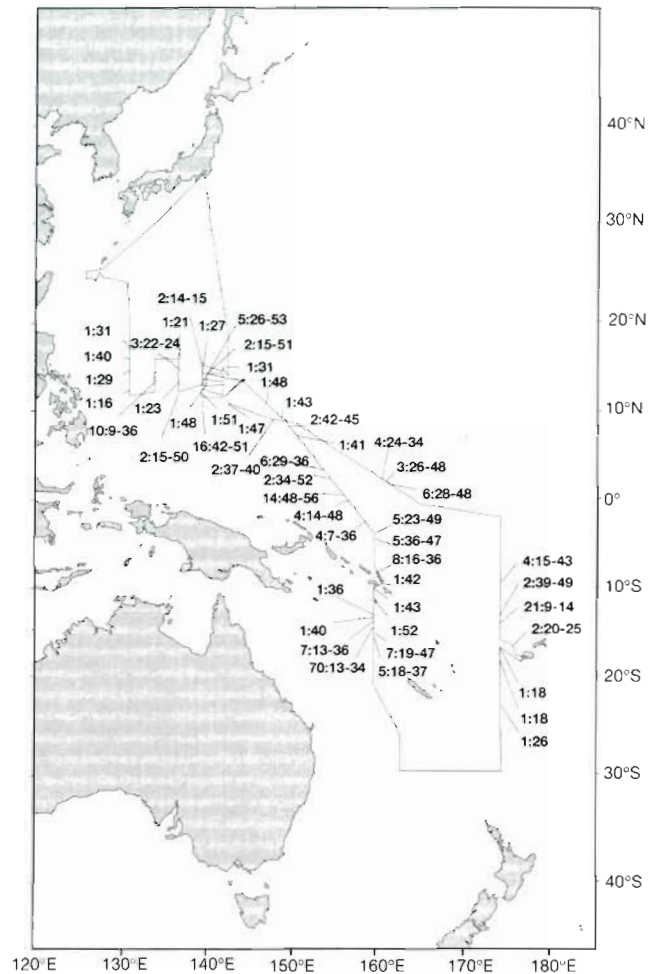


Fig. 1. Chart and sampling locations of anguillid leptocephali, except for 128 *Anguilla japonica*, collected on the research cruise during 7 July to 27 September 1995 of RV 'Hakuho-Maru', Ocean Research Institute, University of Tokyo. Number of leptocephali collected and their ranges of total length (mm) are shown for each sample location

A. borneensis, *A. celebesensis*, *A. interioris*, *A. megastoma*, *A. bicolor pacifica*, *A. reinhardtii*, *A. obscura*, *A. australis australis*, *A. australis schmidtii* and *A. dieffenbachii* (Aoyama 1998). These sequence data will appear in the DDBJ/EMBL/GenBank nucleotide sequence databases with the accession numbers: AB021748, AB021750–AB021754, AB021756, AB021758, AB021762–AB021764.

Based on this comparison, we established 'specific standard sequences' comprised of 60 sites at which specific substitutions were observed (Table 2). Thereafter, homologous sites obtained from leptocephalus sequences were selected for comparison with these standards and the number of differences were counted by PAUP version 3.0 (Swofford 1991).

RESULTS

Species identification

A portion of the mitochondrial 16S ribosomal RNA gene (412 to 469 base pairs) was determined from all of the 63 anguillid leptocephali analyzed, and the 60 homologous sites were selected for comparison with specific standard sequences. Each leptocephalus sequence corresponded to one of the specific standard sequences only (0 to 3 sites differences), while they were distinguishable from the others with more than 4-fold differences (5 to 28 sites, Table 3). Based on this observation, all of the 63 individuals examined could be precisely identified as follows: 1 *Anguilla japonica*, 9 *A. australis*, 25 *A. bicolor pacifica*, 14 *A. marmorata*, 2 *A. megastoma* and 12 *A. reinhardtii* (Table 1). Other species which might occur around this area are *A. celebesensis*, *A. borneensis*, *A. obscura* and *A. dieffenbachii*, but were not found.

Among 63 leptocephali, 44 were identified morphologically, and of these, 35 (79.5%) agreed with the identification by molecular characters (Table 1).

Distribution of anguillid leptocephali

Mapping of locations of identified leptocephali revealed that their horizontal distributions showed a specific patchiness (Fig. 2). In the North Pacific, 17 *Anguilla bicolor pacifica* and 6 *A. marmorata* were distributed from the equator to 10°N, and 10°N to 20°N, respectively. In the South Pacific, 2 *A. megastoma* were found near 10°S and 8 *A. marmorata* from the equator to 10°S. Nine *A. australis* and 12 *A. reinhardtii* were found from 10°S to 20°S. The total length distribution of these specimens did not exhibit significant differences between species (Mann-Whitney *U*-test, $p > 0.05$, Fig. 3).

Table 1. Results of morphological and genetic identification of anguillid leptocephali collected during the research cruise of RV 'Hakuho-Maru' Ocean Research Institute, University of Tokyo, in 1995. Species names are abbreviated as follows: jap: *A. japonica*, rei: *A. reinhardtii*, obs: *A. obscura*, meg: *A. megastoma*, mar: *A. marmorata*, die: *A. dieffenbachii*, bip: *A. bicolor pacifica*, aua: *A. australis*

| Fish no. | Date | TL (mm) | Morphologically identified as | Genetically identified as | Sampling location |
|----------|--------|---------|-------------------------------|---------------------------|---------------------------|
| 01 | 18 Jul | 39.6 | mar | mar | 15° 59' 8 N, 130° 59' 9 E |
| 02 | 18 Jul | 29.4 | mar | mar | 14° 60' 0 N, 131° 00' 2 E |
| 03 | 19 Jul | 16.3 | mar | mar | 12° 60' 0 N, 131° 00' 1 E |
| 04 | 21 Jul | 36.0 | mar | mar | 14° 00' 1 N, 133° 59' 9 E |
| 05 | 23 Jul | 28.1 | jap | jap | 14° 00' 7 N, 136° 57' 7 E |
| 06 | 23 Jul | 22.0 | mar | mar | 14° 60' 0 N, 137° 00' 1 E |
| 07 | 23 Jul | 24.2 | mar | mar | 14° 60' 0 N, 137° 00' 2 E |
| 08 | 24 Jul | 49.6 | bip | bip | 13° 00' 1 N, 136° 59' 9 E |
| 09 | 24 Jul | | bip | bip | 14° 03' 9 N, 139° 59' 3 E |
| 10 | 25 Jul | 50.5 | bip | bip | 15° 00' 1 N, 140° 00' 3 E |
| 11 | 5 Aug | 40.0 | bip | bip | 08° 56' 4 N, 147° 58' 7 E |
| 12 | 7 Aug | 27.6 | bip | bip | 01° 41' 6 N, 160° 27' 5 E |
| 13 | 7 Aug | 24.0 | bip | bip | 01° 41' 6 N, 160° 27' 5 E |
| 14 | 7 Aug | 27.8 | bip | bip | 01° 41' 6 N, 160° 27' 5 E |
| 15 | 7 Aug | 47.9 | bip | bip | 01° 25' 9 N, 160° 54' 0 E |
| 16 | 8 Aug | 48.0 | bip | bip | 01° 09' 4 N, 161° 21' 5 E |
| 17 | 8 Aug | 41.2 | bip | bip | 01° 09' 4 N, 161° 21' 5 E |
| 18 | 8 Aug | 32.7 | bip | bip | 01° 09' 4 N, 161° 21' 5 E |
| 19 | 8 Aug | 28.1 | bip | bip | 01° 09' 4 N, 161° 21' 5 E |
| 20 | 13 Aug | 35.8 | <i>Anguilla</i> sp. | mar | 09° 56' 8 S, 174° 59' 7 E |
| 21 | 13 Aug | 42.8 | <i>Anguilla</i> sp. | meg | 09° 56' 8 S, 174° 59' 8 E |
| 22 | 15 Aug | 39.2 | <i>Anguilla</i> sp. | mar | 13° 00' 1 S, 174° 60' 0 E |
| 23 | 3 Sep | 21.1 | meg or rei | rei | 14° 30' 9 S, 160° 03' 4 E |
| 24 | 3 Sep | 26.5 | meg or die | aua | 15° 30' 0 S, 160° 00' 2 E |
| 25 | 3 Sep | 47.3 | mar | mar | 15° 04' 3 S, 160° 04' 4 E |
| 26 | 3 Sep | 33.7 | meg or die | rei | 14° 30' 9 S, 160° 03' 4 E |
| 27 | 3 Sep | 20.5 | meg | aua | 15° 04' 3 S, 160° 04' 4 E |
| 28 | 3 Sep | 26.3 | rei | rei | 14° 30' 9 S, 160° 03' 4 E |
| 29 | 3 Sep | 25.0 | rei | rei | 15° 04' 3 S, 160° 04' 4 E |
| 30 | 3 Sep | 26.0 | rei | rei | 14° 30' 9 S, 160° 03' 4 E |
| 31 | 3 Sep | 22.5 | meg or rei | aua | 14° 30' 9 S, 160° 03' 4 E |
| 32 | 3 Sep | 26.4 | rei | rei | 15° 04' 3 S, 160° 04' 4 E |
| 33 | 3 Sep | 22.4 | rei | aua | 14° 30' 9 S, 160° 03' 4 E |
| 34 | 4 Sep | 22.0 | rei | rei | 14° 30' 9 S, 160° 03' 4 E |
| 35 | 4 Sep | 22.8 | rei | aua | 14° 30' 9 S, 160° 03' 4 E |
| 36 | 4 Sep | 34.0 | rei? | rei | 14° 30' 9 S, 160° 03' 4 E |
| 37 | 4 Sep | 22.4 | meg or rei | rei | 14° 30' 9 S, 160° 03' 4 E |
| 38 | 4 Sep | 28.6 | rei | rei | 14° 30' 9 S, 160° 03' 4 E |
| 39 | 4 Sep | 32.2 | aua | aua | 14° 30' 9 S, 160° 03' 4 E |
| 40 | 4 Sep | 22.0 | meg or die | rei | 14° 30' 9 S, 160° 03' 4 E |
| 41 | 4 Sep | 20.0 | meg or die | rei | 14° 30' 9 S, 160° 03' 4 E |
| 42 | 4 Sep | 28.4 | meg or rei | aua | 14° 30' 9 S, 160° 03' 4 E |
| 43 | 4 Sep | 30.0 | aua | aua | 14° 30' 9 S, 160° 03' 4 E |
| 44 | 4 Sep | 32.2 | meg? | aua | 14° 30' 9 S, 160° 03' 4 E |
| 45 | 5 Sep | 41.7 | mar or obs | meg | 10° 03' 4 S, 161° 02' 0 E |
| 46 | 6 Sep | 36.3 | mar | mar | 05° 57' 5 S, 160° 01' 1 E |
| 47 | 7 Sep | 48.3 | obs | bip | 02° 02' 7 S, 157° 35' 9 E |
| 48 | 7 Sep | 25.2 | mar or obs | mar | 03° 52' 7 S, 158° 59' 5 E |
| 49 | 7 Sep | 48.3 | obs | bip | 02° 02' 7 S, 157° 35' 9 E |
| 50 | 7 Sep | 26.3 | mar or obs | mar | 03° 52' 7 S, 158° 59' 5 E |
| 51 | 7 Sep | 49.0 | obs | bip | 04° 59' 6 S, 160° 00' 0 E |
| 52 | 7 Sep | 21.3 | mar | mar | 02° 02' 7 S, 157° 35' 9 E |
| 53 | 7 Sep | 36.2 | mar | mar | 03° 52' 7 S, 158° 59' 5 E |
| 54 | 8 Sep | 54.1 | <i>Anguilla</i> sp. | bip | 01° 01' 1 S, 156° 49' 0 E |
| 55 | 8 Sep | 32.6 | bip | bip | 01° 51' 4 N, 154° 32' 0 E |
| 56 | 8 Sep | 50.9 | <i>Anguilla</i> sp. | bip | 01° 01' 1 S, 156° 49' 0 E |
| 57 | 8 Sep | 49.9 | <i>Anguilla</i> sp. | bip | 01° 01' 1 S, 156° 49' 0 E |
| 58 | 8 Sep | 29.3 | bip | bip | 01° 51' 4 N, 154° 32' 0 E |
| 59 | 8 Sep | 50.9 | <i>Anguilla</i> sp. | bip | 01° 01' 1 S, 156° 49' 0 E |
| 60 | 8 Sep | 51.0 | <i>Anguilla</i> sp. | bip | 01° 01' 1 S, 156° 49' 0 E |
| 61 | 9 Sep | 34.1 | bip | bip | 02° 19' 6 N, 154° 09' 9 E |
| 62 | 10 Sep | 41.9 | obs | bip | 08° 18' 7 N, 149° 42' 9 E |
| 63 | 10 Sep | 41.3 | obs | bip | 06° 32' 0 N, 151° 01' 1 E |

Table 2. Specific standard sequences obtained from mitochondrial 16S rRNA gene of adult Pacific freshwater eels *Anguilla* spp.

| Species | No. of site | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|-------------------------------|-------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|---|---|---|
| | 116 | 129 | 130 | 135 | 154 | 155 | 170 | 177 | 218 | 239 | 240 | 241 | 243 | 252 | 253 | 254 | 256 | 259 | 260 | 261 | 264 | 265 | 266 | 273 | 274 | 275 | 276 | 282 | 288 | 318 | | | | |
| <i>A. japonica</i> | T | T | T | T | G | C | A | T | T | G | T | T | A | A | C | C | A | G | G | A | C | G | C | C | C | C | C | C | C | G | | | | |
| <i>A. celebesensis</i> | . | . | . | . | A | . | . | C | . | A | C | C | A | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | A | | | | |
| <i>A. borneensis</i> | . | . | . | . | A | . | . | A | . | A | C | A | . | . | . | . | . | . | A | . | T | A | . | . | . | . | . | . | . | A | | | | |
| <i>A. interioris</i> | . | C | . | . | A | . | . | C | . | A | G | C | . | . | . | . | G | . | . | . | . | . | . | . | . | . | . | . | . | . | | | | |
| <i>A. marmorata</i> | . | G | . | C | . | . | . | C | . | A | G | C | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | A | | | | |
| <i>A. megastoma</i> | . | . | C | . | A | . | G | . | . | A | C | G | . | G | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | A | | | | |
| <i>A. dieffenbachii</i> | . | . | . | . | . | . | . | . | . | A | C | . | G | . | T | T | . | . | . | . | . | . | . | . | . | . | . | . | . | A | | | | |
| <i>A. reinhardtii</i> | . | . | . | . | . | . | . | . | . | A | C | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | A | | | | |
| <i>A. australis australis</i> | . | C | . | G | . | . | . | C | . | A | C | . | . | . | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . | A | | | | |
| <i>A. australis schmidtii</i> | G | . | C | G | . | . | . | C | C | A | C | . | . | . | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . | A | | | | |
| <i>A. obscura</i> | C | . | . | . | . | . | . | . | . | A | C | . | . | G | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | A | | | | |
| <i>A. bicolor pacifica</i> | . | C | . | . | . | . | . | . | . | A | C | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | A | | | | |
| | 320 | 322 | 327 | 342 | 343 | 344 | 346 | 347 | 348 | 350 | 351 | 354 | 355 | 356 | 358 | 359 | 360 | 362 | 368 | 372 | 373 | 376 | 379 | 380 | 401 | 410 | 412 | 414 | 416 | 419 | 453 | | | |
| <i>A. japonica</i> | G | A | G | A | G | A | G | C | T | A | T | C | A | G | A | C | G | A | G | T | C | T | G | C | C | G | A | T | C | A | T | A | | |
| <i>A. celebesensis</i> | A | G | A | . | . | . | . | C | . | C | . | A | . | A | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| <i>A. borneensis</i> | A | G | A | . | . | . | . | A | . | A | C | A | . | A | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| <i>A. interioris</i> | A | G | . | T | A | . | . | A | . | A | C | A | . | A | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| <i>A. marmorata</i> | A | G | . | T | A | . | . | A | . | A | C | A | . | A | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| <i>A. megastoma</i> | A | G | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| <i>A. dieffenbachii</i> | A | G | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| <i>A. reinhardtii</i> | A | G | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| <i>A. australis australis</i> | A | G | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| <i>A. australis schmidtii</i> | A | G | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| <i>A. obscura</i> | A | G | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| <i>A. bicolor pacifica</i> | A | G | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |

DISCUSSION

Species identification

Aoyama (1998) revealed that intra-species variation (less than 0.4%) in the mitochondrial 16S ribosomal RNA gene sequence was much less than inter-species variation for 2 species of freshwater eels, *Anguilla marmorata* and *A. celebesensis*, and suggested that specific standard sequences in this gene could be established from only 1 specimen of each species. Indeed, a sequence obtained from a leptocephalus agreed closely with 1 of the specific standard sequences with only slight variation (less than 3 sites).

The present study could not clearly distinguish the subspecies *Anguilla australis australis* and *A. australis schmidtii*, as recently reported by Dijkstra & Jellyman (1999). Morphological differences between these subspecies were very slight and added further doubt to the validity of recognizing the 2 subspecies (Castle 1963, Tesch 1977, Jellyman 1987, Aoyama 1998). Furthermore, recent genetic investigation of all species of anguillid eels revealed that genetic differences between the adults of both subspecies could be entirely included in intra-species variation (Aoyama 1998). In consideration of the above, it can be concluded that the genetic difference observed between *A. australis australis* and *A. australis schmidtii* is attributed to intra-species variation of *A. australis*.

The concordance between molecular and morphological identification was 79.5%. Critical misidentifications by morphological characters used in the present study were mostly for leptocephali smaller than 30 mm total length (TL) (Table 1). The number of myomeres is the most important for the species identification of leptocephali (Jespersen 1942). However, it is well

known that the number of myomeres varies somewhat within species, and this number is difficult to determine in small leptocephali with morphological characteristics being not fully developed (Mochizuki 1996). A molecular key is applicable to any developmental stages from eggs to adults. Species identification of small anguillid leptocephali cannot be made without consideration of molecular data, particularly in areas where several species occur sympatrically, as in the South Pacific.

Distribution and migration of anguillid leptocephali

The horizontal distributions of the anguillid leptocephali studied here are closely related to the freshwater habitat of the adults by way of ocean currents (Fig. 2), as previously inferred by Tsukamoto & Aoyama (1998). For example, *Anguilla marmorata* leptocephali have a patchy distribution in the North Equatorial Current, and probably disperse to eastern Asia by the North Equatorial Current and further by the Kuroshio Current. The *A. marmorata* leptocephali occurring patchily in the South Equatorial Current would be transported to northern New Guinea, New Caledonia and Fiji by the South Equatorial Current. This patchiness corresponds well to North Pacific and western South Pacific populations, respectively, as revealed by the molecular analysis of *A. marmorata* throughout the Indo-Pacific (Ishikawa 1998).

Anguilla bicolor pacifica leptocephali found near the boundary between the Equatorial Counter Current and the South Equatorial Current showed a different distribution to those of *A. marmorata*, although their adults occur sympatrically. This suggests the existence of different currents which transport leptocephali from 2 different areas to the same coastal region.

Table 3. Number of differences between standard specific sequences and homologous sites of anguillid leptocephali. Species names are abbreviated as follows: jap: *A. japonica*, cel: *A. celebesensis*, rei: *A. reinhardtii*, obs: *A. obscura*, meg: *A. megastoma*, mar: *A. marmorata*, int: *A. interioris*, die: *A. dieffenbachii*, bor: *A. borneensis*, bip: *A. bicolor pacifica*, aus: *A. australis schmidtii*, aua: *A. australis australis*

| Fish no. | jap | cel | rei | obs | meg | mar | int | die | bor | bip | aus | aua |
|----------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 01 | 25 | 12 | 16 | 7 | 17 | 0 | 8 | 17 | 27 | 8 | 20 | 18 |
| 02 | 25 | 12 | 16 | 7 | 17 | 0 | 8 | 17 | 27 | 8 | 20 | 18 |
| 03 | 25 | 12 | 16 | 7 | 17 | 0 | 8 | 17 | 27 | 8 | 20 | 18 |
| 04 | 26 | 13 | 17 | 8 | 18 | 1 | 9 | 18 | 28 | 9 | 19 | 17 |
| 05 | 0 | 20 | 18 | 23 | 19 | 26 | 24 | 24 | 25 | 20 | 27 | 25 |
| 06 | 25 | 12 | 16 | 7 | 17 | 0 | 8 | 17 | 27 | 8 | 20 | 18 |
| 07 | 25 | 12 | 16 | 7 | 18 | 0 | 8 | 17 | 27 | 8 | 20 | 18 |
| 08 | 20 | 10 | 13 | 5 | 16 | 8 | 6 | 16 | 21 | 0 | 20 | 18 |
| 09 | 20 | 10 | 13 | 5 | 16 | 8 | 6 | 16 | 21 | 0 | 20 | 18 |
| 10 | 20 | 10 | 13 | 5 | 17 | 8 | 6 | 16 | 21 | 0 | 20 | 18 |
| 11 | 21 | 11 | 14 | 6 | 18 | 9 | 7 | 17 | 22 | 1 | 21 | 19 |
| 12 | 20 | 10 | 13 | 5 | 16 | 8 | 6 | 16 | 21 | 0 | 20 | 18 |
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| 17 | 20 | 10 | 13 | 5 | 17 | 8 | 6 | 16 | 21 | 0 | 20 | 18 |
| 18 | 21 | 11 | 14 | 6 | 18 | 9 | 7 | 17 | 22 | 1 | 21 | 19 |
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| 21 | 24 | 16 | 14 | 21 | 3 | 22 | 24 | 21 | 25 | 21 | 21 | 19 |
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| 26 | 18 | 12 | 0 | 15 | 10 | 16 | 16 | 10 | 16 | 13 | 12 | 12 |
| 27 | 26 | 18 | 11 | 21 | 16 | 19 | 20 | 10 | 24 | 19 | 1 | 1 |
| 28 | 18 | 12 | 0 | 15 | 9 | 16 | 16 | 10 | 16 | 13 | 12 | 12 |
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| 34 | 20 | 14 | 2 | 17 | 12 | 18 | 18 | 12 | 18 | 15 | 14 | 14 |
| 35 | 27 | 21 | 14 | 22 | 19 | 22 | 23 | 13 | 26 | 22 | 3 | 3 |
| 36 | 21 | 15 | 3 | 17 | 12 | 18 | 18 | 13 | 19 | 15 | 15 | 15 |
| 37 | 19 | 12 | 1 | 16 | 9 | 17 | 15 | 11 | 17 | 14 | 13 | 12 |
| 38 | 19 | 12 | 1 | 16 | 9 | 17 | 15 | 11 | 17 | 14 | 13 | 12 |
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| 63 | 20 | 10 | 13 | 5 | 16 | 8 | 6 | 16 | 21 | 0 | 20 | 18 |

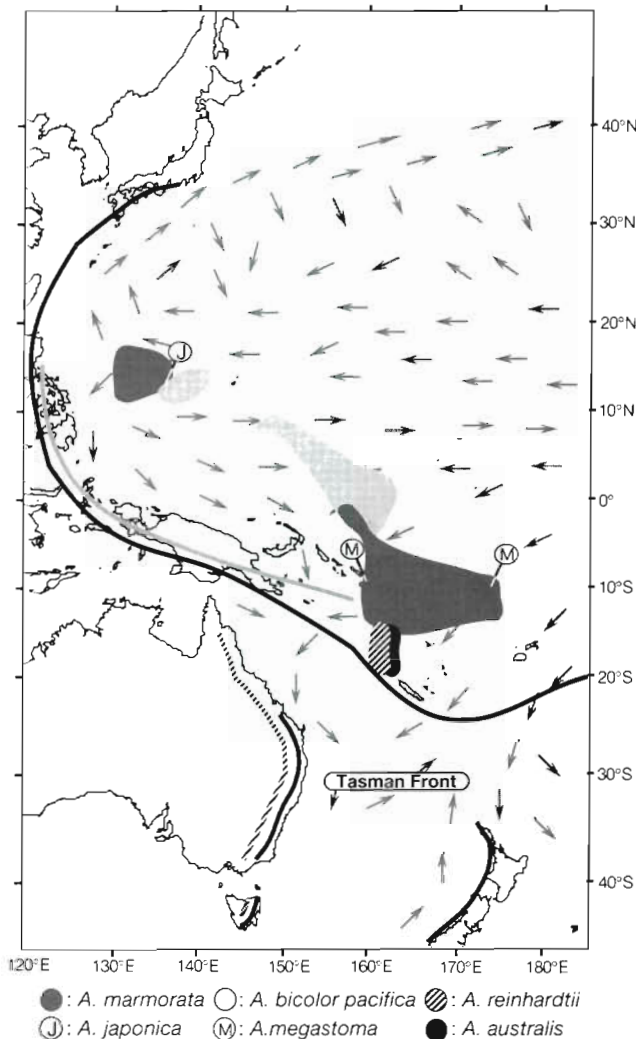


Fig. 2. Distribution of anguillid leptocephali in the western Pacific and their adult freshwater distributions (correspondingly shaded bars)

The distributions of leptocephali of *Anguilla reinhardtii* and *A. australis* completely overlap and the difference in their TLs was not significant (Fig. 3). These findings suggest that they could be transported to the same area by overlapping dispersal routes, although their adult distributions do not completely coincide. The northern part of the east coast of Australia is populated by only *A. reinhardtii*, and in the southern part both species live sympatrically (Jellyman 1987, Sloane 1984). A previous study in New South Wales (Ege 1939), where both species are found, suggested that the TLs of recruiting glass eels of *A. reinhardtii* (46 to 65 mm) were slightly less than *A. australis* (47 to 73 mm). These data indicate that *A. reinhardtii* has a shorter larval duration and detrans from the East Australian Current earlier than *A. australis*, resulting in the solitary recruitment of *A. rein-*

hardtii to the northern part of Australia. Some of *A. reinhardtii* which have relatively long larval duration within intraspecies variation might be transported to the southern part of Australia and further to New Zealand, as has been recently revealed (Jellyman et al. 1996, McDowall et al. 1998).

These observations strongly suggest that leptocephali of freshwater eels in the western Pacific have specific dispersal routes from their adult spawning areas to their freshwaters. Even if a part of their larval dispersal routes overlap, there is likely to be a mechanism to transport leptocephali/glass eels to the specific distributional ranges of their adults. Specific larval durations might be the key to the nature of such movements, as previously suggested for the Atlantic eels (Schmidt 1923, 1925).

In New Zealand, where *Anguilla dieffenbachii* and *A. australis* are in general sympatric, glass eels of both species migrate to estuaries from August to November (Jellyman 1987). Assuming that they spawn in different locations but that they utilize partly overlapped dispersal routes, the leptocephali might appear sympatrically. However, *A. dieffenbachii* leptocephali were not collected in the present study. Jellyman (1987) suggested that *A. dieffenbachii* might have its spawning area nearer to land than *A. australis* since the maturation index of female silver eels migrating to the sea is higher in the former (8.12) than in the latter (3.49, Jellyman 1987). Although the lack of leptocephali of *A. dieffenbachii* in the present collections

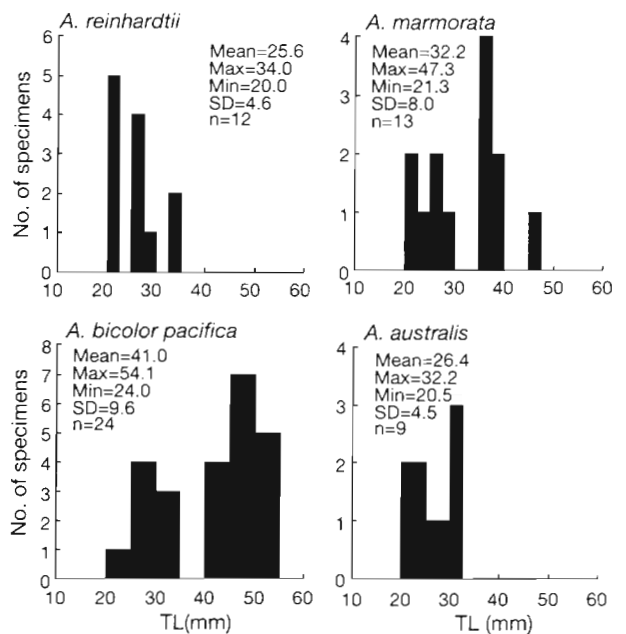


Fig. 3. Distribution of total length of anguillid leptocephalus examined in the present study. One *Anguilla japonica* 28.1 mm and 2 *A. megastoma* 42.8, 41.7 mm were removed from the figure

may not mean that they are absent from the area, it seems reasonable to suppose that the migration route for *A. dieffenbachii* lies out of the area searched here. The absence of leptocephali of *A. celebesensis*, *A. borneensis* and *A. obscura* in the present study might be due to a mismatch of their migration route and/or spawning time.

Spawning area of *Anguilla australis*

Based on the collection of 4 leptocephali, with additional information on oceanic hydrography, size of glass eels and their arrival times in freshwater, several hypotheses concerning the possible spawning area of *Anguilla australis* have been proposed. Castle (1963) and Tesch (1977) speculated that the area might be between Fiji and Tahiti, while Schmidt (1928) placed it on the western side of New Caledonia. Jellyman (1987) suggested that the spawning area for *A. australis* lies across the area of divergence of the South Equatorial Current, 150 to 170° W and 5 to 15° S. Larvae in the northern part of the spawning area would then tend to be transported to the western Tasman Sea, and hence to the Australian coast, while those in the southern part might be dispersed to New Zealand. In the present study, however, no anguillid leptocephali were collected south of New Caledonia, while all of the *A. australis* were caught north of New Caledonia, suggesting that the *A. australis* recruiting to New Zealand follow a different route. It is likely that a part of *A. australis* dispersing to the northeast coast of Australia via north of New Caledonia could recruit further to New Zealand, using the eastward zonal flow of the Tasman Front latitudinally across the Tasman Sea at about 30° S. It might reflect the difference of arrival time of glass eels, February to November in Australia and August to November in New Zealand (Jellyman 1987, Sloane 1984).

If it is assumed that the growth rate of *Anguilla australis* is the same as those calculated for *A. japonica*, i.e. 0.43 mm d⁻¹ (Tsukamoto et al. 1994), the leptocephali of *A. australis* collected in the present study (at about 14° S and 160° E, average 26.4 mm TL) are therefore likely to be ca 54 d old. This suggests that these leptocephali could be transported ca 1866 km westward from a possible eastern spawning area, by the South Equatorial Current (40 cm s⁻¹, Jellyman 1987). Furthermore time needs to be allowed for the transport of developing eggs.

In consideration of the above, the location of the spawning area for *Anguilla australis* might be inferred to agree with Castle's speculation (Castle 1963). Further investigations in this area will be required to determine more confidently the precise spawning area of *A. australis*.

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