Acta Zoologica Taiwanica 12(1):9-19(2001)

Validation of the Occurrence of Short-finned Eel *Anguilla bicolor pacifica* in Natural Waters of Taiwan

Yu-San Han, Chih-Wei Chang, Jie-Teng He and Wann-Nian Tzeng*

Institute of Zoology, College of Science, National Taiwan University, Taipei 106, Taiwan, R.O.C.

ABSTRACT

Previous studies indicated that elvers of the short-finned eel *Anguilla bicolor pacifica* Schmidt occurred in the estuaries of Taiwan. However, no studies have validated that juveniles and adults exist in Taiwan's natural waters. We report that six short-finned eels, ranging 32-68 cm in total length, were collected from the Kaoping and Tungkang Rivers of southern Taiwan during May 1998 to July 1999. Polymerase chain reaction (PCR) amplification and sequencing of the mitochondrial cytochrome b gene (mt*Cyt-b*) confirmed this species to be *A. bicolor pacifica.* Accordingly, *A. bicolor pacifica* is a native eel species of Taiwan.

Key words: Short-finned eel, *Anguilla bicolor pacifica*, Species identification, PCR, Mitochondrial cytochrome b gene.

INTRODUCTION

The life history of catadromous eels, genus *Anguilla*, is very interesting but still mysterious. The eel spawns in the deep tropical/subtropical oceans. Their leaf-like larvae, leptocephali, are transported and dispersed by ocean currents, and metamorphose into glass eels after several months of drifting. Glass eels become pigmented elvers in estuaries and grow up in fresh water to be juveniles that are often called yellow eels. Eels metamorphose from a yellow (non-migratory) to a silver (migratory) form before beginning their spawning migration. Silver eels migrate long distances back to spawn and die in the deep ocean (Tesch, 1977).

According to Ege (1939) and Castle and Williamson (1974), anguillid eels are classified based on skin color, morphometric characters, dentition, and vertebral counts into 18 species and subspecies: *A. japonica* Temminck and Schlegel, 1846; *A.anguilla* (Linnaeus, 1758); *A. rostrata* (LeSueur, 1817); *A. dieffenbachii* Gray, 1842; *A. reinhardti* Steindachner, 1867; *A. marmorata* Quoy and Gaimard, 1824; *A. megastoma* Kaup, *1856*; *A. celebesensis* Kaup, 1856; *A. borneensis* Popta, 1924; *A. mossambica* (Peters, 1852); *A. interioris* Whitley, 1938; *A. obscura* Günter, 1871; *A. bicolor bicolor* McClelland, 1844; *A. bicolor pacifica* Schmidt, 1928; *A. australis australis* Richardson, 1841; *A. australis schmidti* Phillips, 1925; *A. nebulosa labiata* Peters, 1852;

*Corresponding author: Tel: 886-2-23630231 ext. 3357; Fax: 886-2-23639570;
E-mail: wnt@ccms.ntu.edu.tw

and *A. nebulosa nebulosa* McClelland, 1844. Two are found in the northern Atlantic region and the rest in the Indo-Pacific zone. Based on the ano-dorsal length (distance between the verticals from the beginning of the dorsal fin to the anus) relative to the total length (expressed as a percent), eels are divided into long-finned and short-finned forms. Thirteen species and subspecies belong to the first category, and the remainder to the second one.

Four species of anguillid eels have been reported in Taiwan based on morphometric indices, namely *A. japonica*; *A. marmorata*; *A. bicolor pacifica*; and *A. celebesensis* (Tzeng, 1982, 1983a, 1983b; Tzeng and Tabeta, 1983; Shen, 1993). The former two have records of adult forms, but the latter two have only elver records. *A. japonica* is the most abundant, which contributes to 93%-99% of the total catch of elvers , followed by *A. marmorata* (1%-7%) (Tzeng *et al*., 1995a). The other two species are very rare and have been recorded only as elvers (Tzeng, 1982; Tzeng and Tabeta, 1983). It is not known whether they can grow to juveniles and adults in the natural waters of Taiwan. Based on the ano-dorsal length, it is easy to distinguish the short-finned eel *A. bicolor pacifica* from the other three long-finned eel species in Taiwan. In this study, we found juvenile and adult short-finned eels, and validated the species by molecular tools. This is the first formal record of the juvenile/adult *A. bicolor pacifica* being found in native waters of Taiwan.

MATERIALS AND METHODS

Specimen collection and morphometric measurements

The eels were collected by local fishermen mainly in the Kaoping and Tungkang Rivers (Fig. 1), but also from other rivers from May 1998 through July 1999. They were captured by bamboo traps or by hollow plastic water pipes, and were killed by freezing. The total length (cm), predorsal length (cm), preanal length (cm), horizontal and vertical diameters (mm) of the left eye, and body weight (g) were measured after thawing. The fin difference index (FDI%) (Ege, 1939) and ocular index (OI) (Pankhurst, 1982) were calculated respectively as:

$$\mathbf{FDI\%} = 100\% \times [\,|\,A - B\,|\,/\,L], \qquad (1)$$

where A is preanal length (cm), B is predorsal length (cm), and L is eel total length (cm).

$$\mathbf{OI} = 100 \times [((A + B)/4)^2 \times \pi / L], \qquad (2)$$

where A and B are horizontal and vertical diameters (mm) of the left eye, and L is eel total length (mm).

Histological examination of gonads

Gonads of one short-finned eel (AB6) were fixed in Bouin's solution for more than 48 h. After being dehydrated in an ethanol series and embedded in paraffin, the gonads were sectioned at a thickness of 5 ìm and stained with hematoxylin and eosin (H & E). Mean diameters of oocytes were calculated from 20 randomly chosen oocytes in which the nucleus was visible and large under a light microscope. The maturation stage of the ovary was classified according to Yamamoto *et al*. (1974).

DNA extraction and PCR amplification of mtDNA

Liver tissues of one short-finned eel (AB6, Table 1) were chosen for DNA extraction. The extraction method was carried out with a phenol/chloroform standard protocol. The two oligonucleotide primers used for PCR amplification of the mitochondrial cytochrome b gene (mt*Cyt-b*), named *Cyt-b*1 5'-TGCTAACGATGCCCTAGTGG-3') and *Cyt-b*2 (5'-CTAGTCAACCTACTAATGGG-3'), were designed based on the conserved regions of deposited cytochrome b sequences of the genus *Anguilla* (GenBank/

Table 1. Sample information and morphometric index of *A. bicolor pacifica*.

	May 1998		October 1998			July 1999
	AB1	AB2	AB3	AB4	AB5	AB6
Collecting area	Kaoping River		Tungkang River			Kaoping River
Total length (cm)	54.8	48.2	32.2	35.7	64.8	68.0
Body weight (g)	308.8	198.4	56.0	91.8	505.7	545.8
Predorsal length (cm)	25.0	18.8	13.7	14.2	26.4	27.8
Preanal length (cm)	23.4	19.7	13.7	14.6	26.1	27.7
FDI%[a]	2.9	1.9	0.0	1.1	0.5	0.1
Ocular index (OI)	---	---	2.8	4.66	8.7	4.2

a FDI%: fin difference index, distance between the verticals from the beginning of the dorsal fin to the anus, expressed in percent of total length.

EMBL). The locations of *Cyt-b*1 and *Cyt-b*2 are at nucleotides 39-58 and 1098-1117, respectively, in the open reading frame of mt*Cyt-b*. The PCR was performed by Taq polymerase according to the supplier's protocol (Promega, USA). Briefly, after an initial 5-min denaturing step at 94 ℃, 30 cycles of amplification were performed using the cycle profile of 94℃ for 30 s, 60 ℃ for 1 min, and 72 ℃ for 1.5 min. Elongation was extended to 10 min at 72 ℃ after the last cycle. The PCR product was sequenced commercially (Mission Biotech, Taipei, Taiwan), by a big dye-terminator kit and analyzed on polyacrylamide gels with an ABI 377 automated sequencer (Perkin-Elmer Applied Biosystems). The acquired DNA sequence was then aligned with those of anguillid species deposited in GenBank using the Clustal W (v1.81) network service of the European Bioinformatics Institute of EMBL. The phylogenetic tree was constructed by the Neighbor-joining method using the program MEGA 2 (Kumar *et al*., unpublished). The distance used for the phylogenetic analysis was calculated by Kimura's two-parameter model. The robustness of the phylogenetic analysis was tested by 1000 bootstrap replicates.

RESULTS

Sample distribution and species identification

A total of six juvenile/adult short-finned eels (AB1-6) were found in the Kaoping and Tungkang Rivers of southern Taiwan (Table 1, Fig. 2). We also collected eels in rivers elsewhere in Taiwan a few times, however, no juvenile/adult short-finned eels were found in these samples. The reason may be due to its low population size and our sparse collecting efforts in other rivers. To confirm the species identification of the short-finned eels, one specimen (AB6) was chosen for DNA sequencing; the other five short-finned eels were corrupted because of inappropriate preser-

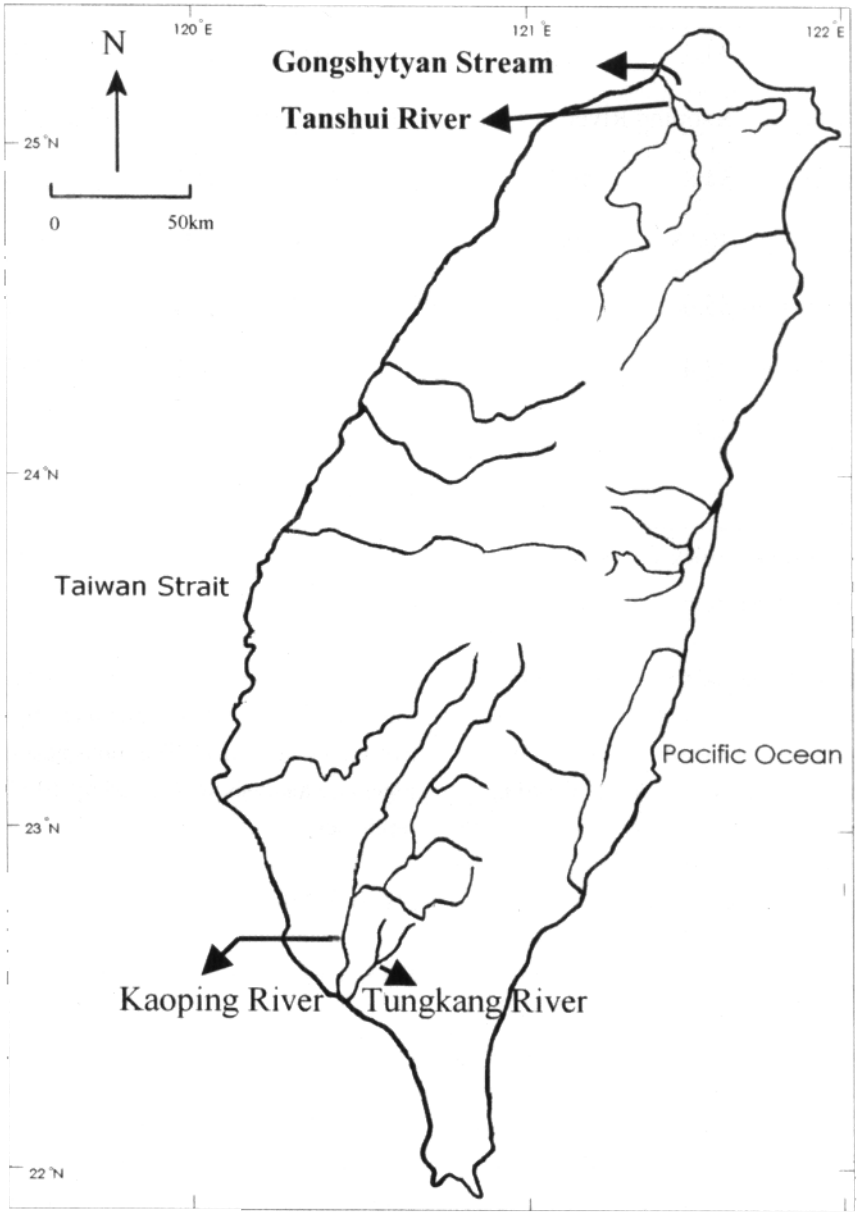

Figure 1. Sampling sites of juvenile/adult short-finned eels.

vation and were not used for DNA identification. PCR products of mt*Cyt-b* of this eel were aligned with those of anguillid species deposited in GenBank/EMBL. No indels were found in the sequence. Topology of the phylogenetic tree constructed by the Neighbor-joining method showed that the short-finned eel was clustered into the group of *A. bicolor pacifica* with robust bootstrap values (Fig. 3). The sequence of the mt*Cyt-b* gene of this eel was 100% identical to that of *A. bicolor pacifica*. Therefore, the eel species of the short-finned eel was validated to be *A. bicolor pacifica*.

Morphometric and gonadal characters

The total length and body weight of the short-finned eels ranged between 32.2 and 68.0 cm and 56.0 and 545.8 g, respectively (Table 1). The FDI%, measured by the distance between the verticals from the beginning of the dorsal fin to the anus (ano-dorsal length) relative to the total length, ranged between 0% and 2.9% (mean 1. 1%). It can be easily distinguished from *A. celebesensis* (9.0%), *A. japonica* (9.2%), and *A. marmorata* (16.3%) (Ege, 1939). The ocular index of the short-finned eels was between 2.8 and 8.7 (OI values of AB1 and AB2 were lost). Gonadal histology showed that the oocytes of the short-finned eel (AB6) were intermingled with adipocytes (Fig. 4), which differ from those of *A. japonica* (non-intermingled type). The mean oocyte diameter was about 80 ìm, and oocytes were in the previtellogenic stage (Fig. 4). On the other hand, mesenteric fat in the short-finned eels was abundant, similar to that in *A. marmorata* but contrasting with that in *A. japonica* (Han *et al*., unpublished data).

DISCUSSION

By comparison of morphometric characters and vertebral counts, Tzeng and Tabeta (1983) first identified short-finned elvers (glass eels) distributed in the northern and southern Taiwan (Gongshytyan stream, Shihting River, Shuangchi River, and Tungkang River) to be *A. bicolor pacifica*. However, since only elvers but not juvenile/adults were found, we were not sure if this species can inhabit the natural waters of Taiwan. Tzeng *et al*. (1995b) found two adult short-finned eels in the Gongshytyan Stream and Tanshui River in northern Taiwan (Fig. 1), but lacked species identification. A photo of adult *A. bicolor pacifica* is presented in the book, Fishes of Taiwan, but it lacks a description (Shen, 1993). Since the vertebral counts and FDI% of the three short-finned eel species and subspecies around the world, i.e., *A. obscura*, *A. bicolor bicolor*, *A. bicolor pacifica*, *A. australis australis*, and *A. australis schmidti*, partially overlap, molecular characterization may be required to unambiguously distinguish these eel species. In this study, we caught six short-finned eels in the juvenile or adult stage, thus confirming their existence in the natural waters of Taiwan. Sequence analysis of the mt*Cyt-b* gene validated the short-finned eels to be *A. bicolor pacifica*. So, combining evidence of the catch records of elvers (Tzeng and Tabeta, 1983) and juveniles/adults (Tzeng *et al*., 1995b; Shen, 1993; and this study), we conclude that *A. bicolor pacifica* is a native eel species of Taiwan.

The geographical distributions of the five short-finned eel species and subspecies around the world are as follows: *A. bicolor pacifica*, a tropical eel in the equatorial zone of the western Pacific, is distributed from the east coast of Borneo and the northern part of New Guinea to Luzon in the Philippines where it occurs in abundance. *A. bicolor bicolor*, a tropical eel of the Indian Ocean, is distributed mainly along the coast of East Africa, Malagasy, the Indian hinterland, Burma, Sumatra, Java, and Northwest Australia. *A. obscura*, also a tropical eel of the western equatorial and southern Pacific Ocean, is distributed mainly in New Guinea, Fiji, and

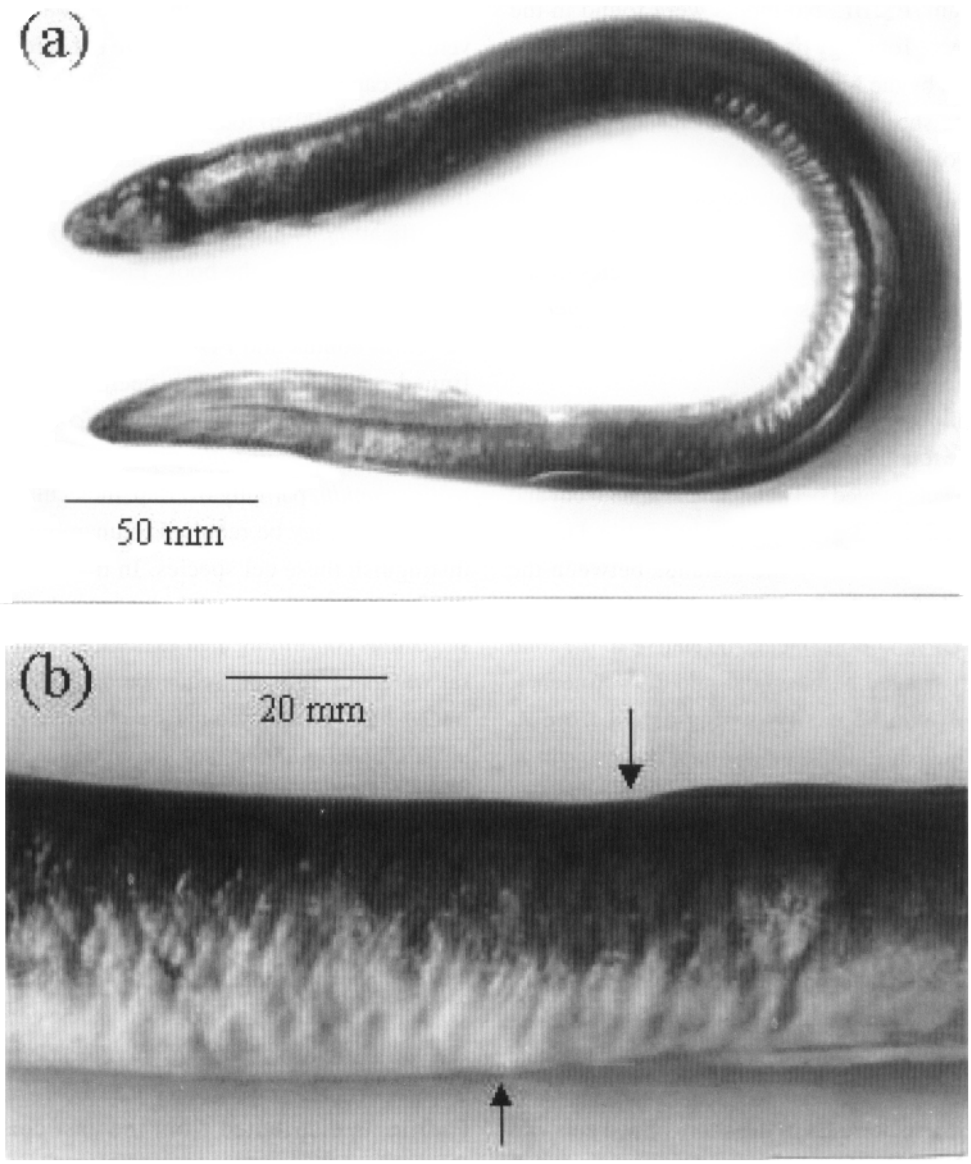

Figure 2. Photographs of the adult short-finned eel *A. bicolor pacifica* (AB1, No. NTUM 08691) found in May 1998 (Table 1) in the Kaoping River of southern Taiwan. (a) Top view of the eel. (b) The arrows indicate positions of the predorsal (upper) and preanal (down) fins, respectively. Scale bar: 20 mm.

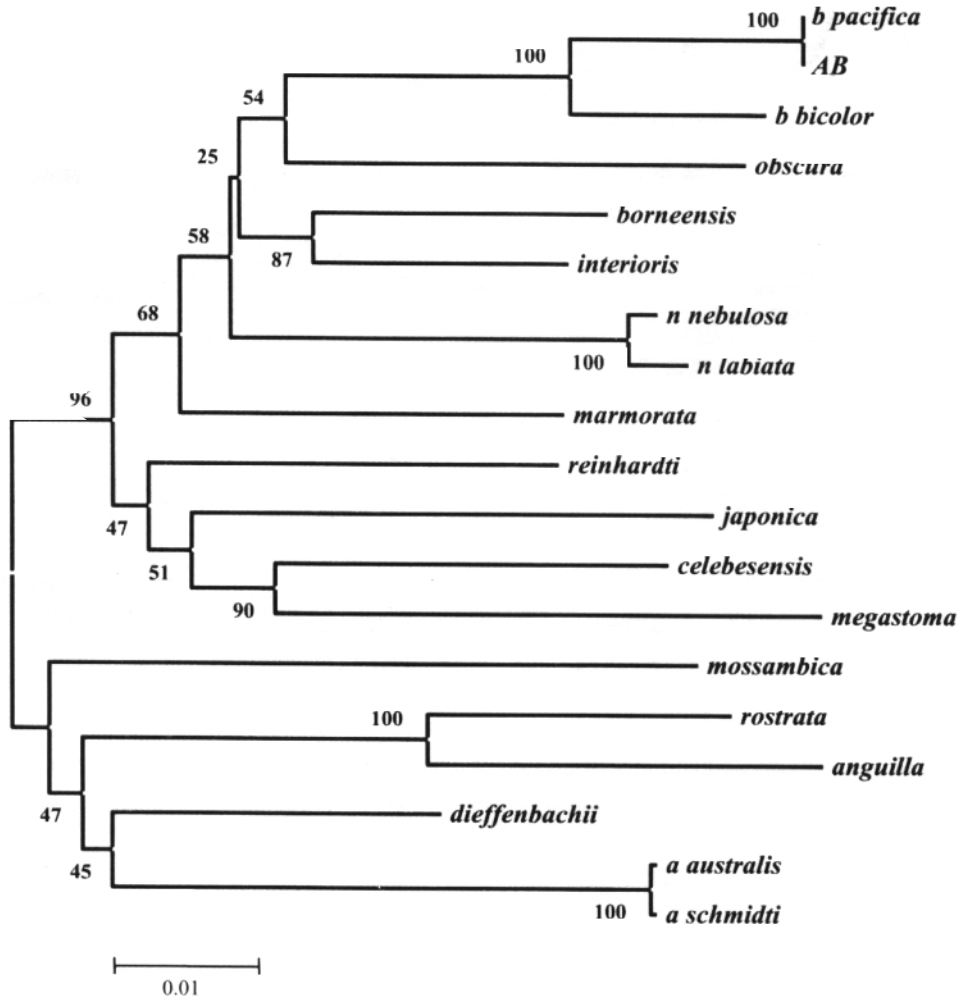

Figure 3. Topology of phylogenetic tree constructed by the Neighbor-joining method. DNA sequences of mt*Cyt-b* of *A. bicolor pacifica* (AB) and 18 sequences obtained from GenBank/EMBL of 18 known eel species and subspecies were used for analysis. The numbers indicate bootstrap values of 1000 replicates. Accession numbers: *A. dieffenbachii*, AF006711; *A. rostrata*, AF006716; *A. anguilla*, AF006714; *A. interioris*, AB021773; *A. obscura*, AB021781; *A. japonica*, AF006702; *A. japonica*2, AF006703; *A. reinhardti*, AF006706; *A. megastoma*, AB021771; *A. marmorata*, AF074863; *A. borneensis* (synonym of *A. malgumora*), AF006718; *A. bicolor bicolor*, AF006710; *A. bicolor pacifica*, AF006708; *A. mossambica*, AF074864; *A. australis australis*, AF006712; *A. australis schmidti*, AB021769; *A. celebesensis*, AB021777; *A. nebulosa labiata* (synonym of *A. bengalensis labiata*), AF074866; and *A. nebulosa nebulosa*, AB021783. All branch lengths are directly proportional to genetic distance (Kimura's two-parameter model).

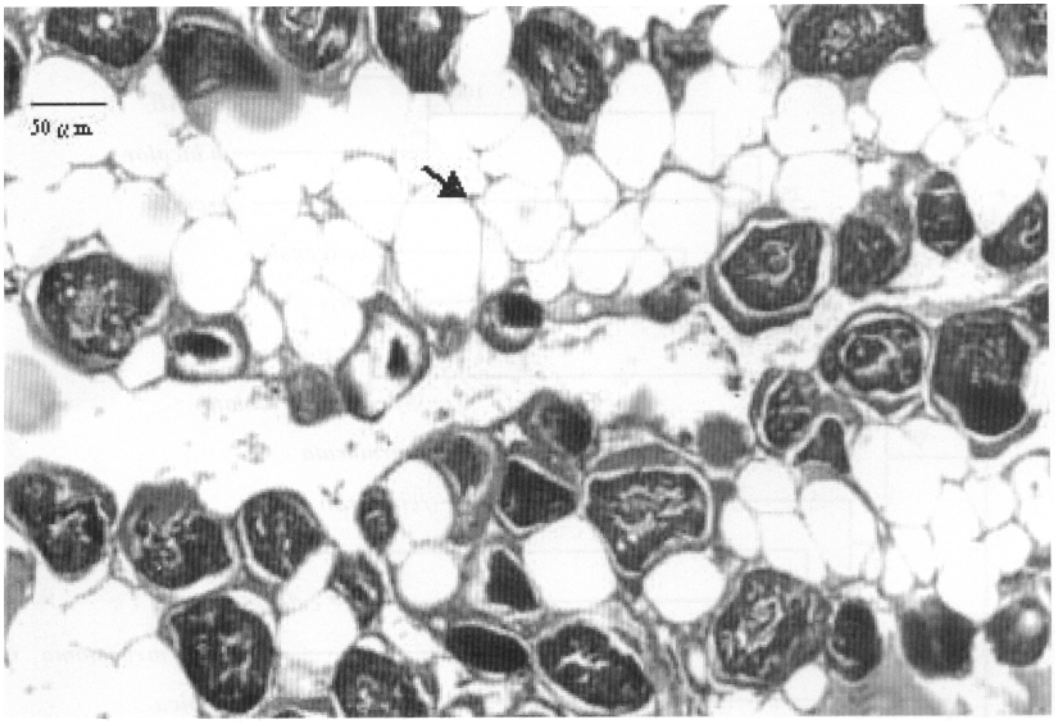

Figure 4. Microscopic histology of gonads stained with hematoxylin and eosin in *A. bicolor pacifica* (AB6) collected in July 1999 (Table 1). The arrow indicates the adipocytes. Scale bar: 50 μm.

Tahiti. Of *A. australis australis* and *A. australis schmidti*, both temperate eels of the southern Pacific Ocean, the former is distributed mainly in southeastern Australia, and the latter in New Zealand (Tesch, 1977). From a zoogeographical point of view, the species of short-finned eels whose distribution is closest to Taiwan is *A. bicolor pacifica*, which fits in with the results of this study. Hence, the existence of *A. bicolor pacifica* in Taiwan extends the northernmost distribution of this species.

The ocular index of Japanese eel in Taiwan is between 1.87 and 6.90, and is significantly larger in the silver eel than in the yellow eel (Han *et al.*, unpublished data). The ocular index of the six short-finned eels ranges between 2.8 and 8.7 (Table 1), which is comparable to that of Japanese eels. The large OI value, e.g., AB5 and AB6, may represent the silver stage compared to the Japanese eel. In addition, the OI value of specimen AB6 was 4.2, and it also had a mean oocyte diameter of 80 μm (Fig. 4), which was comparable to the silver/yellow stage of Japanese eels. We infer that the metamorphosis of female *A. bicolor pacifica* might occurs around a total length of 65 cm. In addition, the oocytes in the ovary of *A. bicolor pacifica* (AB6, Fig. 4) were intermingled with adipocytes, the same pattern as those of American eels (Wenner and Musick, 1974) but differing from those of Japanese eels (figure not shown). Mesenteric fat is rich in the abdomen of *A. bicolor pacifica*, the same with American eels but in contrast to Japanese eels, in which mesen-

teric fat is sparse.

Exotic human-introduced eels for aquaculture use may occasionally escape from culture ponds into natural waters. This has been proven in the case of *A. rostrata*, which has been found inhabiting in the Kaoping River (Han *et al*., unpublished data). Imported elvers have mainly consisted of *A. anguilla* and *A. rostrata* (both long-finned eels) since 1969 and 1977, respectively (Li, 1997). No other records of imported eel species are known. Hence, it seems quite impossible that *A. bicolor pacifica* is an exotic eel. Moreover, the catch records of *A. bicolor pacifica* elvers in estuaries also exclude this possibility, for the developmental stages of these elvers were very young, just soon after their upstream migration. If they were elvers escaped from culture ponds, then their developmental stages should be advanced (Tzeng and Tabeta, 1983). Additionally, since the routes of eel migration are believed to be species specific (Tsukamoto, 1992), it would be hard for exotic eel species to settle down and complete their life cycles. Therefore, we are certain that *A. bicolor pacifica* found in Taiwan is a native eel species.

In conclusion, we have identified the existence of juvenile/adult *A. bicolor pacifica* in the natural waters of Taiwan by a molecular method. Combined with the record of elver catches, we validate that *A. bicolor pacifica* is the third native eel species of Taiwan, in addition to the other two well-known species, *A. japonica* and *A. marmorata*.

ACKNOWLEDGEMENTS

This study was financially supported by the National Science Council, Republic of China (grant no. NSC-89-2313-B002-077 to W. N. Tzeng). The authors are grateful to Dr. H. T. Yu and Mr. C. H. Yu for technical support with molecular identification.

REFERENCES

Castle, P. H. J. and G. R. Williamson (1974) On the validity of the freshwater eel species *Anguilla ancestralis* Ege from Celebes. Copeia 1974: 569-570.

Ege, V. (1939) A revision of the genus *Anguilla* Shaw, a systematic, phylogenetic and geographical study. Dana Report 16: 1-256.

Li, L. S. (1997) Aquaculture. Kaohsiung: Chien-Cheng Publishing Co., pp 84. (in Chinese)

Pankhurst, N. W. (1982) Relation of visual changes to the onset of sexual maturation in the European eel *Anguilla anguilla* L. J. Fish Biol. 21: 127-140.

Shen, S. C. (1993) Fishes of Taiwan. Taipei: Department of Zoology, National Taiwan University, pp 97. (in Chinese)

Tesch, F. W. (1977) The Eel. Biology and management of anguillid eels. London: Chapman and Hall.

Tsukamoto, K. (1992) Discovery of the spawning area for Japanese eel. Nature 356: 789-791.

Tzeng, W. N. (1982) New record of the elver, *Anguilla celebesensis* Kaup, from Taiwan. Bioscience 19: 57-66. (in Chinese)

Tzeng, W. N. (1983a) Species identification and commercial catch of the anguillid elvers from Taiwan. China Fish. Month. 366: 16-23. (in Chinese)

Tzeng, W. N. (1983b) Seasonal and long-term changes of the catch of anguillid elvers in Taiwan. J. Fish. Soc. Taiwan 10: 1-7. (in Chinese)

Tzeng, W. N. and O. Tabeta (1983) First record of the short-finned eel *Anguilla bicolor pacifica* elvers from Taiwan. Bull. Jpn. Soc. Sci. Fish. 49: 27-32.

Tzeng, W. N., J. J. Hsiao, H. P. Shen, Y. T. Chern, Y. T. Wang and J. Y. Wu (1995a) Feeding habit of the Japanese eel, *Anguilla japonica*, in the streams of northern Taiwan. J. Fish. Soc. Taiwan 22: 279-302. (in Chinese)

Tzeng, W. N., P. W. Cheng and F. Y. Lin (1995b) Relative abundance, sex ratio and population structure of the Japanese eel *Anguilla japonica* in the Tanshui River system of northern Taiwan. J. Fish Biol. 46: 183-201.

Wenner, C. A. and J. A. Musick (1974) Fecundity and gonad observations of the American eel, *Anguilla rostrata*, migrating from Chesapeake Bay, Virginia. J. Fish. Res. Board Can. 31: 1387-1391.

Yamamoto, K., M. Omori and K. Yamauchi (1974) Oogenesis of the Japanese eel. Bull. Jpn. Soc. Sci. Fish. 40: 9-15.

(Received Jun 26, 2001; Accepted Aug. 6, 2001)

確認臺灣天然水域中存在太平洋雙色鰻 (*A. bicolor pacifica*)

韓玉山、張至維、何杰騰、曾萬年
國立臺灣大學動物學研究所

摘 要

至目前爲止只知道臺灣北部及南部的河口曾經紀錄過太平洋雙色鰻 (*A. bicolor pacifica*) 的玻璃鰻幼鰻 (glass eel)。本報告進一步於 1998 年 5 月至 1999 年 7 月，於南臺灣高屏溪及東港溪中發現短鰭鰻成鰻之蹤跡。利用聚合酶連鎖反應放大粒線體細胞色素 b 基因之序列比對之後，確認其爲太平洋雙色鰻。本結果證實太平洋雙色鰻爲一臺灣原生種鰻魚。

關鍵詞：短鰭鰻、 太平洋雙色鰻、 種類鑑定、 聚合酶連鎖反應、 粒線體細胞色素 b 基因