

Species Composition and Seasonal Occurrence of Recruiting Glass Eels (*Anguilla* spp.) in the Hsiukuluan River, Eastern Taiwan

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Nico Jose Leander, Kang-Ning Shen, Rung-Tsung Chen, and Wann-Nian Tzeng (2012) Species composition and seasonal occurrence of recruiting glass eels (*Anguilla* spp.) in the Hsiukuluan River, eastern Taiwan. *Zoological Studies* 51(1): 59-71. There are 16 species and 3 subspecies of freshwater eel (*Anguilla* spp.) found in the world. Among them, 4 species, *A. japonica*, *A. marmorata*, *A. bicolor pacifica*, and *A. celebesensis* were reported from Taiwan. *Anguilla japonica* is an important aquaculture species and is abundant on the west coast of Taiwan, while the rest are tropical species and are more abundant on the east coast. In addition, *A. celebesensis*, which was identified in Taiwan in the past, appears to be the new species *A. luzonensis* (*A. huangi*) described from northern Luzon, the Philippines. To clarify these issues, the species composition, relative abundances, and seasonal occurrences of anguillid eels on the east coast of Taiwan were investigated based on 1004 glass eel specimens collected from the estuary of the Hsiukuluan River, eastern Taiwan in 2005-2009. Eel species were identified using morphological characters such as caudal fin pigmentation patterns, the position of the origin of the dorsal fin, and body proportions. The reliability of the morphological method for species identification was checked by a DNA analysis. *Anguilla marmorata* was the most abundant eel species in the Hsiukuluan River, making up 98.4% of the total catch, while there were very few *A. bicolor pacifica* (1.6%) and *A. japonica* (< 1%). *Anguilla marmorata* recruited mainly to the estuary during spring to summer but was found year-round, while *A. bicolor pacifica* recruited mainly during autumn. Results of the DNA analysis did not support the occurrence of *A. luzonensis* and/or *A. celebesensis* based on differences in the distance between the origin of the dorsal and anal fins as a percent of total length (ADL/%TL). *Anguilla celebesensis*, which was identified in the past, was not found in this study and might just be the newly described eel species, *A. luzonensis*, or just a phenotypic variation of *A. marmorata*. Differences in abundances and geographic distributions of these eel species were explained by their temperature preferences, species origins, and current systems in the coastal waters of Taiwan. <http://zoolstud.sinica.edu.tw/Journals/51.1/59.pdf>

Key words: Japanese eel, Giant mottled eel, Indonesian shortfin eel, Glass eels, Temporal and spatial distribution

Freshwater eels (of the genus *Anguilla*) have been a constant source of fascination to humans, and despite decades of intensive research, many aspects of their biology still remain a mystery. There are 16 species and 3 subspecies of freshwater eels in the world (Ege 1939, Castle and Williamson 1974, Watanabe

et al. 2009), and among them, 4 species are reported from Taiwan: Japanese eel *A. japonica*, giant mottled eel *A. marmorata*, Indonesian short fin eel *A. bicolor pacifica*, and Indonesian mottled eel *A. celebesensis* (Tzeng 1982 1983a, Tzeng and Tabeta 1983, Han et al. 2001). *Anguilla japonica* is an important aquaculture species and

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is more abundant on the southern, western, and northern coasts of Taiwan. *Anguilla marmorata* in Taiwan is rarely studied because it was on the endangered species list until 2009. On the other hand, the 2 other species are very rare (Tzeng et al. 1995, Tzeng and Chang 2001). *Anguilla japonica* is a temperate species, while the other 3 species are tropical ones (Tesch 1977). Recently, a new eel species, *A. luzonensis*, was found in the Pinacanauan River, a tributary of the Cagayan River in northern Luzon, the Philippines (Watanabe et al. 2009). This new species has variegated skin pigmentation and broad maxillary bands of teeth similar to those of *A. celebesensis*; thus, it is difficult to distinguish these 2 species morphologically, although they show statistically significant differences in 6 proportional characters and 2 meristic characters (Watanabe et al. 2009). *Anguilla luzonensis* inhabits the Philippines and can possibly extend to adjacent areas such as Taiwan. It was hypothesized that the spawning area of this species is in the North Equatorial Current (NEC) region of the western North Pacific (Aoyama 2009). Shortly afterwards, Teng et al. (2009) also described a new species of anguillid eel from an aquaculture farm in Taiwan which was named *A. huangi*. The eels at this aquaculture farm were reportedly imported from Luzon, the Philippines. These 2 new species have overlapping morphological characters and geographical distributions with *A. celebesensis*. Both new species are genetically distinct from all known anguillid eel species (Minegishi et al. 2009, Teng et al. 2009, Watanabe et al. 2009).

In Taiwan, fishermen collect glass eels (elver) of Japanese eel in estuaries for aquaculture. They classify glass eels into white and black types according to the pigmentation pattern on the tail bud and caudal fin (Tzeng 1983a 1985, Tzeng and Tabeta 1983). White-type eels are comprised solely of *A. japonica*, while black-type eels are supposedly comprised of *A. marmorata*, *A. bicolor pacifica*, *A. celebesensis*, and possibly the new species as well. White-type elvers are usually collected for aquaculture, and several studies were conducted on this species including ones which examined its metamorphosis, estuarine arrival (Tzeng 1990, Cheng and Tzeng 1996), fluctuations in estuarine recruitment in relation to environmental conditions (Tzeng 1984a 1985, Chen et al. 1994, Tzeng 2006, Han et al. 2009), exploitation rates (Tzeng 1984b), population genetic structure (Tseng et al. 2006, Chang et al. 2007, Han et al. 2010), population dynamics, and fishery management (Lin

and Tzeng 2008, Lin et al. 2009). However, little information is available on the tropical black-type eel population, which might be due to the fact that fishing for and aquaculture of *A. marmorata* were illegal on the island before Apr. 2009 because it was listed as an endangered species according to the *Wildlife Conservation Act of Taiwan*. Studies of species identification, catch composition, and recruitment dynamics are essential for fisheries management of tropical eels.

This study aimed to clarify the species composition and seasonality of tropical eels recruiting in eastern Taiwan. Because *A. celebesensis* and *A. luzonensis* are more or less morphologically similar, it was hypothesized that they can be distinguished from *A. marmorata* using morphological measurements such as the ano-dorsal fin length (ADL) in relation to the total length (ADL/%TL), with the latter having a larger ADL/%TL than the former. In addition, to understand if the *A. celebesensis* specimen described by Tzeng (1982) was misidentified with the new recently described species (Teng et al. 2009, Watanabe et al. 2009), their morphometric characters and mitochondrial DNA cytochrome (Cyt) *b* sequences were investigated, and *Anguilla* eel species in Taiwan were revised.

MATERIALS AND METHODS

Sample collection

The anguillid glass eel specimens used in this study were collected by the Taiwan Endemic Species Research Institute (TESRI) of Nantou County, Taiwan. Samples were collected monthly in 2005 and 2007-2009 at 2 stations in the lower reach of the Hsiukuluan River, eastern Taiwan (Fig. 1, Table 1). The Hsiukuluan River is the largest river in eastern Taiwan with a length of 81 km and a drainage area of 1790 km² (Shiao et al. 2003). The 1st station was located in the river mouth, and a traditional triangle net was used for glass eel collection; the 2nd station was located 2 km upstream where a fish way trap was set up in an artificially dug water channel that was about 20 m long, 1.5 m wide, and 25 cm deep. Glass eels were collected together with amphidromous shrimp (Chen et al. 2009). After collection, specimens were immediately preserved in 75% ethanol. Environmental parameters such as dissolved oxygen (DO), salinity (conductivity), pH, water temperature, water velocity, and turbidity were

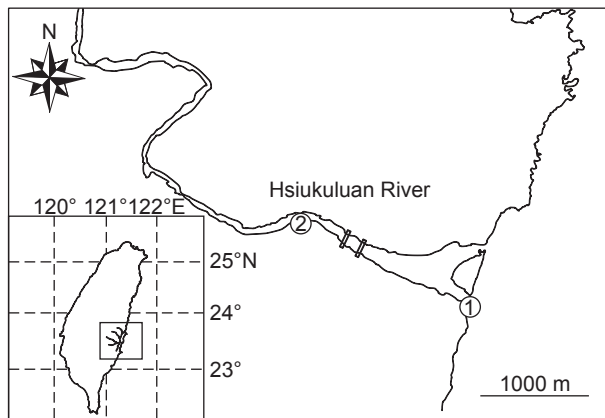


Fig. 1. Map showing glass eel sampling stations (1 and 2) in the lower reach of the Hsiukuluan River in eastern Taiwan. The bars indicate the Juisiu and Long Rainbow Bridges.

also recorded at every sample collection.

Morphometric measurements and species identification

Glass eel species were identified using morphological characters and pigmentation patterns summarized in figure 2 which was modified from Tzeng (1982 1983a) and Tzeng and Tabeta (1983). Morphological characters, including total length, pre-dorsal fin length (PDL), pre-anal fin length (PAL), and ADL, were measured to the nearest 0.1 mm (Fig. 3). Total length (TL) was determined by measuring the distance between the tip of the snout and the end of the tail, while the PDL was determined by measuring the distance from the tip of the snout to the origin

Table 1. Species composition of *Anguilla* glass eels collected in the lower reach of Hsiukuluan River in eastern Taiwan in 2005-2009. (A) and (B) indicate possible proportions of *A. marmorata* and *A. luzonensis* and/or *A. celebesensis* (uncertain). Values in parentheses indicate the number of individuals used for the measurement of ADL/%TL to discriminate *A. marmorata* and *A. luzonensis* and/or *A. celebesensis* (uncertain) and number of individuals used for molecular identification in A and B

Sampling period		Sample size			Species composition					
		Stn. 1	Stn. 2	Total	<i>A. japonica</i>	<i>A. bicolor pacifica</i>	<i>A. marmorata</i> (A) + uncertain (B)	A	B	Unidentified
2005	June	0	151	151	0	0	151	-	-	-
2007	July	7	19	26	0	0	26 (26)	24	1	1
	Sept.	0	8	8	0	0	8 (8)	6	1	1
2008	Apr.	0	1	1	0	0	1 (1)	1	0	0
	May	0	48	48	0	0	48 (48)	46	1	1
	June	31	23	54	0	0	54 (14)	12	0	2
	July	0	13	13	0	0	13 (13)	11	1	1
	Aug.	56	0	56	0	0	56	-	-	-
	Sept.	4	1	5	0	0	5 (5)	4	1	0
	Oct.	36	0	36	0	0	36 (22)	17	2	3
	Nov.	280	0	280	2	10	268 (67)	56	5	6
	Dec.	62	0	62	1	2	59 (54)	38	9	7
	2009	Jan.	1	0	1	1	0	0	0	0
Feb.		1	0	1	0	0	1	-	-	-
Mar.		42	2	44	0	0	44 (24)	19	0	5
Apr.		64	0	64	1	0	63 (60)	53	5	2
May		17	0	17	0	0	17 (9)	9	0	0
June		61	0	61	0	1	60 (48)	41	1	6
July		69	0	69	0	0	69 (18)	17	0	1
Aug.		4	0	4	0	0	4 (4)	2	0	2
Sept.		1	2	3	0	0	3 (3)	2	0	1
Total		736	268	1004	5	13	985 (424)	358 (2)	27 (4)	39

Stn., station.

of the dorsal fin. The PAL was determined by measuring the distance from the tip of the snout to the origin of the anal fin. The ADL, on the other hand, was the difference in distance between the origin of the dorsal and anal fins in percent of the total length (ADL/%TL). In addition, tail bud and caudal fin cutaneous pigmentation patterns, which appear during the glass eel pigmentation process, were also used for species identification. Developmental stages from glass eel to elver were also determined according to the extent (or absence) of skin pigmentation over the head, tail, and other body regions following methods described by Strubberg (1913), Bertin (1956), and Tesch (1977 2003).

Glass eels were classified into long- and short-finned types according to the value of ADL/%TL. Individuals with ADL/%TL values of < 5% were classified as short-finned eels (the pre-dorsal fin origin is closer to the anus than jaw), while individuals with ADL/%TL values of > 5% were classified as long-finned eels (the pre-dorsal fin origin is closer to the jaw than anus) (Ege 1939, Tesch 2003). *Anguilla bicolor pacifica* is a short-finned eel with pigmentation in the tail bud that extends to the caudal fin, while the rest are long-finned eels. The 3 long-finned eel species were then separated according to the cutaneous pigmentation patterns on the posterior part of the body. *Anguilla japonica* has no pigmentation at this stage, while both *A. marmorata* and *A. luzonensis* and/or *A. celebesensis* have more or less the same pigmentation patterns in the tail bud. *Anguilla marmorata* and *A. luzonensis* and/or *A. celebesensis* were then separated according to ADL/%TL values. The ADL/%TL was reported to differ between these species but there is some

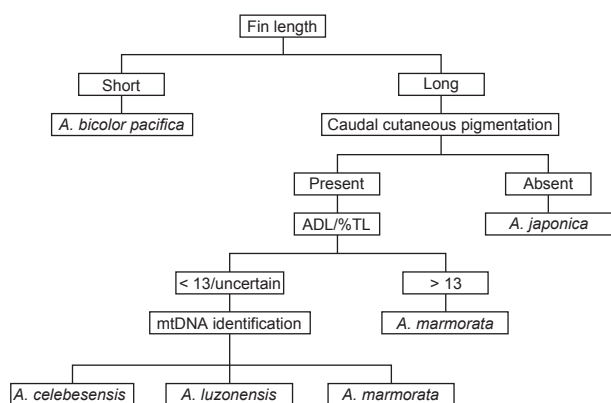


Fig. 2. Schematic diagram of the methods used for anguillid glass eel species identification.

degree of overlap (Tzeng 1982, Teng et al. 2009, Watanabe et al. 2009). Individuals with ADL/%TL values of > 13 were classified as *A. marmorata*, while those with values of < 13 were classified as *A. luzonensis* and/or *A. celebesensis*. Furthermore, the reliability of using ADL/%TL to discriminate *A. marmorata*, *A. luzonensis*, and/or *A. celebesensis* was tested by molecular identification. In total, 6 individuals with minimum, medium, and maximum values of ADL/%TL were chosen for the DNA analysis.

DNA extraction, polymerase chain reaction (PCR) amplification, and phylogenetic analysis

Total genomic DNA was extracted from muscle tissues of individuals with minimum, medium, and maximum values of ADL/%TL using a DNA purification and extraction kit. A pair of oligonucleotide primers, H15341 (5'-TGCTAACGATGCCCTAGTGG-3') and L151341 (5'-CTAGTCAACCTACTAATGGG-3') was used to amplify a fragment of Cyt *b* using PCR amplification (Han et al. 2002). PCR amplification was carried out in a 25- μ l reaction mixture containing 0.5 μ l template DNA, 2.5 μ l 10x reaction buffer, 0.5 μ l dNTP, 1 μ l of each forward and reverse oligonucleotide primers, 0.25 μ l DNA Taq polymerase, and 19.25 μ l double-distilled water. The thermal profile consisted of initial denaturation at 94°C for 3 min followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 50-55°C for 1 min, and extension at 72°C for 30 s, with a final extension at 72°C for 10 min. PCR products were electrophoresed on a 1% agarose gel and stained with ethidium bromide (EtBr) for band characterization via ultraviolet trans-illumination. Sequencing reactions were performed using an ABI PRISM 377 Auto DNA Sequencer (Applied Biosystems, Foster City, CA, USA).

The generated sequences were compared to the mitochondrial (mt)DNA Cyt *b* sequences of all known species and subspecies of *Anguilla*

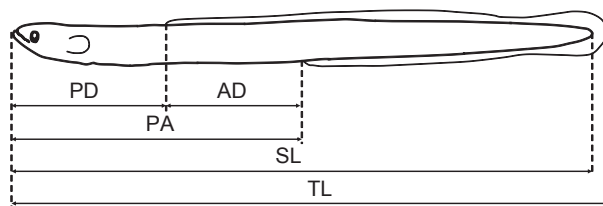


Fig. 3. Diagram showing morphometric measurements of the glass eel. PD, pre-dorsal length; AD, ano-dorsal length, PA, pre-anal length, SL, standard length, and TL, total length.

retrieved from GenBank (accession nos.: AB038556, AB469437, and AP007233-49) to determine their phylogenetic relationships using the Neighbor-joining (NJ) method with the Kimura two-parameter model as implemented in MEGA 4.1 (Tamura et al. 2007). The resultant topology was assessed by bootstrapping with 1000 replications.

Data analysis

The morphometric datasets were subjected to normality and equal variance tests because of the unequal sample sizes. If the dataset passed the test, significant differences were examined using a one-way analysis of variance (ANOVA) followed by pairwise multiple comparisons using the Holm-Sidak method. On the other hand, if the dataset failed the test, significant differences were examined using a Kruskal-Wallis ANOVA on ranks followed by multiple comparisons using Dunn's method. Holm-Sidak and Dunn's post-hoc tests were conducted to detect pairwise differences between species with an overall alpha level of 0.05. All statistical analyses were carried out using SigmaStat software vers. 3.5 (Systat Software, San Jose, CA, USA). In addition, morphological data of *A. celebesensis* measured by Tzeng (1982) were compared to the new species, *A. luzonensis*, to check if they were synonymous. Also, vertebral counts determined for *A. celebesensis* (in Tzeng 1982), *A. luzonensis* (in Watanabe et al. 2009), and *A. huangi* (in Teng et al. 2009) were compared.

RESULTS

Species composition

In total, 1004 anguillid eels were collected and examined in this study (Table 1). Species were preliminarily identified using the caudal pigmentation pattern and ADL/%TL values (Table 1). Cutaneous pigmentation patterns on the caudal part of glass eels differ among species and can be classified into 3 types (Fig. 4): type 1 lacks pigmentation on both the tail bud and caudal fin, i.e., *A. japonica* (Fig. 4A); type 2 has large patches of small (stellate) melanophores on the caudal fin, i.e., *A. bicolor pacifica* (Fig. 4B); and type 3 has a large patch of diffused melanophores on the tail bud, i.e., *A. marmorata*, *A. luzonensis*, and/or *A. celebesensis* (Fig. 4C). Pigmentation patterns alone cannot be used to distinguish these 3 pigmented tropical eel species so a subset

(424 individuals) was chosen to discriminate the species. Individuals with ADL/%TL values of > 13 were classified as *A. marmorata* (A) while those with ADL/%TL values of < 13 were classified as *A. luzonensis* and/or *A. celebesensis* (uncertain or B) (Table 1). On the other hand, unidentified individuals were labeled as uncertain. Individuals with ADL/%TL values of > 13 totaled 358 individuals, while those with values of < 13 totaled only 27 individuals. Based on pigmentation patterns and morphometric analyses, very few *A. japonica* (5 individuals) or *A. bicolor pacifica* (13 individuals) specimens were identified. A subset

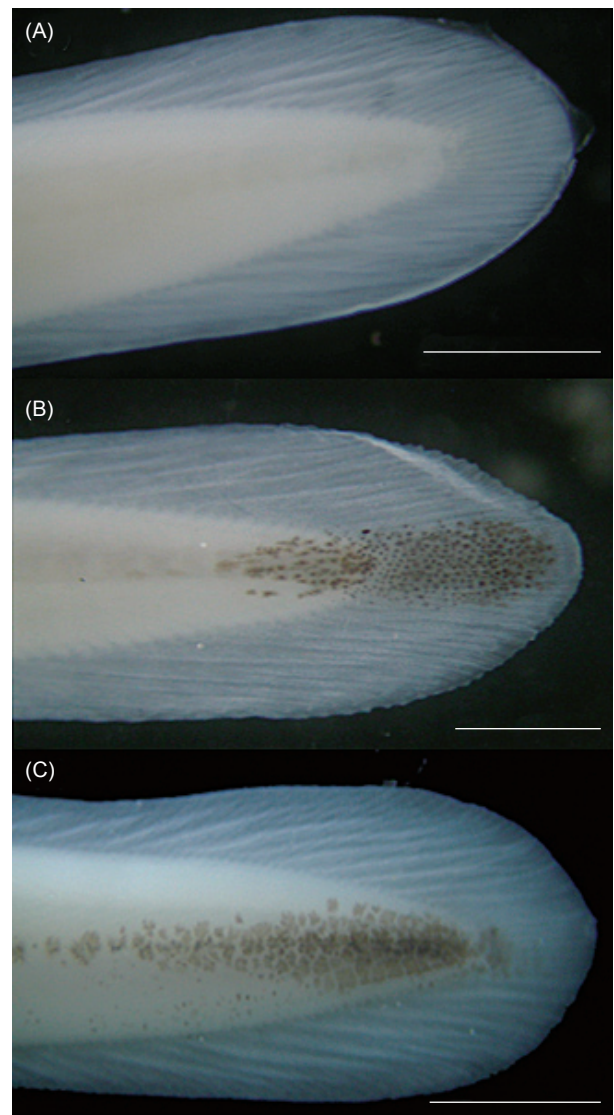


Fig. 4. Caudal fin and tail bud pigmentation patterns of anguillid glass eels. (A) *Anguilla japonica*, (B) *A. bicolor pacifica*, and (C) *A. marmorata*, *A. luzonensis*, and/or *A. celebesensis*. Scale bar = 1.5 mm.

of samples with ADL/%TL values of < 13 and of > 13 were chosen for DNA analyses to check the reliability of using ADL/%TL to discriminate *A. marmorata*, *A. luzonensis*, and/or *A. celebesensis*.

Molecular identification

The phylogenetic tree of all currently recognized species and subspecies of *Anguilla* constructed using the entire mitochondrial DNA genome is shown in figure 5A. The resultant topology clearly indicates clustering of *A. luzonensis* and *A. huangi*, and this was strongly supported by 100% bootstrap probability. Also, the phylogenetic tree supported the occurrence of the new eel species that is genetically distinct from all known species and subspecies of *Anguilla*. In addition to this, the complete mtDNA genome sequence homology test using the BLAST algorithm (Zhang et al. 2000) showed 99% similarity between *A. luzonensis* and *A. huangi*, indicating a very high degree of genetic similarity between them.

On the other hand, the phylogenetic analysis did not support the occurrence the new species and/or *A. celebesensis* in Taiwan based on species-specific differences in ADL/%TL values.

Sequences of eel species with ADL/%TL values of < 12%, of 12%-13%, and of > 13% all clustered with *A. marmorata* in the phylogenetic tree with 100% bootstrap probability (Fig. 5B), indicating that the proposed species-specific differences in ADL/%TL may just be a phenotypic variation of *A. marmorata*, and the use of ADL/%TL to distinguish *A. luzonensis* and/or *A. celebesensis* might not be reliable.

Comparison of morphometric characters among eel species

Based on groupings according to caudal cutaneous pigmentation patterns, morphometric measurements of different species were determined. Because the molecular analysis did not support the occurrence of *A. luzonensis* and/or *A. celebesensis*, individuals with ADL/%TL values of < 13 were now classified as *A. marmorata* and were not included in the morphometric comparisons. Ranges and mean TLs differed among eel species (Table 2). TLs of long-finned eels ranged 46.47-58.04 (mean \pm standard deviation: 49.43 \pm 2.32) mm in *A. marmorata*, 45.45-57.79 (53.01 \pm 4.54) mm in *A. japonica*, and 40.40-47.00 (44.50 \pm 3.04) mm in *A. celebesensis*

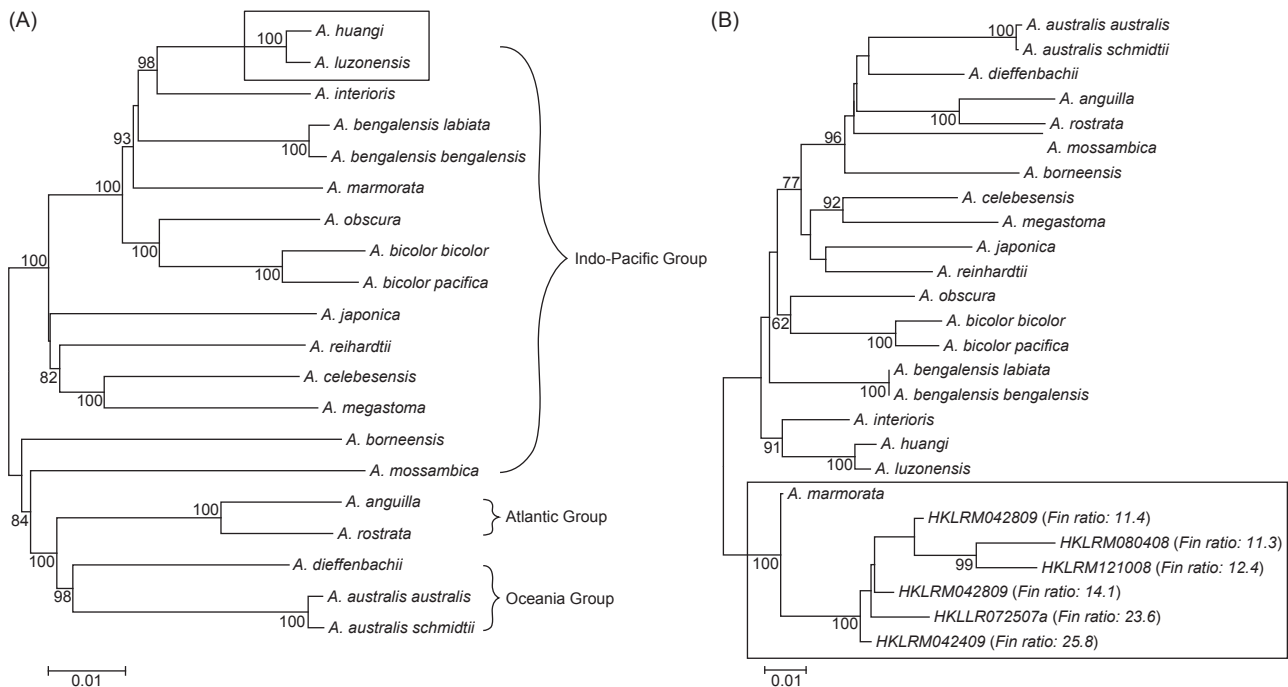


Fig. 5. Phylogenetic tree of the genus *Anguilla* inferred from the entire mitochondrial genome (A) and cytochrome *b* (B) sequences. Individuals with maximum (25.8% and 23.6%), medium (14.1% and 12.4%), and minimum (11.3% and 11.4%) values of the ratio of the ano-dorsal length to the percent total length (ADL/%TL) are also shown (B). Bootstrap probabilities are indicated near the nodes.

(Tzeng 1982). On the other hand, the TL of *A. bicolor pacifica* ranged 45.86-49.22 (47.29 ± 1.06) mm. TLs of all 4 species examined significantly differed (ANOVA, $p < 0.05$). Length-frequency distributions of all species are presented in figure 6. *Anguilla japonica* had the largest mean TL, followed by *A. marmorata*, *A. bicolor pacifica*, and *A. celebesensis*.

The PDL of the short-finned eel *A. bicolor pacifica* ranged 16.52-18.55 (17.82 ± 0.63) mm, the largest among all eel species examined (Table 3). In long-finned eel species, the PDL ranged 12.04-15.05 (13.21 ± 1.59) mm in *A. japonica*, 9.00-14.40 (11.43 ± 0.94) mm in *A. marmorata*, and 12.50-13.70 (13.03 ± 0.50) mm in *A. celebesensis*. Significant differentiation in PDL was observed among species (Kruskal-Wallis: $H = 36.51$, $d.f. = 3$, $p < 0.001$; Table 3). No significant differentiation was observed between *A. bicolor pacifica* and *A. japonica* or between *A. celebesensis* and *A. marmorata* ($p > 0.05$; Table 2). *Anguilla marmorata* was observed to have the smallest PDL, while *A. bicolor pacifica* had the largest. PAL, on the other hand, did not significantly differ between short- and long-finned eel species ($p > 0.05$; Table 2).

The PAL of *A. bicolor pacifica* ranged 16.77-18.74 (18.04 ± 0.60) mm, while those of *A. japonica*, *A. marmorata*, and *A. celebesensis* ranged 15.85-19.46 (18.54 ± 1.56), 17.02-23.92 (19.13 ± 1.32), and 15.80-19.40 (17.65 ± 1.58) mm, respectively. No significant differentiation in PAL was observed among species (ANOVA, $p > 0.05$; Table 3).

According to the ADL, anguillid eels collected were classified into a short-finned eel, i.e., *A. bicolor pacifica*, with a mean (± SD) ADL of 0.22 ± 0.11 (0.08-0.37) mm, which was significantly smaller than those of long-finned eels, *A. japonica*

(mean, 5.33 ± 1.43; range, 3.81-7.02 mm), *A. marmorata* (7.70 ± 0.95; 6.20-9.91 mm), and *A. celebesensis* (4.63 ± 1.16; 3.30-5.70 mm; Tzeng 1982) (Table 2). *Anguilla marmorata*

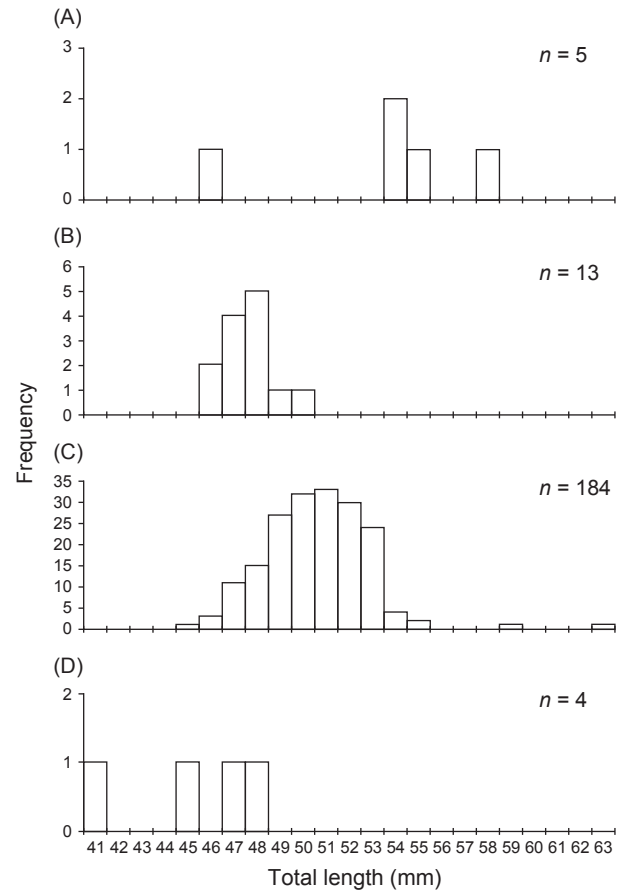


Fig. 6. Length frequency distribution of glass eels of *Anguilla japonica* (A), *A. bicolor pacifica* (B), and *A. marmorata* (C) caught in the lower reach of the Hsiukuluan River of eastern Taiwan and *A. celebesensis* (D) (Tzeng 1982). n = sample size.

Table 2. Comparison of morphometric characters among the 5 *Anguilla* species. All values are in millimeters except for ADL/%TL. Values inside parentheses beside the species names indicate the sample sizes used for morphometric comparisons. Different superscript letters indicate a significant difference at $p < 0.05$

	Mean ± S.D. (range) mm			
	<i>A. japonica</i> (5)	<i>A. bicolor pacifica</i> (13)	<i>A. marmorata</i> (30)	<i>A. celebesensis</i> ¹ (4)
Total length	53.01 ± 4.54 (45.45-57.79) ^d	47.29 ± 1.06 (45.86-49.22) ^b	49.43 ± 2.32 (46.47-58.04) ^c	44.50 ± 3.04 (40.40-47.00) ^a
Pre-dorsal fin length	13.21 ± 1.59 (12.04-15.05) ^b	17.82 ± 0.63 (16.52-18.55) ^b	11.43 ± 0.94 (9.00-14.40) ^a	13.03 ± 0.50 (12.50-13.70) ^a
Pre-anal fin length	18.54 ± 1.56 (15.85-19.46) ^a	18.04 ± 0.60 (16.77-18.74) ^a	19.13 ± 1.32 (17.02-23.92) ^a	17.65 ± 1.58 (15.80-19.40) ^a
Ano-dorsal fin length	5.33 ± 1.43 (3.81-7.02) ^a	0.22 ± 0.11 (0.08-0.37) ^a	7.70 ± 0.95 (6.20-9.91) ^b	4.63 ± 1.16 (3.30-5.70) ^b
ADL/%TL	10.03 ± 2.43 (8.19-12.97) ^a	0.43 ± 0.22 (0.17-0.79) ^a	15.57 ± 1.77 (13.27-20.35) ^b	10.30 ± 1.97 (8.17-12.13) ^b

¹ Measurements from Tzeng (1982).

had the largest ADL followed by *A. japonica*, *A. celebesensis*, and *A. bicolor pacifica*. Significant differentiation in ADL was observed among species (Kruskal-Wallis, $H = 40.18$, $d.f. = 3$, $p < 0.001$; Table 2). No significant difference was observed between *A. celebesensis* and *A. marmorata* or between *A. bicolor pacifica* and *A. japonica* ($p > 0.05$; Table 2).

The ADL/%TL value of *A. marmorata* ranged 13.27%-20.35% ($15.57\% \pm 1.77\%$) while values of the other eel species ranged 0.17%-0.79% ($0.43\% \pm 0.22\%$) in *A. bicolor pacifica*, 8.19%-12.97% ($10.03\% \pm 2.43\%$) in *A. japonica*, and 12.13%-18.17% ($10.30\% \pm 1.97\%$) in *A. celebesensis*. Significant differentiation in ADL/%TL values was observed (Kruskal-Wallis, $H = 40.16$, $d.f. = 3$, $p < 0.001$; Table 2). No significant difference was

found between *A. celebesensis* and *A. marmorata* or between *A. japonica* and *A. bicolor pacifica* ($p > 0.05$; Table 2).

Figure 7 indicates that the short-finned eel *A. bicolor pacifica* can easily be distinguished from *A. marmorata*, *A. japonica*, and *A. celebesensis* using body proportions, especially the PDL and ADL in relation to TL. The PDL/TL (%) (Fig. 7A) was 36.02%-37.69% (mean, 37.68%) in the short-finned eel *A. bicolor pacifica*, while in the long-finned eel species it ranged 19.37%-24.81% (23.12%) in *A. marmorata*, 26.49%-26.04% (24.92%) in *A. japonica*, and 27.68%-30.94% (29.28%) in *A. celebesensis*. On the other hand, ADL/TL (%) more-significantly differed between short- and long-finned eel species ($p < 0.05$, Table 2; Fig. 7). The ADL/TL (%) (Fig. 7B) was 0.17%-0.75% (0.46%) in the short-finned eel, *A. bicolor pacifica*, while in the long-finned eel species it ranged 13.34%-17.07% (15.58%) in *A. marmorata*, 8.38%-12.15% (10.05%) in *A. japonica*, and 8.17%-12.13% (10.40%) in *A. celebesensis*.

Comparisons of vertebral counts between the new species *A. luzonensis* (Watanabe et al. 2009) and *A. huangi* (Teng et al. 2009) and the supposedly *A. celebesensis* specimens described by Tzeng (1982) are shown in table 3. Numbers of vertebrae showed overlapping counts. Total vertebral counts ranged 101-110 (105.3) in *A. celebesensis*, 103-107 (104.8) in *A. luzonensis*, and 103-106 (104.9) in *A. huangi*. Abdominal vertebral counts ranged 39-42 (34.3) in *A. celebesensis*, 40-42 (41.1) in *A. luzonensis*, and 40-41 (40.6) in *A. huangi*. Caudal vertebral counts, on the other hand, ranged 62-68 (65) in *A. celebesensis* and 61-66 (63.8) in *A. luzonensis*. Caudal vertebral counts of *A. huangi* were not available.

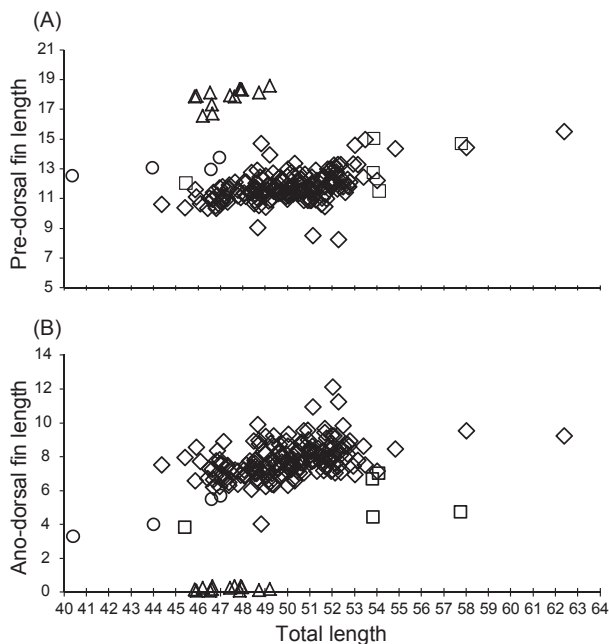


Fig. 7. Relationships among the pre-dorsal fin length, ano-dorsal fin length, and total length in glass eels of *Anguilla japonica* (\square), *A. bicolor pacifica* (Δ), *A. marmorata* (\diamond), and *A. celebesensis* (\circ from Tzeng 1982).

Developmental stages

Anguillid eels immigrating to the Hsiukuluan River were at the transition stage from glass eel

Table 3. Comparison of vertebral counts of *Anguilla celebesensis*, *A. luzonensis*, and *A. huangi*

Species	Vertebral counts		
	Total	Abdominal	Caudal
<i>A. celebesensis</i> ^a	101-110 (105.3)	39-42 (34.3)	62-68 (65)
<i>A. luzonensis</i> ^b	103-107 (104.8)	40-42 (41.1)	61-66 (63.8)
<i>A. huangi</i> ^c	103-106 (104.9)	40-41 (40.6)	N/A

^aTzeng (1982), ^bWatanabe et al. (2009), ^cTeng et al. (2009). N/A, not available.

to elver with various pigmentation stages (Table 4). At station 1 (river mouth), the majority of individuals caught were at stage V_A (62%) followed by stages V_B (34.1%), VI_{A1} (2.2%), and VI_{A2} (1.7%). These stages were characterized by the respective absence (stage V_A) or presence (stage V_B) of a cerebral nerve-cord spot and pigmentation on the tail and caudal fin (Bertin 1956). The pigmentation stage suggested that the glass eels had recently arrived at the river mouth. On the other hand, at station 2 (approximately 2 km upstream from the river mouth), the majority of individuals caught were at stage VI_{A1} (35.2%) followed by stages V_B (27.8%), VI_{A2} (13%), VI_{A4} (11.1%), VI_{A3} (7.4%), V_A (3.7%), and VI_B (1.9%). Stages V_B-VI_{A4} (elver stage) were characterized by a progressive pigmentation on the dorsolateral part of the elver.

Seasonal occurrence

Relative abundances of glass eels varied with sampling stations, months, and years (Table 1). Four anguillid eel species were identified based on caudal pigmentation patterns and morphometric measurements. *Anguilla japonica* is a temperate species, and relatively few (< 1%) were found in eastern Taiwan, although it is abundant on the west coast of Taiwan. *Anguilla marmorata* was most abundant among the 3 tropical eel species, making up 98.4% of the total catch, followed by a very few (1.3%) *A. bicolor pacifica*. But because the phylogenetic analysis did not support the occurrence of the new species and/or *A. celebesensis*, the relative abundance of *A.*

marmorata should be more than 98.4%.

It was found that different eel species recruit to the Hsiukuluan River at different times of the year: *A. marmorata* recruited mainly during spring and summer but was found year-round, while *A. bicolor pacifica* recruited during autumn. The temperate species, *A. japonica*, recruited mainly during winter.

DISCUSSION

Pigmentation stage in relation to upstream migration

The pigmentation stage of glass eels differed among sampling stations. It was observed that the stages of glass eels became more advanced as they moved upstream. Station 1 is brackish water to seawater, while station 2 is fresh water. It is still not known whether these changes in pigmentation stages are due to the effect of salinity change, a time-delay effect of recruitment, or both. But the effect of a time delay can be checked by the difference in the number of otolith daily increments of elvers between stations.

Why is *A. japonica* abundant on the west coast while *A. marmorata* is abundant on the east coast of Taiwan?

The species composition of glass eels recruiting to the Hsiukuluan River, eastern Taiwan, was dominated by *A. marmorata* which greatly

Table 4. Pigmentation stages of different *Anguilla* eel species collected from 2 stations in the lower reach of the Hsiukuluan River, Taiwan

Station	Species	n	Pigmentation stage						
			V _A	V _B	VI _{A1}	VI _{A2}	VI _{A3}	VI _{A4}	VI _B
1	<i>A. japonica</i>	5	5	0	0	0	0	0	0
	<i>A. bicolor pacifica</i>	12	11	1	0	0	0	0	0
	<i>A. marmorata</i>	144	85	53	4	2	0	0	0
	Uncertain	18	10	7	0	1	0	0	0
	Total	179	111	61	4	3	0	0	0
	% composition			62.0	34.1	2.2	1.7	0	0
2	<i>A. japonica</i>	0	0	0	0	0	0	0	0
	<i>A. bicolor pacifica</i>	1	0	0	0	0	0	0	0
	<i>A. marmorata</i>	44	2	11	17	7	1	5	1
	Uncertain	9	0	3	2	0	3	1	0
	Total	54	2	14	19	7	4	6	1
	% composition			3.7	27.8	35.2	13.0	7.4	11.1

differs from that reported from rivers in southern, western, and northern Taiwan where *A. japonica* dominates (Tzeng 1982 1983b, Tzeng and Chang 2001). The low relative abundance of *A. japonica* in this study was doubtful at first, because it might have been due to sampling bias due to fisherman sorting out *A. japonica* for aquaculture before handing the glass eel samples to the researchers. But a recent study by Han et al. (pers. comm.) in the same river system also revealed that the relative abundance of *A. japonica* was very low (< 1%). But why does the dominant species of recruiting glass eels differ between the east and west coasts of Taiwan? This scenario can be further explained by the different geographical distributions, temperature preferences, and habits of the different eel species. During the leptocephalus stage, *A. marmorata* and *A. japonica* might not have different distributions because they use the same spawning ground to the west of Mariana Island, and their larvae are transported by the NEC and Kuroshio Current to their destinations (Kuroki et al. 2009, Miller et al. 2009). But after metamorphosing from leptocephalus to glass eels, the temperate *A. japonica* migrates with the cold China Coastal Current to the west coast of Taiwan (Cheng and Tzeng 1996), while the tropical species, *A. marmorata*, *A. bicolor pacifica*, and the newly described *A. luzonensis* prefer the east coast which is influenced by the warm Kuroshio Current. Previous studies also revealed that *A. japonica* elvers were more abundant on the northern, western, and southern coasts of Taiwan than the east coast (Tzeng 1996, Tzeng and Chang 2001). Those reports indicated that species-specific differences in geographical distribution of glass eels were closely correlated to the coastal current systems which differ between the east and west coasts of Taiwan (Tzeng 1996). Also, the species-specific recruitment, abundance, and distribution of *A. marmorata* and *A. japonica* in Taiwan can also be explained by differences in the duration of their leptocephalus stage, age at metamorphosis, and growth rate. The somatic growth rate is faster and the age at metamorphosis is younger in *A. marmorata* than *A. japonica* (Leander et al. unpubl. data). Thus, the former can recruit earlier at a younger age. This must be the reason why *A. marmorata* can recruit abundantly in northern Luzon, the Philippines and along the east coast of Taiwan, while very few *A. japonica* are known to recruit there, because the latter is still in the leptocephalus stage and drifting with the Kuroshio Current. The drifting *A. japonica* leptocephali

metamorphose into glass eels beyond Taiwan, and some of them then enter the westward branch of the Kuroshio Current that takes them to continental waters of East Asia where they migrate with the cold, southerly flowing China Coastal Current to the northern, western, and southern coasts of Taiwan. This scenario was validated by the peak catch of elvers that coincided with the period of the lowest winter temperatures when the northeastern monsoon-driven China Coastal Current was strongest (Tzeng 1985) and from the daily ages of elvers arriving at estuaries along the west coast of Taiwan being older in the south than in the north (Cheng and Tzeng 1996). In addition to this, Tzeng and Chang (2001) also suggested that the comparatively abundant freshwater discharges and wider shelf area along the west coast of Taiwan can potentially attract elvers to migrate upstream, unlike on the east coast where conditions might be less attractive for inshore migration and recruitment of elvers because the warm Kuroshio is very close to the shore, the salinity is higher, and the continental shelf is narrower. Compared to *A. marmorata*, the abundance of *A. bicolor pacifica* was very low, such that they can be considered an occasional species. This is because the origin or the spawning ground of these eel species is far from Taiwan.

Differences in habitat use and seasonal occurrence

Previous studies indicated that the peak catch of *A. japonica* elvers in Taiwan occurred during winter from Nov. to Feb. (Tzeng 1983b 1996, Tzeng et al. 1995), while the peak catch of *A. marmorata* elvers in the Hsiukuluan River in eastern Taiwan occurred mainly during spring and summer (Lin 2001, Han et al. unpubl. data). This difference in the recruitment season also supports different temperature preferences of these 2 species. In addition, the habitat use of the adult *A. japonica* and *A. marmorata* living sympatrically in a river also differs (Shiao et al. 2003) with the former usually occupying lower reaches of the river while the latter occupies upper reaches.

Differences in recruitment patterns of the different species of glass eel might be due to differences in their spawning season and migration. In temperate eel species, spawning occurs over a limited period, i.e., Feb. to Apr. in *A. rostrata* (McCleave et al. 1987), Mar. to June in *A. anguilla* (McCleave and Kleckner 1987), Apr. to Nov. in *A. japonica* (Tsukamoto 1990), Aug. to

Dec. in *A. dieffenbachi* (Jellyman 1987), and Sept. to Feb. in *A. australis* (Jellyman 1987). With these limited spawning periods, recruitment of their glass eels is therefore limited to certain seasons. On the other hand, tropical eel species have a spawning season that persists almost throughout the year, and this year-round spawning behavior may extend the period of recruitment of their glass eels to estuarine habitats to year round, as was described in previous studies (Tabeta et al. 1976, Arai et al. 1999a b 2001, Shen and Tzeng 2007). In addition, fluctuations in daily catches of glass eels in estuaries are greatly influenced by the spawning duration, oceanic currents, and differences in early life history traits such as the age at metamorphosis and age at recruitment, as well as environmental cues such as the moon phase, tidal currents, and water temperature (Tzeng 1985, Cheng and Tzeng 1996, Wang and Tzeng 1998 2000).

Misidentification of *A. celebesensis* in Taiwan

Previous studies (Tzeng 1982, Tzeng and Tabeta 1983) reported the occurrence of *A. celebesensis* in natural waters of Taiwan, but it was never found again in any recent studies. It is possible that *A. celebesensis* glass eel specimens described in previous studies from the northern Philippines, Taiwan, and southern China (Tabeta et al. 1976, Tzeng 1982, Ozawa et al. 1989, Arai et al. 1999b 2003, Lue et al. 1999) were misidentified and were probably *A. luzonensis*. The leptocephali of *A. celebesensis* have never been identified in the NEC region, and its spawning area was found to be located in the Celebes Sea and Tomini Bay of Indonesia which is far from the NEC region (Kuroki et al. 2006, Watanabe et al. 2009). Also, recent studies indicated that *A. celebesensis* appears to have short spawning migrations and larval durations (Miller et al. 2009).

The newly described eel species, *A. luzonensis* and *A. huangi*, were synonymous because they showed a very high degree of similarity in morphometric characters, which was also strongly supported by the phylogeny of all currently recognized species and subspecies of *Anguilla* constructed using the entire mtDNA genome sequence for which sequences of *A. luzonensis* and *A. huangi* exhibited 99%-100% similarity. Because it is agreed that both names refer to the same species, therefore these names should be formally synonymized. According to Article 23 of the *International Code of Zoological Nomenclature*, the precedence between 2 or

more names is determined by the dates on which the works containing the names or acts were published, unless that name has been invalidated or another name is given precedence by any provision of the code or by any ruling of the commission. In other words, the 1st nomenclatural act or the 1st published name is given precedence, and in the case of the new eel species, the name *luzonensis* was published in Mar. 2009 while *huangi* was published in Nov. 2009, so the name *luzonensis* has priority and precedence over the 2nd name. Also, *luzonensis* is a better name for an eel species discovered in Luzon, the Philippines.

It seems that the new species was found in Taiwan before but was probably misidentified as *A. celebesensis* in 1982 because of the similarity in pigmentation patterns and almost identical morphometric characters (Table 3). Numbers of vertebrae (total, abdominal, and caudal vertebrae) measured in *A. celebesensis* by Tzeng (1982), *A. luzonensis* by Watanabe et al. (2009), and *A. huangi* by Teng et al. (2009) also showed overlapping counts (Table 4). Unfortunately, further comparisons using genetic approaches are not possible because *A. celebesensis* specimens described by Tzeng (1982) are no longer available. But with the obvious similarities in pigmentation patterns, morphometric measurements (i.e., PDL, PAL, and ADL/%TL), and vertebral counts, there is no doubt that *A. celebesensis* found by Tzeng (1982) in natural waters of Taiwan might be the new eel species, *A. luzonensis*.

In summary, there are 3 species of anguillid eels found on the east coast of Taiwan based on species-specific pigmentation patterns and morphometric measurements. But the results of the genetic analysis did not support the occurrence of a 4th species (*A. luzonensis* and/or *A. celebesensis*) indicating that the use of species-specific differences in ADL/%TL to distinguish the new species is not reliable. Further investigations on the species composition of recruiting anguillid eels on a wider scale are warranted to validate the occurrence of *A. luzonensis* and/or *A. celebesensis* in natural waters of Taiwan. The newly described *A. luzonensis* might have been misidentified as *A. celebesensis* in previous studies because these species are almost identical in terms of morphological features. Geographical distributions differ among glass eels species. In the Hsiukuluan River, eastern Taiwan, the species composition of the recruiting anguillid eels was dominated by *A. marmorata* with *A. japonica* and *A. bicolor pacifica* as minor species, which greatly

differs from what was previously reported in rivers of northern, western, and southern Taiwan where *A. japonica* dominates. The recruitment season of *A. marmorata* is mainly from early summer to autumn but can occur almost year round, while that of *A. japonica* is during winter. *Anguilla bicolor pacifica* mainly recruits during autumn. These results suggest that tropical eels have a unique geographical distribution and recruitment season which greatly differs from those of the temperate eel, *A. japonica*. This information is essential for fishery regulation and management implementation.

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