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Habitat Partitioning and its Possible Genetic Background Between Two Sympatrically Distributed Eel Species in Taiwan

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The geographical distributions of the Japanese eel (*Anguilla japonica*) and Giant-mottled eel (*A. marmorata*) overlap in many regions in East Asia and therefore suffer from interspecific competition in the same rivers. After a long period of adaptation, the Japanese eel and Giant-mottled eel may exhibit habitat partitioning in the rivers to diminish the interspecific competition between them. In this study, we conducted a field investigation in the Fengshan River in Taiwan to survey the habitat distributions of the Japanese eel and Giant-mottled eel throughout a river. Moreover, we investigated whether their habitat distributions are related to their swimming and upstream migration. Thus, the mRNA expression levels of several candidate genes that may be associated with the swimming and upstream migration of eel were examined in the glass eels of the Japanese eel and Giant-mottled eel. Field investigation indicated that the Japanese eel mainly inhabited the lower and middle reaches of the Fengshan River, but the Giant-mottled eel was distributed over the middle to upper reaches. The mRNA expression levels of *fMYH*, *dio2*, *gria3*, and *neurod1* were higher in the Giant-mottled eel than in the Japanese eel, implying that Giant-mottled eels might have better swimming bursts and more active upstream migration than Japanese eels. These results suggest that there is a habitat partition at which these two eel species coexist in a river, and their habitat distributions may be linked to their swimming bursts and upstream migration. Determining the habitat distributions of freshwater eels is important for developing applicable plans for eel conservation and resource management.

Key words: Giant-mottled eel, Habitat partitioning, Interspecific competition, Japanese eel and upstream migration.

BACKGROUND

Freshwater eels (genus *Anguilla*) are typical catadromous fish; they spend most of their growth phase in freshwater rivers and estuaries and finally migrate to their spawning grounds in the ocean to reproduce (Aoyama et al. 2018; Chen et al. 2018; Higuchi et al. 2018; Tesch 2003). In the world, there are a total of 19 species and subspecies of freshwater eels (Arai 2016; Tesch 2003; Watanabe et al. 2009). In Taiwan, *A. japonica* (Japanese eel), *A. marmorata* (Giant-mottled eel), and *A. bicolor pacifica* (Shortfin eel) are three common species of freshwater eels, and among these the resources of the Japanese eel and Giant-mottled eel are more abundant than that of the Shortfin eel (Han et al. 2012; Tzeng and Tabeta 1983). According to the previous study, the geographical distributions of the Japanese eel and Giant-mottled eel were found overlapped in many regions in East Asia, including Taiwan, southeast of China, and south of Japan (Han et

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al. 2012). The Japanese eel and Giant-mottled eel would have a great chance to coexist in same rivers at these regions, and they may even share the same niches, use the same habitats, and forage for the same prey, thus causing serious interspecific competition (Tzeng et al. 1995). After a long period of adaptation, freshwater eels coexisting in the same rivers may evolve a substantial survival strategy, such as habitat partitioning, to avoid/reduce interspecific competition.

Recently, several studies have revealed that the habitat distributions of freshwater eels across a river can be affected by extrinsic environmental factors such as salinity, water temperature, rainfall, river size, and carrying capacity, and different freshwater eel species living together in the same rivers might have different habitat usage (Arai and Abdul Kadir 2017; Briones et al. 2007; Jellyman et al. 2003; Shiao et al. 2003). However, the effects of environmental factors on the habitat distributions of freshwater eels are variable and hard to evaluate in different areas. In addition to the environmental factors, other intrinsic factors like upstream migration, genetic differences or body coloration of eels are also possibly related to their habitat distributions.

Several studies have proposed physiological functions associated with eel upstream migration. First, excellent climbing and swimming capacities are indispensable for eels to successfully cross steep slopes in a river. Climbing and swimming capacities link to skeletal muscle performance, swimming mode, and swimming speed (Podgorniak et al. 2015a). In fish, there are two types of skeletal muscle, slow and fast (Kiessling et al. 2006), and understanding the composition and metabolism of skeletal muscle can provide insights into muscle physiological manifestations during the upstream migration or different swimming modes (Altringham and Ellerby 1999; Garenc et al. 1998; Martinez et al. 2003). Additionally, thyroid activity is thought to influence eel migration behavior (Edeline et al. 2004 2005). These studies indicated that glass eels migrating upstream have higher expression levels of T4 (thyroxin) and T3 (triiodothyronine) hormones in their body. Furthermore, Podgorniak et al. (2015a b) found that cognitive functions, such as spatial learning and memory acquisition, play a critical role in eel upstream migration (Podgorniak et al. 2015a b). These cognitive functions may assist migrating eels in searching for their migratory pathways during the upstream migration.

The nature eel stock has drastically decreased over the past few decades owing to overfishing, habitat destruction, and changes in the ocean environment (Chen et al. 2014; Tsukamoto et al. 2009); therefore, management and conservation of natural eel stock are imperative. Knowing the habitat distributions of eels would help for developing methods to manage and conserve natural eel stock. Different eel species might have different habitat preference after entering the rivers, and they might not change their habitats unless they encounter a severe anthropogenic effect such as the introduction of invasive species or water pollution (Dudgeon et al. 2006). When the glass eels metamorphosing from the leptocephali arrive at the estuary, most would start an upstream migration to the freshwater habitats (Han et al. 2016b; Tesch 2003). As a result, the glass eel stage is very important for upstream migration, and the distance different eel species can migrate upstream may be determined at this stage.

The purpose of the present study is to investigate the habitat distributions of the Japanese eel and Giant-mottled eel across a river, as well as the relationship between their habitat distributions and upstream migration. A field investigation in the Fengshan River in Taiwan was executed to survey the habitat distributions of Japanese and Giant-mottled eels. Moreover, the mRNA expression levels of six candidate genes were preliminarily analyzed in the glass eels of both species to estimate their differences in upstream migration.

MATERIALS AND METHODS

Field investigation

The field investigation was carried out in the Fengshan River located in northwestern Taiwan from April 2014 to August 2017. We determined six sampling sites from the lower reaches to the upper reaches of the river. (Fig. 1), and recorded detailed coordinates of these sampling sites (Table S1). Eel sampling was performed for one hour at each sampling site during the daytime for each investigation. The method of eel sampling was electrofishing, which only incapacitated the eels temporarily. The electrofishing at each sampling site was executed within a region measuring 100 meters by the width of the river. The survey with electrofishing was permitted by the Fisheries Agency of the Council of Agriculture, Executive Yuan (Taiwan), for the purpose of scientific research.

Sample collection

All captured eels from the field investigation were transported to our laboratory and anesthetized with 300 ppm tricaine methanesulfonate (MS-222; Sigma-Aldrich, Missouri, USA) for 10 minutes. After anesthesia, the morphological parameters were measured to identify the species (Han et al. 2001). In addition, glass eels for the experiment of

reverse transcription-quantitative PCR (RT-qPCR) were captured at the estuary of the Yi-Lan River in northeastern Taiwan in November 2016. The glass eels of Japanese eel (n = 10) and Giant-mottled eel (n = 10) were then transported to our laboratory and euthanized with an overdose of tricaine methanesulfonate (MS-222; Sigma-Aldrich, Missouri, USA). The glass eel specimens (~150 mg per specimen) were washed with double distilled water (ddH_2O) before being preserved in *RNALater* RNA Stabilization Reagent (QIAGEN, Valencia, CA, USA). After that, the specimens were kept in -20°C until total RNA extraction.

RNA extraction and RT-qPCR

The total RNA of whole-body glass eel specimens was extracted using Trizol® Reagent (Invitrogen, Carlsbad, CA, USA) in compliance with the manufacturer's procedures. Purified RNA was quantified using an ND-1000 spectrophotometer (Nanodrop, Wilmington, DE, USA). The cDNA of each specimen was reverse transcribed from 1 μg of total RNA using iScript cDNA Synthesis kits (Bio-Rad) according to the manufacturer's protocol.

In this study, a total of six genes in relation to

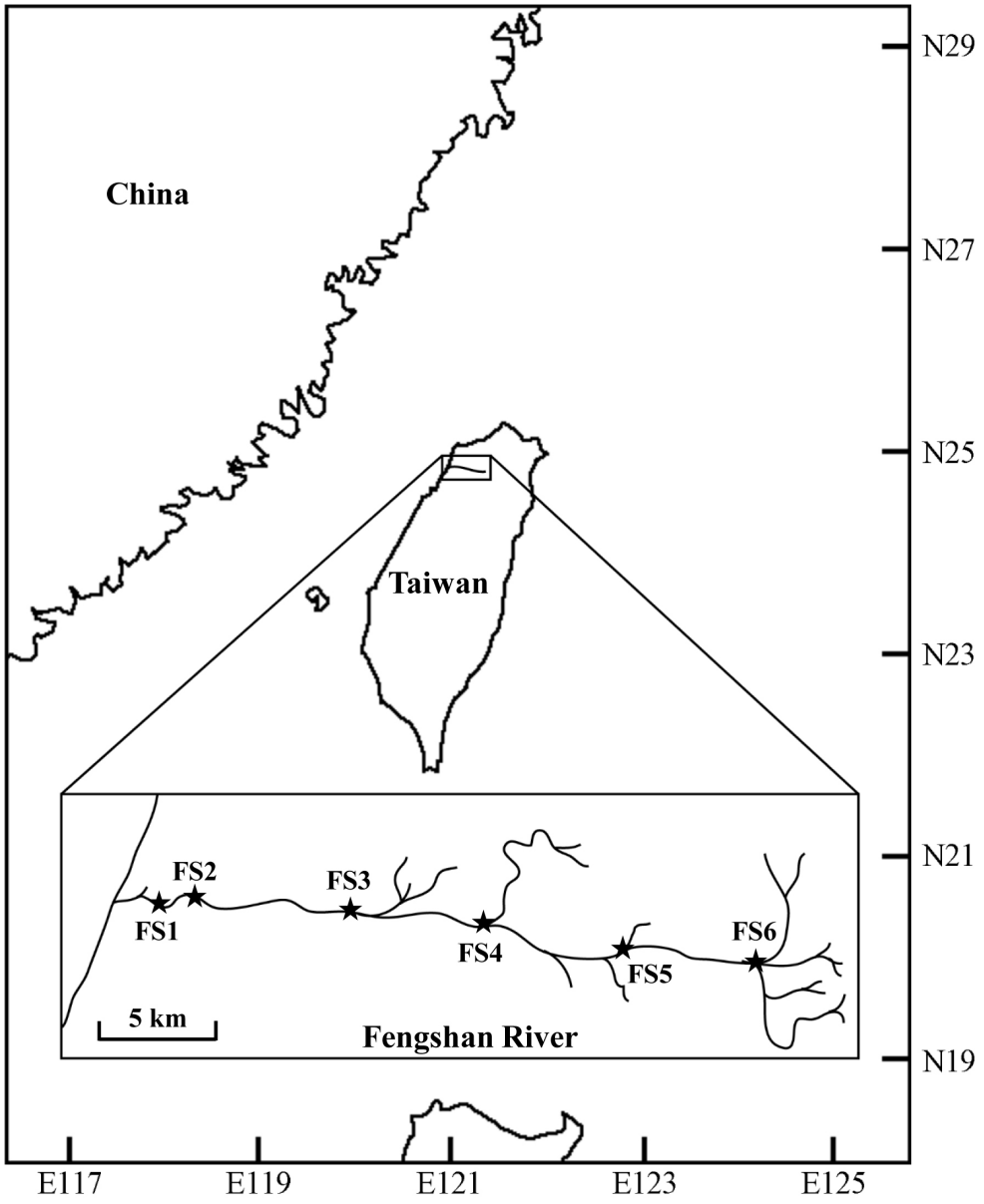

Fig. 1. Map of the sampling sites in the Fengshan River of Taiwan. The Fengshan River is located in northwestern Taiwan, East Asia. FS1 and FS2 are in the lower reaches, FS3 and FS4 are in the middle reaches, and FS5 and FS6 are in the upper reaches of the Fengshan River. This figure was drawn by using Adobe Illustrator CS6 (Adobe Systems Incorporated, USA).

skeletal muscle performance, the concentration of active thyroid hormone and cognitive functions of the brain were subjected to gene expression analysis in the glass eels of Japanese and Giant-mottled eels. In fish, skeletal muscles are categorized as slow-twitch or fast-twitch muscle, both of which are composed of myofibrils containing actin and myosin filaments (Altringham and Ellerby 1999; Kiessling et al. 2006). Therefore, slow-twitch myosin heavy chain (*sMYH*) and fast-twitch myosin heavy chain (*fMYH*) could be used to evaluate the composition of skeletal muscles because they are part of muscle myofibrils (Emerson and Bernstein 1987). Then, the gene *dio2* encoding the iodothyronine deiodinase 2, which is responsible for converting the thyroxin (T4) hormone into the biologically active form 3, 5, 3'-triiodothyronine (T3), was selected (Kohrle 2000; Politis et al. 2018). Previous studies showed that the concentration of active thyroid hormone and the expression of *dio2* are highly associated with upstream migration of the glass eels of the European eel and revealed that the expression of *dio2* significantly increases with age throughout ontogeny in European eel larvae, also displaying its importance in early development (Edeline et al. 2004 2005; Podgorniak et al. 2015a; Politis et al. 2018). Additionally, three genes related to cognitive brain functions were selected. First, the gene *gria3* encodes glutamate receptor 3, which is a kind of AMPA receptor (an ionotropic transmembrane receptor for glutamate) and usually mediates fast excitatory synaptic transmission in the central nervous system in many vertebrates, including mammals, reptile, birds, and teleost fish (Chen et al. 2001; Gecz et al. 1999; Hoppmann et al. 2008). AMPAR signaling plays a significant role in neuron outgrowth, neuronal plasticity of dendritic spines, cell survival of neurons and development of the nervous system, essential for the normal cognitive functions (Catsicas et al. 2001; Drian et al. 2001; Fischer et al. 2000; Hoppmann et al. 2008). Second, the gene *s100b* encodes S100B, a calcium-binding protein predominantly found in astrocytes, oligodendrocytes, and neural progenitor cells and highly conserved between the mammalian animals and the teleost (Kraemer et al. 2008; Thelin et al. 2017). The S100B protein can promote neurogenesis and neuronal plasticity, perform neuro-modulating actions and enhance processes involved in memory and learning (Thelin et al. 2017). Third, the gene *neurod1* encodes the neurogenic differentiation factor 1 protein (NeuroD1), which is generally considered to be the principal marker of cell proliferation and neural differentiation in the brains of teleost fishes (Sorensen et al. 2013). The NeuroD1 is very critical for neuron survival, differentiation, and maturation, and influences spatial learning and memorization (Gao et al. 2009).

Here, we obtained the transcripts of six candidate genes from our published online transcriptome database (http://molas.iis.sinica.edu.tw/4eels/). Detailed information on each transcript was included in the supplementary materials (Table S2 and Table S3). The primer pairs of these candidate genes for the RT-qPCR were designed using Primer 3 software (Table 1). The acidic ribosomal phosphoprotein P0 (*arp*), which steadily expressed in the eels, was used as a housekeeping gene (Sudo et al. 2013). The specificity of each primer pair was checked by PCR and electrophoresis, and the PCR product was validated by DNA sequencing. All the primer pairs were acceptable for use in RT-qPCR.

The RT-qPCR was performed on a Bio-Rad MyiQ real-time PCR system (Bio-Rad, Hercules, CA, USA) within a 25 μL mixture containing 12.5 μL of 2x SYBR green supermix (Bionova, Fremont, CA, USA), 1 μL of each primer, 1 μL of cDNA template (10x dilution), and 9.5 μL of ddH_2O. The RT-qPCR conditions were as follows: (1) pre-incubation, 95°C for 10 min; (2) amplification (40 cycles), 95°C for 30 s, 58°C for 45 s, and 72°C for 45 s. The RT-qPCR data were analyzed in two steps. First, the *arp* was used for normalization by subtracting its C_T value from the C_T value of each target gene ($\geq C_T$). Second, in every category (based on different genes), Japanese eels were used as control samples for normalization by subtracting their mean $\triangle C_T$ value from the $\triangle C_T$ value of each glass eel sample ($\triangle\triangle C_T$). The normalized mRNA expression was calculated as $2^{-(\triangle\triangle C_T)}$ and presented as the mean ± SD.

Statistical analyses

All RT-qPCR data were analyzed using SAS statistical software (version 9.1; SAS Institute Inc., Cary, North Carolina), and they were tested for normality and homogeneity of variance before conducting the statistical analyses. Statistical analyses on the RT-qPCR data were carried out by a *t*-test. For all statistical results, a *p*-value of < 0.05 was considered a significant difference.

RESULTS

Field investigation in the Fengshan River

The results of field investigation were summarized in table 2, and a total of 264 eel samples are collected from six sampling sites. We determined that all samples were either Japanese eel or Giant-mottled eel in the yellow or silver eel stage. Based on the results of the

field investigation, we further calculated the percentage of Japanese and Giant-mottled eels in each sampling site (Fig. 2). At the FS1 (n = 96) and FS2 (n = 111) sampling sites in the lower reaches, all captured eels were Japanese eels (100%). At the FS3 (n = 3) sampling site in the middle reaches, 66% of the captured eels were Japanese eels, and 34% were Giant-mottled eels. At another sampling site in the middle reaches, FS4 (n = 41), 68.3% of the captured eels were Japanese eels, and 31.7% were Giant-mottled eels. At the FS5 (n = 9) sampling site near the upper reaches, 33.3% of the captured eels were Japanese eels, and 66.7% were Giant-mottled eels. At the FS6 (n = 4) sampling site in the upper reaches, all captured eels were Giant-mottled eels (100%).

Expressional analysis of genes related to upstream migration

For the skeletal muscle components, the expression of *sMYH* was higher in the Japanese eel than in the Giant-mottled eel ($p < 0.05$) (Fig. 3a), while the expression of *fMYH* in the Giant-mottled eel was over one thousand times higher than that in the Japanese eel ($p < 0.05$) (Fig. 3b). In addition, the expression level of *dio2* (Fig. 3c) in the Giant-mottled eel was also significantly higher than that in the Japanese eel ($p < 0.05$). For the cognitive function-related genes, the expression of *gria3* in the Giant-mottled eel was significantly higher than that in the Japanese eel ($p < 0.05$) (Fig. 3d). However, no significant expressional difference in *s100b* was found between the Japanese and Giant-mottled eel (Fig. 3e). Finally, the expression level of *neurod1* in the Giant-mottled eel was three times higher than that in the Japanese eel ($p < 0.05$) (Fig. 3f).

DISCUSSION

Based on the results of the field investigation, there seems to be an apparent habitat partition between the Japanese eel and Giant-mottled eel since no Japanese eels were caught at the upper reaches and no Giant-mottled eels were caught at the lower reaches. The biological evolutionary implications of these results are worth exploring. In this study, we thought that the burst swimming and upstream migration of freshwater eels might be related to their habitat distributions. The eels with better swimming bursts and more active upstream migration are more likely to take advantage

Table 1. Primer pairs of target genes for RT-qPCR. Primer pairs (*i.e.*, "aj-f" and "aj-r") of *sMYH*, *fMYH*, *s100b*, and *neurod1* were only used for the Japanese eel (*A. japonica*). Another set of primer pairs (*i.e.*, "am-f" and "am-r") of *sMYH*, *fMYH*, *s100b*, and *neurod1*, were only used for the Giant-mottled eel (*A. marmorata*). The other primer pairs (*i.e.*, "f" and "r") of *dio2*, *gria3* and *arp* were used for both species

Gene	Primer sequences	Amplicon size (bp)	PCR efficiency
sMYH	aj-f: 5′- AGCTACAGACTGAAAATGGG - 3′	108	99.8%
	aj-r: 5′- GGTCTTCAATTTGCTGAGTG - 3′		
	am-f: 5′- GAATGGATCTTGAGAGGGC - 3′	91	99.6%
	am-r: 5′- TGCTGCTTGTCATTCTCTAG - 3′		
fMYH	aj-f: 5′- CAAAAGAAAGCAGAACCAGG - 3′	111	99.8%
	aj-r: 5′- GTTGTCATTCCTCACAGTCT - 3′		
	am-f: 5′- CAGAAACTGGAATCAAGGGT - 3′	110	99.2%
	am-r: 5′- TCCTTGACTCACCTCTCATA - 3′		
dio2	f: 5′- GTACCACCGAGTCATATAGC - 3′	114	99.4%
	r: 5′- TCCCTCCTCAACAGAAAATG - 3′		
gria3	f: 5′- CCTTGGCCTATGAAATTTGG - 3′	115	99.7%
	r: 5′- TCATCAATGTCCTCCAGATG - 3′		
s100b	aj-f: 5′- GCCATTTACAAGCAGGTTAC -3′	91	99.9%
	aj-r: 5′- GGCTCCACTGTAGAATTCAA -3′		
	am-f: 5′- GCTCTCCATCAAACTGTCTA - 3′	113	99.8%
	am-r: 5′- GAGACAAGCACAAACTGAAG - 3′		
neurod1	aj-f: 5′- ATGCAAGGGATTATCTCAGC - 3′	113	99.8%
	aj-r: 5′- CTGTTTGCATATGAGAGGGT - 3′		
	am-f: 5′- ATGATAGTCGCGCATGATAG - 3′	118	99.6%
	am-r: 5′- CCTTGGCACTTATTTACCGT - 3′		
arp	f: 5′- GTGCCAGCTCAGAACACTG - 3′	107	99.8%
	r: 5′- ACATCGCTCAAGACTTCAATGG - 3′		

of the habitats in the upstream areas to avoid/reduce interspecific competition. Therefore, according to our genetic analysis, we speculate that Giant-mottled eels might have better swimming bursts and more active upstream migration than Japanese eels to enable them to reach the upstream areas. Additionally, there were some check dams located at the middle and middle-upper reaches of the Fengshan River, which may stop the Japanese eel from reaching the upstream areas and separate the main habitats of these two eel species. However, more studies are required to confirm why the Giant-mottled eel does not appear in the downstream areas; perhaps this is associated with the interspecific competition, carrying capacity of the environment, and salinity.

On the other hand, it is unfortunate that the sample sizes of two sampling sites, FS3 and FS6, were relatively insufficient. There are several reasons that may have occurred. First, the number of cemented revetments and water pollution can affect the number of eels in a river (Dudgeon et al. 2006; Itakura et al. 2015). There were more cemented revetments at the FS3 sampling site than other sampling sites, and the water quality here was also worse owing to the industrial wastewater from nearby chemical and electronic factories, probably leading to low eel resource and affecting the sample size in our investigation. Moreover, in northwestern Taiwan, the Japanese eel accounts for almost 80% of eel resources, while the Giant-mottled eel only accounts for around 20% (Han et al. 2012). Furthermore, the freshwater eels are nocturnal, but our investigation was performed in the daytime, possibly explaining the lower catches. Additionally, only Giant-mottled eels were ever caught in the upstream areas

Table 2. Data from the field investigation, including sampling date, location and number of *Anguilla japonica* and *A. marmorata* specimens

Sampling site	FS1		FS2		FS3		FS4		FS5		FS6	
Date (yyyy/mm/dd)	Aj	Am	Aj	Am	Aj	Am	Aj	Am	Aj	Am	Aj	Am
2014/04/02	4	0	12	0	0	0	*	*	*	*	*	*
2014/07/16	6	0	9	0	1	1	*	*	*	*	*	*
2014/09/23	0	0	3	0	0	0	0	0	0	0	0	0
2014/11/06	23	0	42	0	0	0	0	0	0	0	0	0
2015/06/08	2	0	2	0	1	0	1	0	0	0	0	0
2015/07/22	*	*	*	*	*	*	0	0	0	0	0	1
2015/10/04	7	0	4	0	0	0	*	*	*	*	*	*
2015/10/07	*	*	*	*	*	*	4	1	1	1	0	1
2015/11/11	0	0	0	0	*	*	1	0	*	*	0	0
2015/11/23	3	0	3	0	0	0	1	0	0	0	0	0
2015/12/09	2	0	4	0	*	*	0	1	*	*	*	*
2015/12/21	3	0	1	0	0	0	1	0	*	*	*	*
2016/04/12	2	0	5	0	0	0	1	0	0	0	0	0
2016/04/26	*	*	*	*	0	0	0	0	2	2	0	0
2016/05/18	1	0	0	0	*	*	0	0	0	0	0	0
2016/07/06	0	0	2	0	0	0	2	0	*	*	*	*
2016/07/27	0	0	4	0	*	*	*	*	*	*	*	*
2016/08/18	0	0	1	0	0	0	0	0	0	1	0	1
2016/10/18	*	*	4	0	0	0	6	0	0	1	*	*
2016/11/02	4	0	10	0	*	*	0	0	*	*	*	*
2016/11/08	15	0	0	0	0	0	0	1	0	0	0	0
2016/11/24	0	0	0	0	0	0	1	4	0	0	0	0
2016/12/05	0	0	1	0	0	0	3	3	0	0	0	0
2016/12/26	11	0	2	0	*	*	2	2	0	0	0	0
2017/04/17	12	0	2	0	0	0	2	1	0	0	0	0
2017/05/01	0	0	0	0	0	0	1	0	0	1	0	0
2017/08/24	*	*	*	*	0	0	2	0	0	0	0	1
Total	96	0	111	0	2	1	28	13	3	6	0	4

Aj: *Anguilla japonica* (Japanese eel), Am: *Anguilla marmorata* (Giant-mottled eel). The symbol "*" means no eel sampling (electrofishing) conducted at that time.

of the Fengshan River, according to local fishermen. Consequently, in spite of the low sample size from the FS6 sampling site, our data from this area still suggest that the Giant-mottled eel was mainly distributed over the upper reaches and its resource in the Fengshan River in northwestern Taiwan was relatively low.

According to many previous studies, there are usually at least two dominant eel species coexisting in the rivers in some tropical/subtropical areas, such as Taiwan, Vietnam, East Luzon Island, East Australia, French Polynesia, Peninsular Malaysia, and India (Arai and Abdul Kadir 2017; Han et al. 2016a; Marquet and Galzin 1991; Menon et al. 1998; Nguyen et al. 2018; Pusey 2004; Shiao et al. 2002 2003). Moreover, the differences in habitat distributions of these dominant eel species across a river could also be observed. For example, Shiao et al. (2003) indicated that the Japanese eel and the Giant-mottled eel were dominant and coexisted in the rivers of Taiwan, and the Japanese eel preferred to live in brackish water (estuaries and the lower reaches), but the Giant-mottled eel mainly inhabited the freshwater environment (middle to the upper reaches) based on the otolith microchemical analysis. This result of habitat partitioning of these two eel species is consistent with our field investigation data. Moreover, the Shortfin eel (*A. bicolor pacifica*) and the Philippine Mottled eel (*A. luzonensis*) were dominant and coexisted in the rivers of East Luzon Island, and the Shortfin eel mainly inhabited the lower reaches, while the Philippine Mottled eel resided in the upper reaches (Han et al. 2016a). Studies discussing the habitat distributions and partitions across a river of freshwater eels generally focused on the effects of environmental factors, including water temperature, water current, salinity, depth of water, river size, and carrying capacity (Arai and Abdul Kadir 2017; Briones et al. 2007; Nguyen et al. 2018; Shiao et al. 2003). These studies indicated that freshwater eels residing in the upper reaches may prefer the environments with low temperature, fast water current, pure freshwater, and deep waters, and the habitat partitions of eels may be influenced by river size and carrying capacity. However, almost no study has further investigated whether the genetic, physiological, and morphological differences

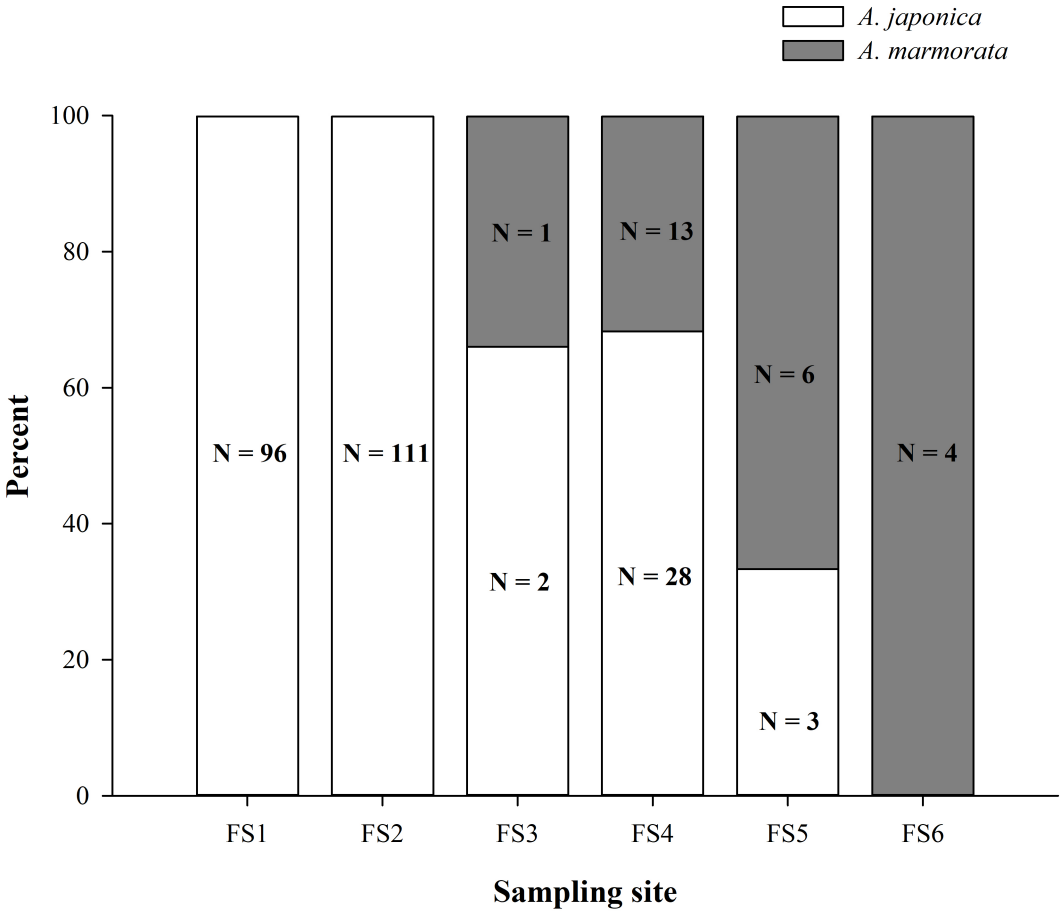

Fig. 2. Species composition and percentage of eels collected from the Fengshan River. The bar charts in white represent *A. japonica*, and those in gray represent *A. marmorata*. The numbers in every bar chart are the total number of captured eels.

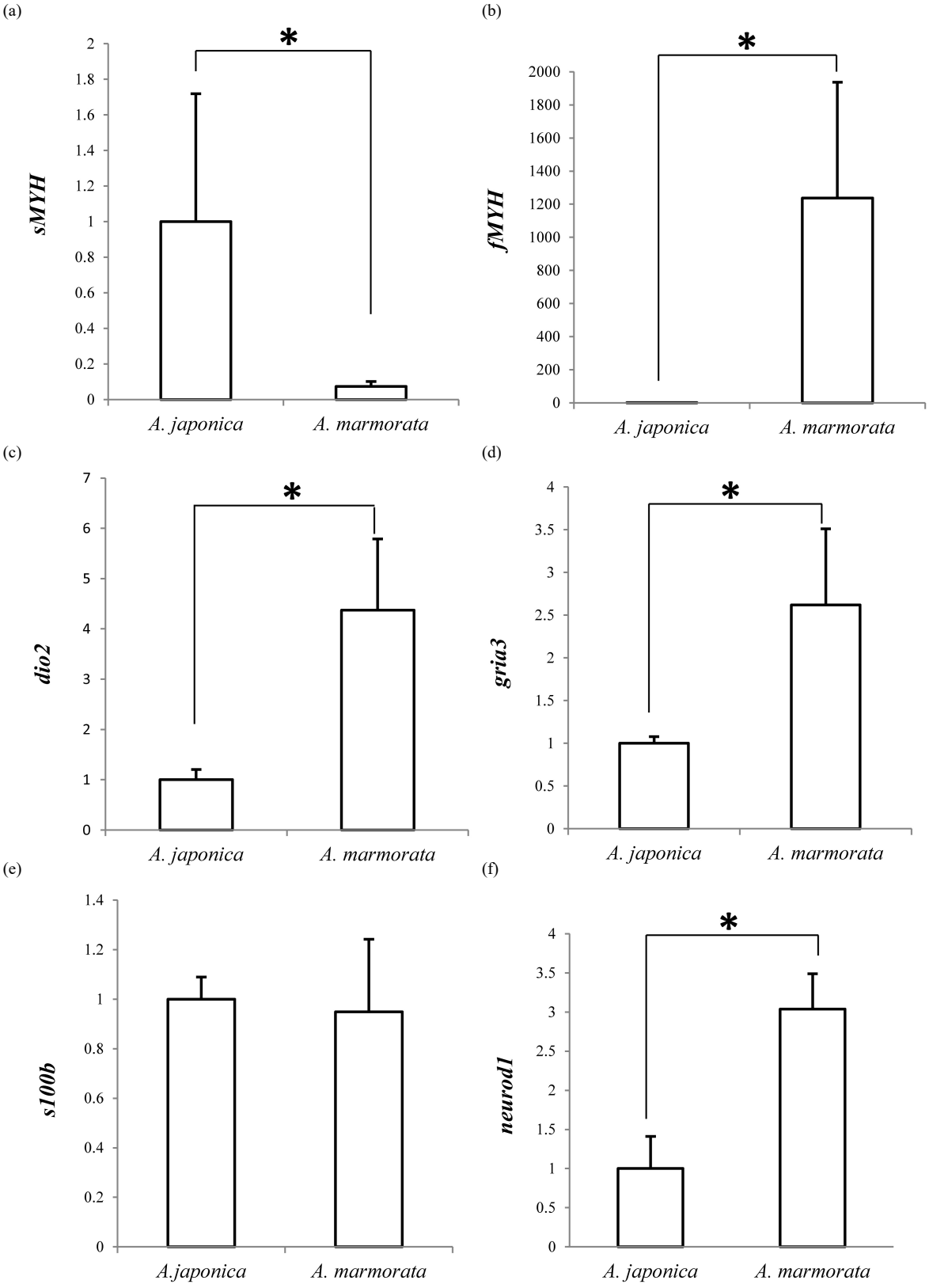

Fig. 3. Relative mRNA expression of *sMYH*, *fMYH*, *dio2*, *gria3*, *s100b* and *neurod1* in the glass eels of *A. japonica* and *A. marmorata*. The mRNA expression levels were presented as mean ± SD. The asterisk (*) shows that the mRNA expression levels between *A. japonica* and *A. marmorata* were significantly different ($p < 0.05$). N = 10 for each eel species.

of freshwater eels drive their habitat partitions, and we believe that more research is needed to concentrate on these issues.

The freshwater eels known to mainly inhabit the upper reaches of the rivers in tropical/subtropical areas are the Giant-mottled eel, Philippine Mottled eel, Speckled longfin eel (*A. reinhardtii*), Polynesian longfinned eel (*A. megastoma*), and Indian-mottled eel (*A. bengalensis bengalensis*); conversely, the freshwater eels primarily residing in the downstream areas are the Japanese eel, Shortfin eel, Southern Shortfin eel (*A. australis australis*), Pacific Shortfin eel (*A. obscura*), and another Shortfin eel (*A. bicolor bicolor*) (Arai and Abdul Kadir 2017; Han et al. 2016a; Marquet and Galzin 1991; Menon et al. 1998; Nguyen et al. 2018; Pusey 2004; Shiao et al. 2002 2003). Interestingly, we discovered that freshwater eels with the same main habitats have a closer phylogenetic relationship, based on previous studies of molecular phylogeny (Aoyama et al. 2001; Minegishi et al. 2005), and they also have similar body coloration. The freshwater eels inhabiting the upper reaches have marbled skin, which may help them blend in with the environmental substrates of the upper reaches, such as cobble, gravel, and fallen leaves, to avoid the detection by the predators or fishermen. On the other hand, the freshwater eels residing in the lower reaches have uniformly-colored skin, probably allowing them to hide in the sandy and muddy environments of the lower reaches. In addition to the morphological difference, freshwater eels that are phylogenetically closely related may generate similar physiological functions like osmoregulation, swimming, and upstream migration to help them find suitable habitats after a long-term adaptation. As a result, most of the freshwater eels inhabiting the upper reaches might have better swimming burst and active upstream migration, and worse osmoregulation, but the freshwater eels residing in the lower reaches might have better osmoregulation, worse swimming bursts, and passive upstream migration.

Another point worth discussing is that there are many rivers with only a single eel species, especially in the temperate and subtropical areas (Greene et al. 2009; Itakura et al. 2015; Jessop et al. 2008; Shiao et al. 2003). This point is usually associated with the biogeographical distributions of freshwater eels and habitat environments of rivers. Different eel species have different biogeographical distributions and prefer different habitat environments. Temperate areas contain only temperate eels, including the Japanese eel, European eel (*Anguilla anguilla*), and American eel (*Anguilla rostrata*) (Tesch 2003). Therefore, almost all the rivers in the temperate areas contain only a single eel species, and the habitat distribution of eels across a river might mainly depend on the river's number of artificial constructions, carrying capacity, and size (Greene et al. 2009). It may be that temperate eels use habitats from the lower to the upper reaches. On the other hand, some small and steep rivers in the subtropical areas only have the Giant-mottled eel because of the habitat environments (Shiao et al. 2003). The habitat environments in the lower reaches of these small rivers are very similar to those in the middle and the upper reaches. Thus, the habitat distribution of Giant-mottled eels in these small rivers might be from the lower to upper reaches owing to the lack of interspecific competition and the similar habitat environments.

In our study, an expressional analysis was performed on six candidate genes, *sMYH*, *fMYH*, *dio2*, *gria3*, *s100b*, and *neurod1*, in the glass eels of the Japanese eel and Giant-mottled eel, preliminarily to assess the possible differences in swimming, expression of active thyroid hormone, and cognitive functions related to their upstream migration. These candidate genes have been studied in many vertebrates. For instance, the gene *gria3* encodes a kind of AMPA receptor (glutamate receptor) in zebrafish that mainly functions at early developmental stages of the CNS (Hoppmann et al. 2008). Moreover, another study also indicated that AMPA receptors are up-regulated in the embryogenesis of pelagic mahi-mahi (*Coryphaena hippurus*) (Xu et al. 2017). Additionally, the glutamate receptors were proposed to have a positive effect on synaptic plasticity in mammalian animals, vital for neural communication, learning, and memorization (Debanne et al. 2003). For freshwater eels, the *gria3* was originally viewed as a candidate gene related to the upstream migration of European eels; but later no significant difference in the expression of *gria3* between climbing-experienced and no climbing-experienced European eels could be found (Podgorniak et al. 2015a). However, the *gria3* was recently found to be differentially expressed in the brains of resident and migratory rainbow trout (*Oncorhynchus mykiss*) juveniles (Hale et al. 2016). In the present study, our results showed that the expression of *gria3* in Giant-mottled eel was higher than that in the Japanese eel. This suggests that the expression of *gria3* affects the migration of these migratory fishes, but the acting mechanism of *gria3* on upstream migration of eels still requires further investigation.

The gene *s100b* encodes a calcium-binding protein, S100, which is involved not only in cell differentiation and cell cycle progression, but also promoting neurogenesis and neuronal plasticity, performing neuro-modulating actions, and enhancing processes involved in memory and learning (Thelin et al. 2017). In addition, several studies indicated that

the regulatory function of S100B is concentration-dependent (Rothermundt et al. 2003; Thelin et al. 2017). Lower concentrations of S100B protein could be beneficial, but higher concentrations could have harmful effects, such as neuronal dysfunction or cell death. A similar situation was also found in the turquoise killifish (*Nothobranchius furzeri*): the S100B protein was over-expressed as neural cells aged in adults (Tozzini et al. 2012). However, the expression of *s100b* had no significant difference either between Giant-mottled eel and Japanese eel or climbing-experienced and no climbing-experienced European eels (Podgorniak et al. 2015a). These results suggest S100B protein may operate in freshwater eels' neuronal plasticity, neuro-modulating actions, memory, and learning, etc., but it probably has no positive or negative effect on their upstream migration.

The gene *neurod1* encodes the NeuroD1 protein, involved in neuron survival, differentiation, and maturation associated with learning and memorization (Munoz et al. 2007), and it is also the indicative marker of cell proliferation and neural differentiation in teleost fishes (Gao et al. 2009). After exposing the Atlantic salmon (*Salmo salar*) juveniles to enriched habitat environments, the mRNA expression of *neurod1* in the forebrain was up-regulated (Salvanes et al. 2013). Moreover, the learning abilities of Atlantic salmon juveniles assessed in a spatial task was improved (Salvanes et al. 2013). These results suggested that environmental enrichment could stimulate the expression of *neurod1* in Atlantic salmon juveniles and further improve their cognition and learning. Here, the mRNA expression in *neurod1* was higher in the Giant-mottled eel than the Japanese eel, implying that Giant-mottled eels might have better spatial learning ability than the Japanese eel, which could enhance its upstream migration.

The gene *dio2* encodes iodothyronine deiodinase 2 (DIO2), responsible for converting the thyroxin (T4) hormone into the biologically active form 3, 5, 3'-triiodothyronine (T3) (Politis et al. 2018; Sorensen et al. 2013). In the European eel, the thyroid hormone is thought to regulate the mechanisms leading to the colonization of continental habitats (Edeline et al. 2004 2005). Moreover, Imbert et al. (2008) demonstrated that the juveniles of European eel categorized as "upstream climbers" have higher T3 expression than inactive juveniles. Furthermore, the differential expression of *dio2* between climbing-experienced and no climbing-experienced European eel juveniles was determined (Podgorniak et al. 2015a). In addition, Aubin-Horth et al. (2009) found that the gene *dio2* is up-regulated more in the brains of early migrating than late migrating Atlanic salmon, providing more evidence that the expression of *dio2* is an influential factor for the migration of migratory fishes. In our study, the expression of *dio2* was higher in Giant-mottled eels than in Japanese eels, suggesting that there may have more active thyroid hormone in the Giant-mottled eel to drive its upstream migration.

Muscle myosin, composed of two heavy chain subunits, two alkali light chain subunits, and two regulatory light chain subunits, is known for muscle contraction (Emerson and Bernstein 1987). In fish, skeletal muscles can be categorized into slow-twitch and fast-twitch muscle. Slow-twitch muscle myofibrils are called red fibrils because they are rich in mitochondria and red myoglobin proteins, while fast-twitch muscle myofibrils, by contrast, are referred to as white fibrils, containing a high level of stored glycogen and fewer mitochondria and myoglobin (Kiessling et al. 2006). Slow-twitch muscles are generally linked to long-duration swimming and low-intensity activities, and the fast-twitch muscles are associated with powerful bursts and rapid movement (Kiessling et al. 2006). In the present study, the Giant-mottled eel showed higher expression of *fMYH* but lower expression of *sMYH* compared to the Japanese eel. This result suggests that the Giant-mottled eel might have stronger swimming bursts to promote their crossing over check dams during the upstream migration.

This study is the first to discuss the possible relationship between habitat distributions of eels and their genetic differences associated with upstream migration, and provides a new perspective on surveying the habitat distributions of all freshwater eels across a river. Now, surveys are needed to determine the compositions and habitat distributions of freshwater eels in the rivers of many tropical and subtropical areas. Realizing the habitat distributions and habitat partitions of freshwater eels is crucial for formulating plans for eel conservation and resource management.

CONCLUSIONS

In conclusion, there is a habitat partition between the Japanese eel and Giant-mottled eel in the Fengshan River, and Japanese eels mainly inhabit the lower and middle reaches, while Giant-mottled eels are distributed over the middle to upper reaches. On top of that, five candidate genes, *sMYH*, *fMYH*, *dio2*, *gria3*, and *neurod1*, are differentially expressed in the glass eels of the Japanese eel and Giant-mottled eel, preliminarily suggesting that there may be innate differences in swimming bursts and upstream migration between them, which probably affect their habitat distributions. Nevertheless, more comprehensive studies on genetic

expression, protein performance, hormone performance, and migratory behavior are needed to further investigate the innate differences in physiological functions and upstream migration between the Japanese eel and Giant-mottled eel.

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REFERENCES

Altringham JD, Ellerby DJ. 1999. Fish swimming: patterns in muscle function. J Exp Biol **202:**3397–3403. PMID: 10562522

Aoyama J, Nishida M, Tsukamoto K. 2001. Molecular phylogeny and evolution of the freshwater eel, genus *Anguilla*. Mol Phylogenet Evol **20:**450–459. doi:10.1006/mpev.2001.0959.

Aoyama J, Wouthuyzen S, Miller MJ, Sugeha HY, Kuroki M, Watanabe S, Syahailatua A, Tantu FY, Hagihara S, Trie T, Otake T, Tsukamoto K. 2018. Reproductive ecology and biodiversity of freshwater eels around Sulawesi Island Indonesia. Zool Stud **57:**30. doi:10.6620/ZS.2018.57-30.

Arai T. 2016. Taxonomy and Distribution. *In*: T. Arai (ed.) Biology and Ecology of Anguillid Eels p1-20. CRC Press.

Arai T, Abdul Kadir SR. 2017. Diversity, distribution and different habitat use among the tropical freshwater eels of genus *Anguilla*. Sci Rep **7:**7593. doi:10.1038/s41598-017-07837-x.

Aubin-Horth N, Letcher BH, Hofmann HA. 2009. Gene-expression signatures of Atlantic salmon's plastic life cycle. Gen Comp Endocr **163:**278–284. doi:10.1016/j.ygcen.2009.04.021.

Briones AA, Yambot AV, Shiao JC, Iizuka Y, Tzeng WN. 2007. Migratory pattern and habitat use of tropical eels *Anguilla* spp. (Teleostei: Anguilliformes: Anguillidae) in the Philippines, as revealed by otolith microchemistry. Raffles B Zool Suppl **14:**141–149. Accession Number: WOS: 000244303400018.

Catsicas M, Allcorn S, Mobbs P. 2001. Early activation of Ca^{2+}-permeable AMPA receptors reduces neurite outgrowth in embryonic chick retinal neurons. J Neurobiol **49:**200–211. doi:10.1002/neu.1075.

Chen JZ, Huang SL, Han YS. 2014. Impact of long-term habitat loss on the Japanese eel *Anguilla japonica*. Estuar Coast Shelf S **151:**361–369. doi:10.1016/j.ecss.2014.06.004.

Chen SC, Chang CR, Han YS. 2018. Seaward migration routes of Indigenous Eels, *Anguilla japonica*, *A. marmorata*, and *A. bicolor pacifica*, via satellite tags. Zool Stud **57:**21. doi:10.6620/ZS.2018.57-21.

Chen YC, Kung SS, Chen BY, Hung CC, Chen CC, Wang TY, Wu YM, Lin WH, Tzeng CS, Chow WY. 2001. Identifications, classification, and evolution of the vertebrate alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor subunit genes. J Mol Evol **53:**690–702. doi:10.1007/s002390010256.

Debanne D, Daoudal G, Sourdet V, Russier M. 2003. Brain plasticity and ion channels. J Physiology-Paris **97:**403–414. doi:10.1016/j.jphysparis.2004.01.004.

Drian M, Bardoul M, Konig N. 2001. Blockade of AMPA/kainate receptors can either decrease or increase the survival of cultured neocortical cells depending on the stage of maturation. Neurochem Int **38:**509–517. doi:10.1016/S0197-0186(00)00103-0.

Dudgeon D, Arthington AH, Gessner MO, Kawabata ZI, Knowler DJ, Leveque C, Naiman RJ, Prieur-Richard A, Soto D, Stiassny MLJ, Sullivan CA. 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. Biol Rev **81:**163–182. doi:10.1017/S1464793105006950.

Edeline E, Bardonnet A, Bolliet V, Dufour S, Pierre E. 2005. Endocrine control of *Anguilla anguilla* glass eel dispersal: effect of thyroid hormones on locomotor activity and rheotactic behavior. Horm Behav **48:**53–63. doi:10.1016/j.yhbeh.2005.02.001.

Edeline E, Dufour S, Briand C, Fatin D, Elie P. 2004. Thyroid status is related to migratory behavior in *Anguilla anguilla* glass eels. Mar Ecol Prog Ser **282:**261–270. doi:10.3354/meps282261.

Emerson CP, Bernstein SI. 1987. Molecular Genetics of Myosin. Annu Rev Biochem **56:**695–726. doi:10.1146/annurev.bi.56.070187.003403.

Fischer M, Kaech S, Wagner U, Brinkhaus H, Matus A. 2000. Glutamate receptors regulate actin-based plasticity in dendritic spines. Nat Neurosci **3:**887–894. doi:10.1038/78791.

Gao ZL, Ure K, Ables JL, Lagace DC, Nave KA, Goebbels, Eisch AJ, Hsieh J. 2009. Neurod1 is essential for the survival and maturation of adult-born neurons. Nat Neurosci **12:**1090–1092. doi:10.1038/nn.2385.

Garenc C, Silversides FG, Guderley H. 1998. Burst swimming and its enzymatic correlates in the threespine stickleback (*Gasterosteus aculeatus*): full-sib heritabilities. Can J Zool **76:**680–688. doi:10.1139/z97-236.

Gecz J, Barnett S, Liu JJ, Hollway G, Donnelly A, Eyre H, Eshkevari HS, Baltazar R, Grunn A, Nagaraja R, Gilliam C, Peltonen L, Sutherland GR, Baron M, Mulley JC. 1999. Characterization of the human glutamate receptor subunit 3 gene (GRIA3),

a candidate for bipolar disorder and nonspecific X-linked mental retardation. Genomics **62:**356–368. doi:10.1006/geno.1999.6032.

Greene KE, Zimmerman JL, Laney RW, Thomas-Blate JC. 2009. Chapter 7, American eel (*Anguilla rostrata*). Atlantic coast diadromous fish habitat: a review of utilization, threats, recommendations for conservation, and research needs. Atlantic States Marine Fisheries Commission Habitat Management Series No. 9, Washington, D.C.

Hale MC, McKinney GJ, Thrower FP, Nichols KM. 2016. RNA-seq reveals differential gene expression in the brains of juvenile resident and migratory smolt rainbow trout (*Oncorhynchus mykiss*). Comp Biochem Phys D **20:**136–150. doi:10.1016/j.cbd.2016.07.006.

Han YS, Lin YF, Wu CR, Iizuka Y, Castillo TR, Yambot IU, Mamalangkap MD, Yambot AV. 2016a. Biogeographic distribution of the eel *Anguilla luzonensis*: dependence upon larval duration and oceanic currents. Mar Ecol Prog Ser **551:**227–238. doi:10.3354/meps11728.

Han YS, Tzeng WN, Huang YS, Liao IC. 2001. Silvering in the eel: changes in morphology, body fat content, and gonadal development. J Taiwan Fish Res **9:**119–127.

Han YS, Wu CR, Iizuka Y. 2016b. Batch-like arrival waves of glass eels of *Anguilla japonica* in offshore waters of Taiwan. Zool Stud **55:**36. doi:10.6620/ZS.2016.55-36.

Han YS, Yambot AV, Zhang H, Hung CL. 2012. Sympatric spawning but allopatric distribution of *Anguilla japonica* and *Anguilla marmorata*: temperature- and oceanic current-dependent sieving. PLoS ONE **7(6):**e37484. doi:10.1371/journal.pone.0037484.

Higuchi T, Watanabe S, Manabe R, Kaku T, Okamura A, Yamada Y, Miller MJ, Tsukamoto K. 2018. Tracking *Anguilla japonica* Silver Eels Along the West Marina Ridge Using Pop-up Archival Transmitting Tags. Zool Stud **57:**24. doi:10.6620/ZS.2018.57-24.

Hoppmann V, Wu JJ, Soviknes AM, Helvik JV, Becker TS. 2008. Expression of the eight AMPA receptor subunit genes in the developing central nervous system and sensory organs of zebrafish. Dev Dynam **237:**788–799. doi:10.1002/dvdy.21447.

Imbert H, Arrowsmith R, Dufour S, Elie P. 2008. Relationships between locomotor behavior, morphometric characters and thyroid hormone levels give evidence of stage-dependent mechanisms in European eel upstream migration. Horm Behav **53:**69–81. doi:10.1016/j.yhbeh.2007.06.011.

Itakura H, Kaino T, Miyake Y, Kitagawa T, Kimura S. 2015. Feeding, condition, and abundance of Japanese eels from natural and revetment habitats in the Tone River, Japan. Environ Biol Fish **98:**1871–1888. doi:10.1007/s10641-015-0404-6.

Jellyman DJ, Bonnett ML, Sykes JR, Johnstone P. 2003. *In*: D. A. Dixon (ed.) Biology, Management, and Protection of Catadromous Eels. p 63-78. American Fisheries Society Symposium 33, Bethesda, Maryland, USA.

Jessop BM, Cairns DK, Thibault I, Tzeng WN. 2008. Life history of American eel *Anguilla rostrata*: new insights from otolith microchemistry. Aquat Biol **1:**205–216. doi:10.3354/ab00018.

Kiessling A, Ruohonen K, Bjørnevik M. 2006. Muscle fibre growth and quality in fish. Archiv Tierzucht Dummerstarf **49:**137–146.

Kohrle J. 2000. The deiodinase family: selenoenzymes regulating thyroid hormone availability and action. Cell Mol Life Sci **57:**1853–1863. doi:10.1007/PL00000667.

Kraemer AM, Saraiva LR, Korsching SI. 2008. Structural and functional diversification in the teleost S100 family of calcium-binding proteins. BMC Evol Biol **8:**48. doi:10.1186/1471-2148-8-48.

Marquet G, Galzin R. 1991. The eels of French Polynesia: taxonomy, distribution and biomass. Lar Mer **29:**8–17.

Martinez M, Guderley H, Dutil JD, Winger PD, He P, Walsh SJ. 2003. Condition, prolonged swimming performance and muscle metabolic capacities of cod *Gadus morhua*. J Exp Biol **206:**503–511. doi:10.1242/jeb.00098.

Menon NG, Balachandran K, Scariah KS. 1998. The eels and eel fisheries of India. Marine Fisheries Information Service, Technical and Extension Series **157:**10–14.

Minegishi Y, Aoyama J, Inoue JG, Miya M, Nishida M, Tsukamoto K. 2005. Molecular phylogeny and evolution of the freshwater eels genus *Anguilla* based on the whole mitochondrial genome sequences. Mol Phylogenet Evol **34:**134–146. doi:10.1016/j.ympev.2004.09.003.

Munoz EM, Bailey MJ, Rath MF, Shi Q, Morin F, Coon SL, Møller M, Klein DC. 2007. *NeuroD1*: developmental expression and regulated genes in the rodent pineal gland. J Neurochem **102:**887–899. doi:10.1111/j.1471-4159.2007.04605.x.

Nguyen AT, Tsukamoto K, Lokman PM. 2018. Composition and distribution of freshwater eels *Anguilla* spp. in Vietnam. Fish Sci **84:**987–994. doi:10.1007/s12562-018-1239-9.

Podgorniak T, Angelini A, Blanchet S, de Oliveira E, Pierron F, Daverat F. 2015a. Climbing experience in glass eels: A cognitive task or a matter of physical capacities? Physiol Behav **151:**448–455. doi:10.1016/j.physbeh.2015.08.001.

Podgorniak T, Milan M, Pujolar JM, Maes GE, Bargelloni L, De Oliveira E, Pierron F, Daverat F. 2015b. Differences in brain gene transcription profiles advocate for an important role of cognitive function in upstream migration and water obstacles crossing in European eel. BMC Genomics **16:**378. doi:10.1186/s12864-015-1589-y.

Politis SN, Servili A, Mazurais D, Zambonino-Infante JL, Miest JJ, Tomkiewicz J, Butts IAE. 2018. Temperature induced variation in gene expression of thyroid hormone receptors and deiodinases of European eel (*Anguilla anguilla*) larvae. Gen Comp Endocr **259:**54–65. doi:10.1016/j.ygcen.2017.11.003.

Pusey B. 2004. Freshwater fishes of north-eastern Australia. CSIRO Publishing. ISBN: 9780643092082.

Rothermundt M, Peters M, Prehn JHM, Arolt V. 2003. S100B in brain damage and neurodegeneration. Microsc Res Techniq **60:**614–632. doi:10.1002/jemt.10303.

Salvanes AGV, Moberg O, Ebbesson LOE, Nilsen TO, Jensen KH, Braithwaite VA. 2013. Environmental enrichment promotes neural plasticity and cognitive ability in fish. Proc R Soc B **280:**20131331. doi:10.1098/rspb.2013.1331.

Shiao JC, Iizuka Y, Chang CW, Tzeng WN. 2003. Disparities in habitat use and migratory behavior between tropical eel *Anguilla marmorata* and temperate eel *A. japonica* in four Taiwanese rivers. Mar Ecol Prog Ser **261:**233–242. doi:10.3354/meps261233.

Shiao JC, Tzeng WN, Collins A, Iizuka Y. 2002. Role of marine larval duration and growth rate of glass eels in determining the distribution of *Anguilla reinhardtii* and *A. australis* on Australian eastern coasts. Mar Freshwater Res **53:**687–695. doi:10.1071/MF01037.

Sørensen C, Johansen IB, Øverli O. 2013. Neural plasticity and stress coping in teleost fishes. Gen Comp Endocrinol **181:**25–34. doi:10.1016/j.ygcen.2012.12.003.

Sudo R, Suetake H, Suzuki Y, Aoyama J, Tsukamoto K. 2013. Profiles of mRNA expression for prolactin, growth hormone, and somatolactin in Japanese eels, *Anguilla japonica*: the effect of salinity, silvering and seasonal change. Comp Biochem Phys A **164:**10–16. doi:10.1016/j.cbpa.2012.09.019.

Tesch FW. 2003. The eel. Blackwell Science, Oxford, U.K. doi:10.1111/j.0022-1112.2004.00509a.x.

Thelin EP, Nelson DW, Bellander BM. 2017. A review of the clinical utility of serum S100B protein levels in the assessment of traumatic brain injury. Acta Neurochir **159:**209–225. doi:10.1007/s00701-016-3046-3.

Tozzini ET, Baumgart M, Battistoni G, Cellerino A. 2012. Adult neurogenesis in the short-lived teleost *Nothobranchius furzeri*: localization of neurogenic niches, molecular characterization and effects of aging. Aging Cell **11:**241–251. doi:10.1111/j.1474-9726.2011.00781.x.

Tsukamoto K, Aoyama J, Miller MJ. 2009. Present status of the Japanese eel: resources and recent research. American Fisheries Society Symposium No. 58. p 21–35, Bethesda, Maryland.

Tzeng WN, Hsiao JJ, Shen HP, Chern YT, Wang YT, Wu JY. 1995. Feeding habit of the Japanese eel *Anguilla japonica* in the streams of northern Taiwan. J Fish Soc Taiwan **22:**279–302.

Tzeng WN, Tabeta O. 1983. First record of the short-finned eel *Anguilla bicolor pacifica* from Taiwan. Bull Japan Soc Sci Fish **49:**27–32.

Watanabe S, Aoyama J, Tsukamoto K. 2009. A new species of freshwater eel *Anguilla luzonensis* (Teleostei: Anguillidae) from Luzon Island of the Philippines. Fish Sci **75:**387–392. doi:10.1007/s12562-009-0087-z.

Xu EG, Mager EM, Grosell M, Stieglitz JD, Hazard ES, Hardiman G, Schlenk D. 2017. Developmental transcriptomic analyses for mechanistic insights into critical pathways involved in embryogenesis of pelagic mahi-mahi (*Coryphaena hippurus*). PLoS ONE **12(7):**e0180454. doi:10.1371/journal.pone.0180454.

Supplementary Materials

Table S1. Detailed coordinates of the sampling sites in Feng-Shan River. (download)

Table S2. Detailed information of the transcripts of candidate genes of *Anguilla japonica*. (download)

Table S3. Detailed information of the transcripts of candidate genes of *Anguilla marmorata*. (download)