



Composition and distribution of freshwater eels *Anguilla* spp. in Vietnam

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Abstract

A sampling survey to determine the composition and distribution of freshwater eel species was conducted in 12 provinces along the coast of Vietnam from October 2014 to February 2015. The sampled provinces spanned the country from central Vietnam to the south, but freshwater eels were only found in the central part of Vietnam. Species identification of 77 specimens, collected from different habitats (rivers and estuaries), was performed by analysis of both morphological characters and nucleotide sequences of mitochondrial cytochrome oxidase subunit I (COI). The 77 specimens were morphologically and genetically identified as 58 *Anguilla marmorata*, 17 *A. bicolor pacifica*, and two *A. japonica*. This is the first definitive survey about the composition and distribution of freshwater eels in Vietnam where distribution has been hitherto unknown.

Keywords *Anguilla* · Distribution · DNA · Freshwater eels · Vietnam

Introduction

Nineteen freshwater eel species/subspecies of the genus *Anguilla* (family Anguillidae) have been recorded globally, with the notable exceptions of the South Atlantic and the west coasts of North and South America (Ege 1939; Castle and Williamson 1974; Watanabe 2003). Freshwater eels live in a wide range of ecological types, spanning tropical, subtropical, and temperate regions around the world (Tesch 2003). Based on their distribution and ecology, eels are divided coarsely between two groups: temperate and tropical eels (Aoyama 2009; Tseng 2012). Although freshwater eels are distributed in more than 150 different countries (IUCN 2014), their populations have faced dramatic declines in recent decades. Temperate species with high commercial

value such as *A. anguilla*, *A. rostrata*, and *A. japonica* are especially at high risk (Haro et al. 2000; Casselman 2003, Dekker 2003; Tatsukawa 2003; Tsukamoto et al. 2009). According to the IUCN (2014), these species are all threatened. Particularly, *A. anguilla* is now listed as critically endangered and *A. rostrata* and *A. japonica* are considered to be endangered species with decreasing population trends. It is further notable that the population trends of most tropical species are still unknown (IUCN 2014), but their situation appears to be no better than that of their temperate congeners. For example, the status of *A. borneensis* was assessed as vulnerable, whereas two other tropical species (*A. bicolor* and *A. bengalensis*) have been re-categorized as “near threatened” from “least concern” (IUCN 2014).

It is likely that the threats to tropical eels and the number of tropical eel species threatened will continuously increase in the near future due to the increasing demand of tropical species to replace temperate eels in aquaculture, an industry that is exclusively reliant on wild-caught juvenile (seed) eels (Jacoby et al. 2015). Indeed, as populations of *A. japonica* and *A. anguilla* decline, and as their exploitation is increasingly restricted, many tropical eel species (*A. mossambica* from Africa, *A. marmorata* from Southeast Asia, *A. bicolor* from the Philippines, and *A. obscura* and *A. megastoma* from the Central and South Pacific) are now being exploited to

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stock eel farms in East Asian countries (Crook and Nakamura 2013; Crook 2014; Jacoby et al. 2015).

Apart from over-exploitation, a large variety of other factors may contribute to the dramatic decline in anguillid abundance. These factors include habitat destruction in the form of dams and weirs (Jansen et al. 2007), changes in oceanic conditions by climate change (Hanel et al. 2014; Pacariz et al. 2014), increasing risk of diseases and parasites (Fazio et al. 2012) and predation (Wahlberg et al. 2014), and exposure to pollutants (Robinet and Feunteun 2002). However, it is difficult to definitively determine the reasons for the anguillid abundance declines because of their long and complicated life cycle and because the distribution of freshwater eels is not limited by country boundaries. This poses huge challenges for conservation; apart from the necessity to elucidate the reasons for population decline, conservation of freshwater eels requires accurate information about their distribution and composition in a specific area. However, a number of regions still lack such data; Vietnam is a typical example of a tropical country for which information about the geographical distribution and abundance of freshwater eels is severely lacking.

Although research into this void was conducted by Hoa and Phung (2003) and Dat and Ty (2008), their studies do not withstand scrutiny because of a lack of peer review, the unknown number of samples collected, and the exclusive use of morphological characters for species identification according to Ege (1939). The taxonomy of the genus *Anguilla* proposed by Ege (1939) has long been accepted, but the differences in morphology between species have been considered insufficient to precisely identify all species. This was demonstrated by Watanabe et al. (2004), who re-examined the *Anguilla* taxonomy by Ege (1939). Due to the limitations of these studies, Vietnam is still a “black box” in the map of worldwide distribution of freshwater eels in comparison with neighboring countries such as the Philippines, Malaysia, and Indonesia.

The price of eel exports in Vietnam has increased rapidly in the last 3 years. According to the Vietnamese Directorate of Fisheries in 2015, the average export price of eels in the first few months of 2015 was around 500,000 VND/kg, which is equivalent to 25 USD/kg. This price is higher than that of 2014 by 100,000–150,000 VND/kg (5–7 USD/kg) (Fistenet 2015). Additionally, as of February 2015, an eel processing factory was being constructed in Bac Lieu province, Mekong Delta, Vietnam, in the form of a co-investment between Vietnam and Korea, and it is expected to process about 7 tonnes of eel/day (Baclieu 2015). Eel aquaculture can thus be a promising industry to bring great economic benefit to Vietnamese farmers. However, if there is no appropriate management policy and conservation, then there is a high possibility for native freshwater eels in Vietnam to face the threat of extinction due to overfishing and invasion of

introduced eel species from aquaculture. Notably, the management and conservation policies need to be developed on the basis of data on geographic distribution and composition of eel species, but such data in Vietnam are sparse, patchy, and anecdotal. Moreover, the conservation and management of anguillid eels requires broad cooperation between countries (Jacoby et al. 2015). The establishment of input data related to distribution and species composition in Vietnam is therefore urgent, not only for eel conservation in Vietnam but also for efforts at a regional and/or global scale.

Employing morphological characters and molecular genetic characters for species identification, the aim of this research is therefore to provide the first reliable information about distribution and composition of freshwater eels in Vietnam for eel conservation, fishery management and aquaculture.

Materials and methods

Sampling location

The sampling was conducted in 12 provinces (from the middle part to the south of Vietnam) during 5 months from October 2014 to February 2015 (Fig. 1; Table 1). These provinces were chosen because of either the presence of eel farms or the existence of wild eels in these locations according to anecdotal sources. Sampling sites with minimum human disturbance at each province were determined based on the instruction of local, experienced fishermen. Each sampling expedition lasted about 3 days with about seven fishing hours/day from 16:00 to 3:00 because this time period coincides with feeding activities of eels. The wild eels, ranging in total length from 44 mm to 1.4 m, were caught by electric shocker, trawl net and fish trap fences, depending on habitat. Electric shocker was used to catch eels in streams, irrigation dams and reservoirs, whereas trawl net and fish trap fences were utilized for rivers and lagoons, respectively.

Sampling protocol

Eels were anesthetized with Aqui-S (10 ml/m³ of water) before measuring morphological characters and collecting tissues for DNA analysis. The morphological characters recorded included color marking of skin (plain or mottled patterns), total length (TL) (measured as the distance from the tip of the lower jaw, mouth closed, to the tip of the caudal fin) and anal-dorsal distance in percentage of TL (AD%) [distance between the verticals through the anus and origin of dorsal fin; see Watanabe et al. (2004)]. These three characters were considered to be the most valuable traits in separating freshwater eel species into different groups by

Fig. 1 Sampling localities of eels in Vietnam

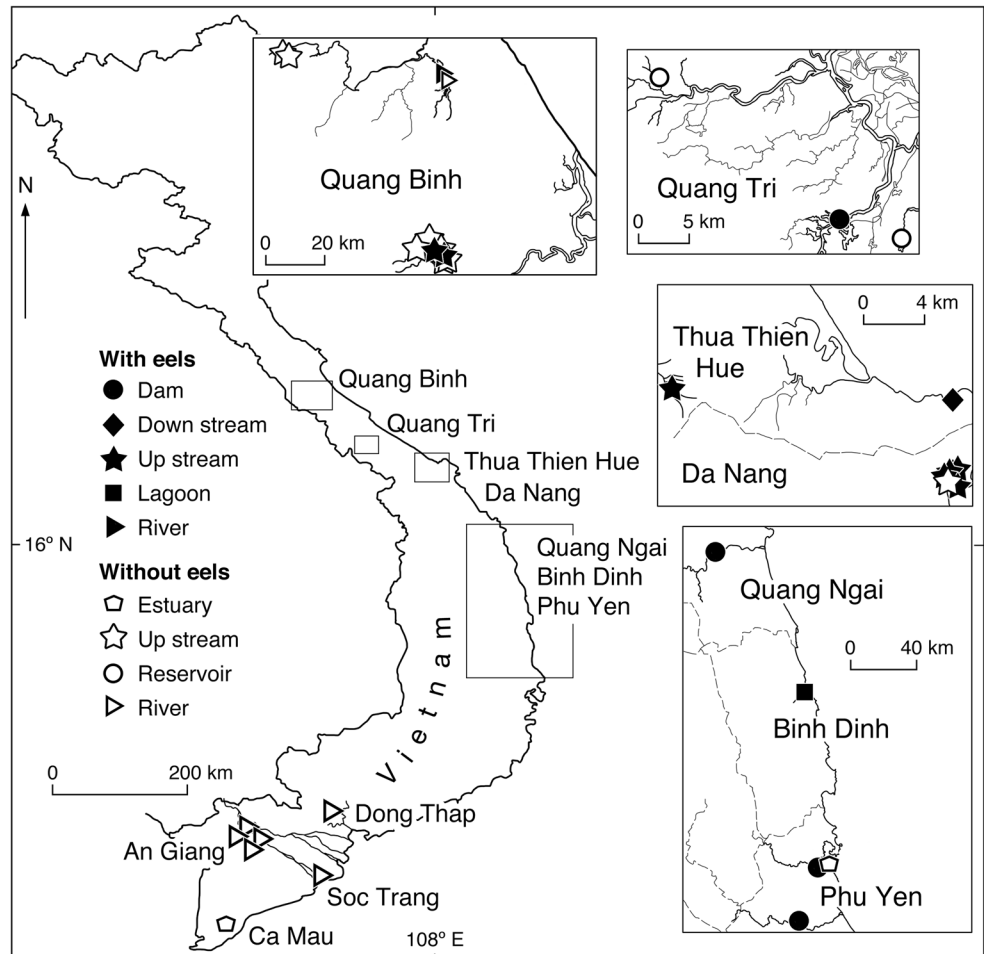


Table 1 Three taxonomic groups of freshwater eels can be identified on the basis of Ege (1939) and Watanabe et al. (2004)

Group	Color marking	Origin of dorsal fin	Candidate species
First group	Variegated	Longfin	<i>A. bengalensis bengalensis</i> , <i>A. bengalensis labiata</i> , <i>A. celebesensis</i> , <i>A. interioris</i> , <i>A. luzonensis</i> , <i>A. marmorata</i> , <i>A. megastoma</i> , <i>A. reinhardtii</i>
Second group	Non-variegated	Shortfin	<i>A. australis australis</i> , <i>A. australis schmidti</i> , <i>A. bicolor bicolor</i> , <i>A. bicolor pacifica</i> , <i>A. obscura</i>
Third group	Non-variegated	Longfin	<i>A. anguilla</i> , <i>A. borneensis</i> , <i>A. dieffenbachii</i> , <i>A. japonica</i> , <i>A. mossambica</i> , <i>A. rostrata</i>

both Ege (1939) and Watanabe et al. (2004) (Table 1). After morphological measurement, small pieces (5 mm²) of pectoral fin tissue of all specimens were taken and preserved in micro-centrifuge tubes containing ethanol (70%) at 4 °C; eels were then handed over to the eel fishermen or returned to the wild. Tissues were shipped to the University of Otago, New Zealand for DNA analysis.

DNA extraction and sequencing

Total DNA was isolated using an adaptation of the method used by Walsh et al. (1991). Briefly, 5 mm² of tissue was

placed in 1.5-ml tubes containing 400 µl of 5% Chelex and 2 µl of proteinase K (20 mg/ml). These tubes were incubated overnight at 50–65 °C in a hot block and then heated to 90 °C for 10 min before centrifuging at 14,000 rpm for 7 min. The supernatant was collected for polymerase chain reaction (PCR) application. A fragment of about 650 bp of the mitochondrial cytochrome oxidase subunit I gene (cox1) was amplified via PCR with a pair of universal primers (Ward et al. 2005): FishF1 (5'TCAACCAACCACAAAGAC ATTGGCAC3') and FishR1 (5'TAGACTTCTGGGTGG CCAAAGAATCA3'). The PCR amplification was carried out using an Eppendorf EpGradient S PCR machine under

the following conditions: initial denaturation at 95 °C for 2 min, followed by 35 alternating cycles of 35 s at 95 °C for denaturation, annealing at 50 °C for 30 s and extension at 72 °C for 1 min. A total of 5 µl of PCR product was run out on a 1% agarose gel and visualized using 2.5 µl of SYBR® Safe DNA Gel Stain (Invitrogen — Life Technologies) and viewed under UV light (Unitech Gel Doc). Subsequently, the correct-sized bands (650 bp) were cut out and purified through a MEGAquick-spin™ Total Fragment DNA Purification Kit (iNtRon Biotechnology) following the manufacturer's protocol (Gel DNA extraction). The purified products were sent to Genetic Analysis Services, University of Otago, New Zealand, for sequencing using an ABI 3730xl DNA Analyzer. To verify the identity of annotated sequences, the BLAST program (<http://blast.ncbi.nlm.nih.gov>) was used.

Results

Among the 12 provinces investigated, the presence of wild freshwater eels was recorded in eight provinces located in the central part of Vietnam (Table 2). Based on morphological examination, the collected eels, ranging from 44 to 1400 mm in total length, were divided into three groups except for 11 specimens of glass eels which were identified only by analysis of COI sequences due to the difficulty of morphological examination at this stage. Almost all collected eels were in the yellow stage except for one silver eel sampled at Phu Yen (Table 2). Particularly, 47 specimens with variegated skin, long dorsal fins and AD/TL% ranging from 14.5 to 18% (Fig. 2a) were assigned into the first group. Seventeen specimens with short dorsal fins, without variegated skins and AD/TL% of 0.6–1.5% (Fig. 2b) were assigned to the second group, whereas two specimens with long dorsal fins, without variegated markings and with AD/TL% of 9 and 10.2% (Fig. 2c) were determined to belong to the third group. Using the geographical distribution range of species and subspecies of the genus *Anguilla* as described by Ege (1939), the eels assigned into the first, second and third groups were identified as *A. marmorata*, *A. bicolor pacifica*, and *A. japonica*, respectively. This deduction was reinforced by the results of COI sequencing with the value of the highest identity ranging from 96 to 99% (Table 2). Regarding 11 samples of glass eel, the result of COI sequencing indicated that these samples were *A. marmorata* with a high identity value (99%).

Discussion

This study aimed to provide the first reliable data about the number of freshwater eel species and their distribution in Vietnam. These data are expected to contribute to

development of eel conservation and fishery management policy in Vietnam, important tools in the context of the urgent need for international co-operation to prevent further significant global declines in the abundance of anguillid eels.

During a survey that included numerous expeditions in highly challenging terrain and stretched across 5 months, three freshwater eel species were found in the central part of Vietnam, i.e., *A. marmorata*, *A. bicolor*, and *A. japonica*. No eel species were collected from the southern region during this study. This finding is consistent with geographical distribution range, and the known spawning areas and larval migrating loop of the genus *Anguilla* as recorded in Ege (1939), Arai et al. (2001), Han et al. (2012), Kuroki et al. (2014), and Watanabe et al. (2014). Interestingly, this finding was notably different to previous publications about the composition of freshwater eels in Vietnam. Thus, a survey by Hoa and Phung (2003) found *A. marmorata*, *A. bicolor pacifica*, and *A. nebulosa* in the central part of Vietnam, whereas Dat and Ty (2008) found *A. marmorata*, *A. bicolor bicolor*, and *A. malgumora* in the same region. The finding of *A. nebulosa*, *A. bicolor bicolor*, and *A. malgumora* in Vietnam seems to be very unlikely. According to Ege (1939) and Watanabe et al. (2014), two subspecies of *A. nebulosa* (*A. bengalensis*) and *A. bicolor bicolor* are distributed in the Indian Ocean, whereas *A. malgumora* (*A. borneensis*) is thought to spawn in the Celebes Sea in the vicinity of its freshwater growth habitat (Aoyama et al. 2003). The inconsistency in results of these studies may be due to utilizing only morphological characters for species identification. In particular, Dat and Ty (2008) identified samples based on color marking and distance between the verticals through the anus and relative position of the dorsal fin in relation to total length. This may not be a useful taxonomic key, as these characters overlap in most species (Watanabe et al. 2004). Meanwhile, Hoa and Phung (2003) did not mention any taxonomic characters used in identification of their eel specimens.

The samples in the first group of this study were identified as *A. marmorata* which has the widest distribution among all species in the genus *Anguilla* (Tesch 2003). These results were reinforced by analysis of COI sequences, which upon BLAST searching (Basic Local Alignment Search Tool) displayed the highest sequence identity (97–99%; Table 2) with this species. In addition, 100% of the glass eel samples were identified as *A. marmorata* based on the data of COI sequences, strongly supporting this conclusion. According to Ishikawa et al. (2004) and Minegishi et al. (2008), *A. marmorata* are distributed throughout the Indo-Pacific, ranging from the eastern side of Africa to French Polynesia and presenting a multiple population structure. *A. marmorata* collected in this study likely belong to the North Pacific population and are assumed to have an overlapping spawning area with *A. japonica* (Kuroki et al. 2009). The finding

Table 2 Taxonomic identification of anguillid eels collected in Vietnam from fresh and brackish water sources

Locality and sampling period	N	Morphological characters				Ranges of TL (mm)	Ranges of AD/TL (%)	Developmental stages	Habitat	Group ^a (based on morphological characters)	Species based on DNA sequencing	Highest identity (%)
		Color marking of skin	Origin of dorsal fin	Origin of dorsal fin	Origin of dorsal fin							
Quang Binh (12/2014)	4	Variegated	Longfin	Longfin	335–400	16.1–17.1	Yellow	Upstream	1	<i>A. marmorata</i>	99	
	2	Non-variegated	Shortfin	Shortfin	425–731	1.3–1.4	Yellow	River	2	<i>A. bicolor pacifica</i>	99	
Quang Tri (2/2015)	6	Variegated	Longfin	Longfin	305–390	15.6–18.0	Yellow	Dam	1	<i>A. marmorata</i>	99	
	1	Non-variegated	Shortfin	Shortfin	350	1.1	Yellow	Dam	2	<i>A. bicolor pacifica</i>	98	
Hue (10/2014)	4	Variegated	Longfin	Longfin	210–550	14.8–16.6	Yellow	Upstream and down-stream	1	<i>A. marmorata</i>	99	
	4	Non-variegated	Shortfin	Shortfin	330–580	1.2–1.3	Yellow	Downstream	2	<i>A. bicolor pacifica</i>	99	
Da Nang (10/2014)	11	Variegated	Longfin	Longfin	130–330	15.6–17.1	Yellow	Upstream	1	<i>A. marmorata</i>	99	
Quang Ngai (1/2015)	2	Variegated	Longfin	Longfin	145–225	15.4–15.6	Yellow	Dam	1	<i>A. marmorata</i>	97	
	1	Variegated	Longfin	Longfin	182	14.6	Yellow	Dam	1	<i>A. marmorata</i>	96	
Binh Dinh (1/2015)	5	Variegated	Longfin	Longfin	125–275	15.6–17.3	Yellow	Dam	1	<i>A. marmorata</i>	98	
	3	Variegated	Longfin	Longfin	175–209	14.5–16.6	Yellow	Dam	1	<i>A. marmorata</i>	99	
Phu Yen (1/2015)	7	Non-variegated	Shortfin	Shortfin	315–530	0.9–1.5	Yellow	Lagoon	2	<i>A. bicolor pacifica</i>	99	
	6	Variegated	Longfin	Longfin	395–560	15.3–17.8	Yellow	Lagoon	1	<i>A. marmorata</i>	99	
Nha Trang (1/2015)	2	Non-variegated	Longfin	Longfin	548–550	9–10.2	Yellow	Lagoon	3	<i>A. japonica</i>	99	
	11	Non-pigmentation	Shortfin	Shortfin	44–51		Glass eel	Dam		<i>A. marmorata</i>	99	
Phu Yen (1/2015)	2	Non-variegated	Shortfin	Shortfin	440–530	0.9–1.2	Yellow	River	2	<i>A. bicolor pacifica</i>	99	
	1	Variegated	Longfin	Longfin	1400	17.1	Silver	Dam	1	<i>A. marmorata</i>	99	
Hue (10/2014)	2	Variegated	Longfin	Longfin	145–162	14.7–17.1	Yellow	Upstream	1	<i>A. marmorata</i>	99	
	1	Non-variegated	Shortfin	Shortfin	160	0.6	Yellow	River	2	<i>A. bicolor pacifica</i>	99	
Hue (10/2014)	2	Variegated	Longfin	Longfin	147–193	14.7–17.9	Yellow	Upstream	1	<i>A. marmorata</i>	98	

TL total length, AD anal–dorsal distance

^a1, 2, 3 indicate the first group, the second group and the third group, respectively



Fig. 2 **a** Longfin eel with variegated skin (TL=45 cm); **b** Shortfin eel without variegated skin (TL=33 cm); **c** Longfin eel without variegated skin (TL=55 cm). Scale bar: 5 cm

of *A. japonica* in the present study supports this assumption. Moreover, the geographical distribution of other candidate species in this group is more difficult to reconcile with a presence in Vietnam, and hence, their absence among samples in this study is not surprising. For example, *A. bengalensis* with two subspecies, *A. bengalensis bengalensis* and *A. bengalensis labiata*, are distributed in the east and the west of the Indian Ocean, respectively (Ege 1939; Watanabe et al. 2014). *A. reinhardtii* has a limited distribution in the northwest margin of the southwest Pacific and along the east coast of Australia (Ege 1939; Aoyama 2009). The spawning location of *A. celebesensis* was suggested to be in the Celebes Sea and Tomini Bay, and their larvae are recruited into the freshwater habitat after a short local migration (less than 100 km) (Aoyama et al. 2003). *A. interioris* and *A. megastoma*, two remaining species in this group, are distributed in Papua New Guinea and the South Pacific Ocean, respectively (Ege 1939; Aoyama et al. 1999; Kuroki et al. 2014). Collectively, the samples in this study with variegated skin and longfin were conclusively determined as *A. marmorata*.

Using analogous methods, the samples characterized by short dorsal fins and non-variegated skin were identified as *A. bicolor*. We excluded *A. obscura* and *A. australis* in the

second group because these species are distributed in the South Pacific (Ege 1939). On the basis of recent analyses of both mitochondrial and microsatellite DNA, *A. bicolor* has diverged between Indian and Pacific oceans (Minegishi et al. 2012). The species distributed in the Indian Ocean is known as *A. bicolor bicolor* (Watanabe et al. 2014) and its spawning location is estimated to lie west of Sumatra and east of Madagascar (Jespersen 1942; Robinet et al. 2003; Aoyama et al. 2007; Kuroki et al. 2007). On the back of the above information, specimens characterized by short dorsal fins and without variegated markings were identified as *A. bicolor pacifica* which has a relatively wide distribution range, including the coasts of China, Vietnam, Philippines, Borneo Island, Sulawesi Island, and New Guinea (Tesch 2003). Data from COI sequencing also indicated that these samples were *A. bicolor pacifica* with 99% DNA sequence identity. The finding of *A. marmorata* and *A. bicolor pacifica* in the present study is consistent with a study by Arai et al. (2013) who examined the migration and habitat use of tropical eels in Phu Yen — one of the sampling locations of this study. During the sampling period, it was recognized that *A. marmorata* was predominantly distributed in upstream locations, whereas *A. bicolor pacifica* was found mainly

downstream and in lagoon regions. Interspecific competition was considered to be a key factor in habitat preference between these species (Arai et al. 2013).

Considering the geographical range of the species in the third group, *A. anguilla*, *A. rostrata*, *A. mossambica*, and *A. dieffenbachii* were ruled out as possibilities because they are distributed in the North Atlantic, western Indian Ocean and South Pacific, respectively (Ege 1939). Although *A. borneensis* is found in the North Pacific, this is unlikely to be a match for this sample. This species was suggested to spawn in the Celebes Sea and the larvae migrate back to the freshwater growth habitat which is adjacent to the spawning area (Aoyama et al. 2003). Moreover, the COI sequence data obtained from samples in this study were 99% identical to *A. japonica*. Based on these pieces of evidence, the two specimens assigned to the third group were identified as *A. japonica*.

The finding of two samples of *A. japonica* in the present study is a surprising result although the presence of this species was anecdotally recorded. Based on interviews with fishermen and observations during field expeditions, it is unlikely that *A. japonica* is an introduced exotic species in Vietnam because no aquaculture or commercial fisheries are known for Japanese eels in the sampling areas. Therefore, this study provides the first compelling evidence for the distribution of the Japanese eel in Vietnam.

According to Han et al. (2012), the interaction between water temperature preferences of *A. japonica* and *A. marmorata* glass eels and available oceanic currents are important in explaining the allopatric distribution of these two species. Regardless of this information, the recorded flow of oceanic currents in the South China Sea conducted by Hu et al. (2000) and Xue et al. (2004) shows a high possibility for the recruitment of Japanese eels along the coast of central Vietnam through two main currents; the South Chinese Sea Warm Current (SCSWC) and the Kuroshio intrusion. These currents are thought to originate from the main body of flows from the Kuroshio. The SCSWC is formed from a branch of the Kuroshio in the south and the Kuroshio intrusion flows into the South China Sea through the Luzon Strait (Xue et al. 2004). Both of these currents can reach the coastline of central Vietnam in winter (Hu et al. 2000). It is notable that the North Equatorial Current (NEC) and Kuroshio are the main currents transporting larvae of *A. japonica*, *A. marmorata*, and *A. bicolor pacifica* towards the coastal waters of East Asia from spawning areas (Tesch 2003; Aoyama 2009; Kuroki et al. 2009). Furthermore, the spawning season of *A. japonica* ranges between April and August (Shinoda et al. 2011) and this time period coincides with the activation of the SCSWC and Kuroshio intrusions in the South China Sea during winter (Hu et al. 2000). Therefore, it is likely that the larvae of these species made their way to the central part of Vietnam through either the SCSWC or Kuroshio intrusion.

This is also a likely explanation for the predominant distribution of eels in the central part of Vietnam.

Although Japanese eels were found in Vietnam, opposing temperature preferences for recruitment between *A. japonica* and *A. marmorata* cause a distinct biogeography for these species (Han et al. 2012). This may be one of the main reasons for the low encounter rates of *A. japonica* in Vietnam during these field expeditions. It is also possible that changes in currents associated with global warming play a role. According to Han et al. (2012), *A. japonica* transported to an unsuitable habitat, such as Vietnam, are vagrant and may not actively migrate upstream from estuaries. Interestingly, the fishermen interviewed during the field trip confirmed that *A. japonica* tends to be found only in brackish areas and coincidentally the two *A. japonica* specimens in the present study were collected in lagoons.

This study provides the first evidence on the distribution of eel species in Vietnam. Following intensive fishing expeditions, three species of eel were found, identified as *A. japonica*, *A. marmorata*, and *A. bicolor pacifica*. Further research related to seasonal larval migration of these species should be conducted in order to guide eel conservation and seed eel management for aquaculture in Vietnam.

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