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Authors: Watanabe, Shun, Miller, Michael J., Honryo, Tomoki, and Sasal, Pierre

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Indo-Pacific Eels (*Anguilla marmorata*) From the Caroline Islands Belong to the Micronesia Population Based on Total Number of Vertebrae Counts[1]

Shun Watanabe,[2,3,7] Michael J. Miller,[4] Tomoki Honryo,[5] and Pierre Sasal[6]

Abstract: The Indo-Pacific eel, *Anguilla marmorata* Quoy & Gaimard, has the widest geographic distribution of anguillid eels. At least four populations (North Pacific, Micronesia, Indian Ocean and South Pacific) of this species were estimated to exist by previous morphological and molecular genetic studies. Recent mitochondrial control region haplotype analysis of *A. marmorata* eels from the eastern Caroline Islands and Guam grouped them in two separated lineages with eels from the South Pacific and western Indian Ocean. To examine the composition and possible distribution of the Micronesia population of *A. marmorata* that may mostly be found in the Mariana (Guam) and Caroline (Palau, Yap, Chuuk, Pohnpei, Kosrae) islands in relation to other populations in the Indo-Pacific region, we statistically compared the total number of vertebrae (TV) of specimens collected from throughout most of the range of *A. marmorata* including 32 specimens from Kosrae and Pohnpei islands. The TV for 1298 specimens of *A. marmorata* in combined data from 15 localities ranged from 100 to 110. The mean TV (107.95, 108.03) and range (105–110) of eels from the Mariana and Caroline islands were clearly higher than other localities. The post hoc multiple pairwise comparisons of the 105 pairs of single localities showed that there were 57 significant TV differences between each locality. The most significant differences were between Mariana and Caroline eels and all the other 13 localities. The statistical differences of pooled regional TV data, various genetic studies, and general geography indicate that there are likely six spawning populations of *A. marmorata*. This study indicates that the Caroline Islands are included in the Micronesia population, but further research is needed to understand the spawning area and life history of the Micronesia population of *A. marmorata* that seems to live on a relatively small number of islands in Oceania.

Keywords: Kosrae, Pohnpei, *Anguilla marmorata*, mtDNA lineages, evolutionary history, spawning area

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[2] Department of Fisheries, Faculty of Agriculture, Kindai University, 3327-204 Nakamachi, Nara 631-8505, Japan.

[3] Agricultural Technology and Innovation Research Institute, Kindai University, 3327-204 Nakamachi, Nara 631-8505, Japan.

[4] Department of Aquatic Bioscience, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo, Tokyo 113-8657, Japan (retired).

[5] Uragami Station, Aquaculture Research Institute, Kindai University, 468-3, Uragami, Nachi-katsuura, Higashimuro, Wakayama 649-5145, Japan.

[6] Centre de Recherches Insulaires et Observatoire de l'Environnement (CRIOBE), BP1013, Papetoai, 98729 Moorea, French Polynesia.

[7] Corresponding author (email: swpc@nara.kindai.ac.jp).

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Freshwater eels of the genus *Anguilla* Schrank have now been recognized as comprising 16 species (Ege 1939, Castle and Williamson 1974, Watanabe et al. 2009*a*), three of which are further divided into two subspecies (Ege 1939). To resolve the inconsistencies in the common names used for some anguillid species, Tsukamoto et al. (2020) proposed a standardized list of the common names of species and subspecies in anguillid eels. The Indo-Pacific eel, *Anguilla marmorata* Quoy & Gaimard (often referred to previously as the giant mottled eel), is unique among all anguillids because it has the widest geographic distribution of anguillid eels and is found longitudinally from the east coast of Africa to the Marquesas Islands in the southeast Pacific Ocean and as far north as southern Japan (Ege 1939, Figure 1), and so it was given a new common name of the "Indo-Pacific eel" in that paper. Recently this species was found at Palmyra Atoll in the central Pacific (Handler and James 2006) and even farther to the east in the Galapagos Islands (McCosker et al. 2003, Escobar-Camacho et al. 2023), which may indicate that it has an even wider geographic range than previously thought.

Anguillid eels have the unique life history of catadromy, in which most juvenile feeding and growth occur in freshwater or estuaries before reaching maturity when the adult eels migrate to oceanic waters to reproduce. Most anguillid species are distributed in the Indo-Pacific where some make long spawning migrations like the two Atlantic eels, but other species were found to make short migrations to spawn locally, such as in the Indonesian Seas (Aoyama 2009). Larval sampling surveys were able to find the spawning areas of some species in the Pacific and Indian oceans (Kuroki et al. 2009, 2020, Tsukamoto et al. 2011, Miller and Tsukamoto 2017), but many spawning locations have not been clarified (Miller 2023). There seems to be relatively limited variation in the life histories of anguillid eels other than differences in the sizes of species ranges, adult migration distances and larval durations (Aoyama 2009, Kuroki et al. 2014, Righton et al. 2021). However, *A. marmorata* uniquely developed the ability to expand its species range to overlap with the ranges of most of the other Indo-Pacific anguillid species, possibly because it is a habitat generalist when interacting with other sympatric anguillid species (Miller et al. 2023). The presence of *A. marmorata* in the Indian Ocean and both the North and South Pacific oceans, a species range that overlaps with all species other than those in Australia and New Zealand (Watanabe 2003, 2023), makes it impossible to have only one spawning population.

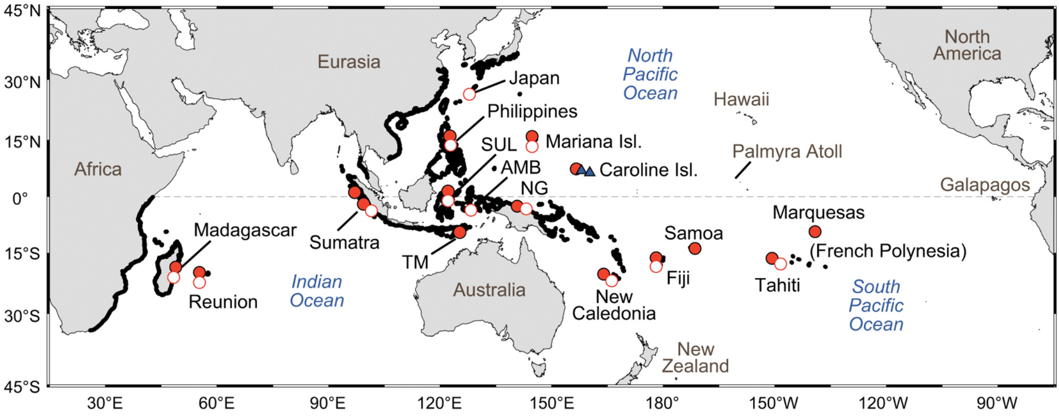

Figure 1. Map showing the range of *A. marmorata* (thick lines on coastlines), with the collection localities of specimens examined by Ege (1939) and Watanabe et al. (2009*b*) shown with solid and open circles, respectively, and the collection localities of Donovan et al. (2012) with triangles. SUL: Sulawesi; AMB: Ambon; NG: New Guinea; TM: Timor.

Because of this wide geographic range, various studies have evaluated the possible population structure of *A. marmorata*. Ege (1939) evaluated the regional morphological variation and suggested that there were three primary races of this species. Ishikawa et al. (2004) recognized five geographically separated populations, which were the North Pacific, Fiji, Tahiti, Madagascar, and Sumatra populations, using 626 base pair segments of the mitochondrial DNA (mtDNA) control region (mtCR) from 162 specimens of *A. marmorata* collected from 10 localities across their distribution range, but specimens from Guam were not included. Genetic differentiation among populations in the Southern Hemisphere was supported by examination of nuclear DNA with amplified fragment length polymorphism (AFLP) analysis performed on 76 individuals from Madagascar, Sumatra, Fiji, and Tahiti (Ishikawa et al. 2004). Furthermore, Minegishi et al. (2008) examined the population structure of *A. marmorata* by analyzing the two mtDNA genes of mtCR and cytochrome b and a few nuclear microsatellites of eels from 13 localities. These results generally indicated that *A. marmorata* has at least four genetically different populations (North Pacific, South Pacific, Indian Ocean, Guam region). The North Pacific population may be fully panmictic (Minegishi et al. 2008), but parts of genomic regions across multiple chromosomes exhibited significant genetic differentiation between the northernmost (Yakushima Island, Southern Japan) and southernmost (Sulawesi, Indonesia) individuals (Hirase et al. 2022), whereas the South Pacific and Indian Ocean populations have metapopulation structures, which both might include more than one spawning population. Interestingly, Guam was suggested to be inhabited by a reproductively isolated population (Minegishi et al. 2008, Watanabe et al. 2008, 2009*b*) without any information about their spawning area or adult or larval migrations. Further analysis of *A. marmorata* in the Indian Ocean that also included nuclear DNA, found that there were two genetically distinct populations in the Indian Ocean, with one in the southwestern Indian Ocean and another on the eastern side along Sumatra Island in Indonesia (Gagnaire et al. 2009). Analysis of *A. marmorata* population structure using AFLP only found three divergent populations that were the North Pacific, the South Pacific, and the southwestern Indian Ocean populations (Gagnaire et al. 2011). By analyzing the genetic diversity of mtCR of *A. marmorata* from several drainages of Vietnam in comparison to existing sequences from other regions, most of the eels in Vietnam were found to belong to the North Pacific population of that species (Nguyen et al. 2022).

However, some aspects of these genetic studies on *A. marmorata* should be interpreted with caution. Differences in types of DNA or genetic analysis techniques have been found to cause mtDNA and AFLP analyses to produce different results (Lucchini 2003, Bensch et al. 2006). There are also problems associated with the maternal inheritance aspect of mtDNA (Rubinoff and Holland 2005) that may cause significant limitations for use in population structure studies on species such as anguillid eels that have long larval durations that probably results in some occasional mixing of larvae and mtDNA lineages among populations over evolutionary time, which could mask some recent population separations. Furthermore, genetic studies usually used mixed individuals of different ages and that could be another problem. Genetic exchange between populations and age differences between individuals may make it impossible to distinguish between populations.

A different method that has been used to examine the population structure of anguillid species such as *A. marmorata* is statistical analysis of total number of vertebrae (TV). *Anguilla marmorata* has the largest range of TV in the genus *Anguilla*, which extends from 100 to 110, with some apparent regional variation (Ege 1939, Watanabe et al. 2008, 2009*b*). Analyses of the TV among eels in all the different regions of the species range indicate that there are at least four populations (North Pacific, Micronesia, Indian Ocean and South Pacific) of *A. marmorata* (Watanabe et al. 2009*b*) as has been indicated previously by molecular genetic research (Minegishi et al.

2008). Analyses of TV have also found statistical differences between *A. australis* subspecies (Watanabe et al. 2006) and *A. megastoma* in the eastern and western sides of the South Pacific (Watanabe et al. 2011).

When considering regional differences in meristic characters, it should be noted that there is a tendency for TV to increase with latitude, a relationship known as Jordan's rule (Jordan 1892, e.g., McDowall 2008), which is often regarded as an association between TV and water temperature (Fowler 1970, Hubbs 1922). Furthermore, various other environmental factors such as salinity also influence TV (Tåning 1952, Lindsey 1988). However, in the case of eels of the genus *Anguilla*, the spawning areas that are known for the temperate species *A. anguilla*, *A. rostrata*, and *A. japonica* (Schmidt 1925, McCleave et al. 1987, Tsukamoto et al. 2011) and the tropical species *A. bicolor*, *A. borneensis*, *A. celebesensis*, and *A. marmorata* (Jespersen 1942, Miller et al. 2002, Aoyama et al. 2003) are all in tropical to subtropical regions with similar water temperatures. In addition, both *A. anguilla* and *A. rostrata* spawn in the same area (McCleave et al. 1987, Miller et al. 2015), but their TV are different with very little overlap (*A. anguilla*: range 110–119, mean 114.7; *A. rostrata*: range 103–111, mean 107.2; Ege 1939, European material: range 104–120, mean 114.6; American material: range 103–112, mean 107.2; Boëtius 1980). Therefore, because of the similar temperatures that occur at the spawning areas of most anguillids, it seems likely that variations of TV in anguillid eels are derived from genetic rather than environmental factors and that differences in TV may be useful for detecting the existence of different populations of *A. marmorata* (Watanabe et al. 2008, 2009*b*). Possible reasons for genetically-based changes in TV ranges within and among species might be related to body elongation for enhanced swimming performance or more random factors such as genetic drift (Tibblin et al. 2016), but this has only begun to be evaluated for anguillid eels (Forsman and Berggren 2016).

One of the most interesting findings of both the TV and the most detailed genetic study of Minegishi et al. (2008) that included both mitochondrial and nuclear DNA, is that the eels collected in Guam of the Mariana Islands formed a separate population, which has been referred to as the Micronesia Population. Micronesia consists of four major archipelagos as follows: Mariana Islands, the Caroline Islands, Gilbert Islands, and Marshall Islands (Figure 2*A*). Myers and Donaldson (2003) reported this species from the Mariana Islands and all of the larger or "high islands" of the Caroline Islands, which include from west to east: Palau, Yap, Chuuk (Truk), Pohnpei, and Kosrae (Figure 2).

To test if the *A. marmorata* eels occurring in the eastern Caroline Islands are part of the same Micronesia population as those in Guam, Donovan et al. (2012) obtained 39 specimens of *A. marmorata* from Kosrae and Pohnpei (Figures 1 and 2) and sequenced their entire mtCR. They also compared the Micronesia eels to 267 previously sequenced haplotypes from across the Indo-Pacific (Minegishi et al. 2008). Their analysis found two distinctive lineages for the eastern Caroline Islands and Guam that were mostly grouped with two clusters of South Pacific Ocean eels and the western Indian Ocean and South Pacific Ocean eels (Figure 3; Donovan et al. 2012). Although that clustering pattern is likely related to the limitations of mtDNA for population studies mentioned above, their findings supported the likelihood of the existence of a Micronesia spawning population.

To further examine if the eels of Guam and the eastern Caroline Islands are actually both part of the Micronesia population *A. marmorata* in the Indo-Pacific, we statistically analyzed the TV of specimens collected from throughout most of the range of *A. marmorata* including 32 specimens collected on Kosrae and Pohnpei islands, which are some of the specimens used by Donovan et al. (2012) and also some data of Ege (1939). Furthermore, another objective of the present study was to determine how TV variation may be related to the findings of population genetic studies.

MATERIALS AND METHODS

The TV data of the specimens of *A. marmorata* used in this study are mainly from

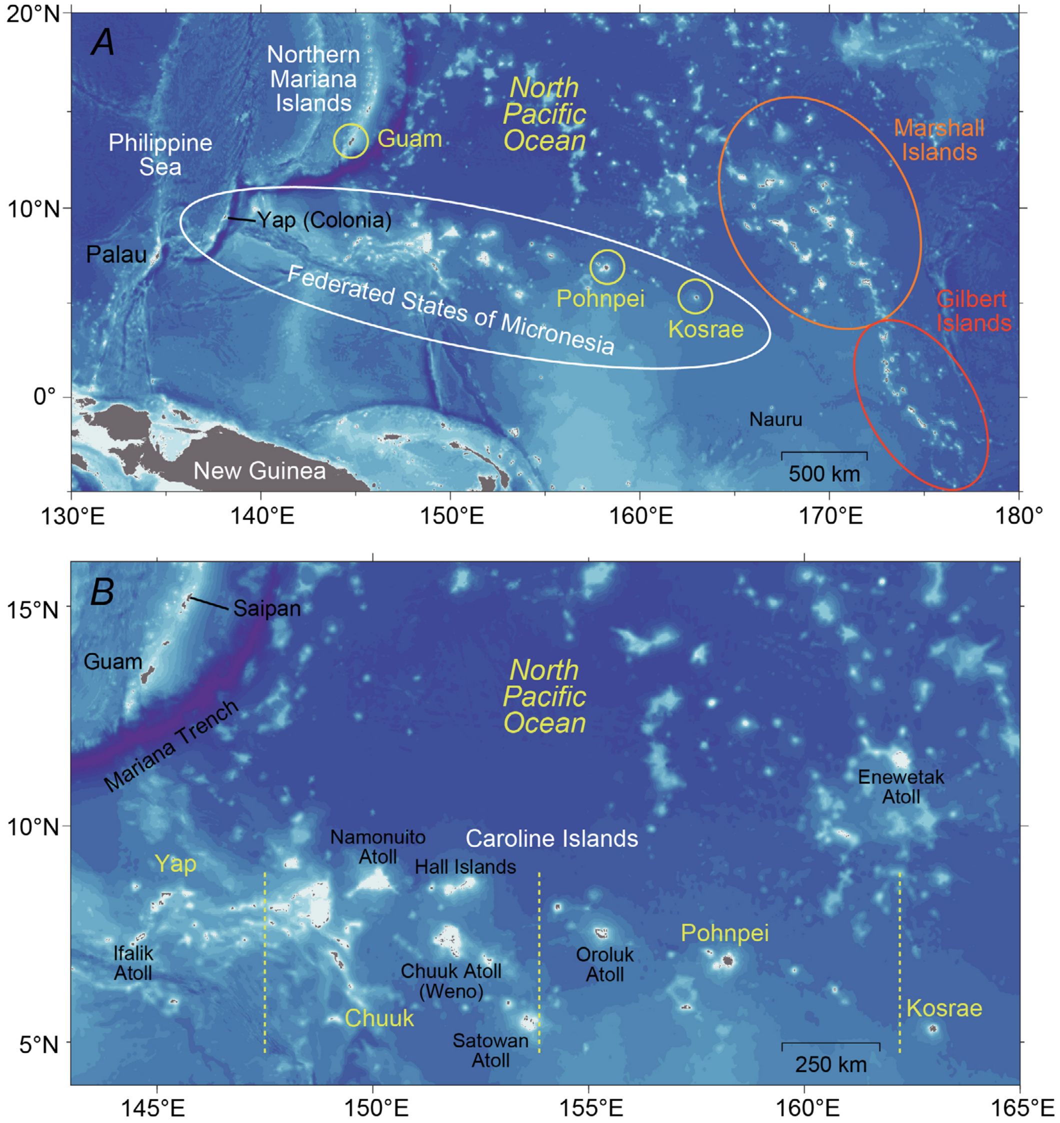

FIGURE 2. Bathymetric maps showing Micronesia and the surrounding region including Palau, Northern Mariana Islands (including Guam), Federated States of Micronesia, Marshall Islands, and Gilbert Islands, with small circles showing locations where *A. marmorata* Micronesia population specimens of this study were from at Guam, Pohnpei, and Kosrae (*A*), and the Caroline Islands region separated (by vertical dashed lines) into island groups (Yap, Chuuk, Pohnpei, Kosrae) (*B*), with some atolls labeled, but only the islands of Chuuk Atoll are large enough to have any surface freshwater.

the studies of Ege (1939) and Watanabe et al. (2009*b*), and newly obtained TV data from the eels used in the study of Donovan et al. (2012). The present study used 19 of 39 specimens of *A. marmorata* used in Donovan et al. (2012) and an additional five specimens of this species, all of which were obtained from collections made in May–June 2007 and June–July 2008 at Pohnpei and Kosrae (Figures 1 and 2) by those authors using

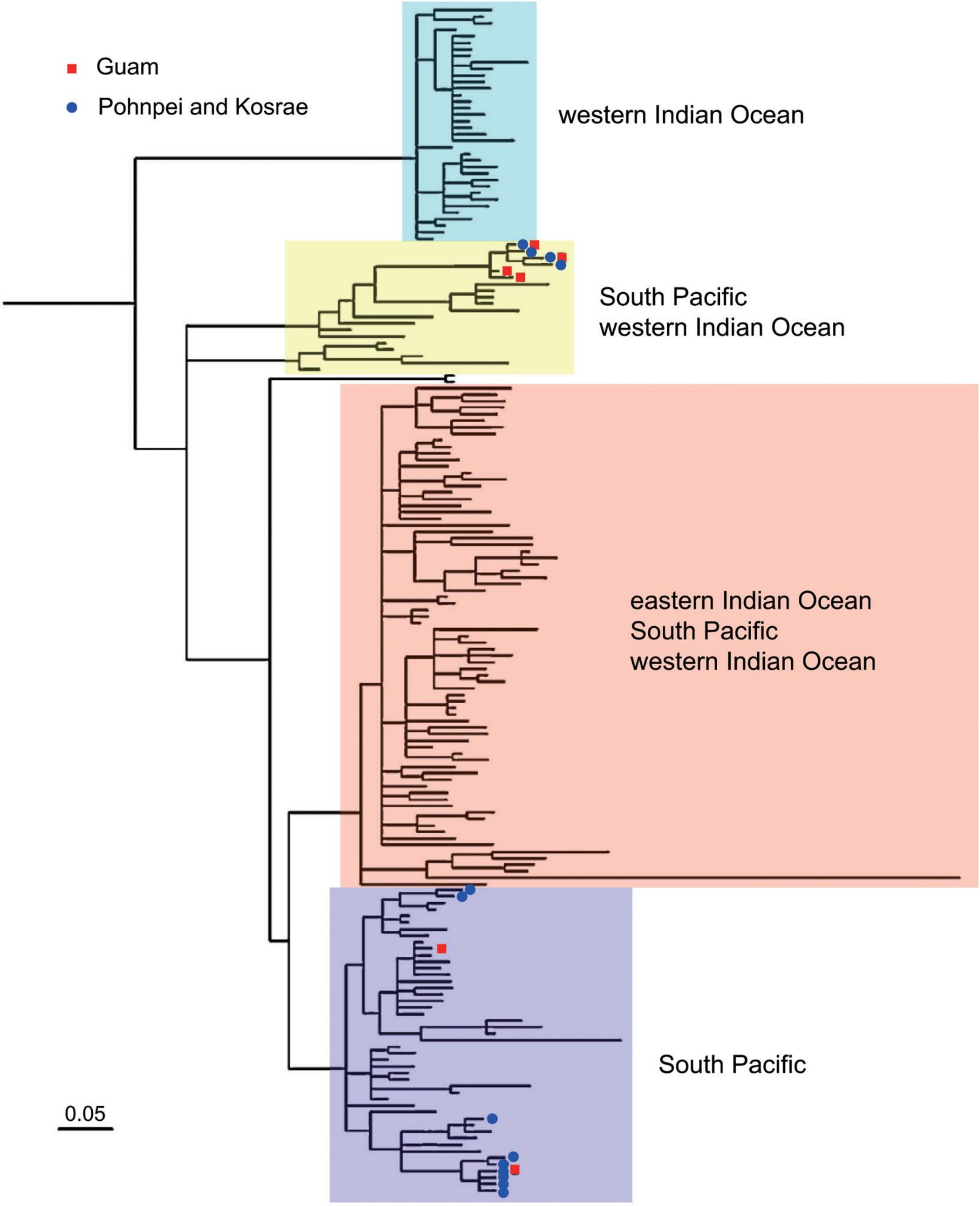

FIGURE 3. Part of a Bayesian tree (excluding the Northern Population of *A. marmorata*) based on 277 mtCR haplotypes of *A. marmorata* from throughout the Indo-Pacific that shows that the eels from Guam (Mariana Islands) and Pohnpei and Kosrae (Caroline Islands) were grouped in separated lineages, which was modified from Donovan et al. (2012).

TABLE 1

Comparison of Frequency Distributions of Total Number of Vertebrae (TV) of *Anguilla marmorata* from Pohnpei and Kosrae Island and Ege' Data That Were Used in This Study

Locality or data set	TV 105	106	107	108	109	110	Total	Mean
Pohnpei Island	1		3	3	3	1	11	107.91
Kosrae Island		2	1	8	2		13	107.77
Ege's data				4	3	1	8	108.63
Caroline Islands	1	2	4	15	8	2	32	108.03

TABLE 2

Frequency Distributions for Total Number of Vertebrae (TV) in *A. marmorata* from 21 Locations in Data of (*A*) Ege (1939) and (*B*) Watanabe et al. (2009*b*): Madagascar (MAD), Réunion Island (REU), Nias Island (NIA), Sumatra (SUM), Timor (TIM), Japan (JPN), Philippines (PHI), Luzon Island (LUZ), Sulawesi (Celebes) (SUL), Ambon Island (AMB), Mariana Islands (MAR), Guam Island (GUA), Caroline Islands (CAR), New Guinea (NGU), Papua New Guinea (PMG), New Caledonia (NCA), Fiji (FIJ), Samoa (SAM), Tahiti (TAH) Marquesas (MQU), and French Polynesia (FPN)

Locality	TV 100	101	102	103	104	105	106	107	108	109	110	Total	Mean
(*A*)													
MAD[b]						1	6	1				8	106.00
REU				1	14	42	52	20	3			132	105.64
NIA				1	5	7	3					16	104.75
SUM[b]					1		3	1				5	105.80
TIM[a]				2	15	12	6		1			36	104.72
PHI[b]				2	2	5	3					12	104.75
SUL[a]	1	1	1	17	45	48	23	5				141	104.59
MAR[b]									3	2		5	108.40
CAR[b]									4	3	1	8	108.63
NGU[a]				2	12	33	44	20	7		1	119	105.79
NCA[a]				1	2	21	28	16	2	1		71	105.93
FIJ[a]				1	1	7	6	5				20	105.65
SAM[a]					7	15	18	7		1		48	105.60
TAH[a]					3	19	60	66	15	1		164	106.45
MQU								4	3		1	8	106.88
Total	1	1	1	27	107	210	252	145	38	8	3	793	105.7
(*B*)													
MAD					3	4	3					10	105.00
REU			1	1	4	20	23	10	1			60	105.62
SUM					6	12	8	4	1			31	105.45
JPN				8	7	12	4					31	104.39
LUZ				17	35	25	8	3				88	104.38
SUL			3	5	10	16	3					37	104.30
AMB			1	3	11	12	12	6				45	105.09
GUA						2	2	8	16	8	3	39	107.90
PNG					2	11	9	1	1			24	105.50
NCA						1	2	3		1		7	106.71
FIJ				2	7	22	17	12	2	1		63	105.63
FPN					2	6	24	10	4			46	106.17
Total			5	36	87	143	115	57	25	10	3	481	105.4

[a] Including the museum specimens.
[b] Only museum specimens.

handlines, spears, Hawaiian slings, and a Smith-Root Model LR-24 electrofisher. There were 24 specimens from Pohnpei ($n = 11$) and Kosrae ($n = 13$) that were radiographed by an X-ray apparatus (MA-60, Hitex Co.) and a Digital X-ray imaging sensor (Naomi-NX04H, RF Co.) to count their TV (Table 1). These TV data are referred to as "Donovan's TV data."

The TV data in Ege (1939) were obtained from Madagascar, Réunion, Nias (off west Sumatra), Sumatra, Timor, the Philippines, Celebes (Sulawesi Island), the Mariana Islands, Caroline Islands, New Guinea, New Caledonia, Fiji, Samoa, Tahiti, and the Marquesas Islands (Figure 1). A total of 793 specimens including TV data from examinations of museum specimens were used for "Ege's TV data" (Table 2*A*). The TV data in Watanabe et al. (2009*b*) were obtained from Madagascar, Réunion, Sumatra, Japan, Luzon Island of the Philippines, Sulawesi Island (Celebes), Ambon Island, Guam, Papua New Guinea, New Caledonia, Fiji, and Raiatea, Tahiti, and Moorea islands in French Polynesia (Figure 1). A total of 481 specimens including TV data were used for "Watanabe's TV data" (Table 2*B*).

The nonparametric Kruskal–Wallis test was applied to the TV data among Pohnpei ($n = 11$) and Kosrae ($n = 13$) specimens whose vertebral counts were examined in this study along with the Caroline Islands ($n = 8$) from Ege's data using Prism (version 8.4.3) for macOS (GraphPad Software, LLC), which showed no significant differences ($P = 0.175$). Therefore, these data were combined as TV data from the Caroline Islands ($n = 32$, Tables 1 and 3).

Donovan's, Ege's and Watanabe's TV data were also combined to have 15 localities in the Indo-Pacific, which resulted in a total of 1298 specimens as shown in Table 3 that were analyzed statistically. The TV data from Nias Island ($n = 16$) in Ege's TV data were included with the TV data from Sumatra in both Ege's

TABLE 3

Comparison of Frequency Distributions of the Combined Data Set of Total Number of Vertebrae (TV) of *A. marmorata* from the 15 Localities Analyzed in This Study: Madagascar (MAD), Réunion Island (REU), Sumatra (SUM), Timor (TIM), Japan (JPN), Philippines (PHI), Sulawesi (Celebes) (SUL), Ambon Island (AMB), Mariana Islands (MAR), Caroline Islands (CAR), New Guinea (NGU), New Caledonia (NCA), Fiji (FIJ), Samoa (SAM), and French Polynesia (FPN)

	TV														
Locality	100	101	102	103	104	105	106	107	108	109	110	Total	Mean	SD	*P*
MAD					3	5	9	1				18	105.44	0.86	0.0068
REU			1	2	18	62	75	30	4			192	105.64	0.99	<0.0001
SUM				1	12	19	14	5	1			52	105.27	1.10	0.0004
TIM				2	15	12	6		1			36	104.72	1.00	0.0004
JPN				8	7	12	4					31	104.39	1.02	0.0009
PHI				19	37	30	11	3				100	104.42	1.02	<0.0001
SUL	1	1	4	22	55	64	26	5				178	104.53	1.13	<0.0001
AMB			1	3	11	12	12	6				45	105.09	1.24	0.0084
MAR						2	2	8	19	10	3	44	107.95	1.14	0.0014
CAR						1	2	4	15	8	2	32	108.03	1.09	0.0039
NGU				2	14	44	53	21	8		1	143	105.74	1.12	<0.0001
NCA				1	2	22	30	19	2	2		78	106.00	1.04	<0.0001
FIJ				3	8	29	23	17	2	1		83	105.64	1.16	0.0002
SAM					7	15	18	7		1		48	105.60	1.05	0.0004
FPN					5	25	84	80	22	1	1	218	106.41	0.93	<0.0001
Total	1	1	6	63	194	354	369	206	74	23	7	1298	105.60	1.38	

Note: SD and *P* show standard deviation and statistically significant values for a normal distribution by Shapiro–Wilk test.

and Watanabe's TV data, because this island is very near Sumatra. The TV data from Tahiti and Marquesas in Ege's TV data and Watanabe's TV data were all combined into the TV data from French Polynesia.

All 15 localities in the combined TV data sets did not have normal distributions (Shapiro–Wilk test, $P < 0.05$, Table 3) using JMP (version 14.2) for macOS (SAS Institute Inc.). The TV data among each location were tested using the nonparametric Kruskal–Wallis test followed by pairwise comparisons with Dunn's test using Prism (version 8.4.3) for macOS (GraphPad Software, LLC).

Based on the difference in TV among 15 localities and the results of molecular genetic studies (Ishikawa et al. 2004, Minegishi et al. 2008, Gagnaire et al. 2009), the validity of the estimated six regional populations (West Indian Ocean, East Indian Ocean, North Pacific, Micronesia, West South Pacific, East South Pacific) was examined using normal distributions (Shapiro–Wilk test) and the nonparametric Kruskal–Wallis test followed by pairwise comparisons with Dunn's test.

RESULTS

The TV for 1298 specimens of *A. marmorata* in the combined data from 15 localities ranged from 100 to 110 and overlapped at least at 105 and 106 vertebrae in all locations (Table 3). The mean TV at each location ranged from 104.39 to 108.03 (Table 3). While the mean TV in Timor, Japan, Philippines, and Sulawesi were lower (104.39–104.72) than other localities, the mean TV in the Mariana and Caroline islands were higher (107.95, 108.03) than other localities (Table 3). The statistical analysis of the TV data found a significant difference among the 15 localities (Kruskal–Wallis test, $P < 0.0001$). The post hoc multiple pairwise comparisons of the 105 pairs of localities showed that there were 57 significant differences (Dunn's test, $P < 0.0001$–0.05) in the TV data between each locality (Table 4). The most significant differences

TABLE 4

Result of Each Pairwise Comparison (P 0.05, Dunn's Test) of the TV of *A. marmorata* at 15 Localities: Madagascar (MAD), Réunion Island (REU), Sumatra (SUM), Timor (TIM), Japan (JPN), Philippines (PHI), Sulawesi (Celebes) (SUL), Ambon Island (AMB), Mariana Islands (MAR), Caroline Islands (CAR), New Guinea (NGU), New Caledonia (NCA), Fiji (FIJ), Samoa (SAM), and French Polynesia (FPN)

		Indian Ocean			North Pacific Ocean						South Pacific Ocean				
Region	Locality	REU	SUM	TIM	JPN	PHI	SUL	AMB	MAR	CAR	NGU	NCA	FIJ	SAM	FPN
Indian Ocean	MAD	NS	NS	NS	NS	NS	NS	NS	****	****	NS	NS	NS	NS	NS
	REU		NS	**	****	****	****	NS	****	****	NS	NS	NS	NS	****
	SUM			NS	NS	*	NS	NS	****	****	NS	NS	NS	NS	****
	TIM				NS	NS	NS	NS	****	****	***	****	*	NS	****
North Pacific Ocean	JPN					NS	NS	NS	****	****	****	****	***	*	****
	PHI						NS	NS	****	****	****	****	****	****	****
	SUL							NS	****	****	****	****	****	***	****
	AMB								****	****	NS	*	NS	NS	****
	MAR									NS	****	****	****	****	***
	CAR										****	****	****	****	**
South Pacific Ocean	NGU											NS	NS	NS	****
	NCA												NS	NS	NS
	FIJ													NS	****
	SAM														**

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, and ****$P < 0.0001$. NS: non-significant.

were between the Mariana and Caroline specimens compared to each of all the other 13 localities, except for between Mariana and Caroline, which resulted in those two localities forming a statistically independent group (Table 4, Figure 4). There were 15 significant differences between Japan, Philippines, and Sulawesi in the North Pacific Ocean region and all localities (New Guinea, New Caledonia, Fiji, Samoa, and French Polynesia) in the South Pacific Ocean region (Table 4). The specimens from French Polynesia had the third highest mean TV (106.41, Table 3) and were significantly different from all 12 other localities except for between Madagascar and New Caledonia (Table 4). The significant differences other than the above were between Réunion and each of Timor, Japan, Philippines, and Sulawesi, between Sumatra and Philippines, between Timor and New Guinea, New Caledonia, and Fiji, and between Ambon and New Caledonia (Table 4).

There were no significant differences between Madagascar and the other 12 localities except for between Madagascar and the Mariana and Caroline islands or between Ambon and 10 other localities except for between Ambon and Mariana, Caroline, New Caledonia, and French Polynesia (Table 4, Figure 4). There were no significant differences among Madagascar, Réunion, Sumatra, and Timor in the Indian Ocean region, except for between Réunion and Timor. There were also no significant differences among Japan, Philippines, Sulawesi, and Ambon in the North Pacific Ocean region or among New Guinea, New Caledonia, Fiji, and Samoa in the South Pacific Ocean region (Table 4, Figure 4). The four localities in the Indian Ocean region

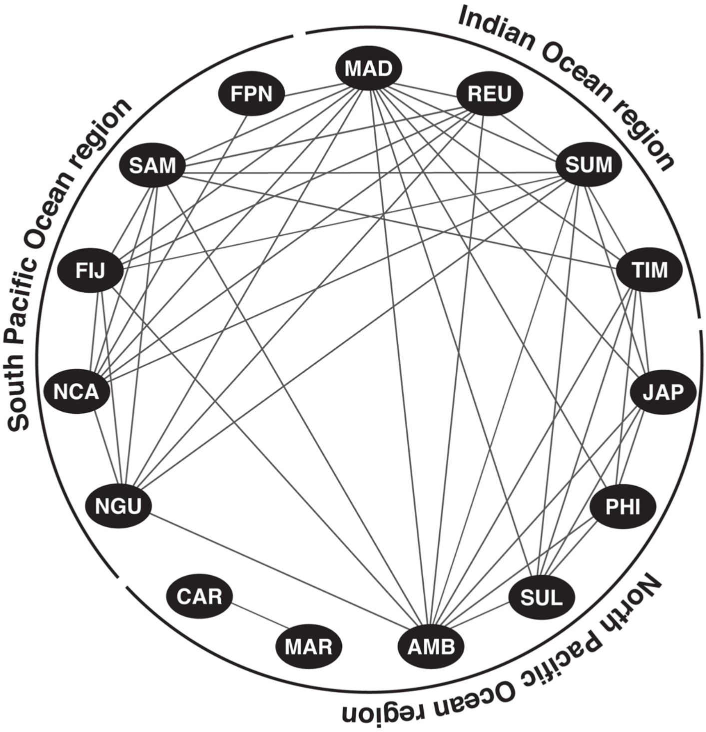

FIGURE 4. Diagrammatic summary of the results of the statistical comparisons of the total number of vertebrae (TV) data of *A. marmorata* between each locality (see Table 4). Lines connecting two localities indicate no statistically significant differences between those localities ($P \geq 0.05$). Localities that are not connected by lines were statistically different ($P < 0.05$). Abbreviations of locations are from Table 4.

(Madagascar, Réunion, Sumatra, and Timor) showed almost no significant differences from the four localities in the North Pacific Ocean region (Japan, Philippines, Sulawesi, and Ambon), except for between Réunion and three localities (Japan, Philippines, and Sulawesi) and between Sumatra and Philippines, or among the four localities in the South Pacific Ocean region (New Guinea, New Caledonia, Fiji, and Samoa), except for between Timor and three localities (New Guinea, New Caledonia, and Fiji) (Table 4, Figure 4).

However, comparisons among many different sites among likely population regions may mask the overall TV patterns statistically,

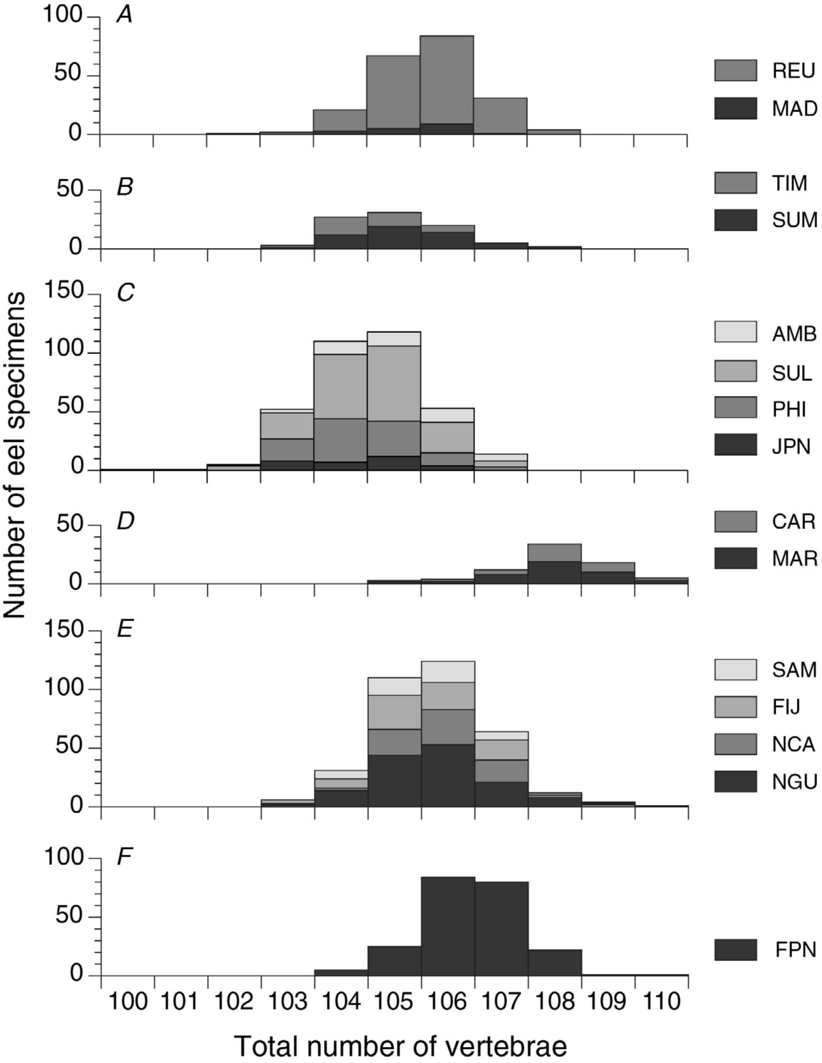

FIGURE 5. Frequency distributions of the combined TV data of six known or likely regional populations of *A. marmorata* in the west Indian Ocean (*A*), the east Indian Ocean (*B*), North Pacific (*C*), Micronesia (*D*), the west South Pacific (*E*), and the east South Pacific (*F*). Abbreviations of locations are from Table 4.

TABLE 5

Comparison of Frequency Distributions of Total Number of Vertebrae (TV) of *A. marmorata* from Six Populations

	TV													
Population	100	101	102	103	104	105	106	107	108	109	110	Total	Mean	SD	*P*
West Indian Ocean			1	2	21	67	84	31	4			210	105.62	0.98	<0.0001
East Indian Ocean				3	27	31	20	5	2			88	105.64	1.09	<0.0001
North Pacific	1	1	5	52	110	118	53	14				354	105.27	1.12	<0.0001
Micronesia						3	4	12	34	18	5	76	104.72	1.11	<0.0001
West South Pacific				6	31	110	124	64	12	4	1	352	104.39	1.11	<0.0001
East South Pacific					5	25	84	80	22	1	1	218	104.42	0.93	<0.0001
Total	1	1	6	63	194	354	369	206	74	23	7	1298	105.60	1.38	

SD and *P* show standard deviation and statistically significant values for a normal distribution by Shapiro–Wilk test.

TABLE 6

Result of Each Pairwise Comparison (*P* 0.05, Dunn's Test) of the TV of *A. marmorata* among Six Populations

Population	East Indian Ocean	North Pacific	Micronesia	West South Pacific	East South Pacific
West Indian Ocean	**	****	****	NS	****
East Indian Ocean		NS	****	****	****
North Pacific			****	****	****
Micronesia				****	****
West South Pacific					****

**$P < 0.01$ and ****$P < 0.0001$. NS: non-significant.

so we also pooled the TV data of sites in regions that might represent different populations with no significant differences among the sites in each region (Figure 4). Based on the above results of this study and the results of molecular genetic studies (Ishikawa et al. 2004, Minegishi et al. 2008, Gagnaire et al. 2009), there is the possibility of six regional populations (West Indian Ocean, East Indian Ocean, North Pacific, Micronesia, West South Pacific, East South Pacific) (Table 5, Figure 5). The six estimated populations in the combined TV data sets (Figure 5) did not have normal distributions (Shapiro–Wilk test, $P < 0.0001$, Table 5), but the statistical analysis of their TV data found a significant difference among them (Kruskal–Wallis test, $P < 0.0001$). The post hoc multiple pairwise comparisons of the 15 pairs among six populations showed 13 significant differences (Dunn's test, $P < 0.0001$–0.01), and that there were no significant differences between West Indian Ocean and West South Pacific or between East Indian Ocean and North Pacific (Table 6).

DISCUSSION

The analysis of the TV data of *A. marmorata* in the present study using data from Ege (1939), Watanabe et al. (2009*b*), and newly obtained data of eels from Pohnpei and Kosrae of the Caroline Islands (Donovan et al. 2012) provide new evidence for the existence of a population of this species that lives on the larger islands of the Micronesia region. The specimens of *A. marmorata* from the Mariana and Caroline islands showed a remarkable difference in TV range and mean from those collected at the 13 other localities and there was no significant difference between the Mariana and Caroline islands specimens. Ege (1939) also showed that the TV of a specimen from Palau (a museum specimen), was 108, which is within the higher TV range of the Mariana and Caroline islands specimens. From a morphological viewpoint, this supports that individuals from the Mariana, Caroline, and Palau islands are included in the same population, which has been referred to as the Micronesia population (Minegishi et al. 2008, Watanabe et al. 2008). The TV data from the Caroline Islands also had the exact same range of TV (105–110) as the eel specimens from Guam and their two means (107, 108) and modal values of 108 were the highest of all the localities. The pooled TV frequency distributions of six likely regional populations in Figure 5 clearly show how different the Micronesia population is. In contrast, all the means (104–106) and modes (104–106) of all the other localities included direct overlaps even among widely separated sites in different ocean basins, and there was a wide range of statistical differences or no differences found in our study and in previous TV or other morphological analyses (Watanabe et al. 2008, 2009*b*).

Therefore, in some respects, the distinctly different TV data for the Micronesia population based on samples from three separate islands show more definitive evidence of it being a highly distinct population than was illustrated in the genetic analyses. For example, the analysis of Donovan et al. (2012) that used sequences of the entire control region mtDNA for 39 eels from Kosrae and Pohnpei and compared them to 267 previously sequenced haplotypes (Minegishi et al. 2008) from across the Indo-Pacific Ocean, found that some of the Mariana and Caroline islands specimens occurred in their own clade of a cluster of South Pacific and Indian Ocean eels (Figure 3). Other Mariana and Caroline Islands specimens were placed in their own branch in a South Pacific cluster, but four others were in different branches of that cluster. The analyses of Minegishi et al. (2008) found a similar pattern of a single distinct clade of only Guam specimens, with other specimens from Guam being placed in other clusters with eels from other regions. Ishikawa et al. (2004) did not include any Micronesia specimens, but were able to find a distinct Northern Population clade, and a distinct Madagascar clade. Eels from other areas were in various clusters and a cluster formed with only Fiji and French Polynesia eels, but other clusters were highly mixed. Gagnaire et al. (2009, 2011) also showed a distinct western Indian Ocean population, which supports the likelihood that those eels spawn on the western side of the Indian Ocean and that there is a different spawning population on the east side.

Therefore, the results in this study, the population genetic study of this species (Minegishi et al. 2008), and all the other gathered information generally indicate that there are undoubtedly at least four regional populations (Indian Ocean, North Pacific, Micronesia, and South Pacific), but it is more likely that there are six spawning populations of *A. marmorata*, with different spawning populations being present in the Indian (east and west) and South Pacific (French Polynesia and western region) oceans based in part on the wide longitudinal zones of those ocean basins and the statistical analysis of the regionally pooled TV data (Table 5, Figure 5). That is also consistent with Ishikawa et al. (2004) that suggested that sequence differences in mtDNA and nuclear DNA with AFLP analysis among geographic samples revealed the existence of four geographic populations around Madagascar, Sumatra, Fiji, and Tahiti in the South Pacific and Indian Ocean regions. Minegishi et al. (2008) showed significant differences in F_{ST} between four of the localities (Madagascar, Sumatra, Fiji, and Tahiti), but Fiji and Tahiti showed no significant genetic differences in mtCR, and Réunion (an additional sampling location in that study) in the western Indian Ocean showed no significant difference with Sumatra in the eastern Indian Ocean in the microsatellite analysis. Gagnaire et al. (2009) supported the existence of three divergent populations (the southwestern Indian Ocean, the South Pacific Ocean, and the North Pacific Ocean populations) in *A. marmorata* and provided a new interpretation for the genetic distinctiveness of Sumatra and Guam. These three populations identified from the AFLP dataset (Gagnaire et al. 2009) mostly correspond to the three main mitochondrial lineages previously found in *A. marmorata* (Ishikawa et al. 2004, Minegishi et al. 2008).

Similar to the DNA studies, the statistical analyses of TV probably have a limited ability to clearly distinguish all the likely spawning populations that may exist in *A. marmorata*. Using individual collection site TV data, significant differences in the TV data were found between the North Pacific Ocean region, except Ambon Island, and South Pacific Ocean regions, while there were few differences between specimens from the Indian and the North or South Pacific Ocean region. The regionally pooled TV confirmed most regional differences (Table 6), but as can be seen in their similar TV distributions in Figure 5, each of the Indian Ocean regional populations were not statistically different from at least one of the Pacific populations (the west Indian Ocean vs. the west South Pacific and the east Indian Ocean vs. North Pacific, Table 6), even though they cannot be part of the same spawning populations. Similarly, the specimens from French Polynesia showed a significant difference of TV from those collected at 10 other localities, but not Madagascar and New Caledonia. French Polynesia was different than all the regionally pooled locations however, which suggests that the nonparametric statistical comparisons may have limitations to find significant differences within overlapping TV distributions. Most importantly though, the regionally pooled data support the hypothesis that there are different spawning populations on each side of both the Indian Ocean and the South Pacific, and there are two spawning populations in the North Pacific.

Although, both molecular genetic and TV studies have some limitations for clearly distinguishing all the likely spawning populations (e.g., some Indian Ocean vs. Pacific Ocean populations were not different for TV), the geography of the six suggested spawning populations seem consistent with the general patterns of ocean currents that would be needed to transport their leptocephalus larvae to their recruitment areas. In the western North Pacific, there must be a spawning area of the Micronesian population in addition to that of the North Pacific population, which appears likely to spawn to the west of the Mariana Islands (Miller et al. 2002, Kuroki et al. 2006, Chow et al. 2017). The spawning area of the Micronesia population is not known, but the North Equatorial Current (NEC) flows westward throughout the year at latitudes from about 8 to 16°N (Reverdin et al. 1994), and part of this water enters the eastward flowing North Equatorial Countercurrent (NECC; Lukas et al. 1991). Therefore, a spawning area in the NEC east of the Mariana and Caroline Islands could also transport leptocephali to the Palau Islands (Figure 2*A*). Eastward transport of leptocephali in the NECC could also explain the presence of this species in other more eastern Micronesian islands, including Palmyra Atoll (Handler and James 2006) (Figure 1). The specimen of *A. marmorata* collected from an artificial freshwater pond Palmyra Atoll (Handler and James 2006) had 110 TV (A. Y. Suzumoto, unpubl. data), which is the maximum number of vertebrae that has been observed in this species that has been seen in the Micronesian population (Tables 2 and 3), except for at Marquesas in Ege's TV data. Therefore, this specimen may have arrived at Palmyra Atoll naturally without assistance from humans. This Palmyra Atoll specimen suggests that freshwater eels have begun to be noticed in new locations, possibly also because of possible recent shifts in oceanic currents (McCosker et al. 2003, Escobar-Camacho et al. 2023).

Other types of information have been obtained about possible spawning populations of *A. marmorata*, but not for the Micronesia population. Net sampling of larvae (Miller et al. 2002, Kuroki et al. 2009) and trawl captures of spawning-condition adults (Chow et al. 2009, Tsukamoto et al. 2011) have found that the North Pacific population of *A. marmorata* spawns in an area in the NEC overlapping with the Japanese eel spawning area. Both pop-up satellite transmitting tags (PSAT; Schabetsberger et al. 2013, 2015) and catches of small larvae (16–18 mm; Miller et al. 2022) have indicated that there is an offshore spawning area of *A. marmorata* to the northeast of Vanuatu (northwest of Fiji) presumably for the western South Pacific population. In contrast, local spawning near Samoa was suggested by PSAT studies

(Schabetsberger et al. 2019, 2021) and the capture of a small larva (7.8 mm) near those islands is consistent with that (Kuroki et al. 2020). However, the distance between those possible spawning areas (northwest of Fiji and near Samoa) and the island archipelagos of French Polynesia (~2,300 km from Samoa to Tahiti), suggest that separate spawning areas likely occur in those far eastern areas even if DNA studies cannot definitively demonstrate that. These various types of information suggest that both long- or medium-distance (North Pacific population, possibly Vanuatu-Fiji region in South Pacific) and local (Samoa, Madagascar, Sumatra) migration and spawning strategies can be used by *A. marmorata*, which are difficult to detect by existing DNA studies or using TV. Only by studying the same cohorts for several years from different places, could research provide a clear answer on genetical structures. However, this kind of data is difficult to obtain.

The patterns found in the DNA studies seem to highlight the limitations of using mtDNA in population structure studies of a relatively old widespread species that has expanded its range across the entire Indo-Pacific. For example, *A. marmorata* does not appear to be one of the oldest species of anguillid eels, but may be about 10 million years old (Myr) (Barth et al. 2020) and seems to have originated within an Indo-Pacific clade after *A. celebesensis* and *A. megastoma* appeared (Minegishi et al. 2005). The long larval duration of anguillid eels, including *A. marmorata* (~3–7 months; Kuroki et al. 2014), would have enabled eventual dispersal of the species across the Indo-Pacific. The maternal inheritance of mtDNA with no recombination (Rubinoff and Holland 2005) has likely resulted in a situation where each regional spawning population retains some ancestral haplotypes, some new ones occur in each population, and some mixing of haplotypes occurs over evolutionary time, which results in the population structure in the South Pacific being obscured in mtDNA studies. Minegishi et al. (2008) suggested that the ancestral population of *A. marmorata* originated somewhere in the western Pacific Ocean and then invaded the central South Pacific and Indian oceans, which is consistent with those 2 ocean regions having similar overall TV ranges as seen in Table 4 and Figure 5. It is possible that the Micronesia population of this species was derived from two lineages with origins in the Indian and South Pacific oceans as suggested by the clustering patterns of Donovan et al. (2012) as seen in Figure 3.

The Micronesia population seems unique in some other respects however, because it is the only anguillid to have colonized the relatively few larger islands of that region. However, *A. bicolor pacifica* has been reported in Palau, the Mariana Islands and Pohnpei along with *A. marmorata* (Myers and Donaldson 2003), whereas in the South Pacific, *A. marmorata* co-occurs with *A. megastoma* and *A. obscura* (Marquet and Galzin 1991). The larger islands such as Palau, Guam, Pohnpei, and Kosrae have been documented to have the same types of aquatic faunas of amphidromous snails, shrimp, and gobies and freshwater insect larvae (Nelson et al. 1996, 1997, Buden et al. 2001, Leberer and Nelson 2001, March et al. 2003, Myers and Donaldson 2003) that form the main part of the diets of anguillids on the islands of Oceania in the South Pacific (Marquet and Lamarque 1986, Miller et al. 2023). Therefore, these islands with freshwater streams (Palau, Yap, Guam, Chuuk, Pohnpei, Kosrae, Figure 2) appear to be the core habitats of the Micronesia population, because there is little apparent surface water in the archipelagos of the Marshall and Gilbert islands (Figure 2*A*), which consist entirely of atoll islands that are part of fringing reefs and only have small ponds or brackish water bodies (Myers and Donaldson 2003).

Although there is basically no clear understanding of all aspects of the life histories of any population of *A. marmorata* yet, the early life histories have been examined in some regions (reviewed by Kuroki et al. 2014). There were many studies of the hatching dates, the leptocephalus durations, and the age at recruitment of glass eels of *A. marmorata* in areas such as Japan, Taiwan, Philippines, and Indonesia for the North Pacific population (e.g., Arai et al. 1999, 2002, Kuroki et al. 2006, 2020), Réunion, Mauritius, and Mayotte for the Indian Ocean population (Robinet et al. 2003, Réveillac et al. 2008), and Fiji for the South Pacific population (Hewavitharane et al. 2020). Helme et al. (2018) documented the seasonal occurrence of recruiting glass eels of *A. marmorata* in two estuaries of French Polynesia (Tahiti and Moorea islands) from 2009 to 2017. The recruitment was seasonal and occurred between November and March during the rainy season, with peaks in December and January. There is no information on the occurrence of glass eels of the Micronesia population, or other aspects of their eels in freshwater. Further research on the spawning areas, distributions of leptocephali, and the recruitment patterns in the Micronesia population will be required to fully understand the population structure and life history of this widespread anguillid species that has established a unique population on a relatively small number of islands of Oceania.

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