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## Species identification and morphological differences of anguillid glass eels recruiting to Viti Levu Island of Fiji in the western South Pacific

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**Abstract:** There are six species of anguillid eels that live in various regions of the western South Pacific but the species compositions of glass eels recruiting to many of the islands of the region are not well known. A total of 1368 anguillid glass eels were collected fortnightly at the mouth of a small river at Namelimeli near Navua in Fiji Islands between April 2015 and June 2016. These were found through DNA barcoding to have a species composition comprising of two longfin eels *Anguilla marmorata* and *A. megastoma*, and one shortfin eel *A. obscura*. 35 glass eels from each species were then selected for morphological studies, it was found that the external morphological characters of anodorsal length ratios, caudal pigmentation patterns, and the internal characters of total, pre-dorsal, and anodorsal vertebral counts were sufficient to classify these three species using morphological characters alone. These findings will simplify research and monitoring techniques of anguillid glass eel recruitment for conservation, fisheries management or aquaculture purposes in the South Pacific.

**Key words:** *Anguilla marmorata*; *Anguilla megastoma*; *Anguilla obscura*; Fiji

Freshwater eels of the genus *Anguilla* consist of 16 species and 3 subspecies that are distributed in the Indo-Pacific and North Atlantic Ocean (Ege 1939; Castle and Williams 1974; Watanabe et al. 2009). Six species occurring within the island groups and continental landmasses of the western South Pacific Ocean, which include *Anguilla marmorata*, *Anguilla obscura*, *Anguilla megastoma*, *Anguilla dieffenbachii*, *Anguilla reinhardtii* and *Anguilla australis* (Jellyman 2003). Each of these species has different geographic distributions. Three of the six species, *A. marmorata*, *A. megastoma* and *A. obscura*, are distributed south of the equator along a belt of island groups in the South West Pacific Ocean (Ege 1939; Beumer et al. 1981; Allen 1991; Marquet and Galzin 1991).

These three species are all listed on the IUCN Red list of threatened species (Jacoby and Gollock 2014; Jacoby et al. 2015). *A. marmorata* is listed as a species of “least concern” (LC), while *A. megastoma* and *A. obscura* are both listed as “data deficient” (DD). Therefore, research on key life history traits and the ecology of glass eels, such as seasonal patterns of recruitment and the species identity of the eels that inhabit freshwater bodies in the region, is essential for the design of appropriate management and conservation programs aimed at efficiently protecting or sustainably utilising these vulnerable species.

Morphological identification of juvenile and adult anguillid eels can be attained through the examination of several internal and external

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morphological characters such as vomerine teeth, body colour, anodorsal length ratios and vertebral counts (Ege 1939). But as glass eel stages are predominantly underdeveloped, species identification is difficult without the recourse to DNA barcoding.

Thus the present study aims to provide diagnostic characters for identification of South Pacific glass eels by morphology alone. We compared five external morphological characters, and four internal morphological characters derived from vertebral counts, of genetically identified glass eel specimens collected at Viti Levu Island, Fiji, western South Pacific.

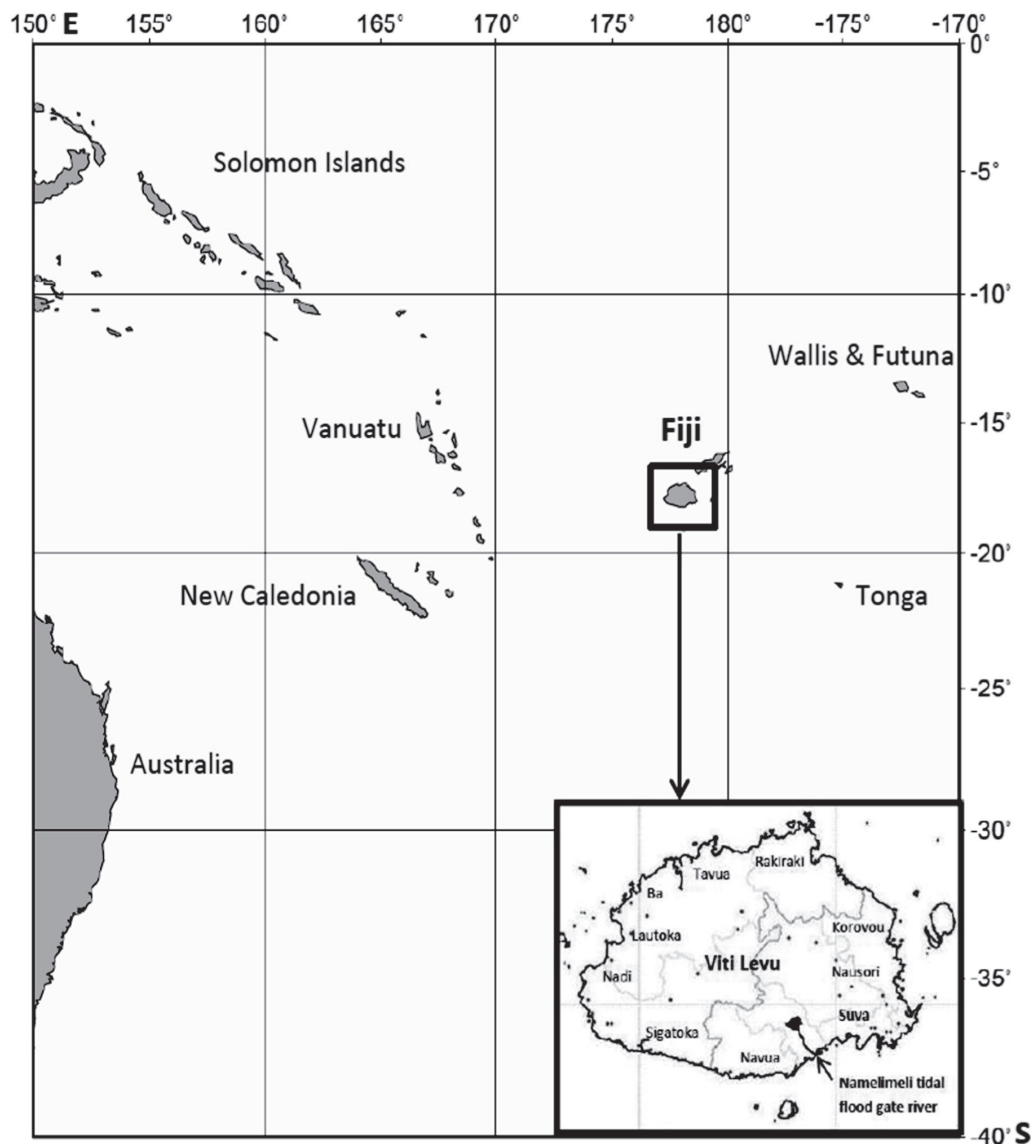
## Materials and methods

### Field sampling

Glass eels were collected from April 2015 to June 2016 during new moon and full moon periods at night at the Namelimeli tidal flood gate river on Viti Levu Island, Fiji (Fig 1). A total of 1368 glass eel capture was undertaken using two methods, a glass eel fyke net, and a light attraction and scoop method.

### Specimens

105 genetically identified glass eels (35 specimens from 3 species, *Anguilla marmorata*,



**Fig. 1.** Map of the location of Fiji within the western South Pacific Ocean and the sampling location at the Namelimeli tidal flood gate river on Viti Levu Island.

*A. megastoma*, and *A. obscura*) were used for the present morphological study and were genetically identified using a partial sequence of mitochondrial DNA 16S rRNA region (Minegishi et al. 2005; Tawa et al. 2012). As sequences within each species were identical the sequences of 10 randomly selected specimens from each species were submitted to the DDBJ (DNA Data Bank Japan) under the accession numbers LC222561-LC222590 and specimens were stored individually in 99.8% ethanol at the Kyushu University Museum (KYUM-PI 4753 - 4782). All glass eels used for morphological comparison comprised of pigmentation stage VA (Strubberg 1913).

#### *Morphological characters examined for comparison*

The five morphometric characters of glass eels of total length (TL), anodorsal length (ADL), horizontal diameter of eye (DE), body depth at anus (BD<sub>A</sub>) were compared across the three species of glass eels as shown in Fig 2. Caudal peduncle and cutaneous caudal fin pigmentation were observed using a binocular dissecting microscope with an external light source. Vertebral counts, predorsal (PDV), preanal (PAV), anodorsal (ADV), and total vertebrae (TV) were examined using Soft-X (Softex Co., Ltd) and counting procedures described by Tabeta et al. (1976) were used.

#### *Statistical Analysis*

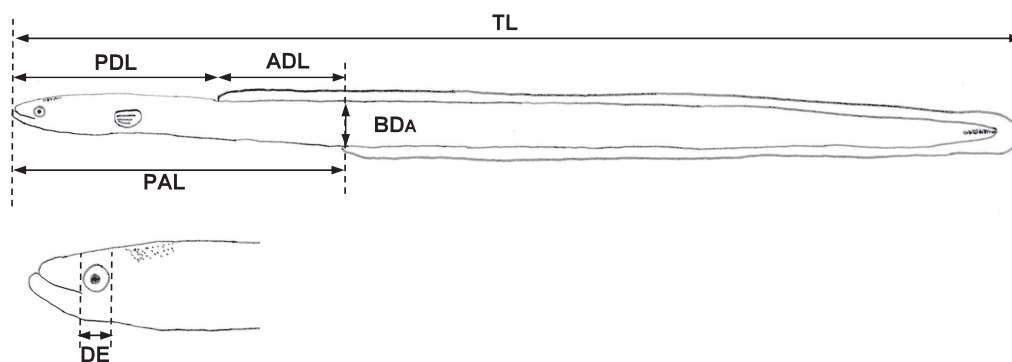
All data sets were examined using one-way analysis of variance (ANOVA) followed by post-hoc tests to detect pairwise differences between

species. All statistical analyses were conducted using IBM SPSS statistics version 22.

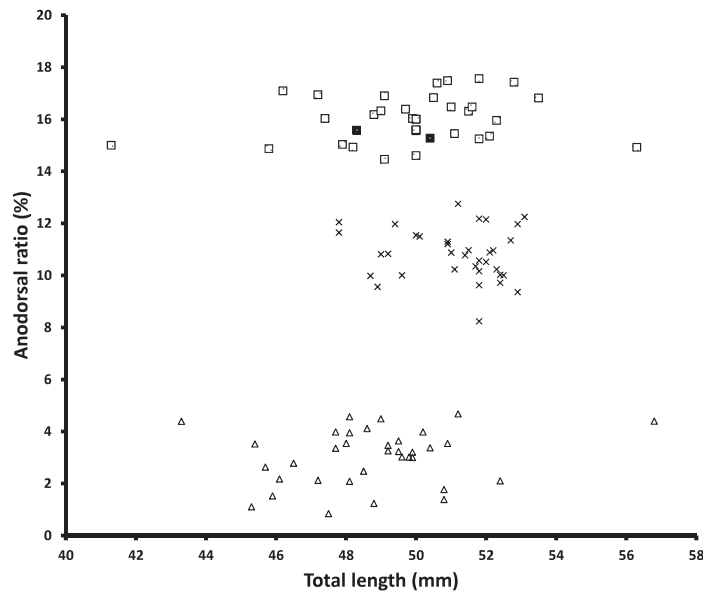
## Results

#### *Morphological characters*

All four of the external morphometric characters assessed in the present study showed significant differences within mean values ( $P < 0.05$ ) between the three species of glass eels *A. marmorata*, *A. megastoma*, and *A. obscura* (Table 1). Out of the four external morphometric characteristics analysed, anodorsal length ratio was the only character that could be used to effectively distinguish between the three species of glass eels (Fig. 3). There was a significant difference between the TL of the three species of glass eels ( $P < 0.05$ ) but, due to a high degree of overlap between the species, using this feature was not possible for morphological diagnostic characters. *A. megastoma* displayed the longest mean TL, followed by *A. marmorata* and *A. obscura* (Table 1). Body depth at the anus was significantly different between the three species of glass eels ( $P < 0.05$ ) but, again due to a high degree of overlap, assignment of particular specimens to one species or another was not always possible (Table 1). *A. marmorata* had the highest body depth, followed by *A. obscura* and *A. megastoma*. The mean horizontal diameter of eye (DE) was also significantly different between the three species ( $P < 0.05$ ) but again showed a high degree of overlap. *A. marmorata* showed the largest DE followed by *A. megastoma* while the



**Fig. 2.** Diagram showing measurements of total length (TL), pre-dorsal length (PDL), pre-anal length (PAL), anodorsal length (ADL), body depth at anus (BD<sub>A</sub>), and horizontal diameter of eye (DE) that recruited into Viti Levu Island, Fiji.



**Fig. 3.** Relationship between total length (TL) and anodorsal length ratio in *Anguilla marmorata* (□), *A. megastoma* (×) and *A. obscura* (Δ) glass eels.

smallest DE was displayed by *A. obscura* (Table 1). Total vertebral counts (TV), predorsal vertebral counts (PDV) and, anodorsal vertebral counts (ADV), were the most conspicuous internal morphological characteristics observed in the present study (Tables 2, 3, 4). The TV of *A. megastoma* were significantly lower ( $P < 0.05$ ) and had no overlap with counts from *A. marmorata* and *A. obscura*. The TV was therefore a useful character to differentiate *A. megastoma* from the other two species. There was a large overlap in the range of TV values between *A. marmorata* and *A. obscura*

however, so these two species cannot be differentiated unless other characteristics are used. The PDV and ADV between *A. obscura* and *A. marmorata* were significantly different ( $P < 0.05$ ) and the range of values are completely separate and therefore morphological differentiation can be easily made using either one of these two characters (Tables 3, 4). The ADV of *A. marmorata* and *A. megastoma* showed a high degree of overlap so differentiation of these two species is not possible using ADV alone (Table 4). The PDV values for *A. marmorata* and *A. megastoma* partially overlap, so differentiation

**Table 1.** Ranges and mean values ( $\pm$  SD) of 4 external morphological measurements in *Anguilla. marmorata*, *A. megastoma* and *A. obscura* glass eels that recruited into Viti Levu Island, Fiji

Species/number of samples	Characters	TL (mm)	ADL (%)	DE (mm)	BD <sub>A</sub> (mm)
<i>A. marmorata</i> (n=35)	Range	41.3–56.3	14.0–17.0	0.55–0.77	1.91–2.60
	Mean	49.9	16.0	0.67	2.27
	SD	2.5	0.9	0.04	0.19
<i>A. megastoma</i> (n=35)	Range	47.8–53.1	8.2–12.0	0.58–0.78	1.45–2.17
	Mean	51.1	10.8	0.65	1.85
	SD	1.5	1.0	0.04	0.17
<i>A. obscura</i> (n=35)	Range	43.3–56.8	0.8–4.7	0.47–0.68	1.52–2.27
	Mean	48.7	3.0	0.61	2.00
	SD	2.4	1.1	0.04	0.17

TL, total length; ADL-ratio, distance between dorsal fin origin and anus related to the total length; DE, horizontal diameter of eye; BD<sub>A</sub>, body depth at anus.

**Table 2.** Counts of the total numbers of vertebrae (TV) in glass eels of *Anguilla marmorata*, *A. megastoma* and *A. obscura* that recruited into Viti Levu Island, Fiji

Number of vertebrae	Species		
	<i>A. marmorata</i>	<i>A. megastoma</i>	<i>A. obscura</i>
102	4		9
103	10		7
104	12		13
105	9		2
106			4
107			
108		1	
109		16	
110		8	
111		2	
112		3	
113		2	
114		3	
Mean	103.7	110.2	103.6
SD	1.0	1.7	1.2

**Table 3.** Counts of the number of pre-dorsal vertebrae (PDV) in glass eels of *Anguilla marmorata*, *A. megastoma* and *A. obscura* that recruited into Viti Levu Island, Fiji

Number of vertebrae	Species		
	<i>A. marmorata</i>	<i>A. megastoma</i>	<i>A. obscura</i>
15	3		
16	1		
17	6		
18	10		
19	7		
20	7	2	
21	1	1	
22		4	
23		10	
24		9	
25		9	
26			
27			
28			
29			1
30			4
31			8
32			5
33			10
34			4
35			3
Mean	18.2	23.4	32.2
SD	1.5	1.4	1.6

**Table 4.** Counts of the anodorsal vertebrae (ADV) in glass eels of *Anguilla marmorata*, *A. megastoma* and *A. obscura* that recruited into Viti Levu Island, Fiji

Number of vertebrae	Species		
	<i>A. marmorata</i>	<i>A. megastoma</i>	<i>A. obscura</i>
1			2
2			11
3			8
4			10
5			4
6			
7			
8			
9			1
10			7
11			8
12	1		6
13	2		9
14	0		4
15	4		
16	11		
17	9		
18	5		
19	2		
20	0		
21	1		
Mean	16.5	11.8	3.1
SD	1.7	1.4	1.1

**Table 5.** Counts of the number of pre-anal vertebrae (PAV) in glass eels of *Anguilla marmorata*, *A. megastoma* and *A. obscura* that recruited into Viti Levu Island, Fiji

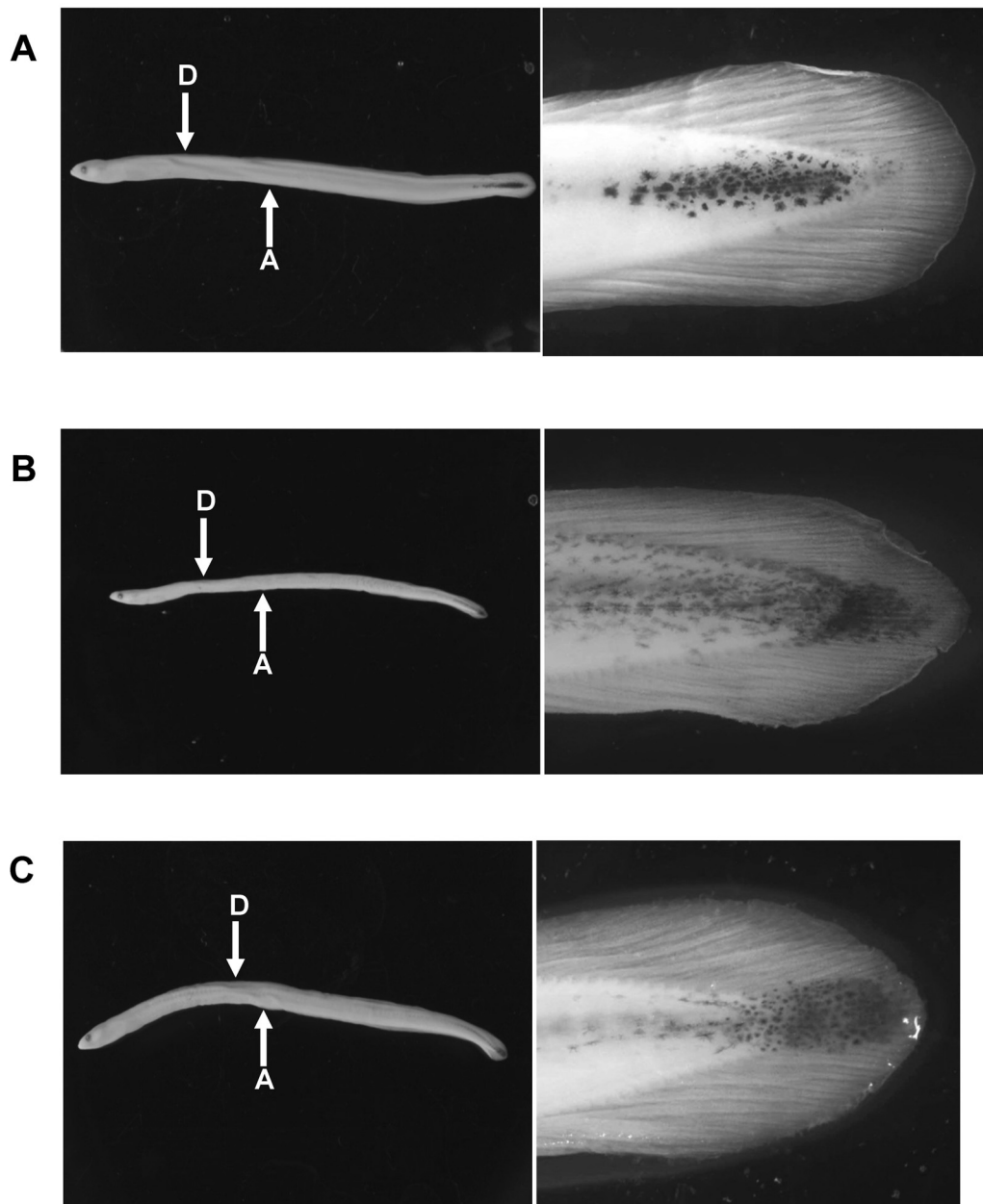
Number of vertebrae	Species		
	<i>A. marmorata</i>	<i>A. megastoma</i>	<i>A. obscura</i>
31	2		
32	1		
33	2	5	3
34	13	7	8
35	7	9	12
36	5	7	3
37	4	4	6
38	1	3	2
39			1
Mean	34.7	35.2	35.3
SD	1.6	1.5	1.5

between these two species is not possible using PDV alone. The PAV were not significantly different between the three species of glass eels ( $P = 0.166$ ) (Table 5).

*Caudal peduncle and cutaneous caudal fin pigmentation patterns*

The caudal peduncle and cutaneous caudal fin pigmentation patterns of each species of glass eel showed distinctive patterns during early

glass eel stages (Fig. 4) that allow each species of glass eels to be easily differentiated. *A. marmorata* glass eels have a dense belt of melanophores on the caudal peduncle which are condensed along the mediolateral line (Fig. 4A). The melanophores are not spread out over the caudal peduncle, and very few occur on the base of the dorsal or anal fin regions. Furthermore, very little pigmentation extends onto the caudal fin. This contrasts with *A. megastoma*, which



**Fig. 4.** Photographs of the lateral side of the body (right) and caudal fin and caudal peduncle pigmentation patterns (left) of glass eels. A, *Anguilla marmorata* (50.4 mm TL, KYUM-PI4753); B, *A. megastoma* (52.1 mm TL, KYUM-PI4763); C, *A. obscura* (51.9 mm TL, KYUM-PI4773). Arrows with letter D show origin of the dorsal fin; arrows with letter A show the anus. KYUM: Kyushu University Museum.



has pigmentation on both the caudal peduncle and caudal fin regions of the glass eels (Fig. 4B). On the caudal peduncle melanophores are spread out along the entire surface, and they extend to the bases of the dorsal fin and anal fin regions. On the caudal fin, melanophores form a dense mat near the tip of the caudal peduncle but the pigmentation only extends to approximately 50-60% of the caudal fin length. Glass eels of *A. obscura* have a dense mat of melanophores predominantly occurring on the caudal fin, extending from the tip of the caudal peduncle to almost the entire length of the caudal fin (Fig. 4C). There is very little extension of the pigmentation onto the caudal peduncle of *A. obscura* glass eels.

## Discussion

### *Morphometric characters and caudal fin pigmentation patterns*

The present study identified three anguillid species, *A. marmorata*, *A. megastoma* and *A. obscura* of glass eels from Viti Levu Island in Fiji and compared the morphological characters of genetically identified specimens of glass eels. This comparison shows that morphological identification between the three species is possible, using two external morphological characters and three internal morphological characters derived from vertebral counts.

The two differentiable external morphological characters are ADL ratios and pigmentation patterns of the caudal peduncle and cutaneous caudal fin (Table 1, Fig. 4). The useful internal morphological characters were TV, ADV and PDV (Tables 2, 5, 3). ADL ratios was the morphometric character that most readily enabled the three species found in Fijian glass eel catches to be differentiated.

*A. obscura* is the only shortfin eel species identified in the present study, and is a western equatorial and northern western South Pacific Ocean species (Tzeng and Tabeta 1982). The ADL values of the *A. obscura* in Fiji are similar to those reported by Ege (1939), Castle and Williamson (1974), Tabeta et al. (1976), and Watanabe et al. (2004). Additionally, Jellyman

(1991) reported ADL values for *A. obscura* in Lake Te Rotonui, Mitiaro, Cook Islands as having a mean of  $5.2 \pm 1.1$  and a range of 3.1–7.2. These values are slightly higher than those recorded in the present study. Jellyman (1991) used specimens which were of relatively large yellow eel stage (mean 538mm for netted eels and mean 647mm for gaffed eels). The discrepancy may be caused by differences in development between glass eel and yellow eel stages.

*A. marmorata* is a longfin eel species with a wide distribution extending from East Africa to Indonesia and across to Tahiti in the South Pacific Ocean (Ishikawa et al. 2004). The ADL values for *A. marmorata* observed in the present study are similar to those reported by Ege (1939), Castle and Williamson (1974), Tabeta et al. (1976), and Watanabe et al. (2004). There is very little information yet available about ADL of glass eels in the western South Pacific, so our results add new information about *A. marmorata* in this region.

*A. megastoma* is also a longfin eel found in the Pacific (Watanabe et al. 2011) but there is very little information about this species due to only small numbers of specimens being obtained by previous researchers. Watanabe et al. (2004) reported obtaining only 14 specimens of *A. megastoma*, so the 35 specimens analysed in the present study represent a significant addition to existing knowledge. The ADL values we obtained for *A. megastoma* closely match those of Ege (1939) and Watanabe et al. (2004).

Ege (1939) had reported a second shortfin species, *A. australis*, as present in Fiji, however this was on the basis of a single specimen (Beumer 1985). Ege (1939) himself questioned the validity of this specimen, and recognised the need for additional material before the presence of *A. australis* in Fiji could be confirmed. Beumer (1985) did not find any further evidence for presence of *A. australis* in Fiji, and neither has the present study. Therefore further work is required to ascertain positively whether *A. australis*, or indeed any other anguillid species besides the current three, occur as adults in the island groups of the South West Pacific Ocean.

Pigmentation patterns of caudal peduncle and cutaneous caudal fin (Fig. 4) was another external morphological character that was effective and easy to use in differentiating glass eels as they recruited into the Fiji Islands. Therefore, using both the characters of ADL and pigmentation patterns of the caudal peduncle and cutaneous caudal fin together (Table 1, Fig. 4) provides a strong basis for morphological differentiation of glass eel stages of the three species that occur as adults in Fiji. The caudal peduncle and cutaneous caudal fin pigmentation patterns of *A. marmorata* have been previously reported by several researchers (Ege 1939; Gagnair 2009; Leander et al. 2012; Robinet et al. 2003). Caudal fin pigmentation patterns of *A. marmorata* observed in the present study are very similar to these other descriptions. *A. obscura* also had very similar caudal fin pigmentation patterns, as previously reported by (Edge 1939). Furthermore, Leander et al. (2012), Reveillac et al. (2009) and Robinet et al. (2003) reported that *A. bicolor bicolor* and *A. bicolor pacifica* both have similar pigmentation to that of *A. obscura*. There is no information that either of these other two short fin species occurs in the western South Pacific. *A. bicolor bicolor* have been reported to be dispersed around the Indian Ocean while *A. bicolor pacifica* in the Indo-west Pacific Ocean (Ege 1939). Therefore pigmentation patterns of the caudal peduncle and cutaneous caudal fin is a valid character to differentiate *A. obscura* in the western South Pacific. The caudal peduncle and cutaneous caudal fin pigmentation patterns for the early glass eel stage of *A. megastoma* are distinct from those seen in the glass eel stages currently described for other species.

Morphometric characters such as horizontal diameter of eye (DE), body depth at anus (BD<sub>A</sub>), and total length (TL) exhibiting significant differences between the three species of glass eels, but were not useful for distinguishing the species due to the large overlap in ranges of these characters between species. Very few studies have reported DE and BD<sub>A</sub> for the glass eels stages of anguillids, although many previous studies have compared DE and BD<sub>A</sub> on

larger specimens at the yellow eel stage (Jellyman 1991; Watanabe et al. 2006, 2009). The mean total lengths of *A. marmorata* and *A. obscura* glass eels in this study are similar to those reported by Beumer (1985) for glass eels collected in the Vunidawa River, Fiji. However, Beumer (1985) did not report finding any *A. megastoma* glass eels in his collections, so our results provide the first record of *A. megastoma* glass eels in Fiji.

#### *Vertebral counts*

The TV and the ADV of *Anguilla* have been important characters used for species identification in the past (Ege 1939; Robinet et al. 2003; Watanabe et al. 2004; Budimawan and Lecomte-Finiger 2005). The TV of *A. megastoma* here are similar to those reported by Watanabe et al. (2011). For adult *A. megastoma* specimens from Samoa, the TV counts ranged between 108 and 114 (the same as the present study) while our mean TV is slightly higher at 110.2 compared with 108. Watanabe et al. (2011) compared the TV data of *A. megastoma* from four different localities in the western South Pacific, and from this they concluded there may be two populations present in the region. Due to insufficient sample sizes of *A. megastoma* from Fiji and New Caledonia in the Watanabe et al. (2011) study, it was also stated that the population of *A. megastoma* from these two regions may be similar to the population found in Samoa. The present study provides a larger sample size of eel specimens from Fiji. Our results for TV of *A. megastoma* are consistent with those for the Samoan *A. megastoma* specimens assessed by Watanabe et al. (2011). This further strengthens the case for existence of two separate populations of *A. megastoma* in the South West Pacific Ocean. Analysis of the TV counts for specimens of *A. obscura* from five localities in the Western South Pacific (mean 104, range 100–108) did not indicate population structure, which indicates there may be only a single population in the western South Pacific (Watanabe et al. 2011). The present study adds further support to this view, by producing similar TV values of mean and range for *A.*



*obscura*. The TV compared from samples of *A. marmorata* collected from 13 localities in Indo-Pacific region by Watanabe et al. (2008) indicate that the Northern Pacific population is highly divergent from all other populations. Further, *A. marmorata* found in Fiji and Tahiti may belong to two different populations (Ishikawa et al. 2004). The TV values of the present study are similar to those recorded by Watanabe et al. (2008), and may also support the idea of two separate populations of *A. marmorata* in the western South Pacific.

For effective conservation and management strategies to be implemented, more emphasis is required on studies of migration and recruitment patterns of these catadromous fishes. The present study provides the ability to readily and reliably identify newly recruiting glass eels to species level, without need for recourse to genetic techniques, this will help broaden eel research and allow effective monitoring techniques in the South Pacific.

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## 南太平洋フィジーのビティレブ島に来遊したウナギ属シラスの種同定と形態的差異

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2015年4月～2016年5月に南太平洋のフィジー、ビティレブ島ナブア地区近郊の小河川の河口部において採捕された1368個体のウナギ属シラスのうち、DNAバーコーディングによって *Anguilla marmorata* (オオウナギ)、*A. megastoma*, *A. obscura* に種同定された105個体(各35個体)を用いて、これらの形態的差異を明らかにした。体部比、脊椎骨構成、尾部の黑色素沈着を検討したところ、これら3種は全長に対する背鰭-臀鰭間長比と尾部の色素沈着状態を用いることにより形態的に識別可能であることが示された。本研究によって、南太平洋におけるウナギ属魚類の保全、資源管理、養殖に必要なシラスウナギ加入量のモニタリングを容易に実施できるようになる。