

Nonnative Occurrence of *Anguilla marmorata* in Hawai‘i: Identification Using Morphological and Molecular Characters¹

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Introduction

Anguilla eels (Pisces: Anguilliformes: Anguillidae) are found throughout the Asia-Pacific Rim and South Pacific, as well as western Europe and the American East Coast. Some species are highly prized as food; most are considered nuisances in the freshwater rivers they inhabit, feeding on a variety of fishes and shrimps. All *Anguilla* species are typically catadromous, i.e., the adults leave their freshwater rivers and streams and migrate upwards of thousands of kilometers to marine breeding areas (Aoyama *et al.* 2001). Larval eels passively make their way back to source rivers by subtropical currents to complete their life cycle. While adults leaving the streams may be relatively few in number, offspring may number in the thousands upon their return. Considered top carnivores, Anguilla eels pose a potentially serious threat to freshwater native stream animals in Hawai‘i. It is highly likely that Hawaiian stream organisms would have little defense against predation by an individual eel, or worse, a population of these eels. Anguilla eels can cross dry land to travel from pond to pond, either in search of additional food or more favorable water conditions.

A freshwater eel of a species previously unrecorded from the wild in the Hawaiian Islands was captured on Maui during the summer of 2002. The specimen was identified using morphological characteristics, with DNA analysis of two mitochondrial genes being employed to confirm identification.

Materials and Methods

Specimen collection

On 23 June 2002, Maui resident Patrick Domen speared a 1.02-m, 3.2-kg eel in a large freshwater pond in south Maui. The eel was frozen and delivered to Skippy Hau, Hawaii State Department of Land and Natural Resources-Division of Aquatic Resources-Maui, who transported it to Honolulu, 19 July 2002, to the Bishop Museum for identification. After a tissue sample was removed for DNA analysis, the specimen was fixed in 10% formalin and preserved in 75% ethanol, and cataloged in the fish collection of the Bernice Pauahi Bishop Museum (BPBM), with permission of the collector, as BPBM 39092.

DNA analysis

Genomic DNA was extracted from muscle of the unidentified eel specimen using the protocol of Sambrook and Russell (2001). Voucher DNA is held at the Pacific Center for Molecular Biodiversity, Bishop Museum (Accession No. PCMB B177). Polymerase chain reaction

1. Contribution 2006-013 to the Hawaii Biological Survey.

(PCR) amplifications were performed in 50 μ L of a solution containing approximately 10 ng of genomic DNA, 400 μ M of each dNTP, 1.5 unit Taq Polymerase (Promega), 2 μ M $MgCl_2$, each primer at 1 mM, and buffer. Primer sequences for the mitochondrial genes were taken from Palumbi (1996), as follows (5'-3'): 12S forward, AAA CTG GGA TTA GAT ACC CCA CTA T; 12S reverse, GAG GGT GAC GGG CGG GCG GTG TGT; 16S forward, CGC CTG TTT ATC AAA AAC AT; 16S reverse, CCG GTC TGA ACT CAG ATC ACG T. PCR cycling parameters (PTC 100, MJ Research Watertown, Massachusetts) for the initial double-stranded amplification were 94 °C for 1 min, 50 °C for 1 min, and 72 °C for 1 min, repeated for 45 cycles, with final extension of 72 °C for 5 min. The PCR product was gel extracted using QIAQuick Gel Extraction Kit (Qiagen Inc., California), and quantified on a 1.5% agarose gel using ethidium bromide. Cycle sequencing of 15–45 ng of the double-stranded PCR product was carried out with each primer and BigDye terminators (v. 3.1, ABI Biosystems) diluted to half concentration using 2.5x buffer. Sequences were determined by the Brigham Young University Sequencing Center on an automated sequencer. Both sequences have been submitted to GenBank (Accession Nos. AY207028 and AY207029).

Nucleotide sequences were aligned using ClustalX (default parameter settings; Thompson *et al.* 1997) then by eye. A nucleotide-nucleotide BLAST search (Altschul *et al.* 1990) was performed for the eel 12S and 16S rRNA sequences using GenBank (Benson *et al.* 2002). The most closely related sequences were downloaded, and a consensus of the 279 (12S) and 56 (16S) most parsimonious trees was constructed using PAUP version 4.0b10 (Swofford 2002) using *Stemonidium hypomelas* (Gilbert) as an outgroup. Trees were produced to clearly indicate the identification of the Maui eel, rather than to suggest the phylogeny of the genus *Anguilla*. Such studies have already been completed (Aoyama *et al.* 2001, Lin *et al.* 2001, Bastrop *et al.* 2000).

Results

Visual examination of the eel revealed it to be a male of advanced age (5+ years). Herald (1975) suggested *Anguilla anguilla* (Linnaeus), the European Anguilla eel, may attain a length of 1.5 m (5 ft) in 12 years for females, or 0.5 m (20 in) in 4–8 years for males. The gut was mostly vacant except for a bolus of greenish gray sludge near the vent, containing the remains of at least one small crustacean, *Macrobrachium lar* (Fabricius) (pincer length, 14 mm), and an otolith-like piece suggesting fish as a diet item. The eel possessed characters consistent with *Anguilla marmorata*, the Giant Mottled Eel, as diagnosed in Smith (1999): slightly projecting lower jaw; scales present (embedded in the skin); dorsal fin origin nearer gill opening than vent; dark brown mottled with lighter brown, with a yellowish-white belly. In Hawai'i, the only fish that could possibly be confused with the Maui eel would be a large individual of the family Congridae (conger eels). Both families attain a large size (to 1.5 m) and have very prominent pectoral fins. *Conger* differs from *Anguilla* in possessing a slightly inferior lower jaw and lacking scales entirely, and is typically a uniform gray color with white ventrally, or irregularly-banded with white and dark brown (this latter pattern more frequently exhibited at night). Due to the unexpected nature of this occurrence of *Anguilla* in Hawai'i, and to eliminate any possibility of misidentification of an aberrant specimen of *Conger*, DNA analysis seemed appropriate to ensure a positive identification.

Comparison with sequences published in GenBank (Table 1) resulted in the 427 base-pairs of the 12S and 628 base-pairs of the 16S mitochondrial rRNA sequences of the eel matching exactly with sequences of *Anguilla marmorata*, confirming the visual identification of the eel (Figures 1, 2). Although there were single nucleotide differences between some of the *Anguilla marmorata* GenBank accessions, the source location of the Maui eel was not able to be identified with certainty (Table 1, Figures 1, 2).

TABLE 1. Closest sequence matches to the 12S and 16S rRNA eel sequences using the GenBank nucleotide-nucleotide BLAST search

Species	GenBank accession	Collection location	Number of bp different	Reference
12S 427 base pairs				
<i>Anguilla marmorata</i>	AB021890	Indonesia	0	Aoyama pers. comm.
	AF266485	S Africa	0	Lin <i>et al.</i> 2001
	AF266484	Taiwan	1	Lin <i>et al.</i> 2001
	AF417308	not available	1	unpublished
<i>Anguilla interioris</i>	AB021886	New Guinea	1	Aoyama pers. comm.
<i>Anguilla malgamora</i>	AF266499	Philippines	2	Lin <i>et al.</i> 2001
	AF266498	Philippines	2	Lin <i>et al.</i> 2001
<i>Anguilla reinhardtii</i>	AF266487	Australia	4	Lin <i>et al.</i> 2001
16S 628 base pairs				
<i>Anguilla marmorata</i>	AJ244817	Taiwan	0	Bastrop <i>et al.</i> 2000
	AJ244816	Taiwan	1	Bastrop <i>et al.</i> 2000
	AJ244818	Taiwan	1	Bastrop <i>et al.</i> 2000
	AB021760	Indonesia	1	Aoyama <i>et al.</i> 2001
	AJ244819	Taiwan	3	Bastrop <i>et al.</i> 2000
<i>Anguilla obscura</i>	AB021762	Fiji	8	Aoyama <i>et al.</i> 2001
<i>Anguilla bicolor</i>	AB021757	Philippines	9	Aoyama <i>et al.</i> 2001

Discussion

Anguilla marmorata Quoy & Gaimard is a tropical freshwater eel, found between 24°N and 33°S. *Anguilla marmorata* is distributed throughout the Indo-Pacific, from East Africa and India to French Polynesia, north to southern Japan (Smith, 1999), east to the Galápagos (McCosker *et al.*, 2003). It is recorded as being native to French Polynesia (notably Tahiti), Fiji, and Samoa. It is not indigenous to the Hawaiian Islands. Long-time aquarists in Honolulu can remember when *Anguilla* spp. were imported into Hawai'i, primarily as live food items with a few as part of the aquarium trade (1950s–1960s), prior to the eels being prohibited for import in 1974 (HRS 150A-6; Hawaii Administrative Rules, 1995, Chapter 4-71). While most were believed to have been consumed, some were kept as display animals in koi (carp) ponds. Escapes have never been documented and until now *Anguilla* have not been reported from any Hawaiian stream.

Although larvae of freshwater *Anguilla* species are distributed by oceanic currents, it is unlikely that this individual found on Maui arrived without human intervention. Ziegler (2002) commented that the nonoccurrence of Anguillidae in the Hawaiian Islands is somewhat surprising given its widespread nature elsewhere in the western Pacific. The isolation of the Hawaiian Islands, both geographically and in terms of oceanic current flow, plus factors of deep-ocean salinity, have been, and continue to be, sufficient barriers preventing this family from reaching the Hawaiian Islands. Although *Anguilla* has been confirmed from the Galápagos (McCosker *et al.*, 2003) and Palmyra Atoll in 2003 (Handler & James, 2006) it is still unlikely that this eel could naturally find its way to the Hawaiian Islands.

The western Pacific region (Japan, the Philippines, to Indonesia), from which the Maui eel originated given the sequences available in GenBank, is inhabited by a single genetic population of *Anguilla marmorata* (J. Aoyama, pers. comm. 2002). As a result, the exact locality within the western Pacific region cannot be confirmed, particularly as the 12S and 16S rRNA regions of *Anguilla marmorata* are not sufficiently sensitive for such a determination.

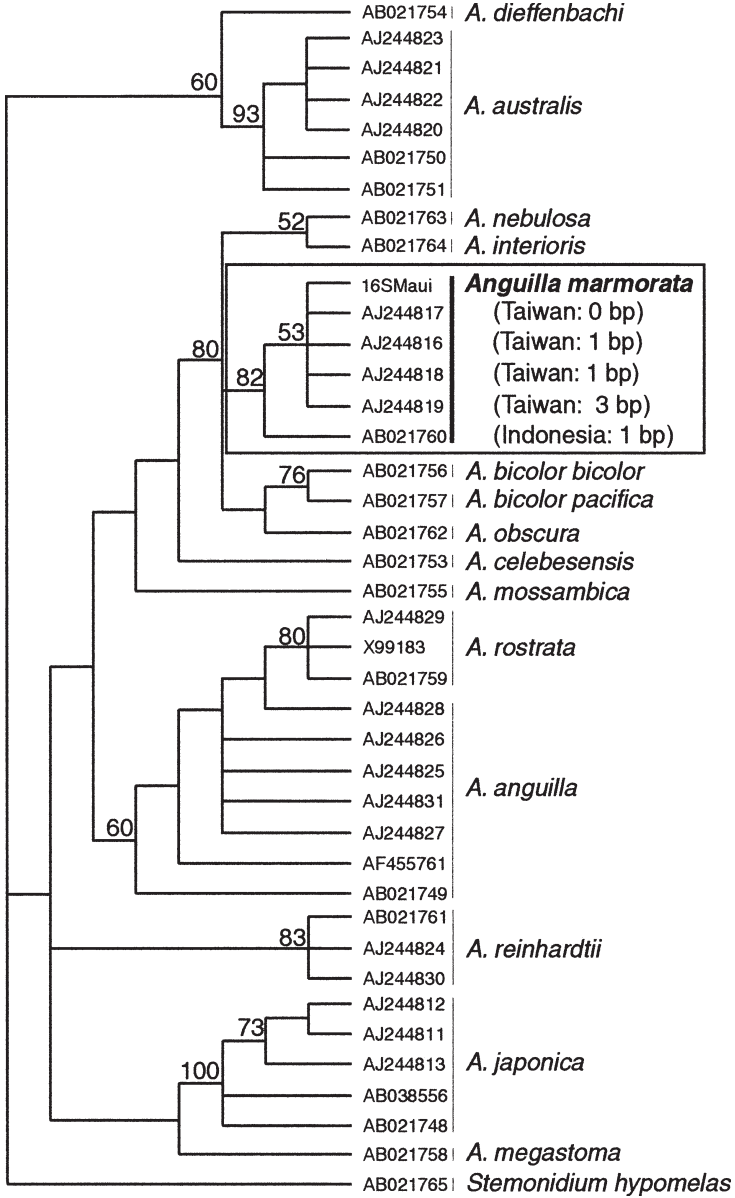


Figure 1. Consensus of 279 most parsimonious trees for 12S rRNA mitochondrial gene sequences closely matching that of the Maui eel. Base-pair (bp) differences from the Maui specimen have been indicated. Parsimony bootstrap values above 50% are shown.

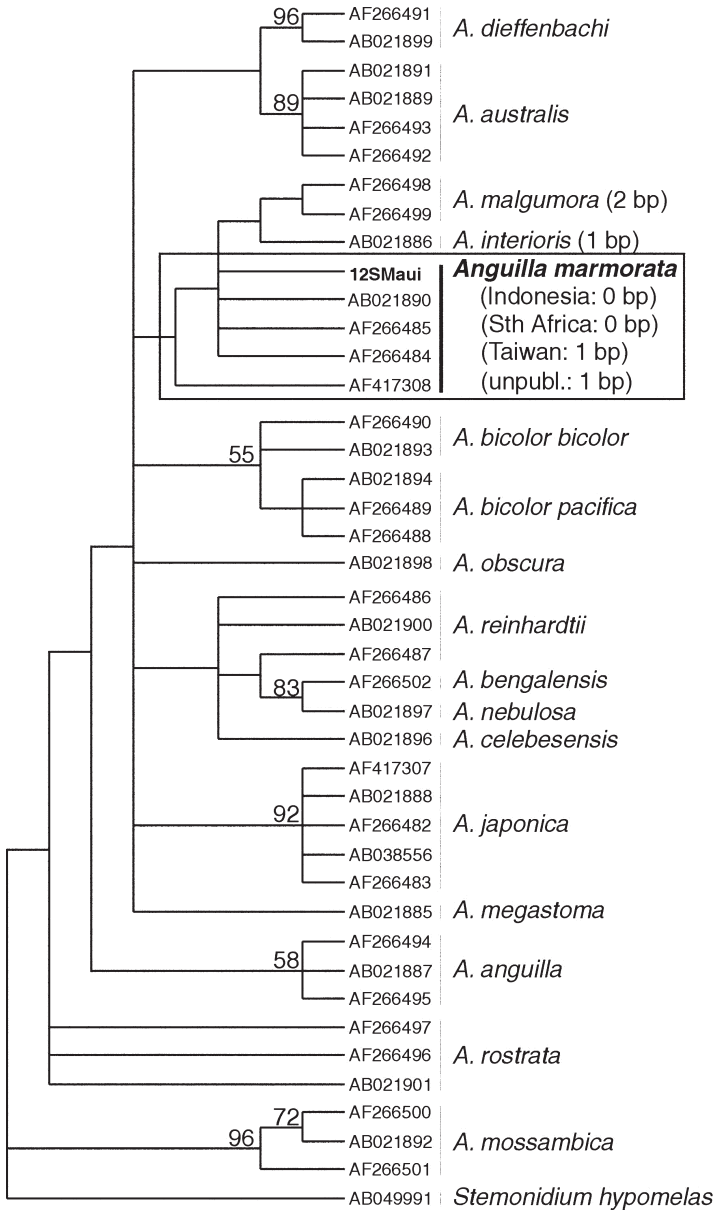


Figure 2. Consensus of 56 most parsimonious trees for 16S rRNA mitochondrial gene sequences closely matching that of the Maui eel. Base-pair (bp) differences from the Maui specimen have been indicated. Parsimony bootstrap values above 50% are shown. The Maui eel falls within the monophyletic group of *Anguilla marmorata*.

There is immediate concern regarding the discovery of *Anguilla marmorata* in a Hawaiian stream, as the species is active at night and feeds on a wide variety of prey, including arthropods (crustaceans and insects), amphibians, and fishes. Continued survey and monitoring of alien introductions, and the maintenance of education programs for Hawai'i residents and visitors which emphasize the importance of not introducing non-native species into native ecosystems, are essential for keeping the Hawaiian environment in balance. In this regard, DNA analysis can play a significant role in species identification and in elucidating the potential origin of undesirable organisms.

Acknowledgments

We thank Patrick Domen and the Domen family; Skippy Hau and Mike N. Yamamoto, Hawaii State DLNR-DAR; John E. Randall, Richard L. Pyle, and Loreen R. O'Hara, Bishop Museum; Glenn Y. Takeshita, aquarist; Domingo Cravalho, Plant Quarantine Branch, Hawaii State Department of Agriculture; and John E. McCosker, California Academy of Sciences.

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