

# Examining the global phylogeography of a little known species: the pygmy killer whale (*Feresa attenuata*)

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## Introduction

- Pygmy killer whales are typically found in tropical and subtropical areas, in deep open ocean environments, and at times around oceanic islands.
- They are naturally rare, elusive, and cryptic.
- Conservation status is largely unknown, however there have been mass strandings, and there are reports of these animals associated with fisheries bycatch.
- Few density estimates exist.
- Current knowledge of their life history is based on a mark-recapture study, satellite telemetry of five animals, and stranding events (1, 2, 3,4).
- To date there are no genetic studies on taxonomy or phylogeography of these animals.

- Phylogeographic characteristics are displayed with a Median Joining Network (MJN) created with PopArt (7).
- Population differentiation ( $F_{ST}$  and  $\Phi_{ST}$ ) was analyzed using strataG excluding the sample from Fiji.
- Nei's  $d_A$  (a measurement of nucleotide divergence) was calculated with strata G excluding the sample from Fiji.

## Results

- 25 haplotypes were identified.
- When using the 671bp fragment for analyses, haplotypes 13 and 15 collapsed into 1 haplotype.
- 36 variable sites were identified with the 671 bp fragment (Table 1). 37 variable sites were identified with the 960 bp fragment.
- Haplotypic diversity  $h=0.93$ .
- Mean nucleotide diversity  $\pi = 0.0065$ .

Haplotype	1	16	29	32	69	80	81	89	107	137	175	239	246	253	260	264	275	276	284	296	300	328	341	362	364	365	417	423	426	472	512	574	587	607	610	613	
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3	C	.	.	.	.	.	.	G	.	T	C	C	T	.	.	.	.	.	.	.	.	.	C	C	C	.	G	.	A	.	.	.	.	.	.	C	
6	.	.	.	.	.	.	.	G	.	T	C	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A
7	.	.	.	.	.	.	.	G	.	T	C	C	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A
8	.	.	.	.	.	.	.	G	.	T	C	C	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	A
14	.	T	.	.	.	.	.	G	.	T	C	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A
11	.	.	.	.	C	.	.	G	G	T	C	.	.	.	A	.	.	G	.	.	.	G	.	C	.	.	.	.	.	.	.	.	.	.	.	A	
19	.	.	.	.	C	.	.	G	T	C	.	.	.	.	A	T	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	A
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22	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	A
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Table 1. Variable Sites

Haplotypes are arranged according to the median joining network layout. This table utilizes the 671bp fragment, the haplotype from Fiji (19) is included. Dots indicate that the site is identical to haplotype 16 which is the reference sequence. ^ Haplotypes 13 and 15 are collapsed and retain the name of haplotype 13.

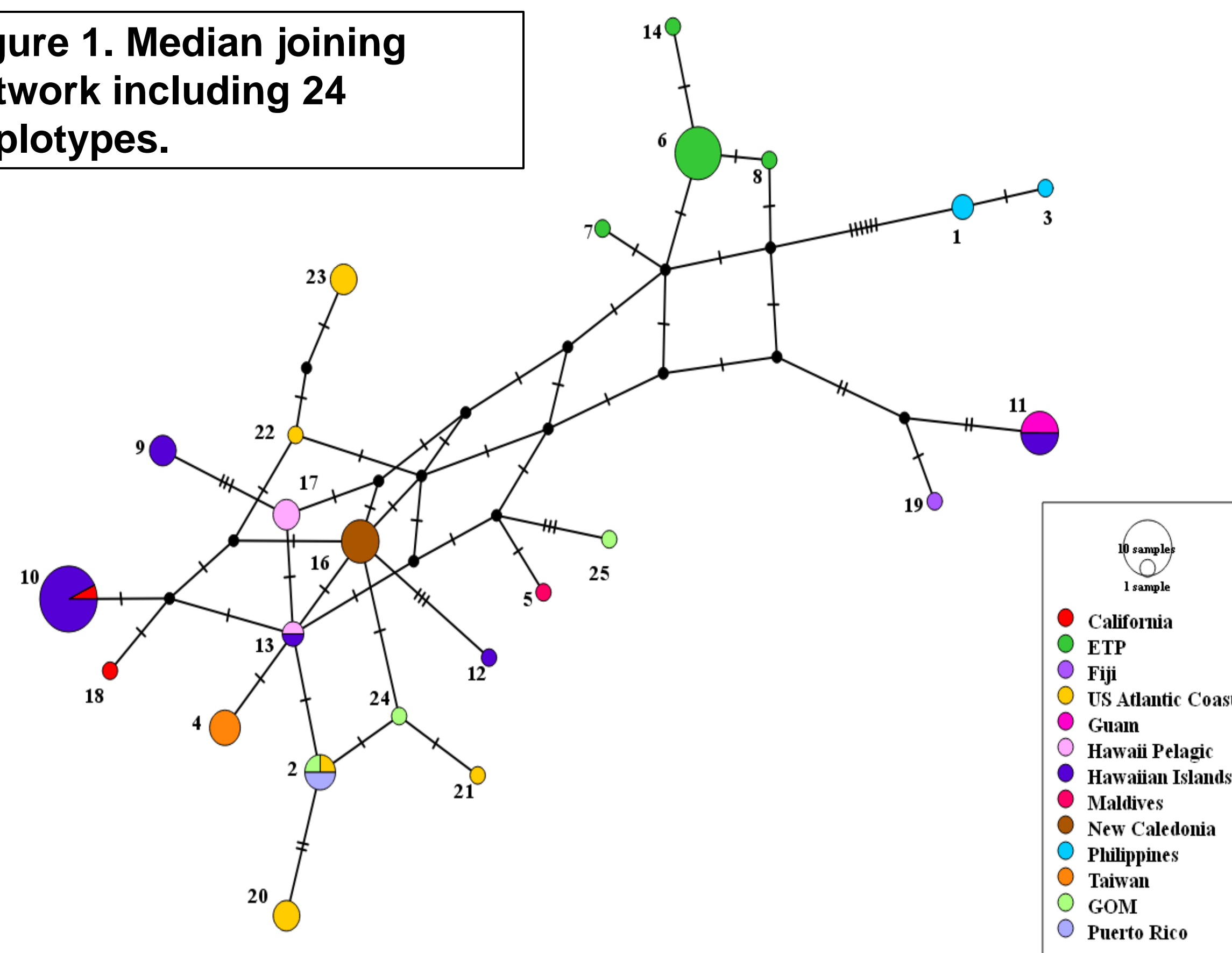
## Sample Information and Methods

- Seventy-one tissue samples either from biopsied animals or stranded individuals were analyzed.
- Geographic distribution of samples is as follows:
 

Philippines n=3	Taiwan n=4
Maldives n=1	Guam n=3
Hawaii Pelagic n=4	Hawaiian Islands n=21
California n=2	New Caledonia n=6
Fiji n=1	US Atlantic Coast n=9
Gulf of Mexico n=3	Puerto Rico n=2
Eastern Tropical Pacific (ETP) n=12	

- A 960 bp fragment of the mtDNA control region was sequenced for all samples except the sample from Fiji, which had a sequence length of 671bp.
- Variable sites within the sequences were analyzed with SeqAssessor (5). Haplotype and nucleotide diversities were analyzed with R scripts using strata G (6).

Figure 1. Median joining network including 24 haplotypes.



- The global MJN indicates strong phylogeographic structure, with all haplotypes from the ETP being closely related to each other in a cluster distinct from all other samples (green circles in Figure 1).
- The most striking result is that the animals from the Philippines, represented by two haplotypes, appear to be very different from all other samples (light blue circles in Figure 1).
- We identified five fixed nucleotide differences between the Philippines samples and all other pygmy killer whales (yellow boxes in Table 1).

Table 2. Pairwise population differentiation values for three geographically separated regions.  $F_{ST}$  is shown below the diagonal and  $\Phi_{ST}$  is above. Associated p-values are in parentheses.

	ETP (n=12)	Hawaiian Is. (n=21)	N Atlantic (n=14)
ETP	**	0.59 (0.001)	0.63 (0.001)
Hawaiian Is.	0.43 (0.001)	**	0.21 (0.001)
N Atlantic	0.33 (0.001)	0.24 (0.001)	**

## Conclusions

- The net nucleotide divergence between the Philippines and the other regions ( $d_A = 0.009$ ) is higher than that between many recognized cetacean subspecies. This indicates a revision of this species taxonomy is warranted.
- The MJN,  $F$  statistics, and Nei's  $d_A$  (0.004) indicate that the ETP is strongly differentiated from the other pygmy killer whales globally (Figure 1 and Table 2).
- There appears to be greater differentiation within the North Pacific than between ocean basins (Table 2).
- Our results indicate that there is undescribed structure in pygmy killer whales and that further study is warranted. This is especially true given that information about this species is sparse, and that their conservation status is unknown.



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- SeqAssessor Software (Karen K. Martien, available upon request).
- The R package strataG (F.I. Archer, available upon request).
- PopArt website: <http://popart.otago.ac.nz>

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