

Resolution of the taxonomic status of Chilean and Californian jack mackerels using mitochondrial DNA sequence

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Phylogeographic reconstructions using mitochondrial DNA sequences from both the control region and the cytochrome b gene demonstrated that two disjunct populations be recognized as *Trachurus murphyi* and *Trachurus symmetricus*. The species have been isolated for at least 250 000 years.

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The genus *Trachurus*, jack mackerels, comprises a group of *c.* 14 marine fish species occurring in Pacific, Atlantic, Indian and Mediterranean waters. Five of these species have been recorded in temperate waters of the Pacific Ocean: *Trachurus japonicus* (Temminck & Schlegel), *Trachurus novaezelandiae* Richardson and *Trachurus declivis* (Jenyns) in the western Pacific, and a pair of anti-tropical species, *Trachurus symmetricus* (Ayres) in the north-eastern Pacific, and *Trachurus murphyi* (Nichols) in the south-eastern Pacific (Shaboneyev, 1980). Although the systematic position and the taxonomy of most of these species seem to be reasonably clear (Ben Salem, 1995), the situation for the two anti-tropical eastern Pacific forms is unresolved.

Trachurus symmetricus was first described in California waters by Ayres (1855). Later, Nichols (1920) recognized a new species, *T. murphyi*, from Peruvian waters (Fowler, 1945), and misidentified the Chilean form as *T. symmetricus*. Mann (1954) proposed that since the morphological differences between the two forms were so slight, that they should be considered as sub-species, [*i.e.* *T. symmetricus symmetricus* (Ayres) in the north, and *T. symmetricus murphyi* (Ayres) in the

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south]. Based on morphometric and meristic analysis, Berry & Cohen (1972) and Ben Salem (1995) subsequently recommended that *T. murphyi* and *T. symmetricus* should be considered as distinct species. Using isozyme markers, Stepien & Rosenblatt (1996) found little genetic divergence between populations of jack mackerel from the Pacific North and South America. This led them to conclude that the taxa belonged to the same species, suggesting the existence of gene flow across the tropics. In a recent review, Oyarzún (1998) emphatically concluded that there is no basis for recognizing the sub-species status of the south-eastern Pacific population, and asserted that the correct name for the species in this area is *T. symmetricus*. To assist in the resolution this controversy, phylogeographic reconstruction of the two disjunct populations using DNA sequences from both the mitochondrial control region (CR) and the cytochrome b gene (Cytb) was performed in the present study.

Specimens of *T. symmetricus* and *T. murphyi* were collected from the north-eastern (California) and south-eastern Pacific (Chile), respectively. Specimens of *T. neozelandae* and *T. declivis* were obtained from New Zealand. Total genomic DNA was extracted from muscle tissue using a standard phenol/chloroform protocol, and subjected to the polymerase chain reaction (PCR). The complete Cytb (1140 bp) was amplified and sequenced for each of the four Pacific species. Complete CR and Cytb sequences for *T. japonicus* (AP003092) were obtained from GenBank. The complete CR (833 bp) of 95 jack mackerel specimens from Chile and 28 specimens from California were amplified and sequenced with two primers designed from tRNA-Pro and tRNA-Phe sequences of carangids (tRNA-T1: CAGAAAAGGAGACTCTAACTCCTAAA and tRNA-T2: TGCTTGCGGGGCTTTCTA). The CR from a single specimen of *T. neozelandae*, and one specimen of *T. declivis* were also sequenced. Sequence alignments were accomplished using Clustal X (Thompson *et al.*, 1997), and were checked by eye. Because of its close phylogenetic relationship with the genus *Trachurus* (Reed *et al.*, 2002), the Cytb sequence of *Decapterus punctatus* (Cuvier) (GenBank AY050732) was chosen as the out-group. For analysis of evolutionary divergence between haplotypes, maximum likelihood-based phylogenetic (ML) relationships were estimated using PAUP* (Swofford, 2002), implementing a heuristic search of tree space for CR sequence and exhaustive search for Cytb sequence. The simplest ML model that best explained the data was estimated using the Akaike Information Criterion (AIC) in the programme MODELTEST (Posada & Crandall, 1998). Bootstrap re-sampling was performed using 1000 replicates.

The results from the MODELTEST indicated that the Tamura–Nei model (Tamura & Nei, 1993) with a proportion of invariable sites (TrN + I) was the most descriptive model of evolution for both Cytb and CR data sets.

Cytochrome b: the ML based phylogenetic tree generated from the Tamura–Nei distance matrix (TrN + I), rooted by *D. punctatus*, showed two distinct mitochondrial clusters [Fig. 1(a)]. The first cluster included the three species from the western Pacific region (65% bootstrap support), and the second cluster includes the two eastern Pacific taxa (92% bootstrap support). Nucleotide divergence between these two main clusters varied between 4.9 and 5.7%, and between 0.9 and 1.6% among taxa from the same cluster (Table I).

Control region: 13 and nine haplotypes, respectively, were found in the north-eastern and south-eastern Pacific samples. The unrooted phylogenetic tree based

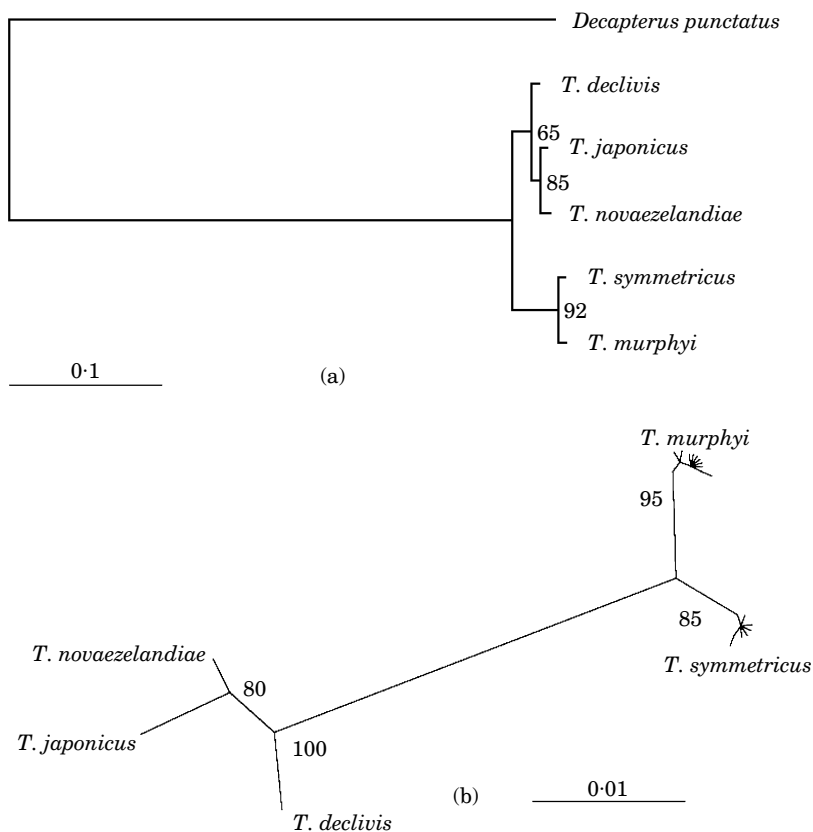


FIG. 1. Phylogenetic relationships among jack mackerel species. (a) Rooted ML phylogenetic tree from complete cytochrome b gene sequences of Pacific species of *Trachurus* (ln-likelihood score = -2446.7, AIC = 4903.3525). (b) Unrooted ML phylogenetic tree from complete control region sequences of Pacific species of *Trachurus*, and relationships among *T. murphyi* and *T. symmetricus* haplotypes. In both cases, numbers above branches are bootstrap values (only values >60% are shown).

on CR sequences divided the taxa into the same two clusters, which correspond to the east and west Pacific [Fig. 1(b)]. The east Pacific cluster was strongly supported by a 100% bootstrap score, as was the separation of the haplotypes from California (85%) and Chile (95%). Nucleotide divergence varied from 6.5

TABLE I. Pair-wise maximum likelihood distance matrix from cytochrome b gene sequences (under the TrN + I model) for species of *Trachurus* and *Decapterus punctatus*

	<i>T. japonicus</i>	<i>T. declivis</i>	<i>T. novaezelandiae</i>	<i>T. symmetricus</i>	<i>T. murphyi</i>
<i>T. declivis</i>	1.47				
<i>T. novaezelandiae</i>	1.09	1.67			
<i>T. symmetricus</i>	5.33	4.94	5.21		
<i>T. murphyi</i>	5.59	4.93	5.73	0.90	
<i>D. punctatus</i>	35.01	33.95	34.83	36.30	37.79

to 7.7% between the two main clusters, and from 1.4 to 2.6% between the west Pacific species. Divergence between North and South Pacific haplotypes ranged from 1.9 to 2.7%; variation among haplotypes from the same hemisphere was 0.1–0.5%. Under one 'standard' mtDNA clock calibration [*c.* 2% sequence divergence per Myr for Cytb and 5% for the CR (Johns & Avise, 1998)], the mtDNA lineages between *T. murphyi* and *T. symmetricus* separated *c.* 250 000 years ago.

Both cytochrome b and control region sequences showed a clear separation between the east and west Pacific taxa, which cluster into a well-supported group, clearly separated from the closely related species pair, *T. murphyi* and *T. symmetricus*. This result confirms the idea that these two east Pacific species are sister taxa. They are characterized by low Cytb divergence (mean \pm s.d. $0.88 \pm 0.28\%$) when compared to divergence values observed among other congeneric pairs of teleosts (Johns & Avise, 1998). Limited divergence, however, has been commonly found between many other pairs of sister-species (Johns & Avise, 1998; Baric *et al.*, 2003). The low divergence observed between *T. murphyi* and *T. symmetricus* may have accumulated after recent separation or have been present between previously separated lineages that are evolving at slower rates, as proposed by Cantatore *et al.* (1994). Regardless of this, the observed genetic distance is very close to those noted among groups of west Pacific taxa [Fig. 1(a)], all of which are recognized as 'good' species (Shabonev, 1980).

Clustering of D-loop haplotypes of *T. murphyi* and *T. symmetricus* also strongly supports monophyletic groups. There is strict allopatric distribution: no haplotype is shared between fishes from both hemispheres. In addition, the genetic divergence between the two taxa (1.7 to 2.4%) is much higher than that observed among haplotypes within each taxon (0.1 to 0.5%).

This study resolves the controversy regarding the taxonomic status of jack mackerel from California and Chile. Divergence between forms is similar to that observed among well-recognized west Pacific species pairs and haplotypes are distinctly clustered into two groups, reflecting their anti-tropical distribution. These results indicate the absence of gene flow between jack mackerel populations across the eastern Pacific tropics, and suggest that they have been isolated for at least 250 000 years. Jack mackerels from the North Pacific should be recognized as *T. symmetricus*, and those from the South Pacific as *T. murphyi*.

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