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Mitochondrial genealogy of Western Australian: applications of inbreeding coefficients and coalescent analysis for separating temporal population processes

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ABSTRACT

In Australian populations of barramundi *Lates calcarifer*, phylogenetic reconstruction of mtDNA sequences provided evidence of significant historical levels of gene flow, despite the substantial structuring of contemporary populations. The geographical pattern of mtDNA sequences among the populations was not congruent with previous evidence of a major disjunction between western and eastern populations of barramundi in Australia.

Keywords: F statistics; coalescent theory; mtDNA; barramundi phylogenies; gene flow.

INTRODUCTION

The usefulness of DNA-level polymorphism as a tool in fisheries science has been recognized for some time (Hallerman & Beckmann, 1988). As with many population genetic studies, fisheries surveys typically characterize the allele frequencies of a population at a given point, but may fail to elucidate the evolutionary progression of the population. For example, if a single population becomes subdivided, subsequent divergence in gene frequencies may be measured by the standardized variance in gene frequencies among populations (F_{ST}; Wright, 1965). F_{ST} values are often used to estimate gene flow, or genetically effective migration among populations from the theoretical relationship between gene flow and genetic differentiation (Wright, 1931). A long-standing problem in population genetics, however, is the extent to which gene frequency data can be used to separate genetic processes responsible for historical branching events, from demographic processes which influence geographical structure (Felsenstein, 1982).

The historical relationship among gene lineages and their comparison to geographical distributions (phylogeography, Avise, 1992) may be investigated through the study of animal mitochondrial DNA (mtDNA). Mitochondrial DNA is predominantly maternally inherited and therefore non-recombining in animals, including fish (Meyer, 1994). As such, mtDNA offers an insight into the maternal descent, or genealogy of populations. Estimates of genetic divergence and gene flow among populations from a coalescent or genealogical approach differ from those provided by F_{ST}, in that gene frequencies are ignored and historical genetic exchange can be inferred directly from the phylogenetic tree (Slatkin & Maddison, 1989; Barton & Wilson, 1995). Further, it has been argued (Edwards, 1993; Milligan *et al*., 1994) that unlike the traditional F_{ST} approach, genealogical analysis does not confound the effects of genetic factors (such as mutation) and demographic factors (such as population size and migration) on genetic markers, and is

therefore important in inferring the primary determinants of the distribution of alleles among populations.

Knowing the genealogy of a population offers fisheries managers an opportunity to understand better the evolutionary processes which have led to genetic differences among populations. This may assist decisions on whether fisheries require management on a multi-stock, rather than a single-stock, basis (Ovenden, 1990). Such rationales have been demonstrated clearly where the results of mtDNA studies have been considered in combination with historical geological events (Avise, 1994).

Barramundi *Lates calcarifer* (Bloch) is a tropical euryhaline species with a broad Indo-West Pacific range including northern Australia. Genetic differences among barramundi populations have been described for the Australian fishery eastwards from the Ord River (Fig. 1) in Western Australia (Shaklee *et al*., 1993; Keenan, 1994). Keenan (1994) thought the generally low levels of heterozygosity found in Australian barramundi were due to founder effects of populations that have recolonized rapidly many tropical Australian estuaries during the most recent changes in sea levels. According to this scenario, primary eastern and western source populations of barramundi were split by the Australia/New Guinea land bridge. At the eastern and western margins of the species' range, genetic drift caused loss of heterozygosity, but at the point of contact between the source populations, genetic heterogeneity increased (Keenan, 1994).

In this paper we demonstrate how diversity-based analyses of mitochondrial sequences (i.e. F_{ST} analogues) and coalescent analysis can provide different perspectives on population genetic structure and gene flow. The mitochondrial genealogies of representative, but broadly dispersed barramundi

populations across Northern Australia are used to reconstruct the zoogeographical history of Western Australian barramundi populations in the recent period.

MATERIALS AND METHODS

FIELD AND LABORATORY PROCEDURES

The sampling regime of Australian barramundi was biased in a westwards direction to sample polymorphism in the Kimberley populations in the Western Australian Ord and Fitzroy Rivers (Fig. 1), and to reveal polymorphic differences between those fish and adjacent but distant stocks. Thus, 14 fish were sampled from each of the Ord (ORD) and Fitzroy Rivers (FIT), 10 fish from Darwin (DAR) and five fish from Cairns (CAI) (Fig. 1). One barramundi sample from New Guinea provided an outgroup for phylogenetic analysis.

Caudal fin clips were taken from adult and juvenile barramundi. Tissue samples were shaken in a vortex in 250 µl 0·1% Triton X-100 (Sigma) for 1 min. Fifty microlitres of 100-mg ml^{-1} proteinase K (Sigma) were added to the tube and the mixture heated for 1 h at 50) C with occasional mixing. Twenty-five microlitres of SET buffer (5% SDS, 50 mM EDTA, 500 mM Tris pH 8) (Sigma) were added to the reaction and a single extraction of total genomic DNA was performed with 500 µl 1 : 1 phenol : chloroform/isoamyl alcohol (Sigma). The aqueous layer was removed to a new tube and 25 µl of 3 M sodium acetate (Sigma) and 250 µl isopropanol (BDH) added. DNA was precipitated from the solution at -20º C for 30 min and then pelleted at 13 000 rpm for 15 min at room temperature. The DNA pellet was washed once with 70% ethanol (BDH), dried and resuspended in 50 µl sterile water.

Primers described by Chenoweth *et al*. (1988a) were used to amplify a 290-bp fragment within Region 1 of the barramundi mitochondrial control region. Polymerase chain reactions (PCR) consisted

of polymerase reaction buffer (67 mM Tris–HCl pH 8·8, 16·6 mM $(NH_4)_2SO_4$, 0·45% Triton X-100, 2 mg ml^{-1} gelatin) (Biotech), 0·05 µM of each primer (Research Genetics), 0·5 units of *Taq* polymerase (Biotech) 250 µM dNTPs (Biotech), 2 mM $MgCl_2$ (Sigma) and 20 ng of target DNA in a 10-µl reaction. Amplifications were done in capillary tubes on a MJ research minicycler. Thermocycling conditions were an initial denaturing step of 94º C for 5 min followed by 35 cycles of 94 for 30 s, 40 for 30 s, 72 for 60 s and a final step of 72 for 5 min. Five µl of each reaction was run on a 2% agarose gel in 1 x TAE buffer (Sambrook *et al*., 1989) at 70 V for 30 min using pUC19 *Hpa*II (Biotech) standards of known fragment size. DNA was visualized by staining with ethidium bromide. PCR products were cleaned following the manufacturer's protocol (QIAquick). Double-stranded DNA was quantified fluorescently using the minigel method (Sambrook *et al*., 1989).

Thermal cycle sequencing involved the mixing of fluorescently labelled dideoxynucleotide triphosphates, template DNA and PCR primers to make a 10-µl reaction (Applied Biosystems). The sequencing reactions were done in capillary tubes on a MJ research minicycler. Sequencing conditions involved 96º C for 30 s, followed by 50 for 15 s and 60 for 4 min, and the cycle was repeated 25 times.

Excess dideoxynucleotide triphosphates were removed from samples by ethanol precipitation (Applied Biosystems). For each sample, 25 µl of template suppression reagent (Applied Biosystems) was added, briefly centrifuged, and denatured in a Perkin Elmer 9600 thermocycler preheated to 95º C for 2 min. Each sample was transferred to an ABI 310 Genetic Analyzer and run for 80 min. The double-stranded sequences were aligned, and the light strand sequence was resolved using the Sequence Navigator 1.0.1 software package (ABI Prism). Light strand sequences were aligned by eye using MacClade 3.03 (Maddison & Maddison, 1992).

PHYLOGENY OF SEQUENCES

Genetic distances between barramundi sequences were estimated from Kimura's (1980) two-parameter corrective model which accounts for the observation that base transitions and transversions occur at different rates (we used a transition : tranversion ratio of 8 : 1; see results), but assumes equal frequencies of base change over the length of the sequence. PHYLIP 3.5 (Felsenstein, 1993) was used to estimate pairwise genetic distances between all barramundi sequences. We used PHYLIP 3.5 to construct Fitch–Margoliash and neighbour joining trees from the matrix of genetic distance between sequences, and very similar trees were produced. Since these trees were also similar to minimum-length trees produced by maximum parsimony using PAUP (Swofford, 1993), only the Fitch–Margoliash tree is presented here.

GENETIC DIVERSITY WITHIN AND BETWEEN POPULATIONS

Diversity within populations was measured by the number of mitochondrial haplotypes, genotype (haplotype) diversity (*h*) calculated by the methods of Nei (1987), the number of polymorphic sites, and Nei's (1987) estimate of nucleotide diversity (π) calculated using DnaSP 2.0 (Rozas & Rozas, 1997). Transition/transversion ratios were estimated by direct counts.

Diversity among populations was measured by the average number of nucleotide differences (*k*; Tajima, 1983), and the average number of nucleotide substitutions per site (d_{xy}; Nei, 1987), calculated using DnaSP 2.0.

Excoffier *et al*. (1992) describe an analysis of molecular variance (AMOVA), derived from a matrix of squared distances among all pairs of genotypes, which produces variance estimates that reflect the correlation of genetic diversity at different levels of hierarchical subdivision. AMOVA 1.05 (Excoffier *et al*., 1992) was used to estimate φ_{ST}, which is analogous to F_{ST} (Reynolds *et al*., 1983).

Genetic distances between sequences were estimated from Kimura's (1980) two-parameter model as previously described. To test for population subdivision among all four Australian barramundi populations, we compared all genotypes. To test for significant population subdivision between Darwin and Kimberley populations, and then between only Kimberley populations, the Cairns and then Darwin samples were removed systematically from the analysis. One thousand permutations for each analysis were performed to give statistical significance levels. To estimate gene flow (*Nm*) for each hierarchical test of population subdivision we used the formula of Wright (1931), but corrected for the haploid nature of the mitochondrial genome:

$$Nm_f = 0{\cdot}5(F_{ST}^{-1} - 1)$$

where Nm_f is female gene flow, following Hudson *et al*. (1992).

GENEALOGICAL ANALYSIS

An alternative estimation of gene flow by methods other than F_{ST} analogues is based upon the coalescent model (Hudson, 1990), and estimates by calculation of coalescent events, how closely a given phylogeny constructed for mtDNA sequences approximates the random-mating island model of population genetic structure. The model (Slatkin & Maddison, 1989) assumes that mtDNA samples have been taken from distinct geographical areas, so each sampling location is regarded as a state character. A parsimony reconstruction of geographical states, following the procedure of Slatkin & Maddison (1989), and implemented with MacClade 3.03, gave the minimum number of migration events (s) consistent with the data. We used the Fitch–Margoliash phylogeny, with the PNG sequence deleted, in the analysis. Hudson *et al*. (1992) and Edwards (1993) have demonstrated that estimates of gene flow using the method of Slatkin & Maddison (1989) are comparable across tree-making methods. The phylogeny was resampled one thousand times to compare the observed s value with its null distribution assuming panmixis.

The standard error of the estimate of s was calculated by jackknifing over the 22 different Australian genotypes (see below). This involved constructing new phylogenies after the systematic removal of each genotype and recalculating s values.

To estimate *Nm* from *s*, genotypes from the Darwin and Kimberley samples were removed randomly to equalize sample sizes and the smallest sample (Cairns) was discarded (following Slatkin & Maddison, 1989; see also Edwards, 1993). *s* was recalculated and scaled for two sampling locations. The resulting value was used to obtain an approximate estimate of *Nm* by interpolation from Table 1 in Slatkin & Maddison (1989).

RESULTS

DIVERSITY WITHIN POPULATIONS

We found 22 different genotypes among the 43 Australian barramundi mtDNA control region sequences, each being 231 nucleotide bases in length (Table I). Four genotypes were present in the fish sampled from Darwin and nine in each of the two Kimberley rivers. The five fish sampled from Cairns were represented by only a single genotype. Five fish from the Fitzroy River and six fish from the Ord River shared the only mtDNA genotype that was present in more than one geographical location.

Like most studies of mtDNA, transitions outnumbered transversions. The large transitional basis in Ord River fish (28 : 1) may indicate a relatively high rate of mutation occurring within that population. There were 46 polymorphic sites among all sequences. The Darwin sample contained only four polymorphic sites, much fewer than the Fitzroy River sample (17 sites) or the Ord River sample

(25 sites). Genotype diversity (*h*) was high for all populations. There were large differences in within-population nucleotide diversity (π) among populations (Table I), but the only significant difference was between the Darwin and Ord River populations ($P<0{\cdot}05$, with a Bonferroni correction).

DIVERSITY AMONG POPULATIONS

The average number of nucleotide differences per site and the average number of nucleotide substitutions per site, between populations, indicated the distinctiveness of the Darwin population and the similarity of the Kimberley and Cairns populations (Table II), which may be an artifact of the small sample size and monomorphic nature of the Cairns sample.

There was significant population structuring when all four populations were analysed (Table III), with 56% of the observed nucleotide diversity due to differences among populations. Further, when Cairns fish were removed from the analysis, a φ_{ST} value of 0·526 confirmed the well-defined genetic structuring among the Kimberley and Darwin populations. Under the island model, these values are equivalent to a low predicted gene flow among populations of 0·39–0·45 individuals per generation. There was little genetic structuring between the Ord and Fitzroy River populations in Western Australia, with φ_{ST} not significantly different from 0 and an estimated gene flow of at least five individuals per generation.

PHYLOGENETIC DISTANCE AND THE RETENTION OF ANCESTRAL LINEAGES

The Cairns fish formed a separate group, but were associated with a major clade that incorporated 75% of Kimberley fish (Fig. 2). The Darwin population grouped with the other significant clade containing the remaining 25% of Kimberley fish. These clades were quite distinct, with about 3% sequence divergence. Within the Cairns clade, that group, and a single divergent fish (ORD 8) were

separate from a second clade that contained 85% of the Fitzroy River fish and 57% of the Ord River fish. In this clade, FIT 4, 9, 10, 13 and 14 shared genetic identity with ORD 1, 2, 3, 5, 12 and 13. The PAUP analysis produced 14 minimum length trees that all shared the major structure of Fig. 2, but differed in their placement of genotypes within the Darwin/Ord/Fitzroy River clade.

Although only the Ord and Fitzroy River populations shared identical sequences (Fig. 2), the sharing of ancestral sequences among populations was more widespread. For example, the Fitzroy and Ord genotypes in the first clade were related more closely to the Cairns genotypes than to the other genotypes from the Kimberley. In the second clade, ORD 4, 9, 10 and 14 were related more closely to the Darwin genotypes than to FIT 11, ORD 7 and FIT 8.

GENEALOGICAL ANALYSIS OF BARRAMUNDI PHYLOGENIES

Slatkin & Maddison's (1989) algorithm estimated a minimum number of five between-population migration steps (s=5±0·07 S.E.) in the barramundi phylogeny whether constructed using the Fitch–Margoliash algorithm or maximum parsimony. This was significantly less (P<0·001) than the value (s=23) predicted from a panmictic population structure. Estimated Nm_f among Darwin and Kimberley barramundi populations ranged between 1·0 and 2·0 individuals migrating among populations per generation in different resamplings, substantially greater than the Nm_f estimate of 0·45 provided by φ_{ST}.

DISCUSSION

CHARACTERISTICS OF BARRAMUNDI mtDNA

The large amount of polymorphism thought to characterize the mtDNA control region of fish (Bernatchez *et al*., 1992; Meyer, 1994) is also a feature of barramundi populations. Estimates of mtDNA variation (*h* and π) for barramundi were generally higher than those reported for a range of coastal and marine species including the catadromous eel *Anguilla rostrata* (Le Sueur) (see Table 2 in Avise, 1992), but are similar to values estimated by Chenoweth *et al*. (1998*a*) for the mtDNA control region in Australian barramundi populations. The absence of mtDNA variation in the population from Cairns may be due to the small sample size, although Chenoweth *et al*. (1988*b*) also reported low mtDNA diversity in eastern Australian populations of barramundi, possibly resulting from a population bottleneck during recent evolution. The overall transition/transversion ratio (8 : 1) complies with the general model of piscine mtDNA evolution (5–10 : 1; Meyer, 1994), but with two dominant features; first, mutation rates in this section of the mtDNA control region are very high, and second, the differences between populations suggest that they may be evolving at different evolutionary rates (Kocher *et al*., 1989; Meyer *et al*., 1990; Fajen & Breden, 1992), although our estimates are based on small sample sizes and standard errors may be large.

POPULATION SUBDIVISION AND GENE FLOW

Although the relatively low number of individuals and populations sampled risks underestimating existing genetic diversity, the φ_{ST} estimate of 0·56 for all Australian populations indicates a substantial population genetic structure for Australian barramundi (see Lynch & Crease, 1990: table 4; Bernatchez *et al*., 1992). This inference still holds when Cairns fish are removed from the analysis (φ_{ST} T=0·53), but not when the analysis is restricted to only Kimberley populations (φ_{ST} =0·08). All frequency-based estimates of gene flow outside the Kimberley predict *Nm* of less than 0·5, suggesting

that populations may differentiate through genetic drift (Wright, 1931). Our estimates of φ_{ST} are one to two orders of magnitude larger than the allozyme-based F_{ST} estimates for eastern and northern Australian barramundi populations (0·004–0·064) given by Shaklee & Salini (1985), Shaklee *et al.* (1993) and Keenan (1994), but are similar to mtDNA sequence-based φ_{ST} estimates for the same populations given by Chenoweth et al. (1998*a*; 0·328). These results may indicate that population subdivision is greater for mtDNA than for nuclear genes, which is a common empirical finding (Hallerman & Beckmann, 1988; Billington & Hebert, 1991; Nielsen *et al*., 1996; Chenoweth & Hughes, 1997). Birky *et al*. (1983) showed that the haploid nature and maternal inheritance of mtDNA produced a fourfold smaller effective population size than for nuclear genes in the same population. For barramundi, which are sequentially hermaphroditic, the reduction in effective population size will not be as great, but in any case, the magnitude of the difference between the estimates of φ_{ST} for mtDNA and of F_{ST} for allozyme loci seems much greater than can be accounted for by differences in effective population sizes between nuclear and mitochondrial genes.

The phylogenetic analysis of relationships among genotypes showed a somewhat different picture to that derived from the φ_{ST} analysis. The new information provided by the phylogeny showed that although extant sequences were largely confined to different populations, thereby giving high estimates of population subdivision and low estimates of gene flow, ancestral sequences were more widely spread among the Darwin and Kimberley populations. Theoretically, this pattern can arise either because of historical gene flow, or because of the retention of ancestral genotypes among populations (Slatkin & Maddison, 1989). Slatkin & Maddison (1989) simulated the relationship between the expectation of s and t/N_e, the ratio of the number of generations since divergence of two populations and effective population size for mitochondrial genes, assuming that divergence is caused solely by random drift with no migration. Scaling our value of s=5 for two populations, we obtain t/N_e~0·25. Assuming a generation time for barramundi of 5 years (Keenan, 1994) and allowing a separation time of 200 000 years between populations (see next section), then N_e=160 000. That is, given the likely separation time of the populations, an effective population size of 160 000 is required

to retain sufficient ancestral mtDNA genotypes to give the observed value of *s*. This is much greater than the estimated N_e of 2000–3500 for Australian barramundi (Keenan, 1994), especially considering the reduced effective population size of mtDNA compared with nuclear genes. This suggests that historical gene flow is a much more likely explanation of the observed mtDNA phylogeny than is the retention of ancestral genotypes.

The estimated gene flow from the phylogeny (Nm=1–2, calculated assuming an island model of population structure) suggests, in contrast to the gene flow estimated from the variance of gene frequencies among populations, that genetic drift is not likely to be a significant force in promoting genetic differentiation. The apparent discrepancy between substantial genetic structure indicated by the variance in gene frequencies among populations, and the number of reconstructed migration events, may be explained partly by high maturation rates in the mtDNA region analysed. The control region of mtDNA is thought to evolve five to 10 times faster than single copy nuclear DNA (Moritz *et al*., 1987). This may obscure the history of migration of mtDNA genotypes, unless the phylogeny of the mtDNA lineages is tracked. The coalescent analysis assumes that the intraspecific phylogeny shown in Fig. 2 is accurate, and represents the true phylogeny of mtDNA sequences. We found very similar trees using both neighbour-joining and parsimony methods. Although repeatabilty is not necessarily a reliable indicator of accuracy (Hillis & Bull, 1993), our resampling of the phylogeny indicated little variance in *s*.

RECENT HISTORY AND ZOOGEOGRAPHY OF WESTERN AUSTRALIAN BARRAMUNDI

Climates of the world have been characterized by a series of glacial–interglacial cycles for at least 2·5 million years (Berggren *et al*., 1980), and most probably well before this (Aplin *et al*., 1993). A glacial peak *c*. 125 000 years BP is thought to have reduced northern Australian sea levels to at least 150 m below present levels (Chappell, 1983; Shackleton, 1987), thus forming a land bridge between Australia and New Guinea (Fig. 1). A further glacial peak *c*. 18 000 years BP is thought to have had

similar effects (Chappell, 1983), with sea levels often 50–60 m below present levels during the period 70 000–10 000 years BP (Torgersen *et al*., 1983, 1985; Fig. 1). Only during the peaks of the warmest periods, 7000 years BP to present and *c.* 115 000 years BP, were sea levels sufficiently high to open the Torres Strait (Keenan, 1994), and for the remainder of the time, perhaps 108 000 years or so, the eastern and western populations of barramundi would have been isolated.

Chenoweth *et al*. (1998*b*) identified two mtDNA lineages, with 4% sequence divergence, in Australian barramundi sampled from the eastern and northern range of the species (but not extending as far west as the Ord River). They interpreted these lineages as arising from the separation of eastern and western populations with the closure of the Torres Strait. The lineages were not totally restricted to particular sides of the Torres Strait, but showed opposing clines in frequency, which Chenoweth *et al*. (1998*b*) interpreted as secondary introgression via contemporary gene flow. Using the divergence rate of ATPase genes as a calibration, Chenoweth *et al*. (1998*b*) suggested that the lineages separated *c.* 335 000 years ago.

We found a similar level of divergence (3%) between the two major mtDNA lineages in this study (Fig. 2). Using the calibration of Chenoweth *et al*. (1998*b*), the two major clades in our study separated *c.* 203 000 years ago, again prior to the last opening of the Torres Strait. However, the geographical pattern of our genealogy does not fit easily the vicariant scenario of Chenoweth *et al*. (1998*b*). The major separation in our genealogy is between all the Cairns and 75% of the Kimberley genotypes on the one hand, and all the Darwin and 25% of the Kimberley genotypes on the other hand. In the study of Chenoweth *et al*. (1998*b*), the eastern lineage had the highest frequency in Cairns and the western lineage had the highest frequency in Darwin. It seems unlikely that this pattern could be explained by extensive gene flow between eastern and western populations after the most recent opening of Torres Strait. The extensive mtDNA differences between some Kimberley and Darwin genotypes may have arisen separately through isolation by distance over the broad

geographical range of the western lineage independent of the vicariant scenario of Chenoweth *et al*. (1998*b*). Gene flow between northern and southern regions of the western lineage may have been restricted further by the deepening of the Ord River basin and the gulf above Darwin as the sea levels rose to 50–60 m below present levels towards the end of the glacial maximum (Fig. 1). Further phylogeographical explorations of Kimberley populations of barramundi are required to test these hypotheses.

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Figure 1. Regions of northern Australia and southern New Guinea showing two depth contours (adapted from Keenan, 1994), and sampling locations.

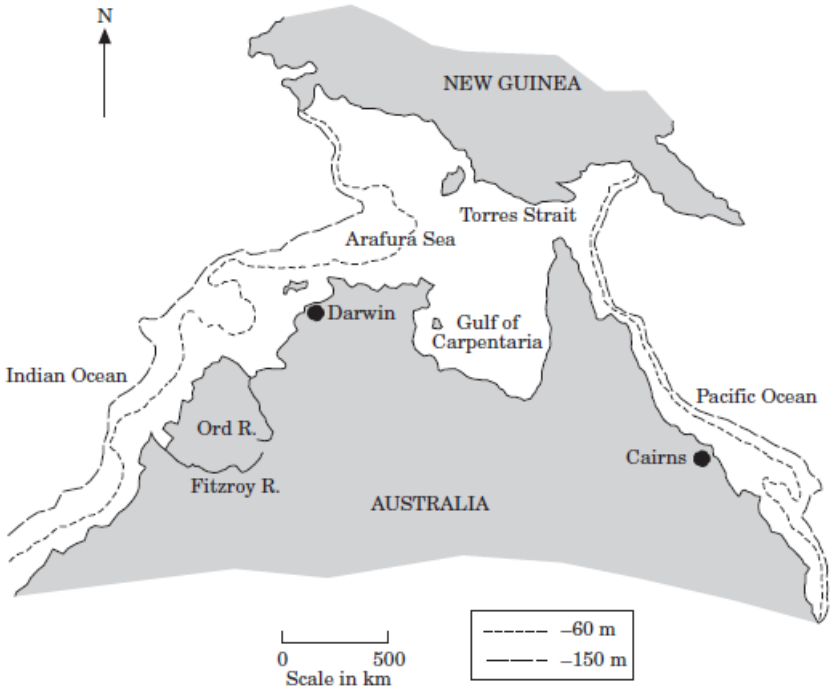

Figure. 2. Fitch–Margoliash tree showing reconstructed phylogenetic relationships among 22 mtDNA control region genotypes for Australian barramundi. Scale bar represents 1% sequence divergence, estimated after Kimura (1980).

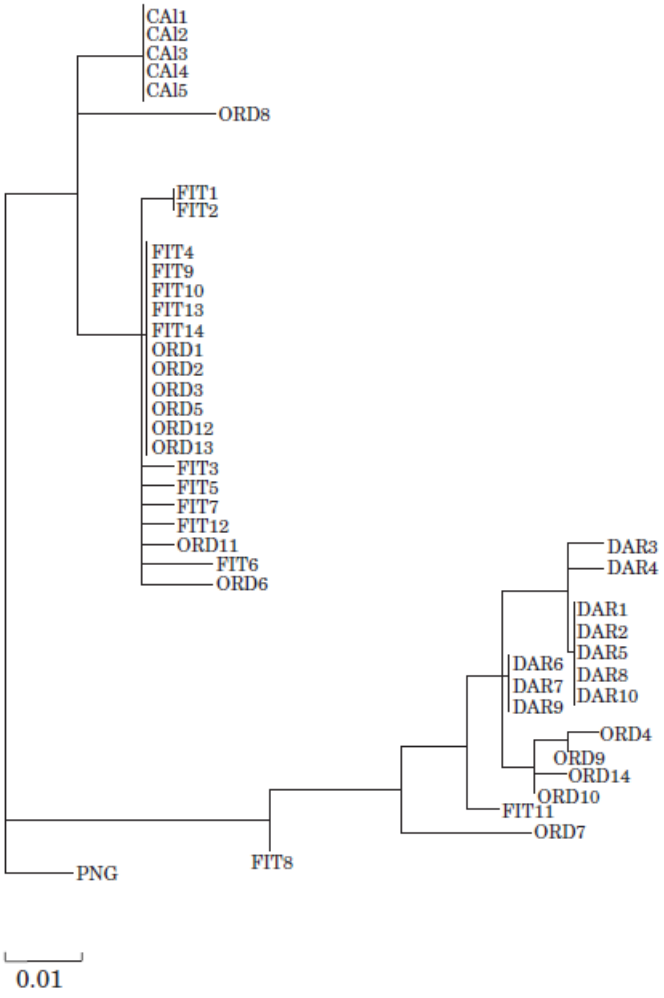

Table I. Estimates of genetic diversity in the mtDNA control region within four Australian populations of *Lates calcarifer*

Population	Sample size	No. of genotypes	T/T ratio	No. of polymorphic sites	h ± S.E.	π ± S.E.
Cairns	5	1		0	0	0
Darwin	10	4	9·5 : 1	4	0·711 ± 0·239	0·006 ± 0·001
Fitzroy River	14	9	4 : 1	17	0·897 ± 0·045	0·017 ± 0·006
Ord River	14	9	28 : 1	25	0·836 ± 0·057	0·040 ± 0·006
Total	43	22	8 : 1	46	0·911 ± 0·111	0·033 ± 0·002

T/T ratio, Ratio of transitions to transversions; *h*, haplotype diversity; π, nucleotide diversity.

TABLE II. Estimates of genetic diversity in the mtDNA control region between populations of barramundi in Australia; average number of nucleotide differences per site (k) above diagonal and average number of nucleotide substitutions per site (d_{xy}) below diagonal

	Cairns	Darwin	Fitzroy River	Ord River
Cairns	—	9·4	4·12	8·31
Darwin	0·08	—	7·62	9·00
Fitzroy River	0·02	0·05	—	5·45
Ord River	0·03	0·05	0·02	—

TABLE III. Estimates of φ_{ST} and gene flow (Nm) for different groupings of Australian barramundi populations

Populations	φ_{ST}	Nm
Cairns *v.* Darwin *v.* Ord *v.* Fitzroy	0·563***	0·39
Darwin *v.* Ord *v.* Fitzroy	0·526***	0·45
Ord *v.* Fitzroy	$0·084^{NS}$	5·45

***Indicates φ_{ST} is significantly greater than 0 (P<0·001).