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GENETIC AND DEMOGRAPHIC VARIATION AMONG DIFFERENT COLORATIONS OF THE EASTERN SOUTH PACIFIC FISH "JERGUILLA" (*APLODACTYLUS PUNCTATUS* VALENCIENNES, 1832) (PERCIFORMES: APLODACTYLIDAE)

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ABSTRACT

Colour variation and molecular diversity of *Aplodactylus punctatus* were addressed with DNA sequence data. Mitochondrial cytochrome b and region control sequences were obtained from representatives of three Chilean populations of this species. Substantial levels of molecular diversity were detected, although there are not relation between the haplotypes obtained and geographical distribution or colour. We consider factors such as geographic distribution, population size, dispersal, secondary contact, and phylopatry as potential causes of the high level of mtDNA nucleotide diversity in this species.

Key words: DNA mitochondrial, South Eastern Pacific, Littoral fishes, Chile.

RESUMEN

Variación genética y demográfica entre las distintas coloraciones de la "Jerguilla" del Pacífico Suroriental (*Aplodactylus punctatus* Valenciennes, 1832) (Perciformes: Aplodactylidae). La variación en color y la diversidad molecular de *Aplodactylus punctatus* se estudió con secuencia de ADN. El citocromo mitocondrial b y la región de secuencia de control se obtuvieron de tres poblaciones chilenas representativas de esta especie. Aunque se detectaron niveles sustanciales de diversidad molecular no se encontró relación entre los haplotipos encontrados y la distruibución geográfica o coloración de la "jerguilla". Consideramos como causas potenciales de los altos niveles de diversidad de los nucleotidos mtADN en esta especie, factores tales como distribución geográfica, tamaño de la población, dispersión, contacto secundario y filopatría.

Palabras clave: ADN mitocondrial, Pacífico Suroriental, Peces litorales, Chile.

INTRODUCTION

A great deal of morphological and behavioural variation exists in natural populations of most species, yet empirical evidence for the role of this variation in establishing genetic differentiation and, ultimately, speciation remains limited (reviewed in Lynch 1989). Biological species concepts have had become more inclusive during the last 50 years as taxonomists, ecologists, evolutionary biologists and palaeontologists have brought morphological, distributional, reproductive and phylogenetic perspectives to their definition. Currently, one can choose from at least four different models emphasizing shared or divergent characters interpreted in static or dynamic schemes (Gosling, 1994). However, it is clear that allopatric speciation through vicariant events and colonizations have played a key role in the diversification of terrestrial, aquatic animals as well as plants (Avise, 1994) .

Calls for a more pluralistic paradigm (Mishler & Donoghue, 1982) are a pragmatic recognition that speciation is a dynamic process that has not followed the same pathway for all organisms nor attained a single state for those that share common characters (Avise, 1994). In fact, even in the absence of geographical barriers, it is thought that variation can lead to partial or complete reproductive isolation between sympatric populations through a reduction in gene flow (Bush, 1994). The challenges inherent in identifying instances of sympatric speciation have contributed to ongoing debates concerning its importance. For instance, in marine science the usual presumption is that the large range of larval stages and the high fecundity of marine organisms are associated with high gene flow over vast distances (Williams & Benzie, 1993). However, the full extent of cryptic speciation as a result of limited gene flow is only just beginning to be appreciated (Knowlton, 1993). Research on morphological diversification and speciation in aquatic systems (Pigeon *et al.*, 1997: Nagel & Schluter, 1998) has highlighted the potential for the establishment of reproductive isolation and possible speciation in sympatry. In addition to these empirical studies, a series of recent theoretical treatments has attempted to identify evolutionary conditions that promote sympatric speciation (Dieckmann & Doebeli, 1999; Kondrashov & Kondrashov, 1999). Often minor morphological variations, such as colour patterns, has been identified as possible conducive of sympatric speciation.

Many fish have sexually dimorphic colouration, or changes in colour reflecting moods of individuals (Thresher & Moyer, 1983). But more intriguing are some species with permanent polychromatism, which is unrelated sex, like *Aplodactylus punctatus*. This species is distributed from Paita, Perú to Concepción, Chile (Chirichigno 1974), it is an herbivorous fish living in association with large brown algae (v. gr. *Lessonia trabeculata*) (Nuñez & Vásquez 1987, Cáceres *et al.* 1993, Angel & Ojeda 2001), and because of the presence of different colorations of the body, mainly green mottled with black spots and yellow with black spots, they were considered as different species, but recent revision of the genus (Burridge, 2000, Russel, 2000) recognize only *Aplodactylus punctatus*.

In an effort to measure and describe preliminarly the population structure that might be congruent with possible sympatric speciation in the Chilean populations of *A. punctatus*, we amplified and sequenced the first half of the cytochrome b gene and the most variable segment of mitochondrial DNA (mtDNA), the control region. Our combined sequence data analyses were used in an effort to clarify the relative roles of geographical and ecological factors (polychromatism) in promoting reproductive isolation within *A. punctatus*.

MATERIALS AND METHODS

Fresh specimens of *A. punctatus* were collected in 2001 from the following areas of Chile: Antofagasta (23° 29'S), Coquimbo (29° 57'S) (northern-centre) and Concepción (36° 50'S) (southern). Tissue of heart and muscle were stored in ethanol and frozen at –20°C prior to DNA extraction, which was performed following a previously published extraction protocol (Quinteiro *et al.*, 2000). The first half of mtDNA control region was amplified with new primers; TRA-pro 5'TCCCACCTCTAACTCCCAAAGCTAG-3' y DA-L 5'GGCCGTGAAWTAGGAACCARATG and the first half of cytochrome b gene with the universal primers L141841 y H15149 (Kocher *et al.*, 1989) under previously published reaction conditions (Burridge, 2000). Sequencing reactions was followed the protocol described in Quinteiro *et al.* (2000). The DNA sequences were aligned with Clustal W program, and PAUP (versión 4.0b2; Swofford, 1998) was used for the phylogenetic analysis.

RESULTS

Cytochrome b

The molecular variability of the first half of the cytochrome b gene was explored preliminarily, two individuals were analysed from the sampling sites of Coquimbo (22 P, "Queen" coloration (yellowish)) and Coquimbo (15P, "Green" coloration). It was sequenced a 438 bp fragment with one variable position between both haplotypes (position 260: G↔C). In relation with the haplotype reported by Burridge (2000; AF133060) two variable positions were obtained, both in third positions (288: G↔C and 294: C↔G). The new sequence obtained in the present study (15 p) has been deposited in the GenBank, access code AY074890.

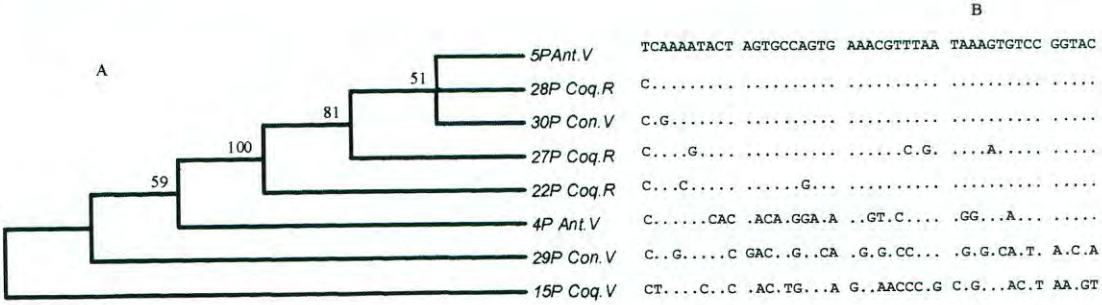
Control region

In order to further explore genetic differentiation, we sequenced a 360 bp fragment of the mtDNA control region from a random subset (n = 8; from Antofagasta, Coquimbo and Concepción) of *A. punctatus* specimens, including those used for the cytochrome b sequenced previously. Base composition was strongly biased: adenine and thymine were represented roughly equally, occurring at mean (± SE) percentages of 36.4 ± 0.28% and 31.0 ± 0.16%, respectively, while cytosine occurred at 17.1 ± 0.29% of sites, and guanine at only 15.4 ± 0.18% of sites. These biases are similar to those found in the mtDNA control-region of other fishes, (Bernatchez & Danzmann 1993). The numbers of parsimonious and variable characters were of 12 and 45, respectively (Fig. 1A). A considerable level of diversity was detected between the 8 haplotypes sequenced, with a mean of 4.5 % (range 1.0 – 8.0) of sequence divergence (model utilised Tamura & Nei, 1993; Table 1).

TABLE 1. Genetic-distance matrix (Tamura – Nei model) of *A. punctatus* mtDNA control region sequences. Alphanumeric codes internally assigned. Coq; Coquimbo, Con; Concepción; Ant; Antofagasta, V; Green coloration's, R; Queen coloration's. Haplotypes sequences have been deposited in the GenBank, access. code AF467432, AF467433, AF467434, AF467435, AF467436, AF467437, AF467438 and AF467439.

Haplotypes	1	2	3	4	5	6	7
1. 5p Ant. V							
2. 4P Ant. V	0.05						
3. 15P Coq. V	0.08	0.07					
4. 30P Con. V.	0.01	0.05	0.08				
5. 29P Con. V.	0.06	0.06	0.07	0.06			
6. 22P Coq. R	0.01	0.06	0.08	0.01	0.07		
7. 27P Coq. R	0.01	0.05	0.08	0.01	0.07	0.02	
8. 28P Coq. R	0.01	0.05	0.07	0.01	0.06	0.01	0.01

The neighbour-joining (NJ) tree (Saitou and Nei, 1987) indicated that all 8 haplotypes were closely related. There are not well-differentiated clades and no obvious association with geographical locations or potential taxonomic distinctions with polychromatism base (Fig. 1B). The overall pattern of the minimum spanning network shown in Figure 2 is similar to that suggested by the NJ analyses, and includes 'starlike' elements with several haplotypes closely related to the most common haplotype However, a number of alternative relationships were suggested by the network.



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TCAAAATACT AGTGCCAGTG AAACGTTTAA TAAAGTGTCC GGTAC
C......... .......... .......... .......... .....
C.G....... .......... .......... .......... .....
C....G.... .......... ......C.G. ....A..... .....
C...C..... ......G... .......... .......... .....
C......CAC .ACA.GGA.A ..GT.C.... .GG...A... .....
C..G.....C GAC..G..CA .G.G.CC... .G.G.CA.T. A.C.A
CT....C..C .AC.TG...A G..AACCC.G C.G...AC.T AA.GT
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FIGURE 1. A) Neighbor-joining tree of the relationships between *A. punctatus* mtDNA control region haploytpes. The numbers at each node represent bootstrap proportions based on 1000 replications. Alphanumeric abbreviations as in Table 1. B) Variables characters observed in the first half of the mtDNA control region.
Alphanumeric abbreviations as in Table 1.

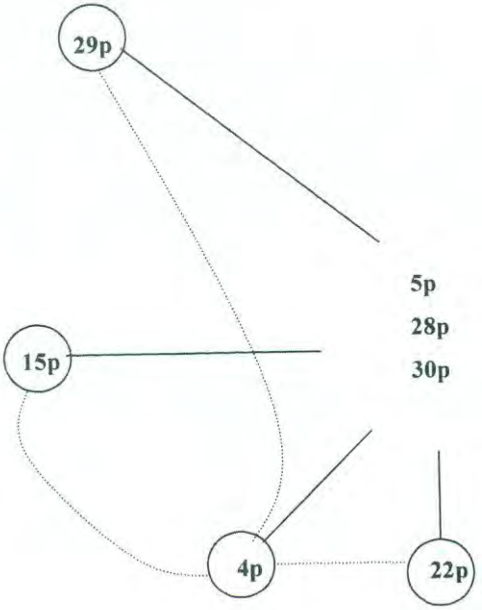

FIGURE 2. Minimum spanning network among mtDNA control region haplotypes, based in a 95% parsimonious criterion. Dashed lines indicate groupings in alternative minimum spanning network. Alphanumeric abbreviations as in Table 1.

DISCUSSION

Molecular Diversity

An important result of this investigation is the substantial molecular diversity detected at intraspecific level. This result indicated a number of key points such that there is not genetic segregation between the coloration analysed and that one of the populations evaluated of *A. punctatus*; which represents only one panmitic unit. This elevated molecular diversity is accompanied with a considerable evolutive divergence between haplotypes. Although there are not relationship between the obtained haplotypes and geographical distribution or coloration, these characteristics are more associated generally with pelagic organisms.

The unusually high levels of mtDNA diversity detected in *A. punctatus* may be interpreted in a number of ways. First, it may simply reflect an ancient population divergence coupled with morphological stability. An alternative explanation is that gene flow may be facilitated by male dispersal, while the female *A. punctatus* may be strongly philopatric. Such scenario would lead to the divergence of maternally inherited mtDNA, without any associated divergence in nuclear DNA or morphology. However, there is no evidence for such gender-biased dispersal in this species. A third alternative is an unusually rapid rate of mtDNA evolution, but this possibility is more unlikely given that others members of Suborder, such as Cheilodactylidae, seems to present a normal rate of evolution. Likewise the origin of the genus *Aplodactylus* seems to be recent (Burridge, 2000).

In general, species with large evolutionary effective population sizes are thought to harbor higher levels of molecular diversity than species with historically low or unstable populations sizes (Avise, 1994). Therefore high levels of mtDNA diversity may indicate that *A. punctatus* is an ancient lineage that has historically maintained very large population sizes, preventing lineage extinction and allowing the gradual accumulation of mtDNA diversity. Nonetheless, this alternative does not accord with the ecological and population characteristic of *A. punctatus*. In fact, Cáceres *et al.* (1993) have indicated that populations from near Antofagasta have low individuals abundance rather than southern populations near Valparaíso. Also, *A. punctatus* has a diet based mainly on *Lessonia trabaculata* (Nuñez & Vásquez, 1987, Cáceres *et al.*, 1993), which probably limits its abundance and latitudinal distribution.

A final possibility that we can propose is that ours results indicate a historical population divergence that has been eroded with subsequent gene flow. Moreover, changes in climatic or oceanographic conditions may have resulted in increased dispersal and mixing of isolated populations.

Coloration variation

Although the methodologies underlying studies of possible cases of sympatric speciation (Meyer, 1993; Pigeon *et al*., 1997; Dieckmann & Doebeli, 1999) differ in several important respects, there are common patterns consistent with classical studies (Maynard Smith, 1966) identifying two key characteristics that are conductive to sympatric speciation. Variation in an ecological trait (such as differences in resource use or polychromatism) is necessary for disruptive selection against intermediates to take place (Losos, 2000). Assortative mating drives this disruptive selection and leads the divergent subpopulations along separate evolutionary trajectories resulting in speciation in as few as 300 generations (Dieckmann & Doebeli, 1999). However, and spite of this theoretical framework and of the natural colour variation observed in *A. punctatus*, the absence or fixed differences between the coloration of this species suggested that the colour morphotypes represent a single polymorphic species in accordance with the current taxonomy. While often it has been proposed that the permanent polychromatism represents distinct species (Domeier, 1994), this does not apply to marine fish where almost all situations can be observed, including cryptic species (distinct species with similar colour morphs) or distinct species interbreeding and producing hybrids, or intermediate cases such *Hypoplectrus unicolor* (Walbaum) (Graves & Rosenblatt, 1980). In *H. unicolor* the authors did not find genetic differentiation and concluded that colour morphs interbred within the same species. Therefore, it is possible that the observed cases of *A. punctatus* represent a marine fish generality of colour variation and not the exemption of sympatric speciation. However, still is it possible that colour variation in *A. punctatus* can represents other types of segregation related, for instance, to depth or complex behavioural. In *Acathochromis polyacanthus*, white and black-white morphotypes occurred at different depths while their allozymic structures did not distinguish the colour morphotypes (Planes & Doherty, 1997). The rockfish species *Sebastes chrysomelas* (Jordan & Gilbert) and *S. carnatus* (Jordan & Gilbert) are morphologically and meristically identical, but differ in colour morphs and habitat (Larson, 1980). mtDNA control region sequences (G. Bernardi, pers. comm. Italy, 2002) failed to show fixed differences, suggesting that the two species are colour morphotypes of a single species. In *Paracirrhites arcatus* (Cuvier), it has been hypothesised that the patterns of colour frequencies represent trade-off between intraspecific communication and predation risk that vary with different coral structure and fish densities (DeMartini & Donaldson, 1996).

Further analysis is required to determine the origin of the colour morph variation in *A. punctatus* and to confirm alternative explanations like complex behaviour and depth as the source of the observed variations.

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