Cytochrome b and Island speciation of the Genus *Astatheros*, from Bocas del Toro, Panama

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Abstract

Two cichlid populations each found on separate islands in the Bocas del Toro archipelago, Panama were identified as members the genus *Astatheros*. DNA from each population was isolated and purified and the mitochondrial gene cytochrome b was used as a molecular marker. Resulting sequences were compared with already published cytochrome b sequences of *Astatheros* from an internet database. Phylogenetic analyses showed that the two cichlid populations were divergent from other populations of the genus and indicated that they are conspecific, despite morphological differences.

Introduction

The family Cichlidae is a diverse group of fresh water perciforms commonly found in the neotropics and tropical Africa with one genus in India and Sri Lanka. Cichlids are known for explosive radiation and are one of the most specious families of the subphylum Vertebrata (Meyer 1993). Meyer suggested that the key to their evolutionary success is the presence of pharyngeal jaws that function independantly allowing the oral jaws to become highly specialized (Meyer 1993). There are over 600 known species of cichlid in the Americas from South America to Texas, (Bussing 1998). Cichlids are believed to have colonized Central America from South America during the late Pliocene which coincides with the rise of the Isthmus of Panama (Fig 1) and underwent an explosive radiation shortly thereafter (Martin and Bermingham 1998). According to Bussing the migration of ichthyofaunas from the Northwestern corner of South America west into southern Central America is little understood. Cichlids are hardy and tolerant of a wide range of water conditions. Bussing (1985) believes they may have migrated along coastlines, possibly from estuary to estuary. However it is doubtful that cichlids “island
hop” because even the most resilient of Central American cichlids can only resist full strength seawater for brief periods. The systematics of Central American cichlids are not formally agreed upon (Roe et al. 1997). *Astatheros bussingi*, found in the River Sixaola drainage, Panama was until recently, grouped with the species *Astatheros alfari* (Bussing 1998). The genus *Astatheros* is a recently resurrected taxon that was reinstated after molecular data determined that a number of species were not monophyletic with the rest of the genus *Amphilophus* (Roe. et al. 1997). However the genus *Astatheros* is still sometimes included with the genus *Amphilophus* (Kullnader 2003). In the Bocas del Toro archipelago (Fig 2) two islands contain two undescribed cichlids presumed to belong to the genus *Astatheros*.
Molecular phylogenetic analyses of Central American Cichlids using the mitochondrial gene cytochrome b (cyt-b) have included members of the genus Astatheros (Roe et al 1997; Martin and Bermingham 1998; Farias et al 2001). These cyt-b sequences are published and readily available through an internet species database Genbank [www.ncbi.nlm.nih.gov/Genbank](http://www.ncbi.nlm.nih.gov/Genbank). The purpose of this study was to compare cyt-b sequences of cichlid specimens collected from isla Colón and isla Popa to those of already published sequences of the genus Astatheros.

**Materials and Methods**

All cichlids were collected using cast nets or fishing line under permits issued by the Panamanian National Authority of the Environment (June 2005). Water chemistry data was collected using a hand held pH/temperature meter. Whole specimens were preserved
in 70% EtOH for at least 48 hours before being vacuum sealed at ambient temperature for transport. After transport specimens were kept frozen at -20°C. Genomic extraction was carried out using the DNeasy Genomic Isolation Kit (Qiagen, Valencia, CA). The entire cyt-b gene was amplified by PCR using primers in the flanking glutamine (5’-TGACTTGAAR AACCA YCGTT) and threonine (5’-GGAAT TCACC TCTCC GGTTTACAAG AC) tRNA genes (Martin and Bermingham 1998). PCR was carried out with Taq DNA polymerase (Qiagen), using 30 cycles at the following conditions: 95°C for 40s, 50°C for 40s, 72°C for 90s. Gel electrophoresis confirmed successful PCR amplification of the cyt-b gene. PCR products were cloned into the pDrive cloning vector using a PCR cloning kit (Qiagen). DNA plasmids containing the cyt-b gene were transformed into E. coli TOP10 and plated onto LB agar containing x-gal (40µg/mL) and ampicillin (150µg/mL). Bacterial colonies containing the cyt-b gene were confirmed by PCR analysis. Plasmid isolations were performed using the QIAspin Miniprep Kit (Qiagen). Sequencing reactions were carried out using M13F and M13R primers (SeqWright DNA Technology Service Houston, TX). Nucleotide variation was observed and phylogenies reconstructed using Vector NTI software (Invitrogen, Carlsbad, CA). Sample sequences were compared against published sequences of four known Middle American cichlids that are indigenous on the Atlantic versant of the Panama-Costa Rica area: Astatheros rhytisma, A. bussingi, A. alfari and A. altifrons. The Genbank locus numbers for each of these sequences are: AF145131, AF145130, AF145129, AF009948, AF009946, U97158, U97164 (Martin and Bermingham 1998; Farias et al. 2001; Roe et al 1997). Guide Tree calculation is based on a sequence distance method and utilizes the Neighbor Joining (NJ) algorithm (Saitou, N. and Nei, M. 1987).
Results and Discussion

Both island varieties are unique to a particular island (Fig 2) and have different color morphology. The Isla Popa variety resembles *A. rhytisma* (Lopez 1983) and the Isla Colón variety resembles *A. bussingi* (Loiselle 1997). The fish collected on Isla Colón (Fig 3) were collected at altitudes between 60 to 120m in elevation, in streams with a coral limestone substrate and a pH of 7.5. Fish on Isla Popa (Fig 4) were caught in streams near sea level with tea colored water (pH 6.0) and a substrate of decomposing organic matter. Both streams had water temperature measured from 24-25°C. No cichlids have been found on any other island of Bocas del Toro and indigenous peoples, who rely on these fish for food, confirmed that they are only found on these two islands. On the Isthmus of Panama *A. bussingi* and *A. rhytisma* are also found at differing elevations. Although they often share the same habitat, *A. bussingi* normally is found at higher elevations than *A. rhytisma* (Bussing 1998).
The two island cichlids might be *A. bussingi* and *A. rhytisma* since geologic reconstructions of the islands during the last glacial period suggest that all of the islands in Bocas del Toro were connected by land to the isthmus of Panama (Bermingham, pers. Comm.).

The genetic distances between sequences were organized into phylogenetic trees of maximum parsimony or minimum evolution (Saitou N. and Nei, M. 1987) shown in Figure five. In cichlids the mutation rate of mitochondrial DNA has been estimated at 1-1.2% sequence divergence per million years (Bermingham et al. 1997). *A. bussingi* and *A. bussingi* II share 99.8% sequence identity with *A. alfarsii* (River Sixaola population) suggesting that the two groups have been separated for approximately 200,000 years, these fish are now considered to be the same species (Bussing 1998). The isla Colón cichlid and the isla Popa cichlid share 99.0% sequence identity suggesting at most a million years of divergence and an isolation event in the late Pleistocene. However further analysis and repeat sequencing is recommended for these populations due to small sample size, small coverage and inadequate surveys of the island Ichthyofauna. The use of a second molecular marker would also be advised as there are many shortcomings to the use of cyt-b (Meyer, 1994).
Conclusions

The two undescribed cichlids from Bocas del Toro appear to belong to the genus *Astatheros* and the same mitochondrial clad comprising a single species even after almost a million years of divergence. They show sufficient genetic distance to be considered distinct from any other member of the genus on the Isthmus of Panama. Further study of these fish would be extremely valuable in understanding allopatric speciation in fluvial cichlids of the neotropics. Also there are many other genera of cichlids that have overlapping distributions with the genus *Astatheros* and why they are not found on islands is unknown. Also, why no cichlids whatsoever are found on Isla Cristobal and Isla Bastimentos (both large islands with substantial bodies of fresh water) is equally
perplexing. Continuing research of cichlid faunas in the Bocas del Toro archipelago will most likely offer new insights into the evolution of cichlids.

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References


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