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Systematics and historical biogeography of Greater Antillean Cichlidae

Prosanta Chakrabarty *

University of Michigan Museum of Zoology, Fish Division, 1109 Geddes Avenue, Ann Arbor, MI 48109-1079, USA

Abstract

A molecular phylogenetic analysis recovers a pattern consistent with a drift vicariance scenario for the origin of Greater Antillean cichlids. This phylogeny, based on mitochondrial and nuclear genes, reveals that clades on different geographic regions diverged concurrently with the geological separation of these areas. Middle America was initially colonized by South American cichlids in the Cretaceous, most probably through the Cretaceous Island Arc. The separation of Greater Antillean cichlids and their mainland Middle American relatives was caused by a drift vicariance event that took place when the islands became separated from Yucatan in the Eocene. Greater Antillean cichlids are monophyletic and do not have close South American relatives. Therefore, the alternative hypothesis that these cichlids migrated via an Oligocene landbridge from South America is falsified. A marine dispersal hypothesis is not employed because the drift vicariance hypothesis is better able to explain the biogeographic patterns, both temporal and phylogenetic.
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"The geology is in many respects uncertain, the phyletic analysis inadequate and the fossil record wretched. We have if not the worst case scenario definitely a very bad one."

E.E Williams (1989) discussing the state of Caribbean biogeography.

1. Introduction

The biogeographic history of the Greater Antilles has been a contentious issue among biologists and geologists. Both disciplines have major camps that support different hypotheses. Biologists have relied on geological reconstructions to frame their hypotheses about the movement of organisms. Modern geological reconstructions that explain the presence of the biota on the Greater Antilles fit into two major categories. One category suggests South American origins from an Oligocene landbridge that connected South America to the islands (Iturralde-Vinent and MacPhee, 1999). The other category suggests Middle American origins from a period of coalescence between these islands and Yucatan in the early Cenozoic (Pitman et al., 1993; Pindell, 1994; updated from Malfait and Dinkelman, 1972; Tedford, 1974). Biologists have argued that these reconstructions explain the colonization of the Greater Antilles (Dávalos, 2004; Murphy and Collier, 1996; Rosen, 1975). Biologists have also argued that marine dispersal explains the presence of the biota on these islands (Glor et al., 2005; Hedges, 1996; Hedges et al., 2002; Martin and Bermingham, 1998). Cichlids have played a major role on all sides of these arguments (Briggs, 1984, 2003; Rivas, 1986; Sparks and Smith, 2005). However, until now, no phylogenetic assessment of these fishes has been done.

Cuba has two cichlid species (*Nandopsis tetracanthus* and *N. ramsdeni*) and Hispaniola one extant (*Nandopsis haitiensis*) and one fossil species (*N. woodringi*). *Nandopsis vombergae* (Ladiges, 1938) is a junior subjective synonym of *N. haitiensis* and will not be discussed (Chakrabarty, 2006).

The islands that compose the Greater Antilles do not all share a geological history. The islands of Cuba, Hispaniola, Puerto Rico, and Jamaica form the Greater Antilles. However, geologically, Jamaica does not share an arc history

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* Fax: +1 734 763 4080.
  E-mail address: pchakrab@umich.edu.

with the other Greater Antillean islands (Pindell and Barrett, 1990). Because cichlids are only on Cuba and Hispaniola, these islands will be the setting for the reconstructions described here.

The complex geological history of the Caribbean will be described before biogeographic hypotheses are tested (see also Table 1). The Caribbean region formed as a product of the separation of Gondwana and Laurasia, particularly the separation of North and South America 170 million years ago (Iturralde-Vinent and MacPhee, 1999; Pindell, 1994). Most geologists agree that the Caribbean plate originated in the Pacific (Pindell, 1994; Pindell and Barrett, 1990; Ross and Scotese, 1988; but see Meschede and Frisch, 1998). Landmasses that originated with the formation of this plate include parts of Cuba, the Cayman Ridge, Hispaniola, Puerto Rico, and the Virgin Islands (Pindell and Barrett, 1990). These landmasses collectively formed what will be called here the Cretaceous Island Arc (following the convention of Iturralde-Vinent and MacPhee, 1999). As this arc drifted eastward it became positioned between North and South America. During periodic dry periods 70 to 80 million years ago this arc may have served as a functional landbridge which could have acted as a corridor between the two continents (Iturralde-Vinent and MacPhee, 1999). The Cretaceous arc broke-up at the end of the Cretaceous with its remnants forming the Paleogene arc (Iturralde-Vinent and MacPhee, 1999; Kerr et al., 1999). The Paleogene arc contained parts of Cuba and Hispaniola. In the Paleogene (early Cenozoic), this arc drifted into a position that connected it to northern Middle America. Geological reconstructions by Pitman et al. (1993) argue that the connection between the Paleogene arc and Middle America may have lasted until 49 million years ago. It is this period of coalescence that could have allowed faunal exchange between these landmasses (named here as the Paleogene arc drift vicariance scenario). The separation between the North America Plate and the Caribbean Plate is the Cayman Trough which lies between Cuba and Yucatan. The Cayman Trough began to form in the Eocene (Pindell and Barrett, 1990; Pindell et al., 1988). Since the Eocene, Cuba, and Hispaniola (as remnants of the Paleogene arc) drifted 1100 kilometers to their current positions. Cuba and Hispaniola separated 20 to 25 million years ago with the formation of the Oriente Fault (Pindell, 1994).

Table 1
Taxa sequenced, with GenBank and UMMZ catalogue numbers

Taxon	UMMZ #	S7 GenBank Accesion No.	Tmo-4C4 GenBank Accesion No.	16S GenBank Accesion No.	COI GenBank Accesion No.
Middle America					
Archocentrus centrarchus	243177	DQ119165	DQ119164	DQ119162	DQ119163
Archocentrus multispinosus	243207	DQ119253	DQ119224	DQ119166	DQ119195
Archocentrus nigrofasciatus	243200	DQ119254	DQ119225	DQ119167	DQ119196
Archocentrus octofasciatus	243175	DQ119255	DQ119226	DQ119168	DQ119197
Amphilophus citrinellus	243174	DQ119256	DQ119227	DQ119169	DQ119198
Amphilophus lyonsi	243179	DQ119257	DQ119228	DQ119170	DQ119199
'Cichlasoma' salvini	243182	DQ119258	DQ119229	DQ119171	DQ119200
Herichthys carpintis	243199	DQ119259	DQ119230	DQ119172	DQ119201
Hypsophrys nicaraguensis	243188	DQ119260	DQ119231	DQ119173	DQ119202
Parachromis managuensis	243204	DQ119261	DQ119232	DQ119174	DQ119203
Parachromis dovii	243205	DQ119262	DQ119233	DQ119175	DQ119204
Parachromis motaguensis	243183	DQ119263	DQ119234	DQ119176	DQ119205
Petenia splendida	243170	DQ119264	DQ119235	DQ119177	DQ119206
Thorichthys aureus	243202	DQ119265	DQ119236	DQ119178	DQ119207
Tomocichla sieboldii	243171	DQ119266	DQ119237	DQ119179	DQ119208
Vieja synspila	243203	DQ119267	DQ119238	DQ119180	DQ119209
Vieja tuyrense	243180	DQ119268	DQ119239	DQ119181	DQ119210
Greater Antilles					
Nandopsis ramsdeni	245137	DQ119269	DQ119240	DQ119182	DQ119211
Nandopsis tetracanthus	245598	DQ119270	DQ119241	DQ119183	DQ119212
Nandopsis haitiensis	243287	DQ119271	DQ119242	DQ119184	DQ119213
South America					
Apistogramma bitaeniatum	243211	DQ119272	DQ119243	DQ119185	DQ119214
Bujurquina vittata	243206	DQ119273	DQ119244	DQ119186	DQ119215
'Cichlasoma' festae	243201	DQ119274	DQ119245	DQ119187	DQ119216
Geophagus steindachneri	243208	DQ119275	DQ119246	DQ119188	DQ119217
Heros appendiculatus	243189	DQ119276	DQ119247	DQ119189	DQ119218
Hypselecara temporalis	243197	DQ119277	DQ119248	DQ119190	DQ119219
Uaru amphiacanthoides	243176	DQ119278	DQ119249	DQ119191	DQ119220
India					
Etroplus maculatus	245135	DQ119279	DQ119250	DQ119192	DQ119221
Madagascar					
Paratilapia polleni	243192	DQ119280	DQ119251	DQ119193	DQ119222
Paretroplus kieneri	243195	DQ119281	DQ119252	DQ119194	DQ119223

A major alternative to the Paleogene arc drift vicariance scenario proposes a South American origin for the Greater Antillean fauna. Iturralde-Vinent and MacPhee (1999) propose a short-lived landbridge between the Greater Antilles and northwest South America circa 32 million years ago. The authors name this Early Oligocene landbridge GAARlandia (from Greater Antilles + Aves Ridge). One consequence of this alternative hypothesis is that the Greater Antillean Island chain would have had a more recent connection with South America than with Middle America.

These alternative biogeographic hypotheses will be tested under a phylogenetic framework. The relationships among the cichlids of Middle America, South America, and the Greater Antilles will elucidate the history of the origins of these fishes.

2. Materials and methods

2.1. Acquisition of DNA dataset

A molecular phylogeny of 30 cichlid taxa (listed in Table 1) was completed using portions of nuclear genes S7 and Tmo-4C4, as well as portions of mitochondrial genes cytochrome *c* oxidase subunit I (COI) and 16S. The final data set was 2278 aligned positions. Primers S7RPEX1F 5′-TG GCCTCTTCCTTGGCCGTC-3′ and S7RPEX2R 5′-AA CTCGTCTGGCTTTTCGCC-3′ were used to amplify and sequence the first intron in the nuclear S7 ribosomal protein gene, yielding sequences of 774 aligned positions (Chow and Hazama, 1998; Lavoué et al., 2003). Primers Tmo-f2-5′ 5′-ATCTGTGAGGCTGTGAACTA-3′ (Lovejoy, 2000) and Tmo-r1-3′ 5′-CATCGTGCTCCTGGGTGACAAAG T-3′ (Streelman and Karl, 1997) were used to amplify and sequence a portion of the nuclear gene Tmo-4C4, yielding sequences of 299 aligned positions. Primers 16S ar-L 5′-CG CCTGTTTATCAAAAACAT-3′ and 16S br-H 5′-CCGG TCTGAACTCAGATCACGT-3′ (Koucher et al., 1989; Palumbi, 1996) were used to amplify and sequence a fragment of mitochondrial large ribosomal subunit 16S, yielding sequences of 614 aligned positions. Primers COIfor 5′-T TCTCGACTAATCACAAAGACATYGG-3′ and COIrev 5′-TCAAARAAGGTTGTGTTAGGTTYC-3′ were designed in this study (modified from the primers of Folmer et al., 1994) to amplify and sequence a segment of mitochondrial gene COI, yielding sequences of 591 aligned positions.

Tissue samples were taken from specimens preserved as vouchers in the University of Michigan Museum of Zoology (UMMZ) Fish Division. Voucher and GenBank accession numbers are listed in Table 1. Locality data for specimens can be obtained by searching the UMMZ fish collection catalogue. All specimens are either wild caught or purchased from a breeder raising wild caught individuals and selling their young (Jeff Rapps; http://www. tangledupincichlids.com/). Fish tissues are preserved in 95% ETOH and stored in −80 °C. Tissue extraction was done using a Qiagen DNeasy Tissue Kit following the manufacturer's protocol. PCR amplifications were done for 30–35 cycles. Denaturation of 20 s at 95 °C was followed by annealing for 15 s at temperatures of 60 °C (S7), 50 °C (Tmo-4C4), 45 °C (COI). Extension times varied from 1 min 30 s, to 2 min. This extension was followed by a terminal extension for 7 min at 72 °C. PCR amplification of 16S follows the protocol of Sparks (2004). PCR product was isolated on 1% agarose gels. Bands were removed from the gel under a UV light and extracted using Qiagen Gel Extraction Kits following the manufacturer's protocol. Sequencing was completed by the University of Michigan Sequencing Core Facility. DNA sequences were edited from chromatograms and aligned manually in Sequence Navigator (Elmer, 1995).

2.2. Phylogenetic analyses and support indices

Parsimony analyses were completed in PAUP* 4.0b (Swofford, 2002). Heuristic searches were performed with 10,000 random addition replicates. Bremer support (Bremer, 1995) was calculated using TreeRot v.2 (Sorenson, 1999). Jackknife resampling (100 replicates, 100 search replicates) and the parsimony ratchet (to verify PAUP* results) were performed in NONA (Goloboff, 1993) and WinClada (Nixon, 1999). The outgroup, *Paratilapia polleni,* was used to root all trees.

Likelihood analyses were performed in MrBayes 3.01 (Huelsenbeck and Ronquist, 2001). MrModeltest (Nylander, 2002) was used for parameter estimation for each gene and in combination using the hierarchical log-likelihood ratio tests. Four Markov Chains were run for six million generations, sampling every 500 generations. Burn-in time of 1.5 million generations was determined from where likelihood scores reached stationarity. Trees were filtered in PAUP* 4.0b (Swofford, 2002) under a maximum likelihood optimality criterion to recover the best trees under that framework.

Parametric bootstrapping was implemented to statistically test a South American origin of the Greater Antillean cichlids. Trees were searched under the topological constraint that the Greater Antillean cichlids must have a sister relationship with South American cichlids. Using the best fit model of sequence evolution selected from Modeltest (Posada and Crandall, 1998), branch lengths were optimized on the constrained tree. SG Runner (Wilcox, 2005) and Seq-Gen (Rambaut and Grassly, 1997) were then used to simulate 1000 data sets on the constrained topology using the same model of sequence evolution. The optimal tree for each dataset was found using PAUP* as was the optimal trees for each dataset under the constraint of the Greater Antillean cichlids being South American. Tree lengths were compared across constrained and unconstrained trees for each dataset. Significance was assessed by comparing the difference in the actual data set to the simulated datasets.

2.3. Date estimation and calibration

In the absence of a strict molecular clock a penalized likelihood approach was used for estimating divergence times. Penalized likelihood combines likelihood based substitution models with a penalty term to allow varying (but constrained) rates of change across a phylogeny. By incorporating the likelihood term of the substitution model this method also remains consistent with the method used for recovering the tree topology. Penalized likelihood was implemented in R8s 1.7 (Sanderson, 2003) to estimate divergence dates of internal nodes.

Determining the optimal level of constrained variation across branches (termed 'rate smoothing') is accomplished through cross validation (Sanderson, 2002). Cross-validation iteratively removes a terminal branch and compares estimated values for that branch to the observed value. The cross validation score is the differences in observed and estimated branch lengths that are summed across the tree. The lowest cross validation score is the optimal value. The additive penalty function was applied to penalize squared differences in rates across branches. This penalty function is the appropriate option when calibration points are deeper in the tree than the nodes to be estimated (Sanderson, 2004).

R8s has the advantage over other programs estimating divergence times in allowing calibration points to be set as minimum, maximum or fixed ages rather than only fixed ages (see Heads, 2005 for a discussion). Because of the nature of the geological and fossil evidence available for this study, the flexibility allowed by not fixing absolute dates on calibration points was essential.

Three calibration points were chosen to put a temporal scale on the phylogenetic hypothesis. The minimum age of the Greater Antillean cichlids was placed at 5 million years (node B, Fig. 1), because *Nandopsis woodringi* is an extinct member of the Greater Antillean endemic genus *Nandopsis*. This species, described from Las Cohobas, Haiti (Cockerell, 1924), is Late Miocene in age (11.6–5.3 million years ago; see Chakrabarty, 2006 about incorrect dates in literature). Because there is a paucity of fossils that can be placed on lineages in the current phylogeny, calibration points from Gondwanan vicariance events were also used. Cichlids are distributed mainly on former Gondwanan fragments (India, Madagascar, South America, and Africa). The relationships among members of this family reflect the break up of Gondwana (Sparks and Smith, 2005; see review of cichlid phylogenies in Chakrabarty, 2004). Traditional molecular clock evidence is equivocal (Kumazawa et al., 2000; Vences et al., 2001) and there is some question about the methods used (Chakrabarty, 2004; Sparks and Smith, 2005). The use of Gondwanan vicariance ages as calibration points to test a Greater Antillean vicariance scenario allows the use of rates to estimate dates. Without these independent calibration points the penalized likelihood approach would lack maximum age calibration points. These points are necessary to prevent nodal age estimates from estimating ages infinitely back in time. The super-continent of Gondwana began to break up circa 165 million years ago. This age is the minimum age for the origin of Cichlidae if they were present before fragmentation. For this reason a 165 million year fixed calibration point is placed at the base of the phylogeny. A second fixed calibration point is placed in the separation of India and Madagascar that took place 88 million years ago. This separation is represented on the phylogeny by the separation of the Indian genus *Etroplus* and the Malagasy genus *Paretroplus*. Both these fixed calibration points are associated with outgroup lineages. Their function is to serve as anchor points from which dates can be estimated for divergences in the Neotropical ingroup.

All the nucleotide data were treated simultaneously for the penalized likelihood approach as they were in the phylogenetic methods (both parsimony and maximum likelihood). The advantage of multigene data sets is that they contain more information than single gene data sets, much of the information pertaining to divergence times will be lost if the dataset is reduced to a single gene or part of a single gene (Thorne and Kishino, 2002; Yang and Yoder, 2003). Rather than disregard large portions of the dataset by pruning taxa or removing gene sequences the assumption of a constant rate of evolution was relaxed. Constant rate analysis is not rigorous because it does not recognize the uncertainty in divergence time estimation (Thorne and Kishino, 2002). Evolutionary rates differ over time and among genes but as a phylogeny shows, these genes share a common set of divergence times.

3. Results

3.1. Model selection, likelihood assumption set

For parametric bootstrapping and all maximum likelihood PAUP* analyses, ModelTest selected the following parameters for the combined dataset: GTR + G + I model of sequence evolution, with four rate categories, base frequencies ($A = 0.266$, $C = 0.257$, $G = 0.199$, and $T = 0.278$), rate heterogeneity according to the gamma distribution with a shape (α) = 0.511, and the proportion of invariable sites (pinv) = 0.3143.

For analyses using MrBayes, parameter estimation was selected from MrModelTest to be partitioned by genes to have six substitution sites under a GTR model. Both 16S and Tmo-4C4 were selected to have a proportion of the sites invariable while the rates for the remaining sites are drawn from a gamma distribution. Both COI and S7 were selected to have rates at every site drawn from a gamma distribution. The GTR matrix, gamma distribution, nucleotide state frequencies, proportion of invariant sites, and the transition/transversion ratio were all unlinked across data partitions.

Fig. 1. Phylogeny of Neotropical cichlid taxa inferred from S7, Tmo-4C4, 16S, and COI sequences. Topology shown is the most parsimonious tree with geographic regions reconstructed on the phylogeny under parsimony character optimization in MacClade 4.0 (Maddison and Maddison, 1992). Bayesian posterior probabilities values that are significant (⩾95%) are shown below nodes. Above each node Jackknife values are given if 80 percent or above followed by a front slash and Bremer support values if 3 or above. Fixed calibration points are shown (all are associated with outgroup taxa). Letters at nodes correspond to the estimated dates given in Table 2.

Table 2
Estimated dates for nodes of interest with associated reference letters on the phylogeny (Fig. 1)

Geological event	Age of event	Estimated divergence time millions of years ± standard deviation	
		Oldest node	Youngest node
Separation of Paleogene Arc (Cuba and Hispaniola) from Yucatan at opening of Caymen Trough	Middle Eocene, as late as 49 million years ago (Pitman et al., 1993)	50 ± 5 **A**	43 ± 5 **B**
Period when Cretaceous Arc (Greater Antilles, adjacent regions) may have served as landbridge between North and South America	Late Cretaceous, 70–80 mya (Iturralde-Vinent and MacPhee, 1999)	66 ± 6 **C**	55 ± 5 **D**
Separation of Eastern Cuba and Western Hispaniola through the formation of the Oriente Fault	Oligocene or Miocene, 20–25 mya (Pitman et al., 1993)	43 ± 5 **B**	25 ± 5 **E**
Separation of South America from rest of Gondwana	Final separation, 106–84 mya (Pitman et al., 1993)	87 ± 5 **F**	76 ± 6 **G**

3.2. Phylogenetic analyses and support

Combined analyses of gene fragments from S7, Tmo-4C4, 16S, and COI resulted in a single most parsimonious tree that was fully congruent with the maximum likelihood analysis (Fig. 1). A tree length of 2682 was obtained with a consistency index of .516, a retention index of .464, and a rescaled consistency index of .239. The consistency index excluding uninformative sites was .409. Five hundred and fifty-nine characters were parsimony informative. The score
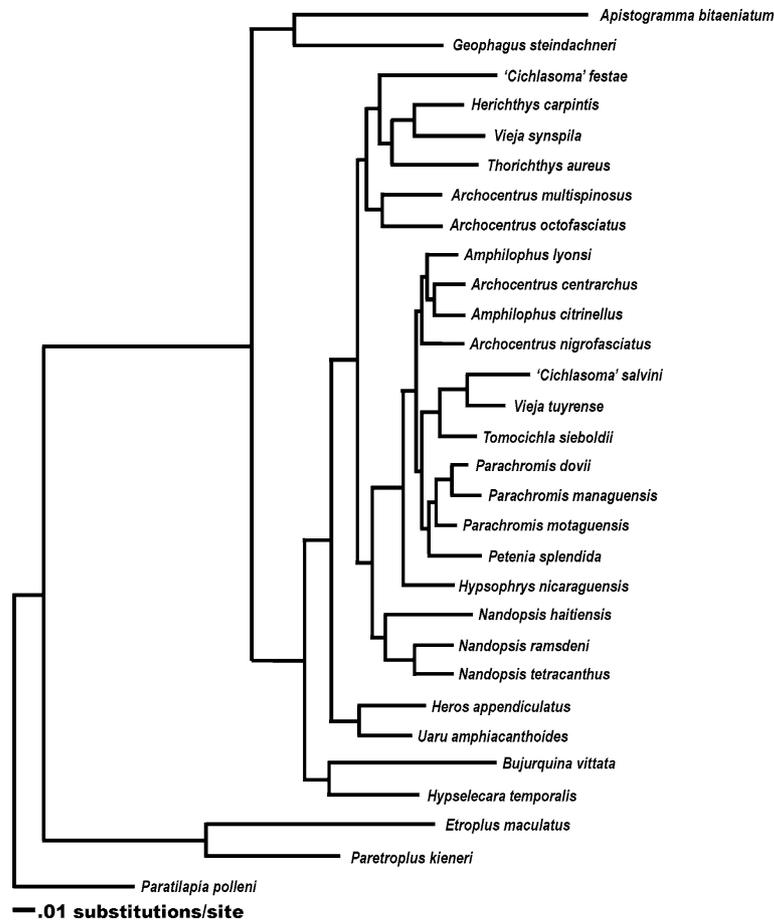
Fig. 2. Maximum likelihood phylogeny of Neotropical cichlid taxa inferred from S7, Tmo-4C4, 16S, and COI sequences.

of the best tree found under the maximum likelihood framework was 16836.29518 (Fig. 2).

The Greater Antillean cichlids are recovered as a monophyletic group. The Cuban cichlids, *Nandopsis tetracanthus* and *N. ramsdeni*, are sister to the Hispaniolan species. The Greater Antillean cichlids are nested within a large clade of mainly Middle American cichlids. The sister group to the Greater Antillean cichlids is a large group of widespread Middle American species.

The phylogenetic tree shows one reversal of a Central American cichlid now endemic to South America. That species, 'Cichlasoma' *festae*, is phylogenetically recovered as a Middle American cichlid (from the parsimony optimization in Fig. 1). This species and the remaining Middle American cichlids form a clade that is nested within the sampled South American species.

The null hypothesis that the Greater Antillean cichlids have sister group relationships with South American cichlids was rejected under parametric bootstrapping. The unconstrained tree (the most parsimonious tree) was 131 steps shorter than the best tree in which the clade of Greater Antillean cichlids was constrained to be South American. This value was significantly greater than can be attributed to chance ($p < .001$). The null hypothesis was similarly rejected under the maximum likelihood framework ($p < .01$).

3.3. Estimated dates

Ages were estimated under the optimal smoothing value of 1.3e-05. Table 2 shows the recovered estimated divergence times for the nodes of interest. Two sets of ages are given, one for the node associated with the youngest age that can be attributed to a group, and one for the node associated with the oldest possible age. Arguably, the more conservative age estimate is that of the youngest age because it is closest to the node of interest; however, there is no way to determine which of these ages can be attributed to a particular clade. Ages estimated for important ingroup nodes include the following: for Cuban cichlids (node E, Fig. 1), an origin 25 million years ago (mya) with a standard deviation of 5 million years. The clade of Greater Antillean cichlids (node B) had an estimated origin 43 mya with a standard deviation of 5 million years. The separation of the Greater Antillean cichlids and its Middle American sister group (node A) is estimated to have taken place 50 mya with a standard deviation of 5 million years. The separation between the South American clade of *Heros appendiculatus* and *Uaru amphicanthoides* with its mainly Middle American/Greater Antillean sister group (node C) is estimated to have taken place 66 mya with a standard deviation of 6 million years. The oldest age estimated for

the entire Neotropical ingroup (node F) is an origin 87 mya with a standard deviation of 6 million years.

4. Discussion

The Greater Antillean cichlids are phylogenetically a clade of Middle American cichlids whose separation from Middle America took place through an early Cenozoic vicariance event. The phylogenetic pattern recovered shows Middle American origins for the Greater Antillean clade (Fig. 1). The estimated ages for the origin of this clade correspond to the time of separation of the Paleogene arc and the Yucatan peninsula. Therefore, the Paleogene arc drift vicariance scenario is supported by the phylogenetic pattern and its temporal scale. In this scenario, a vicariance event separated populations of an ancestral Middle American species that inhabited a contiguous area shared by the Paleogene arc and Yucatan. The drifting of the Paleogene arc led to the allopatric speciation event that gave rise to the Greater Antillean cichlids.

There are no close South American relatives recovered for the Greater Antillean cichlids, rejecting the GAARlandia hypothesis, which predicted South American sister group relationships. The temporal scale fit on the phylogeny also does not correspond to the geological events assumed in the GAARlandia hypothesis. The Greater Antilles could only have been connected to South America through GAARlandia 32 mya, an age at least six million years too young according to the temporal scale of the recovered phylogeny.

Another vicariant event, caused by the separation of Cuba and Hispaniola, was also revealed by the temporal analysis. The 20 to 25 million year old separation of these two islands is concordant with the time of separation of the Cuban and Hispaniolan cichlids.

The age of the Middle American cichlid fauna is also predicted. Bussing (1985) suggested a late Cretaceous or early Tertiary origin for Middle American cichlids based on the patterns of the endemic fauna of this region. Bussing's (1985) hypothesis appears to be corroborated by the estimated divergence dates, which correspond to the periods when the Cretaceous arc could have served as a corridor between North and South America (Iturralde-Vinent and MacPhee, 1999). Middle America (essentially just Yucatan in the Cretaceous) would have been colonized during this period before break-up of the Cretaceous arc. Cichlids would have then dispersed onto the Chortis block (Honduras-El Salvador-Southern Guatemala) when it connected with Yucatan in the Eocene. Likewise, as the remainder of modern lower Central America formed, cichlids would have dispersed south onto these regions. Therefore, it appears that the Cretaceous arc served as a corridor for cichlids to cross from South America to Yucatan; however, this arc apparently never functioned to maintain a refugium-like habitat for cichlids as the Paleogene arc did.

This Cretaceous scenario opposes the Miocene marine dispersal view of some researchers (Martin and Bermingham, 1998; Myers, 1966) to explain the origins of the Middle American Cichlidae. The Miocene dispersal evaluation of Myers (1966) was based mainly on the presence of the fossil *Nandopsis woodringi* on Hispaniola. Martin and Bermingham (1998) used a traditional molecular clock to conclude that the origins of Middle American cichlids can be explained by a Miocene dispersal event. Perdices, Doadrio and Bermingham (2005), using a traditional molecular clock, concluded that synbranchid eels also dispersed at this time. Dispersal can occur at any time but the reason that these radiations took place at nearly the same time was not explored. A Miocene radiation of these fishes would have required crossing of a significant marine barrier between northern South America and nuclear Central America. Cichlids are not known to cross marine barriers to colonize landmasses (Riseng, 1997; Sparks and Smith, 2005). However, the possibility that members from a single species from each group was able to cross this barrier can never be ruled out.

No phylogenetic pattern can reject a marine dispersal hypothesis for the origin of Greater Antillean cichlids, as such hypotheses cannot be refuted. The phylogenetic relationships found in the current study are no exception. However, the period of dispersal attributed to cichlids by the temporal analysis shows dispersal took place during coalescence of now separated landmasses. Dispersal over freshwater corridors at geological coalescence times is more plausible than the marine dispersal route at the same time. Despite the ability of some cichlids to tolerate saltwater, it appears to be a significant barrier to most (Sparks and Smith, 2005). There is no need to employ a marine dispersal hypothesis when the drift vicariance hypothesis is better able, both temporally and phylogenetically, to explain the biogeographic relationships.

The endemic South American species recovered as phylogenetically Middle American does not effect the biogeographic hypotheses presented in this study. This species, 'Cichlasoma' festae, is nested well within Middle American cichlids. The youngest age that can be attributed to its clade (composed of itself and sister group) is 47 mya with a standard deviation of 5 million years. This period corresponds to a time when the Aves Ridge may have connected Middle America to South America (Pitman et al., 1993). It may be at this time that this species dispersed from Middle America to South America. Notably, Hulsey et al. (2004) also recovered 'Cichlasoma' festae nested within Middle American cichlids.

Complete sampling of the extant Greater Antillean Cichlidae reveals for the first time their Middle American origins. The temporal scale fit to this phylogeny also provides insights about three events that were important in their origins: (1) the arrival of species in Yucatan in the Cretaceous (2) followed by a drift vicariance event between the arc composed of Cuba and Hispaniola with Yucatan (3) and finally the separation of Cuba and Hispaniola. The fit

between the estimated divergence dates and these geological events cannot be ignored.

Other groups that share a congruent pattern with cichlids (in having Middle American-Greater Antillean relationships) include: the snake genus *Epicrates* (Kluge, 1988), legumes in part (Lavin et al., 2003), the gar *Atractosteus* (Wiley, 1976), the livebearer tribe Girardiini (Rosen, 1975, 1985; Rosen and Bailey, 1963), and three *Gambusia* species groups (*puncticulata, nicaraguensis, punctata*; Fink, 1971a,b; Lydeard et al., 1995; Rauchenberger, 1988). Murphy and Collier (1996) recover a phylogenetic pattern and temporal scale corresponding to vicariance origins for the aplocheiloid genus *Rivulus* in the Greater Antilles. They used a 70–80 mya calibration point associated with the period that the Cretaceous arc functioned as a corridor between North and South America. The present study finds evidence for that event in the phylogeny of Neotropical cichlids.

Lacking an ability to look into the past we must attempt to reconstruct it as best we can. As biologists build stronger evidence of particular relationships, geologists must follow suit to substantiate or eliminate possible reconstructions that explain those patterns. Reciprocal illumination may work slowly across disciplines but the field of historical biogeography demands that both biologists and geologists keep pace with each other.

Acknowledgments

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