Molecular Phylogenetics and Evolution 60 (2011) 183–191

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

A phylogenetic hypothesis for *Crocodylus* (Crocodylia) based on mitochondrial DNA: Evidence for a trans-Atlantic voyage from Africa to the New World

Robert W. Meredith [a,*], Evon R. Hekkala [b], George Amato [c], John Gatesy [a,*]

[a] Department of Biology, University of California, Riverside, CA 92521, USA
[b] Department of Biological Sciences, Fordham University, Rose Hill Campus, Bronx, NY 10458, USA
[c] Sackler Institute for Comparative Genomics, American Museum of Natural History, 79th Street at Central Park West, New York, NY 10024, USA

ARTICLE INFO

Article history:
Received 12 January 2011
Revised 9 March 2011
Accepted 9 March 2011
Available online 1 April 2011

Keywords:
Crocodile
Crocodylus
Phylogeny
Mitochondrial
Genome
Dispersal

ABSTRACT

The phylogenetic relationships among extant species of *Crocodylus* (Crocodylia) have been inconsistently resolved by previous systematic studies. Here we used nearly complete mitochondrial (mt) genomes (~16,200 base pairs) for all described *Crocodylus* species, eight of which are new to this study, to derive a generally well-supported phylogenetic hypothesis for the genus. Model-based analyses support monophyly of all Asian + Australian species and paraphyly of *Crocodylus niloticus* (Nile crocodile) with a monophyletic New World clade nested within this species. Wild-caught Nile crocodiles from eastern populations group robustly with the four New World species to the exclusion of Nile crocodiles from western populations, a result that is also favored by parsimony analyses and by various subpartitions of the overall mt dataset. The fossil record of *Crocodylus* extends back only to the Late Miocene, while the earliest fossils assigned to *C. niloticus* and to New World *Crocodylus* are Pliocene. Therefore, in combination with paleontological evidence, mt DNA trees imply a relatively recent migration of *Crocodylus* from Africa to the Americas, a voyage that would have covered hundreds of miles at sea.

1. Introduction

The genus *Crocodylus* includes approximately a dozen extant crocodylian species that inhabit tropical and semi-tropical regions of Asia, Australia, Africa, and the New World (Brochu, 2003; McAliley et al., 2006). Although a variety of studies have addressed phylogenetic relationships in this clade, a robust, fully-resolved phylogenetic hypothesis is currently lacking (Brochu, 2000; Brochu et al., 2010; Brochu and Densmore, 2001; Gatesy et al., 2004; McAliley et al., 2006; Willis et al., 2007; Gatesy and Amato, 2008; Meganathan et al., 2010).

The absence of convincing support can be attributed to several factors. First, although morphological characters have been coded and incorporated into a cladistic matrix, along with some evidence from extinct representatives (Brochu, 2003), the full scope of phenotypic variation in this taxon has not yet been placed in a phylogenetic context. Brochu (2000) suggested that the addition of scalation traits, and other anatomical character systems that exhibit extensive variation within *Crocodylus*, could be informative. Second, crown-group *Crocodylus* is a relatively young clade thought to have diversified no earlier than the Miocene (Brochu, 2000). Given the moderate evolutionary rates of nuclear genes that have been applied to crocodylian relationships, relatively few phylogenetically informative sites are apparent within *Crocodylus*, and inferred relationships vary widely in different nuclear gene trees (McAliley et al., 2006; Willis et al., 2007; Willis, 2009; Meganathan et al., 2010). Third, for most published datasets, the sampling of *Crocodylus* species has been incomplete (e.g., McAliley et al., 2006). Concatenation of the incomplete datasets into much larger supermatrices has provided moderate support for some nodes within *Crocodylus*, but such compilations further highlight the spotty sampling of comparative data, and the need for phylogenetic matrices with fewer missing data (Brochu and Densmore, 2001; Gatesy et al., 2004; Gatesy and Amato, 2008). Lastly, interspecific hybridization and the presence of cryptic species have been hypothesized recently within *Crocodylus*, and these patterns further complicate attempts at resolving phylogenetic relationships in the genus. In particular, evidence for interbreeding between various New World *Crocodylus* species has been recorded (Ray et al., 2004; Weaver et al., 2008), matings of different *Crocodylus* species are common in captivity (e.g., Fitzsimmons et al., 2002), and a substantial genetic break is apparent between *Crocodylus niloticus* (Nile crocodile) specimens from eastern and western

Abbreviations: mt, mitochondrial; bp, base pairs; MP, maximum parsimony; ML, maximum likelihood; PBS, partitioned branch support; AU, approximately unbiased; SH, Shimodaira–Hasegawa; KH, Kishino–Hasegawa.

* Corresponding authors. Fax: +1 951 827 4286.
E-mail addresses: robert.meredith@email.ucr.edu (R.W. Meredith), john.gatesy@ucr.edu (J. Gatesy).

1055-7903/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.ympev.2011.03.026

populations in Africa (Schmitz et al., 2003; Hekkala et al., 2010; submitted for publication).

Analysis of large mitochondrial (mt) DNA sequence datasets offers a promising approach to addressing some of the outstanding systematic issues within *Crocodylus*. Mitochondrial genomes provide a bounty of phylogenetic information, albeit from a set of tightly linked, rapidly evolving genes. Previous work has shown that large mt DNA datasets are capable of yielding robustly supported resolution across a range of divergences within Crocodylia (Janke et al., 2005; Roos et al., 2007), and among *Crocodylus* species in particular (e.g., Feng et al., 2010; Meganathan et al., 2010). Here, we combine published mt genomes with eight newly-generated partial mt genome sequences, ~16,200 base pairs (bp), and use this dataset to build a mt DNA tree for all currently recognized extant species of *Crocodylus*.

2. Materials and methods

Our study includes all currently recognized species of *Crocodylus* with multiple exemplars of *C. niloticus*, *Crocodylus porosus*, *Crocodylus siamensis*, and *Crocodylus palustris*; the genera *Osteolaemus* and *Mecistops* were chosen as outgroups to *Crocodylus* based on previous phylogenetic work (Gatesy et al., 2004; Gatesy and Amato, 2008; Feng et al., 2010; Meganathan et al., 2010). Mitochondrial genomes have been published for most of these crocodylian species, but we added new data from eight specimens (Table 1). Tissue/blood from four wild-caught *C. niloticus* were collected by E. Hekkala, T. Shine, and R. Fergusson; these samples included representatives from both eastern and western populations of the Nile crocodile (*sensu* Schmitz et al., 2003; Hekkala et al., 2010, submitted for publication): Gatesy Lab ID #32 Gambia (Gambia River), Gatesy Lab ID #33 Mauritania (guelta Linsherbe), Gatesy Lab ID #36 Madagascar (S.E. near Fort Dauphin; Hekkala ID# MADSE333), Gatesy Lab ID #37 Zimbabwe (Sengwa, Lake Kariba; Hekkala ID# SEN8). The four other *Crocodylus* specimens utilized in this study were from St. Augustine Alligator Farm Zoological Park (St. Augustine, Florida, USA): *Crocodylus acutus* (St. Augustine ID# CA1), *Crocodylus rhombifer* (St. Augustine ID# 880002), *Crocodylus intermedius* (St. Augustine ID# 91277), *Crocodylus novaeguineae* (St. Augustine ID# 97041). Genomic DNAs were extracted from tissues using standard methods (proteinase K, phenol/chloroform, ethanol precipitation) or by utilizing the Qiagen DNeasy extraction kit.

Table 1
Sources of mt DNA sequences utilized in this study.

Species	Genbank number	Source
Crocodylus acutus SAAFZP[a]	JF502241*	This study
Crocodylus intermedius SAAFZP	JF502242*	This study
Crocodylus rhombifer SAAFZP	JF502247*	This study
Crocodylus moreletii	Meganathan	Meganathan et al. (2010)
Crocodylus niloticus 1 "eastern" Madagascar	JF502246*	This study
Crocodylus niloticus 2	DQ273697	Ji et al. (2006)
Crocodylus niloticus 3 "eastern" Zimbabwe	JF502245*	this study
Crocodylus niloticus 4	NC_008142	Janke et al. (2005)
Crocodylus niloticus 5 "western" Gambia	JF502243*	This study
Crocodylus niloticus 6 "western" Mauritania	JF502244*	This study
Crocodylus novaeguineae SAAFZP	JF502240*	This study
Crocodylus mindorensis	GU144287	Feng et al. (2010)
Crocodylus johnstoni	Meganathan	Meganathan et al. (2010)
Crocodylus palustris 1	Meganathan	Meganathan et al. (2010)
Crocodylus palustris 2	GU144286	Feng et al. (2010)
Crocodylus porosus 1	DQ273698	Li et al. (2007)
Crocodylus porosus 2	NC_008143	Janke et al. (2005)
Crocodylus siamensis 1	DQ353946	Ji et al. (2008)
Crocodylus siamensis 2	EF581859	Unpublished
Mecistops cataphractus	NC_010639	Unpublished
Osteolaemus tetraspis 1	EF551001	Unpublished
Osteolaemus tetraspis 2	NC_009728	Roos et al. (2007)

[a] SAAFZP = St. Augustine Alligator Farm Zoological Park.
* New to this study.

Overlapping PCR primer pairs were designed based on published *Crocodylus* mt genomes (DQ273697, NC_008142, DQ273698, NC_008143, DQ353946, EF581859) and used to amplify all mt DNA regions except for the terminal, repetitive segment of the D-loop (~16,200 bp total). All primer sequences are given in Supplementary online material 1. PCR reactions were run using Denville Scientific Inc. Ramp-Taq DNA polymerase in 50 ul reactions with the following thermal cycling parameters: initial denaturation at 95 °C for 2 min; 45 cycles of 1 min at 95 °C (denaturation), 1 min at 50 °C (annealing), and 1 min at 72 °C (extension); final extension at 72 °C for 10 min. When the primary PCR reaction failed to yield a usable product, nested PCR amplifications were performed using the aforementioned regime. One microliter of the original PCR product was used as template DNA in the nested PCR reactions. PCR products were run on 1% agarose gels, excised, and cleaned with Bioneer AccuPrep Gel Purification Kits. Cleaned PCR products were sequenced in both directions on an automated DNA sequencer (ABI 3730xl) at the University of California – Riverside Core Instrumentation Facility. Contigs were assembled with Sequencher 4.8. Eight partial mt genome sequences were generated (Table 1).

The new sequences were manually aligned to 14 previously published mt genomes (11 *Crocodylus* spp., 1 *Mecistops cataphractus*, 2 *Osteolaemus tetraspis*; Table 1) using Se–Al (Rambaut, 1996). The alignment length was 16,287 bp (Supplementary online material 2). Genes were identified based on a consensus crocodylid mt genome derived from previously annotated Genbank submissions. Regions of the mt genome that could not be directly assigned to a specific gene or to the D-loop were excluded from all analyses (249 bp). Sites in the mt alignment were partitioned a priori into classes based on general evolutionary constraints, and Bayes factors were calculated to determine the optimal partitioning scheme (Brandley et al., 2005). Five partitioning schemes were assessed: one partition (one model for all sites), two partitions (protein-coding sequence, all other sites), four partitions (1st codons, 2nd codons, 3rd codons, all other sites), six partitions (1st codons, 2nd codons, 3rd codons, tRNAs, rRNAs, D-loop), and eight partitions (1st codons, 2nd codons, 3rd codons, tRNA stems, tRNA loops, rRNA stems, rRNA loops, D-loop). jModelTest (Posada, 2008; Guindon and Gascuel, 2003) was used to determine the best fit model of molecular evolution for each partition as suggested by the Akaike Information Criterion. The marginal likelihood of each partitioning scheme was approximated using the harmonic mean estimated by MrBayes V3.1.1 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) and the stepping-stone approach (Fan et al., 2011; Xie et al., 2011) implemented in Phycas (Lewis et al., 2008). Bayes factors strongly favored eight partitions over all other compared partitioning schemes.

Eight datasets were subsequently analyzed: (1) complete (all data, partitioned by the three codon positions, tRNA loops, tRNA stems, rRNA loops, rRNA stems, D-loop), (2) codon (protein-coding regions, partitioned by codon position), (3) 1st codon position, (4) 2nd codon position, (5) 3rd codon position, (6) 1st and 2nd codon positions (partitioned by codon), (7) tRNAs (partitioned by stems and loops), (8) rRNAs (partitioned by stems and loops). tRNA consensus stem and loop positions were based on Ji et al. (2008) and

annotated Genbank mt genomes. In some cases, the tRNAs from annotated genomes had additional 5′ and 3′ nucleotides when compared to Ji et al. (2008). These extra bases were treated as loops in our partitioning schemes that included tRNAs. Consensus rRNA secondary structures, stems and loops, were predicted using the RNAalifold server (Vienna RNA WebServer; Hofacker, 2003) with default settings and all aligned sequences as input. Within the *Crocodylus* mt genome, several pairs of genes overlap each other. Therefore, each overlapping region was assigned to only one of the genes in phylogenetic searches (Supplementary online material 2).

For the model-based analyses (see below), each partition was given a particular model of sequence evolution as suggested by the Akaike Information Criterion of jModelTest (Posada, 2008; Guindon and Gascuel, 2003). Models chosen were as follows: GTR + Γ + I (1st codon position, 2nd codon position, and 3rd codon position); TVMef + Γ + I (tRNA stems); HKY + Γ + I (tRNA loops); TVM + Γ + I (rRNA stems); TVM + Γ (rRNA loops); TrN + Γ + I (D-loop). In cases where the model selected using jModelTest could not be implemented in a particular program, the next more general model was used (e.g., GTR for TVM).

PAUP* 4.0b10 (Swofford, 2002), RAxML v. 7.2.7-ALPHA (Stamatakis, 2006), and MrBayes V3.1.1 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) were used to perform maximum parsimony (MP), maximum likelihood (ML), and Bayesian analyses, respectively. Gaps were treated as missing data in all analyses. All character state transformations were weighted equally in MP searches, and MP bootstrap analyses (1000 replications) employed heuristic searches with 100 random taxon-addition sequences and tree-bisection and reconnection branch swapping. For the complete dataset, branch support (Bremer, 1994) and partitioned branch support (PBS; Baker and DeSalle, 1997) were calculated using Treerot (Sorenson, 1999) and PAUP* 4.0b10 (Swofford, 2002); PBS scores for each supported node were determined for the following partitions: 1st codon position, 2nd codon position, 3rd codon position, tRNA stems, tRNA loops, rRNA stems, rRNA loops, D-loop. ML bootstrap analyses employed 500 replicates, randomized MP starting trees, and the fast hill-climbing algorithm with all free parameters estimated. Bayesian analyses employed eight Markov chains (seven hot, one cold), random starting default priors with chain sampling every 1000 generations, and each partition permitted different branch lengths according to a rate multiplier. Analyses were terminated after the average standard deviation of split frequencies for the simultaneous analyses fell below 0.01 (~1.5–5 million generations). Convergence of topology was also tested using AWTY (Wilgenbusch et al., 2004; Nylander et al., 2008).

The approximately unbiased (AU) (Shimodaira, 2002), Shimodaira–Hasegawa (SH) (Shimodaira and Hasegawa, 1999), and Kishino–Hasegawa (KH) (Kishino and Hasegawa, 1989) tests were used to evaluate alternative phylogenetic hypotheses in a ML context. Two a priori hypotheses were tested using non-partitioned versions of the complete and codon datasets: (1) *C. niloticus* monophyly and (2) *C. niloticus* paraphyly (Schmitz et al., 2003). Constrained tree searches were performed using RAxML (v. 7.2.7-ALPHA; Stamatakis, 2006) under the GTR + Γ + I model of sequence evolution and fast hill-climbing algorithm for ten runs with all other free model parameters estimated. The tree with the best ML value was used in subsequent analyses. PAUP* 4.0b10 (Swofford, 2002) was used to determine the site-wise log-likelihoods for the trees with the highest likelihood scores. Statistical tests were performed using Consel (Shimodaira, 2002). In a MP context, alternative topologies, monophyly versus paraphyly of *C. niloticus*, were compared using the Wilcoxon signed rank test (Templeton, 1983) and the winning sites test (Prager and Wilson, 1988). Both were executed in PAUP* 4.0b10 (Swofford, 2002).

3. Results

Bayesian and ML analyses of the complete dataset of 16,038 aligned sites yielded the same topology (Fig. 1) with high support scores at most nodes (Table 2); 14 of 18 clades in the fully resolved tree for *Crocodylus* record Bayesian posterior probabilities of 1.0 and ML bootstrap scores of 100% (Fig. 1). The four New World species cluster in a monophyletic group as do the nine sequences from Asian and Australian species. Within the Asia + Australia grouping, the two mt sequences from *C. siamensis* (Siamese crocodile) were positioned in different subclades. *C. siamensis* 1 (Genbank DQ353946) fell among *C. porosus* (saltwater crocodile) sequences and showed limited sequence divergence from *C. porosus* 1, Genbank DQ273698 (Fig. 1). *C. siamensis* 2 (Genbank EF581859) clustered with the two *C. palustris* (mugger crocodile) exemplars, and this clade was the sister group to the *C. porosus* group. The combined group that included all sequences from *C. siamensis*, *C. porosus*, and *C. palustris* is characterized by moderate support scores in the explicitly model-based analyses (Table 2), and is the sister group to a clade composed of *Crocodylus johnstoni* (Australian freshwater crocodile), *C. novaeguineae* (New Guinea crocodile), and *Crocodylus mindorensis* (Philippine crocodile). The latter two are sister species according to the mt DNA data (Fig. 1).

Perhaps the most striking result recorded in the mt topology is a robust grouping of *C. niloticus* samples 1–4 with all New World *Crocodylus* to the exclusion of *C. niloticus* 5, *C. niloticus* 6, and all other extant *Crocodylus* species (Fig. 1). If interpreted strictly, the mt DNA data imply that the Nile crocodile, a widespread species in Africa, is not monophyletic and that the four New World species – *C. rhombifer* (Cuban crocodile), *C. acutus* (American crocodile), *C. intermedius* (Orinoco crocodile), and *C. moreletii* (Morelet's crocodile) – share a recent common ancestor with eastern populations of *C. niloticus*. Two of the sequences in this "eastern" clade were derived from wild-caught *C. niloticus* from Madagascar and Zimbabwe (*C. niloticus* 1 and 3, respectively); the other two, *C. niloticus* 2 and 4, were Genbank sequences DQ273697 and NC_008142, respectively (Fig. 1). The two wild-caught specimens from Gambia and Mauritania (*C. niloticus* 5 and 6, respectively) group in a "western" clade that is the sister group to "eastern" *C. niloticus* plus all New World *Crocodylus* species. The overall clade composed of six Nile crocodile sequences and the four New World species is well supported according to the ML and Bayesian methods (Fig. 1; Table 2).

Parsimony analysis of the complete mt dataset yielded a strict consensus of minimum-length trees that is generally congruent with the ML/Bayesian tree for *Crocodylus* but shows less resolution among Asian and Australian species (Fig. 2). The sole conflict among methods concerns the placement of *C. rhombifer* and *C. moreletii* within the New World clade, but support scores are weak (Fig. 2; Table 2). In the MP tree, the four New World *Crocodylus* are again nested within *C. niloticus*, closer to the "eastern" clade of Nile crocodiles, and this relationship garnered 100% bootstrap support (Table 2) with a branch-support score (Bremer, 1994) of +55 steps (Fig. 2).

Partitioned branch-support scores (Fig. 2; Baker and DeSalle, 1997) and separate analyses of different data partitions (Fig. 3; Table 2; Supplementary online Tables 1 and 2) were used to assess the distribution of support among different classes of data in the mt genome. Generally, character support for critical nodes was revealed in diverse molecular partitions (all codons, 1st codon, 2nd codon, 3rd codon, 1st + 2nd codons, tRNAs, tRNA stems, tRNA loops, rRNAs, rRNA stems, rRNA loops, D-loop). In the MP tree, PBS scores were nearly uniformly positive, with no conflicts, or only a trivial amount of conflict, at most nodes (Fig. 2). The exception was a PBS score of −7.5 steps for the 1st codon position at the *C. rhombifer* + *C. moreletii* node (Fig. 2), the only clade in the MP

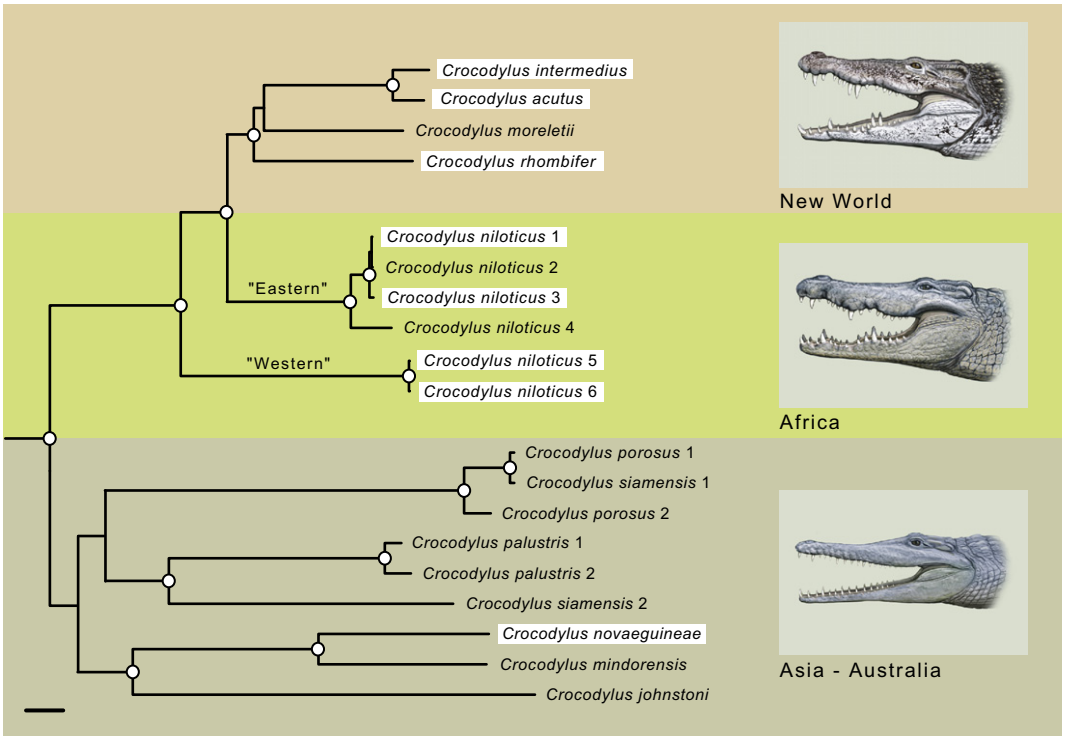

Fig. 1. Maximum likelihood (ML) phylogram for *Crocodylus* based on mt DNA sequences (complete matrix). The topology is identical to that supported by Bayesian analysis; white circles at nodes mark clades with 100% ML bootstrap support and posterior probabilities of 1.0. The scale bar (bottom left) is proportional to 0.01 substitutions per site. New World *Crocodylus* species form a monophyletic group, as do Asian plus Australian *Crocodylus* species, but *C. niloticus* is paraphyletic. According to the mt DNA results, "eastern" Nile crocodiles are more closely related to the four New World species than to "western" Nile crocodiles. The eight new mt DNA sequences generated for this study are indicated by gray rectangles overlaying terminal taxon names. The three background colors in the figure separate New World species of *Crocodylus* (represented by the painting of *C. rhombifer* – top), African lineages (*C. niloticus* – middle), and Asian–Australian species (represented by *C. johnstoni* – bottom). The tree includes multiple representatives for some species, and these are numbered (*C. niloticus* 1 = specimen #36 from Madagascar, *C. niloticus* 2 = Genbank DQ273697, *C. niloticus* 3 = specimen #37 from Zimbabwe, *C. niloticus* 4 = Genbank NC_008142, *C. niloticus* 5 = specimen #32 from Gambia, *C. niloticus* 6 = specimen #33 from Mauritania, *C. porosus* 1 = Genbank DQ273698, *C. porosus* 2 = Genbank NC_008143, *C. siamensis* 1 = Genbank DQ353946, *C. siamensis* 2 = Genbank EF581859, *C. palustris* 1 = Meganathan et al. (2010), *C. palustris* 2 = Genbank GU144286). The tree is rooted with mt sequences from *Mecistops* and *Osteolaemus* (not shown).

Table 2
Bootstrap and posterior probabilities summaries for codon and complete data sets. Bayesian posterior probabilities are reported as the average of the two independent runs. Bold = clade found in best MP or ML tree(s); MP = maximum parsimony; ML = maximum likelihood.

Clade	Data sets					
	Codon			Complete		
	MP	ML	Bayesian	MP	ML	Bayesian
Crocodylus	**100**	**100**	1.00	**100**	**100**	1.00
C. johnstoni + *C. mindorensis* + *C. novaeguineae* + *C. porosus* + *C. siamensis* + *C. palustris*	16	**82**	0.99	7	**69**	0.98
C. johnstoni + *C. mindorensis* + *C. novaeguineae*	43	**100**	1.00	56	**100**	1.00
C. mindorensis + *C. novaeguineae*	**100**	**100**	1.00	**100**	**100**	1.00
C. porosus + *C. siamensis* + *C. palustris*	**57**	**92**	1.00	47	**91**	1.00
C. porosus (DQ273698) + *C. siamensis* (DQ353946)	**100**	**100**	1.00	**100**	**100**	1.00
C. siamensis (EF581859) + *C. palustris*	**99**	**100**	1.00	**100**	**100**	1.00
C. niloticus + *C. rhombifer* + *C. moreletti* + *C. acutus* + *C. intermedius*	**100**	**100**	1.00	**100**	**100**	1.00
C. niloticus (West African)	**100**	**100**	1.00	**100**	**100**	1.00
C. niloticus (East African)	**100**	**100**	1.00	**100**	**100**	1.00
C. niloticus (Monophyly)	0	0	0.00	0	0	0.00
C. niloticus (East African) + *C. rhombifer* + *C. moreletti* + *C. acutus* + *C. intermedius*	**100**	**100**	1.00	**100**	**100**	1.00
C. rhombifer + *C. moreletti* + *C. acutus* + *C. intermedius*	**95**	**99**	1.00	**100**	**100**	1.00
C. moreletti + *C. acutus* + *C. intermedius*	29	**77**	0.99	24	**68**	0.92
C. acutus + *C. intermedius*	**100**	**100**	1.00	**100**	**100**	1.00

strict consensus that is incompatible with the ML/Bayesian tree (Fig. 1). Partitioned branch support at the node that joins New World *Crocodylus* with "eastern" *C. niloticus* shows consistent, positive character support from seven data partitions with no conflicts (Fig. 2). Separate MP, ML, and Bayesian analyses of different mt partitions also strongly favor this grouping in nearly all cases (Fig. 3; Table 2; Supplementary online Tables 1 and 2).

The sequence divergence between members of the "eastern" and "western" clades of *C. niloticus* is extensive, and the support for Nile crocodile paraphyly is significant according to a variety of statistical tests. Uncorrected pairwise distances between "eastern" and "western" Nile crocodiles for the complete dataset range from 5.6–5.7%, with 15.0–15.2% divergence at 3rd codon sites. For comparison, uncorrected distances among the four species of New

World *Crocodylus*, which are nested within *C. niloticus*, are 1.1–4.6% for the complete dataset and 3.0–12.6% at 3rd codons (also see branch lengths in Figs. 1 and 3). The optimal ML tree for the complete dataset was a significantly better fit to the mt DNA data in comparison to the best ML tree that retained a monophyletic *C. niloticus* (AU test: $P = 0.00003$, SH test: $P = 0.0004$, KH test: $P = 0.0004$). Likewise, in a MP context, Nile crocodile paraphyly was a much better fit to the complete dataset according to the Wilcoxon signed rank test ($P = 0.0109–0.0001$) and the winning sites test ($P = 0.0154–0.0001$). The same basic results hold for the codon dataset (AU test: $P = 0.0003$, SH test: $P = 0.002$, KH test: $P = 0.002$, Wilcoxon signed rank test: $P = 0.0001$, winning sites test: $P = 0.0002$).

4. Discussion

ML and Bayesian analyses of partial mt genomes yielded a fully resolved tree for all extant species of *Crocodylus* (Fig. 1). Separate analyses of subpartitions from the 16,038 bp complete matrix showed that the support for the overall tree comes from diverse

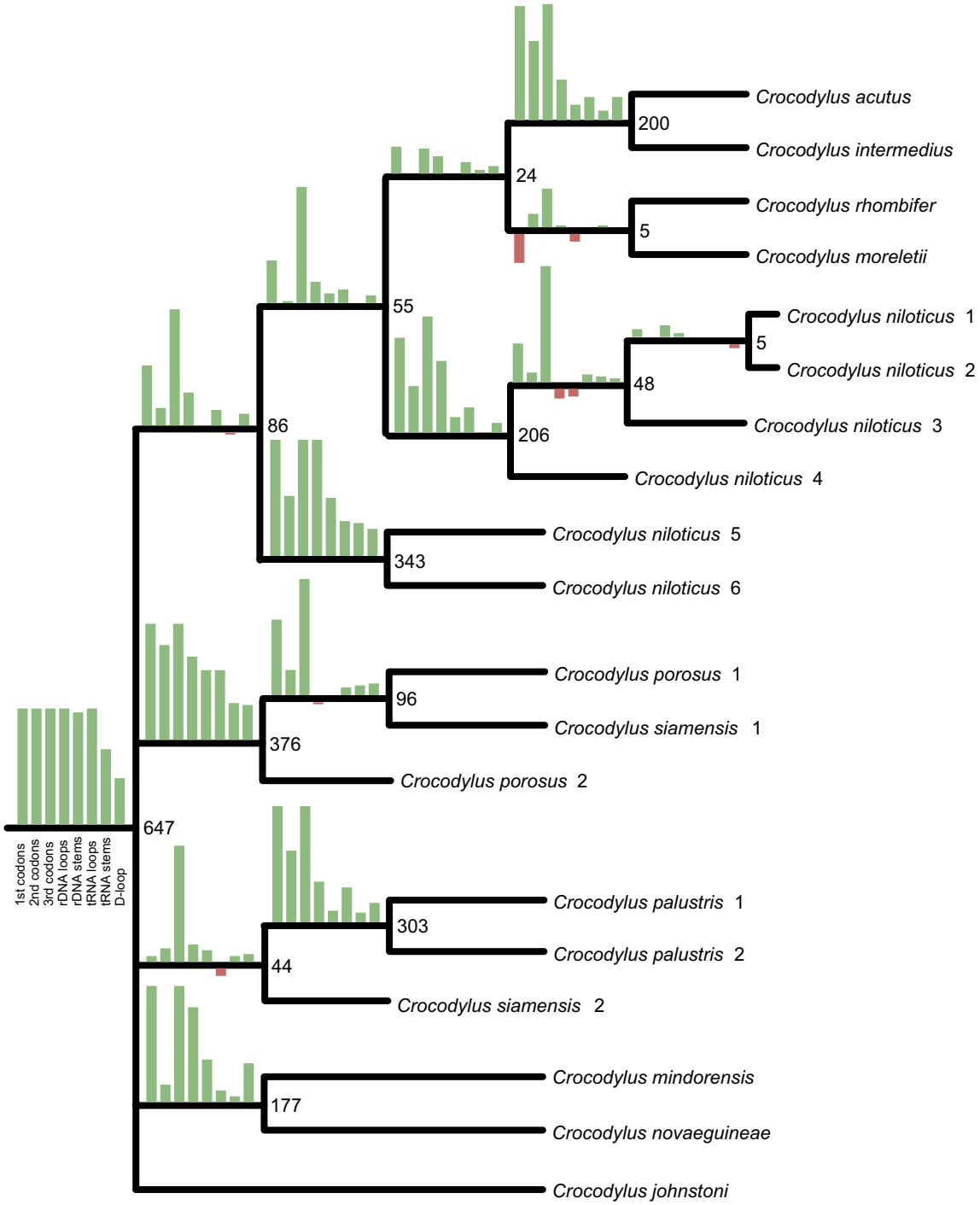

Fig. 2. Strict consensus of minimum-length parsimony trees for the complete matrix with branch support and partitioned branch support (PBS) at nodes. Branch-support scores are shown as numbers to the right of nodes. PBS scores for eight partitions (see basal branch for key) are represented by green bars above internodes (positive) and red bars below internodes (negative); a PBS score of zero for a partition has no bar. The shortest bars represent PBS scores of 0.5 steps. PBS scores that are $\geqslant 30$ steps are represented by bars that are 30 units. Multiple individuals from the same species are numbered as in Fig. 1.

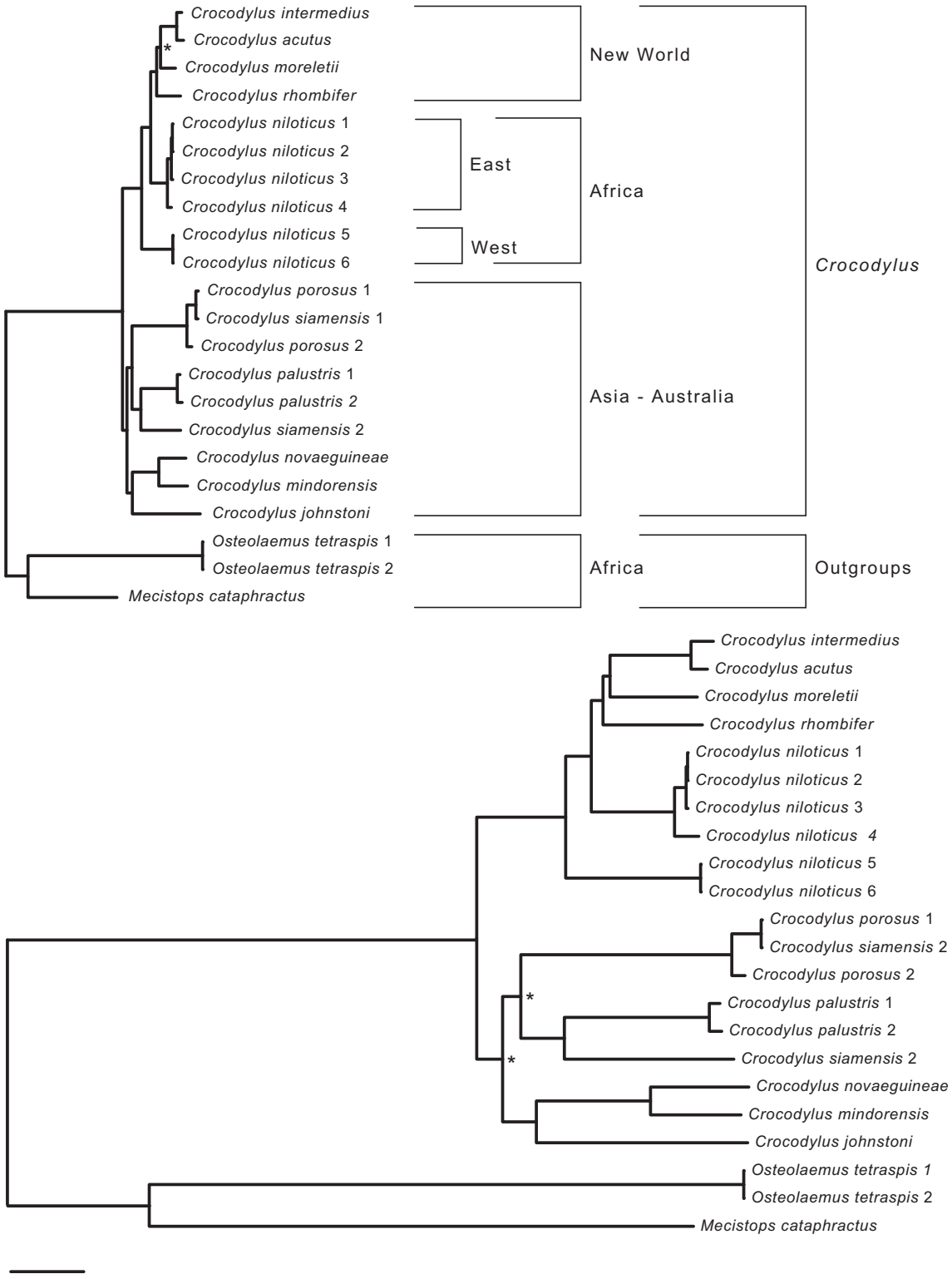

Fig. 3. Bayesian 50% majority rule phylograms for 1st + 2nd codons (top) and 3rd codons (bottom). Branch lengths in the two trees are drawn to scale (scale bar = 0.06 substitutions per site); note the much longer branches in the tree based on 3rd codons. Asterisks at nodes mark the three clades characterized by posterior probabilities less than 0.95. The trees include multiple representatives for some species, and these generally are numbered as in Fig. 1 but with *Osteolaemus* 1 = Genbank EF551001 and *Osteolaemus* 2 = Genbank NC_009728.

classes of mt data (Fig. 3; Table 2; Supplementary online Tables 1 and 2). Although not as well resolved as the explicitly model-based methods, MP analysis of the complete dataset corroborated all of the strongly supported clades in the ML/Bayesian tree (Fig. 1), and PBS scores again indicate that character support comes from across the mt DNA molecule (Fig. 2).

Because distantly related taxa have been included in many previous analyses of crocodylian mt genomes, phylogenetic analyses often have been restricted to data partitions that have evolved at relatively slow rates, in particular 1st and 2nd codon positions of protein coding genes, ~7500 nucleotides (e.g., Janke et al., 2005; Roos et al., 2007; Janke, 2008; Feng et al., 2010). Here, we focused

on relationships at a finer taxonomic level, explored the utility of more rapidly evolving mt DNA partitions, and included a greater percentage of the mt genome in our phylogenetic estimates. Bayesian/ML analyses of 1st + 2nd codons yielded the same topology as the complete matrix (all three codons, rRNAs, tRNAs, D-loop), as did analysis of the three codons combined and separate analysis of just 3rd codons. These sub-analyses demonstrate that rapidly evolving 3rd codons exactly replicate the phylogenetic pattern supported by the more conservative 1st + 2nd codons (Fig. 3; see Supplementary online Table 2 for support scores). By utilizing different models for subpartitions of the complete matrix in ML searches, and by allowing different models and branch lengths in Bayesian analyses for partitions that have evolved at very different rates, we accommodated at least some of the rate heterogeneities in the crocodylian mt genome.

Our overall phylogenetic hypothesis for *Crocodylus* is highly consistent with the analysis of Brochu and Densmore (2001), which was based on a combination of morphological evidence, restriction fragment data, and mt DNA sequences (12S rDNA, ND6 to cyt*b*). *C. palustris* was not included in Brochu and Densmore (2001), but relationships among the remaining ten *Crocodylus* species in their trees are identical to our overall topology (Fig. 1). Published MP supermatrix trees (Gatesy et al., 2004; Gatesy and Amato, 2008) also are highly congruent with our overall results (Figs. 1 and 2), however in both supermatrix studies, *C. porosus* grouped with *C. palustris* to the exclusion of *C. siamensis*. The mt genome trees of Feng et al. (2010) and Meganathan et al. (2010) included fewer species of *Crocodylus* than the current analysis, but matched our overall ML/Bayesian tree (Fig. 1). Smaller datasets also have supported many of the clades consistently resolved by the mt DNA data, including *C. acutus* + *C. intermedius* (Densmore and White, 1991 – restriction fragments; Poe, 1996 – combined morphological and molecular data; McAliley et al., 2006 – mt D-loop; mt ND6 to cyt*b*; Li et al., 2007 – mt D-loop), all New World *Crocodylus* (McAliley et al., 2006 – *C-mos*; Willis, 2009 – *TTR*; Brochu, 2000; Brochu et al., 2010 – morphology; Li et al., 2007 – mt D-loop), all New World *Crocodylus* plus *C. niloticus* (McAliley et al., 2006 – mt ND6 to cyt*b*; Brochu, 2000; Brochu et al., 2010 – morphology; Li et al., 2007 – mt D-loop), *C. mindorensis* + *C. novaeguineae* (Densmore and White, 1991 – restriction fragments; Poe, 1996 – combined morphological and molecular data; McAliley et al., 2006 – mt ND6 to cyt*b*), *C. mindorensis* + *C. novaeguineae* + *C. johnstoni* (McAliley et al., 2006 – mt ND6 to cyt*b*), *C. palustris* + *C. porosus* + *C. siamensis* (Densmore and White, 1991 – restriction fragments; Li et al., 2007 – mt D-loop), and all Asian + Australian *Crocodylus* (Brochu, 2000; Brochu et al., 2010 – morphology).

In addition to generally robust support and congruence with previous systematic studies, our analyses revealed two instances where *Crocodylus* species were not monophyletic according to mt data (Figs. 1–3). In the first case, the two *C. siamensis* sequences did not cluster. *C. siamensis* 1 (DQ353946; Ji et al., 2008) groups within *C. porosus* and shows very limited divergence from the *C. porosus* 1 sequence (DQ273698; Li et al., 2007), only 0.2% pairwise distance (Fig. 1). The uncorrected pairwise distance between the two *C. siamensis* sequences in the dataset, DQ353946 and EF581859, is substantially greater (8.4%). Partial mt rDNA and cyt*b* sequences from an additional *C. siamensis* sample in our lab (St. Augustine ID# S166/162) revealed a very close match to *C. siamensis* 2 (EF581859) and high divergence from the *C. siamensis* 1 sequence. Perhaps the simplest explanation for the grouping of *C. siamensis* 1 within *C. porosus* is that the Siamese crocodile sample sequenced by Ji et al. (2008) was a *C. siamensis* × *C. porosus* hybrid; these species have been crossed frequently in captivity for the skin trade (Fitzsimmons et al., 2002). Our results (Figs. 1–3) are relevant to previous systematic work that has attempted to place *C. siamensis*. In particular, a recent study (Meganathan et al., 2010) robustly supported a sister group relationship between *C. porosus* and *C. siamensis* among extant *Crocodylus* (also see Feng et al., 2010); this relationship should be considered much less secure given our trees based on a broader sample of mt DNA sequences. Additional data, preferably from wild-caught *C. siamensis*, are required to resolve the discrepant phylogenetic positions of mt sequences from this critically endangered species.

The Nile crocodile, *C. niloticus*, also was not monophyletic in our optimal trees (Figs. 1–3). A marked genetic break in this species between eastern and western populations has been documented previously in surveys of mt DNA (Schmitz et al., 2003; Hekkala et al., submitted for publication), nuclear gene sequences (Hekkala et al., submitted for publication), and microsatellites (Hekkala et al., 2010). With sampling from 13 populations of the Nile crocodile, Schmitz et al. (2003) reported high genetic distances, 4.3–5.4%, between East and West African populations for ~425 bp of mt 12S rDNA, and suggested a division of *C. niloticus* into two species. In the present study, a larger sample of molecular data per individual permitted more robust estimates of phylogenetic patterns and divergence among mt lineages of *C. niloticus*. Partial mt genome sequences show 5.6–5.7% uncorrected distance between wild-caught eastern and western Nile crocodiles. As in Schmitz et al. (2003), *C. niloticus* is not monophyletic in mt trees, but for the >16,000 bp examined here, *C. niloticus* paraphyly is very strongly supported (Table 2; Supplementary online Tables 1 and 2). Wild-caught representatives of eastern populations (*C. niloticus* 1 and 3) and the two published mt genome sequences (*C. niloticus* 2 and 4; Janke et al., 2005; Ji et al., 2006) cluster with New World *Crocodylus* to the exclusion of individuals from western populations of the Nile crocodile (*C. niloticus* 5 and 6) (Figs. 1–3).

Monophyly of mt DNA sequences from *C. niloticus* was rejected by multiple statistical tests (AU, SH, KH, Wilcoxon signed rank, winning sites), suggesting significant support for paraphyly of this named species. Some authors have given great weight to such tests in assessments of phylogenetic support for alternative topologies in analyses of mt genomes (e.g., Janke et al., 2005; Roos et al., 2007; Janke, 2008), but this represents a somewhat simplistic view. The tight linkage of all mt genes and the presumed rarity of recombination in the vertebrate mt genome suggest that, given certain circumstances, different nucleotide positions in the mt genome do not represent independent pieces of phylogenetic evidence for the reconstruction of evolutionary history (Maddison, 1997). As has been noted in previous studies of crocodylian phylogeny, it would be naïve to assume the impossibility of mt DNA introgression across species boundaries, deep coalescence, or other processes that yield conflicts between gene trees and species trees (Gatesy et al., 2003; Gatesy and Amato, 2008; but see Janke, 2008 for an alternative view). Thus, the statistical rejection of *C. niloticus* monophyly documented here simply represents robust support for Nile crocodile paraphyly in a mt tree for *Crocodylus* (Fig. 1), and might not represent robust support for the species tree of *Crocodylus*. Additional systematic evidence from the nuclear genome, morphological characters, and extinct taxa is required to determine whether the mt DNA result is a quirk or a general evolutionary pattern that is corroborated by independent character data. That being said, our mt DNA trees present a striking pattern that warrants discussion.

The preferred topologies suggest what, at first glance, might be considered an unlikely biogeographic scenario. Specifically, the nesting of all four New World species of *Crocodylus* within *C. niloticus* implies a relatively recent migration from Africa to the Americas. Given that the earliest, phylogenetically substantiated occurrences of crown *Crocodylus* are at most Late Miocene, the oldest fossils assigned to *C. niloticus* are Pliocene, and that the oldest New World *Crocodylus* also are Pliocene (Miller, 1980; Brochu, 2000; Delfino et al., 2007; Brochu et al., 2010), the simplest

interpretation is that an African *Crocodylus* crossed the Atlantic quite recently, when this oceanic barrier was already hundreds of miles wide (Ford and Golonka, 2003; Scotese, 2004; Bandoni de Oliveira et al., 2009). Previously, Brochu (2001), among others (Densmore, 1983; Taplin and Grigg, 1989), have noted that the young geological age of *Crocodylus* suggests a long trans-oceanic journey to the Americas, so the geographic scope of this dispersal event has been considered feasible in past reconstructions of crocodylian biogeography. In addition, molecular and paleontological data also imply trans-Atlantic dispersals by several other vertebrate taxa (Mausfeld et al., 2002; de Queiroz, 2005; Bandoni de Oliveira et al., 2009).

The anatomical and physiological attributes of *Crocodylus* make these reptiles viable candidates for such a long journey at sea. Crocodiles are large-bodied with a relatively low metabolism, and *C. porosus* individuals have been recorded traveling up to 590 km in 25 days via oceanic currents (Campbell et al., 2010). Like *C. porosus*, Nile crocodiles have lingual salt excreting glands, and some populations of *C. niloticus* occupy estuarine habitats (Taplin and Grigg, 1989). Furthermore, the phylogenetic relationships of Crocodylia, in combination with geographic and temporal data from the fossil record, provide compelling evidence for long-distance dispersals across saltwater barriers throughout the evolutionary history of this clade (Brochu, 2001 and references therein). Thus, a relatively recent trans-Atlantic crossing of "*C. niloticus*" to the New World – with a subsequent diversification that produced *C. acutus*, *C. rhombifer*, *C. moreletii*, and *C. intermedius* – would seem to be the most parsimonious explanation given our phylogenetic results (Figs. 1–3) and the known fossil record of *Crocodylus* (Brochu, 2000; Brochu et al., 2010).

Acknowledgments

NSF grant EAR-0228629 helped to fund this work. A. de Queiroz, C. Hayashi, A. Larson, and two anonymous reviewers gave helpful comments. P. Meganathan and D. Ray sent published mt genome sequences. R. Fergusson and T. Shine provided tissue specimens from wild-caught *Crocodylus niloticus*; St. Augustine Alligator Farm Zoological Park donated additional tissue samples. H. Lee assisted in DNA extraction and sequencing. Carl Buell executed paintings of crocodiles in Fig. 1.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2011.03.026.

References

Baker, R., DeSalle, R., 1997. Multiple sources of character information and the phylogeny of Hawaiian drosophilids. Syst. Biol. 46, 654–673.

Bandoni de Oliveira, F., Cassola Molina, E., Marroig, G., 2009. Paleogeography of the South Atlantic: a route for primates and rodents into the New World. In: Garber, P., Estrada, A., Bicca-Marques, J., Heymann, E., Strier, K. (Eds.), South American Primates: Comparative Perspectives in the Study of Behavior, Ecology, and Conservation. Springer Science + Business Media, New York, pp. 55–68.

Brandley, M., Schmitz, A., Reeder, T., 2005. Partitioned Bayesian analyses, partition choice, and the phylogenetic relationships of scincid lizards. Syst. Biol. 54, 373–390.

Bremer, K., 1994. Branch support and tree stability. Cladistics 10, 295–304.

Brochu, C., 2000. Phylogenetic relationships and divergence timing of *Crocodylus* based on morphology and the fossil record. Copeia 3, 657–673.

Brochu, C., 2001. Congruence between physiology, phylogenetics, and the fossil record on crocodylian historical biogeography. In: Grigg, G., Seebacher, F., Franklin, C. (Eds.), Crocodilian Biology and Evolution. Surrey Beatty and Sons, Chipping Norton, NSW, pp. 9–28.

Brochu, C., 2003. Phylogenetic approaches toward crocodylian history. Ann. Rev. Earth Planet. Sci. 31, 357–397.

Brochu, C., Densmore, L., 2001. Crocodile phylogenetics: a summary of current progress. In: Grigg, G., Seebacher, F., Franklin, C. (Eds.), Crocodilian Biology and Evolution. Surrey Beatty and Sons, Chipping Norton, NSW, pp. 3–8.

Brochu, C., Njau, J., Blumenschine, R., Densmore, L., 2010. A new horned crocodile from the Plio-Pleistocene hominid sites at Olduvai Gorge, Tanzania. PloS ONE 5 (2), e9333. doi:10.1371/journal.pone.0009333.

Campbell, H., Watts, M., Sullivan, S., Read, M., Choukroun, S., Irwin, S., Franklin, C., 2010. Estuarine crocodiles ride surface currents to facilitate long-distance travel. J. Anim. Ecol. 79, 955–964.

Delfino, M., Böhme, M., Rook, L., 2007. First European evidence for transcontinental dispersal of *Crocodylus* (late Neogene of southern Italy). Zool. J. Linn. Soc. – Lond. 149, 293–307.

Densmore, L., 1983. Biochemical and immunological systematics of the order Crocodilia. Evol. Biol. 16, 397–465.

Densmore, L., White, P., 1991. The systematics and evolution of the Crocodilia as suggested by restriction endonuclease analysis of mitochondrial and nuclear ribosomal DNA. Copeia 1991, 602–615.

de Queiroz, A., 2005. The resurrection of oceanic dispersal in historical biogeography. Trends Ecol. Evol. 20, 68–73.

Fan, Y., Wu, R., Chen, M.-H., Kuo, L., Lewis, P.O., 2011. Choosing among partition models in Bayesian phylogenetics. Mol. Biol. Evol. 28, 523–532.

Feng, G., Wu, X., Yan, P., Li, X., 2010. Two complete mitochondrial genomes of *Crocodylus* and implications for crocodilians phylogeny. Amphibia – Reptilia 31, 299–309.

Fitzsimmons, N., Buchan, J., Lam, P., Polet, G., Hung, T., Thang, N., Gratten, J., 2002. Identification of purebred *Crocodylus siamensis* for reintroduction in Vietnam. J. Exp. Zool. 294, 373–381.

Ford, D., Golonka, J., 2003. Phanerozoic paleogeography, paleoenvironment, and lithofacies maps of the circum-Atlantic margins. Mar. Petrol. Geol. 20, 249–285.

Gatesy, J., Amato, G., Norell, M., DeSalle, R., Hayashi, C., 2003. Combined support for wholesale taxic atavism in gavialine crocodylians. Syst. Biol. 52, 403–422.

Gatesy, J., Amato, G., 2008. The rapid accumulation of consistent molecular support for intergeneric crocodylian relationships. Mol. Phylogenet. Evol. 48, 1232–1237.

Gatesy, J., Baker, R., Hayashi, C., 2004. Inconsistencies in arguments for the supertree approach: supermatrices versus supertrees of Crocodylia. Syst. Biol. 53, 342–355.

Guindon, S., Gascuel, O., 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst. Biol. 52, 696–704.

Hekkala, E.R., Amato, G., DeSalle, R., Blum, M.J., 2010. Molecular assessment of population differentiation and individual assignment potential of Nile crocodile (*Crocodylus niloticus*) populations. Conserv. Genet. 11, 1435–1443.

Hekkala, E.R., Shirley, M.H., Amato, G., Austin, J.D., Charter, S., Thorbjarnarson, J., Vliet, K.A., Houck, M.L., DeSalle, R., Blum, M.J., submitted for publication. Splitting an ancient icon: mummy DNA resurrects a cryptic Nile crocodile. Mol. Ecol.

Hofacker, I.L., 2003. Vienna RNA secondary structure server. Nucleic Acids Res. 31, 3429–3431.

Huelsenbeck, J., Ronquist, F., 2001. MrBayes: Bayesian inference of phylogenetic trees. Bioinformatics 17, 754–755.

Janke, A., 2008. The importance of data sampling and analysis in resolving crocodylian relationships. Mol. Phylogenet. Evol. 49, 407–408.

Janke, A., Gullberg, A., Hughes, S., Aggarwal, R., Arnason, U., 2005. Mitogenomic analyses place the gharial (*Gavialis gangeticus*) on the crocodile tree and provide pre-*K*/*T* divergence times for most crocodilians. J. Mol. Evol. 61, 620–626.

Ji, X., Wu, X., Li, Y., Yan, P., Amato, G., 2006. The mitochondrial genome of *Crocodylus niloticus* with implications for phylogenetic relationships among crocodilian species. Acta Zool. Sin. 52, 810–818.

Ji, X., Wu, X., Yan, P., Amato, G., 2008. Complete sequence and gene organization of the mitochondrial genome of Siamensis crocodile (*Crocodylus siamensis*). Mol. Biol. Rep. 35, 133–138.

Kishino, H., Hasegawa, M., 1989. Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data and the branching order in Hominoidea. J. Mol. Evol. 29, 170–179.

Lewis, P.O., Holder, M.T., Swofford, D.L., 2008. Phycas: Software for Phylogenetic Analysis. Storrs (CT): University of Connecticut. <http://www.phycas.org/>.

Li, Y., Wu, X., Ji, X., Yan, P., Amato, G., 2007. The complete mitochondrial genome of salt-water crocodile (*Crocodylus porosus*) and phylogeny of crocodilians. J. Genet. Genomics 34, 119–128.

Maddison, W., 1997. Gene trees in species trees. Syst. Biol. 46, 523–536.

Mausfeld, P., Schmitz, A., Böhme, W., Misof, B., Vrcibradic, D., Frederico, C., Rocha, D., 2002. Phylogenetic affinities of *Mabuya atlantica* Schmidt, 1945, endemic to the Atlantic Ocean archipelago of Fernando de Noronha (Brazil): necessity of partitioning the genus *Mabuya* Fitzinger, 1826 (Scincidae: Lygosominae). Zool. Anz. 241, 281–293.

McAliley, L., Willis, R., Ray, D., White, P., Brochu, C., Densmore, L., 2006. Are crocodiles really monophyletic? – Evidence for subdivisions from sequence and morphological data. Mol. Phylogenet. Evol. 39, 16–32.

Meganathan, P., Dubey, B., Batzer, M., Ray, D., Haque, I., 2010. Molecular phylogenetic analyses of genus *Crocodylus* (Eusuchia, Crocodylia, Crocodylidae) and the taxonomic position of *Crocodylus porosus*. Mol. Phylogenet. Evol. 57, 393–402.

Miller, W., 1980. The Late Pliocene Las Tunas local fauna from southernmost Baja California, Mexico. J. Paleontol. 54, 762–805.

Nylander, J.A., Wilgenbusch, J.C., Warren, D.L., Swofford, D.L., 2008. AWTY (are we there yet?): a system for graphical exploration of MCMC convergence in Bayesian phylogenetics. Bioinformatics 24, 581–583.

Poe, S., 1996. Data set incongruence and the phylogeny of crocodilians. Syst. Biol. 45, 393–414.

Posada, D., 2008. JModelTest: Phylogenetic model averaging. Mol. Biol. Evol. 25, 1253–1256.
Prager, E., Wilson, A., 1988. Ancient origin of lactalbumin from lysozyme: analysis of DNA and amino acid sequences. J. Mol. Evol. 27, 326–335.
Rambaut, A., 1996. Sequence Alignment Editor. Oxford University. <http://evolve.zps.ox.ac.uk/software/index.html>.
Ray, D., Dever, J., Platt, S., Rainwater, T., Finger, A., McMurry, S., Batzer, M., Barr, B., Stafford, P., McKnight, J., Densmore, L., 2004. Low levels of nucleotide diversity in *Crocodylus moreletii* and evidence of hybridization with *C. acutus*. Conserv. Genet. 5, 449–462.
Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.
Roos, J., Aggarwal, R., Janke, A., 2007. Extended mitogenomic phylogenetic analyses yield new insight into crocodilian evolution and their survival of the Cretaceous – Tertiary boundary. Mol. Phylogenet. Evol. 45, 663–673.
Schmitz, A., Mausfeld, P., Hekkala, E.R., Shine, T., Nickel, H., Amato, G., Böhme, W., 2003. Molecular evidence for species level divergence in African Nile crocodiles *Crocodylus niloticus* (Laurenti, 1986). C.R. Palevol. 2, 703–712.
Scotese, C., 2004. A continental drift flipbook. J. Geol. 112, 729–741.
Shimodaira, H., 2002. An approximately unbiased test of phylogenetic tree selection. Syst. Biol. 51, 492–508.
Shimodaira, H., Hasegawa, M., 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. Mol. Biol. Evol. 16, 1114–1116.
Sorenson, M., 1999. TreeRot, Version 2. Boston University, Boston, MA.
Stamatakis, A., 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22, 2688–2690.
Swofford, D.L., 2002. PAUP*. Phylogenetic Analysis Using Parsimony (* and Other Methods). Sinauer Associates, Sunderland, MA.
Taplin, L., Grigg, G., 1989. Historical zoogeography of the eusuchian crocodilians: a physiological perspective. Am. Zool. 29, 885–901.
Templeton, A., 1983. Phylogenetic inference from restriction endonuclease cleavage site maps with particular reference to the evolution of humans and the apes. Evolution 37, 221–244.
Weaver, J., Rodriguez, D., Venegas-Anaya, M., Cedenõ-Vázquez, J., Forstner, M., Densmore, L., 2008. Genetic characterization of captive Cuban crocodiles (*Crocodylus rhombifer*) and evidence of hybridization with the American crocodile (*Crocodylus acutus*). J. Exp. Zool. 309A, 649–660.
Wilgenbusch, J.C., Warren, D.L., Swofford, D.L., 2004. AWTY: A System for Graphical Exploration of MCMC Convergence in Bayesian Phylogenetic Inference. <http://ceb.csit.fsu.edu/awty>.
Willis, R., 2009. Transthyretin gene (TTR) intron 1 elucidates crocodylian phylogenetic relationships. Mol. Phylogenet. Evol. 53, 1049–1054.
Willis, R., McAliley, L., Neeley, E., Densmore, L., 2007. Evidence for placing the false gharial (*Tomistoma schlegelii*) into the family Gavialidae: inferences from nuclear gene sequences. Mol. Phylogenet. Evol. 43, 787–794.
Xie, W., Lewis, P.O., Fan, Y., Kuo, L., Chen, M.-H., 2011. Improving marginal likelihood estimation for Bayesian phylogenetic model selection. Syst. Biol. 60, 150–160.