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Evolution

Generic affinities among crocodilians as revealed by DNA fingerprinting with a Bkm-derived probe

(restriction fragment length polymorphism/multilocus DNA probe/repetitive DNA probe/phenogram/genetic profile)

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ABSTRACT **Genetic fingerprint profiles have been successfully used for establishing biological relationships, in linkage analysis, and in studies of population structure but have not so far been used for ascertaining phylogenetic relationships among related groups of species and genera. This is largely because these profiles are thought to evolve too rapidly to be informative over large time intervals. However, we show here that among the Crocodilia, whose phylogeny is a debated issue, these profiles can provide phylogenetically useful information. By using the probe Bkm-2(8), DNA fingerprints with distinct bands distributed in the size range 0.5–23.0 kb were obtained for individuals of 18 species belonging to seven of the eight genera of crocodilians. These genetic profiles showed individual-, species-, and restriction enzyme-specific patterns. In addition, striking differences were observed in the copy number of Bkm-related sequences in genomes of different crocodilian species. The qualitative data from DNA fingerprint profiles, and quantitative data on copy number variation in Bkm-related sequences, suggest that these genera belong to two distinct groups, one of which includes *Alligator, Paleosuchus*, and *Caiman*; the other includes *Crocodylus, Osteolaemus, Tomistoma*, and *Gavialis*. A close relationship between *Tomistoma* and *Gavialis* is also suggested by these results.**

Crocodilians are the sole living reptilian representatives of the subclass Archosauria, a highly successful group in the Mesozoic era both in numbers and in diversity. At present, only 8 of the 124 described genera have survived and all of these belong to the same suborder, Eusuchia (1). According to most systematists, there are only 21 extant species, 11 of which belong to *Crocodylus*, which is by far the largest genus.

The natural affinities among living crocodilians have so far been determined primarily on the basis of comparative morphology and paleontological records. However, the resolving power of these approaches has not been adequate to solve certain problematic and confusing relationships within the order Crocodilia. The commonality in life-style of many of the crocodilian taxa may have led to similar adaptative strategies—e.g., convergent skull morphology and head shape. Such convergence in characters, although considered phylogenetically important, has made interpretation of the systematic relationships in crocodilians difficult (2). This has led to the use of other approaches such as cytogenetic parameters (3, 4), analysis of coevolving crocodilian–parasite lineages (5), biochemical and immunological studies of proteins (6–8), and Southern blot and DNA sequence analyses of mitochondrial and nuclear ribosomal DNA (9–11) to resolve the natural affinities and evolutionary history of the living crocodilians.

On the basis of the approaches described above, there is general agreement in aligning *Osteolaemus* with *Crocodylus* and the caimans (*Caiman, Melanosuchus, Paleosuchus*) as the nearest sister taxa of *Alligator*, whereas opinion is divided on the affinities of the two gharial genera (*Gavialis* and *Tomistoma*) to each other and to other crocodilians. Some favor a close relationship of gavials with crocodylids (1, 6, 9, 11), whereas others place them in a separate family/lineage (12).

The use of DNA fingerprinting (13) has recently been shown to be useful in estimating relative genetic variability and in reconstructing the evolutionary relationships of natural populations of genetically isolated mammals (14). In the present study, we have used DNA fingerprinting, with the Bkm-2(8) probe, to study phylogenetic relationships among 18 of the 21 living species belonging to seven of the eight genera of crocodilians. The Bkm sequences were first identified and isolated as a minor satellite DNA from the genomic DNA of the female Indian banded krait (*Bungarus fasciatus*). Since then, it has been demonstrated that the major component of Bkm consists of tandem repeats of the tetranucleotide GATA, which shows extensive restriction fragment length polymorphism in various eukaryotes and can therefore be used as an efficient probe for genetic fingerprinting (15–22).

Our results, based on quantitative as well as qualitative differences in the genetic fingerprint profiles obtained by use of the Bkm-2(8) probe, suggest that the seven crocodilian genera studied belong to two distinct groups; the first group includes *Alligator, Paleosuchus*, and Caiman, and the second group includes *Crocodylus, Osteolaemus, Tomistoma*, and *Gavialis*. The results also suggest that the two gharial genera, *Tomistoma* and *Gavialis*, are closely related.

MATERIALS AND METHODS

Samples. Blood samples were collected from the heart or brain plexus of 203 individuals and stored at −70°C (Table 1).

DNA Fingerprinting. DNA isolation, digestion, gel electrophoresis, Southern blotting, and filter hybridization were done as described by Lang *et al.* (22) and by Aggarwal *et al.* (23).

Slot Blotting. Slot blots were prepared in duplicate for each individual of different species with 60, 180, and 360 ng of DNA onto a Hybond-N membrane, using a Minifold II apparatus (Schleicher & Schuell). The membranes were then hybridized with the ^{32}P-labeled single-stranded Bkm-2(8) probe. To confirm that the quantity of DNA loaded for different individuals was the same, the hybridized blots were melted and rehybridized with a nick-translated ^{32}P-labeled *Xenopus* rDNA probe.

Scoring and Analysis of DNA Fingerprints. Distinct bands representing DNA fragments ranging in size from 1.3 to 23.0 kb were scored in each genetic profile. All bands showing similar sizes and intensities were considered to be identical. Molecular size markers and duplicate samples from the same individual were run on either side of the gel to check for mobility distortion. Samples of a set of individuals representing a genus/species were run in each gel, along with the

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Table 1. Number, source, and origin of crocodilians used for DNA fingerprinting

Crocodilian genera/species (captive locality)	Sex		
	M	F	?
Paleosuchus palpebrosus (a)	1		
Paleosuchus trigonatus (a)	1	1	
Caiman crocodilus yacare (a)	1		
Caiman latirostris (a)	1		
Caiman crocodilus crocodilus (a, b)	7	10	
Alligator sinensis (a)*		1	
Alligator mississippiensis (b, c,† d‡)	3	2	
Gavialis gangeticus (b)	1	2	1
Tomistoma schlegelii (b, e)	3	1	
Osteolaemus tetraspsis (a, b)	3	2	
Crocodylus palustris (b, f)§	46	84	2
Crocodylus porosus (b, f)	3	6	
Crocodylus acutus (a, c)¶		3	1
Crocodylus niloticus (a, b)		5	
Crocodylus moreletii (b)		1	2
Crocodylus rhombifer (a)		1	
Crocodylus siamensis (b, e)	2	4	
Crocodylus cataphractus (e)		1	1

a, Ocala; b, Madras Crocodile Bank (India); c, Gatorama; d, University of North Dakota; e, Miami Zoo; f, Nehru Zoological Park, Hyderabad, India.
*Origin, China.
†Origin, Florida.
‡Origin, Louisiana.
§Origin, different parts of India and captive bred animals at Madras Crocodile Bank.
¶Origin, Jamaica.

samples to be compared, to facilitate the comparison of DNA fingerprints obtained from different gels.

DNA fingerprints were scanned and the fragments were calibrated for size by using a λ *Hin*dIII/*Eco*RI double digest as the molecular size marker on the Biotrac DNA fingerprinting system (Foster and Freeman, Worcestershire, U.K.) using the BIOWORLD program. The inter- and intrageneric/species variability was estimated by calculating the difference value, *D*, in all possible pairwise combinations. The difference value (*D*) between any two DNA profiles was calculated as the number of fragments that were different divided by the total number of fragments present in the two individuals (14). The degree of relatedness within the members of the same species/genera was calculated by subtracting *D* (average of all the *D* values for the species/genera in question) from 1. The degree of divergence between any two genera was arrived at by averaging all the *D* values between individuals of the two genera. The latter values were used to construct a phylogenetic tree using the UPGMA (unweighted pair group method with arithmetic means) option in the NEIGHBOR program (Phylip software, version 3.41) of J. Felsenstein (University of Washington, Seattle).

RESULTS

Qualitative Differences in DNA Profiles. DNA fingerprints, with distinct scorable bands distributed in the size range of 0.5–23.0 kb and showing individual-, species-, and restriction enzyme-specific patterns, were obtained (Figs. 1 and 2).

The average number of total bands in *Paleosuchus, Caiman, Alligator, Gavialis, Tomistoma, Osteolaemus*, and *Crocodylus* was 48.0, 26.0, 28.2, 24.0, 20.0, 27.7, and 25.3 in their *Alu* I profiles and 46.3, 42.7, 40.5, 27.0, 29.0, 32.7, and 27.8 in their *Hinf*I profiles, respectively. The overall signal of hybridization was stronger in *Paleosuchus, Caiman*, and, to a lesser extent, *Alligator*, than in *Gavialis, Tomistoma, Osteolaemus*, and *Crocodylus*. *Gavialis* and *Tomistoma* showed a particularly poor signal. The *Alu* I-digested DNA profiles of *Paleosuchus, Caiman*, and *Alligator* (Fig. 1*A*) and *Gavialis, Tomistoma, Osteolaemus*, and *Crocodylus* (Fig. 1 *B* and *C*) showed a distinct fingerprint divergence among themselves. The maximum number of bands was visible in the DNA profiles of two species of *Paleosuchus*, which were almost evenly distributed along the length of the DNA fingerprint (Fig. 1*A*, lanes 1–3). Similar DNA profiles but with significantly fewer bands were detected in the three species of *Caiman* (Fig. 1*A*, lanes 4–9). On the other hand, DNA profiles of the remaining five genera showed distinctly fewer bands per fingerprint, most of them being <4 kb (Fig. 1). In *Crocodylus* and *Osteolaemus*, there were many major (high intensity) bands interspersed with minor (low intensity) bands (Fig. 1*B*, lanes 9–15; Fig. 1*C*, lanes 1–16), whereas in *Gavialis* and *Tomistoma* such bands were relatively few (Fig. 1*B*, lanes 1–8). These differences in band distribution and band intensities were much more apparent in the corresponding *Hinf*I-digested DNA fingerprints (Fig. 2). *Hinf*I profiles of *Caiman* and *Alligator* showed many bands > 4 kb when compared to their *Alu* I genetic profiles (Fig. 1*A*, lanes 4–15; Fig. 2*A*, lanes 3–13); they closely resembled those of *Paleosuchus* (Fig. 2*A*, lanes 1 and 2) with respect to size distribution and hybridization intensities. In the remaining four genera (*Gavialis, Tomistoma, Osteolaemus*, and *Crocodylus*), the *Hinf*I profiles, although distinct, were similar to their *Alu* I profiles. The *Hinf*I profiles of *Gavialis* and *Tomistoma* (data not shown) showed only a shift in the position of bands relative to their *Alu* I profiles.

Analysis of the fingerprint data also demonstrated that while most of the bands in the genetic profiles were individual specific, there were certain bands that were highly conserved and were probably specific to a species/genus. The *Hinf*I fingerprints of five individuals of *A. mississippiensis*, from two different localities in the United States, were characterized by the presence of a species-specific doublet > 5 kb (Fig. 2*A*, arrowheads, lanes 9–13). No such elements were detected in the corresponding *Alu* I profiles (Fig. 1*A*, lanes 11–15). Conserved bands (small arrowheads) in the fingerprints of individuals belonging to geographically different localities were also present in *Osteolaemus* (Fig. 2*B*, lanes 1–3), *C. acutus* (Fig. 2*B*, lanes 4–7), and *C. siamensis* (Fig. 1*C*, lanes 6–8; Fig. 2*B*, lanes 9–11). The geographically unrelated individuals of *Gavialis* and *Tomistoma* also showed species-specific distribution of high-intensity major bands in their genetic profiles. *Gavialis* profiles showed a seemingly conserved doublet of ≈3.5 kb and a band in the 15-kb range (Fig. 1*B*, lanes 1–4). In *Tomistoma* there were five such bands in the range 1.5–2.2 kb and one major band of 4.2 kb (Fig. 1*B*, lanes 5–8).

When hybridized blots were washed at a higher stringency, the number of bands and the intensity of hybridization was greatly reduced in *Gavialis, Tomistoma, Osteolaemus*, and *Crocodylus*. By contrast, in *Paleosuchus, Caiman*, and *Alligator* the higher stringency of washing had virtually no effect on the overall number and the intensity of bands in the genetic profiles obtained with both the restriction enzymes. The intensity of signal of hybridization, the number of bands obtained, and the sustenance of the pattern of genetic profiles at high stringency of washing in *Paleosuchus, Caiman*, and *Alligator* samples suggested a quantitative difference in the genomic content of Bkm-related sequences in the genera tested.

Quantitative Differences in Bkm-Related Sequences. The quantitative differences in the genomic content of Bkm-related sequences in different crocodilian genera were studied by preparing slot blots with known but equal quantities of total undigested genomic DNA of one individual each of all the species tested and hybridizing them with the labeled Bkm-2(8) probe. After autoradiography, each slot was numbered, cut out, and assayed. The results were verified by studying samples from additional individuals of each species

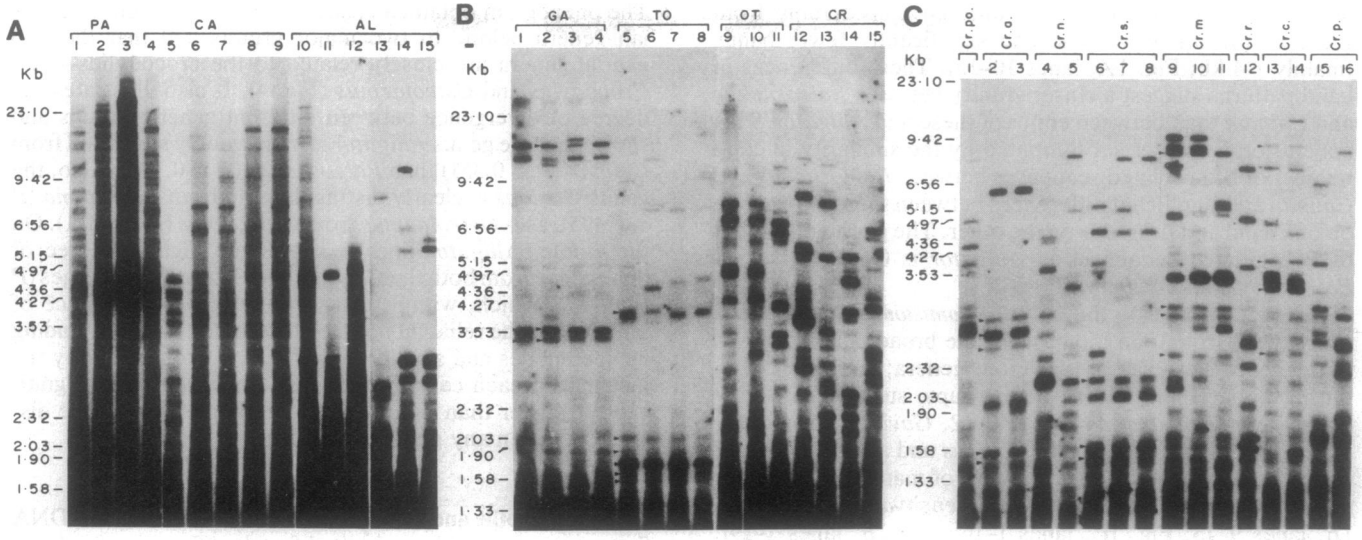

FIG. 1. *Alu* I DNA profiles of different crocodilians developed after hybridization with Bkm-2(8) probe. (*A*) Lanes: 1, *P. palpebrosus*; 2 and 3, *P. trigonatus*; 4, *C. yacare*; 5, *C. latirostris*; 6–9, *C. crocodilus*; 10, *A. sinensis*; 11–15, *A. mississippiensis*. Note overall stronger signal in *Paleosuchus* (PA), *Caiman* (CA), and *Alligator* (AL) compared to the remaining genera shown in *B* and *C*. (*B*) Lanes: 1–4, *G. gangeticus*; 5–8, *T. schlegelii*; 9–11, *O. tetraspsis*; 12, *C. moreletii*; 13, *C. niloticus*; 14, *C. siamensis*; 15, *C. palustris*. Note that there are many more major bands interspersed with minor bands in size range >2 kb in *Osteolaemus* (OT) and *Crocodylus* (CR) compared to *Gavialis* (GA) and *Tomistoma* (TO). (*C*) Lanes: 1, *C. porosus* (Cr.po.); 2 and 3, *C. acutus* (Cr.a.); 4 and 5, *C. niloticus* (Cr.n.); 6–8, *C. siamensis* (Cr.s); 9–11, *C. moreletii* (Cr.m.); 12, *C. rhombifer* (Cr.r.); 13 and 14, *C. cataphractus* (Cr.c.); 15 and 16, *C. palustris* (Cr.p.). Arrowheads indicate probable species-specific marker bands.

wherever possible. In all the species tested, an increase in the concentration of DNA resulted in a concomitant increase in signal strength as indicated by both radioactivity and photodensity. For each of the three DNA concentrations tested, the hybridization signal for *Paleosuchus, Caiman*, and *Alligator* species was invariably 3- to 8-fold higher than that for *Gavialis, Tomistoma, Osteolaemus*, and *Crocodylus* (Fig. 3).

The slot blot results clearly indicated two major groups of crocodilians with respect to the copy number of Bkm-related sequences. In the first group, comprising *Paleosuchus, Caiman*, and *Alligator*, the copy number of Bkm-related sequences in their genome was 3–8 times higher than in the second group consisting of *Gavialis, Tomistoma, Osteolaemus*, and *Crocodylus*. Furthermore, within the first group the copy number of Bkm-related sequences was seemingly the highest in *Paleosuchus*, followed by *Caiman* and *Alligator*, suggesting that *Alligator* lies at the lower boundary of this group. Qualitative differences apparent in the overall band patterns for these genera led to the same conclusions.

Generic Affinities in the *Paleosuchus–Caiman–Alligator* Group. The DNA profiles of *Paleosuchus* and *Caiman* were very similar. In both cases, a large number of bands > 5 kb were obtained with both *Hin*fI and *Alu* I (Fig. 1*A*, lanes 1–9; Fig. 2*A*, lanes 1–8). However, while *Alligator Hin*fI profiles closely resembled those of *Caiman* and *Paleosuchus* with respect to number, size, and distribution of bands (Fig. 2*A*,

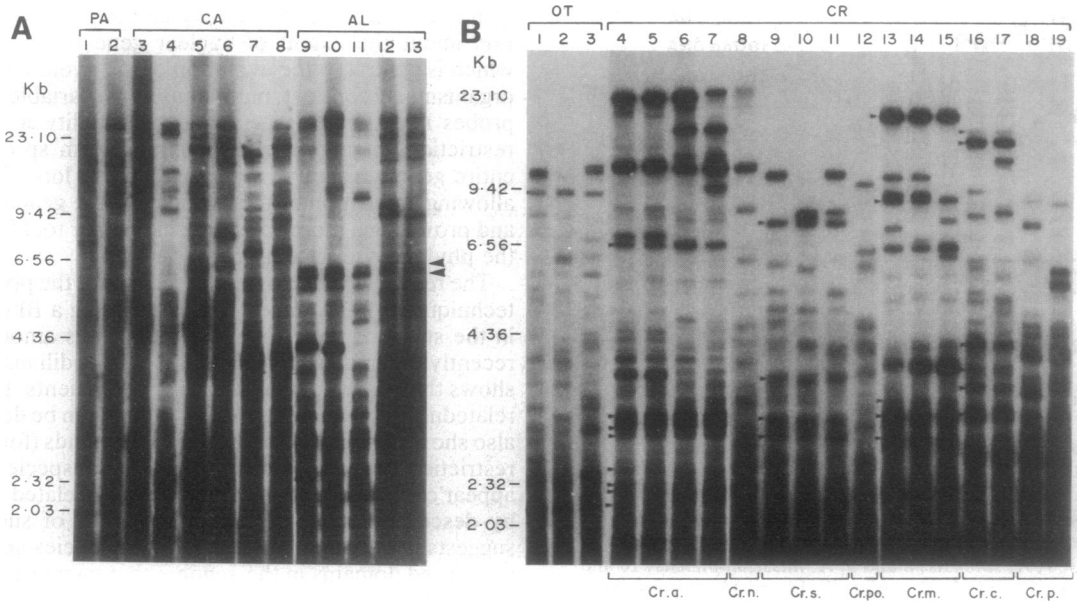

FIG. 2. *Hin*fI DNA profiles of different crocodilians developed after hybridization with Bkm-2(8) probe. (*A*) Lanes: 1 and 2, *P. trigonatus* (PA); 3–8 *Caiman* (CA), 3, *C. c. yacare*; 4, *C. latirostris*; 5–8, *C. c. crocodilus*; 9–13, *A. mississippiensis* (AL). (*B*) Lanes: 1–3, *O. tetraspsis* (OT); 4–19 *Crocodylus* (CR), 4–7, *C. acutus* (Cr.a.); 8, *C. niloticus* (Cr.n.); 9–11, *C. siamensis* (Cr.s.); 12, *C. porosus* (Cr.po.); 13–15, *C. moreletii* (Cr.m.); 16 and 17, *C. catapharactus* (Cr.c.); 18 and 19, *C. palustris* (Cr.p.). Arrowheads indicate probable species-specific marker bands.

lanes 9–13), *Alu* I fingerprints differed considerably from those of the other two and had significantly fewer bands (mainly <4 kb; Fig. 1*A*, lanes 10–15). These differences in band patterns suggest a closer affinity between *Paleosuchus* and *Caiman* than between either of these and *Alligator*. This conclusion is further substantiated by the similarity in copy number of Bkm-related sequences between *Paleosuchus* and *Caiman* and significant differences between these two genera on one hand and *Alligator* on the other. The genomic content of Bkm-related sequences in *Alligator* is 0.5–0.75 that of *Paleosuchus* and *Caiman* (Fig. 3).

Generic Affinities in the *Gavialis–Tomistoma–Osteolaemus–Crocodylus* Group. On the basis of the broad characteristics of the genetic profiles of these four genera, it is possible to further divide them into two subgroups: subgroup I, *Crocodylus* and *Osteolaemus*; subgroup 2, *Gavialis* and *Tomistoma*. The fingerprints of *Osteolaemus* and all the species of *Crocodylus* showed a similar pattern of many high-intensity major bands interspersed with low-intensity minor ones (Fig. 1*B*, lanes 9–15; Fig. 1*C*, lanes 1–16; Fig. 2*B*, lanes 1–19), mostly in the >3-kb size range. By contrast, in the two monotypic genera *Gavialis* and *Tomistoma*, more bands were found in the lower molecular weight range; the remaining bands (>3 kb) were mostly low-intensity minor bands (Fig. 1*B*, lanes 1–8; compare these with lanes 9–15 for *Osteolaemus* and *Crocodylus*).

Phylogenetic Analysis. A phylogenetic tree showing relationships among the seven genera of crocodilians was generated based on coefficients of dissimilarity (data not shown).

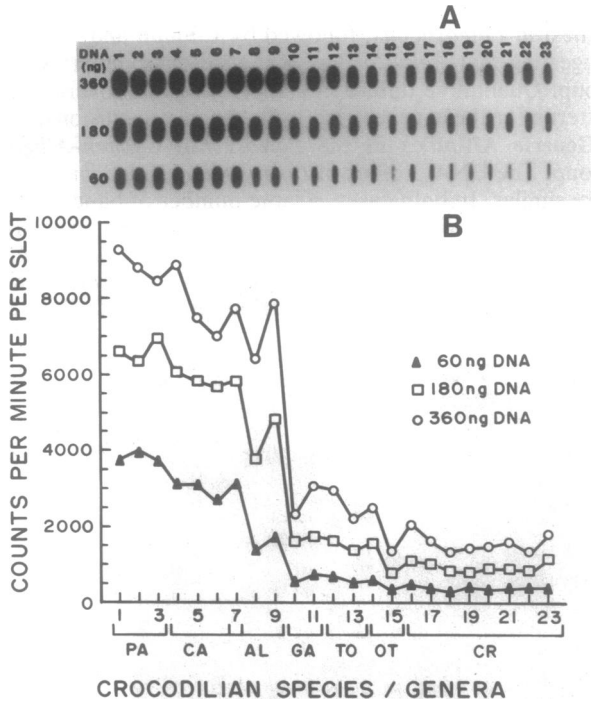

FIG. 3. Quantitative differences in Bkm-related sequences in genomic DNA of different species/genera of crocodilians. (*A*) Slot blot of genomic DNA hybridized with ^{32}P-labeled Bkm-2(8) probe. (*B*) cpm per slot of genomic DNA plotted for different species/genera of crocodilians. PA, *Paleosuchus*; CA, *Caiman*; AL, *Alligator*; GA, *Gavialis*; TO, *Tomistoma*; OT, *Osteolaemus*; CR, *Crocodylus*; Slots: 1, *P. palpebrosus*; 2 and 3, *P. trigonatus*; 4, *C. c. yacare*; 5 and 6, *C. c. crocodilus*; 7, *A. sinensis*; 8 and 9, *A. mississippiensis*; 10 and 11, *G. gangeticus*; 12 and 13, *T. schlegelii*; 14 and 15, *O. tetraspsis*; 16, *C. cataphractus*; 17, *C. rhombifer*; 18, *C. acutus*; 19, *C. niloticus*; 20, *C. siamensis*; 21, *C. moreletii*; 22, *C. porosus*; 23, *C. palustris*. Note the 4–8 times higher signal in *Paleosuchus*, *Caiman*, and *Alligator* compared to the other genera, suggesting that these genera belong to two distinct groups.

The phenogram obtained clearly shows that seven crocodilian genera belong to two major groups and that the two gharial genera are closely related to the crocodylids—i.e., *Crocodylus* and *Osteolaemus* (Fig. 4). It also illustrates the degree of divergence between different generic groups and shows that the genus *Alligator* is more widely separated from *Caiman* ($P = 0.351$) than *Paleosuchus* ($P = 0.328$). Also, the genus *Gavialis* is clearly distinguishable from *Tomistoma* ($P = 0.395$), as is *Osteolaemus* from *Crocodylus* ($P = 0.371$). On the whole, *Alligator* lineage shows the highest degree of divergence from both the *Gavialis* and *Crocodylus* lineages ($P = 0.477$), which, within themselves, show a divergence of only 0.425. The reliability of the phenogram, notwithstanding its deep nodes and smaller internodes, is brought out by the fact that in each case the standard error value was significantly smaller than the respective estimate of pairwise distance between any given two nodes.

DISCUSSION

Genetic Profile and Biological Relatedness. Genetic or DNA fingerprinting provides a method for identification of individuals, confirmation of biological relationships (13), human genetic analysis (24), and demographic studies (14, 25–28). However, it had not until now been used for phylogenetic analysis because the profiles were thought to evolve too rapidly to be informative over large time intervals. In the present investigation, we have used the twin approach of analyzing quantitative differences as well as similarities and dissimilarities in fingerprint profiles to infer phylogenetic relationships among the crocodilians, which as a group have undergone relatively recent divergence compared to their ancient progenitors—i.e., Archosauromorpha.

According to Norell (29), for groups like Crocodilia, which have undergone relatively recent divergence, only those molecular sequences will be phylogenetically informative that behave like fast-clock molecules—i.e., the ones that reflect relatively higher rates of sequence (marker) substitutions/modifications (30), although such sequences may be uninformative regarding relationship of the group with its outgroup taxa because of the possibility of the sequences having progressed to the point of randomization. Mitochondrial DNA markers, which evolve 5–10 times faster than nuclear genes, can be used to reconstruct the phylogenetic history of populations, but they do not provide any information about the extent of nuclear gene flow or variability, which is central to the evolution of the overall makeup of an organism. By contrast, multilocus hypervariable minisatellite probes reveal enormous genetic variability in the form of restriction fragment length polymorphism spread over the entire genome; they evolve rapidly over long time periods, allowing estimation of the overall relative genetic variability and providing a more amenable molecular tool for looking at the phylogeny of closely related groups.

The results presented here demonstrate the potential of the technique of DNA fingerprinting by using a Bkm-2(8) probe in the study of phylogenetic relationships among relatively recently diversified, closely related crocodilians. This study shows that, based on band-sharing coefficients, the degree of relatedness among different individuals can be determined. It also shows that there are a few specific bands (for one or both restriction enzymes) that are unique to a species/genus and appear consistently in all its individuals, related or unrelated by descent or geography. The presence of such elements suggests that there are, perhaps, some species-specific allelic conserved domains in the genome that might serve as potential diagnostic markers to identify a species.

We show here that the true (*Crocodylus*) and dwarf African (*Osteolaemus*) crocodiles are closely related sister taxa, whereas alligators and caimans form a loose assemblage, although *Alligator* is distinct from the two caiman genera

studied. In addition, the present study favors a sister-group relationship between *Tomistoma* and *Gavialis*; these two in turn form a sister group to crocodylids—i.e., *Osteolaemus* and *Crocodylus*. The above conclusions regarding grouping of crocodilians are further substantiated by the phenogram developed from the data on band sharing. The phenogram, besides indicating the probable phylogenetic relatedness of the species/genera involved, also offers a semiquantitative estimate of the degree of genetic divergence. It shows that the *Alligator* lineage is most widely separated from the *Gavialis* and *Crocodylus* lineages ($P = 0.447$), which, within themselves, are closer to each other and relatively less diverged ($P = 0.425$). These measures of relatedness may, however, be slightly inflated because of inherent problems of DNA fingerprinting technique, such as fortuitous comigration of fragments generated by alleles at different loci, as well as limitation in resolving fragments of nearly similar sizes (31). But the fact that fingerprint-based phylogenetic analysis makes use of the variability present in the genotype of the organism lends it more credibility over the findings of traditional approaches that make use of the phenotypic variability, which is influenced greatly by the immediate environment of the organism. Nevertheless, the grouping of crocodilians based on the present study corroborates earlier findings based on traditional disciplines, as well as the more recent biochemical and immunological studies of proteins, restriction fragment length polymorphism, and sequence data of mitochondrial and nuclear ribosomal DNA.

A comparison of the quantitative data pertaining to Bkm-related sequences in the genomes of various crocodilians (Fig. 3) with the available information on the distribution of fossils and living crocodilians through time (32) reveals that *Paleosuchus* and *Caiman*, which show the highest copy number of Bkm-related sequences in their genomes, are also the more recently evolved genera belonging to the *Alligator* lineage. The copy number of Bkm-related sequences in *Alligator*, although less than in the caimans, is distinctly 3–5 times more than in the remaining four genera. It seems that in this lineage, evolution has involved a substantial increase in the copy number of Bkm-like short repeat sequences, involving processes such as amplification and insertion of DNA into chromosomes. This sets apart the *Alligator* lineage from the rest of the crocodilians and also rules out the possibility of its being closely related to the *Gavial* lineage, notwithstanding the stepwise nature of evolutionary changes in the copy number of minisatellite alleles (33).

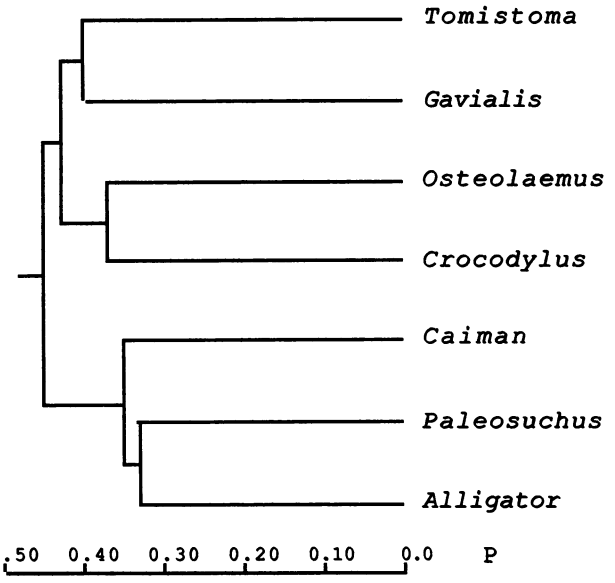

FIG. 4. UPGMA phenogram showing relationships among crocodilian genera based on *Alu* I fingerprinting data. P, probable degree of divergence. The reliability of the phenogram was also tested by generating the most parsimonious tree (results not shown) for a subset of data for 13 individuals belonging to 10 species of four genera, using both the bootstrapping and branch-and-bound options contained in version 3.0 of the PAUP program of David Swofford (Illinois Natural History Survey, Champaign).

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