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# Are crocodiles really monophyletic?—Evidence for subdivisions from sequence and morphological data

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## Abstract

Recently, the phylogenetic placement of the African slender snouted crocodile, *Crocodylus cataphractus*, has come under scrutiny and herein we address this issue using molecular and morphological techniques. Although it is often recognized as being a "basal" form, morphological studies have traditionally placed *C. cataphractus* within the genus *Crocodylus*, while molecular studies have suggested that *C. cataphractus* is very distinct from other *Crocodylus*. To address the relationship of this species to its congeners we have sequenced portions of two nuclear genes (*C-mos* 302 bp and ODC 294 bp), and two mitochondrial genes (*ND6-tRNA*$^{glu}$*-cytB* 347 bp and control region 457 bp). Analyses of these molecular datasets, both as individual gene sequences and as concatenated sequences, support the hypothesis that *C. cataphractus* is not a member of *Crocodylus* or *Osteolaemus*. Examination of 165 morphological characters supports and strengthens our resurrection of an historic genus, *Mecistops* (Gray 1844) for *cataphractus*.

*Keywords:* *Crocodylus*; *Crocodylus cataphractus*; *C-mos*; *ODC*; Mitochondrial ND6; Mitochondrial control region; Systematics; *Mecistops*

## 1. Introduction

The African slender-snouted crocodile, *Crocodylus cataphractus*, has long been a systematic enigma. In one of the earliest systematic treatments of what is now called Crocodylia, Gmelin (1789) indicated that the habitat for *Lacerta gangeticus* (now *Gavialis gangeticus*) included rivers in "Senegal Africae et Gangen Indiae." *Gavialis* has been restricted to the Indian subcontinent throughout historical times, but it is clear from Gmelin's diagnosis that *C. cataphractus*, the crocodylian from Senegal with an elongate, subcylindrical rostrum, would have fallen within *L. gangeticus*, highlighting the morphological gulf between *C. cataphractus* and other *Crocodylus*, reinforcing the need for further systematic analysis.

Various members of the genus *Crocodylus* (the true crocodiles) have been included in a number of phylogenetic studies, but until recently, very little had been written about relationships within *Crocodylus*. That the name "*Crocodylus*" lacked a uniform meaning renders comparisons of different scenarios virtually impossible. Neontologists were necessarily restricted to the 12 recognized living species, but paleontologists assigned fossils ranging throughout the Cenozoic and into the Mesozoic (sometimes as old as the Albian stage of the Cretaceous, between 99 and 112 mya) to *Crocodylus* (Markwick, 1998; Steel, 1973). Explicit diagnoses not reliant on overall head shape were rarely used, and *Crocodylus* was often a default category that simply meant a fossil could not be unambiguously assigned to some other

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genus. Several authors have suggested that the African slender-snouted crocodile (*C. cataphractus)* is the sister taxon to a clade comprising all other members of this genus (Brochu, 1997, 2000; Densmore, 1983; Densmore and Owen, 1989; Gatesy et al., 2003; Gatesy et al., 2004; White and Densmore, 2000). Most of these studies have suffered from limited taxon sampling and/or few representative individuals from the species being compared. To date there have been few studies aimed specifically at the relationship of this species to its congeners and no molecular studies. Recently, Schmitz et al. (2003) in a study on genetic variation within the Nile crocodile, *C. niloticus*, suggested that *C. cataphractus* formed a relationship outside the remainder of *Crocodylus*. However, this portion of their study included just a single *C. cataphractus* sample, two dwarf African crocodile samples (*Osteolaemus tetraspis*) and only three of the eleven recognized extant species of *Crocodylus* (*C. cataphractus*, *C. jonstoni*, and *C. niloticus*).

Herein, using more thorough taxon sampling and much larger sample sizes (especially for the nuclear gene sequences), we report sequence comparisons from both coding and non-coding regions of two nuclear protein-coding genes and from two different regions of the mitochondrial genome (also representing both coding and non-coding sequences) specifically to assess the relationship of *C. cataphractus* to other members of *Crocodylus* and to *Osteolaemus*.

The two nuclear markers sequenced for this study are the proto-oncogene *C-mos* and the gene that codes for ornithine decarboxylase (ODC). *C-mos* is a single-copy gene slightly over 1000 bp in length, contains no introns and codes for a protein (C-mos) involved in oocyte maturation during meiotic metaphase II (Saint et al., 1998; Yew et al., 1993). Due to its relative high degree of conservation, this gene provided the resolution necessary to examine generic level relationships within the Crocodylia.

The *ODC* gene codes for a protein that catalyses the conversion of ornithine to putricine (Friesen et al., 1999) and is involved in the control of cell growth and division (Yao et al., 1995). Comprising some 12 exons and 11 introns, it has a transcription unit 6–8 kb in length. Friesen et al. (1999) characterized a series of PCR primers for this gene spanning a region from intron 6 through intron 8. However, they did not test the amplicons produced with these primers for phylogenetic signal. While the use of the *ODC* gene in phylogenetic analyses has been limited, it has been shown to be comparable to both mitochondrial cytochrome *b* (Allen and Omland, 2003) and control region (Kulikova et al., 2004) sequences at resolving phylogenetic relationships.

Mitochondrial sequence data continue to be widely used in many systematic studies, including crocodylians (Gatesy and Amato, 1992; Gatesy et al., 2003; Gatesy et al., 2004; Ray et al., 2000; Schmitz et al., 2003; White and Densmore, 2000). While most crocodylian mitochondrial datasets have focused on the region that includes the cytochrome *b* gene or the ribosomal DNA genes, we sequenced a region that includes *ND6-tRNA*$^{glu}$*-cytB* (ND6-cytb) genes as well as a portion of the mitochondrial control region. This choice was largely based on recent studies (Ray and Densmore, 2002, 2003; White and Densmore, 2000), which indicate that these sequences are effective markers in the Crocodylia, especially for comparisons involving closely related taxa.

Morphological comparisons were naturally made between *C. cataphractus* and the other slender-snouted species of *Crocodylus* (*C. intermedius* and *C. jonstoni*), but it was generally agreed that these represented independent derivations of a specialized snout morphology (e.g., Meyer, 1984; Mook, 1921; Schmidt, 1924; Sill, 1968). Many slender-snouted crocodylians from throughout the Cenozoic have been referred either to *C. cataphractus* or a putative relative (e.g., Aoki, 1992; Pickford, 1994; Storrs, 2003; Tchernov, 1986), but assignments were often based on overall skull shape and not synapomorphy. Tchernov (1986) argued that the other extant African species of *Crocodylus* (the Nile crocodile *C. niloticus*) was closer to the Indian mugger (*C. palustris*) than to *C. cataphractus.* Based on fossils he presumed to be ancestral to living species, the last common ancestor between *C. cataphractus* and *C. niloticus* was no younger than the Late Eocene, but relationships with other non-African species were not discussed. Aoki (1976, 1992) went further, arguing that *C. cataphractus* was closer to the other living longirostrine crocodylians (*Gavialis* and *Tomistoma*) than to other living *Crocodylus.*

At present, the most comprehensive analyses of morphological data support *Crocodylus* monophyly, but nevertheless place *C. cataphractus* outside a clade including all other extant *Crocodylus* (Brochu, 2000). The vast majority of fossils previously assigned to *Crocodylus* do not belong to the crown genus. Recent work has shown that most fossil *Crocodylus* from the African Neogene are actually closer to *Osteolaemus* (Brochu, 2003, in review). The oldest fossils unambiguously falling within the crown genus are from the Middle Miocene, which is consistent with suggestions from molecular data that *Crocodylus* is a geologically young radiation (Densmore, 1983; Hass et al., 1992). However, morphological support for relationships within *Crocodylus* is comparatively weak, reflecting the emphasis placed on osteological characters by most morphological analyses. Skeletal evidence for deeper crocodylian nodes is extensive, but shallower species-level divergences throughout the clade tend to be supported by more subtle characters, and nodal support tends to be low.

Herein, we provide genetic and morphological evidence for the resurrection of a historical generic name *Mecistops* (Gray 1844) for *C. cataphractus.* As such, *C. cataphractus* will be recognized throughout the remainder of this manuscript as *M. cataphractus.*

## 2. Molecular methods

### *2.1. Blood collection and DNA extraction*

Whole blood was collected from either the ventral caudal sinus (Gorzula et al., 1976) or the dorsal postcranial sinus (Bayliss, 1987) and used as the source of DNA for this