A GEOMETRIC MORPHOMETRIC ANALYSIS OF CROCODYLUS NILOTICUS: EVIDENCE FOR A CRYPTIC SPECIES COMPLEX

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Introduction

Recent molecular studies of *Crocodylus niloticus* indicate that it is actually a cryptic species complex (Schmitz, 2004; Hekkala et al., 2010, 2011; Oaks, 2011). As a result, *C. niloticus* may be split into two species, *C. niloticus* sensu stricto and *C. suchus*. The two species were found to be paraphyletic with respect to New World *Crocodylus*. Hekkala et al. (2011) found that the clear geographic separation currently seen between the two putative species reflects a comparatively recent geographic shift in genotype distributions

This study uses geometric morphometrics to assess whether morphological variation supports a species-level division. Geometric morphometrics can quantitatively test whether morphological variation within *C. niloticus* sensu lato is higher than that of other crocodylid species. A model-based cluster analysis and a geographic analysis are used to identify any morphotypes or distribution to the morphological variation.

Modern crocodylian skull morphology is relatively conservative and overlaps significantly in morphospace. The purpose of this study is not to distinguish species using morphometrics alone, but to use it as a tool to assess the breadth of inter- and intraspecific variation.

Methods

I used two-dimensional geometric morphometrics on skeletonized specimens from museum collections. The complete dataset included 124 skulls from 12 species of modern, fossil, and subfossil members of Crocodylidae. The landmark set consists of 30 landmarks coded unilaterally on the dorsal surface of the skull, which were digitized from photographs using ImageJ (Figure 1; Schneider et al., 2012).

Analyses were run once on all species and once on a dataset that included only *Crocodylus niloticus* s.l. A Procrustes superimposition was performed using tpsRELW, calculating both partial and relative warps (version 1.49; Rohlf, 2010). Morphological changes were visualized using thin-plate splines generated in tpsSplin.

The two datasets were analyzed using *C. niloticus* binned by large-scale hydrologic basins determined using a digital elevation model, which was the most specific locality information that could be ascribed consistently to individuals. This method was chosen because rivers likely represent the primary mode of dispersal for individuals.

Additionally, the relative warps comprising >5% of the variance were *Crocodylus niloticus* s.l. was subject to a model-based cluster analysis to determine how many, if any, distinct morphotypes are present. The cluster analysis was run in R (version 2.9.2) using MCLUST (R Development Core Team, 2008; Fraley and Raftery, 2006).

A permutational multivariate analysis of variance (MANOVA) was then run on the relative warps that represented at least 5% of the variance each using PAST v.2.07 to determine the statistical significance between designated groups (Hammer et al., 2001). Separate analyses were run on crocodylid species and *C. niloticus* s.l. geographical groups, *C. niloticus* s.l.

geographical groups alone, and *C. niloticus* s.l. clusters as determined by the model-based cluster analysis.

Results and Conclusions

Individual species do cluster in morphospace, though there is both a range of variation within species and overlap between species (Figure 2). While geometric morphometrics is extremely effective at quantifying anatomical variation, it is important to consider that shape space is multidimensional and exceedingly complex, so differences may be significant but not apparent in two-dimensional shape space.

A significant amount of variation exists within *Crocodylus niloticus* s.l. when analyzed both interspecifically and intraspecifically. It is by far the most morphologically variable species present in this study. This is certainly in part an artifact of sample size, although *C. porosus* shows as much morphological variation as *C. niloticus* s.l. from the Congo River basin alone, making it impossible to discount the fact that *C. niloticus* s.l. varies at least as much intraspecifically as many crocodylian species vary from each other. *Crocodylus porosus* was the next largest sample size included, has a comparable range of skull sizes, and also has the greatest geographic range of all included species. The fact that morphological variation is comparable to *Crocodylus* from a single river basin in Africa is remarkable.

Despite the extent of variation within the Congo River Basin *C. niloticus* s.l., the sample was statistically significantly different from other regions included in the study. The Congo River Basin was also the only geographic region that included a unique group as indicated by the cluster analysis (Figure 3). While the cluster shows overlap when viewed as a two-dimensional comparison between relative warps, the distinction is highly statistically significantly different ($p<0.0002$). The fact that the cluster is so distinct and yet shows overlap in two-dimensional morphospace shows how complex these anatomical differences are.

Although a morphometric analysis alone is not enough to distinguish a species, the results here are largely congruent with molecular studies of *C. niloticus* s.l. However, the variation exhibited within single geographic regions indicates that the sympatry between the species of *C. niloticus* s.l. is very extensive, or has been in the recent past. The known collection dates for the *C. niloticus* s.l. in this study range from 1825-1960, so the geographic ranges of the morphotypes here likely represent the prior populational distribution elucidated in genetic studies. Whether they are sympatric today in the Congo River Basin is unknown, as no genetic samples from the region have been included (Hekkala, 2011).

Additionally, the variation within the species equals or exceeds that of other crocodylians with similarly expansive geographic ranges. Like *C. niloticus* s.l., species such as *C. acutus* exhibit morphological and genetic variation that merits a reevaluation of their species status (Weaver, 2012). Further investigation is necessary to determine the exact morphological delineation of the species, as the morphological variation across the range of *C. niloticus* s.l. is extremely complex.

In short, the extent of variation within *C. niloticus* s.l. from the Congo River Basin indicates that morphological variation is more complex than populational segregation by river basin, and the two morphotypes present have been sympatric in the recent past.

The shift in population structure merits conservation consideration, as it may be worth considering whether any reintroduction efforts should restore that historical population structure. It is also important to consider the cause of this shift. It is possible that population bottlenecks and subsequent recolonization by a small population of *Crocodylus* contributed to the shift,

although it is also possible that this is the result in changes in habitat and climate played a role.

References

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Figures

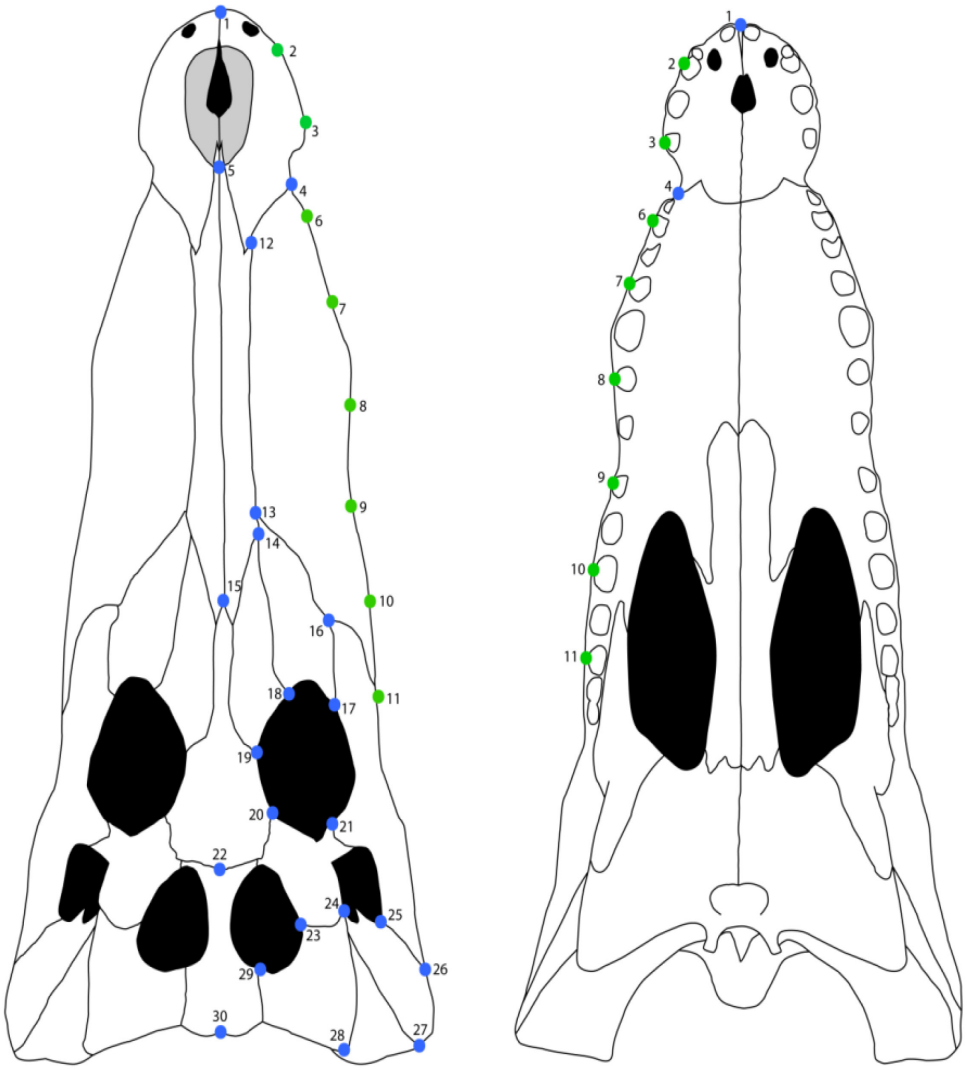


Figure 1. Landmarks used in the morphometric analysis. Alveoli are indicated by green landmarks, which were coded in palatal view and superimposed in dorsal view.

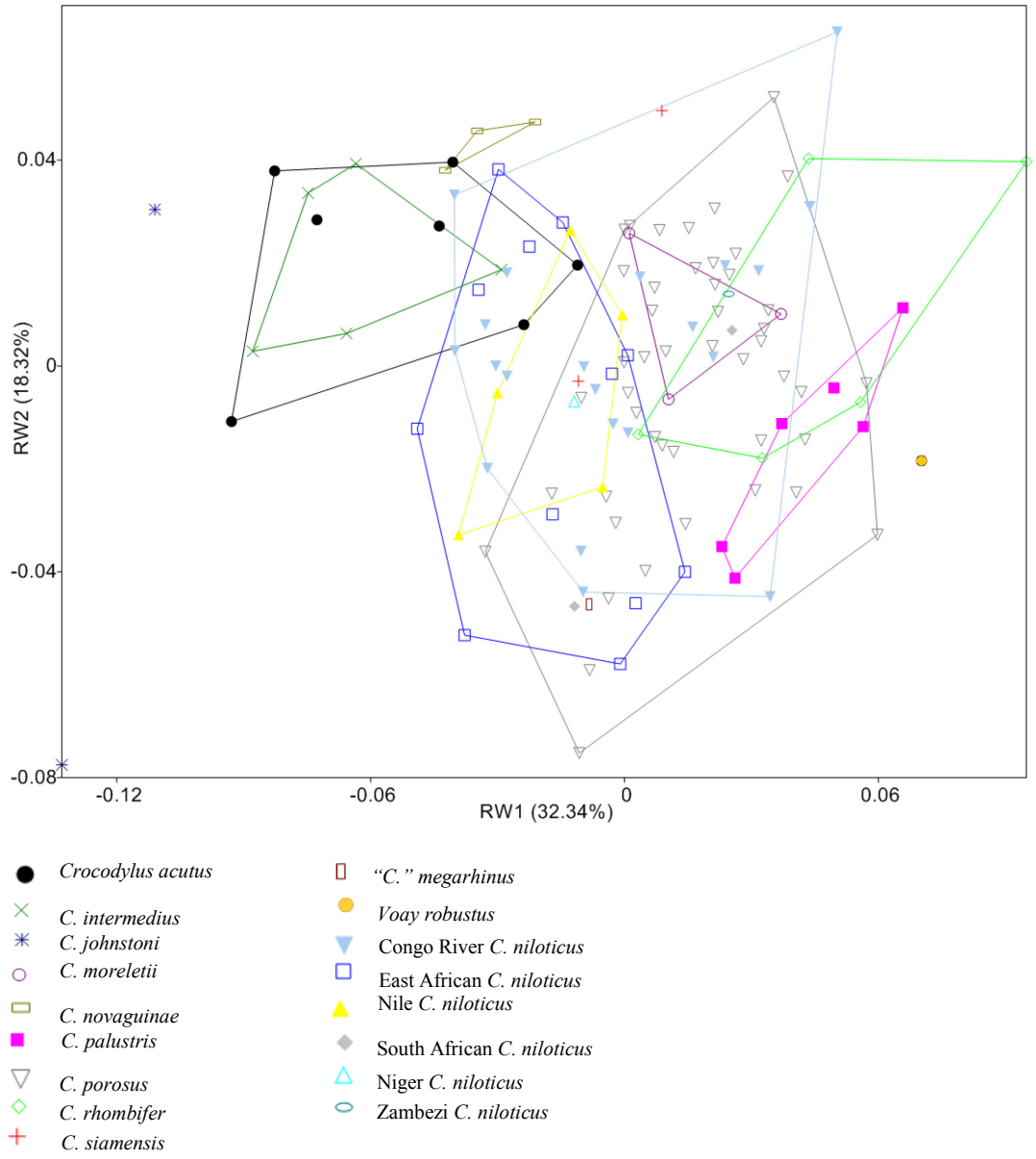


Figure 2. Relative warps 1 and 2 of the interspecific analysis of *Crocodylus*.

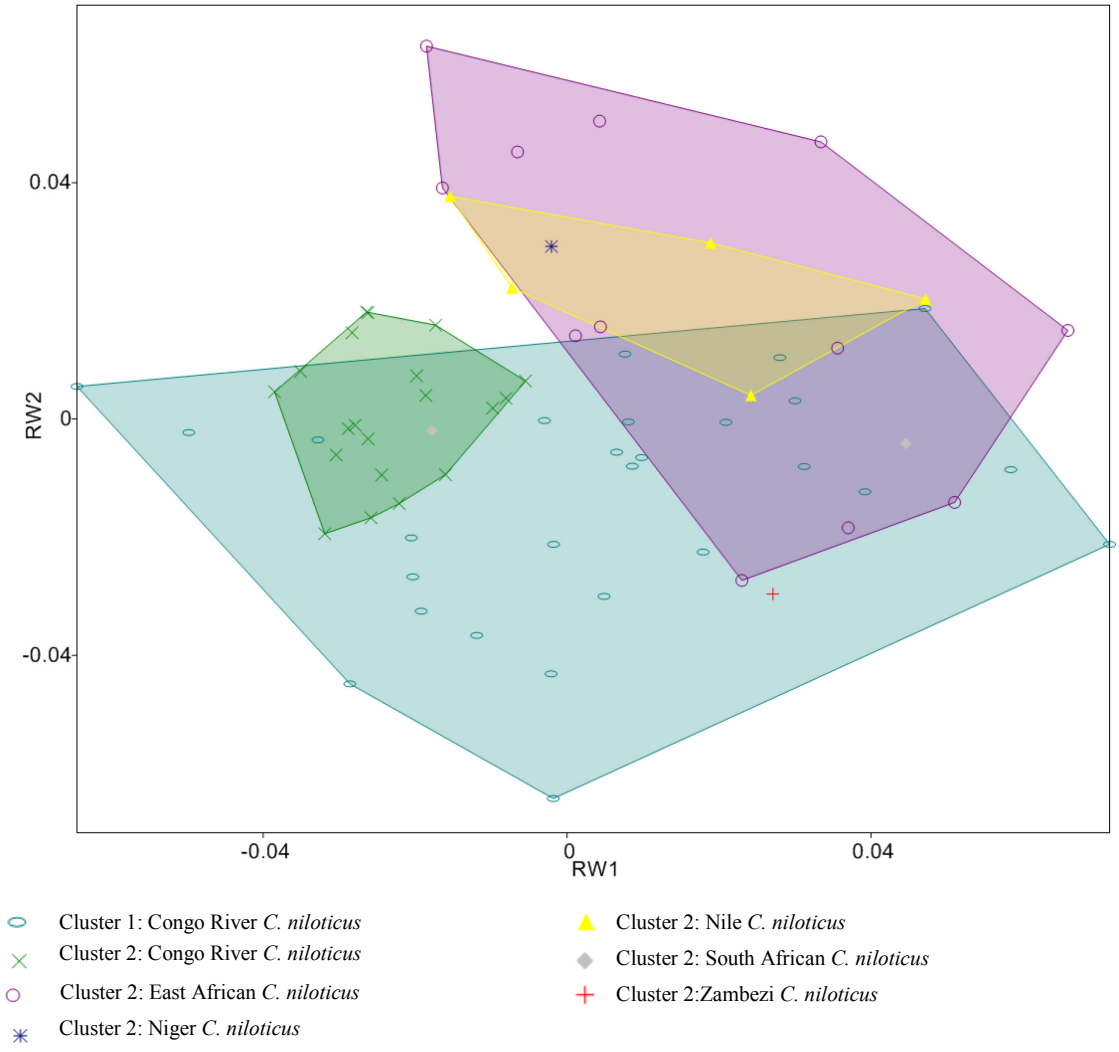


Figure 3. Results of the cluster analysis of *Crocodylus niloticus* s.l.