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Divergent Morphology among Populations of the New Guinea Crocodile, *Crocodylus novaeguineae* (Schmidt, 1928): Diagnosis of an Independent Lineage and Description of a New Species

Christopher M. Murray[1,2], Peter Russo[3], Alexander Zorrilla[4], and Caleb D. McMahan[5]

The freshwater crocodile inhabiting Papua New Guinea, currently recognized as *Crocodylus novaeguineae*, exhibits morphological, molecular, and ecological divergence between the northern and southern versants of the Central Highlands and occupies separate evolutionary trajectories. A robust body of work has long encouraged the formal description of New Guinea crocodiles from the southern versant of the highlands as a distinct lineage with a taxonomy that reflects diagnosed relationships. Here, we use geometric morphometric techniques to assess cranial shape variation between specimens from both versants and add to the diagnostic evidence supporting a more accurate taxonomy. Further, herein, we formally describe the southern variant as a distinct lineage (Hall's New Guinea Crocodile; *Crocodylus halli*, new species).

THE New Guinea Crocodile (*Crocodylus novaeguineae*) is a freshwater species of crocodilian endemic to the island of New Guinea in northern Oceania. The species inhabits both the country of Papua New Guinea in the east and Indonesian West Papua. *Crocodylus novaeguineae* occurs on both the northern and southern side of the Central Highlands, which span east to west dividing the entire island into northern and southern halves. Like most crocodilians, *C. novaeguineae* inhabits various grassy and forested swamps in lowland freshwater areas (Hall and Johnson, 1987) and has maintained both cultural and economic significance in the region for centuries (Hall and Johnson, 1987).

Neill (1971) and, more recently, Hall (1989) have suggested that *Crocodylus novaeguineae* on the northern side of the Central Highlands ("NCN") and those on the southern side ("SCN") are on independent evolutionary trajectories and should be taxonomically recognized. Hall (1989) attempted to affirm the suspicions of Neill and presented compelling morphological and ecological data to do so. Morphologically, the northern and southern hypothesized lineages differed in proportional premaxillary (PXS) to maxillary (MXS) length (NCN: MXS $>$ PXS; SCN: PXS $>$ MXS) and patterns of cervical squamation (NCN: $>$4 post-occipital scutes with lateral contiguity between them, anteromedial nuchal scute separation absent; SCN: 4 post-occipital scutes with lateral discontinuity between them, anteromedial nuchal scute separation present). Ecologically, *C. novaeguineae* south of the Central Highlands nest in the wet season, in synchrony with sympatric *Crocodylus porosus*, whereas north of the Central Highlands, nesting occurs in the dry season. Additionally, variation in reproductive strategy (clutch size and egg size ratios) was diagnosed between NCN and SCN; however, reproductive strategy is highly plastic, even intraspecifically, among crocodilians (Murray et al., 2013). Thus, these character states are not robustly interpretable as diagnostic.

Phylogenetic approaches using molecular data were later tested and interpreted in the unpublished thesis of Gratten (2003) in which NCN and SCN were considered distinct operational taxonomic units in light of Hall (1989). A Bayesian analysis of relationships of Indo-Pacific *Crocodylus* using mtDNA curiously recovered a paraphyletic *C. novaeguineae*, rendered so by the purported Borneo Crocodile *C. raninus*, described from a skull and two preserved juveniles with no known extant population (Muller and Schlegel, 1844). NCN was recovered as more closely related to *C. raninus* than to SCN. This finding was attributed to either extremely recent divergence in NCN or misidentification of a dispersed or introduced NCN to Borneo from which the molecular sample was taken. Oaks (2011) recovered a paraphyletic *C. novaeguineae*; however, all samples of this species were from captive animals and identification of some samples appeared problematic. Thus, our analyses and comparisons herein only include populations of *C. novaeguineae* due to the lack of biologically reasonable comparisons. *Crocodylus novaeguineae* is the only freshwater crocodilian in the region besides the putative *C. raninus*. Little material with robust locality data exists in collections for this species, and in the absence of more specimens and diverse datasets we are unable to make additional comparisons.

An improved analysis of morphological variation among populations of *C. novaeguineae* is warranted, given the ecological and molecular patterns that have slowly emerged. Here, we use multivariate geometric morphometric approaches to gain clarity on the differentiation of populations north and south of the Central Highlands by assessing cranial shape variation across the distribution. We aim to identify diagnostic characters for populations on independent evolutionary trajectories and test whether cranial shape variation corresponds to the hypothesized lineages (a clade north of the central highlands and one south). We predicted that specimens from drainages on the northern side would more closely resemble each other than specimens from drainages on the southern side of the highlands and that shape-based diagnostic characters would be revealed.

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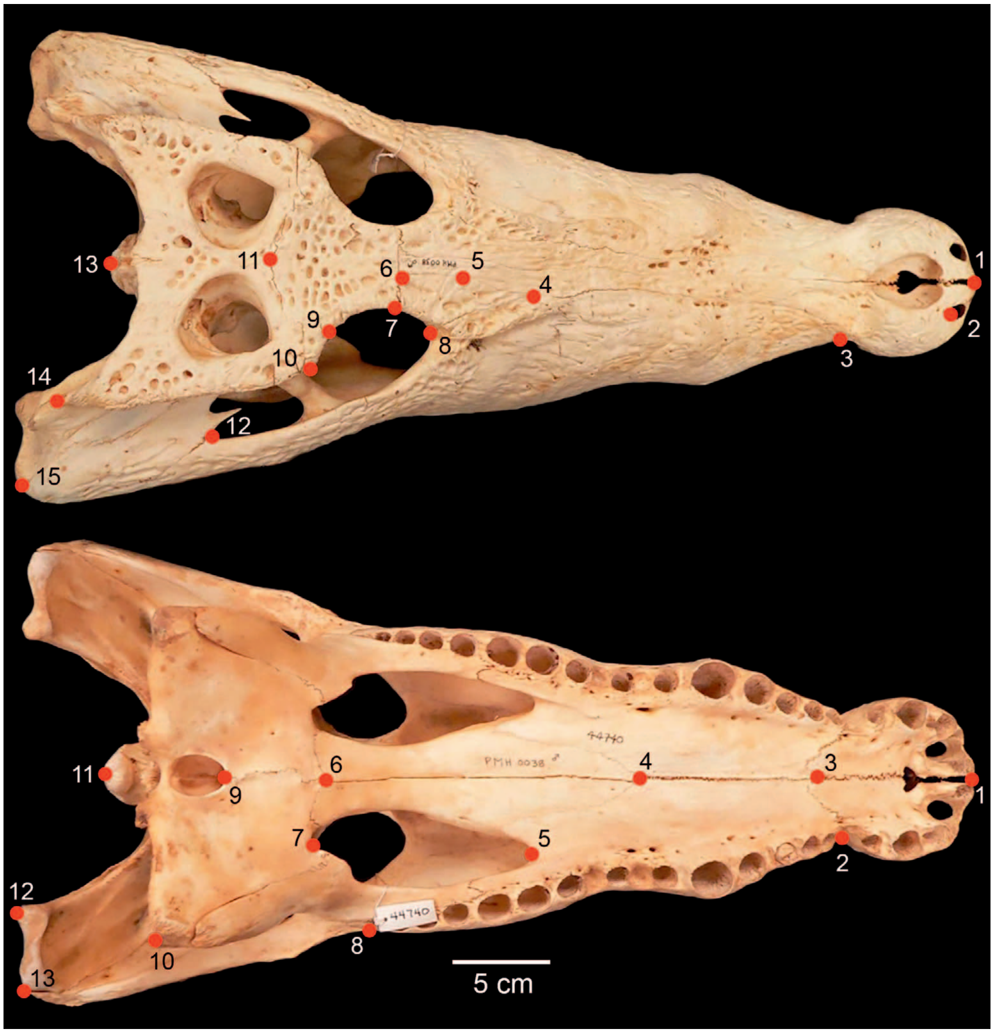

Fig. 1. Homologous landmarks used in geometric morphometric analysis for dorsal (top, $n = 15$ landmarks) and ventral (bottom, $n = 13$ landmarks) views. LSUMZ 44740.

MATERIALS AND METHODS

Skulls from 51 specimens of *Crocodylus novaeguineae* were examined from the Louisiana State University Museum of Natural Science, Florida Museum of Natural History, Field Museum of Natural History, American Museum of Natural History, Museum of Comparative Zoology at Harvard University, Queensland Museum, and Smithsonian National Museum of Natural History. Codes used throughout follow Sabaj (2016). The dorsal and ventral surfaces of each skull were photographed along with a scale bar and specimen ID on a black sheet overlaying a bed of rice to stabilize the skulls during photography. Photographs were taken with a Panasonic Lumix or Nikon D80 digital camera. Specimens characterized by broken or missing structures that rendered landmarks unquantifiable were excluded from analyses. Homologous landmarks were chosen to cover functional areas of the skull as well as the regions suggested by Hall (1989) to be diagnostic or variable. Additionally, both left and right sides of the skull were quantified to provide a field applicable depiction of any recovered diagnostic shape variation.

Homologous landmarks on photos were digitized using TPSDig232 (Rohlf, 2004). Fifteen landmarks on the dorsal cranial surface (Fig. 1, top) and 13 on the ventral cranial surface (Fig. 1, bottom) were plotted. Dorsal landmarks: 1. Anteromedial tip of premaxilla; 2. Posterior margin of right nares; 3. Antero-distal suture of maxilla and premaxilla; 4. Anterior point of prefrontal; 5. Posterior margin of nasal; 6. Posteromedial margin of prefrontal; 7. Postero-distal margin of prefrontal at orbit; 8. Anterior margin of orbit; 9. Distal margin of frontal; 10. Antero-distal margin of postorbital; 11. Posteromedial margin of frontal; 12. Anteriormost point on the suture between the quadratojugal and jugal; 13. Medial point on the occipital condyle; 14. Postero-distal margin of the squamosal; 15. Postero-distal point on the suture between the quadratojugal and quadrate. Ventral landmarks: 1. Anteromedial tip of premaxilla; 2. Antero-distal suture of palatine maxilla and premaxilla; 3. Postero-medial suture of the palatine maxilla and premaxilla; 4. Anteromedial margin of palatine process of palatines; 5. Anteriormost margin of the posterior palatine vacuities; 6. Posteromedial margin of the palatine process of palatines; 7. Posteriormost margin of the orbit; 8. Anteriormost point of the jugal; 9. Anterior margin of the internal nares; 10. Postero-distal margin of the palatine process of pterygoids; 11. Medial point on the occipital condyle; 12. Postero-medial point on the quadrate process; 13. Postero-distal point on the suture between the quadratojugal and quadrate.

Classifier variables were assigned as SCN or NCN of the central highlands and the drainage from which the specimen was collected in order to diagnose morphological differences between SCN and NCN and among populations within those hypothesized clades. Specimens from five drainages were included: Aramia (SCN; $n = 7$), Binaturi (SCN; $n = 2$),

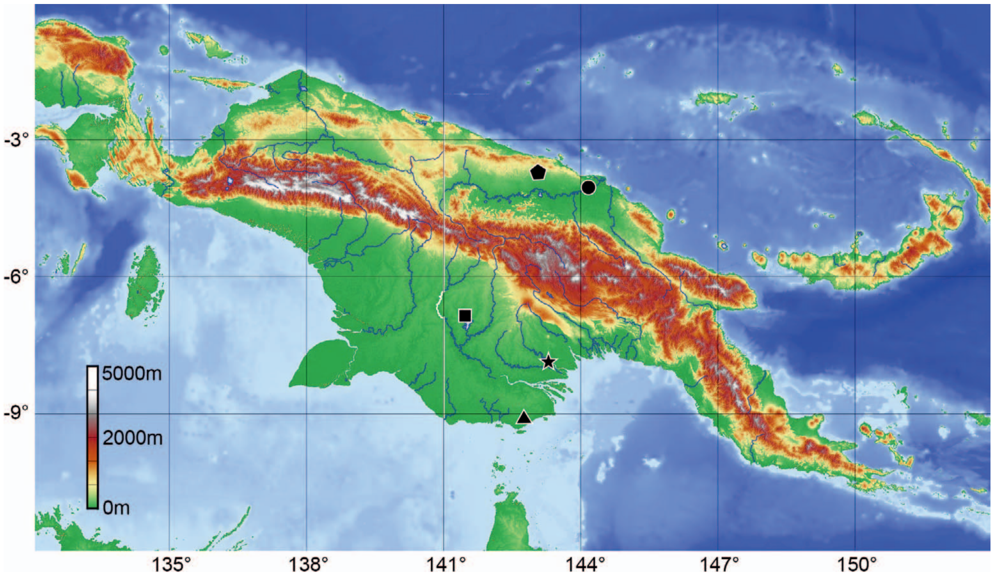

Fig. 2. Map of localities for specimens examined; *Crocodylus novaeguineae* from the Sepik (circle) and Hunstein (pentagon) drainages, and *Crocodylus halli* from Lake Murray (square), Binaturi (triangle), and Aramia (star) rivers.

Hunstein (NCN; $n = 1$), Lake Murray (SCN; $n = 24$), and Sepik River (NCN; $n = 17$; Fig. 2).

Procrustes ANOVAs were performed to assess differences in skull shape between SCN and NCN as well as among drainages. Canonical Variates Analyses (CVA) with 95% confidence intervals were performed to visualize cranial variation within and among drainages in morphospace. All analyses were performed using MorphoJ software (Klingenberg, 2011). Centroid size variation among all drainages was assessed using Procrustes ANOVA to ensure no statistical

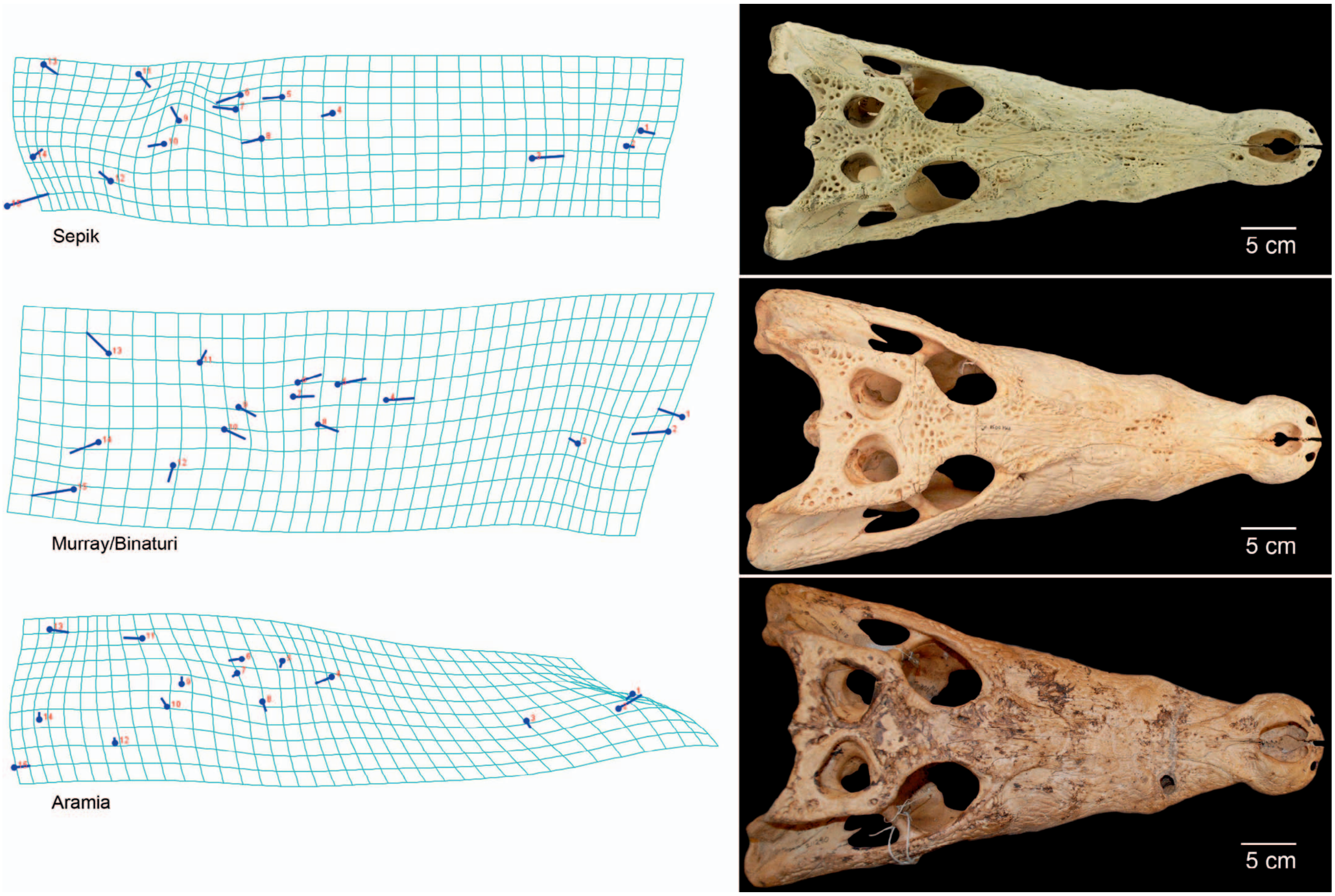

Fig. 3. Dorsal cranial shape variation depicted using transformation grids with corresponding example specimen among drainages in Papua New Guinea. Solid points indicate consensus landmarks for all individuals, and lines extending from points indicate the extent (length) and direction of shape change exhibited by that classifier. Populations in northern drainages (i.e., Sepik River; FMNH 14048; *Crocodylus novaeguineae*) exhibit an extended maxilla and reduced postcranial elements relative to southern populations from Lake Murray/Binaturi (*C. halli*; LSUMZ 44740), exhibiting strikingly shorter maxilla and enlarged postcranial elements, or Aramia River (*C. halli*; USNM 211290), exhibiting a morphology closer to consensus.

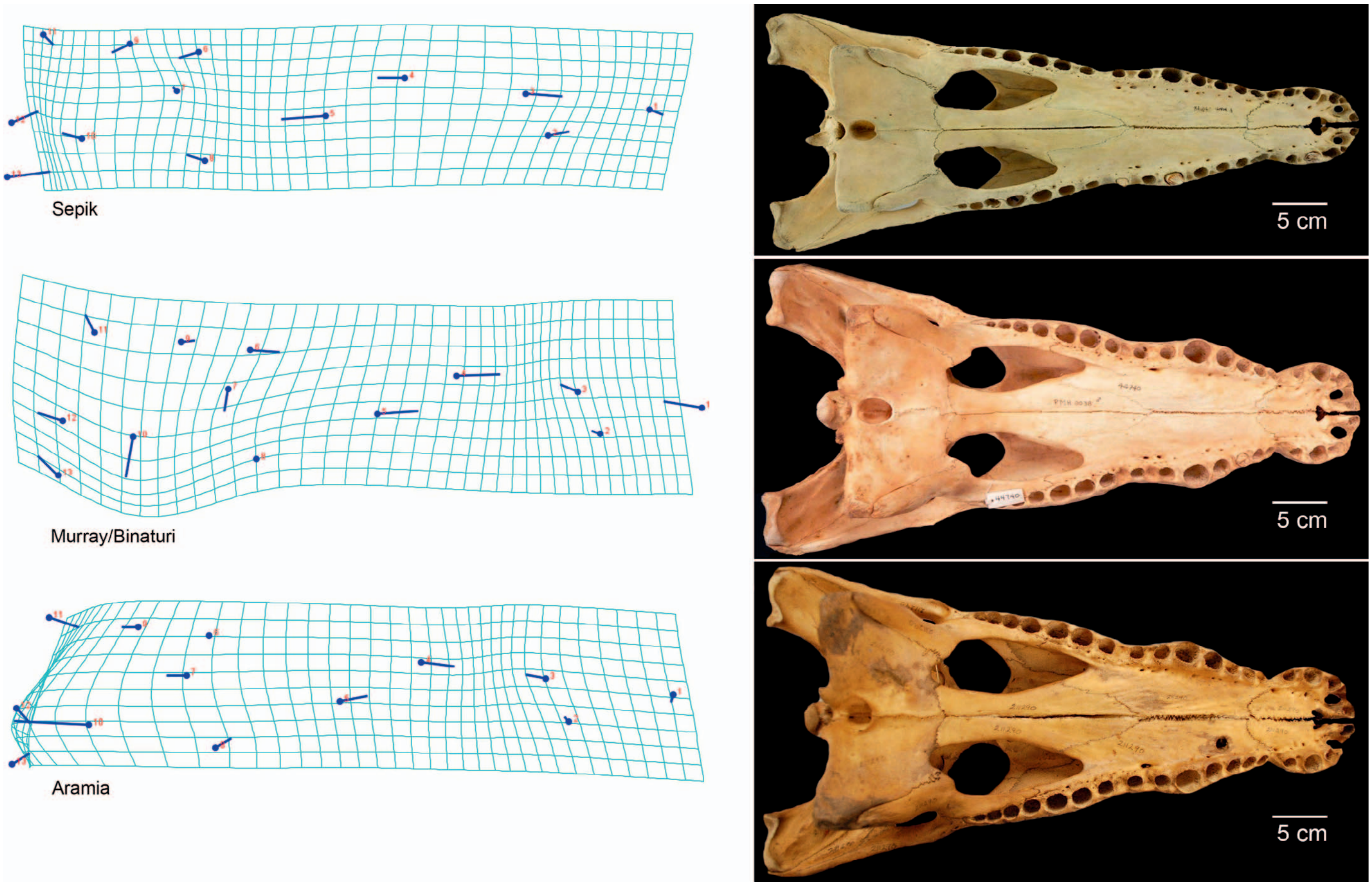

Fig. 4. Ventral cranial shape variation depicted using transformation grids with corresponding example specimen among drainages in Papua New Guinea. Solid points indicate consensus landmarks for all individuals, and lines extending from points indicate the extent (length) and direction of shape change exhibited by that classifier. Populations in northern drainages (i.e., Sepik; FMNH 14048; *Crocodylus novaeguineae*) exhibit an extended maxilla and reduced postcranial elements relative to southern populations from Lake Murray/Binaturi (*C. halli*; LSUMZ 44740) and the Aramia River (*C. halli*; USNM 211290), exhibiting strikingly shorter maxilla and enlarged postcranial elements.

specimen size bias exists among drainages. Landmark-based sources of cranial variation among classifiers were visualized using transformation grids at 3X magnification.

RESULTS

Dorsal cranial morphology significantly varied between NCN and SCN (df = 26, $F = 5.27$, $P < 0.001$) and among drainages (df = 78, $F = 2.71$, $P = 0.0004$), and no size bias was detected in our sampling (df = 3, $F = 0.03$, $P = 0.9936$). Similarly, ventral cranial morphology exhibited the same pattern in shape variation between proposed clades (df = 22, $F = 8.03$, $P < 0.001$), among drainages (df = 110, $F = 4.01$, $P < 0.0001$), and with respect to specimen size consistency (df = 5, $F = 0.51$, $P = 0.7635$). Dorsal variation between proposed northern and southern clades and specimens inhabiting northern versus southern drainages was evident in the length of the premaxilla, maxilla, nasal, postorbital, squamosal, and occipital condyle; further, the width of the squamosal, posterolateral angling of the quadrate, and quadratojugal differentiate the clades (Fig. 3). NCN specimens were characterized by longer premaxilla, frontal and nasal bones, shorter and narrower postorbital and squamosal bones, medially compressed occipital condyle, and antero-medially compressed quadrate and quadratojugal bones relative to SCN (Fig. 3). Ventrally, NCN was characterized by a long maxilla, a short, compressed palatine and pterygoid, and the same antero-medially compressed quadrate and quadratojugal described above relative to SCN (Fig. 4). Among SCN drainages, slight variation was found in the extent and angled shifts of the patterns described above, with specimens from Aramia being closer to consensus than Lake Murray and exhibiting closer proximity of the nare to the tip of the premaxilla. Binaturi specimens were characterized by longer premaxilla and narrower and longer pterygoid relative to other SCN drainages.

Ordination analyses reveal similar trends for both dorsal and ventral morphology. The dorsal CVA elucidates separation in morphospace between SCN and NCN along CV1, which explains 75.5% of the variance (Fig. 5A). Ventral CVA exhibits the same pattern, with CV1 explaining 49% of the variance (Fig. 5B). Within SCN, Binaturi and Lake Murray specimens occupy the same morphospace in both ventral and dorsal CVAs, while Aramia specimens diverge from other SCN specimens along CV2; however, CV2 only explains 13.7% of the dorsal variation and 27.4% of the ventral variation. The proximity of nares to the anterior tip of the premaxilla in Aramia specimens is predominantly responsible for variation along CV2.

DISCUSSION

The Central Highlands of Papua New Guinea are hypothesized to have uplifted less than 10 million years ago (Ma), likely in the last 8–3 million years (Hill and Gleadow, 1989; Toussaint et al., 2014) and contributed to north–south

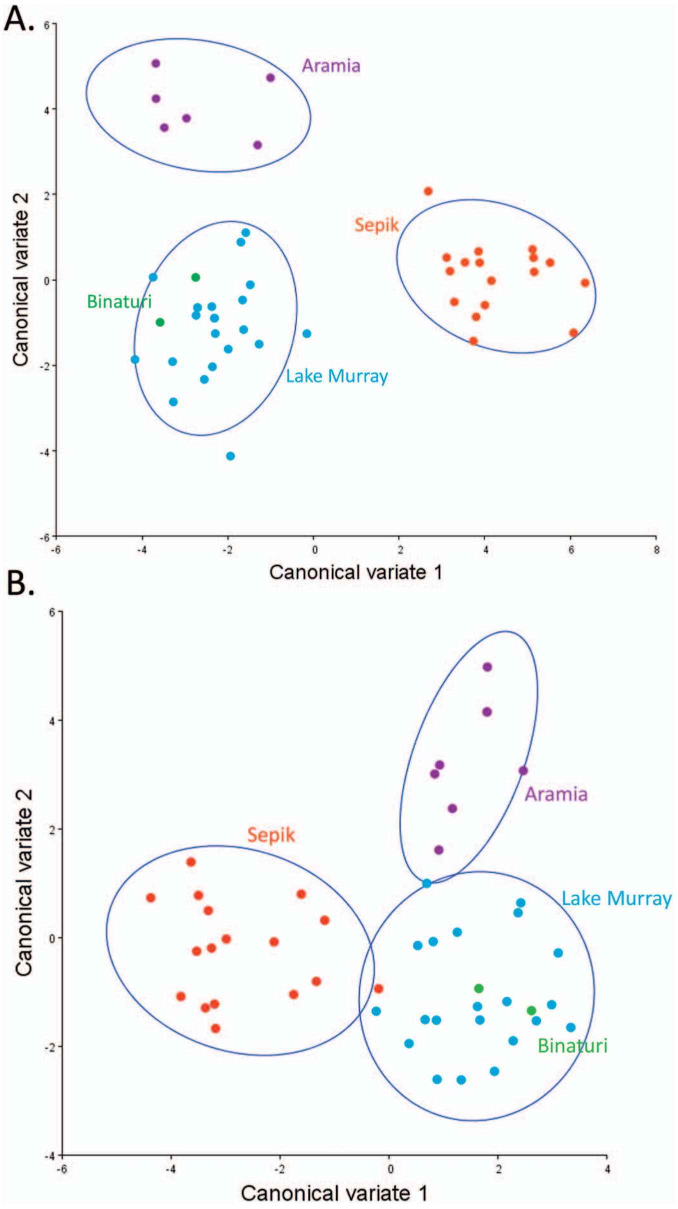

Fig. 5. Canonical variates analysis exhibiting position in morphospace among specimens from SCN (Lake Murray, Binaturi, and Aramia; *Crocodylus halli*) and NCN (Sepik; *Crocodylus novaeguineae*) from dorsal (A; CV1 = 75% variance, CV2 = 13.7% variance) and ventral (B; CV1 = 49% variance, CV2 = 27.4% variance) perspectives.

population divergence of a variety of animals, including fishes (Unmack et al., 2012), arthropods (Toussaint et al., 2014), birds (Deiner et al., 2011), and mammals (Meredith et al., 2010).

While Gratten (2003) did not recover monophyly of mitochondrial haplotypes between northern and southern populations of *C. novaeguineae*, phylogenetic analysis of sequence data do support separate lineages for these two populations (Gratten, 2003). We concur with Gratten (2003) that this is likely due to relatively recent isolation with minimal historical or recent gene flow between the two populations. Furthermore, based on the results of Gratten (2003), the enigmatic Bornean *C. raninus* was recovered sister to and quite similar genetically to NCN *C. novaeguineae*. The single individual from Gratten (2003) was obtained as a juvenile from a crocodile farm in Kalimantan and only putatively assigned to *C. raninus*. Ross (1990) additionally concluded the skull of *C. raninus* is most similar to *C. novaeguineae* from the north coast of New Guinea. The taxonomic status of *C. raninus* warrants further expanded systematic study to clarify validity of this species, as well as its relationship with NCN and SCN *C. novaeguineae*. Additional genetic studies will certainly enhance our understanding of species limits and evolutionary relationships among these crocodilians. Nevertheless, the two populations of *C. novaeguineae* are readily diagnosed from each other.

Three live specimens of *C. novaeguineae* were examined at St. Augustine Alligator Farm in December 2018 in an effort to substantiate observed diagnostic differences based on our analysis of skeletal material. All three individuals were clearly diagnosed as SCN in origin (Fig. 6). Furthermore, several ethno-artifacts and additional skulls on display at St. Augustine Alligator Farm were unambiguously diagnosable as NCN and SCN using characters described herein.

Observed morphological and life history differences based on the present and past studies (Cox, 1985; Hall, 1985, 1989; Hall and Johnson, 1987) lead us to recognize southern populations of *C. novaeguineae* as a separate species from those along the northern portions of Papua New Guinea.

Crocodylus halli, new species

urn:lsid:zoobank.org:act:2B819FD7-B74C-42B4-A85B-7D4B93D96105
Hall's New Guinea Crocodile
Figure 7

Holotype.—USNM 211290, Papua New Guinea, Western Province, Balimo, Aramia River Lagoon, –8.00589 S, 142.938 E, Charles A. Ross, 27 November 1977 [skull with mandibles; sex unknown] (Fig. 7).

Paratypes.—LSUMZ 44734, Papua New Guinea, Western Province, Lake Murray District, Agu River, Philip Hall, 14 January 1981 [skull; female]; LSUMZ 44735, Papua New Guinea, Western Province, Lake Murray District, Lake Murray, Philip Hall, 14 January 1980 [skull; sex unknown]; USNM 211298, Papua New Guinea, Western Province, Balimo, Aramia River Lagoon, –8.00589 S, 142.938 E, Charles A. Ross, 27 November 1977 [skull with mandibles; sex unknown].

Non-type material.—LSUMZ 44722–44732, 44734–44738, 44740–44746, UF 145927, 24, Lake Murray District; MCZ 153039–153040, 2, Binaturi River; USNM 211290, 211293, 211294, 211296, 211298, 211300–211301, 7, Aramia.

Diagnosis.—*Crocodylus halli* is readily separated from *C. novaeguineae* based on a longer frontal bone (versus shorter in *C. novaeguineae*); a shorter maxilla and nasal (versus longer in *C. novaeguineae*); long and wide postorbital and squamosal (versus short and narrow in *C. novaeguineae*); and a wider palatine of pterygoid that extends posteriorly (versus narrow and medially oriented in *C. novaeguineae*). Additionally, *C. halli* possesses no more than four non-contiguous post-occipital scutes, versus four to six contiguous post-occipital scutes in *C. novaeguineae*.

Species description and variation.—Variation in morphology of adult *Crocodylus halli* exists among drainages, with specimens from Lake Murray exhibiting a skull width that is more than half of the skull length (posteromedial squamosal to anteromedial premaxilla), resulting in the

Fig. 6. Live individual of *C. halli* at St. Augustine Alligator Farm Zoological Park.

appearance of a stocky and wide head in adults. Specimens from Aramia River have generally longer maxilla and shorter postcranial elements than Lake Murray individuals, while maintaining shorter maxilla and longer postcranial elements than *C. novaeguineae*. Juveniles retain morphology consistent with ontogenetic constraints, exhibiting a relatively enlarged orbit and compressed postcranial elements.

Distribution.—*Crocodylus halli* occurs within drainages south of the Central Highlands of Papua New Guinea in swamps, rivers, lakes, and occasionally estuaries.

Natural history.—Females of *C. halli* nest in the rainy season (November–April) and lay larger eggs in smaller clutches than *C. novaeguineae,* which nests near end of the dry season (July–November; Cox, 1985; Hall, 1985; Hall and Johnson, 1987).

Etymology.—The specific epithet recognizes the fieldwork and research of Philip Hall whose contributions provided the initial framework for supporting distinctiveness of this species.

MATERIAL EXAMINED

Crocodylus novaeguineae: FMNH 2854 (holotype), 13092, 13966, 14016, 14039–14040, 14043–14044, 14047–14048, 14050, 14053, 34491, AMNH 64425, MCZ 32099-32100, QM 5232, 17, Sepik River; UF 71780, 1, Hunstein.

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Fig. 7. Holotype of *Crocodylus halli*, USNM 211290.

and images from their institutions. We are grateful to John and Jen Brueggen at the St. Augustine Alligator Farm Zoological Park for their generosity and allowing us to examine crocodiles under their care.

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