

CROCODILE SPECIALIST GROUP NEWSLETTER

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IUCN • Species Survival Commission

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COVER PHOTOGRAPH: Adult male Broad-nosed caiman (*Caiman latirostris*) at Criatorio Caiman, Pôrto Feliz, Sao Paulo, Brazil. Photograph: Pablo Siroski.

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The CSG Newsletter provides information on the conservation, status, news and current events concerning crocodilians, and on the activities of the CSG. The Newsletter is distributed to CSG members and to other interested individuals and organizations. All Newsletter recipients are asked to contribute news and other materials.

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Crocodile Park, Malaga, Spain.
The Ebey family, New Mexico, USA.
James Hennessey, The National Reptile Zoo, Ireland.

Editorial

In February 2017, the IUCN Species Survival Commission (SSC) sent out a mass mailing to all current CSG members, inviting them to renew their membership. If you did not receive any communication from the SSC, please advise CSG Executive Officer, Tom Dacey (csg@wmi.com.au) and he will follow up with the SSC to ensure that you receive the membership renewal request.

It is with a great deal of sadness that I inform you that Dr. Effendy Sumarja, from Indonesia, has passed on. In the early 1990s Effendy was responsible within the Ministry of Forestry for upgrading many of Indonesia's wildlife trade issues, to better meet the requirements of CITES. It was a major undertaking, and with crocodilians, Effendy, Widodo Ramono, Hank Jenkins and myself undertook a major review, traveling from one end of Indonesia to the other, discussing crocodilian conservation, sustainable use, management and farming with Government and private stakeholders. Effendy managed this review mission like a military operation, at a remarkable pace. He was a true champion of reform in Indonesia and he will be sadly missed.

The CSG was contacted by Dr. Peter Paul Van Dijk, the new Chair of the CITES Nomenclature Committee, raising the issue of whether the current CITES reference for crocodilians (Wermuth and Mertens 1961) was still suitable, given the many advances, particularly in DNA-based taxonomy, over the last 55 years. The matter was discussed between a number of senior CSG members, and I agreed with the consensus view that we need a Taxonomy and Identification Thematic Group. Dr. Kent Vliet has kindly agreed to chair this new group within the CSG, and those with a special interest should contact Kent or Tom.

At the 23rd CSG working meeting held in Louisiana (May 2014), a special presentation was made to 10-year-old Karin Ebey, who for the 3 years prior to the meeting had contributed financially to the CSG's conservation efforts. Karin has not lost her desire to work with crocodilians, and she and family continue to contribute financially! Karin recently undertook a project on crocodilian morphometrics, using data provided by various CSG members, for which she was awarded first place at the New Mexico Academy of Science Junior Regional Paper Competition (see page 5). She now heads to the State competition with her project, and we wish her the best of luck there and with her future endeavours.

Iran has a small but important population of Muggers (*Crocodylus palustris*). As part of a public education program, Elham Abtin and Asghar Mobaraki have produced a book entitled "Gandou: Marsh Crocodile in Iran" (see page 4).

A meeting of the Siamese Crocodile Task Force is scheduled to be held at the Mahidol University, Nakhon Pathom, Thailand, 1-3 June 2017. A workshop on telemetry will be held on the last day as tracking may become an important element of any reintroduction programs. Details will be posted as soon as they become available.

A workshop on the "Status of Crocodile Conservation in Cuba" will be held at the Hotel Playa Girón, Matanzas, Cuba, 5-9 June 2017. Wildlife Conservation Society and Dr. Frank Mazzotti are funding the participation of Cubans at the workshop, and participants from outside Cuba are welcome to attend. However, there is capacity for only 20-25 non-Cubans. Please contact Natalia Rossi (nrossi@wcs.org) for details and/or assistance for hotel reservations. I have been discussing with Dr. Marisa Tellez the need to get a more holistic view of crocodilian conservation and management in the Central American and Caribbean region, and I believe this can and should be a priority issue at our next CSG Working Meeting (May 2018; see below). It will also be important for the Future Leaders Working Group to meet once again.

Planning for the 25th CSG Working Meeting is now well underway, and it will be held in Santa Fe, Argentina, in May 2018. So now is the time to start saving and make time and travel commitments! The schedule includes a Veterinary Workshop (5 May), CSG Steering Committee meeting (6 May), Working Meeting (7-10 May) and field trips (11 May). The meeting website will be launched very soon.

Professor Grahame Webb, *CSG Chair*.



Yacare caiman (*Caiman yacare*). Photograph: Pablo Siroski.

Books

“Gandou: Marsh Crocodile in Iran”
by Elham Abtin and Asghar Mobaraki

The geography, varying climates, two main mountain ranges (Alborz and Zagros), expansive deserts in the center, and coastlines in the northern and southern parts of Iran have created the basis for a diversity of ecosystems, fauna and flora. Together with cultural diversity and different traditional utilisation of resources, these characteristics make Iran one of the most important countries in the Middle East and West Asia.

Along with the legal protection, specific religious and traditional beliefs are a significant factor in the protection of species and their habitats by local people. A small population of Mugger crocodiles (*Crocodylus palustris*), estimated to comprise about 500 individuals, is distributed in southeastern Iran's Sistan and Baluchestan Province, close to the border with Pakistan. The area is the western-most extent of the distribution of the species.

The main characteristic of the Mugger population in Iran is that it has been divided into several spatially scattered sub-populations. As a management measure, the main parts of crocodile habitat have been designated as a protected area named “Gandou”, which is the local name for the Mugger in Iran (Gandou is a Balouchi word meaning “moving on the belly”). The area is also an “International Wetland Site”.

Muggers occupy a range of natural and artificial waterbodies, especially artificial ponds inside villages, named “Hootak”. Muggers also move between habitats, often crossing roads, and resulting at times in mortality by car strikes. Crocodiles use any available resources as food, including amphibians, birds, dogs and villager's livestock, but they mainly depend on fish. Despite this human-crocodile conflict, the local people respect crocodiles and never harm or hunt them. There have been few fatal attacks by Muggers on humans in Iran.

Gandou: Marsh Crocodile in Iran” (144 pages) is based on the long and extensive fieldwork of the authors (Elham Abtin and Asghar Mobaraki) over more than 20 years. The book provides general information on the world's crocodilians and detailed information on Muggers in Iran. None of our work would have been possible without the ongoing support of the Iranian Department of Environment for our fieldwork and research, as well as the assistance of our colleagues who accompanied us into the field, and last but not least, the local people for their invaluable help.

The book was published by the official support of the Iranian Department of the Environment and financial support of Chabahar Free Zone. We express our thanks to Dr. A.R. Kordi, Head of the Chabahar Free Zone and his colleagues, for their support. In preparing the book, we benefitted greatly from the friendly guidance and advice of experts from around the world, including Prof. Grahame Webb, Charlie Manolis, Colin Stevenson and Aneelm de Silva.

The book is directed towards an audience in Iran, and so is written in Farsi. However, it contains many interesting photographs, some of which were contributed by other CSG colleagues, to whom we extend our thanks (Ruth Elsey, Charlie Manolis, Grahame Webb, Colin Stevenson, Alan Woodward).



Figure 1. Front (left) and back (right) covers of “Gandou: Marsh Crocodile in Iran”.

The book is available (\$US15 plus postage) from the authors (see e-mail contact details below).

Elham Abtin (ala_saly@yahoo.co.uk) and Asghar Mobaraki (amobaraki@yahoo.com), Department of the Environment, Iran.

CSG Student Research Assistance Scheme

The Student Research Assistance Scheme (SRAS) provided funding to 8 students in the January-March 2017 quarter. Two further applications are currently under review.

1. América Jaimes (Venezuela): Characterization of aerobic bacteria from oral and cloacal swabs of the Orinoco crocodile (*Crocodylus intermedius*).
2. Jinhong Zhao (China): Survey of parasites and evaluation of pathopoiesis of parasites to Chinese alligators.
3. Huan Wan (China): Multiple paternity and male reproductive success in the Chinese alligator.
4. Yessenia Sarmiento Marina (Mexico): Population ecology of caimans (*Caiman crocodilus*) and crocodiles (*Crocodylus acutus*) in the eastern portion of the Puerto Arista Estuarine System, Chiapas, Mexico.
5. Emmanuel Amoah (Ghana): Nesting ecology of West African Dwarf crocodile (*Osteolaemus* sp. nov. cf. *tetraspis*) in Chirehin Community Land, Ghana.
6. N'Dede Michel Ahizi (Côte d'Ivoire): Evaluation of the effectiveness of Ivorian protected areas for the conservation of *Mecistops cataphractus*.

7. Yaoua Christine Kouman (Côte d'Ivoire): Dynamics of habitat use by *Mecistops cataphractus* in different protected areas in Côte d'Ivoire.
8. Andres Rodriguez Cordero (Bolivia): Species distribution modelling of crocodilians in Bolivia.

Tom Dacey, *CSG Executive Officer*, (csg@wmi.com.au).

Dr. Dietrich Jelden Awarded SSC Chair's Citation of Excellence

In November 2016 Dr. Dietrich Jelden was presented with the "Species Survival Commission Chair's Citation of Excellence", in recognition of "an astounding and visionary career in wildlife conservation and management at a global level, and specifically for an unparalleled contribution to crocodilian conservation management and sustainable use through the IUCN SSC Crocodile Specialist Group". As CSG members are aware, Dietrich served as CSG Deputy Chair from 1994 until his retirement in October 2016.

Dietrich's exposure to crocodilians came in 1979-1981, when he was employed as a wildlife officer by FAO to work in the FAO/UNDP project in Papua New Guinea. This involved him being based in the remote Sepik River area, and studying the breeding biology of *Crocodylus porosus* and *C. novaeguineae*, which was the basis of his PhD. As Deputy Head of the German CITES Scientific Authority (1985-1993) and Head of the German CITES Management Authority (1994-2106) Dietrich became involved with CITES, and subsequently contributed significantly to crocodilian and other wildlife issues within that forum.

Dietrich's involvement with the CSG is no less significant. He participated in numerous CSG country reviews (eg Indonesia, Thailand, Tanzania, Cambodia, Madagascar, Vietnam), and helped guide the CSG for over 20 years with his extensive knowledge and invaluable advice.



Figure 1. Dr. Jelden (left) is presented with his retirement certificate and the SSC Chair's (Dr. Jon Paul Rodríguez) Citation of Excellence by Secretary of State Jochen Flasbarth of the Federal Ministry of Environment.

We congratulate Dietrich on receiving the SSC Chair's Citation of Excellence. It is well deserved. We also hope that Dietrich will continue his relationship with the CSG, albeit amidst his retirement plans.

Charlie Manolis, *CSG Deputy Chair*.

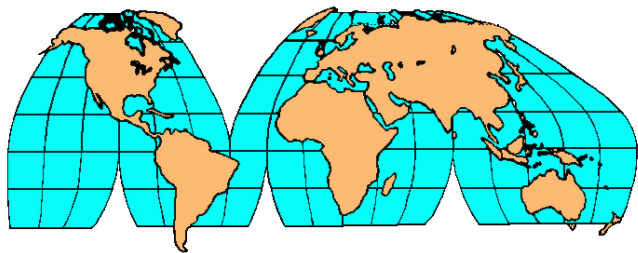
CROCODILIAN STUDIES CONTINUE. I wrote my first letter to the CSG when I was 6 years old [CSG Newsletter 29(2): 23]. My interest in studying crocodilians was acknowledged at the CSG Working Meeting in Louisiana in 2014 [CSG Newsletter 33(2): 12], when I was invited to become a member of the CSG. Now 13 years of age, and in 8th grade, I continue to pursue my interest in studying crocodilians.

This year, in addition to a Science Fair poster, I entered a paper ("A Comparison of Alligator and Crocodile Proportions") and gave a talk in the New Mexico Academy of Science Junior Regional Paper Competition for 6th to 8th graders. My paper was awarded first place and I will now present it at the state competition. I studied the relationship between head-total length ratio (how long the head is proportionally) and rostral proportion (how broad the head is) in four species - *Alligator mississippiensis*, *Crocodylus porosus*, *C. acutus*, and *C. moreletii*. I found that head-total length ratio and rostral proportion are not related to each other, suggesting that the proportions evolved separately. I would also love to study this relationship in the other 20 species of crocodilians. I thank Drs. Ruth Elsey, Grahame Webb, Steve Platt and Thomas Rainwater for sending me data for the study and Charlie Manolis for supporting me over the years. My current dreams are to help save the Gharial and to discover something new in crocodilian science.



Karin Ebey, *Los Alamos, New Mexico 87544, USA*.

Regional Reports



North America

USA

AMERICAN ALLIGATOR (*ALLIGATOR MISSISSIPPIENSIS*) WITH FORELIMB FIBROMA. The state of Louisiana conducts a sanctioned alligator harvest of some 35,000 wild alligators each autumn. On 20 September 2016 an adult alligator was harvested on privately-owned wetlands in Cameron Parish in southwest Louisiana. The alligator was a male and measured 284.5 cm, and had an unusual growth of approximately 9 cm diameter on the ventral surface of the right forelimb (Fig. 1). The alligator otherwise appeared healthy and of normal body condition.

As the alligator was harvested in a remote rural location, the distal part of the limb was removed and frozen. On 26 September, the frozen specimen was transferred to 10% neutral buffered formalin, and subsequently shipped to Louisiana State University School of Veterinary Medicine in Baton Rouge, Louisiana, for histological examination.

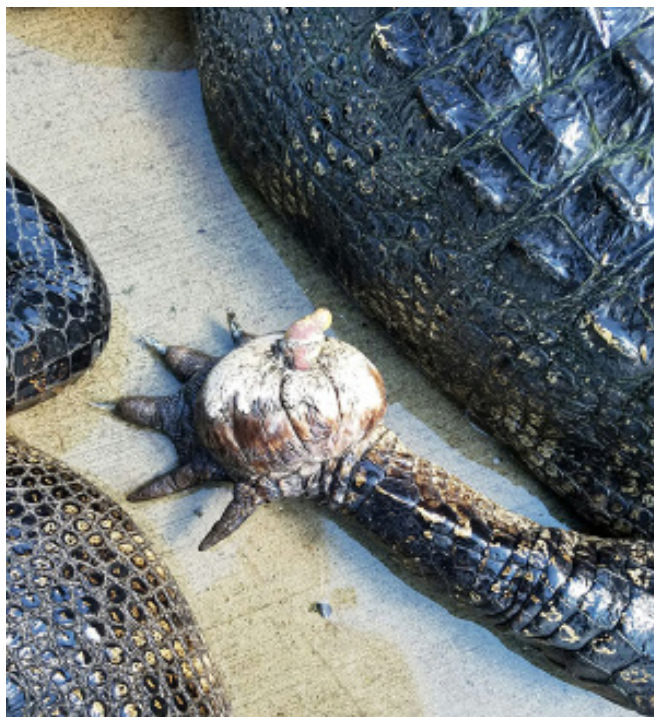


Figure 1. Adult alligator with abnormal growth on right forelimb.

Pathological gross findings revealed the mass was generally firm, but had some focal cavitation thought to be due to postmortem autolysis. It required additional fixation, and was composed of dense collagen with scattered thin low cellularity spindle cells consistent with fibroblasts. Some superficial ulcerations were noted; decalcified sections consisted of disorganized low cellular fibrocollagenous tissue, interpreted as neoplastic lesions. The gross and histological findings were most compatible with a fibroma. The specimen was thought not to be granulomatous due to lack of prominent vasculature or inflammation.

Other (much larger) growths have been reported in wild alligators and include a fibrosarcoma (Elsey and Nevarez 2013) and a fibromyxoma (Elsey *et al.* 2013). Numerous other neoplasms have been described in crocodilians (Huchzermeyer 2003), including fibromas (Youngprapakorn *et al.* 1994). It would be of interest to learn if other types of tumors (benign or malignant) occur in wild or captive crocodilians. Additionally, the investigation of a possible role of environmental contaminants or other etiologies as carcinogenic inducers might be warranted.

Acknowledgements

We thank Ms. Tina Payne for assistance with preservation of the specimen.

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Ruth M. Elsey (*Louisiana Department of Wildlife and Fisheries, 5476 Grand Chenier Highway, Grand Chenier, LA 70643, USA*), Randall Hebert (*1704 Stout Street, Lake Charles, LA 70605, USA*), Javier G. Nevarez (*Louisiana State University School of Veterinary Medicine, Skip Bertman Drive, Baton Rouge, LA 70803, USA*) and Nobuko Wakamatsu Utsuki (*Louisiana State University School of Veterinary Medicine, Skip Bertman Drive, Baton Rouge, LA 70803, USA*).

South Asia and Iran

India

UNIQUE PARENTAL CARE BEHAVIOUR IN GHARIAL (*GAVIALIS GANGETICUS*), NATIONAL CHAMBAL SANCTUARY, INDIA. Parental care has been observed in many crocodilian species and it is presumably the rule in all crocodilians. It includes nest guarding and assisting hatchlings to the water, hatchling guarding, responding to distress calls of young, and occasionally moving creches to new nursery areas. All this is done mainly by the female, but where adults live in pairs or where the female has “disappeared”, males have been reported either helping or taking over the care of the hatchlings (Alvarez del Toro 1974; Garrick and Lang 1977; Bustard 1980).

Kasaua (26° 38.190' N, 78° 59.457' E) and Khera Ajab Singh (26° 39.248' N, 78° 56.429' E) are well known nesting sites for the Gharial (*Gavialis gangeticus*) in the Etawah Range of National Chambal Sanctuary (NCS), Uttar Pradesh, India. Upon learning that hatching had started at these sites, plans were made to visit these sites immediately. On the morning of 28 May 2016, Mr. Rajeev Chauhan, Ms. Perna Bindra and Mr. Suresh Chandra Rajput (Range Officer) visited both sites. On the evening on the same day I visited Kasaua and spent over an hour making direct behavioural observations. I visited Khera Ajab Singh the next day. On both occasions, I noted and made photographic records of the presence or absence of adult Gharials and their behaviour.

At Kasaua there were 15 nests. Hatching had commenced in 9 of them when we arrived, and hatchlings were in the water. When we were about 70-75 m away they remained at the water's edge, with some in the water but most on the dry sand. But as we approached closer, they became alert and one by one started entered the water. A male Gharial (about 5 m total length) was in relatively deep water midstream (river is about 80 m at this point) moved close to the riverbank. By the time we were about 25 m from the water's edge all hatchlings had entered the water, and the male moved in closer to the hatchlings.

Soon after, we saw behaviour not previously recorded. One by one the hatchlings clambered onto the male - from snout to tail (Fig. 1). The male appeared “calm”, but as we approached the water's edge more closely it moved away, with all the hatchlings riding on its back. So as not to alarm the animals further, we retreated, and the male moved back closer to the water's edge, where it remained stationary.

The hatchlings remained on its back for about 35 minutes, and thereafter one by one they got off and swam back to the bank. During this period, two adult females hovered some distance away (20 and 30 m respectively) and played no part in the behavioural interactions.

At Khera Ajab Singh, where 26 nests were recorded, hatching had occurred in 14 nests. At the time of our visit the hatchlings were on the riverbank. As we approached to within 25 m

they slid into the water and began to make distress calls. But unlike the male at Kasaua, the male appeared on the surface, its ghara clearly visible, but it did not come in close to assist the hatchlings. It remained about 20 m from the water's edge, making hissing calls (Fig. 2). The large females remained around 25-30 m away, surfacing frequently.



Figure 1. Adult male Gharial at Kasua, with hatchlings riding on its back and tail.



Figure 2. Adult male Gharial at Khera Ajab Singh, with hatchlings around it.

Lang and Kumar (2015) reported that large male Gharial, with well-developed and prominent ghara often defend quite large creches of young (eg 200-1000 hatchlings from 5-25 nests). Biparental care, especially paternal guarding of young, is likely the usual condition for Gharial.

There was a clear difference in hatchling caring behaviour between the similar sized males at Kasaua and Khera Ajab Singh. The male at Kasaua allowed the hatchlings to ride its back as long as the perception of threat remained - this has never been seen or recorded at these sites previously.

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Science



Recent Publications

Phosri, S., Jangpromma, N., Patramanon, R., Kongyingyoes, B., Mahakunakorn, P. and Klaynongsruang, S. (2017). Protective effect of crocodile hemoglobin and whole blood against hydrogen peroxide-induced oxidative damage in human lung fibroblasts (MRC-5) and inflammation in mice. Inflammation 40(1): 205-220.

Abstract: A putative protective effect of cHb and cWb against H₂O₂-induced oxidative damage was evaluated in detail using MRC-5 cells. In addition, the carrageenan (Carr)-induced mouse paw edema model and the cotton pellet-induced granuloma model were employed to examine the in vivo anti-inflammatory activity of cHb and cWb in mice. It was demonstrated that both cHb and cWb treatments significantly increased cell viability and inhibited morphology alterations in MRC-5 cells exposed to H₂O₂. Orally administered cHb and cWb significantly reduced Carr-induced paw edema volume and cotton pellet-induced granuloma formation. Moreover, cHb and cWb decreased the expression levels of important pro-inflammatory cytokines (IL-6, IL-1 β , and TNF- α), while only cWb was found to increase the expression of the anti-inflammatory cytokine IL-10 significantly. Finally, the activity of antioxidant enzymes (SOD, CAT, and GPx) in the liver improved after cHb and cWb treatment under acute and chronic inflammation. Taken collectively, the results of this study suggest that both cHb and cWb protect against hydrogen peroxide-induced damage in fibroblast cells. Moreover, cHb and cWb were found to exhibit anti-inflammatory activity in both the acute and chronic stages of inflammation and appear to enhance antioxidant enzyme activity and decrease lipid peroxidation in the livers of mice. Therefore, this study indicates that cHb and cWb have great potential to be used in the development of dietary supplements for the prevention of oxidative stress related to inflammatory disorders.

Barksdale, S.M., Hrifko, E.J. and van Hoek, M.L. (2017). Cathelicidin antimicrobial peptide from *Alligator mississippiensis* has antibacterial activity against multi-drug resistant *Acinetobacter*

baumannii and *Klebsiella pneumoniae*. Developmental & Comparative Immunology 70: 135-144.

Abstract: *Alligator mississippiensis* (American alligator), a member of order Crocodylia, lives in bacteria-laden environments but is not often known to succumb to bacterial infections. Their serum has been shown to have antibacterial activity beyond that of human serum, and it is believed that this activity is partially due to cationic antimicrobial peptides (CAMPs). CAMPs are produced by many organisms as part of the innate immune system. CAMPs are attractive possible therapies against multi-drug resistant bacteria, such as those found in biofilm-infected war wounds, because they seldom cause genetic resistance in bacteria and are effective against antibiotic resistant bacteria. In this work, we identified, synthesized, and characterized a cathelicidin and two shorter fragments from the American alligator. We discovered the cathelicidin using Basic Local Alignment Search Tool (BLAST) alignment and by comparing *A. mississippiensis* expressed sequence tags (ESTs) with propeptide cathelicidins of other reptiles. We analyzed the structure using bioinformatics tools and circular dichroism and predicted that the full-length cathelicidin peptide has a mixed structure, with an N-terminal α -helix and a center Pro hinge. In minimal inhibitory concentration (MIC) assays, it was determined that the cathelicidin and the two shorter fragments have strong activity against multiple Gram-negative bacteria, including clinical isolates of multi-drug resistant (MDR) *Acinetobacter baumannii* and carbapenem-resistant *Klebsiella pneumoniae*. Using the ethidium bromide uptake assay, it was found that these peptides permeabilize the bacterial membrane and are less sensitive to salt inhibition than many other known CAMPs. The alligator cathelicidin peptides were not hemolytic against sheep red blood cells at 300 μ g/ml and were not significantly cytotoxic against A549 human lung epithelial cells after 24 h exposure in 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays. These alligator cathelicidin peptides have activity similar to other CAMPs from reptiles such as NA-CATH. It is possible that the alligator cathelicidins play an important role in the innate immune response of *A. mississippiensis*, similar to LL-37 in humans. In addition, due to their activities against MDR bacteria and lack of cytotoxicity, the AM-CATH peptides could be an attractive platform for further development as a potential therapeutic.

Jangpromma, N., Poolperm, N., Pornsri, K., Anwised, P., Kabbua, T., Phosri, S., Daduang, S. and Klaynongsruang, S. (2017). Proteomics profiling and inflammatory factor gene expression in LPS-stimulated RAW 264.7 cells treated with *Crocodylus siamensis* hemoglobin. Chiang Mai J. Sci. 44 (<http://epg.science.cmu.ac.th/ejournal/>).

Abstract: Although we previously reported that *Crocodylus siamensis* hemoglobin (cHb) exhibits anti-inflammatory properties by suppressing nitric oxide (NO) production, the actual underlying mechanism has yet remained elusive. Consequently, this report represents the first study aimed to shed light upon the basic mechanistic details of the anti-inflammatory activities of cHb. In the present study, both 100 and 200 μ g/ml of cHb were found to reduce the production of NO in LPS-stimulated RAW 264.7 cells. Consistent with the NO testing results, it was observed that co-treatment with cHb also significantly decreased inducible nitric oxide synthase (iNOS), likely due to decreased expression levels of cyclooxygenase-2 (COX-2) and proinflammatory cytokines, such as interleukin (IL)-1 β and IL-6 mRNA. Therefore, these results indicate that the mechanism of cHb-induced decrease of NO production might be associated with the transcriptional suppression of iNOS. Moreover, the expression of heme oxygenase-1 (HO-1), an anti-inflammatory enzyme, was evaluated in more detail. It could be shown that HO-1 levels were increased in RAW 264.7 cells co-treated with cHb in a concentration-dependent manner. To elucidate the proteomics response of macrophages treated with LPS in the presence or absence of cHb, several proteins with differential expressions were identified via LC-MS/MS analysis. With respect to the individual functions of these proteins, our data

indicated involvement in various processes during inflammation, such as cellular metabolism, protein fate, oxidative burst, signal transduction and morphogenesis. Consequently, all results of this study directly indicate that cHb exhibits anti-inflammatory activity on LPS-stimulated RAW 264.7 cells via functioning as an activator or suppressor in the expression of inflammatory factor genes and affects several specific proteins related to important pathways of inflammation.

Arrowood, H. and Mvele, C. (2017). Projet Faux Gavial reduces commerce of slender-snouted crocodile in Gabon. 51(1): 13-14.

Capelle, K.D. (2016). Evaluating Survival of Released Ranched American Alligator in Coastal Louisiana. MSc thesis, Louisiana State University, Baton Rouge, USA.

Abstract: Since 1986, Louisiana's American alligator (*Alligator mississippiensis*) ranching program has required the release of alligators produced from eggs collected from wild nests to maintain wild populations. This project assessed long-term harvest data (1991-2010s) to estimate survival of released alligators. First, wildlife and fishery harvest models and general inter-disciplinary survival models were evaluated to determine best fit to the data. Second, once the best fitting model was selected, release length, precipitation and temperature from release sites, and an index of hunter effort were added to investigate influences on survival estimates. Release length was included because over time the proportion and size of ranch-released alligators has been modified. The generalized linear mixed model, with a fixed intercept, and negative binomial probability distribution was selected as the best fitting model based on the minimization of differences between observed and expected values. This baseline model without covariates estimated instantaneous annual survival to be 0.87 and 0.89, for male and female alligators, respectively. The final best fitting model suggested that the larger an alligator is at release (to a certain point, benefits diminishing after 139 cm), the higher the chance of survival, and the longer it will be afield, for both males ($F_{1,169} = 5.62, p < 0.05$) and females ($F_{1,180} = 5.89, p < 0.05$). Mean precipitation was also statistically significant and positively associated with survival of both male ($F_{1,169} = 12.51, p < 0.05$) and female ($F_{1,180} = 12.33, p < 0.05$) alligators, suggesting reduced survival in drier years. Additionally, for male alligators, the coefficient of variation for mean temperature was statistically significant ($F_{1,169} = 7.19, p < 0.05$), suggesting lowered survival during years with more variation in temperature between months. Although not statistically significant, fit of the male model improved with the inclusion of the hunter effort covariate. Inclusion of release length along with environmental and hunter effort covariates improved precision of survival estimates resulting in values of 0.79 for males, and 0.81 for females, suggesting survival was influenced by the included covariates. Survival estimates with included environmental variables were very similar to reported wild alligator survival estimates, suggesting that ranch-released alligators respond to environmental conditions similar to wild alligators and that ranch-released alligator estimates may provide insights into wild alligator ecology.

Iijima, M., Takahashi, K. and Kobayashi, Y. (2016). The oldest record of *Alligator sinensis* from the Late Pliocene of Western Japan, and its biogeographic implication. Journal of Asian Earth Sciences 124: 94-101.

Abstract: The late Cenozoic fossil record of alligators in East Asia is crucial in understanding the origin and past distribution of Asian alligators that are now represented by a single species, *Alligator sinensis*. This study reports a partial skeleton of *A. sinensis* from the Late Pliocene (approximately 3.0 Ma) of western Japan. This Japanese *A. sinensis* is large in size (>200 cm total length), comparable to the maximum size of extant individuals. It demonstrates the oldest record of *A. sinensis* and wider distribution

of this species in the past. Tectonic and geographic history of East Asia suggests that alligators presumably dispersed into Japan before 25 Ma or after 10 Ma, yet finally were wiped out from Japan due to the semi-isolated condition of the Japanese island arc and the deteriorated climate during the Plio-Pleistocene.

Nixon, B., Anderson, A.L., Smith, N.D., McLeod, R. and Johnston, S.D. (2016). The Australian saltwater crocodile (*Crocodylus porosus*) provides evidence that the capacitation of spermatozoa may extend beyond the mammalian lineage. Proceedings of the Royal Society B. Biological Sciences (doi: 10.1098/rspb.2016.0495).

Abstract: Although mammalian spermatozoa only acquire functional maturity as they are conveyed through the male (epididymal maturation) and female (capacitation) reproductive tracts, the degree of post-testicular development necessary to achieve fertilization in other vertebrate species remains far less clear. Indeed, despite reports that the epididymis of birds and reptiles is capable of secreting proteins that bind and modify the sperm surface characteristics, it remains unclear whether capacitation is a pre-requisite for fertilization in these species. Using the ancient reptilian Australian saltwater crocodile as a model, this study was undertaken to explore whether reptile sperm do undergo capacitation-like changes following ejaculation. Our studies revealed that crocodile spermatozoa experienced a rapid and sustained, cyclic-AMP mediated increase in progressive motility following incubation under conditions optimized for the induction of capacitation in mammalian species such as the mouse and human. This response was coupled with elevated levels of phosphorylation associated with both protein kinase A and tyrosine kinase substrates, the latter of which were predominantly localized within the sperm flagellum. In findings that also accord with mammalian spermatozoa, we confirmed a homologue of outer dense fibre 2 as one of the principal substrates for tyrosine phosphorylation. Overall, our findings support the concept that crocodile spermatozoa do undergo a process that is homologous to capacitation in preparation for fertilization of an ovum.

Drumheller, S.K., Wilberg, E.W. and Sadleir, R.W. (2016). The utility of captive animals in actualistic research: A geometric morphometric exploration of the tooth row of *Alligator mississippiensis* suggesting ecophenotypic influences and functional constraints. J. Morphol. 277: 866-878.

Abstract: Captive broad snouted crocodylians are generally thought to have wider, shorter rostra than their wild counterparts. Interpreted to reflect morphological change in response to the conditions of captivity, this qualitative pattern could affect the utility of these animals in a variety of fields of research. However, due to relative ease of access and availability of life history data, captive animals are often utilized in actualistic research. Thus, this issue should be addressed in more detail. Here we explore snout shape variation between captive and wild members of *Alligator mississippiensis* using two-dimensional (2D) morphometric techniques. Several landmark schemes are used to assess the utility of different aspects of morphology in distinguishing the groups. While statistical analyses consistently differentiated between the groups, the area of morphospace occupied by wild members of *A. mississippiensis* generally overlapped with the larger area encompassing the captive specimens. This indicates that the captive condition is not as uniform as previously thought and instead encompasses a large spectrum of morphologies, ranging from the stereotypical broad, shortened snouts to outlines that are indistinguishable from the wild morphotype. These results align well with the interpretation that this change reflects an extreme example of ecophenotypy, since ranched, farmed, or zoo organisms are held in an array of enclosures, ranging from indoor, climate controlled pens to outdoor, more natural areas. This variation in environments should be reflected in different reactions to the animals' surroundings, resulting in a broad spectrum of morphotypes. While wild specimens are still preferred, especially

for fine scale analyses, these results indicate that not all captive members of *A. mississippiensis* exhibit the extreme morphological alterations often cited in the literature. Weighing the conditions in which the animals are held and exploring the possibility of morphological differences against the benefits of using captive specimens should be part of any actualistic study.

Takeuchi Fernandes, V.R., Rodrigues de Souza, M.L. Gasparino, E., Coutinho, M.E., Visentainer, J.V., Spinola Bérnago, A. and Souza dos Reis Goes, E. (2017). Commercial cuts of Pantanal caiman meat according to sex. *Cienc. Rural* 47(2): e20160195.

Abstract: This study evaluated the yield, color, and chemical composition of commercial cuts (tail, sirloin cut, back fillet, and thigh) of Pantanal caiman meat in both sexes. The yield of tail was higher than other cuts, and the yield of females (17.0%) was higher than males (15.9%). The thigh of males had lower protein content (20.8%) compared with other cuts. Females showed a higher lipid content in the tail (2.4%) and thigh (0.8%) compared with males (1.7% and 0.4%, respectively). The tail presented the greatest content of monounsaturated fatty acids (45.2%) and higher n6/n3 ratio (4.6). Although lightness was not different among cuts or between sexes, there were differences in color. Males have more yellowish meat compared with females. Thigh and back fillet were more reddish when compared to sirloin cut and tail, regardless of sex. In conclusion, female tail meat provided greater yield and lipid content than males, and this result was statistically significant. These findings can help producers and consumers alike, better understand yield, quality, and nutritional quality of Pantanal caiman meat.

Kozłowski, H.N., Lai, E.T.L., Havugimana, P.C., White, C., Emili, A., Sakac, D., Binnington, B., Neschadim, A., McCarthy, S.D.S. and Branch, D.R. (2016). Extracellular histones identified in crocodile blood inhibit in-vitro HIV-1 infection. *AIDS* 30(13): 2043-2052.

Abstract: It has been reported that crocodile blood contains potent antibacterial and antiviral properties. However, its effects on HIV-1 infection remain unknown. We obtained blood from saltwater crocodiles to examine whether serum or plasma could inhibit HIV-1 infection. We purified plasma fractions then used liquid chromatography-mass spectrometry to identify the inhibitory protein factor(s). We then analyzed the ability of recombinant proteins to recapitulate HIV-1 inhibition and determine their mechanism of action. *Crocodylus porosus* plasma was tested for inhibition of Jurkat T-cell HIV-1 infection. Inhibitor(s) were purified by reverse-phase chromatography then identified by protein liquid chromatography-mass spectrometry. Anti-HIV-1 activity of purified plasma or recombinant proteins were measured by p24 enzyme-linked immunosorbent assay and luciferase readouts, and mechanism of action was determined by measuring HIV-1 RNA, cDNA and transcription (using IG5 cells). Crocodile plasma contains potent inhibitors of HIV-1 infection, which were identified as histones. Recombinant human histones H1 and H2A significantly reduced HIV-1 infection (IC₅₀ of 0.79 and 0.45 μmol/l, respectively), whereas H4 enhanced JR-FL luciferase activity. The inhibitory effects of crocodile plasma, recombinant H1 or recombinant H2A on HIV-1 infection were during or post-viral transcription. Circulating histones in crocodile blood, possibly released by neutrophil extracellular traps, are significant inhibitors of HIV-1 infection *in-vitro*. Extracellular recombinant histones have different effects on HIV-1 transcription and protein expression and are downregulated in HIV-1 patients. Circulating histones may be a novel resistance factor during HIV-1 infection, and peptide versions should be explored as future HIV-1 therapeutics that modulate viral transcription.

De Carvalho, T.F., Tinoco, H.P., Malta, M.C.C., Pessanha, A.T., Matos, T.T.S., Rocha, C.E., Gomes, P.P.R., Garcia, L.N.N., Frois, D.N., Pereira, M.P.M., Johann, S., Rosa, C.A., Da Paixao, T.A. and

Santos, R.L. (2016). Systemic infection by *Spencermartinsiella* sp. in a Nile crocodile (*Crocodylus niloticus*). *Braz. J. Vet. Res. Anim. Sci.* 53(3): 1-7.

Abstract: A male adult crocodile (*Crocodylus niloticus*) was diagnosed with systemic yeast infection. Histologically, there were extensive areas of necrosis in the lung, which were associated with a diffuse severe lympho-plasma-histiocytic inflammatory infiltrate, with numerous multinucleated giant cells, and myriads of intralesional pseudo-hyphae and yeast like organisms within distended foveolae. Necrotic foci were also observed in the mucosa of the digestive tract, trachea, tunica intima of arteries, liver, and heart, with a marked inflammatory lympho-histiocytic infiltrate, with large numbers of epithelioid macrophages and giant cells, and intralesional and intravascular pseudo-hyphae and yeast-like organisms. Oval yeast structures with 4 to 6 μm in diameter and 5 to 8 μm thick parallel-walled pseudo-hyphae were observed in PAS or GMS stained sections. PCR with DNA template extracted from paraffin embedded tissues amplified the D1/D2 domains of the large subunit rRNA gene, which was sequenced and found to be identical to sequences of a new species, isolated from rotting wood in Brazil, of the genus *Spencermartinsiella*, which its closest relative is *Spencermartinsiella cellulosicola*.

Poapolathep, S., Giorgi, M., Hantrakul, S., Klangkaew, N., Sanyathitiseree, P. and Poapolathep, A. (2017). Pharmacokinetics of marbofloxacin in freshwater crocodiles (*Crocodylus siamensis*) after intravenous and intramuscular administration. *J. vet. Pharmacol. Therap.* 40: 57-61.

Abstract: To evaluate the fate and disposition of marbofloxacin (MBF) in freshwater crocodiles (*Crocodylus siamensis*), MBF was administered either intravenously (i.v.) or intramuscularly (i.m.) at a dosage of 2.0 mg/kg body weight. The concentrations of MBF in plasma were measured using high-performance liquid chromatography equipped with a fluorescence detector. The concentrations of MBF in the plasma were measurable up to 144 h after i.v. and i.m. administration. After the first 45 min, the mean pharmacokinetic profiles produced by the two administration routes were almost identical. No statistically significant differences in the pharmacokinetic parameters between the groups were observed. The half-life was long (about 2.5 days), the volume of distribution was large (about 1.44 L/kg), λ_z was small (0.01 h⁻¹), and the clearance was slow (22.6 mL/h/kg). The absolute i.m. bioavailability (F%) was 105.36%. The dose of MBF administered in this study seems to produce appropriate PK-PD parameters that predict antibacterial success for disease caused by susceptible bacteria. More studies are warranted to evaluate the likely residues after administration of multiple doses.

Zheng, J., Zeng, J., Guo, G., Jiang, J., Yang, N., Wang, P., Zhang, L. and Wang, Y. (2017). An investigation of sudden death in farmed infant Siamese crocodiles during winter and spring in Hainan, China. *Indian Journal of Animal Research* (doi: <http://dx.doi.org/10.18805/ijar.v0i0F.6999>).

Abstract: Sudden deaths without any specific clinical sign occurred among one year old young ones on Siamese crocodile (*Crocodylus siamensis*) farms in Hainan province, China, during winter and spring in 2012-2013. The autopsy showed that the internal organs seemed normal except full of clear ascites in abdominal cavity. There were total 6 bacterial species isolated, *Edwardsiella tarda*, *Proteus penneri*, *Proteus vulgaris*, *Enterobacter asburiae*, *Citrobacter freundii* and *Micrococcus luteus* from either the heart bloods or the spleens and livers. The virulence of isolated strains was tested by intraperitoneal inoculation in female Chinese edible frogs (*Rana rugulosa* or *Hoplobatrachus rugulosus*). The 6-day mortality revealed that *E. tarda* was the most pathogenic, whereas *P. penneri* and *E. asburiae* exhibited no infection at all, and the rest demonstrated similar inconspicuous toxicity. All these findings

suggest that *E. tarda* would be the principal suspect causing death of Siamese crocodile. The sensitivities of the *E. tarda* isolates were tested against 20 kinds of antimicrobial drugs. The results showed that ceftazidime, norfloxacin and amikacin were highly sensitive to the isolates of *E. tarda*.

Poudel, A., Savari, O., Tekin, Z. and Hara, M. (2016). Comparative analysis of islet development. Pp. 49-63 in *Pancreatic Islet Biology*, ed. by A.A. Hardikar. Springer International Publishing.

Abstract: The pancreatic islet plays a critical role in glucose homeostasis. The islet is a highly vascularized micro-organ embedded in the exocrine pancreas, which mainly consists of endocrine hormone-secreting cells: beta cells (insulin), alpha cells (glucagon), delta cells (somatostatin), pancreatic polypeptide (PP) cells, and epsilon cells (ghrelin). In this chapter, we review fetal endocrine pancreatic cell development in various species, providing a glimpse of the evolutionary changes followed by a molecular hierarchy of genes involved in pancreas development and a model of islet formation that recent technological advances have made possible. Considering current concerns, species differences between humans and rodents will be discussed in detail.

Li, H.-L., Deng, Y.-T., Zhang, Z.-R., Fu, Q.-R., Zheng, Y.-H., Cao, X.-M., Nie, J., Fu, L.-W., Chen, L.-P., Xiong, Y.-X., Shen, D.-Y., and Chen, Q.-X. (2017). Evaluation of effectiveness in a novel wound healing ointment - crocodile oil burn ointment. *African Journal of Traditional, Complementary & Alternative Medicines* 14(1): 62-72.

Abstract: Crocodile oil and its products are used as ointments for burns and scalds in traditional medicines. A new ointment formulation - crocodile oil burn ointment (COBO) was developed to provide more efficient wound healing activity. The purpose of the study was to evaluate the burn healing efficacy of this new formulation by employing deep second-degree burns in a Wistar rat model. The analgesic and anti-inflammatory activities of COBO were also studied to provide some evidences for its further use. Materials and methods: The wound healing potential of this formulation was evaluated by employing a deep second-degree burn rat model and the efficiency was comparatively assessed against a reference ointment - (1% wt/wt) silver sulfadiazine (SSD). After 28 days, the animals were euthanized and the wounds were removed for transversal and longitudinal histological studies. Acetic acid-induced writhing in mice was used to evaluate the analgesic activity and its anti-inflammatory activity was observed in xylene-induced edema in mice. Results: COBO enhanced the burn wound healing (20.5 ± 1.3 d) as indicated by significant decrease in wound closure time compared with the burn control (25.0 ± 2.16 d) ($P < 0.01$). Hair follicles played an importance role in the physiological functions of the skin, and their growth in the wound could be revealed for the skin regeneration situation. Histological results showed that the hair follicles were well-distributed in the post-burn skin of COBO treatment group, and the amounts of total, active, primary and secondary hair follicles in post-burn 28-day skin of COBO treatment groups were more than those in burn control and SSD groups. On the other hand, the analgesic and anti-inflammatory activity of COBO were much better than those of control group, while they were very close to those of moist exposed burn ointment (MEBO). Conclusions: COBO accelerated wound closure, reduced inflammation, and had analgesic effects compared with SSD in deep second degree rat burn model. These findings suggest that COBO would be a potential therapy for treating human burns.

Charruau, P., Perez-Flores, J., Cedeño-Vázquez, J.R., Gonzalez-Solis, D., González-Desales, G.A., Monroy-Vilchis, O. and Desales-Lara, M.A. (2016). Occurrence of *Amblyomma dissimile* on wild crocodylians in southern Mexico. *Diseases of Aquatic Organisms* 121(2): 167-171.

Abstract: Ticks are common ectoparasites of amphibians and reptiles but very few reports of such parasites on crocodylians exist worldwide. Herein, we report the first detailed observations of *Amblyomma dissimile* Koch, 1844 on the 3 crocodylian species present in Mexico, with the first report of tick parasitism on *Crocodylus acutus* and the second on *Caiman crocodilus chiapasius*. This is also the first report of *A. dissimile* in the state of Quintana Roo. Proportions of infested individuals found in this study ranged from 0.51 to 1.96%, suggesting that tick parasitism in crocodylians is likely opportunistic and occurs when individuals leave the water for terrestrial activities. Tick parasitism does not represent a major threat to crocodylians. The increasing habitat destruction/fragmentation and cattle expansion in southeastern Mexico, however, could increase tick populations and trigger tick parasitism and tick-borne diseases in herpetofauna and other vertebrates.

Leardi, J.M., Pol, D. and Clark, J.M. (2017). Detailed anatomy of the braincase of *Macelognathus vagans* Marsh, 1884 (Archosauria, Crocodylomorpha) using high resolution tomography and new insights on basal crocodylomorph phylogeny. *PeerJ* 5: e2801.

Abstract: *Macelognathus vagans* Marsh, 1884 from the Late Jurassic Morrison Fm. of Wyoming was originally described as a dinosaur by Marsh and in 1971 Ostrom suggested crocodylian affinities. In 2005, Göhlich and collaborators identified new material of this species from Colorado as a basal crocodylomorph. However, a partial skull found in association with mandibular and postcranial remains was not described. Due to the small size and delicate structures within the braincase, micro CT studies were performed on this specimen. The new anatomical information was incorporated in a phylogenetic dataset, expanding both character and taxon sampling. This new material reinforces the non-crocodyliform crocodylomorph affinities of *Macelognathus* as it bears a large otic aperture, unfused frontals and lacks ornamentation on the dorsal cranial bones. The internal structures also support these affinities as this specimen bears traits (ie heavily pneumatized and expanded basisphenoid; the presence of additional pneumatic features on the braincase; and the occipital-quadrato contact) not present in most basal crocodylomorphs. Furthermore, the presence of a wide supraoccipital and a cranioquadrato passage are traits shared with *Almadasuchus* from the early Late Jurassic of Argentina. *Macelognathus* was recovered as one of the closest relatives of crocodyliforms, forming a clade (Hallopodidae) with two other Late Jurassic taxa (*Almadasuchus* and *Hallopus*). The clade formed by *Almadasuchus* + *Hallopus* + *Macelognathus*, the Hallopodidae, is characterized by a higher degree of suturing of the braincase, posteriorly closed otic aperture (paralleled in mesoeucrocodylians) and cursorial adaptations. Also, the phylogenetic position of this lineage of derived crocodylomorphs as the sister group of Crocodyliformes implies a large amount of unsampled record (ghost lineage), at least 50 million years.

López González, E.C., Larriera, A., Siroski, P.A. and Poletta, G.L. (2017). Micronuclei and other nuclear abnormalities on *Caiman latirostris* (Broad-snouted caiman) hatchlings after embryonic exposure to different pesticide formulations. *Ecotoxicol. Environ. Saf.* 136: 84-91.

Abstract: This study aimed to evaluate the embryotoxicity and genotoxicity of pesticide commercial formulations widely used in soybean crops through the Micronucleus (MN) test and other Nuclear Abnormalities (NAs) in erythrocytes of broad-snouted caiman (*Caiman latirostris*), exposed by topical application through the eggshell. Embryos were exposed (during all incubation: 70 days approximately) to sub-lethal concentrations of two glyphosate formulations PanzerGold® (PANZ) and Roundup® Full II (RU) (500, 750, 1000 µg/egg); to the endosulfan (END) formulation Galgofan® and the cypermethrin (CYP) formulation Atanor® (1, 10, 100, and 1000 µg/egg). Blood samples were taken at the moment of hatching from the spinal vein for the application of the MN test and analysis of other NAs in erythrocytes, as markers of genotoxicity.

Results indicated a significant increase in the frequency of MN for PANZ1000, END 10, CYP 1 and CYP 100 ($p<0.05$), and in the frequency of other NAs including Buds: END 100, 1000 and CYP 10 ($p<0.05$), eccentric nuclei: END 1, 10, 1000, CYP 10, 100, 1000 ($p<0.01$) and END 100 ($p<0.05$), notched nuclei: END 1, 10 ($p<0.01$) and END 1000, CYP 10, 100, 1000 ($p<0.05$), and total nuclear abnormalities: END 1, 10, 100, 1000, CYP 10, 100 and 1000 ($p<0.01$), and the positive control (PC) ($p<0.05$), compared with the negative control. It was demonstrated a concentration dependent-effect in MN frequency only for PANZ ($R^2= 0.98$; $p<0.01$). Our study demonstrated that commercial formulations of pesticides induced genotoxic effects on *C. latirostris*, and NAs are a good indicator of genotoxicity in this species.

Latorre, M.A., Romito, M.L., Larriera, A., Poletta, G.L. and Siroski, P.A. (2016). Total and differential white blood cell counts in *Caiman latirostris* after *in ovo* and *in vivo* exposure to insecticides. *Journal of Immunotoxicology* 13(6): 903-908.

Abstract: Agricultural activities associated mainly with soybean crops affect the natural environment and wildlife by habitat destruction and the extensive use of agrochemicals. The aim of this study was to evaluate immunotoxic effects of the insecticides cypermethrin (CYP) and endosulfan (END) in *Caiman latirostris* analyzing total blood cell count (TWBC) and differential white blood cell count (DWBC) after *in ovo* and *in vivo* exposure. Eggs (*in ovo*) and hatchlings (*in vivo*) from nests harvested in natural habitats were artificially incubated and reared under controlled conditions in the Proyecto Yacare (Gob.Santa Fe/MUPCN) facilities. Exposure of embryos was performed by topication on the eggshell during the first stage of development. The treatments were distilled water (negative control; NC), ethanol (vehicle control; VC), four groups treated with different concentrations of CYP and four groups with END. *In vivo* exposure was performed by immersion; treatments were NC, VC, two groups exposed to CYP and two to END. After embryonic exposure to the insecticides, no differences were found in TWBC or DWBC among the neonates exposed to pesticides versus controls. In the *in vivo* scenario, similar results were obtained for TWBC, but DWBC data showed differences between NC hatchlings and CYP-1 hosts for heterophil, lymphocyte and monocyte levels, and between NC and END-1 hosts for lymphocyte and monocyte levels. Research on the effects of pesticide exposure on this species is of special interest not only to assess the impact on caiman populations, but also to further characterize the species as a potential sentinel of ecosystem health.

Burella, P.M., Simoniello, M.F. and Poletta, G.L. (2017). Evaluation of stage-dependent genotoxic effect of Roundup® (Glyphosate) on *Caiman latirostris* embryos. *Arch. Environ. Contam. Toxicol.* 72(1): 50-57.

Abstract: The agricultural expansion over the past decades, along with the associated increase in the use of pesticides, represents a high risk for many wild species. *Caiman latirostris* is a South American caiman with many features that make it highly vulnerable to pesticide exposure. Considering previous finding on the genotoxicity of the glyphosate-based formulation Roundup® in this species, the aim of this study was to evaluate the possible stage-dependent effect of this compound on *C. latirostris* embryos through the Comet assay (CA), micronuclei (MN), and nuclear abnormalities (NA) tests. Caiman eggs were exposed to three effective concentrations of Roundup® (750, 1250, 1750 $\mu\text{g}/\text{egg}$) in three different stages of the incubation period (total duration 70 ± 3 days at $31 \pm 2^\circ\text{C}$) of approximately 23 days each. A statistically significant difference in DNA damage determined by the CA was found between groups exposed to different concentrations of RU ($p<0.05$) and the negative control, but no difference was observed among the three stages of exposure within any treatment ($p>0.05$). There was no differences in the MN or NA frequencies between the different groups and the negative control ($p>0.05$), nor among the different stages within

each treatment. The results obtained in this study indicate that RU produce DNA damage on *C. latirostris* embryos independently of the developmental stage where the exposure occurs, implying an important risk for the species during all its period of development, when pesticide application is at maximum rate.

Ali, S.H., Rampersad-Ali, N. and Murphy, J.C. (2016). The discovery of Cuvier's Dwarf Caiman, *Paleosuchus palpebrosus* (Reptilia: Alligatoridae) in Trinidad. *Living World, Journal of the Trinidad and Tobago Field Naturalist's Club* 2016: 41-42.

Charrau, P., Pérez-Flores, J.S. and Labarre, D. (2017). Skin parasitism by *Paratrichosoma recurvum* in American crocodiles and its relation with environmental and biological factors. *Diseases of Aquatic Organisms* (doi: 10.3354/dao03071).

Abstract: *Paratrichosoma* spp. are capillarid worms that parasitize the abdominal skin of crocodiles. They are likely not a threat to crocodiles' health but they affect the commercial value of their skin. No successful treatment exists against this parasite, and present knowledge on its life cycle is limited. Herein we report new information on *Paratrichosoma recurvum* occurrence in wild American crocodiles *Crocodylus acutus* from Mexican Caribbean islands and its relation with environmental (water salinity, temperature, climatic events) and biological (body condition) factors. The percentage of parasitized crocodiles (30.3%) is among the highest recorded in wild crocodilian populations. Small (<40.8 cm total length [TL]) and large (>270 cm TL) crocodiles are less parasitized, probably due to the characteristics of their skin or of the parasite life cycle. Two individuals showed to have wormed naturally between their capture and recapture. Thorax-abdomen is the most parasitized body part of crocodiles. The infection risk is not associated with the sex of the crocodile, but there was a difference in the proportion of parasitized crocodiles between sites, which could be related to different environmental conditions. The body condition of a crocodile does not seem to be affected by the parasite. Climatic events and water temperature show no effect on the parasitism of crocodiles but salinity could have one. The infection of crocodiles by *P. recurvum* could depend more on an individual's behavior than on environmental conditions.

Constance, B.B. and Read, M.A. (2016). Chapter 33. Alligator and crocodile attacks. Pp. 686-692 in Auerbach's Wilderness Medicine, ed. by P.S. Auerbach, T.A. Cushing and N.S. Harris. Elsevier Health Sciences.

Sherwin, B.D., Mudge, J.F., Cañas-Carrell, J.E., Lanza, H.A., Rainwater, T.R., Platt, S.G., McMurry, S.T. and Anderson, T.A. (2016). Organochlorine pesticide residues in caudal scutes of Belize Morelet's crocodiles (*Crocodylus moreletii*). *Journal of Herpetology* 50(4): 552-558.

Abstract: Historically, Belize has used large quantities of organochlorine (OC) pesticides for agriculture and disease-vector control, yet few tools exist for noninvasive assessment of OC contaminant loads in Belize wildlife. Crocodile caudal (tail) scutes are clipped as a marking technique in wildlife management programs and may also have utility as a minimally invasive, nonlethal technique to assess contaminant burden. We collected caudal scutes from 96 Morelet's crocodiles in Belize over 2 yr to analyze scute tissues for OCs and to compare the observed OC concentrations among different scute tissue (fat, cartilage, and muscle) and among crocodiles of different age classes, sexes, and collection locations. Organochlorines of the DDT-type subclass were detected in 72 of 96 crocodiles, with methoxychlor detected in all 72 scutes containing OCs and p,p'-DDE, p,p'-DDT, and p,p'-DDD detected in 54, 47, and 20 scute samples, respectively. Organochlorines were more-frequently detected in scutes of adult crocodiles, but methoxychlor

was occasionally observed in juveniles at concentrations two orders of magnitude higher than observed in adults, suggesting maternal offload of methoxychlor to offspring, greater exposure through juvenile habitat and diet, or both. Organochlorines were detected in crocodiles from all sampling locations with more frequent and higher concentrations observed in crocodiles from lagoon habitats than from river habitats. This study demonstrates that scutes can be used as a nonlethal indicator of OCs present in Morelet's crocodiles, a finding which has applications for determining the trophic transfer of OC pesticides through tropical aquatic food webs and for estimating the continuing risk posed to crocodiles and other species by OC pesticides.

Bangma, J.T., Reiner, J.L., Jones, M., Lowers, R.H., Nilsen, F., Rainwater, T.R., Somerville, S., Guillette, L.J. and Bowden, J.A. (2017). Variation in perfluoroalkyl acids in the American alligator (*Alligator mississippiensis*) at Merritt Island National Wildlife Refuge. *Chemosphere* 166: 72-79.

Abstract: This study aimed to quantify concentrations of 15 perfluoroalkyl acids (PFAAs) in the plasma of American alligators (*Alligator mississippiensis*) inhabiting wetlands surrounding the Kennedy Space Center (KSC) in Florida, USA, located at Merritt Island National Wildlife Refuge (MINWR). Approximately 10 male and 10 female alligators ($n_{\text{total}} = 229$) were sampled each month during 2008 and 2009 to determine if seasonal or spatial trends existed with PFAA burden. PFOS represented the highest plasma burden (median 185 ng/g) and PFHxS the second highest (median 7.96 ng/g). While no significant seasonal trends were observed, unique spatial trends emerged. Many of the measured PFAAs co-varied strongly together and similar trends were observed for PFOS, PFDA, PFUnA, and PFDoA, as well as for PFOA, PFHxS, PFNA, PFTrA, and PFTA, suggesting more than one source of PFAAs at MINWR. Higher concentrations of PFOS and the PFAAs that co-varied with PFOS were collected from animals around sites that included the Shuttle Landing Facility (SLF) fire house and the Neil Armstrong Operations and Checkout (O&C) retention pond, while higher concentrations of PFOA and the PFAA that co-varied with PFOA were sampled from animals near the gun range and the old fire training facility. Sex-based differences and snout-vent length (SVL) correlations with PFAA burden were also investigated.

Keenan, S.W. and Summers Engel, A. (2017). Reconstructing diagenetic conditions of bone at the Gray Fossil Site, Tennessee, USA. *Palaeogeography, Palaeoclimatology, Palaeoecology* (<http://dx.doi.org/10.1016/j.palaeo.2017.01.037>).

Abstract: The preservation of vertebrate fossils requires the transformation of the original bioapatite mineral matrix, predominantly hydroxylapatite, into a thermodynamically more stable phase during diagenesis. Fossil bone geochemistry and structure provides a wealth of information on the conditions present during diagenesis, including site redox and pH, and about sedimentation rates during burial. The Gray Fossil Site preserves Late Neogene lacustrine deposits that serve as one of a few records of North American paleoclimate during a critical period of the Cenozoic. Despite having >40 vertebrate species and diverse plant assemblages, none of the prior studies used bone composition to understand what paleoenvironmental and diagenetic conditions must have been like to facilitate bone preservation and fossilization. *Alligator* sp. phalanges were examined using multiple geochemical tools, including Fourier-transform infrared spectroscopy, X-ray diffraction, and electron microprobe analyses. Evidence of bioerosion within thin cortical and isolated trabecular regions of bone suggests biotic utilization of organics (collagen) and/or the mineral(s) following deposition. Chemically zoned and laminated cortical *Alligator* bone is interpreted as preserved lines of arrested growth that developed during life, and supports previous interpretations of a regional seasonal climate. Lastly, the *Alligator* bones are mineralogically heterogeneous as fluorinated

and iron-containing apatite phases. Compared to modern bone, iron concentrations were high, at up to 3 wt%. The uptake of Fe by the *Alligator* bones examined in this study suggests that fluids present during diagenesis that led to bone preservation within the lacustrine sediment at the Gray Fossil Site were likely acidic, anoxic, and reducing.

Sandoval-Hernández, I., Duran-Apuy, A. and Quirós-Valerio, J. (2017). Activities that may influence the risk of crocodile (*Crocodylus acutus*: Reptilia: Crocodylidae) attack to humans in the Tempisque River area, Guanacaste, Costa Rica. *UNICIENCIA* 31(1): 13-22.

Abstract: One of the largest populations of crocodiles in Costa Rica is located at the Tempisque River. The species is threatened by habitat loss and poaching; but its populations have grown due to the protection given by law. The research was conducted in Guanacaste, Costa Rica. We made a characterization of popular knowledge, activities and perceptions of 374 residents of the study area. It was found that 55% believe that the crocodiles are abundant, 70% believe that populations have increased. The most dangerous activities done are recreation, swimming and fishing. There are significant differences between the proportions of response (X^2 : 71, $n = 10$, $p < 0.0001$). These activities are done daily (25%), weekly (30%), monthly (18%) and annually (10%). The risk of attack and the crocodile's density in the river are not recognized. Also, a lack of knowledge about the natural history and ecology of the species is shown. The reasons for attacks are: the aggressiveness of the animals and their density. There are differences in the responses on the reasons of the attacks (X^2 : 35, $n = 8$, $p < 0.0001$). Generally, the crocodile perception is unfavorable.

Melstrom, K.M. (2017). The relationship between diet and tooth complexity in living dentigerous saurians. *Journal of Morphology* (doi: 10.1002/jmor.20645).

Abstract: Living saurian reptiles exhibit a wide range of diets, from carnivores to strict herbivores. Previous research suggests that the tooth shape in some lizard clades correlates with diet, but this has not been tested using quantitative methods. I investigated the relationship between phenotypic tooth complexity and diet in living reptiles by examining the entire dentary tooth row in over 80 specimens comprising all major dentigerous saurian clades. I quantified dental complexity using orientation patch count rotated (OPCR), which discriminates diet in living and extinct mammals, where OPCR-values increase with the proportion of dietary plant matter. OPCR was calculated from high-resolution CT-scans, and I standardized OPCR-values by the total number of teeth to account for differences in tooth count across taxa. In contrast with extant mammals, there appears to be greater overlap in tooth complexity values across dietary groups because multicusped teeth characterize herbivores, omnivores, and insectivores, and because herbivorous skinks have relatively simple teeth. In particular, insectivorous lizards have dental complexities that are very similar to omnivores. Regardless, OPCR-values for animals that consume significant amounts of plant material are higher than those of carnivores, with herbivores having the highest average dental complexity. These results suggest reptilian tooth complexity is related to diet, similar to extinct and extant mammals, although phylogenetic history also plays a measurable role in dental complexity. This has implications for extinct amniotes that display a dramatic range of tooth morphologies, many with no modern analogs, which inhibits detailed dietary reconstructions. These data demonstrate that OPCR, when combined with additional morphological data, has the potential to be used to reconstruct the diet of extinct amniotes.

Gabel, K. and Gabel, M. (2017). Reproductive behavior of crocodile in Kotmi Sonar of Janjgir Champa (C.G.): A review paper. *International Journal of Science and Research* 6(1): 775-777.

Abstract: Crocodile has been categorized as a vulnerable species in the Red List of IUCN and is placed under schedule 1 of the Wild Life Protection Act, 1972. To protect crocodiles a Crocodile Conservation Park has been established by The Govt. of Chhattisgarh in Munda pond of Kotmi Sonar, District Janjgir - Champa, (C.G.) India. The physicochemical conditions of Munda pond provide an optimal conditions for, growth and vital activities for this species, *Crocodylus palustris*. The population of *C. palustris* is gradually increasing up to 378. The establishment of incubation centre, artificial hatchery and other technical facilities helps in the increasing population of *C. palustris*. The potentialities of Crocodile Park showed explored from point of view of knowledge, research activities and ecotourism as well.

Francis, M., Morel, P.C.H., Wilkinson, B.H.P. and Wester, T.J. (2017). Alginate increases water stability whilst maintaining diet digestibility in farmed saltwater crocodiles (*Crocodylus porosus*). Journal of Animal Science (doi:10.2527/jas.2016.0908).

Abstract: Saltwater crocodile (*Crocodylus porosus*) farming in Papua New Guinea is an emerging industry that supplies high-quality skins to the fashion industry. Crocodiles are semiaquatic and fed high-quality feed made from extruded animal byproducts (ie forced through a die at low pressure but not heat treated); however, it disintegrates on contact with water, and this leads to low utilization. Alginate is used extensively in food and pharmaceutical processes because it quickly forms a gel at room temperature; however, its effects on nutrient availability are equivocal, and its utility in crocodile diets is unknown. Extruded chicken byproduct-based crocodile diets were formulated (as-fed) with and without 1.7 and 3.3% Na alginate with either CaCl_2 or CaCO_3 to cross-link. After immersion in water at 30°C for 24 h, feed retained on a 0.5-mm screen was measured to determine DM retention (DMR). Regardless of inclusion level, alginate addition resulted in a 13-fold increase in DMR ($P < 0.05$) when CaCO_3 was used as a Ca^{+2} source; however, CaCl_2 use resulted in a much lower DMR. In a digestibility trial, 10 juvenile crocodiles (2.2 to 2.4 yr of age; 1.2 to 1.9 kg BW) were chosen from farm-raised stocks and fed extruded chicken byproduct-based diets with and without 1.5% Na alginate and 1.9% CaCO_3 . Animals fed 2% BW for 12 d and with excreta collected the last 5 d were slaughtered and had digesta sampled from the ileum. There were no differences in apparent ileal digestibilities of any AA, N (65.0 vs. 55.8%, SE= 12.2%), and OM (46.8 vs. 39.6%, SE= 12.8%) between diets with and without alginate, respectively. Total-tract digestibilities of OM (69.8 vs. 39.2%, SE= 9.1%) and energy (72.2 vs. 44.4%, SE= 8.3%), however, were greater in alginate-containing diets ($P < 0.05$). Our study showed that alginate addition to crocodile feed improved its stability in water and did not impair nutrient digestion. Application of these findings should greatly decrease feed wastage, which ultimately will benefit Papua New Guinea by simultaneously increasing economic returns and decreasing environmental impacts.

Vidal, D., Ortega, F., Gascó, F., Serrano-Martínez, A. and Sanz, J.L. (2017). The internal anatomy of titanosaur osteoderms from the Upper Cretaceous of Spain is compatible with a role in oogenesis. Scientific Reports 7: 42035.

Abstract: Dermal armor is one of the most intriguing features of some titanosaurs, the only sauropod dinosaurs that bore osteoderms. Some studies have revealed cavities of varying sizes inside some titanosaur osteoderms, interpreted as the result of bone remodeling for mineral mobilization. Several hypotheses have been proposed to explain the need for mineral mobilization in titanosaurs. However, rejecting those hypotheses was difficult with hitherto available evidence. The Upper Cretaceous site of Lo Hueco (Cuenca; Spain) has yielded one of the largest titanosaur osteoderm sets available. Observation of pre-existing breakages in the fossils and CT-scanning have revealed a predominant internal channel network for bulb and root osteoderms: most had a very compact spongy bone core,

perfused by large longitudinal branching neurovascular canals. Only few osteoderms from the same bed, which was deposited in a single and short event, had areas with low-density spongy bone. This void-like low-density bone is always associated with internal channels. It is also present in osteoderms of different sizes. This scenario is best explained when considering that Lo Hueco titanosaurs might have used their osteoderms as a source of calcium that was mobilized during oogenesis, although other hypotheses cannot be completely ruled out.

Pollard, A.S., Charlton, B.G., Hutchinson, J.R., Gustafsson, T., McGonnell, I.M., Timmons, J.A. and Pitsillides, A.A. (2017). Limb proportions show developmental plasticity in response to embryo movement. Scientific Reports 7: 41926.

Abstract: Animals have evolved limb proportions adapted to different environments, but it is not yet clear to what extent these proportions are directly influenced by the environment during prenatal development. The developing skeleton experiences mechanical loading resulting from embryo movement. We tested the hypothesis that environmentally-induced changes in prenatal movement influence embryonic limb growth to alter proportions. We show that incubation temperature influences motility and limb bone growth in West African Dwarf crocodiles, producing altered limb proportions which may, influence post-hatching performance. Pharmacological immobilisation of embryonic chickens revealed that altered motility, independent of temperature, may underpin this growth regulation. Use of the chick also allowed us to merge histological, immunochemical and cell proliferation labelling studies to evaluate changes in growth plate organisation, and unbiased array profiling to identify specific cellular and transcriptional targets of embryo movement. This disclosed that movement alters limb proportions and regulates chondrocyte proliferation in only specific growth plates. This selective targeting is related to intrinsic mTOR (mechanistic target of rapamycin) pathway activity in individual growth plates. Our findings provide new insights into how environmental factors can be integrated to influence cellular activity in growing bones and ultimately gross limb morphology, to generate phenotypic variation during prenatal development.

Turner, A.H., Pritchard, A.C. and Matzke, N.J. (2017). Empirical and Bayesian approaches to fossil-only divergence times: A study across three reptile clades. PLoS ONE 12(2): e0169885.

Abstract: Estimating divergence times on phylogenies is critical in paleontological and neontological studies. Chronostratigraphically-constrained fossils are the only direct evidence of absolute timing of species divergence. Strict temporal calibration of fossil-only phylogenies provides minimum divergence estimates, and various methods have been proposed to estimate divergences beyond these minimum values. We explore the utility of simultaneous estimation of tree topology and divergence times using BEAST tip-dating on datasets consisting only of fossils by using relaxed morphological clocks and birth-death tree priors that include serial sampling (BDSS) at a constant rate through time. We compare BEAST results to those from the traditional maximum parsimony (MP) and undated Bayesian inference (BI) methods. Three overlapping datasets were used that span 250 million years of archosauromorph evolution leading to crocodylians. The first dataset focuses on early Sauria (31 taxa, 240 chars.), the second on early Archosauria (76 taxa, 400 chars.) and the third on Crocodyliformes (101 taxa, 340 chars.). For each dataset three time-calibrated trees (timetrees) were calculated: a minimum-age timetree with node ages based on earliest occurrences in the fossil record; a 'smoothed' timetree using a range of time added to the root that is then averaged over zero-length internodes; and a tip-dated timetree. Comparisons within datasets show that the smoothed and tip-dated timetrees provide similar estimates. Only near the root node do BEAST estimates fall outside the smoothed timetree range. The BEAST model is not able to overcome limited sampling to correctly estimate divergences considerably older

than sampled fossil occurrence dates. Conversely, the smoothed timetrees consistently provide node-ages far older than the strict dates or BEAST estimates for morphologically conservative sister-taxa when they sit on long ghost lineages. In this latter case, the relaxed-clock model appears to be correctly moderating the node-age estimate based on the limited morphological divergence. Topologies are generally similar across analyses, but BEAST trees for crocodyliforms differ when clades are deeply nested but contain very old taxa. It appears that the constant-rate sampling assumption of the BDSS tree prior influences topology inference by disfavoring long, unsampled branches.

Gienger, C.M., Brien, M.L., Tracy, C.R., Manolis, S.C., Webb, G.J.W., Seymour, R.S. and Christian, K.A. (2017). Ontogenetic comparisons of standard metabolism in three species of crocodilians. *PLoS ONE* 12(2): e0171082.

Abstract: Due in part to their large size, aggressive temperament, and difficulty in handling, there are few physiological studies of adult crocodilians in the literature. As a result, studies comparing individuals across an ontogenetic series and comparisons among species are also lacking. We addressed this gap in knowledge by measuring standard metabolic rates (SMR) of three species of crocodilians (*Crocodylus porosus*, *C. johnstoni* and *Alligator mississippiensis*), and included individuals that ranged from 0.22 to 114 kg. Allometric scaling of SMR with body mass was similar among the species, but *C. porosus* had significantly higher SMR than did *C. johnstoni* or *A. mississippiensis*. Differences in SMR among species are potentially related to behavioural differences in levels of aggression; *C. porosus* are the most aggressive of the crocodilians measured, and have rates of standard metabolism that are approximately 36% higher at the grand mean body size than those measured for *C. johnstoni* or *A. mississippiensis*, which are among the least aggressive crocodilians.

Blumstein, D.T., Rangchi, T.N., Briggs, T., De Andrade, F.S. and Natterson-Horowitz, B. (2017). A systematic review of carrion eaters' adaptations to avoid sickness. *Journal of Wildlife Diseases* (doi: <http://dx.doi.org/10.7589/2016-07-162>).

Abstract: Species that scavenge on dead animals are exposed to enhanced disease risks. Eight hypotheses have been suggested to explain how scavengers avoid becoming sick from their diet. We conducted a systematic review of the literature and found correlative support for 4 of the 8 hypotheses but limited evidence of systematic studies of the hypotheses. We found no support that using urine to sterilize carcasses, having bald heads, eating rapidly, or food-washing behavior reduced disease risk in carrion eaters. With the exception of food washing, none of these hypotheses have been properly evaluated as an adaptation to avoid sickness from carrion. There is some support for having a specialized microbiome, having enhanced immunologic defenses, avoiding rotten food, and maintaining a low gastric pH to eliminate pathogens. Specialized immunologic defenses and having a low pH have the most support, but the diversity of mechanisms suggests that there is a great opportunity for even more detailed study. Increased knowledge in these mechanisms may provide biomimetic insights to help combat foodborne illnesses and enhance health.

Prajanban, B.O., Jangpromma, N., Araki, T. and Klaynongsruang, S. (2017). Antimicrobial effects of novel peptides cOT2 and sOT2 derived from *Crocodylus siamensis* and *Pelodiscus sinensis* ovotransferrins. *Biochim. Biophys. Acta.* 1859(5): 860-869.

Abstract: In light of the increasing threat of bacterial drug resistance to human health on a global scale, research and development of antimicrobial peptides as a novel class of potent antibiotics has gained considerable attention. The present study focuses on the structural evaluation and membrane interaction of two new cationic

antimicrobial peptides, cOT2 and sOT2, derived from Siamese crocodile (*Crocodylus siamensis*) and Chinese softshell turtle (*Pelodiscus sinensis*) ovotransferrins. Here, cOT1 (+3) and sOT1 (+3) were derived from reptile ovotransferrins by chromatographic purification and characterized by mass spectrometry and N-terminal sequencing analysis. In order to increase the antimicrobial efficacy, two novel peptides, cOT2 (+6) and sOT2 (+5), were designed and synthesized as "naturally-engineered" by primary amino acid sequence extension of cOT1 and sOT1, respectively. These rational designs of modified peptides were assayed in term of antimicrobial activity. These peptides display strong antimicrobial activity against several bacterial strains, eg *Vibrio cholerae*, *Bacillus megaterium*, and *Bacillus pumilus* TISTR 905, with MICs of 7-16.1 µM. In terms of structural conformation in mimic environments, CD spectroscopic analysis of the secondary peptides structure features revealed fairly the similarity on α -helical content with magainin II. Hence, the modes of actions have been speculated as toroidal and carpet model. Furthermore, the disruption of intact bacterial cells induced by cOT2 and sOT2 was investigated by SEM and AFM. The results provided evidence that cOT2 and sOT2 have the potential to cause different morphological changes of bacterial cells and that these effects can be enhanced by increasing the peptide concentration.

Vásquez, P., Vásquez, R., Matayoshi, P., Regal, F., Freitas, D. and Tovar, A. (2017). Estudio poblacional de las especies de crocodílicos en el Perú. Centro de Datos para la Conservación - Universidad Nacional Agraria La Molina/Convenio FDA - Ministerio del Ambiente: Lima. 97 pp.

Schwarz, D., Raddatz, M. and Wings, O. (2017). *Knoetschkesuchus langenbergensis* gen. nov. sp. nov., a new atoposaurid crocodyliiform from the Upper Jurassic Langenberg Quarry (Lower Saxony, northwestern Germany), and its relationships to *Theriosuchus*. *PLoS ONE* 12(2): e0160617.

Abstract: We report a new, small-sized atoposaurid crocodyliiform from the Upper Jurassic of Langenberg, Northeastern Germany. Atoposaurids are small-sized Mesozoic crocodyliiforms of mainly European distribution, which are considered to be phylogenetically close to the origin of Eusuchia. *Knoetschkesuchus langenbergensis* gen. nov. sp. nov. is represented by two well-preserved skulls and additional cranial and postcranial remains representing different ontogenetic stages. 3D reconstructions of a juvenile skull based on micro-computed tomography allow the most detailed description of cranial remains of any atoposaurid hitherto presented. Our new analysis contradicts previous preliminary assignment of the Langenberg atoposaurids to *Theriosuchus*. *Knoetschkesuchus* gen. nov. is characterized in particular by the presence of two dental morphotypes in the maxilla and dentary, slit-like secondary choanae within a narrow groove on the surface of the pterygoid, absence of lacrimonasal contact, presence of an antorbital foramen and an external mandibular fenestra, and proportional characters of the interorbital and intertemporal region. A similar combination of characters allows attribution of *Theriosuchus guimarotae* to *Knoetschkesuchus*, forming the new combination *Knoetschkesuchus guimarotae*. Our analysis provides an osteological basis for the separation of *Theriosuchus* and *Knoetschkesuchus* and helps further delineate generic differences in other closely related crocodylomorphs. Our phylogenetic analysis corroborates inclusion of *Knoetschkesuchus* into Atoposauridae and supports a position of Atoposauridae within Eusuchia.

Finger, J.W., Hamilton, M.T., Glenn, T.C. and Tuberville, T.D. (2017). Dietary selenomethionine administration in the American alligator (*Alligator mississippiensis*): hepatic and renal Se accumulation and its effects on growth and body condition. *Archives of Environmental Contamination and Toxicology* (doi:10.1007/s00244-017-0370-4).

Abstract: Selenium (Se) is an essential trace nutrient, but in excess,

it can induce toxicity. Incomplete combustion of coal produces coal combustion wastes, which are enriched in Se and often disposed of in aquatic basins. While a multitude of studies have investigated Se accumulation in vertebrates, few studies have examined its effects on longer-lived top trophic carnivores, such as the American alligator (*Alligator mississippiensis*). In this study, alligators were fed one of three Dietary Treatments: mice injected with water (controls) or water supplemented with 1000 or 2000 ppm selenomethionine (SeMet). Dietary Treatment significantly affected Se levels in both the liver ($p < 0.0001$; raw mean \pm SE: 1000 ppm group, 35.20 ± 6.32 ppm; 2000 ppm group, 49.97 ± 4.00 ppm) and kidney ($p < 0.0001$; raw mean \pm SE: 1000 ppm group, 101.60 ± 8.64 ppm; 2000 ppm, 96.38 ± 5.81 ppm), which were significantly higher in alligators fed SeMet than in controls. Post-treatment head length, used to control for size variation, was negatively related to both kidney ($p = 0.0142$) and liver ($p = 0.0010$) Se concentrations. Dietary treatment with SeMet significantly reduced body condition (1000 ppm, $p < 0.0029$; 2000 ppm, $p = 0.0075$), but it significantly increased growth (1000 ppm, $p < 0.0001$; 2000 ppm, $p = 0.0316$). Body condition and growth remained unchanged in control alligators ($p > 0.05$). Our results demonstrate alligators are capable of accumulating high levels of Se through trophic transfer. The positive effects of accumulation on growth may demonstrate Se essentiality, whereas the negative effects on condition may demonstrate toxicity. Accumulation also was associated with mortality, further demonstrating toxicity. Future studies should further investigate the physiological effects of Se accumulation in long-lived, top-trophic carnivores.

Rosell, J., Barragán, A., Carbonell, M.D. and Gerique, A.C. (2017). Septicaemia caused by *Citrobacter braakii* infection in a captive Nile crocodile (*Crocodylus niloticus*). Journal of Comparative Pathology (doi: 10.1016/j.jcpa.2016.11.163).

Combrink, X., Warner, J.K. and Downs, C.T. (2016). Nest predation and maternal care in the Nile crocodile (*Crocodylus niloticus*) at Lake St Lucia, South Africa. Behav. Processes 133: 31-36.

Abstract: Information regarding nest predation, nest abandonment, and maternal care in the Nile crocodile (*Crocodylus niloticus*) is largely restricted to anecdotal observations, and has not been studied quantitatively. Consequently, we investigated their nesting biology using camera-traps over four years at Lake St Lucia, South Africa. We obtained 4305 photographs (daylight captures= 90.1%, nocturnal= 9.9%) of 19 nest-guarding females. Of 19 monitored nests, 37% were raided by predators (mean= 12.1 ± 6.2 days subsequent to camera placement). All females returned to their nests following first predation, and on average returned three times between predator raids before nest abandonment. Water monitors (*Varanus niloticus*) and marsh mongoose (*Atilax paludinosus*) were the main egg predators. Nesting raids lasted 5.9 ± 1.6 days. Diurnally females were seldom on the nest, except during cool/cloudy weather or rain, preferring to guard from nearby shade. Females defended nests aggressively against non-human intruders. Five Nile crocodile females were observed liberating their hatchlings from nests. A detailed sequence of a mother excavating and transporting hatchlings revealed 13 excursions between nest and water over 32.5 h. This, after months of continual nest attendance and defence, is illustrative of the high level of maternal care in Nile crocodiles. Camera-trapping is an effective, non-invasive method for further crocodile nesting behaviour research.

Combrink, X., Warner, J.K. and Downs, C.T. (2017). Nest-site selection, nesting behaviour and spatial ecology of female Nile crocodiles (*Crocodylus niloticus*) in South Africa. Behav. Processes 135: 101-112.

Abstract: Nesting biology and ecology have been investigated for Nile crocodiles (*Crocodylus niloticus*), but information on behaviour and movement patterns of nesting females during nest

guarding is scant. Consequently, we investigated the home ranges, nest-site selection strategies, movement patterns, activity levels and nest fidelity of four nesting females using telemetry. Gravid females selected winter basking/breeding areas close (351 ± 2 m) to nest-sites. Mean home range and core-use areas of nesting females were 8539 ± 4752 m², and 4949 ± 3302 m² respectively. Mean home range (0.85 ha) was significantly smaller than those of non-nesting females (108.4 ha) during nesting season. Activity levels and mean daily movements while nesting were $8.1 \pm 2.5\%$ and 213 ± 64 m, respectively, and increased to $47.9 \pm 11.7\%$ and 2176 ± 708 m post-nesting. Overall levels of nest fidelity were $82.8 \pm 11.7\%$ (day $78.1 \pm 15.9\%$; night $87.3 \pm 7.8\%$). Highest nest fidelity recorded during incubation was 99.7% over 96 days. Telemetry data from nesting females were helpful for elucidating spatial and behavioural patterns during the nest guarding period, and provided novel insights into this biologically important event.

Rice, E.S., Kohno, S., St. John, J., Pham, S., Howard, J., Lareau, L., O'Connell, B.L., Hickey, G., Armstrong, J., Deran, A., Fiddes, I., Platt, R.N., Gresham, C., McCarthy, F., Kern, C., Haan, D., Phan, T., Schmidt, C., Sanford, J.R., Ray, D.A., Paten, B., Guillette, L.J. Jr. and Green, R.E. (2017). Improved genome assembly of American alligator genome reveals conserved architecture of estrogen signaling. Genome Res. (doi: 10.1101/gr.213595.116).

Abstract: The American alligator, *Alligator mississippiensis*, like all crocodilians, has temperature-dependent sex determination, in which the sex of an embryo is determined by the incubation temperature of the egg during a critical period of development. The lack of genetic differences between male and female alligators leaves open the question of how the genes responsible for sex determination and differentiation are regulated. One insight into this question comes from the fact that exposing an embryo incubated at male-producing temperature to estrogen causes it to develop ovaries. Because estrogen response elements are known to regulate genes over long distances, a contiguous genome assembly is crucial for predicting and understanding its impact. We present an improved assembly of the American alligator genome, scaffolded with in vitro proximity ligation (Chicago) data. We use this assembly to scaffold two other crocodilian genomes based on synteny. We perform RNA sequencing of tissues from American alligator embryos to find genes that are differentially expressed between embryos incubated at male- versus female-producing temperature. Finally, we use the improved contiguity of our assembly along with the current model of CTCF-mediated chromatin looping to predict regions of the genome likely to contain estrogen-responsive genes. We find that these regions are significantly enriched for genes with female-biased expression in developing gonads after the critical period during which sex is determined by incubation temperature. We thus conclude that estrogen signaling is a major driver of female-biased gene expression in the post-temperature sensitive period gonads.

Kim, K.T., Lee, S.H. and Kwak, D. (2016). Sudden death of a Siamese crocodile (*Crocodylus siamensis*) due to systemic aspergillosis. J. Vet. Med. Sci. 78(11): 1723-1726.

Abstract: A 4-year-old female Siamese crocodile (*Crocodylus siamensis*) housed at a zoo died without any prior clinical signs. During necropsy, numerous scattered, well-demarcated, yellowish-white, firm nodules were observed throughout the liver and lungs. Microscopic examination with periodic acid-Schiff staining revealed granulomatous inflammation in the liver and lungs. Liver granulomas were characterized by the presence of a connective tissue barrier and hyphae, and the centers of the granulomas showed signs of necrosis. Lung samples showed characteristics similar to those observed in the liver samples. The fungus was identified as *Aspergillus fumigatus* based on its appearance on Sabouraud dextrose agar, microscopic examination with lactophenol cotton blue staining and genetic sequencing. Therefore, zoo veterinarians should pay close attention to fungal infections in captive animals.

Tsai, S., Abdelhamid, A., Khan, M.K., Elkarargy, A., Widelitz, R.B., Chuong, C.M. and Wu, P. (2016). The molecular circuit regulating tooth development in crocodilians. *J. Dent. Res.* 95(13): 1501-1510.

Abstract: Alligators have robust regenerative potential for tooth renewal. In contrast, extant mammals can either renew their teeth once (diphyodont dentition, as found in humans) or not at all (monophyodont dentition, present in mice). Previously, the authors used multiple mitotic labeling to map putative stem cells in alligator dental laminae, which contain quiescent odontogenic progenitors. The authors demonstrated that alligator tooth cycle initiation is related to β -catenin/Wnt pathway activity in the dental lamina bulge. However, the molecular circuitry underlying the developmental progression of polyphyodont teeth remains elusive. Here, the authors used transcriptomic analyses to examine the additional molecular pathways related to the process of alligator tooth development. The authors collected juvenile alligator dental laminae at different developmental stages and performed RNA-seq. This data shows that Wnt, bone morphogenetic protein (BMP), and fibroblast growth factor (FGF) pathways are activated at the transition from pre-initiation stage (bud) to initiation stage (cap). Intriguingly, the activation of Wnt ligands, receptors and co-activators accompanies the inactivation of Wnt antagonists. In addition, the authors identified the molecular circuitry at different stages of tooth development. The authors conclude that multiple pathways are associated with specific stages of tooth development in the alligator. This data shows that Wnt pathway activation may play the most important role in the initiation of tooth development. This result may offer insight into ways to modulate the genetic controls involved in mammalian tooth renewal.

Galli, G.L., Crossley, J., Else, R.M., Dzialowski, E.M., Shiels, H.A. and Crossley, D.A. II. (2016). Developmental plasticity of mitochondrial function in American alligators, *Alligator mississippiensis*. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 311(6): R1164-R1172.

Abstract: The effect of hypoxia on cellular metabolism is well documented in adult vertebrates, but information is entirely lacking for embryonic organisms. The effect of hypoxia on embryonic physiology is particularly interesting, as metabolic responses during development may have life-long consequences, due to developmental plasticity. To this end, we investigated the effects of chronic developmental hypoxia on cardiac mitochondrial function in embryonic and juvenile American alligators (*Alligator mississippiensis*). Alligator eggs were incubated in 21% or 10% oxygen from 20 to 90% of embryonic development. Embryos were either harvested at 90% development or allowed to hatch and then reared in 21% oxygen for 3 yr. Ventricular mitochondria were isolated from embryonic/juvenile alligator hearts. Mitochondrial respiration and enzymatic activities of electron transport chain complexes were measured with a microrespirometer and spectrophotometer, respectively. Developmental hypoxia induced growth restriction and increased relative heart mass, and this phenotype persisted into juvenile life. Embryonic mitochondrial function was not affected by developmental hypoxia, but at the juvenile life stage, animals from hypoxic incubations had lower levels of Leak respiration and higher respiratory control ratios, which is indicative of enhanced mitochondrial efficiency. Our results suggest developmental hypoxia can have life-long consequences for alligator morphology and metabolic function. Further investigations are necessary to reveal the adaptive significance of the enhanced mitochondrial efficiency in the hypoxic phenotype.

Porter, W.R., Sedlmayr, J.C. and Witmer, L.M. (2016). Vascular patterns in the heads of crocodilians: blood vessels and sites of thermal exchange. *Journal of Anatomy* 229(6): 800-824.

Abstract: Extant crocodilians are a highly apomorphic archosaur clade that is ectothermic, yet often achieve large body sizes that

can be subject to higher heat loads. Therefore, the anatomical and physiological roles that blood vessels play in crocodilian thermoregulation need further investigation to better understand how crocodilians establish and maintain cephalic temperatures and regulate neurosensory tissue temperatures during basking and normal activities. The cephalic vascular anatomy of extant crocodilians, particularly American alligator (*Alligator mississippiensis*) was investigated using a differential-contrast, dual-vascular injection technique and high resolution X-ray micro-computed tomography (μ CT). Blood vessels were digitally isolated to create representations of vascular pathways. The specimens were then dissected to confirm CT results. Sites of thermal exchange, consisting of the oral, nasal, and orbital regions, were given special attention due to their role in evaporative cooling and cephalic thermoregulation in other diapsids. Blood vessels to and from sites of thermal exchange were studied to detect conserved vascular patterns and to assess their ability to deliver cooled blood to neurosensory tissues. Within the orbital region, both the arteries and veins demonstrated consistent branching patterns, with the supraorbital, infraorbital, and ophthalmotemporal vessels supplying and draining the orbit. The venous drainage of the orbital region showed connections to the dural sinuses via the orbital veins and cavernous sinus. The palatal region demonstrated a vast plexus that comprised both arteries and veins. The most direct route of venous drainage of the palatal plexus was through the palatomaxillary veins, essentially bypassing neurosensory tissues. Anastomotic connections with the nasal region, however, may provide an alternative route for palatal venous blood to reach neurosensory tissues. The nasal region in crocodilians is probably the most prominent site of thermal exchange, as it offers a substantial surface area and is completely surrounded by blood vessels. The venous drainage routes from the nasal region offer routes directly to the dural venous sinuses and the orbit, offering evidence of the potential to directly affect neurosensory tissue temperatures. The evolutionary history of crocodilians is complex, with large-bodied, terrestrial, and possibly endothermic taxa that may have had to deal with thermal loads that likely provided the anatomical building-blocks for such an extensive vascularization of sites of thermal exchange. A clear understanding of the physiological abilities and the role of blood vessels in the thermoregulation of crocodilians neurosensory tissues is not available but vascular anatomical patterns of crocodilian sites of thermal exchange indicate possible physiological abilities that may be more sophisticated than in other extant diapsids.

Cantu, T.M., Bowden, J.A., Scott, J., Pérez-Viscasillas, J.B., Huncik, K., Guille, M.P. and Guille, L.J. Jr. (2016). Alterations in eicosanoid composition during embryonic development in the chorioallantoic membrane of the American alligator (*Alligator mississippiensis*) and domestic chicken (*Gallus gallus*). *Gen. Comp. Endocrinol.* 238: 78-87.

Abstract: Eicosanoids are signaling lipids known to regulate several physiological processes in the mammalian placenta, including the initiation of parturition. Though all amniotes construct similar extraembryonic membranes during development, the composition and function of eicosanoids in extraembryonic membranes of oviparous reptiles is largely unknown. The majority of effort placed in eicosanoid investigations is typically targeted toward defining the role of specific compounds in disease etiology; however, comprehensive characterization of several pathways in eicosanoid synthesis during development is also needed to better understand the complex role of these lipids in comparative species. To this end, we have examined the chorioallantoic membrane (CAM) of the American alligator (*Alligator mississippiensis*) and domestic chicken (*Gallus gallus*) during development. Previously, our lab has demonstrated that the CAM of several oviparous species shared conserved steroidogenic activity, a feature originally attributed to mammalian amniotes. To further explore this, we have developed a liquid chromatography/tandem mass spectrometry method that is used here to quantify multiple eicosanoids in the CAM of two oviparous species at different stages of development. We identified

18 eicosanoids in the alligator CAM; the cyclooxygenase (COX) pathway showed the largest increase from early development to later development in the alligator CAM. Similarly, the chicken CAM had an increase in COX products and COX activity, which supports the LC-MS/MS analyses. Jointly, our findings indicate that the CAM tissue of an oviparous species is capable of eicosanoid synthesis, which expands our knowledge of placental evolution and introduces the possibility of future comparative models of placental function.

Oka, K., Kohno, S., Ohta, Y., Guillette, L.J. Jr., Iguchi, T. and Katsu, Y. (2016). Molecular cloning and characterization of the aryl hydrocarbon receptors and aryl hydrocarbon receptor nuclear translocators in the American alligator. *Gen. Comp. Endocrinol.* 238: 13-22.

Abstract: Aryl hydrocarbon receptor (AHR), a ligand-activated transcription factor, binds to a variety of chemical compounds including various environmental contaminants such as 2,3,7,8-tetrachlorodibenzo-p-dioxin. This receptor regulates expression of target genes through dimerization with the AHR nuclear translocator (ARNT). Since AHR-ARNT signaling pathways differ among species, characterization of AHR and ARNT is important to assess the effects of environmental contamination and for understanding the molecular mechanism underlying the intrinsic function. In this study, we isolated the cDNAs encoding three types of AHR and two types of ARNT from a reptile, the American alligator (*Alligator mississippiensis*). *In vitro* reporter gene assays showed that all complexes of alligator AHR-ARNT were able to activate ligand-dependent transcription on a xenobiotic response element. We found that AHR-ARNT complexes had higher sensitivities to a ligand than AHR-ARNT2 complexes. Alligator AHR1B showed the highest sensitivity in transcriptional activation induced by indigo when compared with AHR1A and AHR2. Taken together, our data revealed that all three alligator AHRs and two ARNTs were functional in the AHR signaling pathway with ligand-dependent and isoform-specific transactivations *in vitro*.

Hamlin, H.J., Edwards, T.M., McCoy, J., Cruze, L. and Guillette, L.J. Jr. (2016). Environmentally relevant concentrations of nitrate increase plasma testosterone concentrations in female American alligators (*Alligator mississippiensis*). *Gen. Comp. Endocrinol.* 238: 55-60.

Abstract: Anthropogenic nitrogen is a ubiquitous environmental contaminant that is contributing to the degradation of freshwater, estuarine, and coastal ecosystems worldwide. The effects of environmental nitrate, a principal form of nitrogen, on the health of aquatic life is of increasing concern. We exposed female American alligators to three concentrations of nitrate (0.7, 10 and 100 mg/L NO₃-N) for a duration of five weeks and five months from hatch. We assessed growth, plasma sex steroid and thyroid hormone concentrations, and transcription levels of key genes involved in steroidogenesis (StAR, 3 β -HSD, and P450scc) and hepatic clearance (Cyp1a, Cyp3a). Exposure to 100 mg/L NO₃-N for both five weeks and five months resulted in significantly increased plasma testosterone (T) concentrations compared with alligators in the reference treatment. No differences in 17 β -estradiol, progesterone, or thyroid hormones were observed, nor were there differences in alligator weight or the mRNA abundance of steroidogenic or hepatic genes. Plasma and urinary nitrate concentrations increased with increasing nitrate treatment levels, although relative plasma concentrations of nitrate were significantly lower in five month, versus five week old animals, possibly due to improved kidney function in older animals. These results indicate that environmentally relevant concentrations of nitrate can increase circulating concentrations of T in young female alligators.

McCoy, J.A., Hamlin, H.J., Thayer, L., Guillette, L.J. Jr. and Parrott, B.B. (2016). The influence of thermal signals during embryonic

development on intrasexual and sexually dimorphic gene expression and circulating steroid hormones in American alligator hatchlings (*Alligator mississippiensis*). *Gen. Comp. Endocrinol.* 238: 47-54.

Abstract: Incubation temperatures experienced by developing embryos exert powerful influences over gonadal sex determination and differentiation in many species. However, the molecular mechanisms controlling these impacts remain largely unknown. We utilize the American alligator to investigate the sensitivity of the reproductive system to thermal signals experienced during development and ask specifically whether individuals of the same sex, yet derived from different incubation temperatures display persistent variation in the expression patterns of sex biased transcripts and plasma sex hormones. Our analysis focuses on assessments of circulating sex steroids and transcript abundance in brain and gonad, two tissues that display sexually dimorphic gene expression and directly contribute to diverse sexually dimorphic phenotypes. Whereas our results identify sexually dimorphic patterns for several target gonadal genes in postnatal alligators, sex linked variation in circulating 17 β -estradiol, testosterone, and expression of two brain transcripts (aromatase and gonadotropin releasing hormone) was not observed. Regarding intrasexual variation, we found that AMH transcript abundance in hatchling testes is positively correlated with temperatures experienced during sexual differentiation. We also describe highly variable patterns of gene expression and circulating hormones within each sex that are not explained by the intensity of embryonic incubation temperatures. The magnitude of sexually dimorphic gene expression, however, is directly associated with temperature for SOX9 and AMH, two transcripts with upstream roles in Sertoli cell differentiation. Collectively, our findings regarding temperature linked variation provide new insights regarding the connections between embryonic environment and persistent impacts on sexual differentiation in a reptile species that displays temperature dependent sex determination.

Guillette, L.J. Jr., Parrott, B.B., Nilsson, E., Haque, M.M. and Skinner, M.K. (2016). Epigenetic programming alterations in alligators from environmentally contaminated lakes. *Gen. Comp. Endocrinol.* 238: 4-12.

Abstract: Previous studies examining the reproductive health of alligators in Florida lakes indicate that a variety of developmental and health impacts can be attributed to a combination of environmental quality and exposures to environmental contaminants. The majority of these environmental contaminants have been shown to disrupt normal endocrine signaling. The potential that these environmental conditions and contaminants may influence epigenetic status and correlate to the health abnormalities was investigated in the current study. The red blood cell (RBC) (erythrocyte) in the alligator is nucleated so was used as an easily purified marker cell to investigate epigenetic programming. RBCs were collected from adult male alligators captured at three sites in Florida, each characterized by varying degrees of contamination. While Lake Woodruff (WO) has remained relatively pristine, Lake Apopka (AP) and Merritt Island (MI) convey exposures to different suites of contaminants. DNA was isolated and methylated DNA immunoprecipitation (MeDIP) was used to isolate methylated DNA that was then analyzed in a competitive hybridization using a genome-wide alligator tiling array for a MeDIP-Chip analysis. Pairwise comparisons of alligators from AP and MI to WO revealed alterations in the DNA methylome. The AP vs. WO comparison identified 85 differential DNA methylation regions (DMRs) with ≥ 3 adjacent oligonucleotide tiling array probes and 15,451 DMRs with a single oligo probe analysis. The MI vs. WO comparison identified 75 DMRs with the ≥ 3 oligo probe and 17,411 DMRs with the single oligo probe analysis. There was negligible overlap between the DMRs identified in AP vs. WO and MI vs. WO comparisons. In both comparisons DMRs were primarily associated with CpG deserts which are regions of low CpG density (1-2CpG/100bp). Although the alligator genome is not fully annotated, gene associations were identified and correlated to major gene class functional categories and pathways of endocrine

relevance. Observations demonstrate that environmental quality may be associated with epigenetic programming and health status in the alligator. The epigenetic alterations may provide biomarkers to assess the environmental exposures and health impacts on these populations of alligators.

Doheny, B.M., Kohno, S., Parrott, B.B. and Guillette, L.J. Jr. (2016). *In ovo* treatment with an estrogen receptor alpha selective agonist causes precocious development of the female reproductive tract of the American alligator (*Alligator mississippiensis*). Gen. Comp. Endocrinol. 238: 96-104.

Abstract: The molecular signaling processes involved the differentiation of the Müllerian duct (MD) into the female reproductive tract, or oviduct, in non-mammalian vertebrates are not well understood. Studies in mammals and birds indicate that steroid hormones play a role in this process, as the embryonic MD has been shown to be vulnerable to exogenous estrogens and progestins and environmental endocrine disrupting contaminants. In a previous study, developmental treatment with an estrogen receptor α (ER α) agonist, 4,4',4''-(4-propyl-[1H]-pyrazole-1,3,5-triyl)trisphenol (PPT), induced significant enlargement of the MD in alligator embryos incubated at a male-producing temperature, which was not observed in embryos treated with an estrogen receptor β (ER β) agonist, 7-bromo-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-ol (WAY 200070), or with 17 β -estradiol (E2). In order to understand the role of estrogen signaling in female alligator oviduct development, we incubated eggs at a female-producing temperature and treated them with E2 and these ER selective agonists, PPT and WAY 200070, just prior to the thermosensitive window of sex determination. At stage 27, one stage prior to hatching, PPT induced significant enlargement of the MD with precocious development of secretory glands and connective tissue differentiation similar to characteristics of mature adult oviduct. PPT treatment *in ovo* increased mRNA expression of ER β , progesterone receptor, androgen receptor and insulin-like growth factor 1 in MD at stage 27, while expression of ER α was decreased. Neither WAY 200070 nor E2 treatment induced these effects seen in PPT-treated MD. The results of this study provide insight into the critical factors for healthy reproductive system formation in this sentinel species, although further investigation is needed to determine whether the observed phenomena are directly due to selective stimulation of ER α or related to some other aspect of PPT treatment.

Zhu, X., Zhang, S., Zhao, S., Zhang, R., Zhou, Y. and Wu, X. (2017). Insulin-like growth factor I (IGF-I) in Chinese alligator, *Alligator sinensis*: Molecular characterization, tissue distribution and mRNA expression changes during the active and hibernating periods. Gen. Comp. Endocrinol. 242: 74-82.

Abstract: The Chinese alligator *Alligator sinensis* is an endangered species endemic to China, up to date, little is known about the regulation of its growth and development. Insulin-like growth factor I (IGF-I) plays a vital role in regulating vertebrate growth and development. In this study, the full-length cDNA of IGF-I in Chinese alligator (caIGF-I) was obtained for the first time, it contains 890-bp nucleotides encoding a 153-amino acid precursor, the mature caIGF-I consists of 70 amino acids by cleaving the signal peptide and C-terminal extension (E domain). The caIGF-I contains all the features of IGF-I peptide with B, C, A, and D domains and the six conservative cysteine residues involved in the stable tertiary structure. Multiple alignment analysis showed that the amino acid sequence of caIGF-I shares high identity with American alligator *Alligator mississippiensis* (100%) and birds (95-97%). Phylogenetic tree analysis of the IGF-I amino acid sequences indicated that alligators cluster into the bird branch. Real-time quantitative PCR technique showed that caIGF-I is widely expressed in all the examined tissues with the highest expression level in liver, higher in pancreas and oviduct while lower in heart, spleen, lung, kidney, stomach, intestines, ovary and muscles. During hibernation, the

caIGF-I expression level decreased significantly in liver, pancreas, oviduct and kidney, while did not significantly change in heart, spleen, lung, stomach, small intestine, ovary and muscles. The mRNA expression changes during the two periods implicate that caIGF-I might play an important role in the regulation of feeding and growth in the Chinese alligator.

He, F., Zarfl, C., Bremerich, V., Henshaw, A., Darwall, W., Tockner, K. and Jähnig, S.C. (2017). Disappearing giants: a review of threats to freshwater megafauna. Wiley Interdisciplinary Reviews: Water (doi: 10.1002/wat2.1208).

Abstract: Charismatic megafauna species may act as both flagship and umbrella species. They influence local environments and biotas, determine related ecosystem processes and functions, and are associated with high levels of biodiversity. However, the intrinsic characteristics of megafauna species including long lifespan, large body size, sparseness and/or rarity, late maturity, and low fecundity, as well as high market value, make them very prone to extinction. Up to now, scientific interest and conservation efforts have mainly focused on terrestrial and marine megafauna, while freshwater species have received comparatively little attention, despite evidence suggesting that freshwaters are losing species faster than marine or terrestrial realms. The high susceptibility of freshwater megafauna to multiple threats, coupled with immense human pressure on freshwater ecosystems, places freshwater megafauna amongst the most threatened species globally. The main threats include overexploitation, dam construction, habitat degradation, pollution, and species invasion. These threats increase mortality, decrease productivity, and reduce fitness, causing the decline of populations and the extinction of freshwater megafauna species. Given the essential ecological and biological roles of freshwater megafauna, further research should focus on their distribution patterns, extinction risks, and population dynamics, thereby improving the knowledge base for conservation planning. Finally, freshwater megafauna-based conservation strategies may raise public awareness for freshwater conservation and therefore benefit a broader range of freshwater species and functions.

Chaeychomsri, W., Yamkong, S., Chaeychomsri, S. and Siruntawinetti, J. (2016). Effects of large volume crocodile blood collection on hematological values of Siamese crocodiles (*Crocodylus siamensis*). Journal of Advanced Agricultural Technologies 3(4): 252-257.

Abstract: Freeze-dried blood from *Crocodylus siamensis* is a natural product which can serve as food supplement. The present study aimed to develop the process for a large volume blood collection without killing animals in order to extend their life and keep them healthy. The process was performed from 20 captive breeding crocodiles. They were randomly divided into control and 3 experimental groups. Ten milliliters of blood were collected weekly from control group for 12 weeks. Group 1, samples were withdrawn 150 ml on week 1, 12 and collected 10 ml weekly on week 2-11. Group 2, samples were withdrawn 150 ml on week 1, 12 and collected 10 ml on week 4 and 8. Group 3, samples were withdrawn 150 ml on week 1 and 12. The results showed that there were no significant differences ($p < 0.05$) in hematological values among the blood samples taken from group 1, 2, 3 and those of the control group at all time intervals. The results of hematological values as well as the results obtained from the behavioral observation revealed that large volume blood collection up to 150 ml had no detrimental effect on crocodile health and behavior. The results from the present study offer a possible alternative to conventional way for commercial crocodile blood collection.

Balaguera-Reina, S.A., Espinosa-Blanco, A.S., Morales-Betancourt, M.A., Seijas, A.E., Lasso, C.A., Antelo, R. and Densmore III, L.D. (2017). Conservation status and regional habitat priorities for the Orinoco crocodile: Past, present, and future. PLoS ONE 12(2):

Abstract: Conservation of large predator species has historically been a challenge because they often overlap in resource utilization with humans; furthermore, there is a general lack of in-depth knowledge of their ecology and natural history. We assessed the conservation status of the Orinoco crocodile (*Crocodylus intermedius*), defining regional habitat priorities/crocodile conservation units (RHP/CCU) and regional research priorities (RRP) for this species. We also estimated a species distribution model (SDM) to define current suitable areas where the species might inhabit and/or that might be successfully colonized. The SDM area obtained with a suitable habitat probability ≥ 0.5 was 23,621 km². Out of 2562 km² are included within protected areas in both Colombia (1643 km²) and Venezuela (919 km²), which represents only 10.8% of *C. intermedius*' potential range. Areas such as Laguna de Chigüichigüe (flood plain lagoon) exhibited an increase in population abundance. In contrast, localities such as the Cojedes and Manapire Rivers reported a significant reduction in relative abundance values. In Colombia, disparity in previous survey methods prevented accurate estimation of population trends. Only one study in this country described an increase over a 13 years span in the Ele, Lipa, and Cravo Norte River populations based on nest surveys. We defined 34 critical areas (16 in Colombia, 17 in Venezuela, and one covering both countries) where we need to preserve/research/monitor and/or generate management actions, 10 RHP/CCU (6 from Venezuela and 4 from Colombia) and 24 RRP (11 from Venezuela, 12 from Colombia, and one in both countries). Caño Guaritico (Creek) and the Capanaparo River in Venezuela and the Ele, Lipa, Cravo Norte River System and the Guayabero River in Colombia were defined as areas with the most optimal conditions for long-term preservation and maintenance of *C. intermedius* populations. We conclude that the conservation status of this species is still critical, which implies the necessity to increase efforts to recover the species, especially in Colombia, to guarantee its survival as a structural and functional component of the ecosystems it inhabits.

Stegmann, G.F., Williams, C.J.A., Franklin, C., Wang, T. and Axelsson, M. (2017). Long-term surgical anaesthesia with isoflurane in human habituated Nile Crocodiles. Journal of the South African Veterinary Association (<https://doi.org/10.4102/jsava.v88i0.1451>).

Abstract: A suitable long-term anaesthetic technique was required for implantation of physiological sensors and telemetric devices in sub-adult Nile crocodiles (*Crocodylus niloticus*) to allow the collection of physiological data. Five Nile crocodiles with a median body mass of 24 kg were used. After manual capture, they were blindfolded and 0.2 mL (1 mg/mL) medetomidine was administered intramuscularly in four of the animals which had an estimated body mass between 20 kg and 30 kg. One crocodile with an estimated body mass of 50 kg received 0.5 mL. For induction, 5 mL propofol (10 mg/mL) was injected intravenously into the occipital sinus. Additional doses were given when required to ensure adequate anaesthesia. Anaesthesia was maintained with 1.5% isoflurane. Ventilation was controlled. Local anaesthesia was administered for surgical incision and external placement of the radio transmitter. Medetomidine was antagonised with atipamezole at the end of surgery. Median heart rate during surgery was 22 beats/min, at extubation 32 beats per min and 30 beats per min the following day at the same body temperature as under anaesthesia. Median body temperature of the animals increased from 27.3°C to 27.9°C during anaesthesia, as room temperature increased from 24.5°C to 29.0°C during surgery. Anaesthesia was successfully induced with intramuscular medetomidine and intravenous propofol and was maintained with isoflurane for the placement of telemetric implants. Intraoperative analgesia was supplemented with lidocaine infiltration. Perioperative physiological parameters remained stable and within acceptable clinical limits. Multiple factors appear to influence these variables during the recovery period, including residual anaesthetic effects, environmental temperature and physical activity.

Heil, O.P. and Langer, D.A. (2017). Identifying the luxury sustainability paradox: Three steps toward a solution. Pp. 125-144 in Sustainable Management of Luxury. Springer: Singapore.

Abstract: In this chapter, we argue about a paradox of sustainability in the context of luxury goods and brands: Intuitively, luxury brands should be more sustainable versus normal brands, since consumer's willingness to pay is high which should allow for highest standards in quality, including sustainability. However, many of the most expensive luxury products appear to exhibit limited sustainability. Examples include sports cars that typically are gas guzzlers, yachts that often carry only a few people but require a high amount of resources to be operated, or fur products that require animals to give their lives for. For example, the Hermes Birkin bag recently received a lot of negative media due to the alleged (mis-)treatment of crocodiles. Hence, albeit a bit counterintuitive, many luxurious products seem less sustainable than they could and probably should these days causing what we call the "Luxury Sustainability Paradox" to surface. In essence, luxury products' superiority appears to not span across all attributes and, paradoxically, to do so may be very challenging if not impossible. In short: being at the absolute top along certain luxury or performance attributes may, paradoxically, preclude top scores on sustainability.

Villegas, A., Mendoza, G.D., Arcos-García, J.L. and Reynoso, V.H. (2017). Nesting of Morelet's crocodile, *Crocodylus moreletii* (Dumeril and Bibron), in Los Tuxtlas, Mexico. Brazilian Journal of Biology (<http://dx.doi.org/10.1590/1519-6984.19015>).

Abstract: We evaluated the nesting by *Crocodylus moreletii* in Lago de Catemaco, Veracruz, southeastern, Mexico. During the nesting and hatching seasons, we searched for nests along the northern margins of the lake and small associated streams. We investigated egg mortality by weekly monitoring each of the nests found, recording sign of predation (tracks and holes dug into the nest) and the effect of water level fluctuations. We not found differences to nest between inland or flooded zones. However, we found that egg size varied among nests. In nests built inland, predation was the major cause of egg mortality whereas flooding resulted in more deaths of eggs in the flooding zone. Flooding killed 25% of eggs monitored in this study. We suggest that to increase nest success in the Morelet's crocodile it is necessary to promote conservation of nesting areas around the lake, recently occupied by urban or tourist developments.

Tian, L., Deng, Y-T., Dong, X., FAN, J-Y., Li, H-L., Ding, Y-M., Peng, Chen, Q-X. and Shen, D-Y. (2017). Siamese crocodile bile induces apoptosis in NCI-H1299 human non-small cell lung cancer cells via a mitochondria-mediated intrinsic pathway and inhibits tumorigenesis. Molecular Medicine Reports 15: 1727-1737.

Abstract: Non-small-cell lung cancer (NSCLC) is a widespread and particularly aggressive form of cancer. Patients with NSCLC and early metastases typically have poor prognosis, highlighting the critical need for additional drugs to improve disease outcome following surgical resection. The present study aimed to determine if Siamese crocodile bile (SCB) had an anti-cancer effect on NCI-H1299 human NSCLC cells. The inhibitory mechanism of SCB was examined in cell culture and nude mice. *In vitro* experimental results revealed that SCB inhibited the proliferation and colony-forming ability of NCI-H1299 cells by arresting cell cycle and inducing apoptosis. The loss of the mitochondrial membrane potential and the release of cytochrome c indicated that SCB treatment may lead to mitochondrial dysfunction in NCI-H1299 cells. At the molecular level, SCB altered the ratio of protein expression of Bax/Bcl-2 and activated associated caspases, suggesting that intrinsic pathway involvement in the SCB-induced apoptosis of NCI-H1299 cells. In the *in vivo* experiments, intraperitoneal injection of SCB for 4 weeks inhibited xenograft tumor growth by 46.8% without observable toxicity in nude mice. Immunohistochemistry analysis of proliferating cell

nuclear antigen and vascular endothelial growth factor also revealed that SCB inhibited cell proliferation and metastasis in NSCLC xenograft tumors. Overall, SCB exerted an anti-cancer effect on NCI-H1299 human NSCLC cells in vitro and in vivo and may have therapeutic potential for the treatment of human NSCLC.

Ballardini, N., Nopp, A., Hamsten, C., Vetander, M., Melén, E., Nilsson, C., Ollert, M., Flohr, C., Kuehn, A. and van Hage, M. (2017). Anaphylactic reactions to novel foods: Case report of a child with severe crocodile meat allergy. *Pediatrics* 2017: e20161404.

Abstract: Availability of “exotic” foods is steadily increasing. In this report, we describe the first case of anaphylaxis to crocodile meat. The patient was a 13-year-old boy with severe immunoglobulin E-mediated allergy to chicken meat. When tasting crocodile meat for the first time, he developed an anaphylactic reaction. Cross-reactivity between chicken and crocodile meat was suspected to have triggered this reaction. Basophil activation and immunoglobulin E testing confirmed the boy’s allergic reaction to crocodile meat proteins. Molecular analysis identified a crocodile α -parvalbumin, with extensive sequence homology to chicken α -parvalbumin, as the main cross-reactive allergen. We conclude that crocodile meat can be a potent food allergen and patients with allergy to chicken meat should be advised to avoid intake of meat from crocodile species. Both foods and people travel around the world and accessibility to exotic foods is steadily growing. As a result, novel allergic cross-reactivities are likely to become a challenge in the management of food allergy and, as our report illustrates, cross-reactivity has to be considered even between foods that might not intuitively be perceived as related.

Mauger, L.A., Velez, A., Cherkiss, M.S., Brien, M.L., Mazzotti, F.J. and Spotila, J.R. (2017). Conservation genetics of American crocodile, *Crocodylus acutus*, populations in Pacific Costa Rica. *Nature Conservation* 17: 1-17.

Abstract: Maintaining genetic diversity is crucial for the survival and management of threatened and endangered species. In this study, we analyzed genetic diversity and population genetic structure at neutral loci in American crocodiles, *Crocodylus acutus*, from several areas (Parque Nacional Marino Las Baulas, Parque Nacional Santa Rosa, Parque Nacional Palo Verde, Rio Tarcoles, and Osa Conservation Area) in Pacific Costa Rica. We genotyped 184 individuals at 9 microsatellite loci to describe the genetic diversity and conservation genetics between and among populations. No population was at Hardy-Weinberg Equilibrium (HWE) over all loci tested and a small to moderate amount of inbreeding was present. Populations along the Pacific coast had an average heterozygosity of 0.572 across all loci. All populations were significantly differentiated from each other with both FST and RST measures of population differentiation with a greater degree of molecular variance (81%) found within populations. Our results suggest *C. acutus* populations in Pacific Costa Rica were not panmictic with moderate levels of genetic diversity. An effective management plan that maintains the connectivity between clusters is critical to the success of *C. acutus* in Pacific Costa Rica.

Clarac, F., De Buffrénil, V., Brochu, C. and Cubo, J. (2017). The evolution of bone ornamentation in Pseudosuchia: morphological constraints versus ecological adaptation. *Biol. J. Linn. Soc.* (doi: <https://doi.org/10.1093/biolinnean/blw034>).

Abstract: Although frequent in vertebrates (eg crocodylians, stem-tetrapods, turtles), the adaptive significance of bone ornamentation, that is the honeycomb-like pattern of pits and ridges that occur on the surface of dermal bones, remains poorly understood. In order to help assess the evolutionary history and ecological correlates of this character, ornamentation was quantified in 69 extant and extinct Pseudosuchia (taxa more closely related to crocodiles than to

birds). This variable was related to the dominant habitat (terrestrial, amphibious, pelagic) of these taxa within a phylogenetic framework covering more than 250 Myr of evolution. The phylogenetic analyses reveal a significant correlation between the degree of bone ornamentation on the skull roof with lifestyle (terrestrial, amphibious, pelagic). A straightforward adaptive interpretation of these results is to be avoided because skull morphology has recently been shown to strongly influence local development of bone ornamentation in Crocodylia. Indeed, ornamentation in long-snouted amphibious forms scores low or nil values on the skull roof while scoring very high values on osteoderms. Our results also show that amphibious forms, whether marine or fluvial, have a high degree of ornamentation, whereas terrestrial and pelagic forms are either not ornamented or have a low level of ornamentation. It is hypothesized that the high development of ornamentation among semi-aquatic pseudosuchians has been positively selected because it improves basking efficiency in semi-aquatic ambush (ie poorly active) predators. This process would have occurred at the Triassic-Jurassic boundary.

Cidade, G.M., Solórzano, A., Rincón, A.D., Riff, D. and Hsiou, A. S. (2017). A new *Mourasuchus* (Alligatoroidea, Caimaninae) from the late Miocene of Venezuela, the phylogeny of Caimaninae and considerations on the feeding habits of *Mourasuchus*. *PeerJ* 5: e3056.

Abstract: *Mourasuchus* (Alligatoroidea, Caimaninae) is one of the most peculiar crocodyliforms due to the skull morphology consisting of a long, wide, dorsoventrally flat rostrum with long, slender mandibular rami. Despite these peculiarities, the systematics, phylogeny and feeding habits of this taxon have not been properly studied. In this paper, we describe a new species of the genus, *Mourasuchus pattersoni* sp. nov., from the late Miocene of the Urumaco Formation of Venezuela. The new species differs from the other *Mourasuchus* species in having a lateromedially wide, dorsoventrally high jugal bone and a circular incisive foramen, which both represent autapomorphies of the new taxon. Phylogenetically, *M. pattersoni* sp. nov. is more closely related to *M. amazonensis* and the specimen UFAC-1424 (formerly attributed to *M. nativus*) than to *M. arendsi* or *M. atopus*, whilst *Mourasuchus* is recovered once more as a monophyletic group. Furthermore, the cladistic analysis performed in this contribution offers a new phylogenetic assessment of Caimaninae, including many taxa described recently for the group. In this study, we also discuss the crocodylian diversity of the Urumaco Formation as well as how paleoenvironment may have contributed toward its evolution. In addition, we provide a discussion of the potential feeding habits of *Mourasuchus*. In this contribution, *Mourasuchus* is regarded as a taxon that likely preferred to prey on small animals. The unusual skull morphology of this group may have evolved to cover a large area with the rostrum, allowing for a more efficient prey capture, while the prey may have consisted predominantly of large amounts of small animals.

Pauwels, O.S.G., Toussaint Biyogho Bi Essono II, Carlino, P., Chirio, L., Huijbregts, B., Leuteritz, T.E.J., Rousseaux, D., Tobí, E., Vigna, C. and Van Neer, W. (2017). *Miscellanea Herpetologica Gabonica VII*. *Bulletin of the Chicago Herpetological Society* 52(1): 1-7.

We present new Gabonese locality records, ecological data or unpublished museum material for *Crocodylus niloticus* (Crocodylidae), *Trionyx triunguis* (Trionychidae), *Agama lebretoni* (Agamidae), *Hemidactylus fasciatus* and *H. mabouia* (Gekkonidae), *Gastropholis echinata* (Lacertidae), *Trachylepis albilabris* (Scincidae), *Afrotyphlops angolensis* (Typhlopidae), *Dipsadoboa viridis*, *Hapsidophrys smaragdinus*, *Toxicodryas pulverulenta* (Colubridae), *Naja melanoleuca* (Elapidae), *Lamprophis olivaceus*, *Psammophis cf. phillipsii* (Lamprophiidae), *Natriciteres fuliginoides* (Natricidae), *Causus lichtensteinii* and *C. maculatus* (Viperidae). We document predation cases by *Hapsidophrys smaragdinus*

on *Hemidactylus mabouia* and *Trachylepis albilabris*, by *Naja melanoleuca* on *Sclerophrys regularis* (Anura: Bufonidae) and by *Psammophis* cf. *phillipsii* on *Phrynobatrachus auritus* (Anura: Phrynobatrachidae), and consumption of *Arius latiscutatus* (Siluriformes: Ariidae) and *Tragelaphus spekii* (Cetartiodactyla: Bovidae) by *Crocodylus niloticus*. We add one, two and one snake species, respectively, to Estuaire, Moyen-Ogooué and Nyanga provinces' reptile lists. We add four reptile species to the list for Wonga-Wongué Presidential Reserve. We refer all records of *Agama agama* in Gabon to *A. picticauda*.

Azeez, O.I., Myburgh, J.G., Meintjes, R.A., Oosthuizen, M.C. and Chamunorwa, J.P. (2017). Histomorphology, ultrastructure and fatty acid composition of the adipose tissue in pansteatitis, the potentials in understanding the underlying mechanism and diagnosis of pansteatitis in the Nile crocodile. *Lipids in Health and Disease* 16: 47 (doi: 10.1186/s12944-016-0405-2).

Abstract: In an effort to characterize the fat body and other adipose tissue in the Nile crocodile and the effects of pansteatitis on the structure and composition of the adipose tissue, we evaluated the regional variation in structure and fatty acid composition of healthy farmed crocodiles and those affected by pansteatitis. Adipose tissue samples were collected from the subcutaneous, visceral and intramuscular fat and the abdominal fat body of 10 4-year-old juvenile crocodiles from Izinhaba Crocodile Farm, Pretoria, South Africa, while pansteatitis samples were collected from visceral and intramuscular fat of crocodiles that had died of pansteatitis at the Olifant River, Mpumalanga, also in South Africa. Histomorphology, ultrastructure and fatty acid composition by fatty acid methyl ester (FAME) analysis were conducted. Histological examination showed regional variations in the adipose tissue especially in the collagen content of the ECM, tissue perfusion and division into lobes and lobules by fibrous capsule. Considerable fibrosis, mononuclear cell infiltration especially by macrophages and lymphocytes and toxic changes in the nucleus were observed in the pansteatitis samples. Regional variation in lipid composition especially in Myristoleic (C14:1), Erucic acid (C22:1n9), and Docosadienoic acid (C22:2n6) was observed. Most of the saturated and trans fatty acids were found in significant quantities in the pansteatitis samples, but had very low levels of the cis fatty acid and the essential fatty acids with C18 backbone. This study demonstrates that there exists some regional variation in histomorphology and fatty acid composition in the healthy adipose tissue of the Nile crocodile. It also showed that pansteatitis in the Nile crocodile might have been triggered by sudden change in energy balance from consumption of dead fish; and probable exposure to toxic environmental conditions with the evidence of up scaled monounsaturated long chain fatty acids composition and toxic changes in the leucocytes observed in pansteatitis in the present study.

Richardin, P., Porcier, S., Ikram, S., Louarn, G. and Berthet, D. (2017). Cats, crocodiles, cattle, and more: Initial steps toward establishing a chronology of ancient Egyptian animal mummies. *Radiocarbon* (doi: <https://doi.org/10.1017/RDC.2016.102>).

Abstract: The ancient Egyptians mummified animals as part of cultic activity from the Late Period into the Roman era (7th century BC to the 4th century AD). Necropolises have provided millions of animal mummies, reflecting the religious fervor of Egyptians with regard to sacred animal cults during this period. Despite the number of sites containing mummies, and the number of mummies themselves, surprisingly little is known with regard to the nuances in the dating of the cults' popularity and activities. As part of a multidisciplinary project, we have conducted a series of radiocarbon dates based on a group of animal mummies from the collection of the Musée des Confluences in Lyon, France. Thus, 63 specimens of animal mummies and their wrappings were analyzed to provide a range of dates for this practice. Results show that some correlations can be made between the popularity of particular species and the time

period in which they were mummified. Monkeys and goats appear to have been among the first mummified species (from 800 BC), while antelopes appear to be a later addition to the corpus (30 BC to 4th century AD), thereby reflecting changes in thought processes, religious beliefs, and economic imperatives over time.

Russo, J., Mateus, O., Marzola, M. and Balbino, A. (2017). Two new ootaxa from the late Jurassic: The oldest record of crocodylomorph eggs, from the Lourinhã Formation, Portugal. *PLoS ONE* 12(3): e0171919.

Abstract: The Late Jurassic Lourinhã Formation is known for its abundant remains of dinosaurs, crocodylomorphs and other vertebrates. Among this record are 9 localities that have produced either dinosaur embryos, eggs or eggshell fragments. Herein, we describe and identify the first crocodyloid morphotype eggs and eggshells from the Lourinhã Formation, from five occurrences. One clutch from Cambelas, composed of 13 eggs, eggshell fragments from Casal da Rola and Peralta, one crushed egg and eggshells from Paimogo North, and four crushed eggs as well as eggshell fragments from Paimogo South. We observed and confirmed diagnostic morphological characters for crocodyloid eggshells and which are consistent with a crocodylomorph affinity, such as the ellipsoidal shape, wedge-shaped shell units, triangular extinction under cross-polarized light, and tabular ultrastructure. This material is distinctive enough to propose two new ootaxa within the oofamily Krokolithidae, *Suchoolithus portucalensis*, oogen. and oosp. nov., for the material from Cambelas, the most complete clutch known for crocodyloid eggs, and *Krokolithes dinophilus*, oosp. nov., for the remaining material. These are the oldest crocodylomorph eggs known, extending the fossil record for this group to the Late Jurassic. Furthermore, except for the clutch from Cambelas, the material was found with theropod eggs and nests, in the other four occurrences, which seem to suggest some form of biological relationship, still unclear at this point.

Van der Ploeg, J., Vermeersch, L., Rodriguez, D., Balbas, M. and Van Weerd, M. (2017). Establishing freshwater protected areas to protect biodiversity and improve food security in the Philippines. Pp. 31-42 in *Marine Protected Areas: Interactions with Fishery Livelihoods and Food Security*, ed. by L. Westlund, A. Charles, S.M. Garcia and J. Sanders. FAO and IUCN: Rome.

Crossley II, D.A., Ling, R., Nelson, D., Gillium, T., Conner, J., Hapgooda, J., Elsey, R.M. and Eme, J. (2017). Metabolic responses to chronic hypoxic incubation in embryonic American alligators (*Alligator mississippiensis*). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* 203: 77-82.

Abstract: Chronic hypoxic incubation is a common tool used to study developmental changes in reduced O₂ conditions, and it has been useful for identifying phenotypically plastic periods during ontogeny in laboratory settings. Reptilian embryos can be subjected to natural hypoxia due to nesting strategy, and recent studies have been important in establishing the phenotypic responses of several species to low developmental oxygen. In particular, the cardiovascular responses of American alligators (*Alligator mississippiensis*) to low developmental oxygen have been detailed, including a substantial cardiac enlargement that may support a higher mass specific metabolic rate. However, embryo mass-specific metabolic demands of hypoxic incubated alligator embryos have not been measured. In this study, alligator eggs were incubated in 10% O₂ (H) or 21% O₂ (N) environments for the entire course of embryonic development. Acute metabolic measures in 21% and 10% O₂ were taken for both H and N groups. We hypothesized that acute 10% O₂ exposure has no impact on metabolic rate of embryonic alligators, and that metabolic rate is unaffected by chronic hypoxic incubation when studied in embryos measured at 21% O₂. Our findings suggest phenotypic changes resulting from hypoxic incubation early in incubation, in

particular relative cardiac enlargement, enable embryonic alligators to sustain metabolic rate during acute hypoxic exposure.

Torralvo, K., Rabelo, R.M., Andrade, A. and Botero-Arias, R. (2017). Tool use by Amazonian capuchin monkeys during predation on caiman nests in a high-productivity forest. *Primates* (doi:10.1007/s10329-017-0603-1).

Abstract: Descriptions of new tool-use events are important for understanding how ecological context may drive the evolution of tool use among primate traditions. Here, we report a possible case of the first record of tool use by wild Amazonian capuchin monkeys (*Sapajus macrocephalus*). The record was made by a camera trap, while we were monitoring caiman nest predation at Mamirauá Reserve in Central Amazonia. An adult individual was registered in a bipedal posture, apparently using a branch as a shovel to dig eggs out of a nest. Caiman eggs are frequently depredated by opportunistic animals, such as the capuchin monkeys. As the Mamirauá Reserve is covered by a high-productivity forest, and caiman eggs are a high-quality food resource seasonally available on the ground, we believe that tool use by capuchins is more likely to be opportunity driven, rather than necessity driven, in our study site.

Polymeropoulos, E.T., Oelkrug, R., White, C.R. and Jastroch, M. (2017). Phylogenetic analysis of the allometry of metabolic rate and mitochondrial basal proton leak. *Journal of Thermal Biology* (<http://dx.doi.org/10.1016/j.jtherbio.2017.01.013>).

Abstract: The mitochondrial basal proton leak (MBPL) significantly contributes to high body temperatures (T_b) and basal metabolic rates (BMR) in endotherms. In endotherms at a given body mass (M), liver MBPL is higher than in ectotherms, supporting the notion that MBPL may partly explain the evolutionary increase in metabolic rate (MR), fostering endothermy. Here, we re-addressed this assumption by performing a phylogenetic analysis comparing all available liver MBPL data for ecto- and endotherms. While MBPL within endotherms negatively scales with M and BMR as shown previously, MBPL of ectotherms does not scale allometrically with M. Phylogenetic analysis reveals that this result is confounded by a positive scaling coefficient for MBPL with M for reptiles. Strikingly, the reptilian MBPL reaches endothermic levels above a body mass of 6.6 kg. Thus, phylogenetic scaling of MBPL supports previous claims of endotherm-like physiological characteristics in large reptiles. It appears that diversification of ancestral ectothermic tetrapods to a body mass of at least 6 kg may have been required to reach a MBPL that is beneficial for sustained high body temperatures. Novel MBPL data for the lesser hedgehog tenrec, a protoendothermic eutherian that displays reptile-like thermoregulatory patterns, fall within the endo- and ectothermic allometric regressions. Finally, we add additional evidence that within endotherms, phylogenetic differences in MR do not correlate with MBPL. Collectively, these data suggest that MBPL does not universally scale with metabolic rate in ecto- or endotherms and that an increasing MBPL with M may have played an important physiological role in the evolutionary history of reptilian thermoregulation.

Company, J. and Pereda-Suberbiola, X. (2017). Long bone histology of a eusuchian crocodyliform from the Upper Cretaceous of Spain: Implications for growth strategy in extinct crocodiles. *Cretaceous Research* 72: 1-7.

Abstract: The long bone histology of a Late Cretaceous eusuchian crocodyliform from the Iberian Peninsula reveals clear variations in the cortical structure which reflects changes in the speed of bone deposition (ie skeletal growth) related to ontogeny. The presence of secondary woven-fibred bone tissue in the perimedullar region of the cortex, and the existence of an external fundamental system in the most external periosteal cortex, which is a proxy for somatic maturity and effective cessation of growth, challenges the former

idea that the growth strategy of extinct crocodylians fit in the typical ectotherm condition, according to which these animals grew slowly during life under an indeterminate growth strategy. The analysed specimen lived for a minimum of 16 years and the highest preserved apposition rates took place in an advanced ontogenetic stage. The study suggests that the general aspects of the modern crocodylian growth strategy were already in place in some lineages by the Cretaceous.

Calverley, P.M. and Downs, C.T. (2017). The past and present nesting ecology of Nile crocodiles in Ndumo Game Reserve, South Africa: Reason for concern?. *Journal of Herpetology* 51(1): 19-26.

Abstract: Knowledge of the distribution and abundance of crocodilian nests and threats facing them is essential in calculating recruitment and determining population trends. We studied the nesting ecology of the Nile crocodiles (*Crocodylus niloticus*) at Ndumo Game Reserve (NGR) in northeastern KwaZulu-Natal, South Africa from 2009 to 2012. Nesting effort in NGR was comparable to other *C. niloticus* populations at 18-22%. Historical data suggest that high water levels completely inundate nesting sites within the reserve once every 10 yr, whereas predation destroys on average <20% of nests annually and can be primarily attributed to water monitor lizards (*Varanus niloticus*). The number of crocodile nests located in NGR remained similar from 1964-2012 despite significant increase in population size. Earlier stocking programs increased the number of *C. niloticus* in the greater Maputo/Phongola floodplain areas, but these numbers may not be sustainable because the majority of *C. niloticus* nests appear to be outside of the reserve in unprotected areas.

Cespedes, H.A., Zavala, K. and Opazo, J. (2017). Evolution of the $\alpha 2$ -adrenoreceptors in vertebrates: ADRA2D is absent in mammals and crocodiles. *bioRxiv* (doi: <https://doi.org/10.1101/106526>).

Abstract: Evolutionary studies of genes that have been functionally characterized and whose variation has been associated with pathological conditions represent an opportunity to understand the genetic basis of pathologies. $\alpha 2$ -adrenoreceptors (ADRA2) are a class of G protein-coupled receptors that regulate several physiological processes including blood pressure, platelet aggregation, insulin secretion, lipolysis, and neurotransmitter release. This gene family has been extensively studied from a molecular/physiological perspective, yet much less is known about its evolutionary history. Accordingly, the goal of this study was to investigate the evolutionary history of $\alpha 2$ -adrenoreceptors (ADRA2) in vertebrates. Our results show that in addition to the three well-recognized $\alpha 2$ -adrenoreceptor genes (ADRA2A, ADRA2B and ADRA2C), we recovered a clade that corresponds to the fourth member of the $\alpha 2$ -adrenoreceptor gene family (ADRA2D). We also recovered a clade that possesses two ADRA2 sequences found in two lamprey species. Furthermore, our results show that mammals and crocodiles are characterized by possessing three $\alpha 2$ -adrenoreceptor genes, whereas all other vertebrate groups possess the full repertoire of $\alpha 2$ -adrenoreceptor genes. Among vertebrates ADRA2D seems to be a dispensable gene, as it was lost two independent times during the evolutionary history of the group. Additionally, we found that most examined species possess the most common alleles described for humans; however, there are cases in which non-human mammals possess the alternative variant.

Caspermeyer, J. (2017). How turtles and crocodiles lost the parietal “third” eye and their differing color vision adaptations. *Mol. Biol. Evol.* 34(3): 776-777.

Carl, N. and Darlington, J. (2017). Extended parental care in Broad-snouted caiman *Caiman latirostris* (Daudin, 1801) (Crocodylia, Alligatoridae). *Herpetology Notes* 10: 127-129.

Alstrup, A.K.O. (2017). Var krokodillenes forfedre varmblodige? [Were crocodiles ancestors endothermic?]. Fauna - Tidsskrift for Norsk Zoologisk Forening (in Norwegian).

Abstract: There are several anatomical and physiological aspects that distinguish crocodiles from other reptiles - including having a four-chambered heart like mammals and birds. This has long puzzled researchers as living crocodilians differ from other ectothermic animals. The reason may be that the ancestors of crocodiles were active, endothermic animals and eventually became ectothermic when they became ambush predators living in water. This hypothesis was proposed by a research team more than a decade ago, and is supported by the presence of the foramen of Panizza between the right and left aortic arches. This foramen indicates a fundamental shift from complete blood separation (endothermic characteristics) to autonomic shunting (ectothermic characteristics). Furthermore, present-day lung function and bone structure indicate that crocodiles may have been endothermic. This article discusses the anatomical, physiological and palaeontological aspects of this hypothesis, but concludes that it will probably not be possible definitely to test the hypothesis.

Bailleul, A.M. and Holliday, C.M. (2017). Joint histology in *Alligator mississippiensis* challenges the identification of synovial joints in fossil archosaurs and inferences of cranial kinesis. Proceedings of the Royal Zoological Society B (doi: 10.1098/rspb.2017.0038).

Abstract: Archosaurs, like all vertebrates, have different types of joints that allow or restrict cranial kinesis, such as synovial joints and fibrous joints. In general, synovial joints are more kinetic than fibrous joints, because the former possess a fluid-filled cavity and articular cartilage that facilitate movement. Even though there is a considerable lack of data on the microstructure and the structure-function relationships in the joints of extant archosaurs, many functional inferences of cranial kinesis in fossil archosaurs have hinged on the assumption that elongated condylar joints are (i) synovial and/or (ii) kinetic. Cranial joint microstructure was investigated in an ontogenetic series of American alligators, *Alligator mississippiensis*. All the presumably synovial, condylar joints found within the head of the American alligator (the jaw joint, otic joint and laterosphenoid-postorbital (LS-PO) joint) were studied by means of paraffin histology and undecalcified histology paired with micro-computed tomography data to better visualize three-dimensional morphology. Results show that among the three condylar joints of *A. mississippiensis*, the jaw joint was synovial as expected, but the otherwise immobile otic and LS-PO joints lacked a synovial cavity. Therefore, condylar morphology does not always imply the presence of a synovial articulation nor mobility. These findings reveal an undocumented diversity in the joint structure of alligators and show that crocodilians and birds build novel, kinetic cranial joints differently. This complicates accurate identification of synovial joints and functional inferences of cranial kinesis in fossil archosaurs and tetrapods in general.

McCurry, M.R., Walmsley, C.W., Fitzgerald, E.M.G. and McHenry, C.R. (2017). The biomechanical consequences of longirostry in crocodilians and odontocetes. Journal of Biomechanics (doi: <http://dx.doi.org/10.1016/j.jbiomech.2017.03.003>).

Abstract: Unrelated clades of aquatic tetrapod have evolved a similar range of skull shapes, varying from longirostrine (elongate and narrow rostrum) to brevirostrine (short rostrum). However, it is unclear which aspects of organismal performance are associated with this convergence in the range of skull shapes. Furthermore, it is not known how fundamental anatomical differences between groups influence these relationships. Here we address this by examining the load bearing capabilities of the skulls of two of the most diverse groups of living aquatic tetrapod: crocodilians and odontocetes. We use finite element analysis to examine the abilities of different cranial morphologies to resist a range of biologically relevant feeding loads

including biting, shaking and twisting. The results allow for form/function relationships to be compared and contrasted between the two groups. We find that cranial shape has similar influences on performance during biting, shaking or twisting load cases at the anterior tooth positions, e.g. brevirostrine species experienced less strain than longirostrine species. The pattern of this form/function relationship is similar for both crocodilians and odontocetes, despite their fundamentally different anatomies. However, when loading teeth at the posterior end or middle of the tooth row the results do not follow the same pattern. Behavioural differences in bite location plays a key role in determining functional abilities in aquatic tetrapod taxa.

Submitted Publications

EARLIEST REPORT ON GALLOPING BY CROCODILIANS. Asymmetrical gaits, so-called “galloping” or “bounding” is seen in some crocodile species, most commonly in *Crocodylus johnstoni* (Webb and Gans 1982; Webb and Manolis 1989; Renous *et al.* 2002). Crocodilian galloping was first described by Cott (1961) in *C. niloticus*. Zug (1974) then analysed galloping in *C. porosus*, using a hatchling (45 cm TL) in captivity. Aoki (1987) reported the first galloping in *Osteolaemus tetraspis* in a captive environment and Whitaker and Andrews (1989) reported on more species, including *C. novaeguineae*, *C. palustris* and *Gavialis gangeticus*. Based on the anatomical differences between Crocodylidae and Alligatoridae, Aoki (1987) suggested that galloping is a characteristic of the crocodylid (including *Gavialis* as close relative of *Tomistoma*). This was later supported by further detailed analysis of crocodilian locomotion by Allen *et al.* (2015), despite a report on a little *Alligator mississippiensis* that “attempted to gallop” on a high-speed treadmill in a laboratory (Reilly and Elias 1998).

Here we introduce the earliest report of galloping, or technically more accurately described as “primitive ricochet jumping” by Bornhauser and Ziswiler (1983) in all crocodilian species. Motoda (1937) documented Kiyota’s “rare experience” in observing *C. porosus* gallop on a small island south of Malakal Island in Palau. Mr. Toshisaburo Kiyota was a local resident of the islands, being passionate on observing wild crocodiles since his settlement from Japan in 1910. He made a number of important field observations on *C. porosus*, which contributed to fauna research of Palau by Prof. Shigeru Motoda at the Palau Tropical Biological Research Center. In his observation, the crocodile was resting on a beach at low tide when Kiyota approached by canoe. As soon as the crocodile noticed Kiyota’s approach, it “stood up in a fright, bounded by the tail down the beach and jumped into the water in a matter of moment” (originally described in Japanese). Although the size of the crocodile was not known, it is likely to be a small individual as Kiyota noted that the crocodile was approximately 72 m away from the water’s edge and it was so fast that it reached the water within a few moments.

Crocodile galloping is a powerful but exhausting activity and it typically goes no more than 30-50 m (Grigg and Kirshner 2015) at a speed of up to 18 kilometres per hour (Webb and Gans 1982). Although the original description of Kiyota’s observation did not call it “galloping” or “asymmetrical gait”, it is worth noting that this is probably the earliest report on crocodilian’s bounding behaviour.

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THE CAIMAN SPIX SUBFAMILY BELONGS TO EVERYONE, AND OPENS A TALE OF COINCIDENCE. In their key to commercial ventral hides, King and Brazaitis (1971) said that if "double osteoderm buttons" are present in the midbelly region of a crocodilian's skin then it is *Melanosuchus*, *Paleosuchus* or *Caiman*. Note that their genus *Caiman* in 1971 was endemic to the New World and consisted of two or three living species depending on whether *Crocodilus yacare* Daudin is considered to be a species on its own (*Caiman yacare*), or alternatively classified as a subspecies of *Caiman crocodilus*. In all cases the modern species *Caiman latirostris* gets counted. Peripherally, according to the expert advice to CITES from King and Burke (1989), there are also the subspecies *C. c. fuscus*, possibly *C. c. chiapasius*, and definitely *C. c. apaporiensis*. Of course, all of these also have the double-button belly skin, as opposed to the single-button kind.

Everyone (possibly except Freiberg and Carvalho 1963) is completely satisfied that *Caiman fissipes* Spix, 1825, is a junior synonym of *Crocodilus latirostris* Daudin, 1801, although as noted in Ross (2005) there was no locality data for Daudin's name. In 1801 he merely distinguished his new *Crocodilus latirostris* as individually different from the common spectacled cayman of Paramaribo, Surinam, and also as explicitly different from D'Azara's long-snouted Paraguayan cayman (*Crocodilus yacare* Daudin, 1801).

Later it was not a Daudin (1801) name, but rather the slightly newer original combination *Caiman fissipes* Spix, 1825, that

Schmidt (1928) judiciously justified and individually and explicitly designated on purpose as the type-species of the genus-group name *Caiman* Spix, 1825. The Spix (1825) book has "Monachii" as its place of publication.

According to Hoogmoed and Gruber (1983) there were two (possibly more) syntypes of *Caiman fissipes*, of which only the skull and mandibles of a half-grown adult skeleton remain in the museum in Munich. This bony head of a single individual was collected by Dr. Johann Baptist Ritter von Spix in South America and then recently it was taxonomically recognized by Hoogmoed and Gruber (1983) as belonging to the iconic broad-snouted caiman species of the Uruguay region, and thus they designated this skeleton's head as the Spix's junior synonym's lectotype.

As the architect of the Caimaninae subfamily that is of direct application and usefulness to CITES for identifying ventral skins by their osteoderms, Huxley (1859a) recognized the Alligatoridae as a full family-level name that had the eponym *Alligator* genus in it (the Alligatorinae), as well as several other genera (the younger Caimaninae). The caymans subfamily consisted in 1859 of two endemic Latin American genera, which are today three (namely *Caiman*, *Melanosuchus* and *Paleosuchus*). The character that united these two or three genera into a subfamily-level group (that collectively could need a scientific name) was their remarkable possession of what is today called in the leather preparation industry as "double-button" belly skins (those with two pieces of dermal bone per large epithelial ventral scale).

The subgenus and the supergenus levels have fallen out of fashion in CITES regulated crocodilian systematics, and rather the hierarchy of subspecies, species, genus, subfamily, family, suborder and order is common today. Any distinctive group consisting of one or more genus-group name(s) can have the ending -inae suffixed onto the oldest applicable genus, and thus have the latter become transformed to newly denote a taxon that is higher than a genus (or supergenus), and lower than a full family.

The three Caimaninae genera and six species (*C. crocodilus*, *C. yacare*, *C. latirostris*, *M. niger*, and *P. trigonatus* and *P. palpebrosus*) all have double-button osteoderms of a special (see below) kind that is not found anywhere in the whole rest of the living Crocodylia, except for in and throughout the subfamily Caimaninae (in which in adults they always occur), and this fact was detailed not only by Huxley (1859a, b), but also by King and Brazaitis (1971), and not surprisingly Brochu (1999) independently (employing a vast array of diverse osteological characters in a cladistic analysis) also found and isolated this collective group (*Caiman*, *Melanosuchus* and *Paleosuchus*) as a definable and distinguishable subset of the alligators family, and in 1999 he named it the "Caimaninae" (verbatim, as detailed below).

It would be awkward to attribute the Caimaninae literally to Huxley (1859a) because his employment of the genus *Caiman* unfortunately followed Natterer into confusion and nomenclatural error. Awkwardly but historically understandably, Huxley's (1859a) two *Caiman* (sic) species are today in *Paleosuchus*. However, King and Brazaitis (1971) knew that the type-species of *Caiman* Spix, 1825, is *Caiman fissipes* Spix, 1825, and that it is today called *Caiman latirostris* (Daudin, 1801). Also, King and Brazaitis (1971) knew that *Paleosuchus* and *Melanosuchus* are younger (newer and thus junior) names in comparison with the older (and more eponymic to "cayman" or "caïman" as a genus) name *Caiman*. Thus, perhaps the Caimaninae that Huxley (1859a) discovered and distinguished as real could today be better called the Caimaninae King and Brazaitis, 1971. For example, on page 18 they say "caimans (*Caiman*, *Melanosuchus*, or *Paleosuchus*)" are for some purposes a very special case.

My objection to Caimaninae Brochu, 1999, is that his subfamily was not based on the lectotype of *Caiman fissipes* Spix (specimen

from southern Brazil and now in Munich), but rather that this new clade-name was anchored in the collection of all specimens reported in 1999 that had been identified to the satisfaction of Brochu as being the “common” (at Paramaribo) caiman (as opposed to the black spectacled caiman *Melanosuchus*, or the broad-snouted spectacled caiman *C. latirostris*, or either of the two smooth-fronted and remarkably unspectacled Caimaninae *Paleosuchus*), namely the spectacled *Caiman crocodilus* individual species whose type-locality is northeastern South America at Atlantic coastal Surinam. It is potentially significant that Daudin (1801, often called 1802) distinguished his *Crocodylus latirostris* as taxonomically separate from today’s *Caiman crocodilus* (Linné) species that Daudin knew (under a different old name) from a Surinamese example in the Paris collection (Ross 2005), namely *Crocodylus caiman* Daudin, 1801.

It was Schmidt (1928) who stabilized the genus *Caiman* and thereby created a meaningful eponym for the Caimaninae Schmidt, 1928, based on cranial characters. It is separately and additionally that I here today stress that some crown Eusuchia have single-button ventral scales, while in contrast the Caimaninae has the double-button condition, and the commercial Caimaninae skin always seems to have substantial amounts of dermal bone(s) in it.

The following quote describes the rectangular bipartite ventral osteoderms of the Caimaninae K.P. Schmidt, 1928, condition in Huxley’s (1859a) words. Each ventral right-angled rectangular and unequally double-osteodermed pair is “composed of two distinct pieces, an anterior and a posterior, which unite together by a transverse serrated suture. The anterior piece or ‘semi-scute’ may attain to three-quarters the length of the posterior, and it has exactly the same width. The anterior semi-scute bears the articular facet and the transverse pitted groove, whose posterior wall is just in front of its hinder edge, or in other words, of the suture, when the two semi-scutes are united” (page 23).

Huxley (1859b) employed the term moiety or moieties for the elements within these double-button osteoderm pairs, but some definitions insist that each of the two parts should be exactly half of the total (which is not true in Huxley’s findings). Thus the term “unequal moieties” is appropriate for his posterior piece being always larger than the anterior element of the pair. Similarly the term demiscute could rigidly mean a piece that is exactly half of the size of a whole scute, while in Huxley’s case there are “unequal demiscutes” in the genera belonging to the Caimaninae.

There is a segmentation series that is active along the whole length of the primitive crocodilian dorsal armor that is “vertebral” (meaning one transverse row of dorsal scales and single-button osteoderms per corresponding individual vertebral centrum of the neck and the backbone and the tail).

Not present today but ancestral in the Crocodylia is the protosuchian character of having “subdorsal” (meaning lateral and ventral only, and all flat) caudal osteoderms that were square in shape and each one’s length was exactly one-half of the length of its corresponding centrum and its dorsal armor row. There were two bones in the subdorsal caudal ossification field, and together they armored the space where a modern eusuchian crocodilian has one subdorsal caudal scale.

In the *Protosuchus* fossil there was a space of granular skin separating the vertebral dorsal ossification field from the demivertebral subdorsal field. This unossified break between the two kinds of tail scutes extended the whole caudal length in the distant past, and a proximal and abbreviated version of such an intervening horizontal space can be seen (apparently persisting vestigially) on some living specimens in some CITES taxa.

I do not yet understand the pavement of square osteoderms that collectively formed the distinctively shaped and positioned “floating” (unattached on all its edges) but solidly-bony plastron

structure located under the belly of *Protosuchus richardsoni*, but each of the included dermal bones appears to be very similar to the square-shaped and essentially flat surfaced osteodermal plates that armor the subdorsal caudal surfaces of the same early and somewhat doglike fossil.

The ancestral Protosuchia were terrestrial archosaurs whose tails were not designed for swimming. Thus there has been a big change in which the vertebral segmentation of the dorsal armor has been extended to the subdorsal caudal scales which are now the same vertebral length as their dorsal-armor counterpart. The derived eusuchian tail has “regular” (same length throughout each band) whorls of scales that encircle the tail on its vertical (not lengthwise) short-axis. Each whorl has parallel edges, and is pretty much parallel with all of the other whorls throughout the entire segmented length of the tail. Some of the scales within a transverse caudal-whorl or vertical band are dorsal, while others are subdorsal, and this modern arrangement produces the strong and efficient swimming instrument that enables the crocodile to dive deep in salt water, and to move so powerfully and dangerously fast when jaws-first ambush attacking terrestrial prey from the water.

The transformation from the old terrestrial tail to the new swimming propulsion design is not yet understood, and I think that it is merely a coincidence that when speaking of the subdorsal caudal scalation of the animal, *Protosuchus* had two osteoderms (one anterior, the other posterior) per vertebral segment, and something similar (again one anterior bone and a second bone posterior to it) happens in the Caimaninae. The difference is that the old scutes were all of the same size and were regularly square in their shape, while in contrast the new ones have the anterior rectangle always shorter than the posterior rectangle, and in all cases both of the two rectangular pieces themselves have the same breadth. Also, they together combine to collectively make a different but also right-angled rectangle.

In the plate of fossil scutes in Huxley (1859b), his Figure 8 (my Figure 1) is the correct right-angled rectangular shape and nature that to him indicated a caiman (Caimaninae) of some sort (and I agree). The fossil species *Crocodylus hastingsiae* was named after the person who collected the Huxley (1859b) Figure 8 on Plate 25 bone at Hordwell, and Huxley (1859a, b) had separately examined a *C. hastingsiae* skull which also looked to him to be an Alligatoridae species (the 4th lower tooth sticks up into a cavity in the palate that receives and hides its tip) in England.

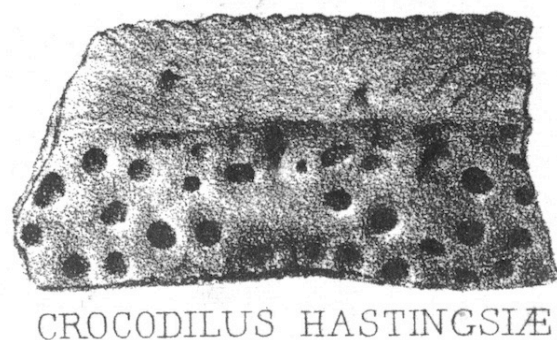


Figure 1. Huxley’s (1859b) Figure 8 on Plate 25 shows part of a rectangular bipartite ventral scute-pair, and to find any crocodilian bone like this hypothetically indicates a Caimaninae species.

Recently Brochu (1999) said that an 1985 Estes and Baez paper reported “a well-preserved postcranial skeleton (UCMP 128400) with bipartite ventral osteoderms” and added that in his own opinion UCMP 128400 “probably represents an ancient caiman” (page 24). Later, Brochu (1999) coined the Caimaninae tax nov.” (p. 66), and under it he mentioned “an undescribed caiman from the Cretaceous Hell Creek Formation of Montana” and “a caiman from the Paleocene of western Texas” (p. 68).

Considering the extraordinarily high number of caiman, alligator and crocodile ventral hides that have been commercially harvested, remarkably little has been learned about the double-button versus single-button ventral scutes dichotomy since 1859. Unfortunately the repeated and reprinted exact same species-account pictures in the 1974 and 2006 books by Karlheinz Fuchs and in the 1983 CITES guide by Wermuth and Fuchs all showed shaved skins, which as King and Brazaitis (1971) had earlier reported can all be expected to be missing some tissue, including some bone when the scales have ossification(s). The anterior edge of the anterior moiety had already been sliced cleanly off and thrown away before the Fuchs photographs were taken. The osteoderms (or what is left of them) are easiest to see clearly on internal views of shaved skins, but the proportions involving the length of the anterior unequal demiscute versus the posterior unequal demiscute are not real.

Researchers with access to fresh or salted crocodilian ventral skins are urged to examine material that has not been shaved, and then to report on the complete extent and the natural relations of ventral osteodermal fields. Further investigation including dissections and cleverly interrupted skeletonizing are needed today to detail the angle that the anterior edge (the articular facet) dives into the animal to permit the scute anterior to it to overlap it.

With reference to my Figure 1, which shows Huxley's (1859b) Figure 8 bone, he said (with my square brackets added) "I have found two examples of this anterior moiety detached [as an isolated bone], one of which is represented in fig. 8 [with its anterior edge horizontal across the top]. It exhibits the articular facet [along the top edge], [and posterior to it on the same bone] the deep rounded sculpture pits of the other [the posterior] moiety of the flat [ventral] scute [pair], and [the fig. 8 bone exhibits] the total absence of even the commencement of a ridge [thus completely lacking any keel], [as is] characteristic of the corresponding [anterior or posterior, who cares] part of the flat ventral scutes [pairs] in Jacare and Caiman [the living Caimaninae], and, finally, its [the Fig. 8 anterior unequal demiscute's] thick posterior edge presents distinct sutural teeth" (page 679) [that can not be seen in this 1859 figured view].

It is relatively unimportant that Huxley mistakenly thought that all of the single-button CITES taxa were devoid of ventral osteoderms. Today we know that many species start life free of ventral osteoscutes, and then develop them as the single-button condition by the time the animals have reached commercial harvesting size. That detail noted, I now frame the hypothesis that when the subdorsal surfaces of the tail are armored with two (same width) rectangular bones per vertebral segment, and the anterior individual ossification is the shorter of the sutured-together pair, then it is a Caimaninae in the Alligatoridae in the Eusuchia, but when both of the same-width moieties or demiscutes have the same length also, then the bony rectangles are each square in shape and the animal is in the Protosuchia (eponym *Protosuchus*).

The protosuchians and the caimans both have rectangular bipartite (per vertebral unit) osteodermal systems, and this coincidence is not restricted to just the lateral and ventral caudal surfaces. Rather, the belly, chest and throat in the Caimaninae have the same double-button osteoderms condition as occurs in the Caimaninae subdorsal tail. Separately the *Protosuchus richardsoni* plastron's osteoscutes were flat thin squares, and there I get lost. It is tempting to follow Huxley (1859a) into interpreting the transverse rows of ventral scales on the body (the collar-to-vent count's region) as being the same segmentation as that observed among the surface scales on the tail, namely vertebral. However, I am decidedly not there yet.

The curious fact remains that in Huxley's caimans the double-button scales on the subdorsal tail matched the double-button scales in the Caimaninae chest and throat, and although the *Protosuchus* plastron was different from the modern condition, the square shape of the

belly osteoderms in this fossil was the same as the square shape of the bones in its subdorsal caudal ossification field.

The question from Freiberg and de Carvalho (1965) concerned the type-locality of *Caiman fissipes* Spix on page 355, and a remark about the Spix (1825) name on page 360, but their real activity was that when naming a new subspecies that had its own type-locality, they became obliged to distinguish it from something known from somewhere else, and they selected a specimen in their own collection. Either way, Hoogmoed and Gruber (1983) listed *Caiman fissipes* under *Caiman latirostris latirostris* (Daudin), thus allowing the 1965 subspecies from Argentina to stand. The Spix (1825) collecting locality (the river San Francisco in Spanish, São Francisco in Portuguese) for the broad-snouted cayman is well within the known range of *Caiman l. latirostris* according to the distribution map in Freiberg and de Carvalho (1965).

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