

**CROCODILE  
SPECIALIST  
GROUP  
NEWSLETTER**

VOLUME 37 No. 3 • JULY 2018 - SEPTEMBER 2018



# CROCODILE

# SPECIALIST

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# NEWSLETTER

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JULY 2018 - SEPTEMBER 2018

IUCN - Species Survival Commission

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**CHAIR:**

Professor Grahame Webb  
PO Box 530, Karama, NT 0813, Australia

**EDITORIAL AND EXECUTIVE OFFICE:**

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**COVER PHOTOGRAPH:** Pure Siamese crocodile (*Crocodylus siamensis*) captured near Hon Me, Kieng Giang Province, Vietnam. See pages 8-14. Photograph: Thomas Ziegler.

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**EDITORIAL POLICY:** All news on crocodilian conservation, research, management, captive propagation, trade, laws and regulations is welcome. Photographs and other graphic materials are particularly welcome. Information is usually published, as submitted, over the author's name and mailing address. The editors also extract material from correspondence or other sources and these items are attributed to the source. If inaccuracies do appear, please call them to the attention of the editors so that corrections can be published in later issues. The opinions expressed herein are those of the individuals identified and are not the opinions of CSG, the SSC or the IUCN unless so indicated.

## CSG Newsletter

The CSG Newsletter is produced and distributed by the Crocodile Specialist Group of the Species Survival Commission (SSC) of the IUCN (International Union for Conservation of Nature).

The CSG Newsletter provides information on the conservation, status, news and current events concerning crocodilians, and on the activities of the CSG. It is available as a free electronic, downloadable copy from "<http://www.iucncsg.org/pages/Publications.html>".

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CSG Executive Office, P.O. Box 530, Karama, NT 0813, Australia.  
Fax: +61.8.89470678. E-mail: [csg@wmi.com.au](mailto:csg@wmi.com.au).

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Matthew Shirley, Florida, USA.

## Editorial

The CSG is a large network of specialists that has been operating for nearly 50 years. With some 624 members in 64 countries, we have witnessed our share of the highs and lows of births, deaths and marriages - but the recent loss of Sergio Medrano Bitar, in July, was both unexpected and particularly sad. Sergio was a long-term Colombian CSG member, a regional Vice Chair for the Latin America and the Caribbean region, a personal friend, and an individual committed to conservation, sustainable use, public education and the whole concept of engaging local people in Colombia's program. He was a strong voice amongst the Colombian farming sector to improve compliance. We will all miss him. See page 4.

In terms of up and coming meetings, the agenda is rapidly filling. A CSG Central America & Caribbean Sub-regional meeting, being organised by Marisa Tellez with help from the Latin America and Caribbean regional office, will be held in Belize, on 26-29 June 2019. This is a geographically complex region and a central goal of the meeting is to get various stakeholders together. See page 6.

Of course in 2020 there will be the full Working Meeting of the CSG, in neighbouring Mexico, where crocodylian conservation, management and research continue to advance. It all heralds well for this region.

A "2nd Forum on Crocodiles in the Philippines" is to be held at the SEAMEO-Southeast Asian Regional Center for Graduate Study and Research in Agriculture, in the University of the Philippines, Los Baños, Laguna, Philippines, on 6-8 March 2019. Again, a great opportunity for regional CSG members to congregate. See page 5.

The 13th meeting of the CITES Animals Committee, held in Geneva on 16-21 July 2018, was attended by various CSG members, both as delegates and observers, including Matthew Shirley, Buddy Baker, Jeb Libscome, Christy Plott and Hesiquio Benitez Diaz. Investigations into compliance issues with captive breeding and ranched specimens continued (Resolution Conf. 17.7 and Decision 17.105), with further deliberations on non-detriment findings. Mexico presented its protocol for implementing a ranching program for *Crocodylus moreletii*.

The 18th meeting of the Conference of the Parties to CITES (CoP18; [www.cites.org/eng/cop/index.php](http://www.cites.org/eng/cop/index.php)) will be held at the Bandaranaike Memorial International Conference Hall ([www.bmich.com](http://www.bmich.com)), Colombo, Sri Lanka, on 23 May-3 June 2019. Draft resolutions and documents, and proposed amendments to the Appendices [under Resolution Conf. 9.24 (Rev. CoP17)] for consideration at CoP18 must be communicated to the CITES Secretariat at least 150 days before the meeting (ie by 24 December 2018).

Human-crocodile conflict continues to become a bigger and bigger issue in many countries, and a central constraint on public and political tolerance of some crocodylian species. In our part of the world alone, with *C. porosus*, the situation is becoming serious. Sebastian Brackhane's work in Timore Leste documented the increasing rate of attacks (about one fatality per month), and the strong cultural context mitigating against culling. The possibility that crocodiles from Australia are implicated is turning the spotlight back on how little we know about sea journeys of this species, one of the unfulfilled goals of Professor Harry Messel's original research program in the 1970s and 1980s!

In India, a similar HCC situation with Saltwater crocodiles is occurring in the Andaman Islands. A popular tourist destination, with snorkelling over inshore reefs a central attraction, the successful rebuilding of the wild crocodile populations is problematic. In July 2018, I participated in a workshop organised by the Wildlife Institute of India to look for solutions. But sustainable, pragmatic and cost-effective solutions, consistent with India's highly protectionist wildlife policies, may be beyond reach.

The same problem is now occurring in the Solomon Islands. Matthew Brien (CSG Regional Vice Chair for Australia and Oceania) represented the CSG at a workshop in September 2018, where Jan van der Ploeg has been assembling a tremendous new data set on HCC and crocodile abundance, working mainly with local people. Once again, the issues are culturally complex, but a commitment to management is now being made.

Congratulations to Phoebe Griffiths and Matthew Shirley, who were recently awarded grants from the National Geographic Society Grants Program on the Recovery of Species on the Brink of Extinction ([/www.nationalgeographic.org/grants/grant-opportunities/species-recovery/](http://www.nationalgeographic.org/grants/grant-opportunities/species-recovery/)). Our congratulations are also extended to Paul Moler, who was awarded the International Herpetological Symposium's Lifetime Achievement Award in June 2018. See page 5.

Natascha Behler, Agata Staniewicz, Rob Suebing, Thomas Zeigler and colleagues recently published their accumulated findings on Siamese crocodiles in Lake Mesangat, Indonesia. This is an important study and establishes a sound foundation for future studies. See page 24 for Abstracts.

Winter CrocFest 2018 will take place on 8 December 2018 at Gatorama in Palmdale, Florida. Jeff Lang's Gharial project has been selected as the beneficiary.

The crocodylian industry is going through tough times, on a global scale, partly due to cyclic overproduction, but also to changes in the marketplace. The major concern for the CSG is that the incentives to conserve wild populations through sustainable use may be jeopardised. The CSG is assembling case histories for the CITES Livelihoods meeting in China (November 2018), and are continuing to gather information to add to that assembled at the last CSG working meeting.

At the last working meeting it was decided that the CSG Newsletter would switch to an exclusively electronic copy in the future, as demand for print copies had tailed off, and it is becoming much more expensive to produce and mail out. Electronic copies of all CSG Newsletters are freely available on the CSG website ([www.iucncsg.org/pages/Publications.html](http://www.iucncsg.org/pages/Publications.html)).

On the sustainable use front generally, I was able to attend, with Rosie Cooney (Chair, Sustainable Use and Livelihoods Specialist Group), an excellent meeting of the regional Oceania Marine Turtle Specialist Group, entitled: "Conservation of Sea Turtles within the Cultural Context of Oceania - Possibilities Beyond Protection" on 19-21 July 2018, in Suva, Fiji. Despite commitments to recovering depleted populations historically, throughout the region, the use of sea turtles was traditionally and culturally important, and now that wild populations have recovered, many people want to reinstate some level of customary use.

The State of Queensland, Australia, has amended its "Nature Conservation (Estuarine Crocodile) Conservation Plan 2018" to include provisions to authorise an individual or corporation to harvest *C. porosus* eggs under a commercial wildlife harvesting licence. There are strict limitations and requirements in place to regulate harvesting, including comprehensive monitoring in all areas where egg harvesting occurs ([www.ehp.qld.gov.au/wildlife/livingwith/crocodiles/egg-harvest/](http://www.ehp.qld.gov.au/wildlife/livingwith/crocodiles/egg-harvest/)).

Let me once again express my thanks to all who have made donations to the CSG. The support of CSG donors, big and small, is critical to the CSG's ability to operate effectively.

Professor Grahame Webb, *CSG Chair*.

### CSG Student Research Assistance Scheme

The Student Research Assistance Scheme (SRAS) provided funding to 5 students in the July-September 2018 quarter, and 8 further applications are currently under review.

1. Alexis Bashonga Bishobibiri (Democratic Republic of Congo): Ecology, conservation and management of birds and crocodiles of the Ruzizi Plains, Democratic Republic of Congo.
2. Ridwane Bio Oure (Benin): Developing adaptive conservation strategies for crocodile species under climate scenarios in Benin (West Africa).

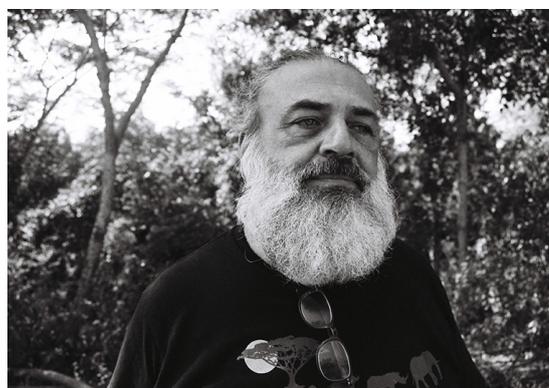
3. Nerike Uys (South Africa): Consumer's acceptance of an innovative crocodile meat product as a sustainable protein source.
4. Joseph Brown (USA): Ecology and conservation of the Critically Endangered Philippine crocodile.
5. Melciellyne Aguilar (Panama): Detection and identification of *Trypanosoma* sp. in wild *Crocodylus acutus* and *Caiman crocodilus* at Monumento Natural Isla Barro Colorado, Lago Gatún, Republic of Panama.

Tom Dacey, *CSG Executive Officer*, ([csg@wmi.com.au](mailto:csg@wmi.com.au)).

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## Obituary

### Sergio Medrano Bitar (1960-2018)



Photograph: Julián Medrano.

On 24 July 2018, Sergio Medrano Bitar (58 y) passed away after a long battle with heart disease, in the company of Mariana (daughter), Ernesto (brother), Olga (girlfriend), Catalina (ex-wife) and close friends. Sergio is survived by his daughter Mariana and son Julián.

Sergio was born on 29 March 1960 in Medellin, Colombia. He graduated as a biologist from the National University of Colombia, with his dissertation on turtles, and was recognized with a national award in ecology. His involvement with crocodylians began with captive breeding programs in Colombia in 1991-1992, and continued in 1993-1995 as part of an advisory group in the Colombian Ministry of Environment, and in 1997-2001 as advisor to the Colombian Association of Zoocria and Sustainable Use of Wildlife (AZOOCOL). Through this latter position Sergio designed the first control tools that allowed evaluation of the *Caiman crocodilus fuscus* captive breeding program in Colombia. Named "Autocontrol", the detailed evaluation was based on infrastructure, reproduction, incubation, management of parents-hatchlings-juveniles, food supply, etc.

In 2001 Sergio established and Biodiversa. He supported the implementation of CSG recommendations to improve the farming industry, including: a) agreements between farmers and Government on reintroduction quotas; b) airport

checking of skins being exported by international certifiers; c) identification of parental stock by microchip; d) scute-clipping of hatchlings; e) study of relationship between belly and flank size and animal size; and, f) the agreement between the Ministry, Autonomous Corporations and farmers on payment quotas, environmental services and reintroduction of caimans.

Also with Biodiversa he implemented a community program called “Canal del Dique”, with the support of some farms and the Cardique Autonomous Corporation, where local families maintain caimans hatchlings to produce skins.

Through the Foundation for the Conservation of Caiman and Crocodiles of Colombia (FunCroco), of which Sergio was a founding member, he supported genetic studies on *Crocodylus acutus* for regulatory purposes. He also developed the first criteria for crocodilian meat for human consumption in Colombia.

As a professor at the University of Applied Science and Environment (in Cartagena), Sergio delivered courses on wildlife production for 6 years, where he was loved by his students for his personality and sense of humour, and whom they referred to as “Chispa”.

During the last years of his life, Sergio was an independent advisor for different farms. He designed and built the thematic park “Vivarium del Caribe”, which opened close to Cartagena about a year ago, where visitors can learn about reptiles, amphibians and crocodile evolution, biology, conservation and sustainable use.

Sergio was a CSG member since the 1990s, and in 2005 he was recognized as Regional Vice Chair in Colombia for the Latin America and the Caribbean region. The goal amongst his current projects was the creation of a group of crocodilian specialists in Colombia, with the subsequent development of interdisciplinary collaboration between researchers in the areas of biodiversity, community management, anatomy, physiology, systematics, ecology and immunology.

His close friends called him “Gordo”, and deeply enjoyed his conversation and personality. Wherever you are Sergio, we remember you and drink a couple of tequilas in your memory

*Alvaro Velasco and Jhon Calderon compiled information from various friends and colleagues, and Julián Medrano reviewed this obituary.*

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### **Paul Moler Honored with Lifetime Achievement Award**

June 2018 marked the 41st meeting of the International Herpetological Symposium (IHS), held in Houston, Texas, USA, in partnership with the East Texas Herpetological Society and the Houston Zoo. IHS honors leaders who have demonstrated significant contributions in the field of herpetology. During the 2018 awards ceremony, CSG member Paul Moler of the Florida Fish and Wildlife Conservation

Commission (FFWCC) was honored with the prestigious Lifetime Achievement Award for his ongoing dedication to the protection of reptiles and amphibians and their habitats. Paul shared some insight to his interest in natural history, conservation, and his long career as a herpetologist with the FFWCC in a recent interview published in Herpetological Review ([www.researchgate.net/publication/327278732\\_PERSPECTIVES\\_IN\\_CONSERVATION\\_An\\_Interview\\_with\\_Paul\\_Moler](http://www.researchgate.net/publication/327278732_PERSPECTIVES_IN_CONSERVATION_An_Interview_with_Paul_Moler)).



Figure 1. Paul Moler with a road-killed American crocodile recovered on US Highway 1, Dade County, Florida, USA, in May 1988. Photograph: Bill Serne.

Jennifer L. Stabile, *President, IHS* ([jens@fieldprojects.org](mailto:jens@fieldprojects.org)).

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### **International Herpetological Symposium (Belize, 19-22 June 2019)**

The primary purpose of the International Herpetological Symposium (IHS) is to provide a forum for the dissemination of information and results of such research pertaining to the natural history, conservation biology, and captive management and propagation of amphibians and reptiles.

Over the years, an increasing number of people with varied interests in herpetology have attended IHS meetings, and this curious mix has allowed IHS to develop its “unique” flavor. Although IHS was historically visualized as an organization with an interesting blend of academia and herpetoculture, the current attendees far exceed those bounds. In short, IHS has something for everyone, and all are welcome. Starting in 2019 IHS will offer special registration rates for youth attendees (17 and under) as well as student discounts.

We encourage you to join us next year at our first international meeting in over a decade, hosted in Belize, on 19-22 June 2019. The call for abstracts is now open, and speaker slots are filling up fast. All local Belizean residents receive a 50% discount on registration costs, with valid ID.

More information on speaker instructions, grants, proceedings, hotel and registration can be found on the IHS website ([www.internationalherpetologicalsymposium.com](http://www.internationalherpetologicalsymposium.com)).

Jennifer L. Stabile, *President, IHS* ([jens@fieldprojects.org](mailto:jens@fieldprojects.org)).

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### **2nd Forum on Crocodiles in the Philippines (Los Baños, Philippines, 6-8 March 2019)**

This 3-day Forum on crocodiles aims to highlight the plight of *Crocodylus porosus* and *C. mindorensis* in the Philippines, and to explore possibilities for furthering crocodile conservation and sustainable management in the country. The Forum is an affirmation of the continuous effort of the Filipino People to conserve their crocodilian species in their natural environments.

Papers may be submitted either as oral or poster presentations, in the following themes: Natural History and Ecology; Husbandry, Veterinary and Health; Conservation Research and Management; and, Encouraging Human-Crocodile Coexistence of Crocodiles in the Philippines. All accepted presentations shall be peer-reviewed for subsequent publication in the Forum Proceedings.

Abstracts (maximum of 250 words) for oral and poster presentations should be submitted electronically no later than 1 November 2018 (contact [2ndcroforumph@gmail.com](mailto:2ndcroforumph@gmail.com) for details on format of abstracts). It is hoped that authors will be notified of the acceptance of papers via e-mail by 30 November 2018.

Registration (\$US150 before 30 November 2018, \$US200 after 30 November 2018, student \$US100) will cover attendance and meals over the 3 days of the Forum (contact [2ndcroforumph@gmail.com](mailto:2ndcroforumph@gmail.com) for details on how to pay registration and tentative program, or download flyer at [www.iucncsg.org](http://www.iucncsg.org)). Details on field trips (after the forum) and accommodation will be made available soon.

Forum Secretariat, [2ndcroforumph@gmail.com](mailto:2ndcroforumph@gmail.com).

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### **CSG Sub-Regional Meeting for Central America and the Caribbean (Placencia Peninsula, Belize, 26-29 June 2019)**

The Belize Forest Department and the Crocodile Research Coalition is hosting a CSG Central American & Caribbean (CAC) Sub-regional meeting, to be held at the Belize Ocean Club, Placencia Peninsula, Belize, on 26-29 June 2019. Marisa Tellez, CSG Regional Vice Chair for the Latin America and the Caribbean (LAC) region, with responsibility for the CAC sub-region, is organising the meeting, with assistance from the LAC office and the host organisations.

The theme of this 4-day meeting is “Fostering Regional Conservation through Collaboration”. The meeting is intended to build the network of key stakeholders in Central America and the Caribbean, and to discuss current conservation and management issues as well as further collaborative research within the sub-region.

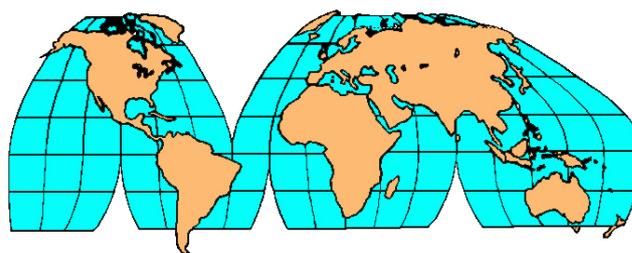
There will be 4 keynote speaker presentations that will discuss topics such as the history of crocodile conservation and management in the sub-region, sustainable use programs, human-crocodile conflict, and discussion of successful conservation and management programs, in addition to submitted presentations. On the last day (29 June) a workshop will be held for students and participants interested in developing their skills in carrying out crocodilian population surveys.

Further details will be posted on the meeting website, which should be available by 31 October 2018.

Marisa Tellez, *Regional Vice Chair, Latin America and the Caribbean and Crocodile Research Coalition* ([marisatellez13@gmail.com](mailto:marisatellez13@gmail.com)).

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## **Regional Reports**



### **East and Southeast Asia**

#### **Laos**

EVIDENCE OF ANOTHER OVERLOOKED SIAMESE CROCODILE (*CROCODYLUS SIAMENSIS*) POPULATION IN KHAMMOUANE PROVINCE, CENTRAL LAO PDR. The Siamese Crocodile (*Crocodylus siamensis*) is one of the most endangered species in Laos. Its populations have severely declined over the past decades and now the species is locally extinct or non-viable at many sites throughout the Range States on mainland southeast Asia (Bezuijen

et al. 2013) after decades of overexploitation. Remaining populations are currently confined to Cambodia, Thailand, Laos and Indonesia, while wild populations in Vietnam have probably been extirpated (Bezuijen et al. 2012).

At the national level, the species is considered “at Risk” in Laos, which is the highest national ranking of extinction threat (Bezuijen et al. 2006). Globally, *C. siamensis* is listed on Appendix I of CITES, and as Critically Endangered on the IUCN Red List (last assessed in 2012; with decreasing population trend; Bezuijen et al. 2012).

Recent field surveys rediscovered the species in Khammouane Province (Ziegler et al. 2015). Similar to most other Siamese crocodile populations in Laos, this population consists of only a few observed individuals. Based on interviews with local people, these remaining individuals seem to have long occupied the area, which strongly suggests their natural origin.

As a result of Cologne Zoo’s conservation activities in Laos, the Ban Soc Crocodile Conservation Area (BSCCA), a protected area in Ban Soc (= Soc Village), was established by the Khammouane Provincial authority in December 2016. The area covers 2007 ha and aims to support the long-term preservation of the *C. siamensis* population near Soc Village.

In June 2017 a training workshop was organized for local villagers around BSCCA to introduce methods for tracking crocodiles (Fig. 1). Cologne Zoo has also provided local people with GPS devices and equipment (eg cameras) for recording the species at other sites in the area. During the 2017 fieldwork we obtained information on the possible occurrence of *C. siamensis* at Nong Boua Ta Lake, near Ka Cham Village, Bualapha District, and approximately 4 km from BSCCA.



Figure 1. Training workshop in Ka Cham Village, held on 9 June 2017, by Thomas Ziegler (right, sitting), Sisomphone Soudthichak (second from right, sitting) and Thanousone Homsaysombath (right, standing). Photograph: Vinh Quang Luu.

We subsequently interviewed 34 randomly-selected people (19 hunters, 15 fishermen) from Ka Cham Village to verify

the information. Around 80% of interviewees confirmed the species’ presence at this new site. Sightings by local people indicate that 2-4 *C. siamensis* individuals are residing in the area, and more importantly, the population appears to be breeding, with both adults and juveniles having been observed.

To confirm information obtained through the interviews we conducted a field survey around Ka Cham Village on 13-29 of May 2018. During the survey, signs of *C. siamensis* (eg faeces and potential resting sites) were identified, and faecal samples (Fig. 2) were collected for genetic screening to confirm species identification and to test the purity using the same protocols performed for the crocodile population from Ban Soc (Ziegler et al. 2015).



Figure 2. Crocodile faeces found on grass at Nong Boua Ta Lake on 13 May 2018. Photograph: Paseun Souvannasy.

Nong Boua Ta Lake (17° 31’ 03.6” N, 105° 34’ 18.0” E), with elevations ranging from ca. 90 to 180 m asl, is now confirmed as a new distribution site for *C. siamensis* (Figs. 3-4). The habitat consists mainly of floating grass mats, and is surrounded by forest, shrubs and bamboo. In the dry season, water levels drop to about 1 m. The inaccessible wetland area, covering approximately 3 ha, is about 30 km from the border with Hin Nam No National Protected Area.



Figure 3. Nong Boua Ta Lake, 13 May 2018. Photograph: Paseun Souvannasy.



Figure 4. Shore area of Nong Boua Ta Lake, 13 May 2018. Photograph: Paseun Souvannasy.

The status of Nong Boua Ta Lake as a local “holy” place (a ritual place protected by a god), benefits the protection of this crocodile population.

Further research on population size, size structure and threats is currently being conducted by our team, and will assist in the formulation of appropriate conservation measures for the population. Additional discussions with Khammouane provincial authorities on crocodile conservation are currently planned. Two options are considered to better protect the habitat and population of the species: 1) establishment of a new conservation area in Ka Cham Village; or, 2) extending the BSCCA to cover both sites (ie at Ban Soc and Ka Cham Villages).

In addition, Cologne Zoo has been collaborating with different agencies and institutions in Laos and Vietnam to undertake genetic screening of both wild and captive *C. siamensis*. The goal of genetic screening was to identify pure-bred individuals for conservation breeding programs, including future release/restocking projects in protected areas in Laos and Vietnam. Preliminary results of the genetic screening of wild and captive individuals in Vietnam and Laos are now available (see Nguyen *et al.* 2018).

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Paseun Souvannasy, Vinh Quang Luu (*Vietnam National University of Forestry, Hanoi, Vietnam*), Sisomphone Soudthichak (*Natural Resources and Environment Department, Khammouane, Lao PDR*), Sengdeuane Wayakone (*National University of Laos, Vientiane, Lao PDR*), Minh Le (*Central Institute for Natural Resources and Environmental Studies and Faculty of Environmental Sciences, Vietnam National University, Hanoi, Vietnam and Department of Herpetology, American Museum of Natural History, New York, USA*), Truong Quang Nguyen (*Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology, Hanoi, Vietnam*) and Thomas Ziegler (*Cologne Zoo, Köln, Germany; ziegler@koelnerzoo.de*).

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GENETIC SCREENING OF SIAMESE CROCODILES (*CROCODYLUS SIAMENSIS*) IN LAOS AND VIETNAM: IDENTIFYING PUREBRED INDIVIDUALS FOR CONSERVATION AND RELEASE PROGRAMS. Historically, the Siamese crocodile (*Crocodylus siamensis*) was widely distributed in mainland Southeast Asia, including Cambodia, Indonesia, Lao PDR and Vietnam. As a result of a >80% reduction in its distribution, due mainly to habitat loss and hunting pressure, the species is listed as Critically Endangered on the IUCN Red List (Bezuijen *et al.* 2012).

The wild population in Vietnam was probably extirpated due to extremely high hunting pressure, and populations in Cambodia, Laos and Thailand have also become highly fragmented and threatened. Most populations consist of less than 10 individuals, and may not be able to survive long into the future (Bezuijen *et al.* 2012). In Laos, recent surveys near Ban Soc, Khammouane Province, discovered an overlooked population of the species (Ziegler *et al.* 2015a). Like other documented populations, this one contains only

a few individuals, which, based on external morphology, could be identified as “pure” Siamese crocodiles. Interviews with local people revealed the population to represent a natural wild population (Ziegler *et al.* 2015a), but molecular testing is required to exclude the possibility that these animals represent escaped farm hybrids. The same concerns apply to the discovery of another very small population in Khammouane Province (Souvannasy *et al.* 2018).

One of the most cost-effective conservation options for the species is restocking diminished wild populations, or to establish populations in suitable habitat where the species existed previously, in combination with other habitat and species conservation measures. In Vietnam, the first population of *C. siamensis* was introduced in Cat Tien National Park in the early 2000s (Polet 2006; Murphy *et al.* 2004). Breeding was subsequently documented, demonstrating initial success of the program (Pahl 2012). The introduction program was carefully implemented by Cat Tien NP through genetic screening of captive individuals using microsatellite markers and mitochondrial genes (FitzSimmons *et al.* 2002).

A major challenge for restocking/reintroduction efforts is to identify purebred individuals from captive populations in the Range States. So far, captive populations in Vietnam and Laos have not been monitored closely to confirm purity of *C. siamensis* held in those facilities. For the reintroduction project at Cat Tien NP, only a few individuals, which morphologically appeared to be pure *C. siamensis*, were genetically screened at Saigon Zoo. Also, only a few individuals were tested at the Lao Zoo (Vientiane), and due to frequent shifts in management, records are difficult to reconstruct.

On the other hand, captive stocks, if managed properly, can play an important role in future restocking/reintroduction efforts in both Laos and Vietnam. To address this issue we carried out molecular screening of 65 *C. siamensis* using mitochondrial and microsatellite markers to determine purity of captive colonies in Laos and Vietnam, and of the wild population reported by Ziegler *et al.* (2015a) in Khammouane Province, Laos.

## Methods

We analyzed a total of 85 samples (see Table 1), comprising:

- 5 Cuban crocodiles (*C. rhombifer*) from Hoyerswerda Zoo (Germany), Terrariet Vissenbjerg (Denmark) and Zagreb Zoo (Croatia).
- 5 samples from two Saltwater crocodiles (*C. porosus*) at Wilhelma Zoo (Germany).
- 3 *C. siamensis* from Plzen Zoo (Czech Republic), descendants of a purebred pair held for some time at Zurich Zoo (Switzerland). The Zurich Zoo animals were progeny of wild-caught parents at Bronx Zoo and Miami Zoo.
- 3 *C. siamensis* from Benidorm Zoo (Spain) - a purebred male (Sample 69, Table 1) from Zurich Zoo (see above), and two females of uncertain status.
- 4 tissue samples from tail scutes, collected in May 2018 from 4 *C. siamensis* at Bau Sau, Cat Tien NP. All captured

individuals were photographed and subsequently released at same site. Extant *C. siamensis* at Cat Tien NP trace back to purebred individuals.

- 62 captive *C. siamensis* (tissue from tail scutes) in Laos (Lao Zoo, Vientiane; Fig. 1) and Vietnam (Hon Me Station, Kien Giang Province (Fig. 2); Saigon Zoo, Ho Chi Minh City; Thu Le Zoo, Hanoi).
- 3 faecal samples from the wild *C. siamensis* population in Khammouane Province, Laos (Ziegler *et al.* 2015a).



Figure 1. Siamese crocodiles at the former Lao Zoo (now Lao Conservation Trust for Wildlife). Photograph: Thomas Ziegler.



Figure 2. Pure Siamese crocodile captured 10 km from Hon Me, Kieng Giang Province, Vietnam (Sample 1, Table 1), near the estuary to the Gulf of Thailand. See also cover photograph. Photograph: Thomas Ziegler.

Where feasible, captive animals were measured (snout-vent length, total length), sexed (palpation), scute clipping recorded if present, scutes cut if not present, and microchips inserted (Fig. 3).

Tissue samples were stored in 70% alcohol. Based on the known purebred individuals from Europe and Vietnam, they were subsequently tested for purity.

The majority of samples (from Lao and Vietnamese facilities and from the wild) were analyzed in the laboratory in Hanoi.

Table 1. Results of genetic screening of 85 crocodiles from Laos, Vietnam and Europe. Samples 2-4, 71-74 = wild; 1, 5-70, 75-85= captive. Siamese crocodiles with a low probability of being purebred are highlighted (in red).

Sample No.	Species	Laboratory No.	Field No.	Proportion <i>C. siamensis</i>	Proportion <i>C. porosus</i>	Proportion <i>C. rhombifer</i>	Locality
1	<i>C. cf. siamensis</i>	Cr1		99.6	0.2	0.2	Kien Giang Province, Vietnam
2	<i>C. cf. siamensis</i>	Cr7	Bs1	99.6	0.2	0.2	Khammouane Province, Laos
3	<i>C. cf. siamensis</i>	Cr8	Bs2	99.6	0.2	0.2	Khammouane Province, Laos
4	<i>C. cf. siamensis</i>	Cr9	Bs3	99.4	0.3	0.3	Khammouane Province, Laos
5	<i>C. cf. siamensis</i>	Cr10	Saigon 1	99.6	0.2	0.2	Saigon Zoo, Vietnam
6	<i>C. cf. siamensis</i>	Cr11	Saigon 2	98.3	0.8	0.9	Saigon Zoo, Vietnam
7	<i>C. cf. siamensis</i>	Cr12	Saigon 3	99.4	0.2	0.3	Saigon Zoo, Vietnam
8	<i>C. cf. siamensis</i>	Cr13	Saigon 4	99.6	0.2	0.2	Saigon Zoo, Vietnam
9	<i>C. cf. siamensis</i>	Cr14	Saigon 5	99.5	0.2	0.2	Saigon Zoo, Vietnam
10	<i>C. cf. siamensis</i>	Cr15	Saigon 6	99.3	0.4	0.3	Saigon Zoo, Vietnam
11	<i>C. cf. siamensis</i>	Cr16	Saigon 7	98.6	1.1	0.2	Saigon Zoo, Vietnam
12	<i>C. cf. siamensis</i>	Cr17	Saigon 8	99.5	0.2	0.3	Saigon Zoo, Vietnam
13	<i>C. cf. siamensis</i>	Cr18	Saigon 9	99.6	0.2	0.2	Saigon Zoo, Vietnam
14	<i>C. cf. siamensis</i>	Cr19	Saigon 10	99.6	0.2	0.2	Saigon Zoo, Vietnam
15	<i>C. cf. siamensis</i>	Cr20	Saigon 11	99.6	0.2	0.2	Saigon Zoo, Vietnam
16	<i>C. cf. siamensis</i>	Cr21	Saigon 12	99.6	0.2	0.2	Saigon Zoo, Vietnam
17	<i>C. cf. siamensis</i>	Cr22	Saigon 13	99.0	0.4	0.5	Saigon Zoo, Vietnam
18	<i>C. cf. siamensis</i>	Cr23	Saigon 14	99.6	0.2	0.2	Saigon Zoo, Vietnam
19	<i>C. cf. siamensis</i>	Cr24	Saigon 15	98.5	0.6	1.0	Saigon Zoo, Vietnam
20	<i>C. cf. siamensis</i>	Cr25	Saigon 16	99.4	0.3	0.3	Saigon Zoo, Vietnam
21	<i>C. cf. siamensis</i>	Cr26	Saigon 17	97.1	1.0	1.9	Saigon Zoo, Vietnam
22	<i>C. cf. siamensis</i>	Cr27	Saigon 18	97.4	1.0	1.6	Saigon Zoo, Vietnam
23	<i>C. cf. siamensis</i>	Cr28	Saigon 19	99.3	0.3	0.4	Saigon Zoo, Vietnam
24	<i>C. cf. siamensis</i>	Cr29	Saigon 20	97.5	2.0	0.5	Saigon Zoo, Vietnam
25	<i>C. cf. siamensis</i>	Cr30	Saigon 21	99.6	0.2	0.2	Saigon Zoo, Vietnam
26	<i>C. cf. siamensis</i>	Cr31	Saigon 22	98.4	0.6	1.0	Saigon Zoo, Vietnam
27	<i>C. cf. siamensis</i>	Cr32	Saigon 23	99.6	0.2	0.2	Saigon Zoo, Vietnam
28	<i>C. cf. siamensis</i>	Cr33	Saigon 24	99.6	0.2	0.2	Saigon Zoo, Vietnam
29	<i>C. cf. siamensis</i>	Cr34	Saigon 25	99.6	0.2	0.2	Saigon Zoo, Vietnam
30	<i>C. cf. siamensis</i>	Cr35	Saigon 26	99.5	0.2	0.2	Saigon Zoo, Vietnam
31	<i>C. cf. siamensis</i>	Cr36	Saigon 27	99.6	0.2	0.2	Saigon Zoo, Vietnam
32	<i>C. cf. siamensis</i>	Cr37	Saigon 28	99.6	0.2	0.2	Saigon Zoo, Vietnam
33	<i>C. cf. siamensis</i>	Cr38	Hanoi 41	99.5	0.2	0.3	Thu Le Zoo, Vietnam
34	<i>C. cf. siamensis</i>	Cr39	Hanoi 30	99.6	0.2	0.2	Thu Le Zoo, Vietnam
35	<i>C. cf. siamensis</i>	Cr40	Hanoi 31	99.2	0.3	0.4	Thu Le Zoo, Vietnam
36	<i>C. cf. siamensis</i>	Cr41	Hanoi 32	99.6	0.2	0.2	Thu Le Zoo, Vietnam
37	<i>C. cf. siamensis</i>	Cr42	Hanoi 33	95.7	2.5	1.8	Thu Le Zoo, Vietnam
38	<i>C. cf. siamensis</i>	Cr43	Hanoi 34	99.6	0.2	0.2	Thu Le Zoo, Vietnam
39	<i>C. cf. siamensis</i>	Cr44	Hanoi 35	99.6	0.2	0.2	Thu Le Zoo, Vietnam
40	<i>C. cf. siamensis</i>	Cr45	Hanoi 36	99.6	0.2	0.2	Thu Le Zoo, Vietnam
41	<i>C. cf. siamensis</i>	Cr46	Hanoi 37	99.6	0.2	0.2	Thu Le Zoo, Vietnam
42	<i>C. cf. siamensis</i>	Cr47	Hanoi 38	99.6	0.2	0.2	Thu Le Zoo, Vietnam
43	<i>C. cf. siamensis</i>	Cr48	Hanoi 39	98.2	1.3	0.5	Thu Le Zoo, Vietnam
44	<i>C. cf. siamensis</i>	Cr49	Hanoi 40	99.6	0.2	0.2	Thu Le Zoo, Vietnam
45	<i>C. cf. siamensis</i>	Cr52	Lao Zoo 5	99.5	0.2	0.2	Lao Zoo, Vientiane
46	<i>C. cf. siamensis</i>	Cr53	Lao Zoo 14	96.7	2.4	0.9	Lao Zoo, Vientiane
47	<i>C. cf. siamensis</i>	Cr54	Lao Zoo 18	98.7	0.4	1.0	Lao Zoo, Vientiane
48	<i>C. cf. siamensis</i>	Cr55	Lao Zoo 19	99.5	0.2	0.2	Lao Zoo, Vientiane
49	<i>C. cf. siamensis</i>	Cr56	Lao Zoo 15	99.4	0.3	0.4	Lao Zoo, Vientiane
50	<i>C. cf. siamensis</i>	Cr57	Lao Zoo 2	99.6	0.2	0.2	Lao Zoo, Vientiane
51	<i>C. cf. siamensis</i>	Cr58	Lao Zoo 4	99.6	0.2	0.2	Lao Zoo, Vientiane
52	<i>C. cf. siamensis</i>	Cr59	Lao Zoo 11	99.4	0.2	0.3	Lao Zoo, Vientiane
53	<i>C. cf. siamensis</i>	Cr60	Lao Zoo 1	88.6	10.9	0.5	Lao Zoo, Vientiane
54	<i>C. cf. siamensis</i>	Cr61	Lao Zoo 10	99.5	0.2	0.2	Lao Zoo, Vientiane
55	<i>C. cf. siamensis</i>	Cr62	Lao Zoo 12	99.4	0.2	0.4	Lao Zoo, Vientiane
56	<i>C. cf. siamensis</i>	Cr63	Lao Zoo 3	99.6	0.2	0.2	Lao Zoo, Vientiane
57	<i>C. cf. siamensis</i>	Cr64	Lao Zoo 16	99.5	0.2	0.3	Lao Zoo, Vientiane
58	<i>C. cf. siamensis</i>	Cr65	Lao Zoo 13	99.6	0.2	0.2	Lao Zoo, Vientiane
59	<i>C. cf. siamensis</i>	Cr66	Lao Zoo 17	99.4	0.2	0.4	Lao Zoo, Vientiane

Table 1 cont'd. Results of genetic screening of 85 crocodiles from Laos, Vietnam and Europe. Samples 2-4, 71-74 = wild; 1, 5-70, 75-85= captive. Siamese crocodiles with a low probability of being purebred are highlighted (in red).

Sample No.	Species	Laboratory No.	Field No.	Proportion <i>C. siamensis</i>	Proportion <i>C. porosus</i>	Proportion <i>C. rhombifer</i>	Locality
60	<i>C. cf. siamensis</i>	Cr67	Lao Zoo 20 (CS20)	<b>99.6</b>	0.2	0.2	Lao Zoo, Vientiane
61	<i>C. cf. siamensis</i>	Cr68	Lao Zoo 21 (CS21)	<b>99.3</b>	0.2	0.5	Lao Zoo, Vientiane
62	<i>C. cf. siamensis</i>	Cr69	Lao Zoo 22 (CS22)	<b>99.5</b>	0.2	0.2	Lao Zoo, Vientiane
63	<i>C. cf. siamensis</i>	Cr70	Lao Zoo 23 (CS23)	<b>99.5</b>	0.2	0.3	Lao Zoo, Vientiane
64	<i>C. cf. siamensis</i>	Cr71	Lao Zoo 24 (CS24)	<b>99.5</b>	0.2	0.3	Lao Zoo, Vientiane
65	<i>C. cf. siamensis</i>	Cr72	Lao Zoo 25 (CS25)	<b>99.6</b>	0.2	0.2	Lao Zoo, Vientiane
66	<i>C. siamensis</i>	Cr95	S1	<b>99.6</b>	0.2	0.2	Plzen Zoo, Czech Republic
67	<i>C. siamensis</i>	Cr96	S2	<b>99.5</b>	0.2	0.2	Plzen Zoo, Czech Republic
68	<i>C. siamensis</i>	Cr97	S3	<b>99.5</b>	0.2	0.2	Plzen Zoo, Czech Republic
69	<i>C. siamensis</i>	Cr98	COS002	<b>99.5</b>	0.2	0.2	Benidorm Zoo, Spain
<b>70</b>	<b><i>C. cf. siamensis</i></b>	<b>Cr100</b>	<b>COS003</b>	<b>97.4</b>	<b>1.7</b>	<b>1.0</b>	<b>Benidorm Zoo, Spain</b>
71	<i>C. siamensis</i>	Cr77	CT.2018.1	<b>99.5</b>	0.2	0.3	Cat Tien NP, Vietnam
72	<i>C. siamensis</i>	Cr78	CT.2018.2	<b>99.5</b>	0.2	0.3	Cat Tien NP, Vietnam
73	<i>C. siamensis</i>	Cr79	CT.2018.3	<b>99.5</b>	0.2	0.2	Cat Tien NP, Vietnam
74	<i>C. siamensis</i>	Cr80	CT.2018.4	<b>99.5</b>	0.2	0.2	Cat Tien NP, Vietnam
75	<i>C. cf. siamensis</i>	Cr99	COS004	<b>99.4</b>	0.3	0.3	Benidorm Zoo, Spain
76	<i>C. porosus</i>	Cp1		0.3	<b>99.4</b>	0.3	Wilhelma Zoo, Germany
77	<i>C. porosus</i>	Cp2		0.3	<b>99.4</b>	0.3	Wilhelma Zoo, Germany
78	<i>C. porosus</i>	Cp3		0.3	<b>99.4</b>	0.3	Wilhelma Zoo, Germany
79	<i>C. porosus</i>	Cp4		0.2	<b>99.4</b>	0.4	Wilhelma Zoo, Germany
80	<i>C. porosus</i>	Cp5		0.2	<b>99.5</b>	0.3	Wilhelma Zoo, Germany
81	<i>C. cf. rhombifer</i>	Crb1		0.3	1.2	<b>98.6</b>	Zagreb Zoo, Croatia
82	<i>C. cf. rhombifer</i>	Crb2		0.2	2.1	<b>97.7</b>	Terrariet Vissenbjerg, Denmark
83	<i>C. rhombifer</i>	Crb3		0.2	0.2	<b>99.5</b>	Hoyerswerda Zoo, Germany
84	<i>C. rhombifer</i>	Crb4		0.2	0.3	<b>99.5</b>	Hoyerswerda Zoo, Germany
85	<i>C. rhombifer</i>	Crb5		0.5	0.8	<b>98.6</b>	Hoyerswerda Zoo, Germany



Figure 3. Taking samples and inserting microchips in captive Siamese crocodiles at Saigon Zoo (left - Thomas Ziegler, middle - Anna Rauhaus, right - Saigon Zoo team). Photograph: Dao T.A. Tran.

GeneJet Genomic DNA Purification Kit (Thermo Fisher Scientific) and QIAamp Fast DNA Stool Mini Kit (Qiagen) were used for extraction. The extraction process was carried out according to the manufacturer's procedure with minor adjustments. Microsatellite loci were amplified using Dream Taq Master Mix and HotStarTaq Master Mix with 5' fluoro-labeled forward primers and regular reverse primers. The primers used were taken from FitzSimmons *et al.* (2001) (Table 2). The PCR volume consisted of 25 µl including 12.5

µl mastermix, 7.5 µl water, 1.5 µl each primer 10 pmol/µl and 1.7 µl mol. The thermal cycle was: initial denaturation of 95°C for 15 minutes with HotStarTaq Master Mix or 5 minutes with Dream Taq Master Mix; 35 cycles at 95°C for

Table 2. Microsatellite primers used in this study (compiled from FitzSimmons *et al.* 2001). F= forward primer, R= reverse primer.

Primer	Sequence
Cj16	F: CAT GCA GAT TGT TAT TCC TGA TG R: TGT CAT GGT GTC AAT TAA ACT C
Cj18	F: ATC CAA ATC CCA TGA ACC TGA GAG R: CCG AGT GCT TAC AAG AGG CTG G
Cj101	F: ACA GGA GGA ATG TCG CAT AAT TG R: GTT TAT ACC GTG CCA TCC AAG TTA G
Cj104	F: TCC TTC CAT GCA TGC ACG TGT G R: GTT TCA GTG TCT GGT ATT GGA GAA GG
Cj119	F: GTT TGC TGT GGA ATG TTT CTA C R: CGC TAT ATG AAA CGG TGG CTG
Cj131	F: GTT TGT CTT CTT CCT CCT GTC CCT C R: AAA TGC TGA CTC CTA CGG ATG G
Cp10	F: GAT TAG TTT TAC GTG ACA TGC A R: ACA TCA AGT CAT GGC AGG TGA G
Cr391	F: ATG AGT CAG GTG GCA GGT TC R: CAT AAA TAC ACT TTT GAG CAG CAG

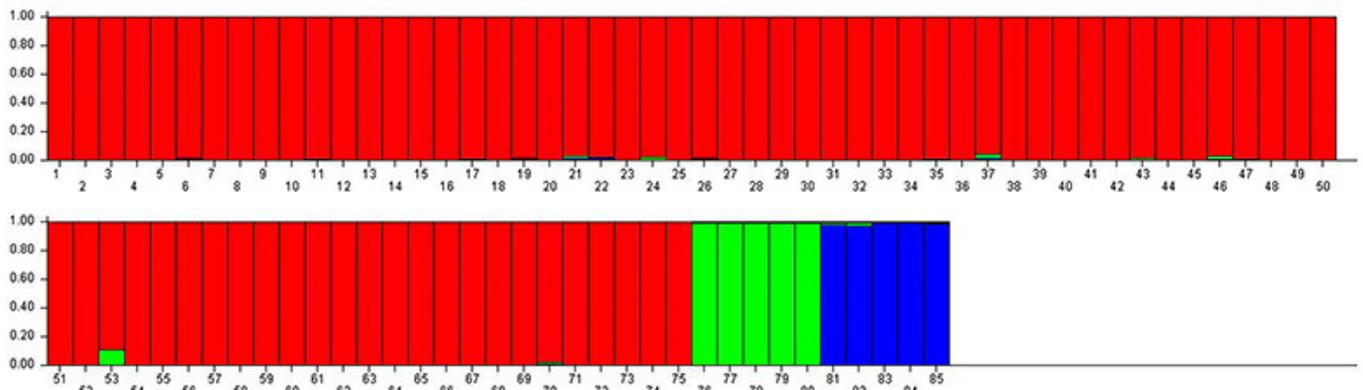


Figure 4. Admixture analysis of 85 individuals with  $K=3$ . Individuals with a low probability of purity are highlighted in Table 1.

30 s, 58-63°C for 45 s, 72°C for 60 s; and final extension at 72°C for 6 min.

A fragment of mitochondrial genes, including tRNA-Thr, tRNA-Pro, tRNA-Phe and a part of the D-loop, was amplified using the primer pair L15463 and H16260 (Glenn *et al.* 2002). Total PCR volume was 21  $\mu$ l including 10  $\mu$ l mastermix, 5  $\mu$ l water, 2  $\mu$ l each primer 10 pmol/ $\mu$ l and 2  $\mu$ l mold. The PCR conditions were: initial denaturation at 95°C for 5 or 15 min; 35 cycles at 95°C for 30 s, 50°C for 45 s, 72°C for 60 s; and final extension at 72°C for 6 min.

PCR products were checked by 1% agarose with 1X TBE buffer, ethidium bromide dye, and UV light. Successful PCR products were purified using GeneJET PCR Purification kit (Thermo Fisher Scientific). The purified products were sent to 1st Base Malaysia for sequencing and genotyping, then edit by the package Sequencher v5.4. Mitochondrial sequences were blasted on GenBank to determine the maternal lineage.

Additional samples from European specimens, including the comparative samples of *C. porosus* and *C. rhombifer* were processed in Germany. DNA extraction and sequencing of mitochondrial DNA followed protocols described in Ziegler *et al.* (2015b). Microsatellite genotyping (same primers and cycling protocols as in Hanoi) was performed on an ABI 3130xl capillary sequencer. Allele scoring was homologized by directly comparing peaks of Siamese crocodiles genotyped in Braunschweig with those genotyped in Hanoi.

Fragment size data were analyzed using Bayesian clustering method as implemented STRUCTURE v2.3.4 (Pritchard *et al.* 2000) to detect potential hybrid individuals. Ten independent runs were performed for each  $K$  value from 1 to 4 using 106 MCMC steps, following burn-in of 100,000 iterations. However, we mainly focused on the results employing three clusters ( $K=3$ ), equivalent to the number of species included in the analysis. Individuals with the genetic proportion of more than 99% similarity to *C. siamensis* were categorized as pure.

## Results and Discussion

All samples were successfully amplified with the mitochondrial gene fragment. Blast analysis showed that

all uncertain samples were *C. siamensis* with a high level of genetic similarity (~99%). Amplifications were positive with all microsatellite loci except Cj131 and Cr391 for some samples of *C. porosus*, *C. rhombifer* and samples of purebred *C. siamensis*. When employing three clusters ( $K=3$  in Structure), pure samples are correctly assigned to distinct groups. The results show that most screened individuals have more than 90% of the genetic characteristics of *C. siamensis* (Fig. 4). In total, 52 of 65 (80%) screened individuals from Laos and Vietnam have a high probability of being pure (ie a genetic proportion greater than 99%; Table 1). Hybrid animals are crosses between female *C. siamensis* and male *C. porosus* or *C. rhombifer*, with a higher proportion attributed to *C. porosus* (Table 1), because all maternal mitochondrial fragments were assigned to the former species.

It is clear that all crocodiles sighted at Ban Soc (Laos) (Samples 2-4 in Table 1) are highly likely to be pure. It is also likely that Samples 2 and 3 belong to the same individual as microsatellite alleles were identical. The genetic differences between Samples 2-3 and Sample 4, support the finding of Ziegler *et al.* (2015a), that more than one individual has survived at the Ban Soc Crocodile Conservation Area. However, this population is very small, and thus in need of further protection - restocking this small population should receive a high conservation priority in the future. This activity can be undertaken in collaboration with the Lao Conservation Trust for Wildlife (previously Lao Zoo), where at least 18 *C. siamensis* are confirmed as pure in our analysis (Table 1).

In the past, a conservation breeding program of the Lao Zoo, in partnership with the Wildlife Conservation Society (WCS), operated for several years, but it was terminated several years ago. One adult female (Sample 53), which historical records identified as pure, and which was kept together in past years with a pure male (Sample 50) in a separate facility, could not be confirmed as being pure in this study. We thus recommend that purebred individuals in captive facilities in Laos and Vietnam be kept separate from hybrids, and are used to build up conservation breeding programs.

Further samples from Lao Zoo are currently being analyzed, and there are plans with Lonnie McCaskill (WCS) and the new managers of the zoo in Vientiane to extend the genetic screening to all Siamese crocodiles held there (Fig. 1) for

improved management of resources and *ex-situ* conservation measures. This finally will also improve these conclusions, as the more samples that are involved the better the outcomes of the study.

This study also allowed the first insights into the composition of European crocodile populations. Of the two female *C. siamensis* held at Benidorm Zoo, one was confirmed to be of hybrid origin, and the other was pure (and could be paired with the pure male at the facility in the future).

Moreover, two Cuban crocodiles (Samples 81 and 82; Table 1) had low proportions of purity and thus should be separated from pure individuals. Cuban crocodile Samples 83-85 were derived from the same parents, however, Sample 85 showed a low proportion of purity (98.6%), so relations/analyses should be double checked/repeated to better resolve this case in the future.

Sample 1 represents an individual brought to the former Hon Me Station of Wildlife at Risk (WAR) in 2012 (Fig. 2). The crocodile had been sighted in the canal near the estuary to the Gulf of Thailand, and was caught by farmers at village “Canal No. 9” of Son Kien Commune, Hon Dat District, Kien Giang Province, about 10 km from Hon Me Station (Ziegler *et al.* 2015c, 2016). Interestingly, this individual proved to have a high probability of being pure. So it is either an escaped farm animal, or, because of its purity, suggests an overlooked wild population in Vietnam - although probability of the latter is quite low. Sadly, local authorities in Kien Giang Province transferred this individual to the Vin Pearl Safari on Phu Quoc Island in 2015, before we were able to insert a microchip. Thus, although this individual is pure, it is unavailable for conservation breeding programs in Vietnam, as it cannot be identified with certainty among other crocodiles held at the Phu Quoc Safari.

All other captive individuals captured and sampled by us in Laos and Vietnam were marked by scute clipping and/or microchipped (Fig. 3), so retrieval of pure individuals for future conservation breeding programs is relatively easy.

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Souvannasy, P., Luu, V.Q., Soudthichak, S., Wayakone, S., Le, M., Nguyen, T.Q. and Ziegler, T. (2018). Evidence of another overlooked Siamese crocodile (*Crocodylus siamensis*) population in Khammouane Province, central Lao PDR. *Crocodile Specialist Group Newsletter* 37(3): 6-8.

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Tham T. Nguyen (*Vietnam National University, Hanoi, Vietnam*), Thomas Ziegler, Anna Rauhaus (*Cologne Zoo, Köln, Germany; ziegler@koelnerzoo.de*), Truong Q. Nguyen (*Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology, Hanoi, Vietnam*), Dao T.A. Tran (*Faculty of Biology-Biotechnology, University of Science, Ho Chi Minh City, Vietnam*), Sengdeuane Wayakone (*National University of Laos, Vientiane, Lao PDR*), Vinh Q. Luu (*Vietnam National University of Forestry, Hanoi, Vietnam*), Miguel Vences (*Zoological Institute, Technical*

*University of Braunschweig, Braunschweig, Germany*), and Minh D. Le (*Central Institute for Natural Resources and Environmental Studies and Faculty of Environmental Sciences, Vietnam National University, Hanoi, Vietnam and Department of Herpetology, American Museum of Natural History, New York, USA*).



## Recent Publications

Miyagawa S., Yatsu R. and Iguchi T. (2018) Environmental Control of Sex Determination and Differentiation in Reptiles. *In: Reproductive and Developmental Strategies. Diversity and Commonality in Animals*, ed. by K. Kobayashi, T. Kitano, Y. Iwao and M. Kondo. Springer: Tokyo. (doi: [https://doi.org/10.1007/978-4-431-56609-0\\_18](https://doi.org/10.1007/978-4-431-56609-0_18)).

**Abstract:** Most vertebrates use a genetic sex determination system, whereas a diverse set of reptile taxa use an environmental sex determination system - more specifically, a temperature-dependent sex determination (TSD) system. The TSD system is where sex is established by the incubation temperature during a critical stage of embryonic development. It has been almost a half century since the first TSD system was found in a lizard species. Thermal effects on sex determination have been described in many other reptile species since then. TSD has been found in all crocodylians and tuataras examined and in most turtles and some lizards. However, clarification is needed about this unique mode of sex determination in reptiles, in particular, factors triggering the intrinsic genetic cascade, which leads either to development of a testis or ovary. In several instances, TSD has evolved separately in reptilian lineage and, therefore, the adaptive significance of TSD is an attractive topic from an evolutionary view. In this chapter, the general background and recent advancements for TSD research in reptiles is discussed.

Adame, M.F., Jardine, T.D., Fry, B., Valdez, D., Lindner, G., Nadji, J. and Bunn, S.E. (2018). Estuarine crocodiles in a tropical coastal floodplain obtain nutrition from terrestrial prey. *PLOS ONE* 13(7): e0200983.

**Abstract:** The estuarine crocodile (*Crocodylus porosus*) is one of the largest and most widespread crocodylians in the world. Although considered an apex species, the role of the estuarine crocodile in aquatic foodwebs is poorly understood; we know what crocodiles ingest, but not what nourishes them. In this study, we used a combination of stable isotope measurements ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{34}\text{S}$ ) and direct feeding observations to identify the source of nutrition of estuarine crocodiles in Kakadu National Park, Northern Australia. Our results show that most crocodiles sampled (size 0.85-4.2 m, with 76% of them being >2.5 m) consume a large variety of prey, however a large proportion of their nutrition is derived from terrestrial prey. Introduced species such as water buffaloes (*Bubalus bubalis*) and pigs (*Sus scrofa*) could contribute between 53 and 84% to the nutrition of the sampled crocodiles. The isotopic composition of large crocodiles (total length >3 m) suggested possible increase in marine prey consumption with size ( $R^2=0.30$ ;  $p=0.005$ ). Additionally, we found crocodiles sampled in the dry season had on average higher terrestrial contributions compared to crocodiles sampled during the wet season ( $84.1 \pm 2.4\%$  versus  $55.4 \pm 7.0\%$ ). Overall, we found that terrestrial prey are important source of nutrition for many crocodiles in this region where introduced herbivorous mammals are abundant.

Solórzano, A., Rincón, A.D., Cidade, G.M., Núñez-Flores, M. and Sánchez, L. (2018). Lower Miocene alligatoroids (Crocodylia) from the Castillo Formation, northwest of Venezuela. *Palaeobiodiversity and Palaeoenvironments* (doi.org/10.1007/s12549-018-0332-5).

**Abstract:** Crocodyliform diversity was particularly high during the middle and late Miocene of South America, with up to 12 species recovered from a single geological unit. Nonetheless, the early Miocene fossil record of low-latitude vertebrates is scarce; hence, crocodylians remain poorly known in the region. The Castillo Formation, located in the northwest of Venezuela, preserves an interesting vertebrate fauna with a well-constrained late early Miocene age. Previous work dealing with crocodylians of this formation only recorded three taxa: the gavialoid *Siquisiquesuchus venezuelensis* and *Gryposuchus* sp. and indeterminate alligatoroid remains. New cranial and mandibular material recently recovered from the Castillo Formation allows us to document four previously unrecognised alligatoroid forms: *Purussaurus* sp., *Caiman* sp., an indeterminate caimanine and an indeterminate alligatoroid. With six taxa, the crocodylian assemblage reveals a previously undocumented relatively high taxonomic diversity in the early Miocene. The Castillo crocodylians show a broad range of morphological disparity and body sizes ranging from small (2.5 m-62 kg) to large (7.5 m-1600 kg) taxa. Thus, crocodylian niche partition, as well as the abundance and variety of resources and environmental heterogeneity of aquatic ecosystems in South America, were already established by at least the early Miocene. The presence of *Caiman* in ~18 Ma strata represents the unequivocally earliest record of the taxon in South America and allows us to propose that the origin of the jacareans is more likely to have occurred during or before the early Miocene, challenging previous molecular hypotheses.

Hinay, Jr., A.A. and Sarol, L.D.C. (2018). *In vitro* inhibition kinetics of *Crocodylus mindorensis* (Philippine crocodile) serum against human immunodeficiency virus Type I Reverse Transcriptase. *Advances in Animal and Veterinary Sciences* (doi: <http://dx.doi.org/10.17582/journal.aavs/2018/6.5.213.218>).

**Abstract:** The study aimed to determine the inhibition kinetics of *Crocodylus mindorensis* (Philippine crocodile) serum against recombinant HIV-1 Reverse Transcriptase (RT) using Enzyme-linked Immunosorbent Assay. The study utilized an experimental design where different concentrations of Philippine crocodile serum were used to determine its quantitative inhibitory effect and mechanism of inhibition against the HIV-1 reverse transcriptase activity. A colorimetric enzyme immunoassay that incorporates digoxigenin and biotin-labeled dUTP onto DNA were used for the assay (Roche Version 13.0, 2010). In addition, the kinetic activity of HIV-1 Reverse transcriptase was studied to determine the mechanism of enzyme inhibition by Philippine crocodile serum. A minimum of 2 mL serum sample from each of the seven (7) purposively selected *Crocodylus mindorensis* were collected at the Animal clinic of the Davao Crocodile Park facility. The results showed that the HIV-1 Reverse transcriptase activity was inhibited by high as  $92.93 \pm 0.72\%$  (CM-07) at 0.5 vol/vol% and as low as  $4.66 \pm 20.76$  at 4 vol/vol% (CM-04) of Philippine crocodile serum. The average half maximal inhibitory concentration (IC<sub>50</sub>) of the seven crocodile serum samples was 1.47% vol/vol. The  $92.93 \pm 0.72\%$  inhibition of 0.5% vol/vol of Philippine crocodile serum has almost the same inhibition as the positive control Nevirapine ( $92.64 \pm 0.20\%$ ). The inhibition kinetics of the Philippine crocodile suggests a potential novel HIV-1 Reverse Transcriptase inhibitor.

Fernandez Blanco, M.V., Cassini, G.H. and Bona, P. (2018). Skull ontogeny of extant caimans: a three-dimensional geometric morphometric approach. *Zoology* 129: 69-81.

**Abstract:** Ontogenetic variation of cranial characters used in crocodylian phylogenetic systematics has never been studied. Furthermore, the relationship between diet and skull morphological

transformation during ontogeny has not been properly explored yet. We quantify the inter- and intraspecific skull morphological variation in extant caiman species focusing on those areas relevant to systematics and, also investigate the relation between diet and morphological changes during ontogeny. We applied a three-dimensional approach of geometric morphometrics on post-hatching ontogenetic cranial series of *Caiman latirostris* and *C. yacare*. In order to incorporate incomplete material, we additionally tested four different methods of missing landmark estimation and apply the thin-plate spline interpolation. We detected morphological changes between species and during ontogeny (snout and pterygoid flanges increase their proportions and, orbits, temporal fenestrae, skull roof and foramen magnum decrease their relative size) that constitutes part of a general morphological change in the cranial ontogeny of crocodylians. Moreover, the negative allometry of the fenestrae and neurocranium and the positive allometry of the splanchnocranium in both caiman species are the plesiomorphic condition, at least, for tetrapods. Shape changes during growth were found to be related to ontogenetic changes in the diet. Dissimilarities between species seem to be related to different mechanical requirements and different use of the habitat. We found inter- and intraspecific variation in some morphological characters with systematic implications (the contact of nasals with naris, the contact of prefrontals in the midline, and the bones that border the suborbital fenestra and the proportion in which one of them participates) that are not currently considered in phylogenetic analyses.

Tisdale, R.K., Lesku, J.A., Beckers, G.J.L. and Rattenborg, N.C. (2018). Bird-like propagating brain activity in anesthetized Nile crocodiles. *Sleep* 41(8) (<https://doi.org/10.1093/sleep/zsy105>).

**Abstract:** The changes in electroencephalogram (EEG) activity that characterize sleep and its sub-states - slow-wave sleep (SWS) and rapid eye movement (REM) sleep - are similar in mammals and birds. SWS is characterized by EEG slow waves resulting from the synchronous alternation of neuronal membrane potentials between hyperpolarized down-states with neuronal quiescence and depolarized up-states associated with action potentials. By contrast, studies of non-avian reptiles report the presence of high-voltage sharp waves (HShW) during sleep. How HShW relate to EEG phenomena occurring during mammalian and avian sleep is unclear. We investigated the spatiotemporal patterns of electrophysiological phenomena in Nile crocodiles (*Crocodylus niloticus*) anesthetized with isoflurane to determine whether they share similar spatiotemporal patterns to mammalian and avian slow waves. Recordings of anesthetized crocodiles were made using 64-channel penetrating arrays with electrodes arranged in an 8 × 8 equally spaced grid. The arrays were placed in the dorsal ventricular ridge (DVR), a region implicated in the genesis of HShW. Various aspects of the spatiotemporal distribution of recorded signals were investigated. Recorded signals revealed the presence of HShW resembling those reported in earlier studies of naturally sleeping reptiles. HShW propagated in complex and variable patterns across the DVR. We demonstrate that HShW within the DVR propagate in complex patterns similar to those observed for avian slow waves recorded from homologous brain regions. Consequently, sleep with HShW may represent an ancestral form of SWS, characterized by up-states occurring less often and for a shorter duration than in mammals and birds.

Beauchamp, J.S., Hart, K.M., Cherkiss, M.S. and Mazzotti, F.J. (2018). Variation in home range size and patterns in adult female American crocodiles *Crocodylus acutus*. *Endangered Species Research* 36: 161-171.

**Abstract:** The American crocodile *Crocodylus acutus* is a threatened species that uses relatively deep, open-water habitats with low salinity. Adult female American crocodiles nest on sandy coastal beaches, islands or human-made berms, assist in the hatching process, and can travel long distances to nesting habitat. We satellite-

tracked 15 adult female American crocodiles in 2 hydrologically distinct areas in Everglades National Park, Florida, USA, to quantify the home range sizes, test for intraspecific differences in home range and core area size and structure, and identify important crocodile high-use areas. Overall home ranges (95% kernel density estimate; KDE) for adult female crocodiles in South Florida ranged from 30.0 to 141.9 km<sup>2</sup> (mean  $\pm$  SD, 84.4  $\pm$  32.3 km<sup>2</sup>), and core areas (50% KDE) ranged from 4.7 to 27.4 km<sup>2</sup> (17.8  $\pm$  7.3 km<sup>2</sup>). We identified patterns in home range and core area overlap, seasonally shifting patterns in core area use, and the Fox Lake complex as an important crocodile high-use area. As the population of American crocodiles continues to grow and expand into new areas, it is important for conservation managers to understand individual crocodile habitat-use patterns and spatial resource requirements.

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Miranda, E.B.P. (2018). Reintroducing apex predators: the perils of muddling guilds and taxocenoses. *R. Soc. open sci.* 5: 180567.

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Sena, M.V.A., Andrade, R.C.L.P., Sayão, J.M. and Oliveira, G.R. (2018). Bone microanatomy of *Pepesuchus deiseae* (Mesoeucrocodylia, Peirosauridae) reveals a mature individual from the Upper Cretaceous of Brazil. *Cretaceous Research* 90: 335-348.

**Abstract:** Peirosauridae is placed within Notosuchia with members found in Cretaceous deposits of Africa and South America. These crocodyliforms were considered most terrestrial predators, according to their morphological features. Our data bring contributions to infer about peirosaurids habits from the bone histology and to inform the autoecology of the *Pepesuchus deiseae* (MN 7466-V). We prepared thin sections of the appendicular bones and an osteoderm from MN 7466-V. Both metacarpals show a broad cortex and a tiny marrow cavity, one has seven cyclical growth marks and Haversian bone in the endosteal region. The ulnare has a highly porous bone with several resorption cavities. The osteoderm is composed of parallel-fibred bone (PFB) forming the basal and external cortex and woven-fibred bone (WFB) constituting the internal core. The tibia shows the presence of external fundamental system (EFS) and the cortex presents WFB and PFB intercalated. The metacarpals exhibit osteosclerosis and the ulnare shows osteoporotic-like bone. In the metacarpals, the occurrence of bone mass increase would have implied rather life in shallow-water environments. The ulnare shows a reduction in bone mass, providing a better maneuverability between its joints with the carpal and ulna. The tibia was the only element indicating the specimen reached the somatic maturity by the presence of EFS. Therefore, this bone was used as a proxy to the maximum growth rate. Currently, the presence of WFB in advanced ontogenetic stage indicates a peculiar feature solely recorded in eusuchian long bones. The resorption cavities in the internal core of the osteoderm probably indicate bone resorption for calcium mobilization during the eggshell formation. In addition, the Haversian bone is an indication that metacarpal A belonged to mature individual, which had accomplished some ovogenetic cycles. In conclusion, our analyses suggest that MN 7466-V was an adult and mature female individual.

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Bombieri, G., Nanni, V., del Mar Delgado, M., Fedriani, J.M., López-Bao, J.V., Pedrini, P. and Penteriani, V. (2018). Content analysis of media reports on predator attacks on humans: Toward an understanding of human risk perception and predator acceptance. *BioScience*. 68(8): 577-584.

**Abstract:** Public tolerance toward predators is fundamental in their conservation and is highly driven by people's perception of the risk they may pose. Although predator attacks on humans are rare, they create lasting media attention, and the way the media covers them might affect people's risk perception. Understanding how mass media presents attacks and how this can affect perception will provide insights into potential strategies to improve coexistence with these species. We collected media reports of predator attacks

on humans and examined their content. Almost half (41.5%) of the analyzed reports contained graphic elements. Differences in framing between species groups or species were found, with sharks and leopards having the highest proportion of graphic reports, whereas canids and bears had the highest number of neutral reports. This bias in coverage, instead of providing insights into the causes of these incidents and possible remedies, may provoke fear and decrease support for predator conservation.

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Choudhary, S., Choudhury, B.C. and Gopi, G.V. (2018). Spatio-temporal partitioning between two sympatric crocodylians (*Gavialis gangeticus* & *Crocodylus palustris*) in Katarniaghat Wildlife Sanctuary, India. *Aquatic Conservation* (<https://doi.org/10.1002/aqc.2911>).

**Abstract:** *Gavialis gangeticus* (Gharial) and *Crocodylus palustris* (Mugger) have a sympatric distribution in the northern and eastern river systems of India, but no single extensive study exists on the mechanisms favouring the coexistence of these species, or explains whether Mugger have a detrimental effect on Gharial. For the effective conservation of gharial within its natural range it is very important to study the impacts of various habitat attributes on its distribution. This study was undertaken to investigate how resources are shared by the species, and the key requirements that lead to the selection of a particular basking and nesting site. Results indicate that the habitat variables that lead to the selection of basking sites differed between the species, and varied between seasons. In winter, habitat variables responsible for the selection of a basking site by gharial were slope, height, soil moisture, presence of sandbar, distance to water, and current land-use pattern, whereas for Mugger the relevant habitat variables were slope and height only. During the summer, habitat use, depth gradient, and distance to water were factors for Mugger, whereas slope, height, soil moisture, the presence of a sandbar, water depth gradient, and distance to water were important habitat attributes for Gharial. Gharial and Mugger had a totally different preference of habitat attributes for nest site selection. Gharial laid eggs near water and only in sandy soil, whereas mugger nests were found far from a water source and no substrate preference was shown. Effective habitat management is imperative for the conservation of gharial. Restocking with individuals bred in captivity might increase the population in the short term, but in order to create a viable population, long-term habitat conservation plans are required.

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Mendes S. Cardia, F., Miloni Santucci, R., Elias Bernardi, J.V., Brandalise deAndrade, M. and Maia de Oliveira, C.E. (2018). Mercury concentrations in terrestrial fossil vertebrates from the Bauru Group (Upper Cretaceous), Brazil and implications for vertebrate paleontology. *Journal of South American Earth Sciences* 86: 15-22.

**Abstract:** In this study we determined total mercury concentrations (THg) in a set of fossils from the Upper Cretaceous Bauru Group, Brazil, and investigated how the incorporation of this element occurs in fossil organisms and in their paleoenvironment. The analyzed fossil specimens were collected from two different locations (Jales and Fernandópolis) of the Adamantina Formation and correspond to samples of teeth, bones, osteoderms, and crocodylomorph eggs (probably laid by Baurusuchidae), and their associated sediments. The samples were submitted to Zeeman atomic absorption spectrometry, showing concentrations ranging from approximately 5 ng.g<sup>-1</sup> to 77 ng.g<sup>-1</sup> in biomineral matrix. The results show mild fluctuations of Hg concentration in the different types of fossils analyzed, with teeth and bones retrieving more Hg than osteoderms, thus being the most suitable for this type of analysis. Adult specimens of Baurusuchidae showed ~30% more Hg than juvenile ones in their biomineralized tissues, which points to a continuous process of Hg accumulation throughout the individual's life history. As for the determination of Hg in sedimentary rocks, which has been previously claimed to be an indication of the relationship between

volcanic activities and some mass mortality events (MMEs), the analysis of Hg in fossils can furnish additional information for future studies regarding the relationship between disturbances in the Hg cycle triggered by volcanic emissions and MMEs. Additionally, the results also suggest that the concentration of Hg in fossil vertebrates can be used as a tool for discussions about ontogenetic stages and, in some very particular cases, identification of female individuals.

Hamilton, M.T., Finger, Jr., J.W., Elsey, R.M., Mastro Monaco, G.F. and Tuberville, T.D. (2018). Corticosterone in American alligator (*Alligator mississippiensis*) tail scutes: Evaluating the feasibility of using unconventional samples for investigating environmental stressors. *General and Comparative Endocrinology* (<https://doi.org/10.1016/j.ygcen.2018.07.008>).

**Abstract:** Baseline plasma corticosterone (CORT) concentrations have been widely used to investigate the effects of stressors in wild and captive crocodylians. However, collecting baseline plasma CORT samples from wild crocodylians may be particularly difficult due to the capture and handling protocols used for large individuals. Thus, it may prove beneficial to use recently modified techniques for extracting CORT deposited in keratinized and non-keratinized tissues to better quantify the effects of long-term stress in crocodylians. In this study, we investigated the feasibility of using American alligator (*Alligator mississippiensis*) tail scute tissues to quantify CORT by collecting blood and tail scutes from 40 alligators before and after a short-term handling stressor. The objective of the current study was to better understand CORT deposition in crocodylian scutes and whether short-term increases in CORT could be detected. We found that CORT can be reliably extracted from alligator scute tissue and quantified using a commercially available enzyme immunoassay. However, there was a significant increase in scute CORT concentrations following an alligator being exposed to a short-term stressor ( $p=0.017$ ), although the magnitude of change was less than observed in plasma samples from the same individuals ( $p=0.002$ ). Furthermore, our results indicate that there was a significant effect of body condition on an alligator's post-stressor CORT concentration ( $p=0.02$ ). While our study is among the first to experimentally examine the usefulness of tissue CORT in crocodylians, a combination of field and laboratory experiments are needed to better understand deposition rates of CORT in scute tissues and to further validate the usefulness of tissue glucocorticoids for evaluating the effects of stress.

Chen, K., Wu, M., Zhang, Y., Zhang, F., Wang, H., Liang, J., Yan, P., Li, E., Yao, L., Xu, J. and Wu, X. (2018). Two introduced crocodile species had changed reproductive characteristics in China. *Animal Reproduction Science* 196: 150-159.

**Abstract:** The purpose of this study was to study the reproductive characteristics of the Nile crocodile and Siamese crocodiles after introduction into China since the time this occurred near the end of the last century. The data for the eggs and young crocodiles (recently hatched crocodiles) of two introduced species were collected at a Sanya crocodile breeding farm in Hainan. The characteristic variables of crocodile eggs were statistically analyzed, and the results indicated that: egg mass of the Nile and Siamese crocodile was significantly correlated with the egg length and width. Regression analyses were used to develop the linear equation between the egg length, egg width and egg mass. There was a strong positive correlation between the egg mass and initial weight of young crocodiles. The linear equation for assessing egg mass and initial weight of young crocodile was developed for regression analyses. There was no significant linear relationship between clutch size and egg characteristics. Mating time of the Nile crocodile in Hainan (November-April) and the spawning season (March-May) are significantly earlier than in the Zimbabwe region of origin. The average of clutch size and the mean size of eggs for Nile crocodiles in their native habitat is greater than the introduced region as indicated by analyzing data using a two-sample t-test. The Siamese crocodile spawning time was similar in

the Hainan and Zimbabwe regions, but the size of clutches and the mean size of eggs in the introduced region were greater than in their native region as indicated by results using a two-sample t-test.

Becker, M., Souza, M.A., Moraes, L.G., Silva, G.S., Antoniassi, N.A.B., Souza, R.L. and Colode, E.M. (2018). Bone quality evaluation of experimental osteometabolic disease in Pantanal alligators (*Caiman yacare*) by High Resolution Computerized Microtomography ( $\mu$ CT). *Pesquisa Veterinária Brasileira* 38(5) (<http://dx.doi.org/10.1590/1678-5150-pvb-5725>).

**Abstract:** Computerized microtomography is the gold standard examination for the evaluation of the three-dimensional bone structure. This experiment was developed to evaluate the structure and bone quality of *Caiman yacare* with metabolic bone disease using high resolution computerized microtomography ( $\mu$ CT). The animals were distributed into four groups: G1 - hyperphosphatemic diet with sun exposure deprivation ( $n=4$ ), G2 - hyperphosphatemic diet with sun exposure ( $n=4$ ), G3 - balanced diet with sun exposure deprivation ( $n=4$ ), and G4 - balanced diet with exposure to sunlight ( $n=4$ ). The parameters for the trabecular bone (Trabecular Number, Trabecular Thickness, Trabecular Separation, Bone Pattern Factor, Fractal Dimension, Euler Number, Structural Model Index, Degree of Anisotropy, Eigenvalues 1, 2 and 3, and Centroids X, Y and Z), and cortical bone (Number of Closed Pores, Volume of Closed Pores, Surface of Closed Pores, Closed Porosity, Volume of Open Pores, Open Porosity and Total Porosity). The overall results showed that the structure and bone quality of group G3 and G4 were better than those of groups G1 and G2, and that the diet factor influenced more than the sun exposure factor. The computerized microtomography allowed to evaluate the quality of the cortical and trabecular bones of the Pantanal alligator tibia with osteometabolic disease. The diet and sun exposure factors influenced individually the results of the  $\mu$ CT parameters between the groups, demonstrating the functional and structural complexity. Thus, these parameters can contribute to the interpretation of the mechanical behavior of bones and correlate them with the risk of lesions and fractures associated with osteometabolic diseases.

Leiva, P.M.L., Labaque, M.C., Fernandez, M.E., Piña, C.I. and Simoncini, M.S. (2018). Physical and chemical characteristics of fertile and infertile eggs of wild *Caiman latirostris*. *Aquaculture* 497: 287-291.

**Abstract:** In oviparous species, physico-chemical characteristics of eggs are an indicator of maternal physiological traits and environmental conditions of the site where the females develop. In this study, we aim to (a) determine the physico-chemical characteristics of eggs from wild *Caiman latirostris* populations; (b) evaluate if fertile and infertile eggs differ in physico-chemical characteristics associated with such reproductive success; and (c) compare the present results with findings in other crocodylian species to identify characteristics that would be associated with reproductive biology and adaptations to their environments. Eggs were collected and identified, and different morphometric variables (percentage of components, and protein, total lipids and fatty acid composition) were determined. Fertile and infertile eggs did not differ in weight or component (eggshell, yolk and albumen) percentages of total egg weight. Fatty acid profiles of yolk differed between fertile and infertile eggs in C14:0, C16:1 and C17:1. However, the comparison of profiles among clutches showed differences in 12 fatty acids. We also observed differences in percentage of egg components respect to other crocodylian species, particularly of albumen, which had a lower proportion in *C. latirostris*. Our results suggest that egg infertility may not be related to maternal lipid investment. We propose using yolk of infertile eggs (which is similar to that of fertile eggs) as a non-invasive method to identify and evaluate the difference in FA composition of eggs for breeding females of various diets.

Zimmerman L.M. (2018). Reptilia: Humoral immunity in reptiles. Pp. 751-772 in *Advances in Comparative Immunology*, ed. by E. Cooper. Springer: Cham.

**Abstract:** Reptiles are a diverse group which include four orders: Crocodylia, Testudines, Tuatara, and Squamata. Though they differ greatly in a number of characteristics, they tend to use their humoral immune response in a similar manner. Compared to mammals, reptiles tend to have a slower and less robust humoral immune response. Reptiles may instead rely on a non-specific component of the humoral immune response: natural antibodies. Phagocytic B cells have also been identified in reptiles, and further studies are needed to explore this additional innate-like function of the humoral response in a variety of reptiles. Because reptiles are ectothermic, temperature can impact their immune responses both during development and as adults. Their immune system is also strongly affected by season. In addition, because reptiles typically have long life spans and can have indeterminate growth, they are an intriguing taxon in which to study aging. Life-history characteristics of reptiles provide many possibilities for understanding the context in which the immune strategy of reptiles evolved and how they may respond to climate change and disease introduction.

Jensen, B. and Smit, T.H. (2018). Examples of weak, if not absent, form-function relations in the vertebrate heart. *J. Cardiovasc. Dev. Dis.* 5(3): 46.

**Abstract:** That form and function are related is a maxim of anatomy and physiology. Yet, form-function relations can be difficult to prove. Human subjects with excessive trabeculated myocardium in the left ventricle, for example, are diagnosed with non-compaction cardiomyopathy, but the extent of trabeculations may be without relation to ejection fraction. Rather than rejecting a relation between form and function, we may ask whether the salient function is assessed. Is there a relation to electrical propagation, mean arterial blood pressure, or propensity to form blood clots? In addition, how should the extent of trabeculated muscle be assessed? While reviewing literature on trabeculated muscle, we applied Tinbergen's four types of causation - how does it work, why does it work, how is it made, and why did it evolve - to better parse what is meant by form and function. The paper is structured around cases that highlight advantages and pitfalls of applying Tinbergen's questions. It further uses the evolution of lunglessness in amphibians to argue that lung reduction impacts on chamber septation and it considers the evolution of an arterial outflow in fishes to argue that reductions in energy consumption may drive structural changes with little consequences to function. Concerning trabeculations, we argue they relate to pumping function in the embryo in the few weeks before the onset of coronary circulation. In human fetal and postnatal stages, a spectrum of trabeculated-to-compact myocardium makes no difference to cardiac function and in this period, form and function may appear unrelated.

Piacentini Pinheiro, A.E., da Costa Pereira, P.V.L.G., de Souza, R.G., Brum, A.S., Lopes, R.T., Machado, A.S., Bergqvist, L.P. and Simbras, F.M. (2018). Reassessment of the enigmatic crocodyliform "*Goniopholis*" *paulistanus* Roxo, 1936: Historical approach, systematic, and description by new materials. *PLoS ONE* 13(8): e0199984.

**Abstract:** The Crocodyliformes are the most represented vertebrate clade in the Upper Cretaceous sequences of the Bauru Group, Paraná Basin. However, some of the species described have an uncertain taxonomic status and phylogenetic position. For instance, "*Goniopholis*" *paulistanus* has been assigned as a *nomem dubium*, due to its description being based on scarce material. The "*G.*" *paulistanus* specimens (ie teeth and a left tibia) were discovered in two different localities in São Paulo state: Mirandópolis and Valparaíso municipalities; where the upper interval of the Adamantina Formation (Early Maastrichtian of Bauru Group) crops out. Revisiting these specimens, we observed multicrenulated teeth

in middle dentary tooth row, a remarkable feature only shared with teleosaurids *Machimosaurus hugii* (Upper Jurassic of Laurasia) and *M. rex* (Lower Cretaceous of Tunisia). This apomorphy was also recognized in new material from the Alfredo Marcondes municipality (Presidente Prudente Formation), which are here also referred to "*G.*" *paulistanus*. We recognized the teeth of "*G.*" *paulistanus* as the lectotype, however the tibia cannot be assigned to a species as it was not collected in association with the teeth. We performed a phylogenetic analysis with a data matrix composed of 388 characters and 86 taxa, analyzed in TNT. The strict consensus tree recovered Neosuchia and Ziphosuchia (Notosuchia + Sebecia) within Mesoeucrocodylia. The species "*G.*" *paulistanus* is valid, as a distinct and new genus within Sebecia, in a polytomy with *Barreirosuchus*, *Pepesuchus*, *Itasuchus* and *Peirosaurus*, forming the clade Itasuchidae. *Stolokrosuchus* is the sister taxon to Itasuchidae, the sister group of all other Sebecia (Peirosauridae (Mahajangasuchidae + Sebecidae and taxa *affinis*)). The clades Ziphosuchia, Sebecia and Itasuchidae are here redefined, and we find the last two clades to be more closely related to terrestrial notosuchids than to semiaquatic neosuchians.

Hickey, A.P., Du, C., Jiang, X., Polsky, G., Rodriguez, M., Kuldilok, K. and Suchato, R. (2018). CDIP: Introducing Crocodile Oil to ASEAN+3. Chapter 7 in *Asian Agribusiness Management*. ([https://doi.org/10.1142/9789813233140\\_0007](https://doi.org/10.1142/9789813233140_0007)).

Leite, K.J. and Fortier, D.C. (2018). The palate and choanae structure of the *Susisuchus anatoceps* (Crocodyliformes, Eusuchia): Phylogenetic implications. *PeerJ* 6:e5372.

**Abstract:** Crocodyliformes is a group with a broad fossil record, in which several morphological changes have been documented. Among known transformations the most iconic is perhaps the series of changes seen in the structural evolution of the choanae. The change in the position of the choanae was important during the evolutionary history of the Crocodyliformes. This structure is relevant in the phylogenetic position of many crocodyliforms. The new skull of *Susisuchus anatoceps* from the Crato Formation of the Santana Group (Lower Cretaceous) is described and the preservation in the ventral view allows character encoding not yet observed for the species. The new specimen shows a typical eusuchian palate for *Susisuchus anatoceps*, in which the choana is fully enclosed by the pterygoid. The *Susisuchidae* clade has been placed in different phylogenetic positions: as a sister group of Eusuchia, advanced Neosuchia and in Eusuchia. In *Isisfordia* there are reports that the choana of this taxon is or is not fully enclosed by the pterygoid. The encoding of the ventral characters of *S. anatoceps* places *Susisuchidae* in Eusuchia. However, this position must be further studied, since the matrices showed fragility in the reconstitution of the Neosuchia-Eusuchia transition.

Henderson, D.M. (2018). A buoyancy, balance and stability challenge to the hypothesis of a semi-aquatic *Spinosaurus* Stromer, 1915 (Dinosauria: Theropoda). *PeerJ* 6:e5409.

**Abstract:** A recent interpretation of the fossil remains of the enigmatic, large predatory dinosaur *Spinosaurus aegyptiacus* Stromer 1915 proposed that it was specially adapted for a semi-aquatic mode of life - a first for any predatory dinosaur. To test some aspects of this suggestion, a three-dimensional, digital model of the animal that incorporates regional density variations, lungs and air sacs was generated, and the flotation potential of the model was investigated using specially written software. It was found that *Spinosaurus* would have been able to float with its head clear of the water surface, although it was laterally unstable and would tend to roll onto its side. Similarly detailed models of another spinosaurid *Baryonyx (Suchomimus) tenerensis* Sereno *et al.* 1998, along with models of the more distantly related *Tyrannosaurus rex* Osborn 1905, *Allosaurus fragilis* Marsh 1877, *Struthiomimus altus* Lambe 1902, and *Coelophysis bauri* Cope 1887 were also able to float in positions

that enabled the animals to breathe freely, showing that there is nothing exceptional about a floating *Spinosaurus*. Validation of the modelling methods was done with floated models of an alligator and an emperor penguin. The software also showed that the center of mass of *Spinosaurus* was much closer to the hips than previously estimated, similar to that observed in other theropods, implying that this dinosaur would still have been a competent walker on land. With its pneumatized skeleton and a system of air sacs (modelled after birds), the *Spinosaurus* model was found to be unsinkable, even with its lungs deflated by 75%, and this would greatly hinder a semi-aquatic, pursuit predator. The conclusion is that *Spinosaurus* may have been specialized for a shoreline or shallow water mode of life, but would still have been a competent terrestrial animal.

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Merchant, M., Savage, D., Cooper, A., Slaughter, M., Perkin, J.S. and Murray, C.M. (2018) Nest attendance patterns in the American alligator (*Alligator mississippiensis*). *Copeia* 106(3): 421-426.

**Abstract:** The behavioral variation in alligator nest attendance has characterized the species as iconic in common lore and perplexed biologists for decades. Here, we quantify patterns in nest attendance among mothers as well as variation in such patterns throughout two nesting seasons. We employed camera traps controlled by circuit boards to capture time-lapse photographs of alligator nest areas for the duration of each nesting season. Data revealed a bimodal pattern of nest attendance over time that significantly varied across incubation days in both 2011 and 2012, and also differed between years. Nest attendance also differed among hours in the diel cycle, and this pattern was the same for both years. Nest visits were frequent immediately after the eggs were laid, and attendance behavior attenuated rapidly after the first week of incubation. Nest visitation then increased near the end of the incubation period with the largest portion of visits recorded during hatching and the maternal movement of hatchlings away from nest sites. While the extent of this pattern varied between years, the pattern itself did not. The majority of attendance behavior occurred during night hours, with little visitation recorded between 1000 and 1600 h. Our study is the first to document temporal variation in alligator nest attendances at daily, seasonal, and annual temporal scales, and our findings suggest nighttime visits during oviposition and hatching periods are consistent among years.

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Bona, P., Ezcurra, M.D., Barrios, F. and Fernandez Blanco, M.V. (2018). A new Palaeocene crocodylian from southern Argentina sheds light on the early history of caimanines. *Proceedings of the Royal Society B* 285(1885). (doi: 10.1098/rspb.2018.0843).

**Abstract:** Caimanines are crocodylians currently restricted to South and Central America and the oldest members are from lower Palaeocene localities of the Salamanca Formation (Chubut Province, Argentina). We report here a new caimanine from this same unit represented by a skull roof and partial braincase. Its phylogenetic relationships were explored in a cladistic analysis using standard characters and a morphogeometric two-dimensional configuration of the skull roof. The phylogenetic results were used for an event-based supermodel quantitative palaeobiogeographic analysis. The new species is recovered as the most basal member of the South American caimanines, and the Cretaceous North American lineage '*Brachychampsia* and related forms' as the most basal Caimaninae. The biogeographic results estimated north-central North America as the ancestral area of Caimaninae, showing that the Cretaceous and Palaeocene species of the group were more widespread than thought and became regionally extinct in North America around the Cretaceous-Palaeocene boundary. A dispersal event from north-central North America during the middle Late Cretaceous explains the arrival of the group to South America. The Palaeogene assemblage of Patagonian crocodylians is composed of three lineages of caimanines as a consequence of independent dispersal events that occurred between North and South America and within South America around the Cretaceous-Palaeogene boundary.

Feng, P. and Liang, S. (2018). Molecular evolution of umami/sweet taste receptor genes in reptiles. *PeerJ* 6:e5570.

**Abstract:** Sensory systems play an important role in animal survival. Changes to these systems may be critical in evolution of species in new environments. Previous studies exploring the correlation between feeding ecology and *Tas1r* evolution mainly focused on mammals and birds, and found that the relationship was complex. However, in reptiles, the correlation between *Tas1r* evolution and dietary preferences is still unclear. Here, we attempted to explore this relationship in representative species of the major groups of reptiles (turtles, snakes, lizards, crocodylians), for which the genome information is known. We first predicted the functionality (intact, partial, or defective) of *Tas1r*, and then related it to the feeding preferences. As a result, we identified 11 *Tas1r1*, 12 *Tas1r2*, and 12 *Tas1r3* genes to be partial or intact and another 22 *Tas1r* genes to be absent or pseudogenized in the 19 reptiles. We found that, as it was revealed in some other vertebrate groups, no correlation existed between feeding ecology and *Tas1r* evolution in reptiles: genomic prediction indicated that the *Tas1r* genes possibly have been lost or pseudogenized in snakes, but in crocodylia and testudines *Tas1r* genes are either intact or partial, regardless of their feeding habits. Thus, we suggest that the driving force of *Tas1r* evolution in reptiles is complex, and the feeding habit of swallowing food whole without chewing or the absence of taste buds in certain species may account for the possible umami/sweet perception loss. In addition, we propose that caution should be taken when predicting gene functionality from the publicly available genome database.

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Von Baczko, M.B., Taborda, J.R.A. and Desojo, J.B. (2018). Paleoneuroanatomy of the aetosaur *Neoaetosauroides engaeus* (Archosauria: Pseudosuchia) and its paleobiological implications among archosauriforms. *PeerJ* 6:e5456.

**Abstract:** The paleoneuroanatomy of pseudosuchian archosaurs is poorly known, based on direct examination of the internal morphology of braincases and a few artificial endocasts. Among aetosaurs, only one endocast has been described almost a century ago by Case (1921) corresponding to *Desmotosuchus spurensis* from the Chinle Formation (Norian) of Texas, US, based on a resin cast. Here, we describe the first natural endocast of an aetosaur, *Neoaetosauroides engaeus* from the Los Colorados Formation (Norian) of NW Argentina, and also developed the first digital endocast of this taxon including the encephalon, cranial nerves, inner ear, and middle ear sinuses. The neuroanatomy of *N. engaeus* exhibits several differences from that of *D. spurensis* despite their phylogenetic proximity, which may be a reflection of their different habits. The information provided by the endocasts of *N. engaeus* about its olfactory region and the orientation of its head, based on the inclination of the inner ear, could support the proposal for an animalivorous diet, instead of an herbivorous one as in most aetosaurs. The new information here obtained contributes to the knowledge of the neuroanatomical diversity of archosauriforms and more specifically among pseudosuchians and their paleobiological roles in the Triassic continental communities.

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Strickland, B.A., Vilella, F.J. and Flynt, R.D. (2018). Long-term spotlight surveys of American alligators in Mississippi, USA. *Herpetological Conservation and Biology* 13(2): 331-340.

**Abstract:** Accurate population estimates and assessments of trajectory are an essential part of harvest management for game species and conservation action plans for protected species. Long-term monitoring can lead to ecological understanding by identifying biotic and abiotic drivers of population dynamics. Spotlight surveys are a widely used method to monitor abundance and size-class structure of crocodylian populations. The American alligator (*Alligator mississippiensis*) has recovered from significant population reductions in the southeastern United States. The Mississippi Department of Wildlife, Fisheries, and Parks (MDWFP) has conducted alligator spotlight surveys since 1971 to monitor

populations. We analyzed this long-term alligator survey dataset to assess possible trends in counts as a proxy for potential population changes. We tested for a positive trend in count data over 46 y and evaluated covariates that could influence counts to assist future survey protocols. Alligator counts during 1971-2016 increased across survey routes in Mississippi. This observed positive response may represent an increase of the alligator population in Mississippi as a result of conservation benefits accrued from improved wetland conditions and species-specific management policies. Evaluation of survey covariates indicated recent rainfall and increasing wind velocity had negative effects on alligator counts while increasing water temperature had a positive effect. Implementing robust survey techniques will improve the reliability of alligator monitoring data and their application to the management of alligator populations. Further, these improved approaches may be useful to other conservation and management agencies as well as for other crocodylian species.

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Pérez-Flores, J. (2018). Predation of an adult female Morelet's crocodile (*Crocodylus moreletii*) by a jaguar (*Panthera onca*) in the Calakmul region, Mexico. *Herpetology Notes* 11: 613-616.

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Serrano-Martinez, A., Knoll, F., Narvaez, I., Lautenschlager, S. and Ortega, F. (2018). Inner skull cavities of the basal eusuchian *Lohuecosuchus megadontos* (Upper Cretaceous, Spain) and neurosensory implications. *Cretaceous Research* (<https://doi.org/10.1016/j.cretres.2018.08.016>).

**Abstract:** The Late Cretaceous fossil site of Lo Hueco (Cuenca, Spain) has yielded a large collection of tetrapod remains, in which crocodyliforms are one of the best represented groups. The crocodyliforms from Lo Hueco helped establish Allodaposuchidae as one of the sister-groups of Crocodylia. A complete skull of the holotype of the allodaposuchid *Lohuecosuchus megadontos* was CT scanned, and all its inner cavities, including those of the brain, nerves, inner ear and blood vessels, as well as the paratympanic sinus system and the paranasal sinuses, were three-dimensionally reconstructed and compared to those of several extant and extinct crocodylians. The endocranial anatomy of *L. megadontos* is congruent with its phylogenetic position as a basal eusuchian. Our work suggests that some of the neurosensory capabilities found in the crown-group Crocodylia such as an acute sense of olfaction and a low frequencies hearing are already present at the base of Eusuchia.

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Farlow, J.O., Robinson, N.J., Turner, M.L., Black, J. and Gatesy, S.M. (2018). Footfall pattern of a bottom-walking crocodile (*Crocodylus acutus*). *PALAIOS* 33(9): 406-413.

**Abstract:** Drone footage of a large American crocodile bottom walking in shallow coastal water off Costa Rica was used to estimate oblique pace and stride lengths and pace angulations from autopodial touchdowns. The crocodile's pes stride lengths were longer but more variable than expected for an individual of comparable size high-walking on land, due in part to punting locomotion, but also to wave action moving the reptile's body. Pace angulations were also larger than expectations for a crocodile high walking on land. These features are also seen in fossil trackways, suggesting that trackways ascribed to "swimming" crocodylomorphs might better be characterized as made by bottom walking and punting reptiles.

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Isberg, S.R., Finger, Jr., J.W. and Thomson, P.C. (2018). Quantification of plasma corticosterone in juvenile farmed saltwater crocodiles (*Crocodylus porosus*) using current Australian Code of Practice guidelines. *Gen. Comp. Endocrinol.* (doi: 10.1016/j.ygcen.2018.08.020).

**Abstract:** Saltwater crocodiles (*Crocodylus porosus*) across three size categories (hatchlings, grower and harvest-size) were repeatedly blood sampled on two farms in the Northern Territory, Australia to

determine reference plasma corticosterone (CORT; crocodylian stress hormone) levels. The mean CORT values for hatchlings (<1 year old), growers (1-3 years) and harvest-size individuals (2+ years) were  $1.65 \pm 0.15$  ng/ml,  $2.73 \pm 0.21$  ng/ml and  $2.19 \pm 0.16$  ng/ml, respectively. No inter-farm differences within the hatchling or harvest-size crocodiles were detected, but growers on Farm 2 had significantly lower plasma CORT than those on Farm 1. However, the grower growth rate coefficients were the same across both farms so the repeated blood sampling design most likely contributed to the difference in CORT values rather than any management procedures. Plasma corticosterone levels significantly increased with time of day. Substantial variation in plasma CORT was observed at each sampling which is not unprecedented in the literature but requires further elucidation. Irrespective, as CORT values were generally low, our results suggest that the farming environment and husbandry practices, as implemented under the Australian industry Code of Practice, are effective as baseline animal welfare measures although they should be viewed as a foundation for further welfare research and not considered static.

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Maraming, P., Maijaroen, S., Klaynongsruang, S., Boonsiri, P., Daduang, S., Chung, J.G. and Daduang, J. (2018). Antitumor ability of KT2 peptide derived from leukocyte peptide of crocodile against human HCT116 colon cancer xenografts. *In Vivo* 32(5):1137-1144.

**Abstract:** Many antimicrobial peptides have been shown to have anticancer activity against human cancer cell lines. Cationic KT2 peptide, derived from white blood cell extract of *Crocodylus siamensis* has antibacterial activity and antitumor activity against human cervical cancer cells, but there are no data on the effect of KT2 peptide on tumor growth in vivo. The anticancer activity of KT2 peptide on human colon cancer xenografts was investigated in nude mice. Tumors in nude mice (BALB/c -nu/nu mice) were induced by subcutaneous injection with HCT116 cells. Twelve days after cancer cell xenograft, mice were treated by intratumoral injection with phosphate-buffered saline or KT2 peptide (25 and 50 mg/kg) once every 2 days for a total of four times and mice were sacrificed at 2 days after the last treatment. KT2 peptide treatment did not lead to significant difference in mouse body weight among groups, but reduced both tumor volume and weight of colon cancer xenografts. Moreover, KT2 peptide increased the expression of apoptotic proteins, such as BCL2-associated X (BAX), cleaved caspase-3, and poly (ADP-ribose) polymerase and reduced that of BCL2 apoptosis regulator in xenograft tumors. This finding suggests that KT2 peptide may inhibit tumor growth via apoptosis induction in this mouse model and supports the antitumor ability of KT2 peptide.

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Maijaroen, S., Jangpromma, N., Daduang, J. and Klaynongsruang, S. (2018). KT2 and RT2 modified antimicrobial peptides derived from *Crocodylus siamensis* Leucrocin I show activity against human colon cancer HCT-116 cells. *Environ. Toxicol. Pharmacol.* 62: 164-176.

**Abstract:** Conventional colon cancer treatments have been associated with side effects. Consequently, the discovery of novel effective and safe therapies is urgently needed. Hence, cationic antimicrobial peptides KT2 and RT2 were evaluated towards human colon cancer HCT-116 cells. The MTT assay indicated that both KT2 and RT2 exhibited anticancer activity with good therapeutic indices, and were found to be non-toxic to non-cancerous Vero cells. The IC50 values of KT2 were determined as 111.96 and 90.25  $\mu$ g/mL while RT2 showed IC50 as 104.07 and 87.84  $\mu$ g/mL after 12 and 24 h treatments, respectively. Moreover, KT2 and RT2 treatment caused a significant reduction in PI3K, AKT1 and mTOR mRNA expression levels, which resulted in suppression either of HCT-116 proliferation or migration. The mechanism involved in apoptosis induction were due to decreased Bcl-2 and XIAP and increased p53, cytochrome c, caspase-2, caspase-3, caspase-8, and caspase-9 mRNA expression levels. These effects increased the level of cell cycle associated gene p21 and decreased cyclin B1 and cyclin D1 expression.

Maraming, P., Klaynongsruang, S., Boonsiri, P., Maijaroen, S., Daduang, S., Chung, J.G. and Daduang, J. (2018). Antitumor activity of RT2 peptide derived from crocodile leukocyte peptide on human colon cancer xenografts in nude mice. *Environ. Toxicol.* 33(9): 972-977.

**Abstract:** RT2, derived from the leukocyte peptide of *Crocodylus siamensis*, can kill human cervical cancer cells via apoptosis induction, but no evidence has shown *in vivo*. In this study, we investigated the antitumor effect of RT2 on human colon cancer xenografts in nude mice. Twenty-four mice were injected subcutaneously with human colon cancer HCT 116 cells. Eleven days after cancer cell implantation, the mice were treated with intratumoral injections of phosphate buffered saline (PBS) or RT2 (0.01, 0.1, and 1 mg/mouse) once every 2 days for a total of 5 times. The effect of a 10-day intratumoral injection of RT2 on body weight, biochemical, and hematological parameters in BALB/c mice showed no significant difference between the groups. Tumor volume showed a significant decrease only in the treatment group with RT2 (1 mg/mouse) at day 6 ( $P < 0.05$ ), day 8 ( $P < 0.01$ ), and day 10 ( $P < 0.01$ ) after the first treatment. The protein expression levels of cleaved poly (ADP-ribose) polymerase (PARP), apoptosis-inducing factor (AIF), and the p53 tumor suppressor protein (p53) in xenograft tumors increased after treatment with RT2 (1 mg/mouse) compared to those in the PBS-injected group. Moreover, RT2 increased the expression of Endo G and Bcl-2 family proteins. Therefore, the peptide RT2 can inhibit tumor growth via the induction of apoptosis in an *in vivo* xenograft model.

Popko L. (2018). Some notes on Papyrus Ebers, ancient Egyptian treatments of migraine, and a crocodile on the patient's head. *Bull. Hist. Med.* 92(2): 352-366.

**Abstract:** Modern literature about the history of migraine treatments often starts with an ancient Egyptian remedy said to be from Papyrus Ebers that involves crocodiles that should be wrapped around the head. A fresh look on this treatment shows the need for revision on many points, including the source of the remedy, its content and meaning, and further implications for the history of Papyrus Ebers.

Phosri, S., Jangpromma, N., Chang, L.C., Tan, G.T., Wongwiwatthanakut, S., Maijaroen, S., Anwised, P., Payoungkiattikun, W. and Klaynongsruang, S. (2018). Siamese crocodile white blood cell extract inhibits cell proliferation and promotes autophagy in multiple cancer cell lines. *J. Microbiol. Biotechnol.* 28(6): 1007-1021.

**Abstract:** Cancer represents one of the most significant threats to human health on a global scale. Hence, the development of effective cancer prevention strategies, as well as the discovery of novel therapeutic agents against cancer, is urgently required. In light of this challenge, this research aimed to evaluate the effects of several potent bioactive peptides and proteins contained in crocodile white blood cell extract (cWBC) against LU-1, LNCaP, PC-3, MCF-7, and CaCo-2 cancer cell lines. The results demonstrate that 25, 50, 100, and 200 µg/ml cWBC exhibits a strong cytotoxic effect against all investigated cell lines ( $IC_{50}$  70.34-101.0 µg/ml), while showing no signs of cytotoxicity towards noncancerous Vero and HaCaT cells. Specifically, cWBC treatment caused a significant reduction in the cancerous cells' colony forming ability. A remarkable suppression of cancerous cell migration was observed after treatment with cWBC, indicating potent antimetastatic properties. The mechanism involved in the cancer cell cytotoxicity of cWBC may be related to apoptosis induction, as evidenced by typical apoptotic morphology features. Moreover, certain cWBC concentrations induced significant overproduction of ROS and significantly inhibited the S-G<sub>2</sub>/M transition in the cancer cell. The molecular mechanisms of cWBC in apoptosis induction were to decrease Bcl-2 and XIAP expression levels and increase the expression levels of caspase-3, caspase-8, and p53. These led to a decrease in the expression level of the cell cycle-associated gene cyclin-B1 and the arrest of cell population

growth. Consequently, these findings demonstrate the prospect of the use of cWBC for cancer therapy.

Phupiewkham, W., Lu, Q., Payoungkiattikun, W., Temsiripong, T., Jangpromma, N., Lai, R. and Klaynongsruang, S. (2018). Development and characterization of an anti-acne gel containing Siamese crocodile (*Crocodylus siamensis*) leukocyte extract. *J. Microbiol. Biotechnol.* 28(5): 707-717.

**Abstract:** Leukocytes are reportedly the first line of the innate immune defense and essential for the control of common bacterial infections. Therefore, in this work, the antibacterial activity of crocodile leukocyte extract against *Propionibacterium acnes* was evaluated, and we also characterized the related activity of skin infection. The leukocyte extract showed the minimum inhibitory concentration to be 100 µg/ml to *P. acnes*. SEM imaging demonstrated that the leukocyte extract adversely affected *P. acnes* cell permeability in a concentration-dependent manner. Furthermore, the crocodile leukocyte extract could significantly reduce proinflammatory markers and decrease inflammatory signs in infected mouse ears. The crude leukocyte extract was further purified using FPLC and RP-HPLC. The resulting fraction F5 was indicated as the anti-acne peptide-containing fraction. The molecular mass of the peptide contained in F5 was calculated to be 4790.5 Da. N-Terminal sequencing revealed the amino acid sequence as GPEPVPALYQ, which displays similarities to immunoglobulin A and leucine-rich repeat neuronal protein. This is the first reported amino acid sequence of a crocodile leukocyte extract that possesses anti-acne activity. To attempt to use it in a prototype cosmetic, an anti-acne gel containing crude crocodile leukocyte extract was formulated, resulting in 7 gel formulations (G1, G2, G3, G4, G5, G6, and G7). The formulations G5, G6, and G7 exhibited 2-fold higher anti-acne activity than G1-G4. Investigation of accelerating stability studies of anti-acne gel formulations G5, G6, and G7 demonstrated that a low storage temperature (4°C) is suitable for maintaining the physical properties and biological activity of the anti-acne gel products.

Joyce, W., Miller, T.E., Elsey, R.M., Wang, T. and Crossley, II, D.A. (2018). The effects of embryonic hypoxic programming on cardiovascular function and autonomic regulation in the American alligator (*Alligator mississippiensis*) at rest and during swimming. *J. Comp. Physiol. B.* (doi: 10.1007/s00360-018-1181-2).

**Abstract:** Reptilian embryos naturally experience fluctuating oxygen levels *in ovo*, and developmental hypoxia has been established to have long-term impacts on cardiovascular function in vertebrates. In the present study, we investigated the impact of developmental 21% (normoxia) and 10% O<sub>2</sub> (hypoxia) on juvenile (4-year-old) American alligator cardiovascular function in animals at rest and during swimming. In both experimental groups, combined right aortic and right subclavian blood flow approximately doubled during swimming. Carotid blood flow increased during swimming in the hypoxia-programmed animals only, and both carotid and left aortic blood flow reached higher values in swimming hypoxic-programmed animals compared to the normoxic group. However, pulmonary blood flow, which increased two to threefold during swimming (in both groups), was higher in normoxic-programmed animals at both rest and swimming. The differences between programming groups were preserved after cholinergic blockade (atropine), but reduced by adrenergic receptor antagonists (propranolol and phentolamine). Propranolol and phentolamine also blunted the incremental increases in blood flows during swimming, which was especially clear in the hypoxia-programmed animals. Alteration in adrenergic control and relative cardiac size (which was increased in hypoxic-programmed alligators) may account for the differences between the experimental groups.

Katsu, Y., Oka, K. and Baker, M.E. (2018). Evolution of human, chicken, alligator, frog, and zebrafish mineralocorticoid receptors: Allosteric influence on steroid specificity. *Sci. Signal.* 11(537). (doi:

**Abstract:** Although multiple steroid ligands of the glucocorticoid, mineralocorticoid, and progestin families bind to and regulate the activity of mineralocorticoid receptors (MRs), the responses to these ligands differ across species. To understand how the different domains of MRs contribute to the ligand-induced activation or inhibition of MR activity, we studied the response to 8 steroids (aldosterone, 11-deoxycorticosterone, 11-deoxycortisol, cortisol, corticosterone, progesterone, 19-norprogesterone, and spironolactone) of human, chicken, alligator, frog, and zebrafish full-length MRs and truncated MRs, which lacked the N-terminal domain (NTD) and DNA binding domain (DBD). Compared to full-length MRs, some truncated MRs were not activated by the steroids, and others required higher steroid concentrations for activation. Progesterone, 19-norprogesterone, and spironolactone did not activate full-length or truncated human, alligator, or frog MRs. However, at 10 nM, these steroids activated full-length chicken and zebrafish MRs, whereas at 100 nM, these steroids had little activity for truncated chicken MRs, but they retained activity for truncated zebrafish MRs. This suggests that regulation of the activation of the chicken MR by progestin resides in the NTD-DBD and that of the zebrafish MR resides in the hinge-LBD. Zebrafish and chicken MRs contain a serine corresponding to Ser810 in human MR, which is required for the antagonist activity of progesterone for human MR, suggesting a previously uncharacterized mechanism of regulation of progestin activation of chicken and zebrafish MRs. These findings suggest that progesterone may be a physiological activator of chicken and zebrafish MRs.

Pacheco-Sierra, G., Vázquez-Domínguez, E., Pérez-Alquicira, J., Suárez-Atilano, M. and Domínguez-Laso, J. (2018). Ancestral hybridization yields evolutionary distinct hybrids lineages and species boundaries in crocodiles, posing unique conservation conundrums. *Front. Ecol. Evol.* 6:138.

**Abstract:** Interspecific hybridization can lead to adaptation and speciation, especially in the context of recent radiations. The emblematic *Crocodylus* (true crocodiles) is the most broadly distributed, ecologically diverse, and species-rich crocodylian genus. Nonetheless, their within-species evolutionary processes are poorly resolved mainly due to their potential for hybridization. Notably, the evolutionary outcomes when hybridization is ancient and involves long-lived species, like crocodiles, remain largely unexplored. Here, we evaluate the genomic admixture between the American (*Crocodylus acutus*) and Morelet's (*C. moreletii*) species, and demonstrate that this hybridization system challenges the definition of species boundaries and poses a triple conservation conundrum: what has been recognized as *C. acutus* is actually two distinct species, therefore its taxonomic reassessment is needed; we identified two evolutionary distinct hybrids lineages, which are genetically discernible from the parental species; the remaining *C. moreletii* populations evidence its likely extinction as a species and/or evolution via hybridization. Hence, the crocodiles' distinct species.

Morato, S.A.A., Batista, V.B.G.V., Martins, A.A.V., da Silva, A.P., Bastos, L.F., Sodre, D.C.A., Tempo, M.M. and Junior, D.S.A. (2018). Distribution, abundance and conservation of crocodylians in Lago Sapucua, Central Amazonia, Para State, Brazil. Pp. 187- 209 in *Herpetofauna da Amazonia Central: Estudos na FLONA de Saraca-Taquera*, ed. by S.A.A. Morato, G.N. Ferreira and M.R.C. Scupino. STCP Engenharia de Projetos Ltda.: Curitiba and Mineracao Rio do Norte S.A.: Porto Trombetas. (In Portuguese).

Rodas-Trejo, J., Ocampo-González, P., Hernández-Nava, J., Mandujano-Camacho, H., Coutiño-Hernández, P.R. and Orantes Zebadua, M.A. (2018). Perception, popular knowledge and exploitation of the wetland crocodile (*Crocodylus moreletii* Duméril & Bibron) for inhabitants of the Flora and Fauna Protection Area of Laguna de Terminos, Campeche, Mexico. *Agroproductividad* 11(6):

**Abstract:** The objective of this study was to understand the exploitation, popular knowledge and perception that inhabitants from three communities in the Flora and Fauna Protection Area of Laguna de Términos (APFFLT) in Campeche, México, have towards the wetland crocodile (*Crocodylus moreletii*). The data were collected through 54 semi-structured interviews with key informants and through the discussion in nine focal groups. Five uses of the wetland crocodile are recognized: medicinal, food, photograph, artisanal and leather. The use of the fat to treat respiratory diseases and to strengthen the immunological system in children is very common in the population. It is mentioned that the commercialization mainly of the skin has decreased due to the vigilance and prohibition by the authorities, with an increase perceived of the populations of *C. moreletii*. It is recommended to establish sustainable management proposals in freedom or captivity living conditions that can generate an economic benefit to the communities.

Augustine, L., Stern, A.G., Rodríguez, G.S. and Fleitas, E.P. (2018). *Crocodylus rhombifer* (Cuban crocodile) subcaudal scale irregularities. *Herpetology Notes* 11: 621-623.

Vyas, R. (2018). Case of polydactyly limb in juvenile Mugger crocodile (*Crocodylus palustris*). *Russian Journal of Herpetology* 25(2): 139-142.

**Abstract:** First, ever recorded case of congenital defects as polydactyly case in wild population of Mugger crocodile (*Crocodylus palustris*) of River Vishwamitri, Gujarat, India.

Somaweera, R., Rhind, D., Reynolds, S., Eisemberg, C., Sonneman, T. and Woods, D. (2018). Observations of mammalian feeding by Australian freshwater crocodiles (*Crocodylus johnstoni*) in the Kimberley region of Western Australia. *Records of the Western Australian Museum* 33: 103-107.

**Abstract:** The dietary preference of most crocodylians is generally thought to be fairly broad. However, the head morphology of slender-snouted crocodylians limits their ability to process large and complex prey. The slender-snouted Australian freshwater crocodile is known to be a dietary specialist consuming small aquatic prey, particularly aquatic arthropods and fish. Here, we report observations of predation events by Australian freshwater crocodiles on medium- and large-sized mammals in the Kimberley region of Western Australia including macropods, a large rodent and an echidna. We discuss the significance of our observations from an ecological and morphological perspective and propose that terrestrial mammalian prey may be a seasonally important prey item for some populations of freshwater crocodiles.

Legorreta-Flores, A., Davila-Tejeda, A., Velásquez-González, O., Ortega, E., Ponce, A., Castillo-Michel, H., Reyes-Grajeda, J.P., Hernández-Rivera, R., Cuéllar-Cruz, M. and Moreno, A. (2018). Calcium carbonate crystal shapes mediated by intramineral proteins from eggshells of ratite birds and crocodiles. Implications to the eggshell's formation of a dinosaur of 70 million years old. *Cryst. Growth Des.* 18(9): 5663-5673.

**Abstract:** In this contribution we want to show how the intramineral proteins affect the crystal morphology of calcite crystals grown in vitro. Intramineral proteins from emu eggshells and two crocodile species were isolated, purified by ultrafast liquid chromatography, and characterized by biochemical and biophysical methods. We saw that the crystal habit of calcite was modified when intramineral proteins were present at certain concentration. Therefore, all crystal species were individually characterized by scanning electron microscopy (SEM) and by electron diffraction with an electron-reduced dosage in transmission electron microscopy. Additionally,

transversal SEM micrographs of eggshells of four types of species, namely, emu, crocodile, hen, and dinosaur (70 million years old), were compared to track the changes produced by the interacting intramineral proteins. Finally, the organic sulfur content analysis of these eggshells was performed by using X-ray absorption techniques like X-ray fluorescence and X-ray absorption near-edge structure. From the analyses of the dinosaur eggshells, these X-ray absorption data showed a very characteristic organic sulfur bonding similar to that semiessential proteogenic amino acid L-cysteine, which implies that there is a possibility of having a very old intramineral protein similar to those found in emu and crocodiles.

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Baynes-Rock, M. (2018). Precious reptiles: Social engagement and placemaking with saltwater crocodiles. *Area* (<https://doi.org/10.1111/area.12484>).

**Abstract:** In this paper I take up the call to expand the boundaries of social and physical landscapes in order to recognise the creative agencies of human and non-human actors. In doing so, I wish to draw attention to the ways in which relations between both individuals and collectives combine to shape multi-dimensional sociality in particular places. The place in question is a crocodile farm in tropical Australia. It is a curious place in that it was fostered by modes of objectification which serve to commoditise and conserve crocodiles at a species level with little attention to individuality. However, the particularity of crocodiles at the farming level compels their human handlers to make concessions to their demands. Crocodiles, by their refusals, attachments and individualities, elicit attention to their needs, which translates into practices and structures that are often at odds with profitability. In this way it is as much social processes as it is practicalities of producing skins which affect the farmed landscape and the beings it produces, creating a nexus of multispecies place-making where individuals matter.

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Zietsman, D. (2018). The Relevance of Ethical Value Perceptions and Supply Chain Information to International Tourists Considering Purchasing Exotic Leather Products. MConsumer Science thesis, University of Pretoria, South Africa. (<http://hdl.handle.net/2263/65957>).

**Abstract:** With this study the researcher investigated the sustainable luxury value perceptions, supply chain information needs, and purchasing intent of American and Chinese tourists for South African manufactured luxury exotic leather products. Online survey data was collected from 1 043 individuals from American (518) and Chinese (525) decent. A non-probability convenient sample method was followed for data collection purposes. A self-administered questionnaire based on the works of Hennigs, Wiedmann, Klarman and Behrens (2013c) and Cervellon and Shammas (2013) was used to test individuals' sustainable luxury value perceptions. A self-developed scale tested the need for supply chain information. Purchasing intent was determined based on the research of Spears and Singh (2004). Data analysis consisted of descriptive and inferential statistics with correlation coefficient determination between constructs. The findings confirmed the "sustainable excellence" perception conceptualised by Hennigs *et al.* (2013c). It was furthermore found that only slight differences existed between American and Chinese consumers sustainable luxury value perceptions. Furthermore, it was found that American and Chinese consumers had a strong need for supply chain information relating to luxury exotic leather products, with a clear distinction existing between their operational and originality supply chain information needs. The findings showed that consumers from both nationalities had a high intention to purchase luxury exotic leather products when visiting South Africa. In addition, significant correlations were found between American and Chinese consumers' sustainable luxury value perceptions, their supply chain information needs, and purchasing intent. Various exotic leather industry stakeholders, such as crocodile and ostrich farmers, tanneries, product designers, manufacturers, retailers, and marketers, can benefit from the findings of this study, to position their products competitively.

Manalo, R.I., Tabayag, E.A. and Baltazar, P.C. (2018). Conservation milestones of the Critically Endangered Philippine crocodile (*Crocodylus mindorensis*, Schmidt 1935). *The Technical Journal of Philippine Ecosystems and Natural Resources* 27(1&2): 30-46.

**Abstract:** Conservation efforts to save the rarest crocodile species in the world, the Philippine Crocodile (*Crocodylus mindorensis*), were exerted through the years from 1891-2016. This study aimed to provide insights for the species conservation management by documenting the milestones that could form part of future conservation programs. Review of historical accounts and published scientific articles identified species milestones in a timeline format. Results showed that as early as 1891, the Philippine crocodile became known to science based on specimens collected from the island of Mindoro (FMNH 11135) and was originally described by Karl Schmidt as *Crocodylus mindorensis* in 1935. It was later considered as a subspecies of the New Guinea Crocodile (*C. novaeguineae mindorensis*) until Philip M. Hall provided new evidence for its designation as a totally separate species in 1989. Wild populations severely declined in the early 1940s to 1980s due to indiscriminate hunting for skin trade and human persecution. This triggered distribution studies to locate and estimate the abundance of extant wild populations. Upon the conclusion of these studies in the early 1990s, the IUCN declared the species as Critically Endangered in 1996. *Ex-situ* conservation breeding program was deemed the only hope for the species in the late 1990s to early 2000s. The successful initiation and continuous development of the collaborative breeding programs have resulted into a restocking of the species to form nucleus populations in its natural habitat from 2009 to 2016. Over the course of 125 years, wild populations have been unearthed and the species was finally released in protected sanctuaries starting in the year 2009.

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Coetzee, B.W.T., Ferreira, S.M. and Maciejewski, K. (2018). Challenges and opportunities for monitoring wild Nile crocodiles with scute mark-recapture photography. *Koedoe* 60(1) (doi: 10.4102/koedoe.v60i1.1505).

**Abstract:** The global conservation status of Nile crocodiles (*Crocodylus niloticus*) was last assessed in 1996. The species presents particular difficulty in monitoring because it can be cryptic, require expertise to handle, and caudal tail tags and transmitters are often lost. Some studies advocate mark-recapture techniques based on photograph identification of the unique scute markings of crocodile tails as a non-invasive means of monitoring their populations. Researchers developed this method with crocodiles in captivity. In this study, we test the technique under field conditions by monitoring crocodiles from 2015 to 2017 in the Sunset Dam in the Kruger National Park. Using a Cormack-Jolly-Seber open population model, we found that the dam may host 15-30 individuals, but that there is a high turnover of individuals and much uncertainty in model outputs. The dam's population thus has high rates of immigration and emigration. The method proved challenging under field conditions, as there was bias in identifying scute markings consistently. The efficient use of the method requires an exceptional quality of photographic equipment. Animal crypsis, however, remains an issue. In this study, we discuss how to improve the mark-recapture photography methodology, especially to adapt the technique for citizen science initiatives. Using scute mark-recapture photography presents challenges under field conditions. These challenges require innovative, practical and analytical solutions to successfully use the technique before monitoring programmes, aimed at ensuring the persistence of crocodiles in the wild, can be implemented.

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Aziz, M.A. and Islam, M.A. (2018). Population status and spatial distribution of Saltwater crocodile, *Crocodylus porosus* in the Sundarbans of Bangladesh. *Bangladesh J. Zool.* 46(1): 33-44.

**Abstract:** The Saltwater crocodile, *Crocodylus porosus* is endangered in Bangladesh, and currently surviving in rivers and channels of the

Sundarbans mangrove forest of the country. Very little information is available on this apex predator in the Sundarbans aquatic ecosystem, therefore a survey was carried out to assess population status and their spatial distribution. By surveying approximately 351 km of rivers of the Bangladesh Sundarbans during daytime between 2014 and 2015, a total of 42 individual crocodiles were recorded, with an overall encounter rate of 0.12 crocodiles (SE= 0.02, 95% CI= 0.08-0.18) per km of rivers. Based on this estimate, it is inferred that a population of approximately 140 crocodiles (95% CI= 90-190) could occur in the Bangladesh Sundarbans. The generalised linear model revealed statistically insignificant negative relationship of relative crocodile abundance with the salinity level ( $\beta = -0.067$ , SE= 0.057,  $p = 0.242$ ) and protection status ( $\beta = -0.208$ , SE= 0.855,  $p = 0.808$ ), and statistically insignificant positive relationship with the distance to human habitations ( $\beta = 0.004$ , SE= 0.039,  $p = 0.914$ ). Disturbance by resource collectors, cargo vessels, and water pollution are the major threats to crocodile populations of the Sundarbans. The results of this study will be useful in future population monitoring to guide conservation management of saltwater crocodile in this important habitat.

McFarland, S.E. (2018). Such beastly behavior! Predation, revenge, and the question of ethics. Exploring animal encounters. In: Exploring Animal Encounters. Palgrave Studies in Animals and Literature, ed. by D. Ohrem and M. Calarco. Palgrave Macmillan: Cham.

**Abstract:** After a woman is dragged from her tent in the night and eaten by a bear in William Kittredge's short story "We Are Not in This Together," Halverson, the story's protagonist, seeks "one bear, for a head, to mount on the wall, to get things even." But what Halverson really searches for is the moment when the bear anticipates his own death: "There had to be time for thinking, and time for the bear, for hoping the animal might dimly sense the thing happening." Likewise when a man checks his crab pots and is pulled under by a crocodile, or a boy is attacked by a tiger, or a young surfer loses her arm to a shark: in each case, their human communities demand revenge. McFarland's chapter explores issues that converge in the behavior of predatory nonhuman animals and vengeful humans: for example, the notion that animals can "murder," the belief that animals must be held accountable for their acts of "violence," and the idea that humans can (and should) enact revenge against other species, demanding that animals pay a penalty for their actions.

Behler, N., Kopsieker, L., Staniewicz, A., Darmansyah, S., Stuebing, R. and Ziegler, T. (2018). Population size, demography and diet of the Siamese crocodile, *Crocodylus siamensis* (Schneider, 1801) in the Mesangat Swamp in Kalimantan, Indonesia. Raffles Bulletin of Zoology 66: 506-516.

**Abstract:** A study of the distribution, abundance and diet of the critically endangered Siamese crocodile (*Crocodylus siamensis*) was undertaken during 2010 and 2011 in the Mesangat wetland, East Kalimantan, Indonesia. It provides the first hard data concerning the ecology of the species outside of mainland Indochina. Crocodiles were captured if possible, weighed, measured and tagged subcutaneously with a Passive Integrated Transponder (PIT tag). Forty-three (43) non-hatchling *C. siamensis* were identified, of which 7 were adults. An estimate of total non-hatchling population size was calculated from individuals marked in 2010 and re-captured in 2011, extrapolated to the area of all locations where *C. siamensis* were seen. The total population size in the survey area was estimated to be approximately 75 individuals. Ninety-one percent (91%) of all *C. siamensis* detections ( $n = 77$ ) occurred outside the forest-type habitat, adjacent to or within two metres of floating grassy mats. The first investigation on stomach contents of the species was undertaken in this study. Diet samples from 13 *C. siamensis* were obtained from stomach lavage and analysed individually. Prey found in stomach contents contained insect remnants, snail shells, fish bones, bird feathers, snake scales and small mammal fur and varied considerably between individuals. The presence of necrophagic ants

suggested that *C. siamensis* consume carrion. The current study confirmed at least one healthy breeding population of *C. siamensis* outside of the mainland of Indochina and provides important data for the species' long-term conservation in Kalimantan.

Staniewicz, A., Behler, N., Dharmasyah, S. and Jones, G. (2018). Niche partitioning between juvenile sympatric crocodylians in Mesangat Lake, East Kalimantan, Indonesia.

**Abstract:** Crocodylians are apex predators and sympatric species are likely to have different ecologies or morphologies in order to minimise competition between species, ie niche partitioning. Here, we examined the ecological niche factors that may affect competition between juvenile Siamese crocodiles (*Crocodylus siamensis*) and Tomistoma (*Tomistoma schlegelii*) in Mesangat Lake - the only documented area where the two species co-exist. This location has also been subjected to recent disturbance from logging and oil palm development. We identified and compared preferred habitats and stomach contents of each species. *Tomistoma schlegelii* were found predominantly in flooded forests, while *C. siamensis* were mainly in open areas. However, overlap in prey choice between juvenile *T. schlegelii* and *C. siamensis* was significantly higher than expected by chance and invertebrates were the most frequent prey items in both species. High overlap in prey choice between the two species suggests that juvenile *T. schlegelii* and *C. siamensis* are generalist predators. Furthermore, the evidence of separation of habitat combined with overlap in prey choice indicates competition-driven niche partitioning between *C. siamensis* and *T. schlegelii*. This paper provides basis for co-ordinated conservation efforts for the two threatened species in this unique ecosystem.

Schmidt, V., Klasen, L., Schneider, J., Hübel, J. and Cramer, K. (2018). Pulmonary fungal granulomas and fibrinous pneumonia caused by different hypocrealean fungi in reptiles. Veterinary Microbiology (<https://doi.org/10.1016/j.vetmic.2018.09.008>).

**Abstract:** In contrast to fungal dermatitis, fungal glossitis and disseminated visceral mycosis, fungal infection of the lung has so far rarely been described in reptiles. Pulmonary fungal granulomas were diagnosed histopathologically within the scope of post mortem examinations. Fragments of the 18S-internal transcribed spacer1-5.8S rDNA (SSU-ITS1-5.8S) and 28S rDNA (LSU), including domains (D)1 and D2 as well as the protein coding gene translation elongation factor 1 alpha (TEF) were used for phylogenetical analysis after isolation of the fungal pathogen by culturing. Ten reptiles, including lizards ( $n = 6$ ), snakes ( $n = 1$ ), crocodylians ( $n = 2$ ) and tortoises ( $n = 1$ ) presented with pulmonary fungal granulomas ( $n = 8$ ) and fibrinous pneumonia ( $n = 2$ ) caused by different non-clavicipitaceous and clavicipitaceous species of the order Hypocreales. *Purpureocillium lavenderum* ( $n = 2$ ) and *Metarhizium robertsii* ( $n = 1$ ) as the etiologic agents of pneumonia in reptile species are described for the first time. Fungal pulmonary granulomas caused by clavicipitaceous fungi ( $n = 6$ ) were all associated with disseminated visceral mycosis as well as oral fungal granulomas ( $n = 4$ ) and/or fungal dermatitis ( $n = 1$ ). Differing infection routes being likely for clavicipitaceous and non-clavicipitaceous fungal pathogens. A potential zoonotic health risk should be taken into account during necropsy or lung sampling in live reptiles with pulmonary fungal granulomas, since human infections, mainly keratitis and sclerokeratitis, caused by *Beauveria bassiana*, *Metarhizium robertsii* and *Purpureocillium lilacinum*, have occasionally been described.

Lott, M.J., Moore, R.L., Milic, N.L., Power, M., Shilton, C.M. and Isberg, S.R. (2018). Dermatological conditions of farmed crocodylians: A review of pathogenic agents and their proposed impact on skin quality. Veterinary Microbiology 225: 89-100.

**Abstract:** The control of pathogens that target crocodylian skin is essential to the long-term success and sustainability of intensive farming operations worldwide. To understand the impact these

pathogens may have on the skin, a brief overview of skin histology is given. A review of the known viral, bacterial, fungal and helminth taxa associated with skin conditions in commercially significant crocodylian species is presented. Best management practices are discussed, with an emphasis on addressing extrinsic factors that influence transmission and pathogenicity. It is argued that, in the past, reduced immune function arising from inadequate thermal regulation was the leading cause of skin disease in captive crocodylians. Consequently, innovations such as temperature control, coupled with the adoption of more stringent hygiene standards, have greatly reduced the prevalence of many infectious skin conditions in intensively farmed populations. However, despite improvements in animal husbandry and disease management, viral pathogens such as West Nile virus, herpesvirus and poxvirus continue to afflict crocodylians in modern captive production systems.

Posso-Peláez, C., Ibáñez, C. and Bloor, P. (2018). Low mitochondrial DNA variability in the captive breeding population of the Critically Endangered Orinoco crocodile (*Crocodylus intermedius*) from Colombia. *Herpetological Conservation and Biology* 13(2): 347-354.

**Abstract:** The Orinoco crocodile (*Crocodylus intermedius*) is a critically endangered crocodile species found only in the Orinoco River basin in Colombia and Venezuela, in northwestern South America. It has mostly failed to recover from human exploitation despite decades of protection and management efforts designed to promote species recovery. Efforts to conserve the Orinoco crocodile include protection of populations in the wild as well as captive breeding/rearing programs. Here, we used mitochondrial DNA sequences to assess genetic variability in the Orinoco crocodile captive-breeding population managed by the Estación de Biología Tropical “Roberto Franco” (EBTRF). We recovered four closely related mtDNA haplotypes (differing by no more than two mutational steps) in 27 wild-caught crocodiles representing the range of the species in Colombia. We suggest that the unstructured haplotype network reveals that the Orinoco crocodile can be managed as a single genetic unit in Colombia. We found some limited evidence of geographical structuring, with a single haplotype (Cin3) restricted to individuals from the northwestern part (Cravo Norte, Department of Arauca) of the species distribution in Colombia. We recovered two of the four haplotypes found in the 27 wild-caught crocodiles in living captive-bred individuals (F1 and F2). Haplotype Cin2, recovered in the female founder Dabeiba, predominated (accounting for almost 91%) among living captive-bred individuals. All remaining captive-bred individuals surveyed contained haplotype Cin1, corresponding to the female founder Lizeth. We suggest that loss of haplotype diversity in the ETBRF captive population is likely if steps are not taken to retain haplotype diversity.

Nizar, N.N.A., Sultana, S., Hossain, M.A.M., Johan, M.R. and Ali, M.E. (2018). Double gene targeting multiplex PCR-RFLP detects *Crocodylus porosus* in chicken meatball and traditional medicine. *International Journal of Food Properties* 21(1): 2037-2051.

**Abstract:** Crocodiles have been hunted and consumed for centuries for skins, nutrients, and medicines. These indomitable trends have overpowered restrictions from wildlife and conservation agencies, continuing the illegal trades of crocodiles across the world. This paper described the development of a very stable, fast, and secured polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay for the confirmed detection of *Crocodylus porosus* under any matrices and decomposing treatments. Two very short-sites (77 and 127-bp) of *atp6* and *cytb* genes of *C. porosus* were controlled digested with *Acil* enzyme; producing distinctive RFLP patterns (83, 54, 44 & 23 bp). The enzyme digested assay was stable following extreme boiling, autoclaving, and microwaving treatments that break down DNA. The sensitivity was tested and validated in model meatballs and it was suitable for detecting 0.01% crocodile meatball matrices. The optimized RFLP assay was used to screen 3 commercial meatballs and 21 traditional medicines

(TM). While no crocodile DNA was found in commercial chicken meatballs, 4/21 TM products were found correctly labelled to contain *C. porosus* DNA. The novel assay demonstrated sufficient merit to be used by regulatory agencies for any forensic and/or archaeological identification of *C. porosus* even under the state of decomposition.

Solanki, G.S., Chalise, M.K. and Sharma, B.K. (2018). Colonization of large wildlife in rehabilitated forests of lowland in Chitwan National Park's Buffer Zone, Nepal. Pp. 343-354 in *Indian Hotspots*, ed. by C. Sivaperuman and K. Venkataraman K. Springer: Singapore.

**Abstract:** The study was conducted in community-managed Baghmara buffer zone forest of Chitwan National Park, Nepal, to identify the colonization of large wildlife in rehabilitated forest areas within the protected area system. Total count of animals and review of secondary sources were employed for data analysis. The number of wildlife species recorded in the pre-community-managed phase was five, and their number was increased to ten during post-community-managed forest during this study. The total population of wildlife species recorded was 7 individuals in initial phase in the year 1995 that increased to 365 individuals after 20 years. The number of species and the size of population colonized were barking deer (n= 12), hog deer (n= 2), sambar deer (n= 23), spotted deer (n= 182), wild boar (n= 5), hanuman langur monkey (n= 1), rhesus monkey (n= 76), mugger crocodile (n= 35), tortoise (n= 25), tiger (n= 1), and rhinoceros (n= 3). Density of ungulates increased from 0.5 animal/km<sup>2</sup> to 104.2 animals/km<sup>2</sup> in post-management phase. There is an increased (P<0.05) in prey population (ungulates), marsh mugger, and tortoise population in post-management phase.

Langsford, C. (2018). From the bush to the cloud: Following the social lives of the skins “Dun” and “Dee”. *A Journal of Social Anthropology & Cultural Studies* 15(1): 11-37.

**Abstract:** Tracing the flows and transitions of some commodities can be incredibly difficult. Like their living counterparts, crocodile skins are unpredictable and multiple, submerging in and out of view. They are disobedient, ‘Other’ objects in the sense used by Ihde (1990), as they regularly work against the classifications and transformation activities of human agents. Like Law and Singleton’s (2004) ‘fire objects’ they are both present and absent as their ‘crocodileness’, their status as animal subjects, moves in and out of focus in different cultural contexts. When faced with such devious objects an ethnographer cannot, like Appadurai, view objects as mere conduits of human meaning and intentionality but instead must make use of multiple methods to capture the voices, actions and effects of these objects as they move through processes of commoditisation. Combining online research, practitioner narratives, object interviews and photography, my research and telling of Dun and Dee’s story aims to explore how the crocodile skins’ physical, relational and symbolic properties as animals/objects/commodities have the capacity to affect the emotions, interpretations and creative efforts of cosplayers and others.

Azeez, O.I., Myburgh, J., Meintjes, R., Bosman, A.-M., Featherstone, J., Oosthuizen, M. and Chamunorwa, J.P. (2018). Pansteatitis induced differential expression of inflammatory and apoptosis genes in adipose tissue: A glimpse into pathophysiology of the condition in the Nile crocodile. *The FASEB Journal*.

**Abstract:** In the quest to understand the pathophysiology of pansteatitis in the Nile crocodile, a condition that was attributed to the recent crocodile die off at the Olifant River, Mpumalanga, the whole transcriptome of the Nile crocodile was sequenced and assembled using whole transcriptome shotgun sequencing data by Illumina RNAseq from RNA obtained from fat body and other adipose tissues. Total RNA, from abdominal fat body and other adipose tissue from normal farmed Nile crocodiles and samples collected from Nile crocodiles that had died of pansteatitis, were

extracted and purified using Qiagen RNA mini prep (Qiagen, USA). RNA samples library were prepped using the Truseq stranded total RNA sample preparation and sequenced on the Illumina Hiseq 2500 with v4 SBS chemistry. Reads were assembled using Tophat (<http://tophat.cbcb.umd.edu/>) and aligned with reference transcriptome from *Crocodylus porosus* using Cufflinks (<http://cufflinks.cbcb.umd.edu/>) for downstream analysis while the differential gene expression analysis was done using Cuffdiff and RSEM (<http://deweylab.github.io/RSEM/>). A total of 13,329 genes were identified out of which 1541 genes related to inflammatory response and apoptosis were identified. A total of 641 genes were up regulated in the pansteatitis samples as compared to normal adipose tissues using a fold change of 16.0. A total of 70 inflammatory genes were up regulated in the pansteatitis samples including, PDCD5 (programmed cell death 5), NFIL3 (nuclear factor, interleukin 3 regulated protein), HSP90B1 (heat shock protein 90kDa beta (Grp94), member 1), VCAM1 (vascular cell adhesion molecule 1), IL1R1 (interleukin 1 receptor, type I), TNIP1 (TNFAIP3 interacting protein 1), JAK1 (Janus kinase 1), TNFAIP2 (tumor necrosis factor, alpha-induced protein 2) IL6ST (interleukin 6 signal transducer (gp130, oncostatin M receptor)) CFD (complement factor D (adipsin) and PPARG (peroxisome proliferator-activated receptor gamma) among others. These genes are responsible for inflammation, apoptosis, regulation of Toll-like receptors in inflammatory response, leucocyte migration and fibrosis in chronic inflammation. We present for the first time in the Nile crocodile, a comprehensive lists of inflammatory response genes that are expressed and up regulated in pansteatitis in the Nile crocodile.

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Srilert, T., Sitthisuan, S., Klaynongsruang, S. and Jangpromma, N. (2018). Antibacterial and antioxidant activity of plasma from crocodile (*Crocodylus siamensis*) and human. In Proceedings of the 6th International Conference on Biochemistry and Molecular Biology.

**Abstract:** *Crocodylus siamensis* is a small freshwater crocodylian. In the wild, crocodiles live in environments with high risk of bacterial infection, but they normally suffer no adverse effects. Even though the immune system of crocodiles has not been well characterized, it was believed that the powerful innate immunity of crocodiles was derived from their blood, especially plasma. In the present study, we have examined both antibacterial and antioxidant properties of *C. siamensis* plasma by comparing those activities with human plasma. In terms of antibacterial properties, broth dilution assay revealed that *C. siamensis* plasma had significantly higher potent effect on *Staphylococcus* spp. than human plasma. At a concentration of 1000 µg/ml, the percentage of *S. aureus* ATCC 25923 inhibition of *C. siamensis* plasma was determined as 97%, while 60% inhibition was observed in human plasma. In addition, 1000 µg/ml *C. siamensis* plasma strongly inhibited growth of bacterial drug-resistant strain (methicillin-resistant *S. aureus* DMST 20652 (MRSA)) with the percentage of inhibition at 99%. However, 1000 µg/ml human plasma was able to inhibit growth of *S. aureus* (MRSA) by only 60%. To investigate antioxidant activity, an ABTS assay was performed, which revealed the ability of crocodile plasma to possess antioxidant activity higher than human plasma. Our data directly indicates that *C. siamensis* plasma is potentially useful as a pharmaceutical agent against disease.

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Roamcharern, N., Payoungkiattikun, W., Jangpromma, N. and Klaynongsruang, S. (2018). Characterization of modified crocodile (*Crocodylus siamensis*) hemoglobin using bis(3,5-dibromosalicyl) fumarate. In Proceedings of the 6th International Conference on Biochemistry and Molecular Biology.

**Abstract:** A variety of cross-linked cell-free hemoglobin (CL-Hb) compounds have been developed with the aim of creating novel hemoglobin-based oxygen carriers (HBOCs). In general, derived CL-Hb using different cross-linking agents exhibits a lower oxygen affinity than native hemoglobin. Therefore, crocodile (*C. siamensis*) hemoglobin (cHb) constitutes an interesting new approach in

HBOC development, as it was reported to possess a higher oxygen affinity than human hemoglobin (hHb). In this study, we performed a modified cHb through cross-linking with bis(3,5-dibromosalicyl) fumarate (DBBF) and aimed to investigate its basically physiological and biological functions as comparing with hHb. The DBBF-cHb plays a different characteristic of cross-linking, since there was the absence of ββ-dimer band on SDS-PAGE. It is important to note that DBBF-cHb exhibited a higher oxygen affinity than DBBF-hHb with P50 value of 20.1 mm Hg (n= 2.5). In conclusion, cHb exhibited oxygen binding characteristics that may benefit the development of a cHb-based oxygen carrier suitable for human use in the future.

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Sosiangdi, S., Tankrathok, A., Klaynongsruang, S. and Jangpromma, N. (2018). Potential antibacterial activity of designed *Crocodylus siamensis* hemoglobin-based peptides. In Proceedings of the 6th International Conference on Biochemistry and Molecular Biology.

**Abstract:** In a previous study, we reported an antibacterial peptide derived from *Crocodylus siamensis* hemoglobin hydrolysate, named QL17 (QAIHNEKVAHGKKVL); however this peptide has a narrow spectrum of activity. To improve the antimicrobial activity of the peptide, it was used as the template to design novel effective peptides. The helical wheel diagram was used to monitor and evaluate hydrophobicity and hydrophilicity after the positional change and substitution of certain amino acids. Lysine (K) and arginine (R) were appropriately selected to extend the hydrophilicity, whereas hydrophobic residues such as leucine (L), isoleucine (I) or tryptophan (W) were used to increase the hydrophobicity. As appropriate, two novel peptides were synthesized and named as IL-K (IKHWKKVWKHWKKKL) and IL-R (IRHWRRVWRHWRRL), which had the same hydrophobicity and net charge at 40% and +7, respectively. Evaluation of the antimicrobial activity by broth microdilution assay revealed that IL-K had a slightly higher inhibition activity than IL-R at concentrations of 12.5, 25, 50 and 100 µg/ml. Both peptides had approximately 2-fold percentage inhibition higher than penicillin and as the QL17 parental peptide against *Klebsiella pneumoniae* and *Staphylococcus aureus*. Our findings suggest that the novel designed peptides are promising to be new antibacterial agents for further development and for use as antibiotics.

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Zafar, M. and Malik, M.F. (2018). A Review on Status and Conservation of Mugger Crocodile. Journal of Advanced Botany and Zoology 6(3) (DOI: 10.5281/zenodo.1288929).

**Abstract:** *Crocodylus palustris* belongs to family Crocodylidae. It is only crocodile which is present in Pakistan. It is also found in India, Nepal, Sri Lanka and Iran. According to IUCN, this specie is vulnerable due to illegal trade for its skin, habitat loss, excessive agricultural activities, construction of dams and roads, shortage of water, drying of streams, deterioration of freshwater ecosystem, incidentally catch in fishing net and hunting. It is listed in Appendix I of CITES and there is prohibition in the trading of this specie. It has variety in its feeding habits, and undergo aestivation and basking to maintain its body temperature. Till now marsh crocodile become extinct in Bangladesh, Bhutan and Myanmar. In eastern Nepal, marsh crocodile was reported in Koshi Tappu Wildlife Reserve. In Pakistan, marsh crocodile is reported at Sindh and coastal areas of Balochistan. In the province of Sindh small number of marsh crocodiles were reported along the Nara Canal in Khairpur, Chotiari reservoir Sanghar, Deh Akro, Naushahro Feroze, New Jatoti Moro, Mangho Pir, Haleji lake Thatta, Shamzoo Park, khar center Karachi and Karachi Zoological Garden. However, in Balochistan in the rivers of Hab, Fitiani, Nari, Dasht, Nahang, Kuch kuar and Hingol. There are 500 crocodiles in the areas of Makhil lake, 1000 in Chotiari Reservoir and 226 in Nara Desert Wildlife Sanctuary. In India, mugger crocodiles have been reported from 10 states and estimated population is about 2500 to 3500. WWF Pakistan and Zoological Society of Pakistan are working for the conservation of Pakistan.

Botha, C.J., Laver, P.N., Singo, A., Venter, E.A., Ferreira, G.C.H., Rösemann, M. and Myburgh, J.G. (2018). Evaluation of a Norwegian-developed ELISA to determine microcystin concentrations in fresh water. *Water Science and Technology: Water Supply* (<https://doi.org/10.2166/ws.2018.118>).

**Abstract:** Cyanobacteria are known for their extensive and highly visible blooms in rivers or dams in Africa. One of the most important cyanobacteria is *Microcystis aeruginosa* which can synthesise various microcystins that may affect the health of humans and animals. Accurate and efficient detection of microcystins in water is thus important for public and veterinary health. Two enzyme-linked immunosorbent assays (ELISA), a commercially-available ELISA kit (Abraxis) and a newly-developed Norwegian ELISA (putatively cheaper and more robust) were used to detect microcystins in fresh water in South Africa. Water samples were collected monthly at two sites, the Hartbeespoort Dam and a crocodile breeding dam. Extremely high microcystin concentrations (exceeding 360 µg L<sup>-1</sup>) were detected in the Hartbeespoort Dam during January 2015, whereas the microcystin concentrations in the crocodile breeding dam peaked during March-April 2015. Both ELISAs were positively correlated when analysing water samples 'as is' and following resin adsorption and methanol extraction. However, following resin adsorption and methanol extraction of the water samples, the correlation between the two assays was much stronger. These results suggest that the two ELISAs provide comparable results. If the Norwegian-developed ELISA can be packaged and made available as a user-friendly kit, it could be used successfully in surveillance programmes to monitor microcystin concentrations in fresh water bodies in Africa.

Ramos Camejo, Y., Abrante Hernández, T. and Hernández Pérez, Z. (2018). Los estudios de aprendibilidad de las Muestras del Mes en el Museo de Historia Natural "Tranquilino Sandalio de Noda" de Pinar del Río. Estudio de caso: Cocodrilo americano. (Studies about learning of the samples of the Month in the Museum of Natural History Tranquilino Sandalio de Noda of Pinar del Rio. Case study: American crocodile). *SAVIA VIII(70)* (<http://repositorio.geotech.cu/jspui/handle/1234/1951>).

**Abstract:** Como fundamento y herramienta para la retroalimentación de las muestras del mes en el Museo de Historia Natural (MHN) "Tranquilino Sandalio de Noda" (TSN), se realizan estudios de aprendibilidad que buscan un involucramiento real y afectivo de la persona con el saber, a la vez, que posibilitan un acercamiento de su público real a la apreciación de los valores históricos-naturales del patrimonio natural. En este estudio se aplicó como instrumento de exploración empírica la encuesta, para determinar los elementos cognitivos y afectivos del público respecto a la temática y al formato expositivo de la Muestra del Mes "Cocodrilo americano". Se utilizó la prueba estadística Chi cuadrado que evaluó la correlación existente entre las variables "explicación del guía" y "conocimiento adquirido" con 75% de confiabilidad. Existe una relación significativa entre las variables "explicación del guía" y "conocimiento adquirido", siendo la explicación del guía representada por un 51.6%, lo que posibilitó en mayor grado, el aprendizaje y contribuyó a potenciar la educación ambiental respecto al cocodrilo americano. Este estudio tributa a la nueva concepción museológica y museográfica del MHN TSN, que asume la exposición como instrumento didáctico para la promoción de una cultura, conciencia y sensibilidad del público en materia ambiental, que facilita la apreciación de dinámicas de los ecosistemas y de procesos en los que el hombre participa. Esta concepción concibe al visitante como sujeto activo, participativo, que interactúa con la exposición, desde el punto de vista físico, cognitivo e interpersonal, utilizando su conocimiento previo y su pensamiento asociativo-valorativo.

Like foundation and tool for the feedback of the samples of the month in the Museum of Natural History (MHN) Tranquilino Sandalio of Noda (TSN), studies about learning ability that look for a real and affective involvement of the person with knowledge

come true, at the same time, that they make an approach of your real public possible to the appreciation of the historic natural values of the natural patrimony. In this study it was applicable as I orchestrate of empiric exploration the opinion poll, in order to determine the cognitive and affective elements of the public in relation to the subject matter and to the expositive format of the sample of the Month American Cocodrilo. He used him the statistical proof Chi-Square that the existing correlation between the variables evaluated the guide's explanation and knowledge acquired with 75% of reliability. A significant relation between the variables is the guide's explanation and acquired knowledge, being the explanation of the guide represented by a 51.6%, what he made possible mainly, learning and it contributed to potentiate the environmental education in relation to the American crocodile. This study pays tribute to the new conception of the MHN TSN, that assumes the exposure like didactic instrument for the promotion of a culture, conscience and sensibility of the public in environmental matter, that facilitates the appreciation of dynamics of the ecosystems and of processes in which the man participates. This conception conceives the visitor like liable asset, communicative, that he interacts with the exposition, from the point of sight physical, cognitive and interpersonal, using her previous knowledge and her associative appraising thought.

Spielmann, G., Gerdes, L., Miller, A., Verhaelen, K., Schlicht, C., Schalch, B., Haszprunar, G., Busch, U. and Huber, I. (2018). Molecular biological species identification of animal samples from Asian buffets. *Journal of Consumer Protection and Food Safety* 13(3): 271-278.

**Abstract:** In 2015, the Bavarian Health and Food Safety Authority (Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit) initiated controls of Asian buffets to verify declaration of animal species. Especially Mongolian buffets, where raw meat of partly exotic animal species is offered with side dishes and sauces, enjoy high popularity in Germany. A total of 27 samples were collected in 5 Bavarian cities including nine mammalian meat, 1 frog, 3 crocodile, 10 fish, 1 squid, 2 shrimp, and 1 mussel sample. All samples were analyzed using molecular biological methods. The animal species was identified by DNA sequencing of the mitochondrial genes cytochrome c oxidase subunit I, cytochrome b or 16S ribosomal DNA with subsequent database mining. From the 27 samples, 5 were objectionable with either wrong or incomplete labelling. These included two fish samples, two samples falsely declared as zebra which were in fact beef, one guanaco sample which was depicted as camel and another guanaco sample which was marketed as llama. The results clearly show the need for continued surveillance of meat species in buffets covering a wide variety of meats and seafood.

Efio, S., Sogbohossou, E.A., Magnon, Z.Y., Houinato, M.R.B., Habiyaemye, M., Sinsin, B.A. and Tossou, C.R. (2018). Human-wildlife conflicts and mitigation measures in Pendjari Biosphere Reserve, northern Benin. *Annales des Sciences Annales des sciences agronomiques* 22(1): 15-31.

**Abstract:** Human-wildlife conflicts are any interactions between human and wildlife with a negative impact for both parties. Understanding these conflicts is necessary to guaranty a better coexistence between human and wildlife and an improvement of wildlife conservation. The current research aims at assessing human-wildlife conflicts and analyzing the management measures developed by local communities around Pendjari Biosphere Reserve in Benin. Data were collected in January and February 2017 through a questionnaire survey of 245 respondents from different socio-professional background. Three main types of conflicts were observed around the Reserve: crop raiding, livestock predation and destruction of fishing materials. The most destroyed crops were maize (15%), cotton (15%) and millet (14%), and sorghum (29%) and baboon was the most important crop raiding species (61% of depredation cases). Regarding livestock, pig (25%) and sheep/goat (23.1%) were the most attacked animals while hyena was the most

important predator reported (40.6% of attacks). Fishing nets and hoop nets were destroyed by crocodile (72.2%) and hippopotamus (27.8%). To reduce these damages, farmers used several measures such as guarding (82%), scarecrows (64.5%), and fires on the outskirts of the fields (67.3%). Herders mostly used livestock guarding (12.7%) and fires or torchlight lit in the enclosures during the night (8.6%). These measures were not efficient to prevent or avoid the damages but they did reduce them. They must be reinforced to reduce the impact of the damages on the agricultural production, the main source of income of local communities.

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Balaguera-Reina, S. (2018). Natural History of the American Crocodile in a Tropical Pacific Island in Panama, Central America. PhD thesis, Texas Tech University, Lovett, Texas, USA.

**Abstract:** Conservation of large predators has long been a challenge for biologists due to the limited amount of information we have about their ecology and generally low numbers in the wild. The last decade has made it clear that to implement sound conservation practices, we must increase our understanding of large predators' ecology, covering all possible aspects. I investigated the natural history of the American crocodile (*Crocodylus acutus*) on Coiba Island, Panama, addressing a comprehensive ecological framework including four specific topics: crocodile reproductive, population, spatial, and trophic ecology. As part of this work, I also developed two novel approaches focused to identify American crocodiles at the individual level based on the dorsal scute pattern (individual identification pattern recognition IIPR) and to estimate population sizes (using spotlight data) based on the sampling distribution (via bootstrapping) with adjusted confidence intervals. American crocodile reproductive ecology works as a primary driver of the spatial patterns found on Coiba Island, which are also highly influenced by environmental conditions such as precipitation. Hierarchization based on age group and sex through space and time was identified in the study area. Dietary niche overlap analysis showed clear ontogenetic dietary partitioning among juveniles, subadults, and adults. However, the general pattern postulated for crocodylians (small individuals eating invertebrates and adults feeding on larger prey) was not supported by the data collected from Coiba Island, indicating that even adult American crocodiles dwelling in coastal areas rely on more on easy-to-catch/abundant prey such as crustaceans. These results have important implications for the way we see the species as a functional entity of the community, including its interactions and more complex roles in the system than thought before. Overall, American crocodile life history varies considerably from insular to mainland populations and from coastal to in-land populations, demonstrating how "flexible" *C. acutus* is in terms of habitat requirements. Data suggest that realized niches in the insular populations "shrink" compared to mainland populations due to a reduced availability of resources and an inherent increase of intra and interspecific competition. Results from the present study allow future management and conservation planning to be based on the comprehensive integration of information on the ecology of a Neotropical crocodylians.

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Eversole, C.B., Henke, S.E., Wester, D.B., Ballard, B.M., Powell, R.L. and Glasscock, S. (2018). Spatial ecology and habitat utilization of American alligators in an urban-influenced ecosystem. *Journal of Urban Ecology* 4(1). (<https://doi.org/10.1093/jue/juy018>).

**Abstract:** Previous studies have explored spatial ecology and habitat use of alligators and other crocodylian species. However, few studies have explored these characteristics in urban environments. We studied an alligator population that occurred in an urban-influenced ecosystem, a habitat that has received little scientific attention. Our objectives were to determine spatial ecology and habitat use of American alligators within this urban system and to provide a template of methodology and analytical techniques that can be used by urban biologists, planners and researchers in order to assess and study urban crocodylian populations. We recorded 653

observations of alligators and their locations during 19 alligator surveys at an encounter rate of 0.6 alligators per km/survey. Results indicated that alligators exhibited clustering patterns of distribution. Thirteen different wetland types occurred within our survey area, but alligators were only observed in 10 of the 13. We found few differences in habitat use among size classes. We observed little segregation between adult and subadult size classes. However, there was spatial segregation between hatchlings and all other size classes, presumably due to female nest site selection. Alligators of all size classes seemingly avoided areas of high human activity; therefore, urbanization can influence alligator distribution and habitat use within wetland ecosystems. We provide methods and information that can be incorporated into future research and management of urban crocodylian populations. Utilizing this information, biologists can identify potential target areas for implementing management strategies, identify habitat and nesting areas, and mitigate human-alligator conflict.

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Hale, M.D., McCoy, J.A., Doheny, B.M., Galligan, T.M., Guillette, Jr., L.J. and Parrott, B.B. (2018). Embryonic estrogen exposure recapitulates persistent ovarian transcriptional programs in a model of environmental endocrine disruption. *Biology of Reproduction* (<https://doi.org/10.1093/biolre/iy165>).

**Abstract:** Estrogens regulate key aspects of sexual determination and differentiation, and exposure to exogenous estrogens can alter ovarian development. Alligators inhabiting Lake Apopka, FL, are historically exposed to estrogenic endocrine disrupting contaminants and are characterized by a suite of reproductive abnormalities, including altered ovarian gene expression and abated transcriptional responses to follicle stimulating hormone. Here, we test the hypothesis that disrupting estrogen signaling during gonadal differentiation results in persistent alterations to ovarian gene expression that mirror alterations observed in alligators from Lake Apopka. Alligator embryos collected from a reference site lacking environmental contamination were exposed to estradiol-17 beta or a nonaromatizable androgen in ovo and raised to the juvenile stage. Changes in basal and gonadotropin-challenged ovarian gene expression were then compared to Apopka juveniles raised under identical conditions. Assessing basal transcription in untreated reference and Apopka animals revealed a consistent pattern of differential expression of key ovarian genes. For each gene where basal expression differed across sites, *in ovo* estradiol treatment in reference individuals recapitulated patterns observed in Apopka alligators. Among those genes affected by site and estradiol treatment were three aryl hydrocarbon receptor (AHR) isoforms, suggesting that developmental estrogen signaling might program sensitivity to AHR ligands later in life. Treatment with gonadotropins stimulated strong ovarian transcriptional responses; however, the magnitude of responses was not strongly affected by steroid hormone treatment. Collectively, these findings demonstrate that precocious estrogen signaling in the developing ovary likely underlies altered transcriptional profiles observed in a natural population exposed to endocrine disrupting contaminants.

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Silva, F.P. (2018). Morphological Aspects Associated with Sexual Dimorphism in Hatchling of *Melanosuchus niger* (Spix, 1825) (Crocodylia: Alligatoridae) in the Reserve of Sustainable Development Mamirauá, Amazonas. MSc thesis, Federal University of Juiz de Fora, Minas Gerais, Brazil (<https://repositorio.ufjf.br/jspui/handle/ufjf/6864>).

**Abstract:** The shape and size of the body are characteristics that differ between adult males and females of crocodylians; however, there is no report about sexual dimorphism for *Melanosuchus niger* hatchling. The present study had as objective to analyze the existence of sexual dimorphism in of *M. niger* hatchling based on morphological data. The specimens analyzed in this study came from an artificial incubation experiment. The eggs were collected at Lake Tracajá on Mamirauá Sustainable Development Reserve. The

gonads were analyzed with macroscopic parameters (shape, texture, color and presence/absence of Müllerian duct) to verify if there was sexual dimorphism. The sexual identification of the hatchling was confirmed by the histological analysis of Gonad-Adrenal-Mesonephro (GAM) complex according to routine procedures. For the analysis of the variation of the form the quantified morphology of the epidermal plates of the hatchling's head was used from 13 landmarks submitted to a geometric morphometry analysis. For this, the heads of the specimens were photographed in dorsal view and the TPSUtil, TPSDig2 and MorphoJ software were used for analysis. Histological analysis served to confirm the animal's sex of this study. There were identified, 38 females and 12 males. In the testes it was possible to observe the presence of the tunica albuginea associated with a little differentiated marrow, where the presence of seminiferous tubules was not observed, only the presence of vacuolated cells. Associated with the mesonephros we observed the presence of the remaining Müllerian duct. In the ovaries, the presence of an evident germinative epithelium, formed by simple cuboid epithelial tissue was observed. In the medullary region the presence of ovarian follicles and marrow gaps were observed. The Müllerian Duct was connected to the mesonephros by the mesosalpinx. It was observed that the macroscopic characteristics of the gonads differ between the sexes. They showed elliptical, smoothly, yellow testes. The ovary presents granular structure with light beige coloration and associated with the presence of Müllerian Duct. Principal component analysis (PCA) showed variation in the shape of epidermal scales of *M. niger* hatchling, indicating the presence of sexual dimorphism. The Principal Component 1 (PC1) explained 52.6% of the shape variation, as the Principal Component 2 (PC2) explained for 14.4%, and the overall variation explained by these two main axes was 67.0%. The analysis of discriminant function confirmed the presence of sexual dimorphism ( $p=0.01$ ). It was observed that the females have the posterior region of the head longer and wider, and the jaw shorter and narrower. While the posterior region of the males head is slightly shorter and narrower, and the jaw longer and wider. The macroscopic and histological characteristics of the gonads were similar to those found in other species of crocodylians. Macroscopic analysis was sufficient to identify the sex of *M. niger* hatchling. In adult crocodylians, sexual dimorphism may be present in the region of the skull related evolutionarily to visual recognition of the genus. However, sexual selection would not be as relevant to young people as to sexually mature individuals. The variation of the shape in both the skull and mandible found in *M. niger* hatchling suggests that males and females may have undergone different ontogenic processes. The lack of work involving the study of sexual dimorphism using a geometric morphometry technique in hatchling of other populations of *M. niger* and/or other species of crocodylians makes difficult direct comparison. However, it is clear the importance of the study of sexual dimorphism in gilts of crocodylians to estimate the sexual proportion, especially if applied in natural populations. Geometric morphometric models can help to develop graphic representations of sexual dimorphism of the head in crocodylians, which makes these results important for conservation programs under natural conditions, since it represents a non-lethal alternative to the techniques used for sexual identification in *M. niger*.

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Macoto Calix, C.J. (2018). Preparation and Preliminary Characterization of *Alligator mississippiensis* Keratin-rich Extracts. MSc thesis, Louisiana State University, Baton Rouge, Louisiana, USA.

**Abstract:** The state of Louisiana has become the lead producer of alligator skins and meat in the United States. More than 300,000 alligators are harvested annually from farm and wild sources. Simultaneously, the industry generates thousands of pounds of waste during the processing of alligators. This waste contains valuable fibrous proteins such as keratins. This study aimed to identify keratin in keratin-rich extracts prepared from alligator backstraps. Alligator backstrap samples were collected from alligator processing facilities located in Springfield, Louisiana. The percentage of protein extracted with 2-mercaptoethanol (2BME),

2BME and dithiothreitol (DTT), DTT, or sodium metabisulfite (SMBS) was calculated. Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE), Matrix-assisted Laser desorption ionization (MALDI), Fourier Transform Infrared Spectroscopy (FTIR), and Scanning Electronic Microscopy (SEM) were used to characterize the AB keratin-rich extract. A multi-step process for the preparation of a keratin-rich extract from alligator backstraps was developed. Reduction with 200 mM dithiothreitol produced the greatest protein solubility (55%). A protein band of 10 kDa was observed when the buffer containing 200 mM DTT was used. The molecular weights (MW) of keratins determined by MALDI did not correlate with SDS-PAGE results suggesting that MALDI analysis was not optimized. FTIR spectra showed peaks for amide A, I, II, and III. The AB keratin-rich extract was rich in glycine and proline which is a characteristic of low-molecular-weight beta-keratins. The AB keratin-rich extract showed a flake-like network structure with some filaments, comparable to previous observations made on 10 kDa beta-keratin from poultry feather. The keratins contained in *Alligator mississippiensis* offer a potential to improve waste management practices and increase revenue in the alligator industry, through their commercialization.

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Hickey, A.P., Du, C., Jiang, X., Polsky, G., Rodriguez, M., Kuldilok, K. and Suchato, R. (2018). CDIP: Introducing crocodile oil to ASEAN+3. Pp. 113-132 in *Asian Agribusiness Management: Case Studies in Growth, Marketing, and Upgrading Strategies*, ed. by R.D. Christy, J.C. Bernardo, A. Hampel-Milagrosa and L. Fu. World Scientific Publishing: Singapore.

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Nixon, B., Johnston, S.D., Skerrett-Byrne, D.A., Anderson, A.L., Stanger, S.J., Bromfield, E.G., Martin, J.H., Hansbro, P.M. and Dun, M.D. (2018). Proteomic profiling of crocodile spermatozoa refutes the tenet that post-testicular maturation is restricted to mammals. *Molecular & Cellular Proteomics* (<https://doi.org/10.1074/mcp.RA118.000904>).

**Abstract:** Competition to achieve paternity has contributed to the development of a multitude of elaborate male reproductive strategies. In one of the most well-studied examples, the spermatozoa of all mammalian species must undergo a series of physiological changes, termed capacitation, in the female reproductive tract prior to realizing their potential to fertilize an ovum. However, the evolutionary origin and adaptive advantage afforded by capacitation remains obscure. Here, we report the use of comparative and quantitative proteomics to explore the biological significance of capacitation in an ancient reptilian species, the Australian saltwater crocodile (*Crocodylus porosus*). Our data reveal that exposure of crocodile spermatozoa to capacitation stimuli elicits a cascade of physiological responses that are analogous to those implicated in the functional activation of their mammalian counterparts. Indeed, among a total of 1119 proteins identified in this study, we detected 126 that were differentially phosphorylated ( $\pm \geq 1.2$  fold-change) in capacitated versus non-capacitated crocodile spermatozoa. Notably, this subset of phosphorylated proteins shared substantial evolutionary overlap with those documented in mammalian spermatozoa, and included key elements of signal transduction, metabolic and cellular remodeling pathways. Unlike mammalian sperm, however, we noted a distinct bias for differential phosphorylation of serine (as opposed to tyrosine) residues, with this amino acid featuring as the target for ~80% of all changes detected in capacitated spermatozoa. Overall, these results indicate that the phenomenon of sperm capacitation is unlikely to be restricted to mammals and provide a framework for understanding the molecular changes in sperm physiology necessary for fertilization.

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Balaguera-Reina, S. and Farfan-Ardila, N. (2018). Are we ready for successful apex predator conservation in Colombia? Human-crocodylian interactions as a study case. *Herpetological Review* 49(1): 5-12.

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For further information on the CSG and its programs on crocodile conservation, biology, management, farming, ranching, or trade, contact the Executive Office (csg@wmi.com.au) or Regional Chairs

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**Task Force/Working Group Chairs:** Future Leaders Program, Dr. Sergio Balaguera-Reina (sergio.balaguera-reina@ttu.edu); Tomistoma Task Force, Bruce Shwedick (Bshwedick@aol.com).