Genital structures and sex identification in crocodiles

Thomas Ziegler¹* & Sven Olbort¹

¹AG Zoologischer Garten Köln, Riehler Straße 173, D-50735 Köln, Germany. * corresponding author: e-mail: tziegler@zoo-koeln.de

Introduction

A suitable sex identification in crocodilians is not only of importance for ecological field work and ethological studies, but also for successful breeding operations. However, sexing based on external morphological features alone is rather difficult particularly in juvenile and mature specimens. In general, males possess relatively thicker tail bases, are stronger built and grow larger than females. But direct comparisons between commensurate sexes are rarely possible, and the aforementioned differences are only applicable in larger specimens. Hence, identifying the sex by external morphology is simplest in the Gharial (*Gavialis gangeticus*) due to a bulb-like appendage sexually mature males reveal on the snout tip, which is lacking in females (e.g. Trutnau 1994).

The most distinct sex character in crocodiles is the male erectile penis that is hidden inside the cloaca in the resting state. For correct sex identification, the male external genital organ of an immobilised crocodile must be felt out and protruded from the cloaca, and subsequently compared to the similar female clitoris. However, as structures in male and female external genitals are very similar especially in juvenile specimens, sexing by genital structures can be rather difficult.

We herein provide a short review of crocodilian genital morphology in general and sex identification based on genital structures in particular. With regard to the terminology and therein relevant definitions pertained, "sex identification" implies diagnostic measures to identify the individual sex, while the term "sex determination" refers to the biological process of sex formation, and which results by the temperature of egg incubation in the crocodile species studied so far (temperature-dependent sex determination [TSD], e.g., Webb et al. 1987, Lang et al. 1989, Lang & Andrews 1994, Thorbjarnarson 1997).

Historical review

Contrary to the female copulation organs in squamate reptiles, the long time overlooked and only relatively recently discovered hemiclitores (e.g., Böhme 1995, Ziegler & Böhme 1996, 1997), external genitals in female crocodilians are known for a long time. For example, morphological structures of the penis and the clitoris of "*Crocodilus lucius*" (= *Alligator mississippiensis*) were already described 150 years ago (Leydig 1857). In that century the crocodile cloaca and its outer genital organs (see Fig. 1) were extensively described by Gadow (1887), Bronn (1890) and Boas (1891). At that time it was yet known that the size of the clitoris of 3 to 4 feet (i.e. 90 to 120 cm) long crocodiles approximately equals that of male copulation organs of similar sized specimens. It was also known that the outer genitals of equal sized sexes almost revealed the same differentiation of the sperm groove and of the terminal genital structures. Gadow (1887) and Bronn (1890) provided first evidence for a sex identification based on genital morphology. Both demonstrated that in larger crocodiles the structurally identical clitoris is smaller-sized compared to the male copulation organ, and former stagnates in growth. Bronn (1890) additionally recognized first differences in the development of the outer genital structures: he found out that the blunt structure below the tip

of the copulation organ (see Fig. 2) was less developed and relatively thinner in equal-sized females, likewise in larger females. It was also well known at that time that both penis and clitoris protrude from the cloaca in early embryonic stages, but disappear into the cloaca some time before the hatching process. Additional historical studies dealing with copulation organs in crocodilians are those by, e.g., Hellmuth (1902), Wiedersheim (1909), Petterer & Neuville (1914), Meisenheimer (1921), Reese (1924), Gerhardt (1933), and McCann (1949).

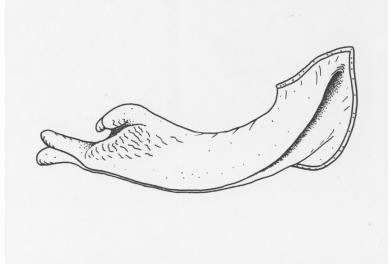


Figure 1. Drawing of a dissected "*Crocodilus vulgaris*" (= *C. niloticus*) penis in lateral view, after Gadow (1887).



Figure 2. Oblique view of an ethanol preserved, ca. 9 cm long *Crocodylus niloticus* penis (ZFMK 59017) from southern Sudan (Keniza). Photo T. Ziegler.

The structure of the copulation organ

The crocodilian phallus is an unpaired organ that is normally hidden inside the longish cloacal slit. It consists of a cone-like process of the anterior ventral cloacal septum. The copulation organ usually is cylindrical, somewhat laterally compressed and buttressed by originally pair wise built connective tissue structures. Spongy tissue with dilated blood lacunas enable the penis to bulge by accumulating blood. Although the shape of the bulging penis is only slightly changed, the protrusion of the copulation organ from the cloaca opening is additionally supported by muscle pressure. A medially located groove stretches dorsally to the anterior tip

of the copulation organ and functions as sperm groove in males. Such a groove is also discernible in the clitoris but remains functionless. The tip of the outer genital organs show structures that are comparable to the mammalian glans (glans penis) and can be distinguished between the lappet-shaped tip on which the sperm groove extends and a blunter structure that is demarcated from the tip by a small groove.

For mating, the penis protrudes from the cloaca and is almost semicircularly bent towards the venter (Fig. 3). In this condition, the sperm groove is turned away from the venter (Fig. 4; see also Webb & Manolis 1989: 32). For photos of the penis of *Crocodylus palustris* during copulation see Gerhardt (1933); for further figures of the species' penis and these of *Alligator mississipiensis*, *Caiman crocodilus apaporiensis*, *Gavialis gangeticus*, and *Paleosuchus palpebrosus* see, e.g., Tonutti (1931), McCann (1949), Whitaker et al. (1980), and Trutnau (1994).



Figure 3. Protruded penis of a 115 cm long *Paleosuchus trigonatus*, photographed under water. Photo S. Olbort.

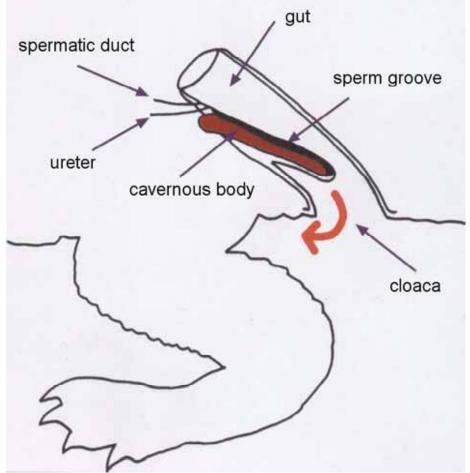


Figure 4. Schematic drawing of the cloaca of a male crocodile, after Boas (1891), Wiedersheim (1909), and Ross (2002). It is well discernible, that the sperm groove of the penis in resting state is directed upwards; once the organ is erected/protruded (red arrow), the sperm groove is downwards directed.

Sex identification by means of outer genital structures

For evaluating the size and the structures of the crocodilian copulation organ, usually the penis or clitoris of a previously immobilized crocodile is protruded by inserting a finger into the cloaca (Chabreck 1967, Brazaitis 1969, Honegger 1978). With the application of this palpation method, the smaller clitoris normally can only be pressed out a few millimetres beyond the cloaca at best, whereas the larger penis should be well palpable and distinctly protruding as a relatively rigid and longish cone-shaped organ, at least in larger specimens. Brazaitis (1969) stated a penetration-depth of eight or less centimetres for 3-4 m large crocodiles, in which the unerected penis is palpable inside the cloaca. However, according to Honegger (1978), palpation with the stretched forefinger only functions with specimens of a minimum total size at 75 cm. To identify the sex in younger crocodiles, a veterinary vaginal speculum serves best to obtain detailed insights into the cloaca and its structures. Another possibility proposed by Whitaker et al. (1980) is the protrusion of the copulation organ by lateral compression of the cloacal region together with a cautious bending of the tail towards the crocodile venter. However, for a successful sex identification, the afore mentioned methods all deserve skills in the recognition of the relative size differences and the different peculiarities of the male and female genital structures.

In general, the crocodilian sex determination and therewith the differentiation of the gonads occurs prior to hatching (Allsteadt & Lang 1995). However, the state of the outer genital organ's differentiation in juvenile crocodiles apparently is species-specific. For example, in *Caiman crocodilus, Crocodylus johnstoni, C. moreletii, C. niloticus, C. palustris, C. porosus,* and *C. siamensis* discernible differences do exist between the penis and clitoris of freshly hatched juveniles (Webb et al. 1984, Hutton 1987, Lang et al. 1989, Lang & Andrews 1994). In contrast, genital morphological sex identification for example in *Gavialis gangeticus* is a rather difficult procedure (Whitaker et al. 1980). After Lal & Basu (1982), a proper sex identification based on the outer genital structures is impossible in juveniles of the latter species and only applicable in Gharial specimens of at least 1 m in total length. Besides *Gavialis gangeticus*, Brazaitis (1969) further mentions the genus *Tomistoma* which likewise can successfully be palpated at a minimum total length of 75 cm to identify its sex.

In a recent paper, Allsteadt & Lang (1995) provided some essential insight in a better understanding of the in part only slight differences between penis and clitoris of juvenile to subadult crocodilians. Both authors exclusively focused on the genital morphological sex identification in 0-24 months old Alligator mississippiensis. Inspection of the outer genitals (which remained inverted in the cloaca) proved to be very reliable, as was subsequently confirmed by macroscopic and histological gonad examination. Penes of hatchlings were always large (with a mean length of 4.1 mm), roundish and red; in contrast, clitores of freshly hatched females were rather whitish-pale and smaller (2.8 mm mean length). In addition to the differences in size, the authors further identified sex differences related to shape and structure of the outer genitals in freshly hatched A. mississippiensis (Figs. 5-6). Success rate of these sex identifications resulted in more than 95%: male sexes were exclusively identified correct; only in few females, which were incubated at relatively high temperatures (32°C), dimensions of their outer genital organs resembled those of males. However, the clitoris length in these females was always smaller than the penis length of males at the same age. Webb et al. (1984) reported on similar observations in Crocodvlus johnstoni hatchlings, viz that males rarely were misidentified as females, and that females with maximum incubation temperature values occasionally were misidentified as males due to their large clitores. Of 4,569 live hatchlings, Webb et al. (1984) correctly identified the sex in 95%, due to the fact that the clitores in hatchlings of C. johnstoni (similar to the condition observed in C. porosus) were significantly smaller than the penes and showed a rather pointed tip (Fig. 7). According to the authors, such differences were observable both in live, freshly dead and preserved specimens. With respect to the post-hatchling development of the outer genitals in A. mississippiensis, Allsteadt & Lang (1995) found out that the penes grew disproportionately faster as compared to the growth of the clitores. At the age of two years, in equal-sized female and male alligators the latter reveal a 3-4 times larger copulation organ.

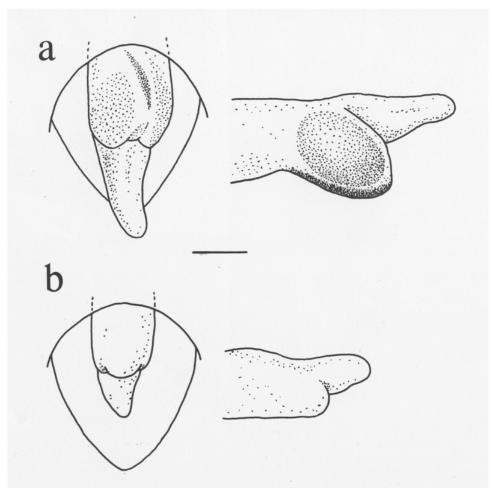


Figure 5. Outer genitals of freshly hatched *Alligator mississippiensis* (a: male, b: female) after Allsteadt & Lang (1995); left: ventral view of cloaca; right: dissected genitals in lateral view. Scale bar: 1 mm.



Figure 6. Penis of a larger Alligator mississippiensis. Photo S. Olbort.

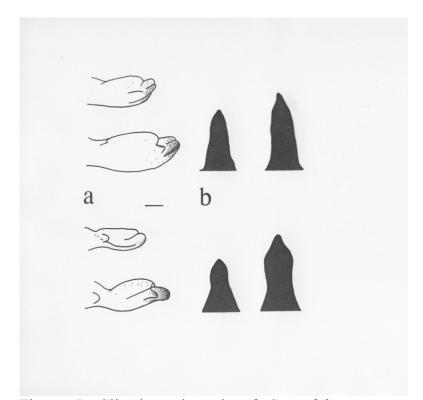


Figure 7. Clitoris and penis of *Crocodylus porosus* hatchlings (above) and *C. johnstoni* hatchlings (below) pointing to the differences in shape and size, after Webb et al. (1984); a) in lateral view (each with the clitoris being depicted above), b) in ventral view (each with the clitoris being depicted on the left). Scale bar: 1 mm.

Outlook

Concluding, further research on the genital morphology related to both sexes is highly recommended. Even studies dealing with temperature-dependent sex determination are still lacking for more than one half of all 23 extant crocodilians (Lang & Andrews 1994, Deeming 2004). Concerning the differentiation and size of copulation organs in adult crocodiles, marginal published notes constitute the majority of information available (except for the above mentioned citations), e.g., a 7 cm long penis of a 1.5 m long caiman, or a 20 cm long penis of an adult Nile crocodile (Trutnau 1994); furthermore, Whitaker et al. (1980) mentioned the 3 cm wide penis of an almost adult *Crocodylus palustris* with 2.5 m total length, that was palpated and subsequently protruded 10 cm beyond the cloaca.

Not before specific differentiation of copulatory organs of the herein not mentioned crocodile species (see Figs. 8-15) are better understood, including age-dependent and individual variation, genital morphology based sex identification will provide more reliable results in the future. Furthermore it must be taken into account, that not only different incubation temperatures influence growth and length of the outer genital organs in crocodiles (e.g., Webb et al. 1984, Allsteadt & Lang 1995), but also human impact on the natural habitat, e.g. juvenile male *A. mississippiensis* from an estrogen-contaminated lake in Florida revealed abnormal reduced outer genitals (Guilette et al. 1994). Contrary to, the male hormones can effect an exceptionally larger growth of outer genital organs in both sexes of juvenile alligators and crocodiles (Forbes 1939, Ramaswami & Jacob 1965).

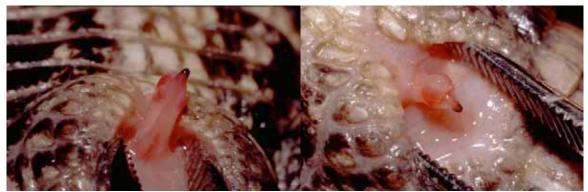


Figure 8. Cloaca of a 40 cm large *Paleosuchus palpebrosus*, that was cautiously spread with blunt tweezers; the size and structural details of the outer genital argue for a male specimen. a (left) view from above, b (right) view from below. Photos T. Ziegler.



Figure 9. Penis (a; left) and clitoris (b; right) of *Paleosuchus palpebrosus*; the male measured 60 cm in total length, the larger female had 49 cm snout vent length. Photos S. Olbort.



Figure 10. Clitoris of an adult Paleosuchus trigonatus. Photo C. Bernhardt.



Figure 11. Penes of *Paleosuchus trigonatus* with (a; left) 100 cm, and (b; right) 115 cm total length. Photos S. Olbort.



Figure 12. Penes of *Alligator sinensis* with (a; left) 120 cm, and (b; right) 90 cm total length. Photos S. Olbort



Figure 13. Clitoris of an Alligator sinensis with 80 cm total length. Photo S. Olbort.



Figure 14. Spread cloaca with about 5 mm large clitoris of an *Alligator sinensis* (with 30.5 cm snout-vent length, and 62.5 cm total length). Photo T. Ziegler.

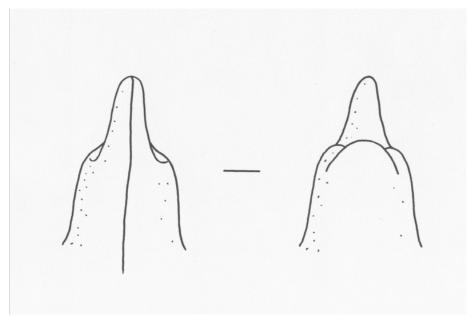


Figure 15. Drawing of the *Alligator sinensis* clitoris depicted in Figure 14; left dorsal, right ventral view. Scale bar: 1 mm.

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