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Crocodilian Phallic Malformations and Anomalies

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Crocodilian Phallic Malformations and Anomalies

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Abstract

Phallic malformations directly impact crocodilian copulatory function and thus could impact reproductive success in individual animals. This case series characterizes malformations observed and the implied reproductive consequences. We assessed a range of phallic malformations observed in saltwater crocodile (*Crocodylus porosus*), Nile crocodile (*Crocodylus niloticus*), broad-snouted caiman (*Caiman latirostris*), and Cuban crocodile (*Crocodylus rhombifer*) during veterinary examinations or postmortem studies and characterized observations into the following categories: amputation, phallic glans malformations, sulcus spermaticus

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Key Words: Caiman, Crocodylus, insemination, intromission, phallic malformations

Introduction

Crocodilian phallic anatomy and associated copulatory functions have been characterized among species in all three extant families of Crocodylia (Johnston *et al*., 2014; Fitri *et al.*, 2018; Moore *et al*., 2020a-c, 2021, 2022). In each taxa, male phalli serve two core copulatory functions: intromission and insemination (Kelly and Moore, 2016). Prior to intromission, coordinated cloacal muscle contractions protrude the phallus from intracloacal storage to an external copulatory position. Suspensory connective tissue attached to the phallic shaft acts as a pivot point rotatating the emerging shaft and glans cranially (Kelly, 2013). The rigid corpus fibrosum of the phallus shaft facilitates intromission that, in turn, places the distal male glans in contact with female reproductive tract openings in the walls of the uroprotodeal fold (Moore *et al.*, 2022). Subsequently, with increased blood pressure, the corpus spongiosum elements within the glans expand the distal phallus into species-specific shapes (examples, Fig. 1) that create a copulatory fit with female cloacal elements and aligns the termination of the spermatazoa-carrying sulcus spermaticus with the vaginal opening to each oviduct (Moore *et al*., 2021, 2022). Further, male-female tissue copulation interactions may advantageously exclude water from the site of semen transfer (Grigg *et al*., 2015). Although many of the species-specific details of insemination mechanism(s) still require further functional study, it appears that the sequence of the copulatory process and related functions of phallic structures are strongly conserved among crocodilian taxa.

Difficulties with any of these steps could impair intromission or insemination and leave a male with reduced or absent reproductive capabilities. These pathologies may be congenital, developmental, or trauma induced. Our case series does not attempt to identify the manifold causes of pathologies in the examples presented or aim to predict frequencies in wild or husbandry situations; we instead focus on highlighting situations where a visual examination of genital morphology could lead to an informed assessment of decreased or eliminated male reproductive function. A proper understanding of the range of abnormalities and malformations of the male genitalia and its potential effect on reproductive capacity is essential for success in any health assessment or breeding program, whether maintaining animals for conservation or improving the efficiency of farming operations.

Materials and Methods

The term crocodilian is a collective term used to refer to members of the Crocodylidae, Alligatoridae, and Gavialidae families. Of the species included in this study, Cuban crocodile (*Crocodylus rhombifer*), saltwater crocodile (*Crocodylus porosus*), and Nile crocodile (*Crocodylus niloticus*) belong to Crocodylidae, while broad-snouted caiman (*Caiman latirostris*) and American alligator (*Alligator mississippiensis*) are members of the Alligatoridae family. Due to species limitations and the opportunistic nature of this case series, Gavialidae spp. were not included in this study.

Nile crocodile tissues were collected at a farming operation (Le Croc; Brits, South Africa) and shipped to the United States for further processing; animal collection, handling, and tissue collection procedures conformed to South African and United States permitting and utilized Institutional Animal Care and Use Committee (IACUC#: Moore 11-2015) approved protocols (CITES certificate #152603) and United States import permitting (CITES schedule II). Animal handling and tissue collection procedures for broad-snouted caiman conformed to IACUC and permitting regulations of the University of the South Institutional Animal Care and Use Committee (IACUC; project #1-2017); approved protocols and subsequent transport utilized Argentinian export (CITES certificate #44150) and United States import permitting (CITES schedule II). Saltwater crocodile and Cuban crocodile data were obtained from opportunistic field photography and communications with veterinarians and field biologists, respectively, from live animals during routine examinations. Cuban crocodile observations came from a CITES-registered captive breeding operation that is authorized to collect and raise Cuban crocodiles per the Cuban government. American alligator tissues were collected under Florida permit (Permit#WX01310) and necropsied under University of Florida approved protocols (IACUC #D525).

Morphological malformations were advantageously observed during either health assessments or experimental necropsy of the four following species:

Saltwater crocodile: Two captive 20-year-old males of good body condition and apparent health were assessed for long-term reproductive failure in 2018. Each male was housed in an individual pen (26 m^2 pens consisting of smooth concrete with a water body in the center of each pen covering 25 percent of the pen surface area) on a Bangledeshi farm as sole breeders with two females. After years of not producing offspring, males were captured for veterinary examination and genital anatomy was photographed. Photographs were shared through field communication following exam.

Nile crocodile: Twenty-two captive 3-year-old juvenile males (Snout-Vent Length [SVL] 93-96 cm; 36-38 in) were necropsied following standard hide collection at Le Croc Breeding Farm and Tannery near Brits, South Africa in 2016. One out of 22 juveniles examined exhibited phallic malformation. Juvenile animals at this facility are group housed in large enclosures with concrete flooring. Histological sectioning, artificial inflation, and 3D reconstruction of serial MRIs were performed as previously published (Moore *et al*., 2021). In short, tissues were washed in 1x phosphate-buffered saline (PBS) then imaged in a Bruker Biospec MRI scanner (Bruker Corporation, Billerica, MA, USA) using a 72 mm diameter Bruker volume RF coil and a 3D FLASH sequence (T/TR/Flip/NA = 6.5 ms/25ms/14°/2) with fat suppression and a total scan time ~ 2 hr. Resolution was 100 by 100 mm isotropic.

Broad-snouted caiman: Twenty-two captive-raised juvenile males (SVL range 48-69 cm; 19-27 in) were necropsied for morphological study (Moore *et al*., 2022) at two Argentinian facilities in May 2018: the Yacaré Project, Santa Fe and Yacaré Pora Ranch, Correntes. Three animals displayed phallic abnormalities. Individuals of up to 10 clutches were co-housed in cement pools with no sex evaluation of the animals until commercial hide harvest. Serial MRI methods match those employed for Nile crocodiles above.

Cuban crocodile: Two adult males were photographed during routine handing including a captive-born male (SVL 2.9 m; 114 in) housed at the Zapata Swamp Farm, Cuba and a wild-caught male (SVL 1.8 m: 71 in) from the Zapata Swamp, Cuba. Opportunistic communications from field biologists yielded photographs from the examination of the two individuals.

Additionally, tissues from an American alligator were utilized to highlight the normal anatomical structures of Alligatoridae. This estimated 4–5-year-old animal was collected by hand at Lake Woodruff Wildlife Refuge, Florida, USA in 2017 and transported to the University of Florida (Gainesville, FL, USA) for necropsy, with normal phallic tissues harvested for anatomic study.

Results

Here we present phallic malformation categories observed among species.

Glans tip malformations:A phallus with a bisected glans tip was observed in one broad-snouted caiman (compare Fig. 2A, B). This malformation would allow semen movement to the distal phallus but lack targeted insemination.

Improper glans inflation: Phallic glans malformation was observed in a broad-snouted caiman, a Nile crocodile, and a Cuban crocodile. While the dense irregular connective tissues of the corpus fibrosum provides shaft rigidity to allow intromission, the corpus spongiosum that laterally flanks the sulcus spermaticus subsequently expands into an inflatable structure that produces a species-specific erect glans shape (Figs. 3A and 4C). An inflated malformed glans results in defect exaggeration as glans aspects deflect from their appropriate copulatory shape (Figs. 2C,D; 3B,C; 4C,D). In histological section (Fig 3D), three-dimensional reconstruction (Fig. 3E), and serial MRIs (Fig. 5A-C), glans corpus spongiosum bisections results in substantial inflation malformations.

Sulcus spermaticus defects: A Cuban crocodile presented a phallus with an eroded sulcus spermaticus at the proximal base (compare Fig. 4A, C to B,E,F), putatively impeding sperm delivery.. The sulcus progressively becomes sclerotic (fibrous scar tissue) and stenotic (narrow) from the base towards the tip.

Phallic amputations: Captive saltwater crocodiles and caiman and wild-caught Cuban crocodile males presented phallic amputations (Fig.6), and these were the most easily observed malformation during examination. Note, extreme amputation cases, such as in saltwater and Cuban crocodile seen here (Fig. 6B, E), could result in animals mistaken for females upon cloacal probing to identify sex.

Discussion

The proper identification and classification of phallic malformations can help to predict functional fertility or infertility as they occur in the animal. By cataloging these abnormalities, we can begin to enumerate how the male genital anatomy can vary. Thus, we can understand the effects of these malformations on breeding fitness and reproductive success.

Amputations, phallic glans malformations, and sulcus spermaticus obstructions were cataloged in this case series and compared to the current literature, taking into consideration applied form and function. Two phallic pathologies not observed in this study but detailed in literature include chronic phallic prolapse and impaired phallic eversion, which will also be discussed.

Amputation: There is some evidence that individuals with persistent paraphimosis can still retain the ability to copulate and inseminate females (Augustine *et al*., 2017). However, amputation of the distal phallus including the glans and distal sulcus spermaticus represents an extreme disruption of copulatory ability. The animal may maintain eversion capability and limited intromission but will lose the ability to produce tissue interactions between the glans and the female proctodeum that may lead to effective insemination (Moore *et al*., 2021, 2022).

Phallic Glans Malformations: Comparing the filiform glans tip from the caiman with the bisected glans tip in Fig. 2B with the normal anatomy of this filiform process seen in members of Alligatoridae, shown in an American alligator in Fig. 1 and 2A, this caiman's malformation would likely impact fertility. We hypothesize that filiform glans tip malformations such as observed in this caiman would have a greater fertility impact than similar bisections of blunt glans tips observed in members of Crocodylidae (Figs. 1, 3A, and C), for functional reasons stated in the introduction.

Glans malformations may impact male fertility by allowing water seepage in the female cloaca, causing impaired glans tip placement, and altered male cryptic signals that stimulate female gamete utilization. Both examples of glans injury presented were collected from farms where concrete bottomed pens are utilzed. This raises concerns of housing and husbandry impacting fertility as animals of two different taxa and located in two different geographic locations displayed similar injuries. Possibly these are abrasion injuries resulting from the concrete bottoms, or they are the result of male-to-male conflict due to the animal concentrations housed in each of these pens. While there is not enough data to determine prevalence of such malformity in farmed crocodilians, particularly those on similar substrates, we posit that similar cases could easily go undocumented.

Leiva *et al*. (2022) documented the frequency of male penile lesions in juvenile broad-snouted caimen raised in a farm situation. In association with the lesions, 10.9 percent of the animals from the sample population had structural defects on the glans cuff. Petechiae were also noted with significant frequency (34.8 percent) on the dorsal or ventral shaft. This case series shows that a malformed glans is not necessarily a rare occurrence in farmed animals, even in young animals (about 1 m; 40 in total length), resulting in malformations as they sexually mature. While many of these individuals are not utilized as breeding stock, those that are expected to go on to copulate may have further difficulty or possible infertility due to these structural defects.

While these housing and husbandry concerns may not be consequential in operations raising animals for leather or meat, this is a problem for facilities raising conservation or collection animals or growing males for farm breeders. Despite raising overall healthy individuals, phallicly-impared males may not be able to reproduce, thus impeding efforts to increase wild breeding populations or simply nullify female reproduction in male-restricted farming facilities. In either case, we propose conducting a physical examination of each male's phallus condition before selecting them for reintroduction or as a potential breeder.

Sulcus Spermaticus Obstruction: Targeted semen delivery is vital for successful copulation. In crocodilians, this is achieved via peristaltic smooth muscle contractions lateral to the sulcus spermaticus and sulcus termination in a glans tip projection. While the Crocodylidae glans tip is blunt, as opposed to filiform in Alligatoridae (compare Figs. 2A and 4C to 3A), both align gamete delivery with oviductal openings during copulation. Therefore, any sulcus spermaticus or glans tip malformation would impede gamete transport and placement, respectively.

The Cuban crocodile in Fig. 4B exhibits significant sclerosis and stenosis along the extent of the sulcus spermaticus. This damage to the sulcus spermaticus would prevent adequate sperm delivery to the distal tip of the glans, impeding fertility via lack of targeted sperm delivery. Excision of the sulcus spermaticus has been utilized as an effective form of birth control in a cohort of mugger crocodiles (*Crocodylus palustris*) without ligating or obstructing the vas deferens (William *et al*., 2015). Therefore, the animal did not lose the ability to create sperm and transport it to the proximal sulcus spermatics, but rather lost the ability to conduct the sperm to the distal glans tip for insemination. This seems to align with the individual in 4B, where successful insemination appears to be effectively impeded.

Prolapse: Prolapse is an additional category of phallic abnormality that would necessitate a breeding examination. We did not observe prolapse in this case series. Prolapse is defined when "inner tissue protrudes through the vent opening, resulting in exposed intestines, cloaca, or [genitalia]" (Hoppes, 2021). In the case of crocodilian male reproductive anatomy, the condition can present as either penile prolapse (*i.e*., paraphimosis), where the everted phallus cannot be retracted into the vent, or cloacal prolapse, where the sum of inner tissues of the cloaca protrudes outside the body.

The Global Federation of Animal Sanctuaries' Standards for Crocodilian Sanctuaries (2019) states that "care [should be] taken to prevent cloacal prolapse… during handling and veterinary care", a condition that can be fatal (Ogunro *et al*., 2018; Vyas and Mistry, 2021), and demands prompt veterinary attention. While Hedley and Eatwell (2014) provide a retrospective study of 56 cases of reptilian cloacal prolapse, none of the examples presented were crocodilians, suggesting that the condition may be more prevalent in squamates and chelonians or underrepresented in literature. Our literature review of cloacal prolapse in crocodilian species identified nine descriptive cases (Nile crocodile: Lankester and Hernandez-Divers, 2005; mugger crocodile: Simon et. al., 2012*;* Vyas and Mistry, 2021;saltwater crocodile: Johnston *et al*., 2014;Cuban crocodile: Augustine *et al*., 2017; gharials (*Gavialis gangeticus*): Mohanty *et al*., 1980; dwarf crocodile (*Osteolaemus tetraspis*): Ogunro *et al*., 2018) with none determining the specific cause of the condition.

Paraphimosis alone may resolve spontaneously (Lankester and Hernandez-Divers, 2005), but can also be recurrent or persistent and cause permanent damage to the phallus (Lankester and Hernandez-Divers, 2005; Johnston *et al*., 2014; Augustine *et al*., 2017). Because crocodilian phalli do not have urinary function, persistent paraphimosis can be treated with phallic amputation at a reproductive cost. In the procedure, significant hemorrhage from the two blood vessels that run parallel to the sulcus on the ventral surface of the distal glans can occur when the damaged portion of the phallus is amputated (Lankester and Hernandez-Divers, 2005). These blood vessels provide the blood supply for inflating the distal glans during copulation (Moore *et al*., 2016) and should be cauterized or otherwise ligated during surgery (Lankester and Hernandez-Divers, 2005).

Persistent or recurrent paraphimosis could be caused by many factors. The simplest cause may be inflammation following trauma to the highly vascularized glans tissues that prevents retraction of the phallus into the vent (Lankester and Hernandez-Divers, 2005). As the phallic eversion mechanism in crocodilians is complex (Kelly, 2013), other kinds of damage can also prevent retraction. Because the crocodilian phallus is everted by cloacal muscle contraction (Kelly 2013), tonic contraction of these muscles could force the phallus out of the vent. Lankester and Hernandez-Divers (2005) posit that paraphimosis could be caused by "neurologic deficits involving the retractor phallus muscles", but did not know that unlike other amniotes, crocodilians lack a retractor muscle on their phallus (Kelly, 2013). Rather, retraction of the crocodilian phallus relies entirely on the elastic recoil of ligamentous rami that are held in tension during phallic eversion (Kelly, 2013); damage to these structures during copulation could lead to persistent phallus prolapse.

The Reproductive Examination: Standardized reproductive examination should be standard practice for crocodilian species of reproductive age, analogous to breeding soundness examinations (BSE) utilized in domestic animal theriogenology. These examinations typically include a physical and functional reproductive examination with the goal of predicting the animal's future reproductive efficacy. While examination for phallic malformations make up a subset of a complete BSE, these examinations would help to identify issues before the loss of investment due to lack of fertility. Implementing BSEs at a young age or even prior to introduction to females for breeding could identify such potential fertility issues. With this information, a physical examination can be instituted to identify any of these abnormalities before spending resources.

In many collections, breeding soundness exams are not commonplace when deciding mating pairs. Rather, these decisions are based on the genetics of the animals. Endless time and resources are expended to transport, maintain, house, and breed these individuals that perhaps will never copulate in their lifetimes. Additionally, several species of crocodilians in zoological collections are IUCN (International Union for Conservation of Nature) red listed. In the case of a valuable animal, identification of potential morphologic issues would allow for prediction of functional capabilities and potential intervention where indicated. While artificial insemination practices have historically been performed in crocodilian species (Larsen and Cardeihac, 1981), these techniques have not reliably been successful or resulted in live offspring. With the absence of consistently efficacious artificial insemination techniques in crocodilians presently, transport of individuals for live cover mating is necessary yet costly. However, if these individuals are not examined for reproductive capabilities before expending these resources, survival of species could be at risk.

It should be noted that as a limitation to this study, only juvenile Nile crocodile and broad-snouted caimen were observed, as these malformation observations were discovered opportunistically over many years of normal morphological studies. These studies focused on farmed juveniles because of availability, especially in species with strict regulation of wild populations. In short, this methodology could potentially have caused us to miss a subset of malformations that occur as the juveniles sexually mature.

Conclusions

In total, 8 individuals representing four crocodilian species from both Crocodylidae and Alligatoridae families exhibiting phallic malformations were observed and described. These malformations were broken down further into categories including phallic glans malformation (three broad-snouted caimen, one saltwater crocodile, one Nile crocodile), sulcus spermaticus obstructive defects (one Cuban crocodile), and amputation (one saltwater crocodile, one Cuban crocodile). Phallic glans malformation was further sub-classified as filiform glans tip malformation versus inability to achieve proper glans inflation. A fourth category, penile and cloacal prolapse, is of importance in the literature and should be considered when assessing for phallic malformations, but was not observed in this case series. Reproductive examinations should be conducted in breeding males routinely to prevent overlooking defects or anomalies from any of the above categories to maximize breeding program success.

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Figure legends

Figure 1. Morphological variance between juvenile male phallic glans of the American alligator (*Alligator mississippiensis*, left) and the Nile crocodile (*Crocodylus niloticus*, right) following artificially inflation of the corpus spongiosum (technique per Moore *et al*., 2021) during necropsy. (A) Lateral view. (B) Distal view. G = glans ridge; GT = glans tip.

Figure 2. Broad-snouted caiman (*Caiman latirostris*) phallic glans malformations. (A) Normal glans morphology-ventral view. (B) Bisected and deflected glans tip (see arrows pointing to distal tips). (C and D) Bisected glans ridge ventral and lateral view, respectively. G = glans ridge; GT = glans tip; SS = sulcus spermaticus. Scale bar = 1 cm.

Figure 3. Malformation of a juvenile Nile crocodile (*Crocodilus niloticus*) phallic glans. (A) Normal glans morphology- lateral view. (B and C) Bifurcated abnormal glans- dorsal and oblique views, respectively (see asterisks). (D) Histological section through bifurcation (solid black arrow) showing corpus spongiosum division. (E) Computer generated, three-dimensional reconstruction of the malformed glans seen in B and C with transparent epithelium showing subjacent structures. G = glans; GT = glans tip; CF = corpus fibrosum (pink); CS = corpus spongiosum (green); SS = sulcus spermaticus (purple).

Figure 4. Cuban crocodile (*Crocodylus rhombifer*) phallic malformations. (A) Normal sulcus spermaticus. (B) Eroded sulcus spermaticus. (C) Normal glans, lateral view. (D) Malformed (arrow) and necrotic glans. (E) Normal glans, distal aspect. (F) Malformed (arrow) glans, distal aspect. G = glans; GT = glans tip; SS = sulcus spermaticus.

Figure 5. Serial MRIs of malformed broad-snouted caiman (*Caiman latirostris*) phallic glans from Fig. 2D. (A through C) proximal to distal axial sections. CF = corpus fibrosum; CS = corpus spongiosum; GT = glans tip; SS = sulcus spermaticus.

Figure 6. Adult male phallic glans amputation examples. (A and B) Saltwater crocodile (*Crocodylus porosus*). (C and D) Broad-snouted caiman (*Caiman latirostris*). (E) Cuban crocodile (*Crocodylus rhombifer*). Cr = crura; SS = sulcus spermaticus.

Figure 1

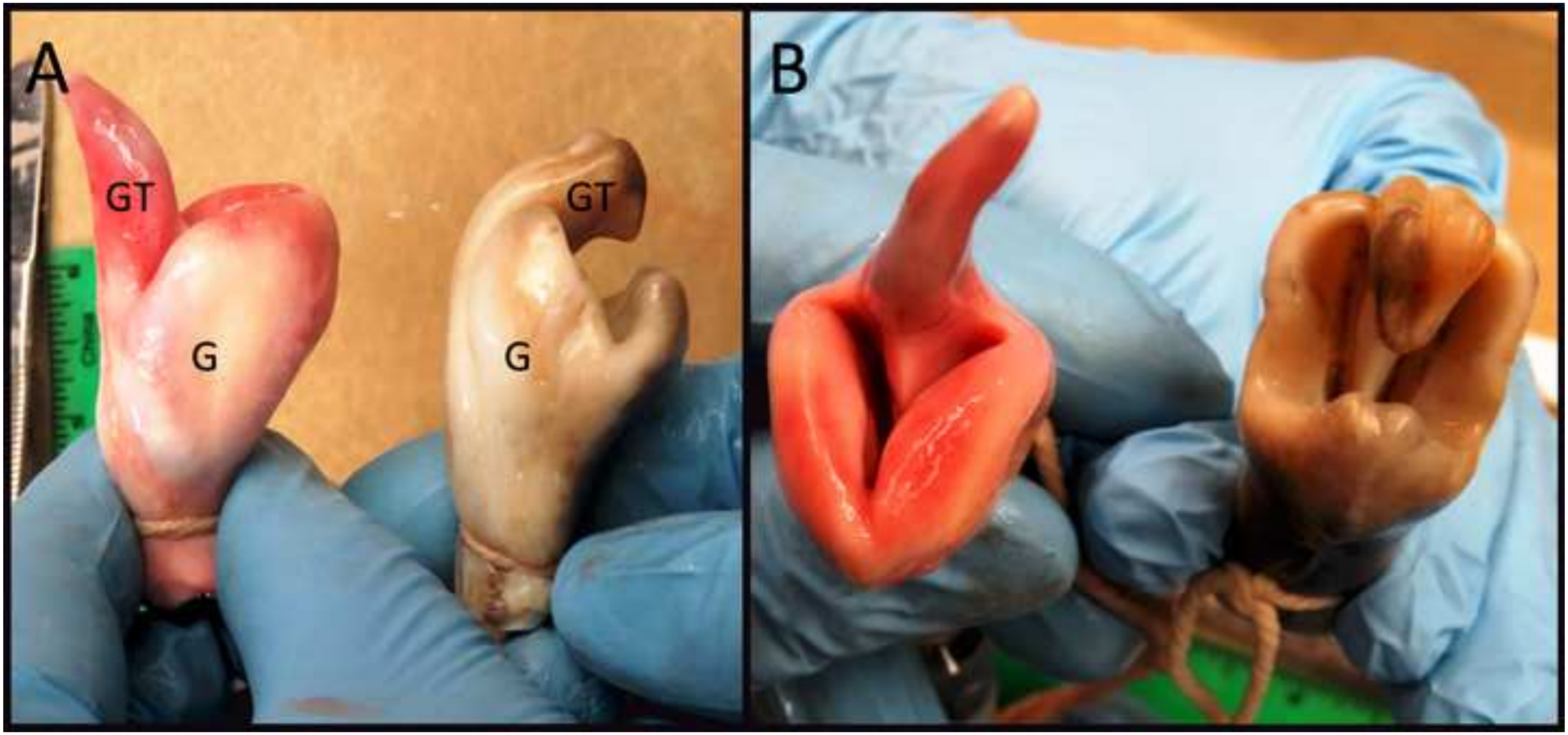

Figure 2

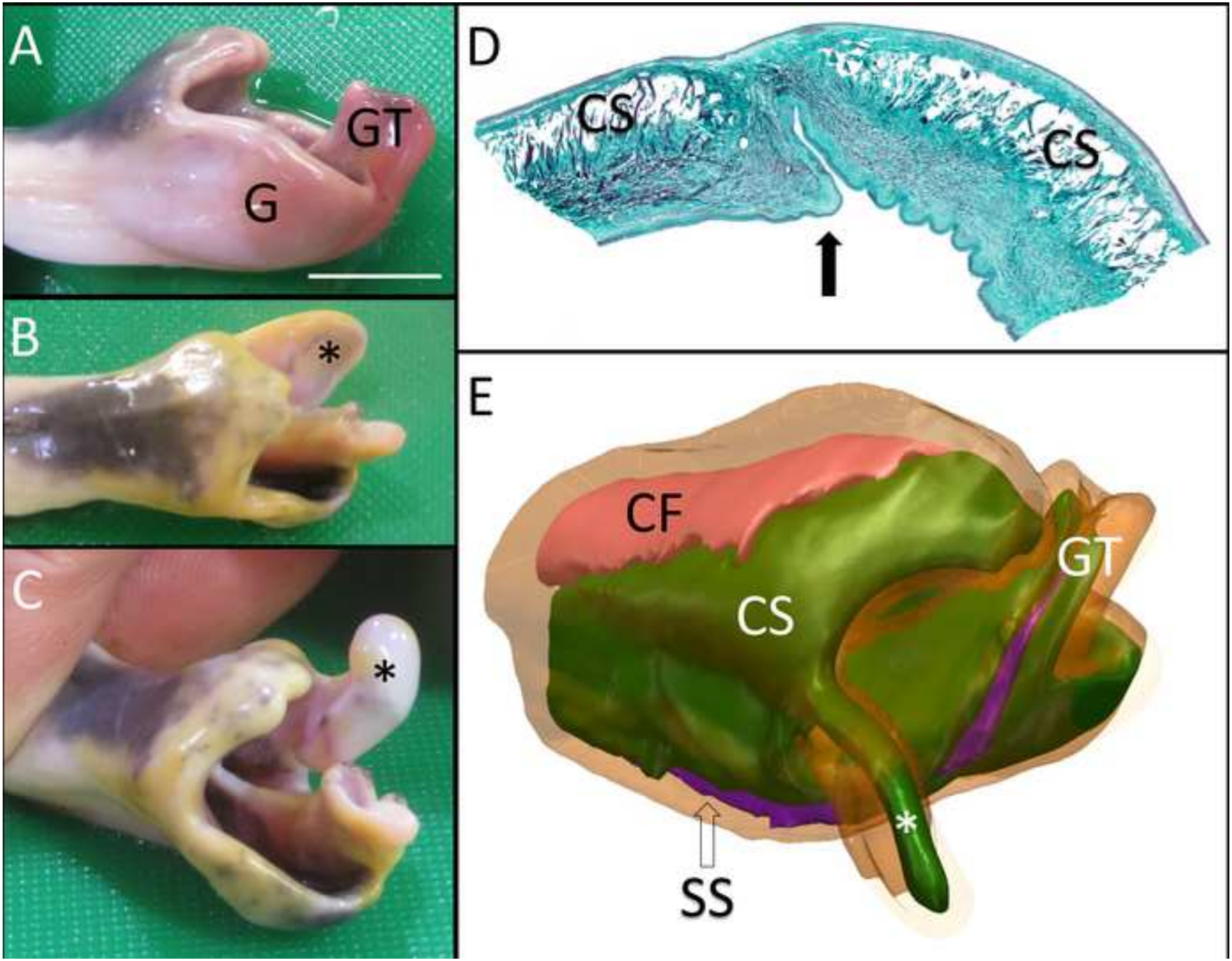

Figure 3

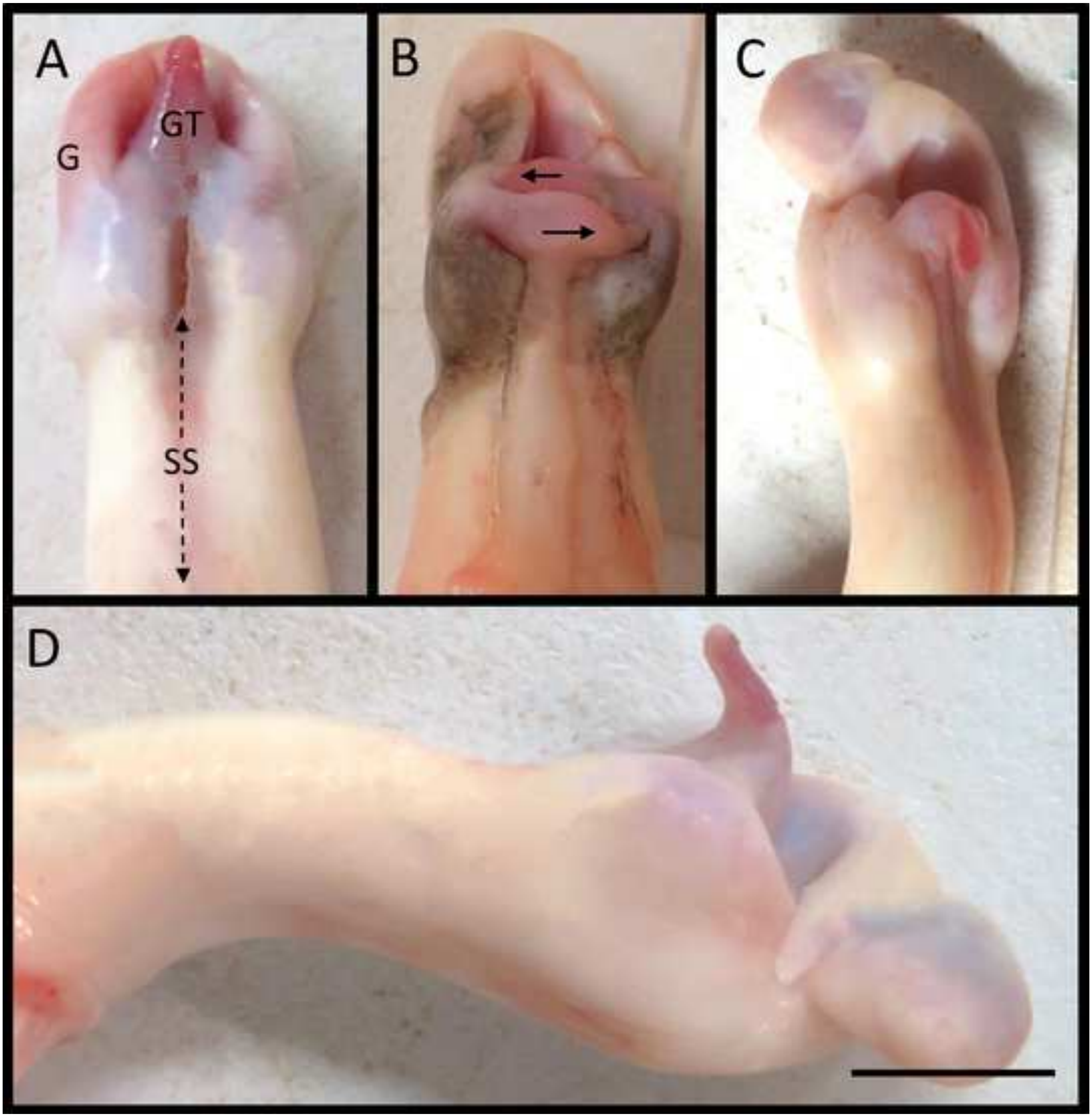

Figure 4

Click here to access/download;Figure;Fig 4 Caiman MRI.tif

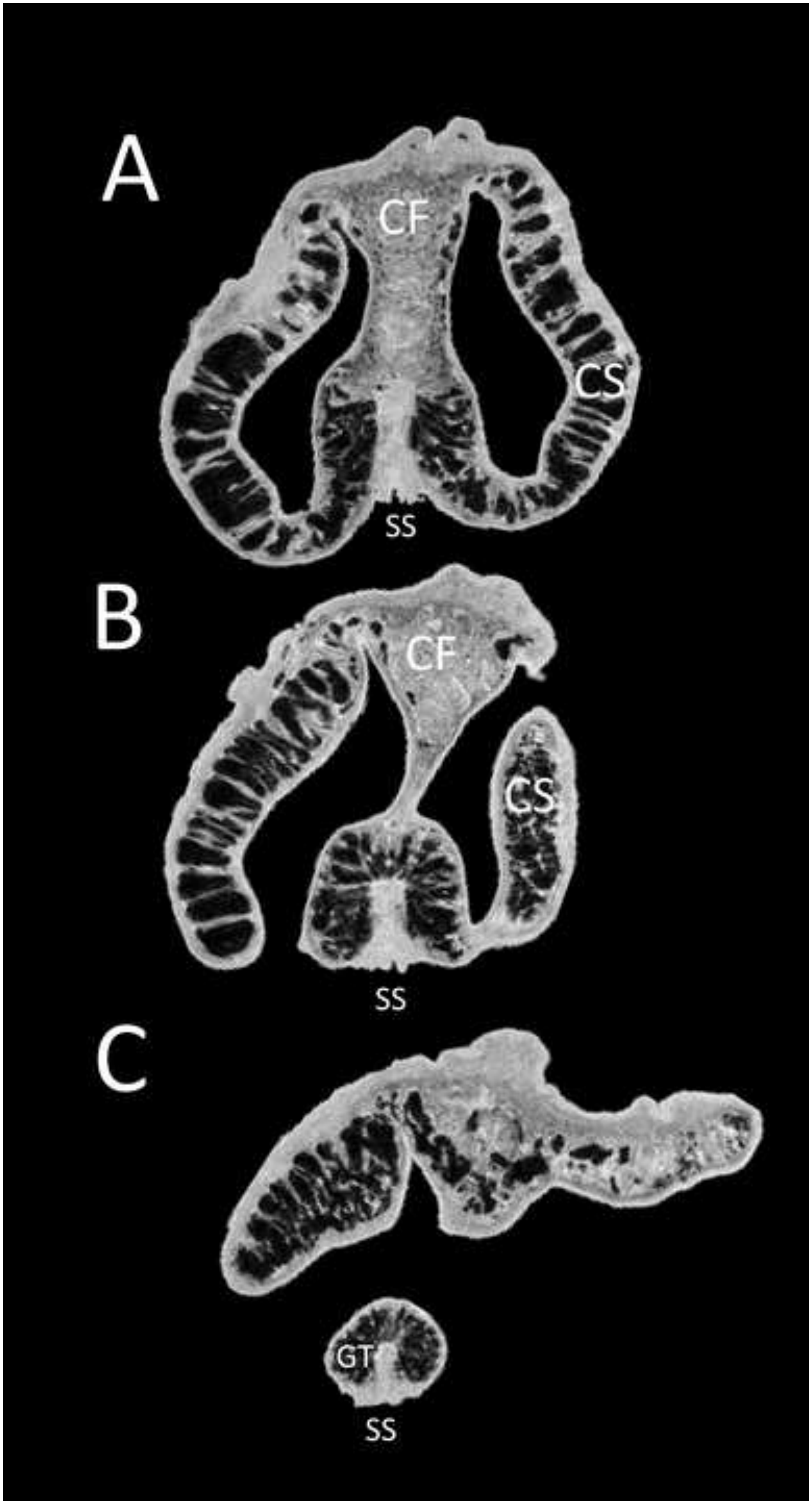

Figure 5

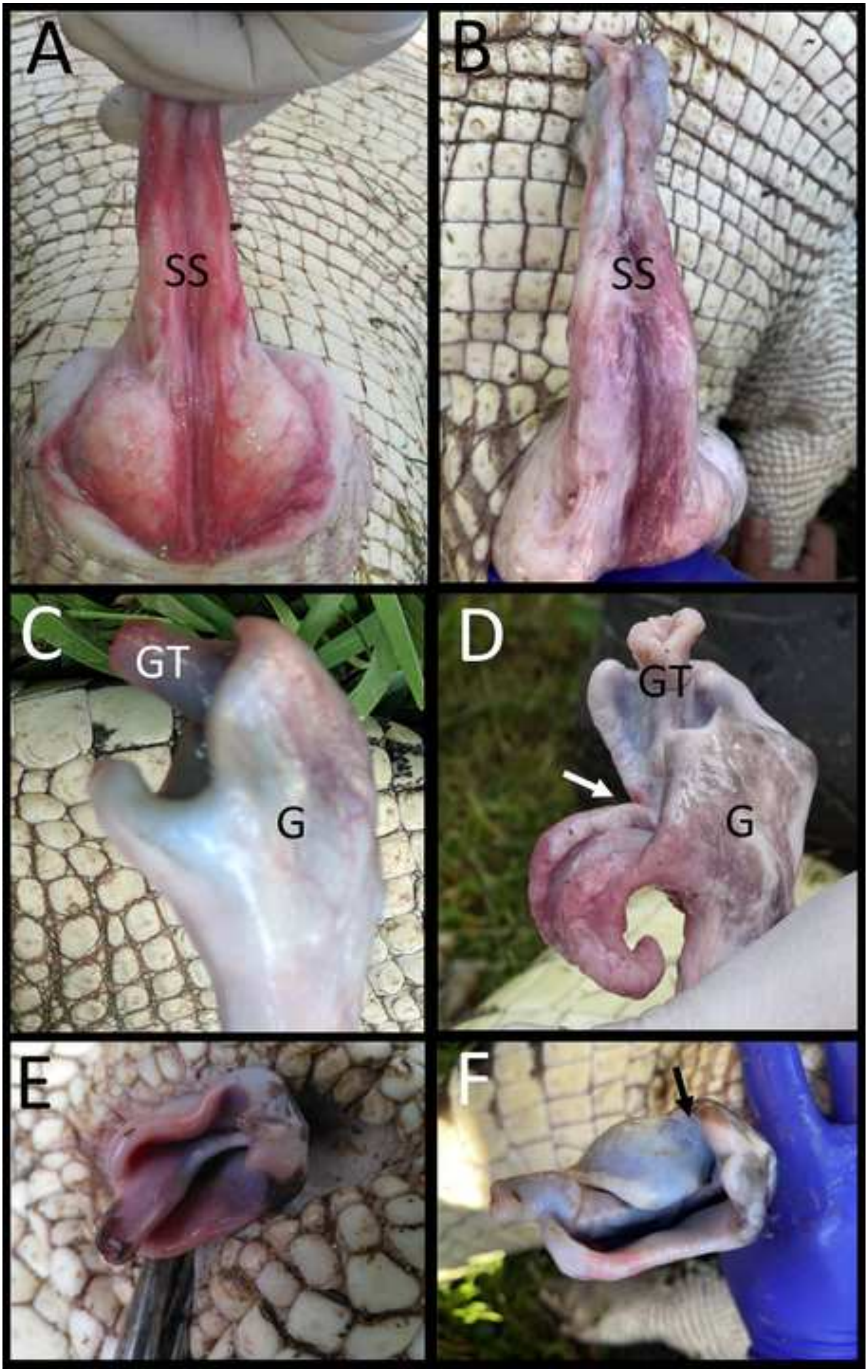

Figure 6