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Preliminary evaluation of the radiographic anatomy, gastrointestinal transit dynamics and sedation response of the Graceful chameleon *(Chamaeleo gracilis)*

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Abstract

Radiographic evaluation remains a primary diagnostic tool in reptile medicine; however, interpretation of reptilian radiographs is often limited by the lack of species-specific anatomical and physiological reference data. This study provides a descriptive assessment of skeletal radiographic anatomy, gastrointestinal transit dynamics and sedation response of the Graceful chameleon (*Chamaeleo gracilis*) under controlled environmental conditions. Four clinically healthy adult chameleons were examined following a two-week acclimatization period. Radiographic imaging was performed in lateral and dorsoventral projections, and gastrointestinal transit time was evaluated using orally administered barium sulfate contrast. Morphometric measurements were obtained from digital radiographs and subjected to descriptive statistics. Sedation was achieved using ketamine (40 mg/kg, intra-muscular) and xylazine (5 mg/kg, intramuscular), with physiological parameters monitored throughout. Radiographs demonstrated well-defined skeletal structures with prominent caudal vertebral elongation. Morphometric analysis revealed a mean body length of 11.13 ± 0.26 cm, head length of 3.00 ± 0.00 cm and tail length of 27.50 ± 0.41 cm. The radius: humerus ratio was 0.86, while the tibia: femur ratio was 1.22, and the tail: body ratio was 2.47, which are consistent with arboreal specialization. Gastrointestinal transit was characterized by rapid oesophageal passage (2.00 ± 0.00 minutes) and gastric filling time (28.0 ± 3.65 minutes), brief gastric emptying (28.0 ± 3.65 minutes) and prolonged small intestine (474.5 ± 9.60 minutes) and colon (594.0 ± 20.8 minutes) transit times, with total gastrointestinal transit time averaging 1103.5 ± 27.7 minutes (18.4 hours). The small intestine and colon accounted for the majority of transit duration (43% and 54%, respectively). Sedation with ketamine and xylazine provided effective immobilization without observable complications. These findings serve as preliminary reference values for radiographic anatomy, gastrointestinal transit time and ketamine-xylazine induced sedation in *C. gracilis*. The data generated will facilitate interpretation of diagnostic images of the chameleon.

Keywords: *Chamaeleo gracilis*; Radiographic anatomy; Gastrointestinal transit time; Contrast radiography; Morphometry; Sedation response.

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Introduction

Diagnostic imaging is a fundamental component of contemporary reptile clinical practice, particularly in species where integumentary adaptations, such as thick dermis, osteoderms or specialized scales, limit the diagnostic utility of physical palpation (Bennett, 1998; Thrall, 2018; Divers and Stahl, 2019). Among available modalities, radiography remains the primary tool for initial evaluation due to its accessibility and effectiveness in assessing skeletal structures and coelomic gas distribution (Krautwald-Junghanns *et al*., 2011). However, the diagnostic value of radiographic imaging depends on the availability of species-specific anatomical and physiological reference data. In many reptilian taxa, such baseline data are incomplete or lacking, increasing the risk of misinterpreting normal anatomical variations as pathology (Mader and Divers, 2013; Zoller *et al*., 2019).

Chameleons (Family: *Chamaeleonidae*) present particular challenges for radiographic interpretation due to their highly specialized morphology. Adaptations to an arboreal life – including zygodactylous feet, elongated appendicular elements and laterally compressed bodies – are well documented in functional and biomechanical studies (Herrel *et al*., 2000; Higham and Anderson, 2013). However, the radiographic correlates of these features, such as bone opacity, cortical thickness and joint spacing, remain insufficiently characterized in many species. This is especially true for the Graceful chameleon *(Chamaeleo gracilis)*, a species widely distributed across West Africa and commonly encountered in both wild and captive settings. Despite its prevalence, there is a lack of standardized skeletal reference data to support objective radiographic assessment.

In addition to skeletal evaluation, gastrointestinal (GI) function is a critical aspect of reptile diagnostics, particularly in cases of suspected impaction or gastrointestinal stasis. As ectothermic organisms, chameleons exhibit digestive kinetics that is strongly influenced by environmental temperature and metabolic rate (Akani *et al*., 2001; Angilletta, 2009; Secor, 2009). Contrast radiography using barium sulfate is widely employed to assess GI motility; however, transit times vary considerably among species and are affected by factors such as diet, hydration status and husbandry conditions (Chirio and LeBreton, 2007; Mans and Hernandez-Divers, 2014). For *Chamaeleo gracilis*, baseline data on gastric emptying and total gastrointestinal transit time are currently unavailable, limiting accurate clinical interpretation of possible gastrointestinal disorders.

The acquisition of high-quality radiographic images in reptiles often necessitates chemical restraint. In many clinical settings, particularly those with limited resources, the ketamine-xylazine combination is frequently employed due to its affordability and availability (Mosley, 2005). However, the use of alpha-2 adrenergic agonists such as xylazine is associated with variable physiological effects in some reptiles, including cardiovascular depression and prolonged recovery, which may differ significantly between species (Ferreira and Mans, 2023; West *et al*., 2014). Despite its widespread use in low-resource clinical settings, the sedative efficacy and safety profile of xylazine-ketamine protocol have not been systematically evaluated in *Chamaeleo gracilis.*

The present study characterized the radiographic skeletal anatomy, established baseline gastrointestinal transit parameters, and evaluated the sedative effects of a ketamine-xylazine protocol in the chameleon (*Chamaeleo gracilis*). It is believed that the generation of these data will provide foundational reference information for clinical diagnosis, and contribute to improved management and conservation of this species.

Materials and Methods

Study Design and Ethical Approval: This study was designed as a prospective experimental investigation to obtain descriptive radiographic and physiological data on *Chamaeleo gracilis*. All procedures were conducted at the Radiology Unit of the Veterinary Teaching Hospital, University of Ibadan, Nigeria, and were performed in accordance with the guidelines of the Institutional Animal Care and Use Research Ethics Committee (ACUREC).

Animals, Sourcing, Housing and Acclimatization: Four clinically healthy adult Graceful chameleons (*Chamaeleo gracilis*), comprising two males and two females, were used in this study. The animals were wild-caught from their natural habitat and transported to the study facility. Body weights ranged from 102 g to 120 g.

The sample size (n = 4) was constrained by ethical and logistical considerations associated with the use of wild-caught chameleons. The study was therefore designed as an exploratory, descriptive investigation aimed at generating preliminary radiographic and physiological data under controlled conditions rather than establishing definitive reference intervals.

The chameleons were allowed an acclimatization period of 2 weeks prior to the commencement of the experiment. During this period, the animals were monitored daily to ensure adaptation to the captive environment and to minimize stress-related physiological alterations. The chameleons were housed individually in well-ventilated enclosures furnished with climbing branches to simulate their arboreal habitat. Ambient temperature was maintained at 28 – 30°C throughout the study period, corresponding to the preferred optimal temperature zone for chameleons (Anderson and Deban, 2010; Herrel *et al*., 2012). Natural photoperiod conditions were maintained. The chameleons were fed a diet consisting primarily of live insects (e.g., crickets and grasshoppers), supplemented with water provided via misting to facilitate hydration. Prior to imaging procedures, animals were fasted for 24 hours to standardize the gastrointestinal contents.

All the chameleons used for the study underwent physical examination and haematological screening before inclusion in the study, to confirm their health status.

Radiographic Imaging Parameters: Radiographic examinations were performed using a digital radiography system. Animals were positioned in lateral and dorsoventral projections, and images were acquired using exposure parameters of 45 kV, 150 mA, and 0.1 seconds, with a focal film distance of 100 cm.

Radiographic Morphometry and Measurement Protocol: Radiographic morphometric analysis was performed using Digimizer Image Analysis Software (DIAS). Measurements were obtained from digital radiographs using standardized anatomical landmarks.

The humerus was measured from the proximal humeral head to the distal condyle. The radius and ulna were measured from the proximal radial head to the distal carpal articulation. The femur was measured from the femoral head to the distal condyle, while the tibia and fibula were measured from the proximal tibial plateau to the distal tarsal articulation. Head length was defined as the distance from the rostral tip of the snout to the occipital region. Body length, expressed as snout–vent length (SVL), was measured from the snout to the cloaca. Tail length was measured from the cloaca to the distal tip of the tail.

To minimize measurement error due to radiographic magnification, a calibrated scale marker of known length was placed within the imaging field at the level of the animal during radiography. All measurements were

corrected for magnification using this reference scale.

Morphometric ratios, including radius: humerus, tibia: femur and tail: body ratios were calculated to assess proportional anatomical relationships independent of body size.

To assess measurement reliability, all morphometric parameters were independently measured by two observers. Inter-observer agreement was evaluated using repeated measurements, and discrepancies were resolved by consensus. This approach was adopted to minimize subjective bias and improve measurement consistency.

Gastrointestinal Contrast Study: For gastrointestinal transit time evaluation, a barium sulfate suspension (25% w/v) was administered orally at a dose of 1 mL per 100 g body weight. Sequential radiographs were obtained at 15 minutes, 30 minutes and at hourly intervals thereafter until complete excretion of the contrast medium was observed.

Animals were maintained within the optimal temperature range during the imaging period to ensure physiological gastrointestinal motility.

To reduce subjectivity in transit assessment, gastrointestinal endpoints were defined radiographically as follows: gastric emptying was recorded when contrast medium was no longer visible within the stomach; small intestinal emptying was defined as the progression of contrast into the colon; and colon emptying was defined as the absence of contrast within the large intestine. All radiographs were separately evaluated by two observers to maintain consistency and objectivity in interpretation.

The timing of radiographic acquisition (15 minutes, 30 minutes and hourly intervals thereafter) was selected to balance temporal resolution with the need to minimize handling stress during repeated imaging. Similar interval-based protocols have been employed in reptile gastrointestinal transit studies (Grosset *et al*., 2014; Mathes *et al*., 2019).

Sedation Protocol and Physiological Monitoring: The chameleons were sedated using ketamine (40 mg/kg, intramuscular) and xylazine (5 mg/kg, intramuscular), administered into the epaxial musculature. Following drug administration, animals were observed for induction time, depth of sedation, and recovery characteristics.

Physiological parameters including heart rate, respiratory rate and cloacal temperature were monitored at 5-minute intervals throughout the sedation period. Sedation was considered adequate when animals achieved sufficient immobilization to allow proper positioning for radiographic imaging.

Sedation depth was assessed using a qualitative scoring approach based on muscle relaxation, response to handling and ability to maintain posture. Adequate sedation was defined as complete immobilization without voluntary movement during positioning. Induction time (time from injection to loss of righting reflex) and recovery time (time to regain spontaneous posture and normal responsiveness) were recorded. No adverse anesthetic events were observed.

Statistical Analysis: Data were analyzed using descriptive statistical methods. Gastrointestinal transit times and morphometric measurements were expressed as mean ± standard deviation (SD), along with minimum and maximum values.

Morphometric ratios were computed to evaluate proportional relationships among skeletal elements and body segments. These ratios were used to assess functional anatomical adaptations associated with arboreal locomotion.

Total gastrointestinal transit time was calculated for each chameleon and expressed

in both minutes and hours. The relative contribution of each gastrointestinal segment to total transit time was calculated as a percentage.

Sedation-related variables were summarized using descriptive statistics. Induction time, duration of sedation, and recovery time were expressed as mean ± standard deviation (SD) alongside observed ranges to characterize central tendency and inter-individual variability.

All statistical analyses were performed using SPSS version 27.0.1.0. Given the small sample size (n = 4), no inferential statistical tests were performed, and results were presented as descriptive, exploratory data.

Results

Radiographic Findings: Plain radiography provided clear visualization of the skeletal structures of *C. gracilis*. Lateral projections (Figure 1) demonstrated the skull, vertebral column, ribs, appendicular skeleton, and elongated caudal vertebrae. The skull appeared well mineralized, and the vertebral column extended into a long caudal series.

Following administration of barium sulfate contrast medium, sequential radiographs allowed visualization of the gastrointestinal tract (Figures 2 and 3), including the oesophagus, stomach, small intestine and colon. The progression of contrast material through the digestive tract was clearly identifiable in dorsoventral projections.

Morphometric Characteristics: Morphometric measurements of the chameleons are presented in Table 1. Body length (snout–vent length) ranged from 10.8 – 11.5 cm, whereas tail length ranged from 27.0 – 28.0 cm. Head length was uniform across all the chameleons used for the study at 3.0 cm. Limb bone measurements were relatively consistent across all the individual animals. The humerus length ranged from 2.3 – 2.5 cm, while the radius/ulna measured 2.0 – 2.2 cm. The femur ranged from 2.3 – 2.5 cm, and the tibia/fibula ranged from 2.8 – 3.0 cm.

Morphometric ratios derived from these measurements are summarized in Table 2. The mean radius: humerus ratio was 0.86, and the tibia: femur ratio was 1.22. The tail: body ratio averaged 2.47, indicating a relatively elongated caudal segment.

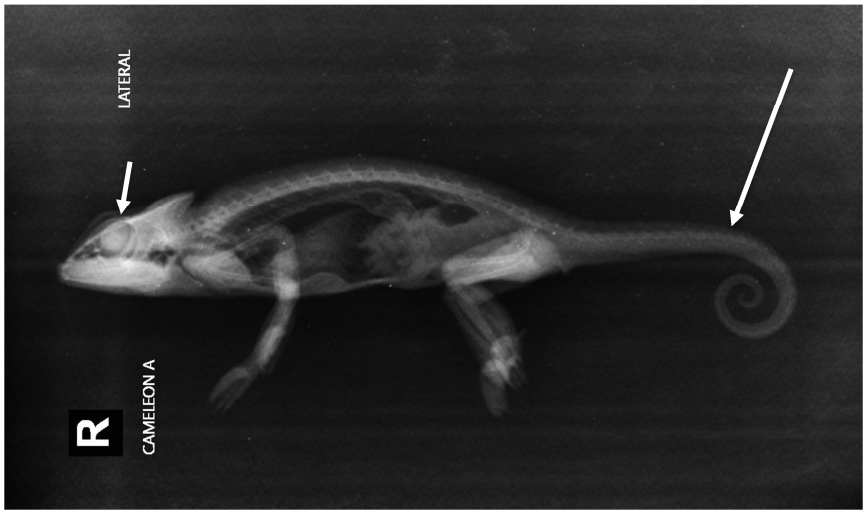

Figure 1. Lateral plain radiograph of *Chamaeleo gracilis* demonstrating normal anatomical features; the short arrow indicates the triangular skull, while the long arrow highlights the prehensile tail.

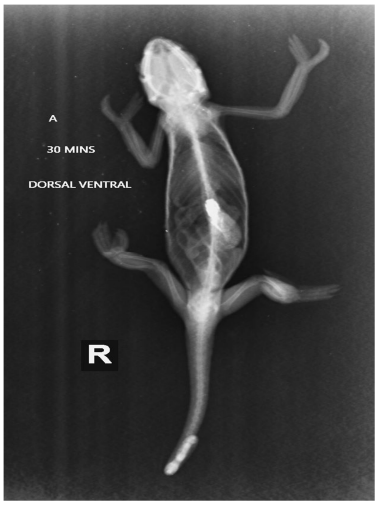

Figure 2. Dorsoventral contrast radiograph of *Chamaeleo gracilis* following oral administration of barium sulfate, demonstrating the distribution of contrast medium within the gastrointestinal tract.

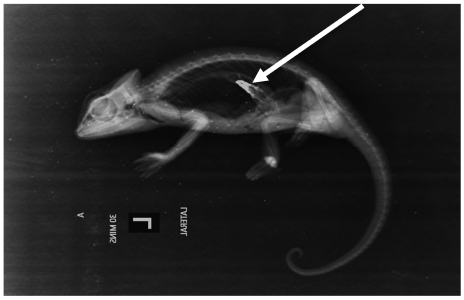

Figure 3. Lateral contrast radiograph of *Chamaeleo gracilis* following oral administration of barium sulfate, with the arrow indicating accumulation of contrast medium within the stomach.

Gastrointestinal Transit Time: Gastrointestinal transit times for each animal are presented in Table 3. Esophageal transit was rapid and consistent across all animals, occurring at 2 minutes following contrast administration. Gastric filling occurred within 4 – 6 minutes, and gastric emptying was observed between 24 and 32 minutes.

Contrast passage through the small intestine occurred within 460 – 482 minutes, while colon emptying occurred between 570 – 620 minutes. Total gastrointestinal transit time ranged from 1079 – 1134 minutes, corresponding to 18.0 – 18.9 hours.

Descriptive statistics for gastrointestinal transit parameters are summarized in Table 4. Mean gastric filling time was 5.0 ± 0.82 minutes, while gastric emptying occurred at 28.0 ± 3.65 minutes. Small intestinal transit time averaged 474.5 ± 9.6 minutes, and colon emptying occurred at 594.0 ± 20.8 minutes.

The mean total gastrointestinal transit time was 1103.5 ± 27.7 minutes, equivalent to approximately 18.4 hours (Table 4). Although variability between individuals was present, the overall temporal pattern of rapid proximal transit followed by prolonged distal passage was consistent across all animals.

Table 1. Appendicular and axial skeletal morphometric measurements of the Graceful chameleon (*Chamaeleo gracilis)*.

Chameleons used, serially numbered	Body length (cm)	Head length (cm)	Tail length (cm)	Humerus length (cm)	Radius and Ulna length (cm)	Femur length (cm)	Tibia and Fibula length (cm)
1	11.5	3.0	27.8	2.5	2.0	2.5	3.0
2	11.0	3.0	28.0	2.3	2.1	2.4	2.9
3	10.8	3.0	27.0	2.4	2.2	2.3	2.8
4	11.2	3.0	27.2	2.5	2.1	2.4	3.0
Mean (SD)	11.13 (0.26)	3.00 (0.00)	27.50 (0.41)	2.43 (0.08)	2.10 (0.07)	2.40 (0.07)	2.93 (0.08)

Table 2: Morphometric ratios of the appendicular skeleton and body segments of the Graceful chameleon (*Chamaeleo gracilis)*, and its implications.

Morphometric ratio	Mean ± SD	Morphological implication
Radioulna-to-humerus ratio	0.86 ± 0.06	Relatively shorter proximal forelimb segment.
Tibiofibula-to-femur ratio	1.22 ± 0.02	Relatively elongated distal hindlimb segment.
Tail length to snout–vent length ratio	2.47 ± 0.06	Elongated caudal segment.

Table 3. Gastrointestinal transit time following oral administration of barium sulfate to the Graceful chameleon (*Chamaeleo gracilis).*

Chameleons, serially numbered	Transit time (minutes)						Total transit time (hours)
	Oesophagus	Gastric filling	Gastric emptying	Small intestine emptying	Colon emptying	Total transit time	
1	2	5	30	480	600	1117	18.6
2	2	6	24	482	620	1134	18.9
3	2	4	32	476	570	1084	18.1
4	2	5	26	460	586	1079	18.0

Table 4. Descriptive statistics of gastrointestinal transit times of the Graceful chameleon (*Chamaeleo gracilis).*

Parameters	Transit time (minutes)				Percentage of the total transit time
	Mean	Standard deviation	Minimum value	Maximum value	
Oesophageal transit time	2.0	0.0	2	2	0.2%
Time to gastric filling	5.0	0.82	4	6	0.5%
Gastric emptying	28.0	3.65	24	32	2.5%
Small intestine transit time	474.5	9.6	460	482	43.0%
Colon transit time	594.0	20.8	570	620	53.8%
Total Gastrointestinal transit time	1103.5	27.7	1079	1134	100%

Relative Contribution of Gastrointestinal Segments: The proportional contribution of each gastrointestinal segment to total transit time is shown in Table 4. The esophageal phase contributed 0.2%, while gastric filling accounted for 0.5% of total transit time. Gastric emptying represented 2.5% of total transit duration. A higher proportion of gastrointestinal transit time occurred within the distal segments: the small intestine accounted for 43% of total transit time, while the colon represented the largest proportion at 53.8% (Table 4).

Sedation Parameters: Sedation using the ketamine-xylazine combination resulted in rapid and consistent immobilization in all the chameleons (Table 5). Induction time ranged from 4 – 6 minutes (mean: 5.0 ± 0.8 minutes). The duration of effective sedation ranged from 45 – 60 minutes (mean: 52.5 ± 6.5 minutes). Recovery time ranged from 20 – 25 minutes (mean: 22.5 ± 2.1 minutes), with all animals regaining normal posture and responsiveness without observable complications. Physiological parameters, including heart rate, respiratory rate and cloacal temperature, remained stable throughout the sedation period (Table 6).

Table 5. Sedation parameters of the Graceful chameleon (*Chamaeleo gracilis*) sedated using ketamine (40 mg/kg) and xylazine (5 mg/kg).

Parameter	Mean ± SD (minutes)	Minimum and maximum values (minutes)
Induction time	5.0 ± 0.8	4 – 6
Duration of sedation	52.5 ± 6.5	45 – 60
Recovery time	22.5 ± 2.1	20 – 25

SD = standard deviation

Table 6. Physiological parameters (heart rate, respiratory rate, and cloacal temperature) of the Graceful chameleon (*Chamaeleo gracilis*) sedated using ketamine (40 mg/kg) and xylazine (5 mg/kg).

Time after drug administration (minutes)	Mean ± standard deviation		
	Heart rate (beats/minute)	Respiratory rate (breaths/minute)	Cloacal temperature (°C)
0 (baseline)	61.0 ± 2.6	21.8 ± 1.7	29.15 ± 0.13
5	57.0 ± 2.6	19.3 ± 1.3	29.05 ± 0.13
10	54.0 ± 2.6	17.3 ± 1.3	28.95 ± 0.13
15	51.5 ± 2.1	15.3 ± 1.3	28.85 ± 0.13
20	49.5 ± 2.1	14.0 ± 0.8	28.78 ± 0.10
25	47.8 ± 1.7	13.0 ± 0.8	28.73 ± 0.10
30	46.5 ± 2.1	12.0 ± 0.8	28.68 ± 0.10
35	45.5 ± 2.1	11.0 ± 0.8	28.60 ± 0.08
40	44.5 ± 2.1	10.5 ± 0.6	28.55 ± 0.13
45	43.5 ± 2.1	10.0 ± 0.8	28.50 ± 0.08

Discussion

Morphometric findings in this study revealed proportional elongation of distal limb segments and a markedly high tail: body ratio (2.47), both of which are consistent with arboreal specialization. The tibia: femur ratio (1.22) in particular, suggests functional elongation of the hindlimb, likely contributing to grasping efficiency and positional stability on vertical substrates (Silva *et al*., 2022). These proportional relationships were readily appreciable on the radiograph, where the appendicular skeleton appeared slender

relative to body size and the caudal vertebral column was notably extended. Compared with more terrestrial lizard species, in which limb proportions tend to be more uniform, these findings reinforce the importance of interpreting skeletal radiographs within a functional and ecological framework rather than relying on generalized reptilian anatomy (Barten, 1996).

Gastrointestinal transit followed a distinct pattern characterized by rapid oesophageal passage (2 minutes), brief gastric emptying (28.0 ± 3.65 minutes), and a prolonged distal phase. The small intestine and colon together accounted for approximately 97% of total transit duration, indicating that the majority of digestive processing occurs beyond the stomach. This distribution is consistent with the metabolic strategy of ectothermic reptiles, in which digestive activity is closely tied to thermal conditions and postprandial energy allocation (Angilletta, 2009; Secor, 2009). The mean total transit time (1103.5 ± 27.7 minutes; approximately 18.4 hours) falls within the broad range reported in other lizard species, although direct comparisons are limited by inter-specific variation (Grosset *et al*., 2014). Notably, the relatively narrow range observed across individuals (1079 – 1134 minutes) suggests that maintaining animals within their preferred optimal temperature zone may reduce variability in gastrointestinal motility (Silva *et al*., 2022). From a clinical perspective, this is relevant, as deviations from expected transit patterns under suboptimal thermal conditions could be misinterpreted as pathological delay.

The ketamine-xylazine protocol produced rapid induction (mean: 5.0 ± 0.8 minutes) and a moderate duration of sedation (mean: 52.5 ± 6.5 minutes), providing a practical window for radiographic imaging. Recovery was similarly predictable (mean: 22.5 ± 2.1 minutes), with all animals returning to normal posture without complications. These findings are consistent with the pharmacological properties of ketamine as a dissociative anaesthetic and xylazine as an alpha-2 adrenergic agonist, which together provide immobilization and muscle relaxation suitable for short diagnostic procedures (Mosley, 2005; Mader and Divers, 2013).

The relatively low variability observed across individuals suggests that maintaining animals within their preferred optimal temperature zone may contribute to stable anaesthetic responses, given the known temperature dependence of drug metabolism in reptiles (Mosley, 2005; Ferreira and Mans, 2023). However, the absence of comparative protocols limits definitive conclusions regarding relative efficacy, and further studies are required to evaluate alternative anesthetic combinations in this species.

From a clinical standpoint, the findings provide practical reference points for interpreting radiographic and contrast studies in *C. gracilis*. Recognition of characteristic skeletal proportions, particularly limb segment relationships and caudal elongation, may assist clinicians in distinguishing normal anatomical variation from pathological conditions such as fractures or metabolic bone disease (Divers and Stahl, 2019). Similarly, an understanding of the distribution of gastrointestinal transit time across anatomical segments may improve the interpretation of contrast studies when evaluating suspected obstruction or motility disorders (Zoller *et al*., 2019). This is particularly relevant in resource-limited settings, where radiography remains the primary imaging modality.

The small sample size (n = 4) represents an inherent limitation and restricts the generalizability of the findings. However, small cohorts are common in wild animals-based research, particularly in exploratory or pilot studies where the primary objective is to generate preliminary data and assess feasibility rather than test formal hypotheses (Piper *et al*., 2023). Ethical frameworks

governing animal research further emphasize minimizing the number of animals used while maintaining scientific validity, reinforcing the use of reduced sample size where appropriate (Pakgohar, 2023).

The relatively low variability observed across individuals suggests that the reported patterns may reflect consistent physiological responses under controlled environmental conditions. Nevertheless, these findings should be interpreted as preliminary and require validation in larger populations, as small sample sizes may limit statistical power and broader inference (Wang *et al*., 2025).

Future studies should incorporate larger sample sizes and include comparisons between captive-bred and wild populations to better define normal variation. The application of advanced imaging techniques such as computed tomography and fluoroscopy would enhance anatomical resolution and allow real-time assessment of gastrointestinal motility (Zoller *et al*., 2019). Additionally, controlled investigations examining the effects of temperature gradients and diet composition on transit time would provide further insight into the physiological determinants of digestive function in chameleons.

Conclusion: This research work provided preliminary radiographic and physiological data on *Chamaeleo gracilis*, contributing baseline descriptive information relevant to clinical imaging. The findings highlight characteristic skeletal proportions associated with arboreal adaptation and demonstrate a gastrointestinal transit pattern dominated by prolonged distal processing. While the ketamine-xylazine protocol facilitated effective immobilization under controlled conditions, further studies are required to validate these observations across larger populations and under varying environmental conditions. These data should therefore be interpreted as an initial framework to guide future research and clinical application.

Conflict of Interest

The authors declare no conflict of interest.

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