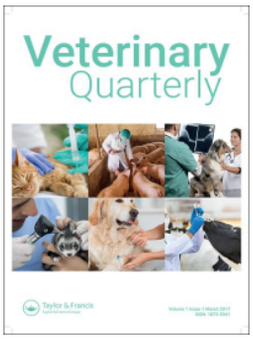

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Endoscopic techniques for early diagnosis of cloacal pathologies and sex determination in blue spotted tree monitor (*Varanus macraei)* and Cuming's water monitor (*Varanus cumingi)*

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ABSTRACT

This article attempts to present, for the first time, the usefulness and feasibility of using endoscopic techniques in the cloacal region of *Varanus cumingi* and *Varanus macraei*. This method can serve both diagnostic and therapeutic purposes, as well as offering an early approach to sex determination through the observation of the urodeum which in males ends in a blind end while in females it consists of two ostia which represent the outlets of the oviducts. In this context, commonly employed sex determination techniques, such as post-cloacal spur detection, have shown unreliability. The study involved the examination of ten specimens, approximately one year old, from a private breeding farm, following a complete clinical evaluation to confirm their general state of health. All subjects underwent sedation, which allowed the evaluation of anatomical structures, the health status of the cloaca and the determination of sex. This study and its findings may provide a critical basis for addressing population declines of these species, particularly for V. macraei, which has already been classified as 'endangered' by the IUCN.

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Varanus macraei; *Varanus cumingi*; cloacoscopy; sex determination

Introduction

Endoscopy allows us to visualize the real state of the organs, highlighting anomalies and pathologies such as obstructions from uroliths and fecal tumors or dystocias which could jeopardize the lives of the breeders as well as long-term reproductive plans; it also proves to be a valid technique for early sex determination and artificial insemination procedures. (Divers 2010; Spadola et al. 2016; Oliveri et al. 2018).

To fully understand this study, it is essential to mention the main anatomical features of the cloaca that will be visualized endoscopically. On the external side, the cloacal fissure has a transverse shape and is operculated by a scale whose perpendicular line divides it into two equal parts. Inside, the cloaca is divided into 3 parts: proctodeum, urodeum and coprodeum (Mader, 2006; Spadola et al. 2021; 2022).

The first portion to highlight is the proctodeum which constitutes the terminal segment of the cloaca, acting as a conduit connecting with the external environment. Proceeding cranially it is possible to observe in specimens of both sexes a papilla that houses the ostia of the ureters, considering that *V. cumingi* and *V. macraei* are part of the *Varanus* without bladder, and also the outlets of the vas deferens in males (Spadola et al. 2021). Just cranial to the urogenital papilla, a septum is visualized that divides the ventral coprodeum from the dorsal urodeum, also called the urorectal septum (Gredler et al. 2014). In female anatomy, the terminal portion of the urodeum culminates with the presence of two ostia, which act as openings for the oviducts, or uteri. In contrast, in males, this terminal section ends in a dead end (Spadola et al. 2021; 2022).

Among reptile breeders, post-cloacal spurs detection (PSSD) is used to determine the sex of some lizard species (Figure 1); this technique involves the recognition of one or more scales, located in the cloacal region, which morphologically appear different between the two sexes. To date, this methodology is not validated as a sexing technique as demonstrated by our studies (Spadola et al. 2022).

Context and objectives of the study

The primary objectives of this study include: (i) examination and description of the cloaca in *V. cumingi* and *V. macraei* using cloacoscopic techniques; (ii) early

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Figure 1. Sex determination using the post-cloacal spurs (black arrows) detection (PSSD) in an adult male of V*aranus macrei*.

diagnosis of pathologies; (iii) the validation of this method for sex determination and its comparison with the determination of the presence of post-cloacal spurs (PPSD); (iv) finalize the technique for conservation and repopulation projects of species at risk of extinction.

Materials and methods

Animals

The study was conducted on ten captive breed monitors: 6 specimens of *Varanus cumingi* and 4 specimens of *Varanus macraei*.

Protocol

In order to ascertain and guarantee the good health of the subjects, a complete clinical examination, a coprological examination and the housing conditions were evaluated.

Since this is a routine clinical procedure for all subjects, the informed consent was completed and signed by the owner.

To prevent any form of stress for the patient and speed up the manipulations, the monitors were subjected to light sedation with Alfaxalone (Alfaxan 10 mg/ml, Jurox Limited), administered intramuscularly at a dose of 15 mg/Kg (Carpenter and Harms 2023; Morici et al. 2018) and anesthetic monitoring for all cloacoscopies.

Instrumentation

The instrumentation used for this procedure is as follows: (i) rigid endoscope (diameter 4 mm, 0°, length 8.5 cm, Olympus medical, Japan) with a working channel connected to a syringe (60 mL, Pic/Artsana solution, Italy); (ii) chamber (Stryker TM 1588); (iii) light source (Stryker TM L10 LED light source); (iv) video system (Stryker TM 1588); (v) monitor (Stryker TM Vision Pro 26" Led Display).

Results

In the species in question the cloaca is made up, proceeding caudocranially, of a first very narrow portion represented by the proctodeum. Cranially to this portion it is possible to appreciate the urodeum which is characterized by the presence of a pair of papillae made up, in the male, of the outlets of the deferens and the ureters, and only by the outlets of the ureters in the female. In the cranial portion the urodeum ends in a blind end in males while in females it consists of the outlet of the oviducts separated by a central septum. Ventral to the urodeum there is a transferential fold that separates the latter from the coprodeum represented in both sexes by the anus. Going beyond the anal orifice it is possible to visualize the rectal chamber which ends with the recto-colic valve. After describing the anatomy of the cloaca we move on to the description of the endoscopic procedure. Upon clinical examination, all subjects had an excellent state of nutrition, no visible lesions and no abnormal behavior either during observation or during manipulation. Coprological examinations of all subjects were negative. The subjects, sedated, were manually restrained and positioned in dorsal recumbency on an electric heating pad (30 °C Bosch PFP 1031; Bosch, Germany) to ensure the correct temperature during the entire procedure.

Once the endoscope was introduced into the monitor's cloaca and irrigated with saline solution (30 °C, 0.9% NaCl, S.A.L.F. Italy), to lubricate and improve vision, a small part of the proctodeum was immediately visualized. This portion is difficult to appreciate because during the procedure it is occupied almost entirely by the end of the instrument. Immediately afterwards, cranially the probe highlighted the papillae on the dorsal wall; these had a conical shape with tapered vertices and pinkish colour, in equal numbers and the apices facing the lumen of the cloacal cavity (Figures 2A, 3A, 3B, 4A). It was also possible to appreciate the transport mechanism of uric acid inside the rectum visualized as a flow of whitish paste that transferred from the apex of the papilla to the rectum (Figure 2A). During the physiological stationing of urination, the papillae are moved ventrally to insert through the anus into the rectum to fit into the coprodeal fossa and facilitate the passage of uric acid into it. In females there was a central septum that separated the two outlets of the oviducts. The central septum and oviduct outlet were not observed in males. In the center of the cloaca a fold can be seen (Figures 2 and 3) which separates the urodeum (dorsal) from the coprodeum

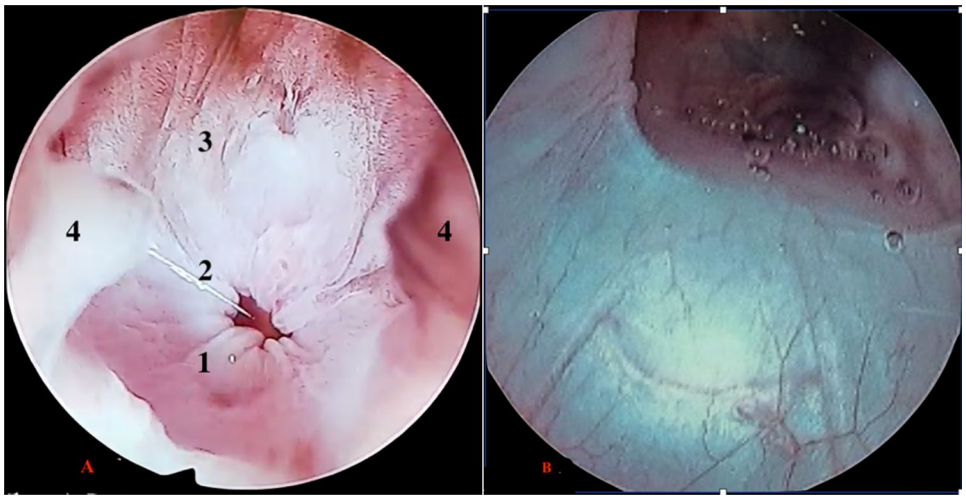

Figure 2. (A) A male cloaca of V. cumingi with transport of uric acid into the rectum. (B) The rectal chamber.
1: coprodeum; 2. urates; 3. anus; 4 urogenital papillae.

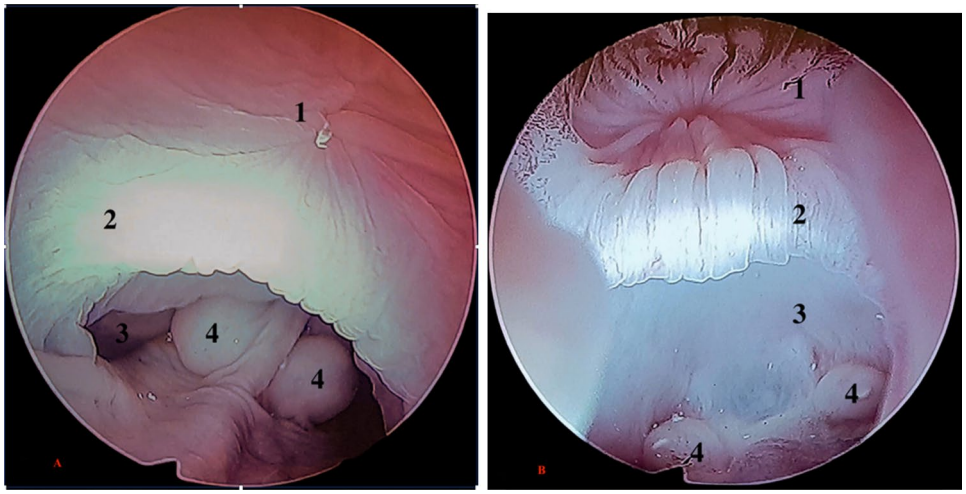

Figure 3. (A) female cloaca of *V. cumingi*. (B) female cloaca of of *V. macraei*.
1. Coprodeum; 2. Septum; 3. Urodeum; 4. Outlets of the oviducts.

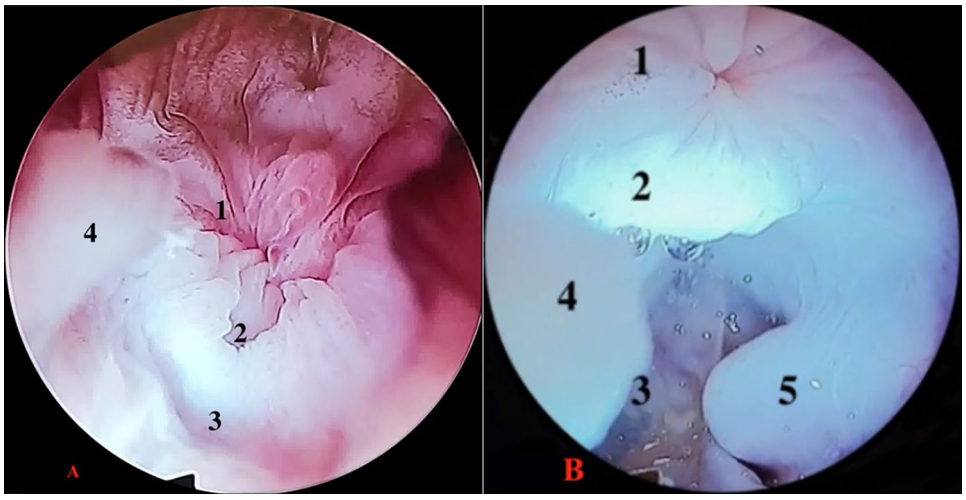

Figure 4. (A) a Male cloaca of a *V. cumingi*; **(B)** a Male cloaca of *V. macraei*.
1. Coprodeum; 2. Septum; 3. Urodeum; 4. Urinary papillae; 5. Neoformation.

(ventral), therefore by moving the probe ventrally it was possible to go beyond the anus and visualize the rectal chamber and the cecocolic valve. In some subjects it was possible to observe the heartbeat, through the caudal vena cava, in the dorsal portion of the rectal chamber (Di Giuseppe, 2017).

Table 1. Comparison of sex determination in V. macraei and V. cuming with the use of two methods: cloacoscopy (CSD) and post-cloacal spurs detection (PSSD).

Species	N°microchip	PSSD	CSD
V. cumingi	901772	Female	Female
V. cumingi	901780	Female	Female
V. cumingi	912340	Female	Male
V. cumingi	901773	Female	Female
V. cumingi	901776	Male	Female
V. cumingi	901774	Female	Female
V. macraei	293341	Female	Female
V. macraei	950328	Male	Male
V. macraei	950160	Female	Male
V. macraei	037967	Female	Female

In summary, the anatomical differences observed between males and females are mainly two: (i) presence of the outlets of the oviducts in the bottom of the urodeum in females and complete absence of the same in males; (ii) the fold between the coprodeum and urodeum in males is less evident than that in females.

During the procedure, the cloacal mucosa appeared bright pink in color, without the presence of continuous solutions, but in some subjects areas with scattered pigmentation were observed.

During the procedure the lizards showed no signs of suffering or discomfort due to the practices carried out.

Of the ten subjects examined, seven were females, in particular five specimens of *V. cumingi* and two specimens of *V. macraei*; these results are in contrast to those of the non-endoscopic sexing method (Table 1) as one specimen was incorrectly sexed. The main feature observed in these subjects was the presence of oviduct outlets in the most caudal segment of the urodeum.

The size of the fold, observed between the coprodeum and urodeum, was significantly larger in female specimens (Figure 3) and represents an additional feature for sex determination.

The remaining 3 subjects were males, in particular one specimen of *V. cumingi* and two of *V. macraei* (Table 1).

Also in this case our results differ from those of the PSSD because 2 specimens were incorrectly sexed. The differences that allow the determination of sex consist in the dead end of the urodeum due to the lack of oviduct ostia and in the reduced size of the fold between the coprodeum and the urodeum. During cloacoscopies carried out on male subjects it was possible to appreciate significant muscle contractions which required further administration of washing solution to make the urodeum visible.

Discussion

The study performed is presented as the first cloacoscopic study ever performed on *V. cumingi* and *V. macraei*. It emerged that 3 out of 10 specimens had been incorrectly sexed (Table 1) using the PSSD, a sexing method used before our study and whose effectiveness in these species has never been verified.

The importance of the sexing method we proposed is evident especially considering the degree of error found using the PSSD, a non-validated method, for sex detection (30%) (Spadola et al. 2022). PSSD does not achieve sufficient efficiency for captive breeding projects.

During the endoscopic procedure it is preferable to position lizards, including varanids, in a ventro-dorsal position to facilitate operator manipulation and image acquisition. By doing so, optimal containment of the animals is guaranteed through manual control of the limbs, preventing potential injuries to both the operators and the animal itself, as well as preventing the animal from hitting the equipment with its limbs.

The importance of having observed substantial differences in subjects of different sexes is truly remarkable and increases the practical applications of this type of project. This method also allows you to save time, energy and resources to be invested only in animals that do not present anatomical anomalies through the illumination and enlargement of the real image and not images to be interpreted as can be the case with ultrasound. To successfully use this diagnostic technique, adequate equipment is required (Divers 2010), in-depth anatomical and physiological knowledge of the cloaca (Mader, 2006; Spadola and Morici 2015), as well as familiarity and experience in use of the endoscope, otherwise they would cause damage to the animal (Spadola et al. 2009).

According to the International Union for Conservation of Nature (IUCN), approximately 21% of known and monitored reptile species are currently at risk of extinction and this trend shows no signs of abating (Alory 2015; Spadola et al. 2022). To counteract this decline it is essential to analyze the environmental problems affecting the habitats in which these reptiles live and increase our knowledge on the morphophysiological level of these species to allow growth in terms of longevity and population size. Among the problems that put the survival of these species at risk (Ziegler et al. 2007), some deserve mention: the exploitation of fauna and flora in international trade, which has reached an overall value of between 1 and 2 million dollars per regarding *V. macraei* (Bennet, 2015), but also habitat loss due to human activities such as deforestation. These are precisely the main reasons why *Varanus macraei* and *Varanus cumingi* are currently in decline. The IUCN (Sy. et al. 2009) has detected a negative trend in the population due to deforestation activities and the imbalance of the delicate habitat in which it lives, namely mangrove forests, which has led to its classification as 'Least Concern'. For species like these (IUDZG/CBSG 1993) conservation and reproduction programs have in fact played a key role in the protection of these populations. However, it is undeniable that the help provided by private entities such as zoos, amateur breeding farms and other organizations plays an important role in the conservation of genetic material, which would otherwise be lost (Spadola et al. 2022). At the same time, research is filling various gaps at the anatomical-physiological level and progressively reaching standards on artificial insemination protocols, also thanks to the diffusion of the endoscopic technique, which is simple, rapid and effective (Oliveri et al. 2018; Spadola et al. 2021).

This technique and the information acquired during this study lay the foundations for the future use of artificial insemination techniques in *V. cumingi* and *V. macraei* useful for future repopulation initiatives for endangered reptile species.

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Disclosure statement

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