The Herpetological Bulletin 156, 2021: 11-13
RESEARCH ARTICLE

https://doi.org/10.33256/hb156.1113

Cloacal anatomy and sex determination in *Tiliqua* sp.

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ABSTRACT - A rigid endoscope was used to examine the cloacae of an adult pair of *Tiliqua gigas gigas*, and single specimens of *Tiliqua gigas evanescens* and *Tiliqua* sp. (Irian Jaya form). Throughout the procedure the animals showed no signs of stress. Clear anatomical differences were observed between the sexes. Females presented the typical two pairs of papillae (ureteral and genital) and males a single pair of urogenital papillae. The observed differences were confirmed when both pairs bred successfully in the following year.

INTRODUCTION

Endoscopy can be used for direct observation of anatomy of the cloaca (Martínez-Silvestre et al., 2015; Oliveri et al., 2016; Spadola et al., 2009). The first report of endoscopy applied to reptiles was published in 1983 (Wood et al., 1983) but it is only in the last few years that endoscopy has become common in routine clinical practice. Normal procedures adopted are laparoscopy and coeloscopy (Schildger et al., 1999; Murray, 2000; Taylor, 2006), but other minimally invasive procedures such as gastroscopy, the assessment of the upper respiratory system, and cloacoscopy are now also routine (Schumacher, 2011; Knotek & Jekl, 2015, Spadola et al., 2009).

Endoscopy of the cloaca using a rigid endoscope with warm saline irrigation enables direct observation of the proctodeum, urodeum, and coprodeum, furthermore the papillae in the urodeum can be used to distinguish between the sexes; in lizards, females typically have two pairs of papillae while male have only one pair (Fox, 1977; Trauth et al., 1987; Vitt & Caldwell, 2009). Nevertheless, evaluation of the cloacal structures is not always easy due to a lack of detailed information on most of the 11,000+ reptile species. Consequently, this study describes the anatomy of the cloaca in both genders of specimens of *Tiliqua*, with attention to the urodeum in order to obtain a detailed description of the male and female cloaca. Such information may then be used for subsequent clinical comparisons or for sex determination.

MATERIALS AND METHODS

An adult pair of *Tiliqua gigas gigas* (3 years old) were investigated first followed by a specimen of *Tiliqua gigas evanescens* (5 years old) and an Irian Jaya form of *Tiliqua* sp. (unknown age but held for 3 years in captivity). The lizards were checked at Messina University Veterinary Teaching Hospital and Pombia Safari Park to ensure that they were in good health before their cloacae were inspected. To do this the animals were constrained manually in dorsal recumbency on an operating table equipped with a heat mat (Fig. 1). The cloaca was investigated with a rigid endoscope of 4 mm diameter and 0° optic inclination, connected to a Telecam DX camera II, all of which were connected to an all-in-one Karl Storz "TELE PACKTM" system, thus ensuring a light source and video control. In order to expand the cloacal chambers and display all its structures, cloacal washing was provided using a 60 ml syringe filled with warm saline solution (NaCl 0.9 %) connected to the endoscope. The entire procedure took about five minutes for each specimen and throughout the procedure the animals showed no signs of stress. Following the example for cloacoscopy in other reptiles (Perpiñán, 2018; Morici et al., 2017; Divers, 2014), we did not sedate the lizards as cloacoscopy is considered a 'non-risky' procedure for reptiles and anaesthesia poses greater risks for reptiles compared to mammals. After the procedure, all the animals were hospitalised and monitored for 24 h. These ethically approved procedures were undertaken as routine clinical practice with the owner's permission.

Figure 1. Containment in dorsal recumbency of *Tiliqua* sp. (Irian Jaya form) during cloacoscopy

RESULTS

During cloacoscopy of the reproductive pair of *T. gigas gigas*, it was possible to highlight the anatomical differences between the sexes. Similar differences between females and males were also identified in the other two *Tiliqua* specimens.

Females

One of the *T. gigas gigas* and the *T. gigas evanescens* proved to be female. The insertion of the endoscope through the cloacal opening allowed us to see first the anatomy of the proctodeum. Proceeding cranially and dilating the walls by irrigation with saline solution, it was possible to observe a general display of the anatomy of urodeum and coprodeum and their relationship. Overcoming the horizontal envelope between coprodeum and urodeum, it was possible to see the urodeum; this was a blind-bottomed cavity whose wall presented four papillae, arranged in parallel and divided by a median crest (Fig. 2). These structures are the urethral papillae (more caudally) and the genital papillae (more cranially). It wasn't possible to see any structure attributable to the urethral ostium. Considering that a urinary bladder has been reported to adjoin the urodeum in the genus *Tiliqua* (Beuchat, 1986), this could suggest the presence of a rudimentary bladder with vestigial urethra, or differences between species of the genus *Tiliqua* not yet anatomically described. Observation of the coprodeum was possible by inserting the endoscope more cranially whilst at the same time it was possible to overcome the anal sphincter by washing with saline solution. From there the rectal ampulla could be observed and, cranially, the rectal colic valve and the caudal portion of the rectal canal. Furthermore, in the rectum the presence of uric acid as a white pulp mass was identified in both investigated animals. The passage of the aforementioned pulp was also documented from the ureteral papilla to the rectum, after cloacal contraction: this mechanism is typical of ophidians and saurians lacking a bladder.

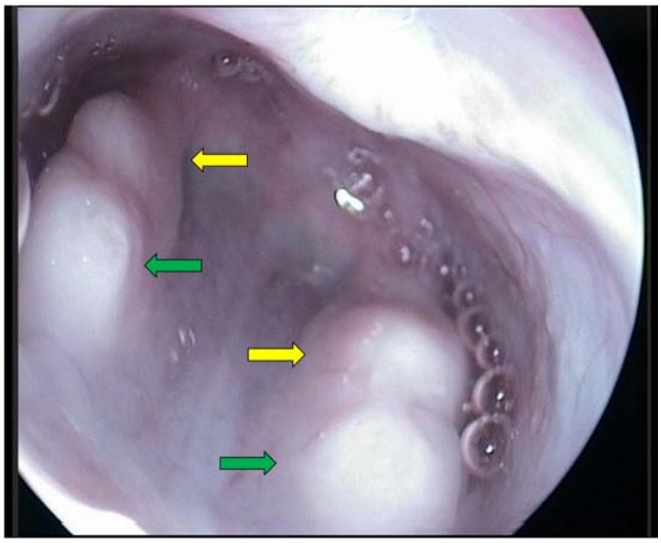

Figure 2. Urodeum of female *Tiliqua gigas gigas*, genital papillae (more cranial- yellow arrows) and urethral papillae (more caudal- green arrows)

Males

One of the *T. gigas gigas* specimens and the *T.* sp. Irian Jaya form were identified as male. During the introduction of the endoscope, it was possible to view the proctodeum, urodeum and coprodeum which appeared in the same general arrangement as for the females and likewise it was possible to dilate the urodeum by proceeding dorsally. The urodeum presented a single pair of urogenital papillae (urethral and genital combined) divided by a median crest, which can be used to diagnose the gender as male (Fig. 3A). During the visualisation of the structures in the urodeum it was possible to observe, as mentioned in females, the sequence of events that effect uric acid excretion and its conveyance through the anal sphincter in the coprodeum, preceded by cloacal contractions (Fig. 3B).

Neither pair of lizards had bred previously and the sex differences observed in this study were confirmed when both pairs bred successfully during the following year.

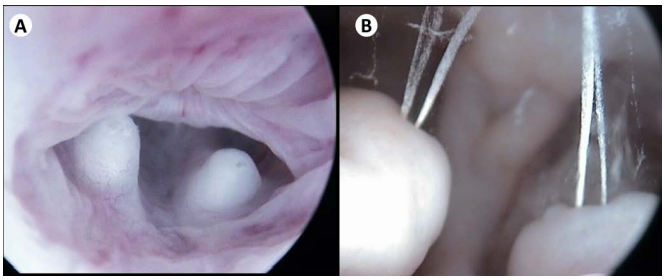

Figure 3. Urodeum of a male *Tiliqua gigas gigas* - **A**. Single pair of urogenital papillae that indicate that the gender is male, **B.** Uric acid discharged from urogenital papillae

DISCUSSION

Tiliqua gigas gigas, *T. gigas evanescens* and *T.* sp Irian Jaya have no obvious external features that distinguish males from females. For the first time, this study has demonstrated that when the urogenital system is viewed using cloacoscopy the presence of two pairs of papillae in the urodeum of females and one pair in males can be used as a reliable guide to sex determination. However, since only four individuals were investigated, intra- and extra-specific anatomical variability cannot be excluded.

Cloacoscopy is a minimally invasive technique that does not require sedation. It can be used to examine the cloaca for sex determination as well as for the diagnosis of common pathologies such as cryptosporidiosis or oviductitis (Rivera, 2008; Spadola & Insacco, 2009; Scullion & Scullion, 2009; Sykes, 2010). Furthermore, it can be used for minimally invasive surgical procedures (Mehler, 2011) of the gastrointestinal and urinary systems, such as removing cloacal calculus or intestinal fecalomas (Mans & Sladky, 2012a & b), obviating long post-operative periods and copious blood loss (Frye, 1972). It may also have a role in conservation medicine by way of assisted insemination as reported in snakes (Quinn et al., 1989; Mattson et al., 2007).

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Accepted: 11 February 2021