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Evaluation of cloacoscopy for sex determination in the Argentine black and white tegu *Salvator merianae*

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INTRODUCTION

Approximately one fifth of reptilian species is listed as Critically Endangered, and another one in five species is classed as data deficient (Alroy, 2015). Their low dispersal ability, together with their great morphological and physiological specialisation, makes reptiles highly sensitive to anthropogenic habitat degradation and climate change (Kearney et al., 2009; Jenkins et al., 2014). Ex situ conservation and captive breeding programmes have therefore become fundamental instruments in reptile conservation (Witzenberger et al., 2011). Moreover, artificial insemination techniques seem to be close to standardisation (Mattson et al, 2007; Molinia et al., 2010; Oliveri et al., 2017). A basic knowledge of reptile reproductive morphology and anatomy is then necessary in order to standardise methods for artificial insemination and early sex recognition (Oliveri et al., 2016). Advanced endoscopic technique has proven to be the ideal method for this purpose, being safe and relatively non-invasive.

Sex recognition in the lizard family Teiidae is challenging. Jaw-muscle dimension (Naretto et al., 2014) and snout-vent distance have been described, but these methods entail a high risk of misdiagnosis (Kratochvíl et al., 2003). Probing of the hemipenal pouches (Hall, 1978; Funk, 2002) may be used in adult tegu lizards, but not in hatchlings. Hemipenal eversion is another possibility but subsequent infection and necrosis of everted hemipenes after injection of saline solution have been reported and eversion with manual massage is challenging in large lizard species with highly muscular tails. It is also dangerous in lizard taxa that perform caudal autotomy and may cause serious injury if it is not performed gently (Reed et al., 2012). Evaluation of the presence of post-cloacal spurs (three slightly protruding scales) is a suggested sex determination technique in lizards of the family Teiidae (Sprackland, 2009). A post-cloacal spur is present on both sides of the tail base in male Argentine black and white tegu. In females, post-cloacal spurs can be also present, but of a smaller dimension than in the male.

Ultrasound imaging and diagnostic endoscopy have both proved to be useful tools for sex determination in lizards. In monomorphic lizards, ultrasound has been used to investigate the development of ovarian follicles and for sex determination (Morris et al., 1996; Schumacher et al., 2001; Gartrell et al., 2002; Prades et al., 2013). However, gonadal ultrasound is only useful when applied to adult, sexually mature lizards (Reed et al., 2012; Stetter, 2006). Cloacoscopy has proved a feasible method of sex determination in many reptile species (Schildger et al., 1989; 1999; Kuchling, 2006; Spadola et al., 2009; Divers, 2010; Innis, 2010; Selleri et al., 2013; Martínez-Silvestre et al., 2015; Perpinan et al., 2016; Spadola et al., 2021). However, to date no information on cloacoscopy of tegus has been published.

The aims of the current study were to i) describe the cloaca in adult male and female Argentine black and white tegu (*Salvator merianae*) using cloacoscopy, and ii) validate this technique for sex determination of hatchlings.

MATERIALS & METHODS

The lizards

A total of 25 Argentine black and white tegus (*Salvator merianae*) were included in the study. Lizards were referred to the Veterinary Teaching Hospital (University of Messina) from private breeders and were divided in two groups according to the age: the first group included six adult tegus (1.8–2.2 kg), while the second group included nineteen 15-day old hatchlings. First, the sex of the adults was determined by the presence of post-cloacal spurs (PSSD) then the results were verified using ultrasonography to detect the ovaries in female tegus (Mylab 40Vet + linear transducer 7.5–12 MHz, Esaote, Italy). The adults were submitted to endoscopy and ultrasound for other clinical reasons and the photographs generated were subsequently used as an aid to identify the sex in the hatchlings. Once the sex was unequivocally determined in all 6 adult tegus, then cloacoscopy (CSD) was undertaken with the hatchlings.

Cloacoscopy

The lizards were restrained manually and positioned in dorsal recumbency on an electric heating pad (30 °C Bosch PFP 1031; Bosch, Germany). For cloacoscopy the following equipment was used - a rigid arthroscope (4 mm diameter, 0°, 8.5 cm length, Olympus medical, Japan) with a working sheath connected via one port to a syringe (60 mL, Pic solutions/Artsana, Italy), camera (Telecam DX-II, Karl Storz, Germany) and a documentation system (TELE PACK, Karl Storz, Germany). The endoscope was gently introduced into the vent of the lizard to visualise the proctodeum, urodeum and coprodeum. To obtain cloacal dilatation and a better visualisation of anatomical structures, there was continuous flushing with sterile saline solution (0.9 % NaCl, S.A.L.F., Italy) combined with 3 ml/L lidocaine chlorohydrate (2 % Lidocaine, Esteve, Italy) from the syringe. After the cloacoscopy, all lizards were kept in the clinic for 24 h to monitor the condition of their health.

One veterinarian (MdG) used PSSD on the 19 hatchling tegus, this was followed by CSD performed with two veterinarians (FS, MM), who were not informed of the results of PSSD. All photographs taken during CSD were then presented to two veterinarians not involved in any of the earlier sex determination work. Since the data were qualitative, numerical statistical testing was not used but instead the reliability of agreement between the results obtained from PSSD, cloacoscopy and final observers was examined with the Fleiss' kappa (K) nominal scale (Fleiss, 1971). The K values range used to measure nominal scale agreement was from 0 to 1.

RESULTS

Details of the cloacoscopy

Using cloacoscopy, the proctodeum of adult tegus was immediately visualised as the endoscope was inserted through the vent. Advancing cranially with the probe, the urodeum (Fig. 1) was seen ventrally. It was present as a small central mucosal fold upon which the urinary papillae could be recognised. In adult female tegus, a mucosal recess was present just beyond the central mucosal fold; this recess was divided in two vaginal pouches by a vertical mucosal slit (Fig. 1A). In adult male tegus the central mucosal fold was not seen (Fig. 1B). In 15-day old hatchling tegus, similar differences between males and females were found (Fig. 2). In the coprodeum the anal sphincter was easily overcome and by passing the endoscope through that the rectal chamber could be seen (Fig. 3). During the whole procedure the lizards did not show any signs of discomfort.

Post-cloacal spur sex determination compared with ultrasonography and cloacoscopy

Sex determination in adult tegus using ultrasonography revealed 3 males and 3 females; by comparison, PSSD suggested the correct sex in five of the six cases (83.3 % accuracy).

In the case of hatchlings, PSSD suggested 7 females and 12 males but CSD revealed 9 females and 10 males. PSSD failed to identify the sex correctly in the case of 4 males and 3 females (Table 1S, see Supplementary Materials), so it was accurate for only 13 or the 19 hatchlings (68.4 % accuracy).

The two blind-trial observers confirmed the results

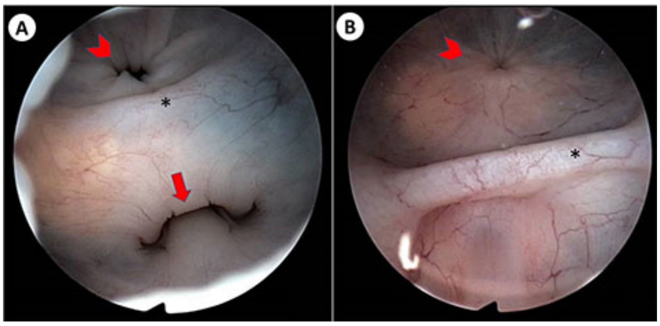

Figure 1. The urodeum of adult *Salvator merianae* visualised by cloacoscopy - **A.** Female, **B.** Male. In the female a mucosal recess (red arrow) is present, and it is divided in the two vaginal pouches by a vertical mucosal slit. In the male the recess was not visualised. An additional horizontal slit (asterisks) divides the urodeum from the coprodeum (arrowheads). When cloacal chambers are not dilated by the saline flush, this horizontal slit covers the 'genital' part of the urodeum, leaving exposed just the central mucosal fold, with the coprodeum connecting directly to the proctodeum.

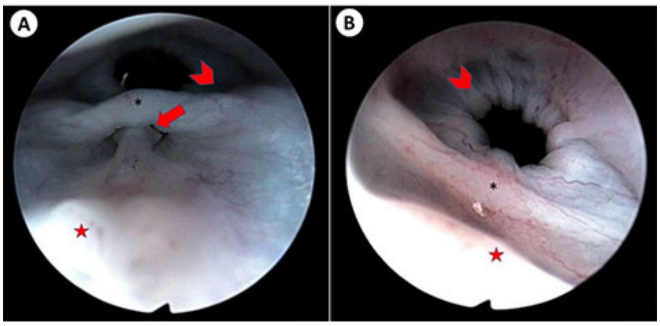

Figure 2. Urodeum of 15-day old hatchling *Salvator merianae* visualised by cloacoscopy - **A.** Female, **B.** Male. Just beyond a mucosal fold (urinary papillae, stars), a mucosal recess (red arrow) and vaginal pouches have been shown even in post-hatchling (15-day old) *S. merianae*. An additional horizontal slit (asterisks) divides the urodeum from the coprodeum (arrowheads). The urodeum differences allow early sex diagnosis.

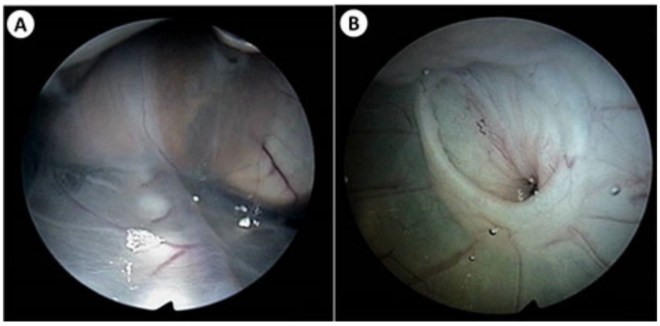

Figure 3. Rectal chamber of adult *Salvator merianae* - **A.** When dilated, the rectal chamber shows the presence of coelomic organs and is highly vascularised, **B.** The rectocolic valve in the rectal chamber

obtained using CSD (Table 1S). There was poor agreement between the PSSD results and those obtained by CSD operators and blind-trial observers (K = 0.479). Cloacoscopic sex determination showed instead perfect statistical agreement between cloacoscopy operators and the subsequent blind-trial observers (K =1).

DISCUSSION

In our experiment, PSSD in young tegus was accurate only in 68.4 % of cases, hence it cannot be considered a reliable method of sex determination in *S. merianae* hatchlings. Instead, cloacoscopy proved to give very accurate sex determination, due to the considerable anatomical differences between sexes. Moreover, cloacoscopic sex determination showed a close statistical agreement between cloacoscopy operators and subsequent blind-trial observers. Cloacoscopy is an important tool in the study of cloacal anatomy. Cloacoscopy and cystoscopy have also been proposed as useful methods of sex determination in young chelonians by indirect visualisation of gonads through the urinary bladder wall (Selleri et al., 2013; Martínez-Silvestre et al., 2015); recently cloacoscopy has been used for sex determination in lizards (Morici et al., 2017), and to aid artificial insemination in snakes (Oliveri et al., 2017; 2016). There is great variability in cloacal morphology between reptile species (Morici et al., 2017; Oliveri et al., 2016; Spadola et al., 2009; 2015; 2016), and the physician should achieve a detailed knowledge of these differences before attempting sex determination through cloacoscopy.

Cloacoscopy has proven to be a safe and minimally invasive technique, comparable in invasiveness to a simple cloacal flushing. Our results demonstrate that CSD represents a highly accurate method for sex determination in *S. merianae*. This method can be considered a useful tool in ex-situ conservation programmes of endangered Teiidae. Moreover, it can be easily used as sex determination method by reptile specialists. We strongly encourage the use of CSD in other squamate species in order to establish accurate references.

REFERENCES

Alroy, J. (2015). Current extinction rates of reptiles and amphibians. PNAS 112(42): 13003–13008.

Divers, S.J. (2010). Reptile diagnostic endoscopy and endosurgery. *Veterinary Clinics of North America: Exotic Animal Practice* 13(2): 217–42.

Fleiss, J.L. (1971). Measuring nominal scale agreement among many raters, *Psychological bulletin* 76(5): 378–382.

Funk, R.S. (2002). Lizard reproductive medicine and surgery. *Veterinary Clinics of North America: Exotic Animal Practice* 5: 579–613.

Gartrell, B.D., Girling, J.E., Edwards, A. & Jones, S.M. (2002). Comparison of noninvasive methods for the evaluation of female reproductive condition in a large viviparous lizard, *Tiliqua nigrolutea*. *Zoo Biology* 21: 253–268.

Hall, B.J. (1978). Notes on the husbandry, behaviour and breeding of captive tegu lizards *Tupinambis teguixin*. *International Zoo Yearbook* 18: 91–95.

Innis, C.J. (2010). Endoscopy and endosurgery of the chelonian reproductive tract. *Veterinary Clinics of North America: Exotic Animal Practice* 13(2): 243–54.

Jenkins, R., Tognelli, M.F., Bowles, P. et al. (2014). Extinction risks and the conservation of Madagascar's reptiles. *PLoS ONE*. 9(8): e100173.

Kearney, M., Shine, R. & Porter, W.P. (2009). The potential for behavioural thermoregulation to buffer ''cold-blooded'' animals against climate warming. *Proceedings of the National Academy of Sciences USA* 106: 3835–3840.

Kratochvíl, L., Fokt, M., Rehák, I. & Frynta, D. (2003). Misinterpretation of character scaling: a tale of sexual dimorphism in body shape of common lizards. *Canadian Journal of Zoology* 81(6): 1112–1117.

Kuchling, G. (2006). Endoscopic sex determination in juvenile freshwater turtles, *Erymnochelys madagascariensis*: Morphology of Gonads and Accessory Ducts. *Chelonian Conservation and Biology* 5(1): 67–73.

Martínez-Silvestre, A., Bargalló, F. & Grífols, J. (2015). Gender identification by cloacoscopy and cystoscopy in juvenile chelonians. *Veterinary Clinics of North America: Exotic Animal Practice* 18(3): 527–539.

Mattson, K.J., De Vries, A., McGuire, S.M., Krebs, J., Louis, E.E. & Loskutoff, N.M. (2007). Successful artificial insemination in the corn snake, *Elaphe gutatta*, using fresh and cooled semen. *Zoo Biology* 26(5): 363–9.

Molinia, F.C., Bell, T., Norbury, G., Cree, A. & Gleeson, D.M. (2010). Assisted breeding of skinks or how to teach a lizard old tricks! *Herpetological Conservation and Biology* 5(2): 311–319.

Morris, P.J. & Alberts, A.C. (1996). Determination of sex in white-throated monitors (*Varanus albigularis*), Gila Monsters (*Heloderma suspectum*) and bearded lizards (*Heloderma horridum*) using two-dimensional ultrasound imaging. *Journal of Zoo and Wildlife Medicine* 27: 371–377.

Naretto, S., Cardozo, G., Blengini, C. & Chiaraviglio, M. (2014). Sexual Selection and Dynamics of Jaw Muscle in Tupinambis Lizards. *Evolutionary Biology* 41: 192–200.

Oliveri, M., Morici, M., Novotný, R., Bartošková, A. & Knotek, Z. (2016). Cloacoscopy in the horned viper (*Vipera ammodytes*). *Acta Veterinaria Brno* 85(3): 251–253.

Oliveri, M., Spadola, F., Morici, M., Bartoskova, A., Di Giuseppe, M. & Knotek, Z. (2017). Artificial insemination in snakes: techniques, challenges and success. Proceedings 3rd ICARE, March 25th-29th 2017, Venice, Italy: 543 pp.

Perpinan, D., Martinez-Silvestre, A., Bargallo, F., Di Giuseppe, M., Oros, J. & Pereira Da Costa T. (2016). Correlation between endoscopic sex determination and gonad histology in pond sliders, *Trachemys scripta* (Reptilia: Testudines: Emydidae). *Acta Herpetologica* 11(1): 91–94.

Prades, R.B., Lastica, E.A. & Acorda, J.A. (2013). Ultrasound features of the kidneys, urinary bladder, ovarian follicles and vaginal sacs of female water monitor lizard (*Varanus marmoratus*, Weigmann, 1834). *Philippine Journal of Veterinary and Animal Sciences* 39: 115–124.

Reed, N.R. & Tucker, A.D. (2012). Determining age, sex and reproductive condition. In *Reptile biodiversity: standard methods for inventory and monitoring*. McDiarmid, R.W., Foster, M.S., Guyer, C., Gibbons, J.W., Chernoff, N. (Eds.),. Los Angeles, California: University of California Press. 151 pp.

Schildger, B.J., Haefeli, W., Kuchling, G., Taylor, M., Tehnu, H. & Wicker, R. (1999). Endoscopic examination of the pleuro-peritoneal cavity in reptiles. *Seminars in Avian and Exotic animals Medicine* 8(3): 130–138.

Schildger, B.J. & Wicker, R. (1989). Sex determination and clinical examination in reptiles using endoscopy. *Herpetological Review* 20: 9–10.

Schumacher, J. & Toal, R.L. (2001). Advanced radiography and ultrasonography in reptiles. *Seminars in Avian and Exotic Pet Medicine* 10: 162–168.

Selleri, P., Di Girolamo, N. & Melidone, R. (2013). Cystoscopic sex identification of posthatchling chelonians. *Journal of the American Veterinary Medical Association* 242: 1744–1750.

Spadola, F. & Insacco, G. (2009). Endoscopy of cloaca in 51 *Emys trinacris* (Fritz et al., 2005): morphological and diagnostic study. *Acta Herpetologica* 4(1): 73–81.

Spadola, F. & Morici, M. (2015). Morphology of the cloacal structure of the Chinese soft-shell turtle in *Pelodiscus sinensis* examined by endoscopy. *Russian journal of Herpetology* 22(3): 175–178.

Spadola, F., Morici, M., Oliveri, M. & Knotek, Z. (2016). Description of cloacoscopy in the loggerhead sea turtle (*Caretta caretta*). *Acta Veterinaria Brno* 85: 367–370.

Spadola, F., Sgroi, P., Lubian, E. & Morici, M. (2021). Cloacal anatomy and sex determination in *Tiliqua* sp. *The Herpetological Bulletin* 156: 11–13.

Sprackland, R.G. (2009). Giant lizards: The definitive guide to the natural history, care, and breeding of monitors, iguanas and other large lizards, T.F.H. Publications. 335 pp.

Stetter, M.D. (2006). Ultrasonography. In: *Reptile medicine and surgery*, 665–674 pp. Mader, D. (Ed.), Saunders/ Elsevier, St. Louis, MO.

Witzenberger, K.A. & Hochkirch, A. (2011). Ex-situ conservation genetics: a review of molecular studies on the genetic consequences of captive breeding programmes for endangered animal species. *Biodiversity and Conservation* 20: 1843.

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