Blue-tongued skinks are viviparous lizards belonging to the *Tiliqua* genus that are growing in popularity in the reptile pet trade and that lack sexual dimorphism. The genus *Tiliqua* includes 7 species of skinks originating from Australia and Indonesia. The 2 most popular species, *Tiliqua gigas* and *Tiliqua scincoides*, each include 3 subspecies. *Tiliqua gigas* is endemic to New Guinea, while *T scincoides* is endemic to Australia. These skinks have several anatomical peculiarities of clinical relevance. One of the most relevant is that the urinary tract of blue-tongued skinks includes a relatively large urinary bladder, which is connected to the cloaca through the urethral opening (Figure 1). The urinary bladders of adult blue-tongued skinks (*Tiliqua scincoides scincoides*), range in body weight between 400 and 580 g, contain on average 58 mL, and could reach a total capacity of 139 mL.

**OBJECTIVE**
To describe a nonsurgical endoscopic technique for sex identification in Indonesian blue-tongued skinks (*Tiliqua gigas*) and to assess accuracy of contrast radiography of the hemipenile/hemiclitoral pouches.

**ANIMALS**
42 clinically healthy Indonesian blue-tongued skinks between 6 months and 3 years old and weighing between 22 and 550 g.

**METHODS**
Cystoscopy was performed under general anesthesia. Gonads were visualized through the transparency of the urinary bladder, and their gross morphology was described. Contrast was applied in the tail pouches before obtaining full-body radiographs. Two radiologists, blinded to the sex of the skink, evaluated the radiographs.

**RESULTS**
Cystoscopy was achieved in all 42 skinks. Visualization of the gonads through the urinary bladder was possible in 41 (98%; 95% CI, 87% to 99%) of the skinks, with 18 of them identified as males and 23 identified as females. Median procedure time was 60 seconds (range, 25 to 180 seconds) and was not associated with procedure order (−0.69; 95% CI, −1.83 to 0.45) or with the weight (0.02 g; 95% CI, −0.07 to 1.0) or the identified sex (11.7; 95% CI, −15.07 to 38.45) of the skink. Radiographs had a sensitivity of 69.6% (95% CI, 47.1% to 86.8%) and a specificity of 75.0% (47.6% to 92.7%) to identify female skinks. All the skinks recovered uneventfully.

**CLINICAL RELEVANCE**
Cystoscopic sex identification is feasible in Indonesian blue-tongued skinks of various age and size. Considering the difficulty in identifying their sex otherwise, this technique could provide a significant improvement in the veterinary care of this species. In this population, contrast radiographs showed limited accuracy for sex identification.

**Keywords:** skink, radiography, blue-tongued skinks, cystoscopy, lizards
Veterinarians working with species with poor or absent sexual dimorphism are often requested to identify the patient’s sex. Sex identification is indeed crucial for reproduction, management, and clinical care of any reptile species. Sex identification helps zoological collections and private breeders planning for reproductive pairs. In species in which intersex aggression is common, sex identification allows one to keep a group that has a male-to-female ratio favoring the numbers of females.

During clinical assessment of individual animals, sex identification is critical for the diagnosis of diseases associated with reproductive organs. Several techniques have been used for sex identification of reptiles that lack obvious sexual dimorphism. Some techniques rely on the assessment of the presence of the phallicus or the hemipenes. A common example is probing the hemipenile pouches or evertting the hemipenes. These procedures, although simple, do not provide consistent results in all species and may result in damage to the hemipenile pouches.

In certain lizards (eg, Varanus spp), hemibacula are mineralized and can be visualized with radiographs. Compared with these techniques, techniques that rely on the visualization of the gonads allow one to reach more definitive conclusions. In other reptiles, investigators have visualized the gonads via endoscopy, either directly by entering the coelom with the endoscope or indirectly by performing a cystoscopy and visualizing the coelom through the transparency of the urinary bladder.

Sexual dimorphism in blue-tongued skinks is considered minimal, and sex identification can be extremely challenging. Although there are subtle differences in body size and shape between adult males and females, the use of morphometrics does not allow a definitive distinction between sexes in all individuals. Recently, 2 medical techniques to identify the sex of blue-tongued skinks were described. One technique consisted in applying radiographic contrast in the hemipenile/hemiclitoral pouches or tail pouches, visualizing the hemipenes after radiographs. The other technique consisted in visualizing the oviductal papillae during cloacoscopic. However, these techniques were tested on a limited number of animals, they rely on secondary rather than primary sexual characteristics, and their accuracy was not confirmed by gonadal visualization.

The purpose of the present study is to describe a nonsurgical endoscopic technique for sex identification in Indonesian blue-tongued skinks and to assess accuracy of contrast radiography of the hemipenile/hemiclitoral pouches for sex identification.

**Methods**

**Study design**

The present article describes the results of a descriptive observational study (cystoscopic sex identification) and of a diagnostic accuracy study (accuracy of radiography of the tail pouches). The article was reported in accordance with the Standards for the Reporting of Diagnostic Accuracy Studies. Ethical approval for the study was granted from the ethical committee of the University of Parma (Protocol No. 1/CESA/2022). The owner was informed that this sex identification technique has not been described before and gave informed consent to allow participation of the animals in the study.

**Animals and housing**

Forty-two clinically healthy captive-bred Indonesian blue-tongued skinks, owned by a private reptile breeder, were presented to the Exotic Animal Service, Clinica Veterinaria Modena Sud, Spilamberto, Italy, for sex identification. The animals were between 6 months and 3 years old and weighed between 22 and 550 g (0.048 to 1.21 lb; median, 184.1 g [0.41 lb]). All the animals were acclimatized for at least 24 hours before the procedure was performed. Light and heat were provided by means of 50-W metal halide (HID UV Lamp; Rep-Tech); temperature in the enclosures ranged from 24 to 27 °C (75.0 to 80.0 °F). Food was withheld 24 hours before the procedure.
The animals had free access to water from a bowl at all times.

**Procedures**

All the animals were premedicated with midazolam administered at 0.7 mg/kg, dexmedetomidine at 0.05 mg/kg, and ketamine at 5 mg/kg combined into a single syringe and delivered via IM injection with a 27-gauge needle in the right triceps. This protocol provided adequate restraint to perform the cystoscopic procedures. All cystoscopic examinations were performed by the same veterinarian, who is board certified in herpetological medicine and surgery (GN). A 2.7-mm, 30° viewing rigid endoscope (Telepack TP100 EN; Karl Storz Endoscopy Italia Srl) housed within a 4.8-mm operative sheath was used for skinks that had a body weight over 300 g. For skinks that had a body weight lower than 300 g, a smaller sheath, the 3.5-mm protective sheath, was used. Before the procedure, the endoscopic equipment was treated by immersion in 2% glutaraldehyde solution for 15 minutes and rinsed with 50 mL of sterile saline (0.9% NaCl) solution. Cystoscopy was performed in a similar fashion as the procedure described in chelonians. The skinks were positioned in ventral recumbence on an elevated Styrofoam platform placed on a surgical table. The endoscope was inserted through the cloaca and directed cranially. The urethral opening was visualized ventral to the rectum, and the endoscope was positioned right in front of it. Warm (30 °C) NaCl (0.9%) solution was infused (1 drop every 2 to 4 seconds) to allow the distension of the urethral opening, while at the same time the endoscope was gently pushed forward. Once the urinary bladder was reached, the endoscope was directed dorsally until visualization of both gonads was achieved. Infusion of saline solution was interrupted when the bladder wall was distended enough to permit the visualization of coelomic organs. Endoscopic photographs of the gonads of each animal were obtained. Procedure time was measured as the time that elapsed from insertion of the endoscope in the cloaca to visualization of the first gonad.

**Gonad morphology**

The description of the gonads was made on the basis of their external endoscopic appearance as described in previous reports for chelonians. Testes were classified by color, shape, and vascular texture. Ovaries were classified by color, shape, and size of the follicles.

**Radiographic technique**

Following cystoscopy, contrast radiographs were performed by 1 operator (AV) blinded to the results of the cystoscopic examination. An 18-gauge, rounded-tip metal probe was gently introduced into the cloaca and directed caudally and parallel to the tail into each one of the hemipenile/hemiclitoral pouches, reaching their maximum depth. A dose of 0.5 to 1 mL of nonionic, water-soluble, iodinated contrast medium (ioversol [300 mg/mL]; Optiray 300; Guebert SpA) was injected in each pouch with a 2.5-mL syringe while the probe was slowly withdrawn. The excess contrast media outlining the cloacal rim was gently cleaned with paper towel. Dorsoventral and left lateral radiographic views were obtained within 5 minutes from contrast administration by means of a digital radiography machine (Aria-X, Demas Srl) with the following settings: kV, 53; mA, 3.2; exposure time, 0.06 seconds. Before the lateral view was obtained, the hind limbs were elevated and bound together with a small piece of tape to prevent their superimpositions with the base of the tail. Radiographs that had at least 1 pouch filled with contrast media were considered adequate.

**Radiographic interpretation**

Each study corresponded to an animal and consisted of 1 DICOM file of a lateral view and 1 DICOM file of a dorsoventral radiographic view, except for 3 studies that consisted of only 1 dorsoventral radiograph. The studies were provided to 2 blinded radiologists in a randomly generated (Research Randomizer; www.randomizer.org) order and after removal of any identifying information. Each radiologist independently evaluated the radiographs and noted the suspected sex of the skinks on an electronic spreadsheet. In general, sexing of skinks was based on 2 previous studies. Skinks in which contrast highlighted short, spiral structures were classified as males, while skinks in which contrast highlighted long, straight structures were classified as females. Once compiled by the radiologists independently, the electronic spreadsheets were collected and compared. Agreement between the radiologists was then calculated as described in the statistical analysis section. Those cases in which there was disagreement were discussed again between the radiologists to achieve a consensus.

**Recovery from anesthesia and follow-up**

Upon the request of the owner, following the completion of the cystoscopic procedure, all skinks except 2 subadult specimens were individually identified with a subcutaneous electronic transponder. Atipamezole (Atidorm; Fatro Industria Farmaceutica Veterinaria SpA) at a dose of 0.5 mg/kg was administered in the left triceps at the end the endoscopic procedure. The skinks were placed individually in a plastic enclosure and closely monitored during recovery from anesthesia. The animals were hospitalized from 7 to 10 days after the procedure before being discharged to the owners.

**Statistical analysis**

Categorical variables were reported as nominators, denominators, and percentages with 95% CIs. Normality of continuous variables was tested with the Shapiro-Wilk test, and results were reported as medians and ranges due to nonnormal distributions. Generalized linear models (GLMs) were built to identify significant associations between time required for cystoscopy and independent variables, including body weight (g), sex (male/female), and order of
execution (1 to 42). First, 3 univariable GLMs were built, 1 with each independent variable. Then, 1 multivariable GLM was built including those independent variables that had a P value of .1 in the previous univariable models. A univariable binary logistic regression model was built to ascertain whether a lighter (white/yellow) rather than a darker (red) coloration of the testicle was associated with body weight (g) in male sexed skinks.

The initial agreement between the 2 radiologists was evaluated via the κ statistic. After the 2 radiologists reached a consensus on the disagreements, diagnostic accuracy of contrast radiography was evaluated using cystoscopy as the reference standard. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall accuracy of radiographs to identify female skinks were calculated and reported with 95% CIs. Each skink was classified based on the presence of agreement between radiography and cystoscopy. Three univariable logistic regression models were built to determine whether agreement between radiography and cystoscopy was influenced by individual characteristics of the skinks, including body weight (g), sex (male/female), and order of execution (1 to 42).

Analyses were performed with the use of IBM SPSS, version 22.0 (SPSS Inc). Two-sided P values inferior to .05 were considered statistically significant.

### Results

#### Cystoscopy and outcome

The urinary bladder was entered in all 42 Indonesian blue-tongued skinks. Cystoscopy allowed visualization of the coelom through the transparency of the urinary bladder in 41 (98%; 95% CI, 87% to 99%) skinks. In a 300-g skink, the urinary bladder was not transparent, and a clear visualization of the coelom or the gonads was not achieved (Figure 2). In addition to the gonads, the intestine, coelomic wall, left and right kidneys, the spleen and left and right adrenal glands were visible in most of the examined skinks. All the skinks recovered from anesthesia, and no clinical complications were observed in the 7 to 10 days of hospitalization. Before hospital discharge, all the animals fed on an insect-based diet at least once.

#### Endoscopic gonadic appearance

Based on the endoscopic appearance of the gonads, 18 of 41 skinks (43.9%) were identified as male, and 23 of 41 (56.1%) were identified as female (Supplementary Video S1; Supplementary Video S2). Ovaries were white (23/23 [100%]), their shape was elongated (9/23 [39.1%]), or flattened (5/23 [21.7%]) with white to beige follicles homogeneous in size (9/23 [39.1%]) or white to beige follicles of different size (14/23 [60.8%]) protruding from the irregular surface (Table 1; Figure 3). Subjectively, older and heavier females showed a larger ovary with more developed follicles than younger and lighter ones.

Testicles were beige with well-developed vascular texture on the serous surface (11/18 [61.1%]), pale red with a fine vascular texture on the serous surface (6/18 [33.3%]), or pale yellow with well-developed vascular texture on the serous surface (1/18 [5.5%]) and were oval (17/19 [89.4%]) or round to oval (1/18 [5.5%]).

#### Cystoscopy duration

Cystoscopy duration was nonnormally distributed (Shapiro-Wilk, P < .001). Median (range) procedure time was 60 (25 to 180) seconds. Cystoscopy duration was not associated with the order of the procedure (–0.69; 95% CI, –1.83 to 0.45; P =.23), with the weight of the skink (0.02; 95% CI, –0.07 to 1.0; Figure 2).

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**Figure 2**—Cystoscopic appearance of the pathological bladder in an adult Indonesian blue-tongued skink. The presence of a diffuse, locally extensive, white opacity (asterisk) precluded visualization of the coelom through the urinary bladder.

**Table 1**—Morphological characteristics of ovaries as visualized via cystoscopy in 23 female Indonesian blue-tongued skinks (*Tiliqua gigas*). Body weight is reported as median (range).

<table>
<thead>
<tr>
<th>No. (%)</th>
<th>Color</th>
<th>Shape</th>
<th>Follicles (size)</th>
<th>Follicles (color)</th>
<th>Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 (21.7%)</td>
<td>White</td>
<td>Flattened</td>
<td>Homogeneous</td>
<td>White to beige</td>
<td>28 (22–32)</td>
</tr>
<tr>
<td>5 (21.7%)</td>
<td>White</td>
<td>Elongated</td>
<td>Variable</td>
<td>White to beige</td>
<td>500 (340–570)</td>
</tr>
<tr>
<td>5 (21.7%)</td>
<td>White</td>
<td>Round to oval</td>
<td>Variable</td>
<td>White to beige</td>
<td>32 (30–34)</td>
</tr>
<tr>
<td>3 (13%)</td>
<td>White</td>
<td>Round to oval</td>
<td>Homogeneous</td>
<td>White to beige</td>
<td>34 (32–95)</td>
</tr>
<tr>
<td>3 (13%)</td>
<td>White</td>
<td>Elongated</td>
<td>Variable</td>
<td>White to yellow</td>
<td>30 (29–310)</td>
</tr>
<tr>
<td>1 (4.3%)</td>
<td>White</td>
<td>Elongated</td>
<td>Homogeneous</td>
<td>White to beige</td>
<td>99</td>
</tr>
<tr>
<td>1 (4.3%)</td>
<td>White</td>
<td>Round to oval</td>
<td>Variable</td>
<td>White to beige</td>
<td>100</td>
</tr>
</tbody>
</table>
When identifying the sex of blue-tongued skinks, a multivariable model was not built. Considering the lack of association, a multivariable model was not built.

Contrast radiographs were obtained in 40 of the 42 skinks (Figure 4). One skink recovered from anesthesia before the radiographs could be obtained. One skink was missing the tail due to previous conspecific aggression. In 3 skinks, only a dorsoventral radiograph was obtained. Diagnostic accuracy statistics were calculated over 39 skinks because 1 of the 40 skinks with available radiographs was not possible to sex through cystoscopy.

Initial agreement between the 2 radiologists was 70% (Cohen’s κ, 0.43). After reaching a consensus on the cases with disagreement, sensitivity to identify female skinks was 69.6% (95% CI, 47.1% to 86.8%) and specificity was 75.0% (95% CI, 47.6% to 92.7%). Assuming a prevalence of 50% females, PPV was 73.6% (95% CI, 53.3% to 87.1%), and NPV was 71.1% (95% CI, 55.5% to 82.9%). Overall accuracy of the technique was 72.3% (95% CI, 55.6% to 85.4%).

Table 2—Morphological characteristics of testicles as visualized via cystoscopy in 18 male Indonesian blue-tongued skinks. Body weight is reported as median (range).

<table>
<thead>
<tr>
<th>No. (%)</th>
<th>Color</th>
<th>Shape</th>
<th>Texture</th>
<th>Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11 (61.1%)</td>
<td>Beige</td>
<td>Oval</td>
<td>Well-developed vascular texture</td>
<td>290 (34–400)</td>
</tr>
<tr>
<td>6 (33.3%)</td>
<td>Pale red</td>
<td>Oval</td>
<td>Fine vascular texture</td>
<td>30.5 (28–230)</td>
</tr>
<tr>
<td>1 (5.5%)</td>
<td>Pale yellow</td>
<td>Oval</td>
<td>Well-developed vascular texture</td>
<td>210</td>
</tr>
</tbody>
</table>

$P = .66$, or with the identified sex (11.7 seconds for males; 95% CI, –15.07 to 38.45; $P = .38$). Considering the lack of association, a multivariable model was not built.

Contrast radiographs

Contrast radiographs were obtained in 40 of the 42 skinks (Figure 4). One skink recovered from anesthesia before the radiographs could be obtained. One skink was missing the tail due to previous conspecific aggression. In 3 skinks, only a dorsoventral radiograph was obtained. Diagnostic accuracy statistics were calculated over 39 skinks because 1 of the 40 skinks with available radiographs was not possible to sex through cystoscopy.

Initial agreement between the 2 radiologists was 70% (Cohen’s κ, 0.43). After reaching a consensus on the cases with disagreement, sensitivity to identify female skinks was 69.6% (95% CI, 47.1% to 86.8%) and specificity was 75.0% (95% CI, 47.6% to 92.7%). Assuming a prevalence of 50% females, PPV was 73.6% (95% CI, 53.3% to 87.1%), and NPV was 71.1% (95% CI, 55.5% to 82.9%). Overall accuracy of the technique was 72.3% (95% CI, 55.6% to 85.4%).

All 3 skinks that had only the dorsoventral radiographs were correctly sexed. Exclusion of these 3 skinks from the analysis resulted in slightly worse diagnostic accuracy of the radiographs: sensitivity to identify female skinks was 68.2% (95% CI, 45.1% to 86.1%), specificity was 71.4% (95% CI, 41.9% to 91.6%), PPV was 70.5% (95% CI, 49.8% to 85.1%), and NPV was 69.2% (95% CI, 52.8% to 81.8%). Overall accuracy of the technique was 69.8% (95% CI, 52.3% to 83.9%).

Agreement between radiographs and the reference standard was not associated with the order of the procedure (OR, 1.05; 95% CI, 0.99 to 1.12; $P = .12$), with the weight of the skink (OR, 1.0; 95% CI, 0.99 to 1.00; $P = .48$), or with the identified sex (OR, 1.31; 95% CI, 0.31 to 5.53; $P = .71$). Considering the lack of association, a multivariable model was not built.
In this study, we report the use of cystoscopy for sex identification of a species of skinks that lacks obvious sexual dimorphism. The technique described herein allowed prompt indirect visualization of the gonads in all but one of the skinks tested, without clinical complications.

So far, limited techniques to identify sex in blue-tongued skinks are reported. A morphometric study in blue-tongued skinks (T. scincoides) found differences with respect to snout-vent length, trunk length, and head width between adult males and females. However, the degree of difference was subtle, with some overlap between males and females. In addition, these differences were not present in young animals. A follow-up study found that morphometric measurements have accuracy of only 70% to identify the sex of eastern blue-tongued skinks (T. scincoides scincoides). Manual eversion of the hemipenes is not considered ideal since a negative result cannot exclude a male, and the process could result in trauma. Ultrasonography is challenging in adult Tiliqua spp due to thick and mineralized scales that limit ultrasound penetration.

Two other medical techniques have been advised to identify the sex of blue-tongued skinks. One of them consists in the injection of contrast media into the hemipenile/hemiclitoral pouches followed by either radiography or CT. This technique has been described in 2 studies. In 1 study, the technique was performed in 3 blue-tongued skinks (T. scincoides) and, in all 3 cases, allowed identification of the hemipenes. However, no female skink was included in that study. Furthermore, sexing was not confirmed by visualization of gonads. The other study of this technique included 20 eastern blue-tongued skinks, 18 of which were adults. Although the results of that study report a perfect accuracy of the technique, the sex of the animals was not confirmed by direct or indirect visualization of gonads. The other technique consists in the visualization of the oviductal openings during cloacoscopy. In a published article including 2 adult Indonesian blue-tongued skinks, the authors identified the presence of the oviductal openings in the urodeum in the female skink; these openings were not visualized in the male skink. Also in that article sexing was not confirmed by visualization of gonads. Considering the limitations of these 3 articles, such as the small number of animals included and the lack of a definitive confirmation of their sex, the evidence behind the use of both of these techniques is weak.

In the present study, we included a larger sample size, and, for the first time, we demonstrated a technique that allows visualization of the gonads, clearly showing a difference between male and female gonads also in juvenile blue-tongued skinks.

Cystoscopic sex identification in reptiles requires 4 steps: (1) identify the urethra in the urodeum, (2) access the urethra and reach the urinary bladder, (3)
visualize the coelom through the transparency of the urinary bladder, and (4) visualize the gonads in the coelom. A rigid endoscope is adapted well to this task in small chelonians because the urethra is extremely short and lies on an imaginary straight line that is parallel to the long axis of the animal, and as such, maneuvering the endoscope toward the left or on the right is rarely needed. The fluid infusion allows the opening of the urethra and the distension of the bladder. A 30° viewing angle is helpful once the bladder is entered since the gonads lie dorsal and slightly caudal to the center of the urinary bladder. In skinks, we found that with the rigid endoscope it was easy to maintain a straight line from the cloaca and visualize the urethra. Gentle saline infusion helped opening the urethra, and a certain amount of fluid was necessary to distend the urinary bladder wall and to increase its transparency. Blue-tongued skinks have an exceptionally voluminous urinary bladder that can contain up to 2% of their body weight. None of the skinks showed adverse effects associated with the iatrogenic distension of the urinary bladder in the days after the procedure. Finally, the 30° viewing angle helped visualize the gonads that lie dorsal to the urinary bladder in skinks, similarly to chelonians.

In this study, we recognized gonads based on their gross morphological appearance. A clear morphological difference in reptile gonads is well demonstrated in the literature. Gross gonad morphology has been used in the past to identify sex in various reptile species, such as Aldabrachelys gigantea and juvenile freshwater turtles (Erymnochelys madagascariensis). In posthatchling Herrmann’s tortoises (Testudo hermanni), the ovaries appear as elongated, white to translucent, slightly convoluted organs containing variably sized, rounded, whitish follicles. Testes are described as oval to rounded, tan-yellow in color, exhibiting a smooth surface characterized by a tight net of blood vessels. In skinks, we noted a change in gonads’ appearance with increasing body size. In general, smaller males had pale red testicles, with a fine vascular texture on the serous surface, while the color changed to white, beige or pale yellow increasing in body size. In a study including 259 dead male eastern blue-tongued skinks, of which 162 were adults, testes were reported as “ovoid white to pale yellow organs.” In that study, a more precise characterization of the color was not reported. Although the darker color observed in our study was not mentioned in that study, it is important to note that the skinks were dead and preserved in 70% ethanol, which could have altered their color. It is possible that the difference in color observed in our study is related to a different sexual maturity between subjects, with the lighter color observed in the testes of heavier skinks indicative of active spermatogenesis and presence of sperm in the seminiferous tubules.

Differences in the appearance of ovaries were noticed also in females, although not formally tested. Heavier females subjectively showed a larger ovary with more developed follicles than lighter ones. During the previtellogenic stage, follicles lack visible structures and exhibit a lighter color compared with the vitellogenic phase. In the vitellogenic phase, follicles increase their vascularization and selectively absorb vitellogenin from the bloodstream, resulting in a brighter yellow to orange coloration due to yolk storage. Hence, the observed color variations could potentially be linked to the varying amounts of yolk present inside the follicles.

There are several reasons that could justify the lower accuracy of contrast radiography of the tail pouches observed in the present study as compared with a previous study. During our study, the operator performing the radiographs was particularly careful at the time of injecting the contrast to avoid any potential damage to the tail pouches. Insertion of the metallic probe at an excessive depth, or injection of the contrast at an excessive pressure, could result in tissue damage and diffusion of the contrast media in the fascial planes of the tail. In some radiographs, only a limited amount of contrast was actually lining the interior of the tail pouches, and this may have contributed to the difficulty in sexing the animals. If radiographs are read immediately after being obtained, additional contrast could be administered in the cases where limited contrast lined the tail pouches, perhaps resulting in higher diagnostic accuracy of this technique. Another potential reason is that in the previous study evaluating accuracy of radiology, the sex of the skink was not confirmed by visualization of the gonads. As such, some degree of bias leading to overestimation of the accuracy of the technique could explain the discrepancy in our results.

The present study had some limitations. Gonads were not examined histologically. However, this limitation is unlikely to be relevant considering that the gonads of the skinks in this study exhibited obvious morphological differences that were consistent with what was observed in other reptile species. Also, endoscopy was performed by a single operator. As such, it is impossible to confirm whether this technique can be performed by individuals with different degrees of endoscopic expertise. Finally, although the sample size is relatively large for a study on a zoological species, follow-up studies including several hundreds of individuals are required to detect rare complications.

Based on our literature search, this is the first report documenting the application of cystoscopy for indirect visualization of gonads and sex identification in Indonesian blue-tongued skinks. Blue-tongued skinks are likely to become one of the most popular species in the reptile pet trade in the next decade, and veterinarians may be asked to identify their sex. Considering the inherent difficulty in identifying the sex of blue-tongued skinks, this technique could provide a significant step forward in the veterinary care of this species.

Acknowledgments

The authors have nothing to declare.
References


Supplementary Materials

Supplementary materials are posted online at the journal website: avmajournals.avma.org