



Australian tree skinks *Egernia striolata*  
Scink *Egernia striolata*

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# Candling as a means to determine sex in the Australian tree skink *Egernia striolata*

## Prosvěcování jako prostředek k určení pohlaví u scinka *Egernia striolata*

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### Abstract

It is important that zoological collections are able to identify the sex of their animals quickly, efficiently and in a welfare-informed manner. Reptiles pose several challenges in terms of sex determination in that sexual dimorphism is often subtle and genitalia are normally internal. While methods such as probing and popping are available, these are generally invasive and can be harmful if practiced incorrectly. The purpose of this study was to trial the use of candling as a means of identifying sex in Australian tree skinks *Egernia striolata*. Four individuals at Sparsholt College's Animal Health & Welfare Research Centre were candled, using a bright LED light shone dorsally through the base of the tail. Of the four individuals, clear evidence of hemipenial shadows were shown in one individual, and the remainder showed no shadows. The skinks were therefore identified as one male and three females. While only a small sample size was available method shows great promise in quickly identifying sex in this species, and the technique may have wider application to other small-bodied lizard species housed in zoological collections.

### Abstrakt

Je důležité, aby v zoologických chovech bylo možné určit pohlaví zvířat rychle, efektivně a s ohledem na jejich welfare. Určování pohlaví u plazů bývá často problematické – pohlavní dimorfismus není mnohdy příliš patrný a pohlavní orgány jsou zpravidla vnitřní. K dispozici jsou sice metody, jako je sondování nebo palpování, ale ty jsou obecně vzato invazivní a při nesprávném postupu mohou zvířeti ublížit. Účelem této studie bylo vyzkoušet prosvěcování jako prostředek k určení pohlaví u australských scinků *Egernia striolata*. Čtyřem jedincům z Animal Health & Welfare Research Centre v Sparsholt College byl prosvěcován kořen ocasu z hřbetní strany pomocí jasného LED světla. U jednoho ze čtyř jedinců byly jasně průkazné stíny hemipenisů, zatímco u ostatních jedinců se neukázaly. Scinkové byli proto určeni jako jeden samec a tři samice. Ačkoli byl k dispozici pouze malý vzorek, metoda je velkým příslibem pro rychlou identifikaci pohlaví u tohoto druhu a tato technika může mít širší uplatnění i u jiných druhů malých ještěřů v zoologických chovech.

### Keywords

*Egernia striolata*, hemipenial transillumination, reptile, Scincidae, sexing, tree-crevice skink, zoological collection

## Introduction

While reptiles are well-represented in zoological collections globally (Brereton & Brereton 2020), they are still not comprehensively studied in comparison to mammals (Melfi 2009, Doody et al. 2021). There remain gaps in the knowledge of reptile anatomy, biochemistry and behaviour (Reed & Tucker 2012), and these gaps can impact the relevance of current husbandry and welfare initiatives. Aspects of social behaviour (Doody et al. 2021) and reproduction are often poorly understood, resulting in use of 'folklore husbandry' techniques in the absence of better tools (Melfi 2009). Reptiles and amphibians would therefore benefit from further study in the zoo setting across a range of topics including behaviour, husbandry and reproduction, especially for endangered species (Molinia et al. 2010).

Reproduction is a particularly challenging area of husbandry, and reptiles present challenges that are not always seen in mammals. Broadly speaking, reptiles may be sexed based on invasive means (such as probing or popping), behaviour (such as egg laying), genetic analysis or physical attributes (such as crests) (Philips et al. 2016, Whiteley et al. 2007). However, reptile sexual dimorphism is often subtle, and signs such as femoral pores may differ in their size or prevalence even between individuals of the same sex (Warner et al. 2011). This increases the chances of misidentification of sex based on physical appearance, and encourages keepers to apply more invasive identification procedures or seek out expensive genetic tests (Reed & Tucker 2012). In cases where genetic testing is not available and there is insufficient experience in an animal collection for traditional sexing techniques, reptiles may be kept in social groups that are not biologically informed or that do not allow the zoo to achieve their breeding aims (Philips et al. 2016).

The Australian tree skink or tree-crevice skink *Egernia striolata* is an interesting species for the study of sociality (Riley et al. 2021). *E. striolata* shows both intra- and inter-population variability in sociality and is therefore a good exemplar species for informing models of social evolution (Riley et al. 2021). Many studies that focus on sociality and ecology of *E. striolata* include in their methodology the identification of sex in the individuals examined (Stewart 1989, Duckett et al. 2012, Riley et al. 2021). The most commonly used method in literature for identifying sex is the hemipene eversion or 'popping' technique to determine the presence or absence of a hemipene (Lancaster et al. 2011, Moore et al. 2022 and Riley et al. 2021). Everting the hemipene can have negative consequences for animal welfare. Reed & Tucker (2012), for example, state that 'eversion of the hemipene can be unnecessary for determining sex and may cause injury if done with excessive enthusiasm'. Vetere et al. (2022) seconds this and states that popping with excessive force can be 'traumatic'. Popping, in this case, carries risks in terms of hemipenal prolapse and bruising (Davis & Leavitt 2007). Complications can also arise when a negative result in 'popping' is diagnosed as a female, which can lead to a misdiagnosis of males as females (Divers 1999, Mader 2006). Ideally, the gold standard would involve a post-mortem dissection to identify sex, but this is not practical for living animals.

Other than 'popping' and dissection, the only other identified method of sex determination in *E. striolata* is based on sexually dimorphic traits (Wright 2014). Wright (2014) describes sexing *E. striolata* as more of an art than a science and suggests looking for more prominent jowls in mature males and a more rounded abdomen in females, particularly if they are gravid. However, these techniques are not foolproof, as jaw size may differ between individuals as a result of size, age and hormonal profile, so there is sometimes difficulty in identifying borderline cases. In a zoological setting, where sustainable populations are necessary for species survival, more reliable methods are needed.

Considering the potential issues with 'popping' and the vague descriptions described for sexual dimorphism, we avoided using invasive procedures for sex determination, which can be stressful for animals. As this species occurs in small social groups of mixed sex in the wild (Wright 2014), the animals were already kept together in a group. On 12 May 2022, a newly born skink was discovered in the enclosure alongside the adults. This led to the conclusion that in the group of adults, both sexes were present. A reliable and definitive way in which to sex the skinks was therefore necessary. The purpose of this study was therefore to identify the sexes of a group of Australian tree skinks, using non-invasive methods. The technique of hemipenal transillumination (or candling), as has been applied to eggs and other reptile species (Davis & Leavitt 2007, Brown 2009), was trialled.

## Material and methods

Research was undertaken at Sparsholt College's Animal Health & Welfare Research Centre during March 2022 (Fig. 1). The research was undertaken on a population of four Australian tree skinks of unknown sex (Tab. 1). The group were acquired from Paris Zoo on 24 July 2021. Sexing had not been previously attempted so at the time of the study, no sexes were known. Measurement of snout to vent length was carried out using callipers (to the nearest 5 mm).

While exact age was unknown, the four individuals were over two years old as shown by Paris Zoo records. Adults and juveniles can be identified from one another using parameters such as those by Riley et al. (2021) (>80mm SVL for mature adults) and by Duckett et al. (2012) (>90mm for mature adults). As per the categories of Riley et al. (2021), all four individual lizards should be considered mature adults. In adults, there should be sexually dimorphic traits visible, yet no clear sex distinction was apparent (Fig. 2).

In candling, a focused bright light emitting diode (LED) light was placed dorsally at the base of the lizard's tail. One author restrained each lizard while the other placed and took an image of the base of the tail ventrally illuminated as similar to Davis and Leavitt (2007). To identify sex, the observers were looking for two dark shadows either side of the tail base as indicators of hemipenal presence.

**Table 1.** Australian tree skink relative size and weight  
**Tabulka 1.** Velikost a hmotnost scinků *Egernia striolata*

ID	Snout to vent length (SVL) (mm)	Weight (g)
RES001	105	30.5
RES002	95	37.0
RES003	95	33.5
RES004	95	30.1



**Fig. 1.** Australian tree skink exhibit at Sparsholt College's Animal Health & Welfare Research Centre

**Obr. 1.** Expozice se scinky *Egernia striolata* v Animal Health & Welfare Research Centre v Sparsholt College

Foto/Photo Gary Miller

## Results

Evidence of hemipenes was shown for only one of the skinks (RES003) (Fig. 3). For this individual, two shadows are clearly evidenced either side of the tail base. This individual was identified as male. RES001, RES002 and RES004 showed no subcutaneous tissue with no shadowing evident. These individuals therefore were identified as females.

The use of candling showed great promise in that it quickly allowed the authors to identify individual animal sex with a minimal amount of handling and restraint per animal. No invasive popping or probing was required, thus further reducing the risk of injury to the animals involved. The output from the candling resulted in clear images in all adults.

It should be noted that the results of the candling were not compared against other strategies for sex determination, such as morphological measures, popping or genetic testing. Ideally, techniques should be compared against a 'gold standard' method of sex determination when they are first used. This method of candling has been used previously on a range of species including the desert night lizard (*Xantusia vigilis*), with 100% correct identification of sex in adults comparison to post-mortem dissection (Davis et al. 2007). Davis et al. (2007) report a slightly lower success rate for juveniles with a SVL of less than 25 mm, so this should be considered in studies in this area.

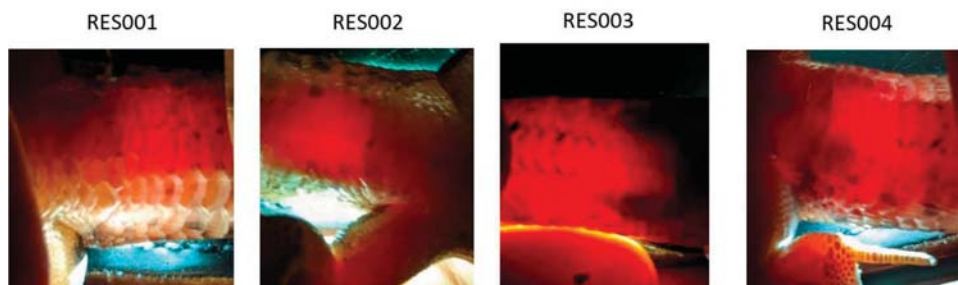


**Fig. 2.** Body size and shape of the four Australian tree skinks  
**Obr. 2.** Velikost a tvar těla čtyř scinků *Egernia striolata*

Foto/Photo Gary Miller

However, the images produced revealed very clear images of the hemipenes for RES003. In this species at least, the use of an incandescent light instead of an LED was not required as Brown (2009) reported, finding LED light potentially too strong for smaller individuals as it 'blasts straight through' the hemipene. For the remaining individuals, there was no evidence of shadows at the base of the tail. Additionally, there are no organs at the base of the tail for this species that are paired either side of the tail. This reduces the risk of a false positive assignment of male for RES003. It was known that at least one male and one female were present in the group on the premise that offspring had been produced.

It should be noted that the commonly-used technique of popping (Lancaster et al. 2011) for Australian tree skinks can result in a false negative test. This can occur in cases where the hemipene fails to evert following manipulation, and results in a male being classified as a female. There are considerable risks associated with misidentification using popping, particularly when trying to sex younger males (Janes et al. 2007, Wright 2014). Further study comparing these methods for juveniles would therefore have value.



**Fig. 3.** Images of the four skinks being candled through the base of the tail to identify the presence of hemipenes  
**Obr. 3.** Snímky čtyř scinků s prosvícenými kořeny ocasu za účelem zjištění přítomnosti hemipenisů

Foto/Photo Gary Miller

## Discussion

Given the ease with which candling can be carried out and the clarity of images produced, there is scope to extend the use of this methods to a much wider range of reptilian species housed in zoological collections. The method is not likely to be useful, however, for large-bodied or heavily scaled lizards, such as the blue-tongue skinks (*Tiliqua* spp.) for whom light is unlikely to successfully permeate through the tail base (McKenzie et al. 2022). However, the technique could have a much wider application across a range of thin-scaled, small-bodied lizards (Davis & Leavitt 2007). There is also potential for application in snakes, which also possess hemipenes.

It should be noted that the small sample size is a limitation of this study. The small sample size is an artefact of *E. striolata* availability: the study colony of five individuals were the only individuals in the United Kingdom at the time of study, with only 61 individuals housed in zoos globally, according to Species360 (2022). In future, however, studies should utilise a much wider number of animals covering both adult and juvenile stages, as previous studies have identified that juveniles are more challenging to identify (Davis & Leavitt 2007). Similarly, use of multiple methods of sex identification, such as additional use of popping, or post mortem examination, would be useful. These were not conducted in this current study from an ethical standpoint.

Future studies could also be used to determine at what stage the sex of a juvenile lizard can be identified. For many reptiles, juveniles pose a challenge for sexing as they often lack the secondary sexual characteristics (such as a widened jaw, femoral pore development or horns) that indicate sex (Warner 2011). Candling, as it illuminates the hemipenes, may allow animal managers to identify reptile sex sooner than other measures. Currently, there is one juvenile housed at the Sparsholt collection. The candling method is due to be trialled for this individual and any others that are successfully bred going forward.

## Conclusion

Overall, candling showed great promise as a tool to identify the sex of Australian tree skinks. The method was much less invasive than the alternative of popping, and less expensive than genetic testing. As such, candling may have potential as a tool for other, related reptile species. While only a small sample size was available for the purpose of this study, this work suggests that candling may have application across a wider range of reptilian species. Where possible, future studies should compare the efficacy of this method against other, existing techniques such as popping, genetic testing and assessment of sex based on reproduction.

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Attempt to determine sex in sexually non-dimorphic group of Australian tree skink  
Pokus o určení pohlaví ve skupině pohlavně nedimorfního scinka *Egernia striolata*

Photo/Foto Gary Miller