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Determining the generation length and level of morphological and genetic differentiation in the Data Deficient glossy grass skink (*Pseudemoia rawlinsoni*)

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ABSTRACT

Context. Human activities are having a significant impact on biodiversity worldwide, to the extent that we are in the midst of the sixth mass extinction event. Although a substantial proportion of species globally have an elevated risk of extinction, some species are poorly known and there is insufficient information available to adequately assess their risk of extinction. **Aims and methods.** One such species is the glossy grass skink (*Pseudemoia rawlinsoni*), a widespread but enigmatic lizard species in south-eastern Australia. In order to improve our knowledge of its life history, and particularly its generation length, we examined museum specimens collected from across the range of the species, supplemented with measurements from field-caught individuals. **Key results.** We estimated that the species reaches sexual maturity in 3 years, at approximately 40 mm snout–vent length. Its generation length was estimated as 5 years. Sexual dimorphism was evident, and female body size was positively related to litter size. Although there was no evidence for substantial variation in morphology across the range of the glossy grass skink, a phylogeographic analysis using mitochondrial DNA sequence data (ND4) revealed the presence of seven genetic sublineages (up to 5.1% genetic divergence) within the species. **Conclusions.** The glossy grass skink appears to be a single, but widespread and genetically variable, species. **Implications.** Our study demonstrates how a targeted, multifaceted study can be effective at rapidly gathering data that can be used to contribute vital information to the assessment of extinction risk in Data Deficient species.

Keywords: Australia, extinction risk, IUCN Red List, life history, lizard, phylogeography, reproductive ecology, Scincidae.

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Introduction

Biodiversity is currently under increasing threat from human-mediated impacts such as habitat loss and fragmentation, invasive species, overexploitation and climate change (Doherty *et al.* 2016; Pecl *et al.* 2017; IPBES 2019). The cumulative impacts of these processes have ushered in a sixth mass extinction event, which is predicted to intensify in the future (Barnosky *et al.* 2011; Ceballos *et al.* 2015, 2020). Predicting the impact of these threats on a species requires detailed information about the species biology, ecology, habitat requirements and likely resilience to each threat (Pimm *et al.* 2014; Johnson *et al.* 2017). Under the International Union for Conservation of Nature (IUCN) Red List of Threatened Species, the extinction risk of species is estimated using a range of parameters, including generation length, population size, geographic range size, number of populations/subpopulations, and population connectivity/fragmentation (Mace *et al.* 2008; IUCN Standards and Petitions Committee 2022). However, where there is insufficient knowledge of these key parameters, it may not be possible to conduct an assessment of the conservation status of the species (IUCN Standards and Petitions Committee 2022).

Species may be classified as Data Deficient due to a lack of knowledge regarding their distribution, biology and ecology (e.g. life history, generation length, habitat requirements), threats to the species, and/or taxonomic status (Bland and Böhm 2016; IUCN Standards and Petitions Committee 2022; Wotherspoon *et al.* 2024). Without this critical knowledge, it is

difficult to determine the threats that the species faces, assess their capacity to withstand and recover from any potential threats, and/or evaluate whether it represents a valid taxonomic entity. Although Data Deficient species are often overlooked by, or are a low priority for, conservation managers (Bland *et al.* 2017), recent studies have highlighted that they are more likely to be threatened than already assessed species (Gumbs *et al.* 2020; Caetano *et al.* 2022; Graham *et al.* 2023). Thus, targeted studies are required to obtain the information necessary to resolve the knowledge gaps, allowing a conservation assessment to be conducted for the species.

The glossy grass skink (*Pseudemoia rawlinsoni*) is an example of a Data Deficient lizard species that has a widespread, but disjunct, distribution in south-eastern Australia (Fig. 1), where the majority of the Australian human population resides (Gillespie *et al.* 2018; Chapple *et al.* 2019; Wilson and Swan 2021). Described in 1988 (Hutchinson and Donnellan 1988), it is an enigmatic species that inhabits moist, densely vegetated wetland habitats (Gillespie *et al.* 2018; Wilson and Swan 2021). State-based conservation listings in the core of the species' range (Victoria: Endangered, South Australia: Vulnerable, Tasmania: Rare) hint at a potential elevated extinction risk for the species (Chapple *et al.* 2019). However, the glossy grass skink is currently listed as Data Deficient on the IUCN Red List (Gillespie *et al.* 2018). This is because, prior to the recent study by Farquhar *et al.* (2023), across the broader range of the species there was uncertainty regarding its distribution (Area of Occupancy), and before it was confirmed by Farquhar *et al.* (2024), it was unknown whether the species was under pressure from infrastructure development and agriculture. However, the species' life history and generation length, number of populations/subpopulations, and its sensitivity to the impacts of invasive species are still largely unknown (Gillespie *et al.* 2018).

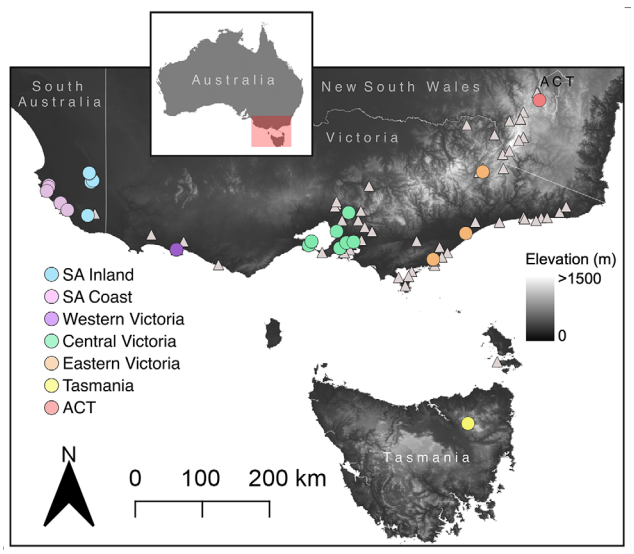

Fig. 1. Locations of glossy grass skink (*Pseudemoia rawlinsoni*) specimens. Coloured circles are locations with both morphological and genetic data, with colours corresponding to genetic lineages. White triangles are locations with only morphology data.

Here we complete a targeted, multifaceted study to rapidly obtain key aspects of the information needed to conduct an IUCN Red List assessment for the glossy grass skink. Using a combination of museum and field-caught specimens, we investigate the life history and reproductive ecology of the species. Specifically, we aim to estimate the generation length of the gloss grass skink, as it is a core component of most IUCN Red List criteria, and a critical determinant of the capacity of a species to respond to, and recover from, threatening processes (Mace *et al.* 2008; IUCN Standards and Petitions Committee 2022). Specifically, generation length is required to determine the timeframe over which a reduction in population size should be assessed (Criterion A; 3 generations or 10 years), decline in the number of mature individuals (Criterion C1; 1, 2, or 3 generations for Critically Endangered, Endangered, and Vulnerable, respectively), and the timeframe for quantitative analyses of extinction probability (Criterion E; 3 or 5 generations for Critically Endangered and Endangered, respectively) (IUCN Standards and Petitions Committee 2022). In addition, we use mitochondrial DNA sequence data (ND4) to conduct a phylogeographic study of the gloss grass skink. This molecular study will provide some preliminary, broad scale information on the level of genetic structuring among populations across the range of the species, and assist with the determination of the number of subpopulations in the species.

Methods

Morphology, life history and reproductive ecology of the glossy grass skink

To quantify the external morphology of the glossy grass skink, we obtained data from both preserved museum specimens and field caught specimens. Firstly, we examined all preserved glossy grass skink specimens from Museums Victoria (n = 88) and the South Australian Museum (n = 18) (Fig. 1). Digital callipers (Mitutoyo 500–763–20 8″/200 mm Coolant Proof Digimatic Calipers; West Heidelberg, Australia) were used to obtain the morphological measurements (in mm) of snout–vent length (SVL), snout-axilla length (SAL), axilla–groin length (AGL), interlimb length (ILL), tail length (TL), tail width (TW), body width (BW), body height (BH), pelvic width (PW), pelvic height (PH), total front limb length (TFL), upper front limb (UFL), lower front limb (LFL), front foot (FF), total hind limb length (THL), upper hind limb (UHL), lower hind limb (LHL), hind foot (HF), head width (HW), head length (HL), head depth (HD), snout length (SL) and eye diameter (ED) (see Supplementary Table S1 for definitions of each trait; Greer 1982; Barter *et al.* 2022). In addition, we conducted scale counts (as outlined in

Taylor 1935) of midbody scale rows, paravertebral scales, subdigital lamellae, nuchals, presuboculars, supraciliaries, supralabials and infralabials (Supplementary Fig. S1).

To examine sexual maturity, and litter size in females, in the preserved specimens, we followed the methods of Barter *et al.* (2022). Briefly, we made a small ventral incision along the left side of the specimen to determine the sex and sexual maturity of each individual. If an existing incision was already present, that was used instead. We considered male specimens to be sexually mature if they had fully developed testes and the ductus deferens appeared to be rough (rather than smooth, as found in juveniles) (Barter *et al.* 2022). Females were assessed for sexual maturity based on the presence of a folded oviduct extending cranially from the ovary (Barter *et al.* 2022). In juvenile females this portion of the oviduct is smooth and not folded (Barter *et al.* 2022). For specimens that appeared to be gravid upon external examination, the incision was made down the midline to count the number of eggs. The reproductive condition (gravid or non-gravid) was recorded for each sexually mature female, along with the litter size. Only adult specimens were used to calculate trait means.

Secondly, we measured field-caught glossy grass skinks collected at six sites across Victoria, Australia, as part of a related study (Farquhar *et al.* 2024). The fieldwork was conducted between October 2021 and April 2022, and COVID-19 related border closures and travel restrictions prevented us sampling populations from outside of Victoria. We measured a total of 70 specimens across these sites, shown here with latitude and longitude: Port Fairy (−38.346409, 142.242085; eight specimens), Queenscliff (−38.268226, 144.628523; 28 specimens), Swan Bay (−38.201634, 144.676130; 20 specimens), Yering (−37.681576, 145.349988; four specimens), Tooradin (−38.211158, 145.427389; one specimen) and Warringine Park (−38.325837, 145.191561; nine specimens) (Fig. 1). Snout–vent length and TL were measured using a ruler (in mm), and digital callipers (Mitutoyo 500–763–20 8″/200 mm Coolant Proof Digimatic Calipers) were used to measure (in mm) HL, HW and HD. Sex was determined by hemipenal eversion in males, or lack thereof in females. We took high-resolution photographs (Canon 5D Mark IV digital camera with a Canon 100 mm f/2.8 L macro lens) of each individual and used a combination of unique external features (scars, missing digits, aberrations in pattern, proportion of tail that was regenerated) and sex to track the identity of each individual over time, and to ensure that we did not measure the same individual twice.

All data analyses were conducted in R (Ver. 4.0.4; R Core Team 2021). To create a summary of all glossy grass skink traits, means, standard errors and ranges were calculated for each morphological measurement. To determine the size at maturity, we recorded the smallest sexually mature individual of each sex. To estimate the age at maturity we created a histogram of SVL of museum and field specimens. Fung and Waples (2017) recommended that, when estimating generation length for reptiles, the information required to develop detailed life history tables is best achieved using the following formula: generation length = age at first reproduction + $z \times$ (maximum age − age at first reproduction), where z represents a constant dependent on survivorship and relative fecundity of individuals in the population (Fung and Waples 2017). We have established a generalised z value of 0.5 due to increased fecundity with size in lizards (Olsson and Shine 1996; Du *et al.* 2005; Meiri *et al.* 2020). Although lizards grow rapidly and indeterminately, their growth rate slows following maturity (Olsson and Shine 1996; Laver *et al.* 2012). However, such increases in fecundity with age are often offset by gradual senescence (Patnaik 1994) and increased mortality with age. Thus, we consider this approach to be appropriate for estimating the generation length of the glossy grass skink.

All body measurements were adjusted to SVL using an allometric growth model (package GroupStruct; Chan and Grismer 2022). We used a Wilcoxon rank sum test to examine sexual dimorphism for each adjusted morphological measurement. Field and preserved specimen measurements were combined in these analyses because the amount of shrinkage endured by preserved specimens will not significantly impact body measurements (Vervust *et al.* 2009; Maayan *et al.* 2022). Additionally, we compared adjusted morphological measurements amongst the populations determined by the genetic groupings using a Kruskal–Wallis rank sum test. To investigate significant differences, we conducted a Dunn test (package dunn.test) with a Bonferroni adjustment. Finally, a linear regression analysis was conducted to determine the relationship between SVL and litter size of preserved specimens.

Assessing the presence of genetic substructuring across the range of the glossy grass skink

We obtained 46 tissue samples of the glossy grass skink across its range in south-eastern Australia (South Australian Museum, $n = 17$; wild caught samples from the 2021/2022 field season, $n = 29$; Table 1, Fig. 1). Genomic DNA was extracted from glossy grass skink liver or tail-tip tissue using a Qiagen DNeasy Blood and Tissue Extraction Kit (Qiagen, Hilden, Germany), following the manufacturer's protocol. For all samples, we amplified the mitochondrial DNA (mtDNA) fragment NADH subunit 4 (ND4; ~700 bp) using the primers ND4 and tRNA-leu, and the PCR protocols as outlined in Haines *et al.* (2014). We used the ND4 mtDNA gene provides similar phylogenetic resolution to other mtDNA coding regions (e.g. Chapple *et al.* 2009), and it was the mtDNA gene that has been most widely used for *Pseudemoia*, allowing us to place our glossy grass skink sequences into context with the broader genus (Haines *et al.* 2014). Additionally, Haines *et al.* (2014), using ND4 and five nuclear genes, provided evidence that the glossy grass skink is a distinct species, with no evidence of hybridisation or introgression with other *Pseudemoia* species. Consequently, we focused on a single

Table 1. Details of the glossy grass skink (*Pseudemoia rawlinsoni*) samples and outgroup sequences used in this study.

Species	Region	Locality	Tissue ID	Specimen ID	GenBank accession
Pseudemoia rawlinsoni	Australian Capital Territory	Mt Bimberi	ABTC40954	SAMR23098	OR469914
	Eastern Victoria	Woodside	ABTC04064	–	KM263322
		Hollands Landing	NMVZ73965	–	OR469929
		Benambra	NMVZ73970	–	OR469928
	Central Victoria	Warringine Park	NMVZ73937	–	OR469930
		Spadonis Nature Reserve	NMVZ73941	–	OR469931
		Warringine Park	NMVZ73944	–	OR469932
		Swan Bay	NMVZ73945	–	OR469933
		Spadonis Nature Reserve	NMVZ73946	–	OR469934
		Spadonis Nature Reserve	NMVZ73949	–	OR469935
		Warringine Park	NMVZ73953	–	OR469936
		Swan Bay	NMVZ73955	–	OR469915
		Swan Bay	NMVZ73956	–	OR469937
		Swan Bay	NMVZ73957	–	OR469941
		Warringine Park	NMVZ73958	–	OR469944
		Waterways	NMVZ73971	–	OR469918
		Warringine Park	NMVZ73973	–	OR469943
		Warneet	NMVZ73974	–	OR469916
		Warneet	NMVZ73975	–	OR469945
		Queenscliff	NMVZ73976	–	OR469946
		Queenscliff	NMVZ73977	–	OR469938
		Queenscliff	NMVZ73978	–	OR469947
		Warringine Park	NMVZ73979	–	OR469948
		Warringine Park	NMVZ73980	–	OR469939
		Tooradin Inlet	NMVZ73981	–	OR469917
		Queenscliff	NMVZ73982	–	OR469942
		Queenscliff	NMVZ73983	–	OR469940
	Western Victoria	Port Fairy	NMVZ73954	–	OR469949
		Port Fairy	NMVZ73963	–	OR469919
		Port Fairy	NMVZ73966	–	OR469950
		Port Fairy	NMVZ73969	–	OR469927
	Tasmania	4.2 km SE of Burns Creek, North Esk River	ABTC57877	SAMR44322	KM263321
	Inland South Australia	Lake Ormerod	ABTC156252	SAMR59775	OR469911
		Lake Ormerod	ABTC156253	SAMR59776	OR469913
		Lake Ormerod	ABTC68946	SAMR53512	OR469912
		5.4 km SSE of Glencoe	ABTC37606	SAMR49405	OR469908
		Bool Lagoon Game Reserve	ABTC54784	SAMR23098	OR469909
		4.1 km N of Bool Lagoon	ABTC68787	SAMR52584	OR469910
		7 km ESE of Kangaroo Hill	ABTC94787	SAMR53753	OR469923
	Coastal South Australia	Silky Tea Tree Swamp	ABTC106240	SAMR57100	OR469924
		20 WNW of Millicent Airport	ABTC37531	SAMR49585	OR469925
		20 WNW of Millicent Airport	ABTC37542	SAMR49589	OR469926
		20 WNW of Millicent Airport	ABTC37543	SAMR49590	OR469922

(*Continued on next page*)

Table 1. (*Continued*).

Species	Region	Locality	Tissue ID	Specimen ID	GenBank accession
		20 WNW of Millicent Airport	ABTC37727	SAMR49594	OR469920
		9 km SW of Millicent	ABTC70044	SAMR53777	KM263323
		9 km SSE of Kangaroo Hill	ABTC94794	SAMR53758	OR469921
Outgroups					
Carinascincus metallicus	NA	Wilsons Promontory, Refuge Cove, Victoria	NMVZ21551	NMVD75207	KM263269
Pseudemoia baudini	NA	Wanna, South Australia	ABTC15402	NMVD60966	KM263201
Pseudemoia cryodroma	NA	Mt Baw Baw, Victoria	ABTC14601	NMVD59893	KM263204
Pseudemoia entrecasteauxii	NA	Mt Kosciusko, New South Wales	ABTC11167	NMVD59933	KM263231
Pseudemoia pagenstecheri	NA	Lake Corangamite, Victoria	NMVZ23650	–	KM263319
Pseudemoia spenceri	NA	Mt Baw Baw, Victoria	NMVZ19287	NMVD74853	KM263324

Specimen codes: SAMR, South Australian Museum; NMVD, Museums Victoria, Melbourne. Tissue ID collection codes: ABTC, South Australian Museum Australian Biological Tissue Collection; NMVZ, Museums Victoria Frozen Tissue Collection.

mtDNA gene in the present study to document the level of intraspecific genetic divergence among populations/regions in the glossy grass skink.

Purifications and sequencing were performed by Macrogen, Inc. (Seoul, South Korea). The sequences were aligned and edited in BioEdit v7.2.5 (Hall 1999). We checked for stop codons using MUSCLE within Geneious v.10.2.6 (Biomatters, Auckland, New Zealand) and none were observed. Three glossy grass skink samples were already sequenced using the same protocol (GenBank numbers: KM263323, KM263322, KM263321; Haines *et al.* 2014), and were used in our study. The ND4 sequences obtained in our study were deposited in GenBank (Table 1). Outgroup sequences for the metallic skink (*Carinascincus metallicus*) and other members of the *Pseudemoia* genus were obtained from Haines *et al.* (2014) and GenBank (Table 1).

We used jModelTest 2.1.10 (Darriba *et al.* 2012) to identify the most appropriate model of sequence evolution based on the Akaike Information Criterion (AIC). We conducted Maximum Likelihood analysis using a RAxML GTR + I + G model with 100 bootstraps (Stamatakis 2014), and a Bayesian analysis using MrBayes 3.2 (Huelsenbeck and Ronquist 2001), in Geneious v.10.2.6 (Biomatters, Auckland, New Zealand) to determine the genetic structuring of glossy grass skink populations across south-eastern Australia. A genetic distancing estimation was calculated using MEGA v.11.0.13 (Tamura *et al.* 2004, 2021) to determine the approximate percentage of genetic variation and time of separation amongst the populations.

Ethical standards

All research was conducted under permission of the Department of Environment, Land, Water and Planning (10010129) and was approved by the Monash University School of Biological Sciences Animal Ethics Committee (30059).

Results

Morphology, life history and reproductive ecology of the glossy grass skink

Our visual examination of the gonads of museum specimens indicated that the smallest sexually mature individual was approximately 40 mm (females: 39.9 mm, males: 38.3 mm [with an outlier individual mature at 35.7 mm]; Fig. 2*a*).

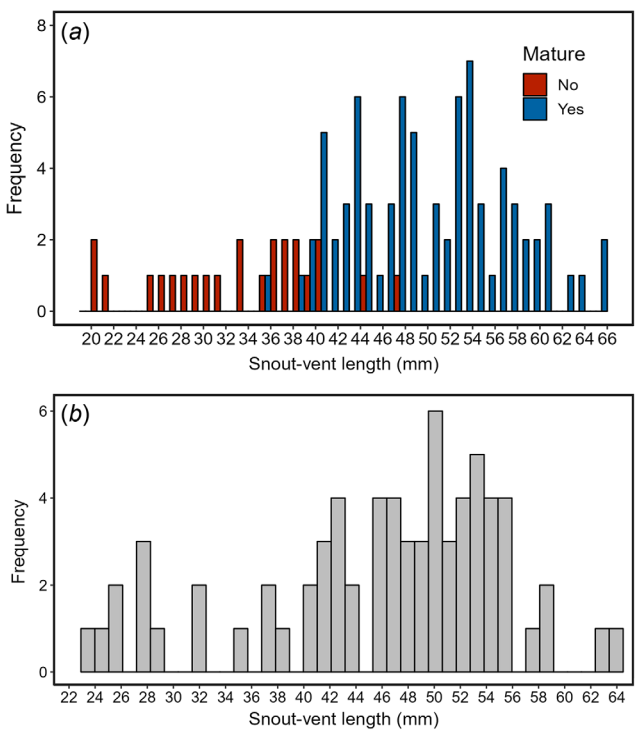

Fig. 2. A histogram of the (*a*) snout–vent length (mm) of all mature (blue) and immature (red) glossy grass skink preserved specimens (n = 103) examined in this study, and (*b*) snout–vent length (mm) of all field caught glossy grass skinks (n = 70) in Victoria, Australia.

Using this estimate for the size at maturity, inspection of the SVL of field caught glossy grass skinks in Victoria suggests that individuals may reach between 23 and 30 mm SVL during their first season, 32 and 39 mm SVL during their second season, and sexual maturity (>40 mm SVL) during their third season (Fig. 2*b*). Assuming an average lifespan of ~7 years for similar-sized skinks in south-eastern Australia (Greer 2022), we used the generation length formula developed by Fung and Waples (2017) to determine the generation length of the glossy grass skink as approximately 5 years ($3 + 0.5 \times (7 - 3) = 5$).

Sexual dimorphism is evident in the glossy grass skink, with males having larger heads (head length, head width, snout length, eye diameter), longer axilla–groin lengths, and longer legs (total front limb length, upper front limb length, lower front limb length, front foot, total hind limb length, upper hind limb length, lower hind limb length, hind foot; Table 2). In contrast, females have larger bodies (paravertebral scale count, interlimb length, body width, body height), longer snout–axilla lengths, and greater tail widths (Table 2). All other body measurements and scale counts were not sexually dimorphic in the glossy grass skink (Table 2).

Table 2. Summary of body measurements (in mm) and scale counts taken for adult preserved and field *Pseudemoia rawlinsoni* specimens.

	Female			Male			Overall			Sexual Dimorphism
Trait	***N***	**Mean ± s.e.**	**Range**	***N***	**Mean ± s.e.**	**Range**	***N***	**Mean ± s.e.**	**Range**	
Snout–vent length	78	51.0 ± 0.75	40.0–65.2	56	49.3 ± 0.68	40.7–60.1	136	50.1 ± 0.53	40–65.2	0.129
Snout–axilla length	42	28.0 ± 0.88	17.2–39.5	36	25.1 ± 0.82	16.8–32.9	80	26.5 ± 0.61	16.8–39.5	**<0.001**
Axilla–groin distance	42	18.9 ± 0.98	13.8–38.3	36	20.7 ± 0.86	15.6–34.3	80	19.7 ± 0.65	13.8–38.3	**<0.001**
Interlimb length	42	31.5 ± 0.83	22.9–42.0	36	28.1 ± 0.68	17.7–35.2	80	29.8 ± 0.58	17.7–42.0	**<0.001**
Tail length	26	79.7 ± 2.01	66.4–105.0	21	79.3 ± 3.08	43.0–104.0	47	79.5 ± 1.75	43.0–105	0.149
Tail width	42	5.1 ± 0.11	3.8–6.6	36	4.7 ± 0.10	3.2–5.8	80	4.8 ± 0.08	3.2–6.6	**0.018**
Body width	42	8.7 ± 0.24	5.9–12.1	36	7.4 ± 0.19	5.5–9.5	80	8.1 ± 0.17	5.4–12.1	**<0.001**
Body height	42	6.1 ± 0.21	3.6–8.6	36	4.9 ± 0.14	3.3–6.4	80	5.2 ± 0.14	3.1–8.6	**0.001**
Pelvic width	42	4.4 ± 0.12	3.1–6.2	36	4.5 ± 0.11	2.8–5.5	80	4.4 ± 0.08	2.8–6.2	0.069
Pelvic height	42	4.8 ± 0.11	3.2–6.2	36	4.8 ± 0.12	3.5–6.0	80	4.8 ± 0.08	3.2–6.2	0.404
Total front limb length	42	11.5 ± 0.20	9.3–15.9	36	12.4 ± 0.21	9.2–14.6	80	11.9 ± 0.15	9.2–15.9	**<0.001**
Upper front limb	42	4.1 ± 0.08	3.3–5.4	36	4.3 ± 0.09	2.7–5.4	80	4.2 ± 0.06	2.7–5.4	**0.001**
Lower front limb	42	3.9 ± 0.08	3.2–5.3	36	4.0 ± 0.08	3.1–5.1	80	4.0 ± 0.05	3.1–5.3	**0.005**
Front foot	42	5.0 ± 0.12	3.3–7.4	36	5.5 ± 0.12	4.2–7.0	80	5.2 ± 0.09	3.3–7.4	**<0.001**
Total hind limb length	42	17.5 ± 0.34	13.8–22.3	36	18.7 ± 0.31	15.3–23.1	80	18.0 ± 0.24	13.8–23.1	**<0.001**
Upper hind limb	42	5.9 ± 0.14	4.5–7.9	36	6.2 ± 0.13	5.1–8.2	80	6.0 ± 0.09	4.5–8.2	**<0.001**
Lower hind limb	42	5.3 ± 0.11	4.1–7.1	36	5.7 ± 0.11	4.2–6.8	80	5.5 ± 0.08	4.1–7.1	**<0.001**
Hind foot	42	8.8 ± 0.19	6.4–12.0	36	9.6 ± 0.17	7.6–11.9	80	9.1 ± 0.13	6.4–12.0	**<0.001**
Head width	78	6.1 ± 0.07	5.2–8.3	56	6.4 ± 0.07	5.4–7.7	136	6.2 ± 0.05	5.2–8.3	**<0.001**
Head length	78	8.6 ± 0.10	5.0–10.9	56	9.4 ± 0.11	7.6–11.3	136	8.9 ± 0.08	5.0–11.3	**<0.001**
Head depth	78	4.6 ± 0.07	3.4–6.3	56	4.6 ± 0.07	3.4–5.6	136	4.6 ± 0.05	3.4–6.3	0.129
Snout length	42	5.4 ± 0.08	4.5–6.9	36	5.8 ± 0.08	5.0–6.9	80	5.6 ± 0.06	4.5–6.9	**<0.001**
Eye diameter	42	1.1 ± 0.02	0.9–1.3	36	1.1 ± 0.02	0.9–1.3	80	1.1 ± 0.01	0.9–1.3	**0.007**
Midbody scales	42	26.8 ± 0.16	26–30	36	26.7 ± 0.18	25–30	80	26.8 ± 0.12	25–30	0.236
Paravertebral scales	42	58.1 ± 0.30	54–62	36	56.4 ± 0.33	53–59	80	57.3 ± 0.24	53–62	**<0.001**
Subdigital lamellae	42	19.2 ± 0.21	17–23	36	20.1 ± 0.24	17–23	80	19.6 ± 0.16	17–23	0.166
Nuchals	42	4.6 ± 0.22	2–8	36	4.3 ± 0.24	2–8	80	4.5 ± 0.16	2–8	0.329
Presubocular	42	2.2 ± 0.06	1–3	36	2.2 ± 0.08	1–3	80	2.2 ± 0.05	1–3	0.996
Supraciliaries	42	6.8 ± 0.07	6–8	36	6.8 ± 0.08	6–8	80	6.8 ± 0.05	6–8	0.433
Supralabials	42	7.0 ± 0.02	7–8	36	7.1 ± 0.04	7–8	80	7.1 ± 0.03	7–8	0.162
Infralabials	42	7.0 ± 0.04	6–8	36	7.1 ± 0.05	6–8	80	7.1 ± 0.03	6–8	0.203

The sexual dimorphism column presents the results of the size-corrected Wilcoxon rank sum exact tests for sexual dimorphism. The sex of two museum specimens was unknown. Significant results are presented in bold.

The mean litter size of glossy grass skink museum specimens was 4.65 ± 0.43 (range 1–9, n = 20). There was a positive association between female SVL and litter size (estimate = 2.58 ± 0.51, t-value = 5.10, $P < 0.001$; Fig. S2).

Assessing the presence of genetic substructuring across the range of the glossy grass skink

Our phylogenetic analysis supports the glossy grass skink being a single species, but with seven distinct genetic lineages across south-eastern Australia: Australian Capital Territory, Eastern Victoria, Tasmania, Central Victoria, Western Victoria, inland South Australia, and coastal South Australia (Fig. 3). The degree of genetic distance amongst lineages varied between 0.92% and 5.13% (Table 3). The seven genetic lineages of the glossy grass skink did not differ in a range of body measurements (SVL, ILL, PW, PH, LFL, THL, UHL, LHL, SL, ED) or scale counts (midbody scales, subdigital lamellae) (Tables S2 and S3). Although significant differences were found among the lineages for a range of body measurements (SAL, AGL, HL, HW, HL, TW, BW, BH, TFL, UFL, FF, HF) and scale counts (nuchals, paravertebral, presuboculars, supraciliaries, supralabials, infralabials), no consistent differences were observed (Tables S2–S22).

Discussion

Our results indicate that the glossy grass skink reaches sexual maturity in approximately 3 years, at a body size of around 40 mm SVL, and has a generation length of 5 years. Our estimate for the size at sexual maturity is consistent with that suggested by Hutchinson and Donnellan (1988) when the species was formally described. Indeed, the life history traits for the glossy grass skink are similar to that reported for other similar-sized viviparous skink species in south-eastern Australia (Hutchinson and Donnellan 1992; Chapple 2006; Atkins *et al.* 2020; Greer 2022). Importantly, our study represents the first detailed determination of the generation length of a *Pseudemoia* species (Greer 2022). Unfortunately, due to a limited number of specimens from alpine regions, we were unable to examine whether the life history traits of the glossy grass skink differed between the lowland (majority of the species range) and highland (alpine regions of Victoria, New South Wales, and the Australian Capital Territory) populations of the species. However, this possibility warrants further investigation as previous studies of viviparous skinks species in south-eastern Australia have documented substantial differences in life-history between high and low elevation populations (Rohr 1997; Wapstra *et al.* 2001; Atkins *et al.* 2020; Van Dyke *et al.* 2021).

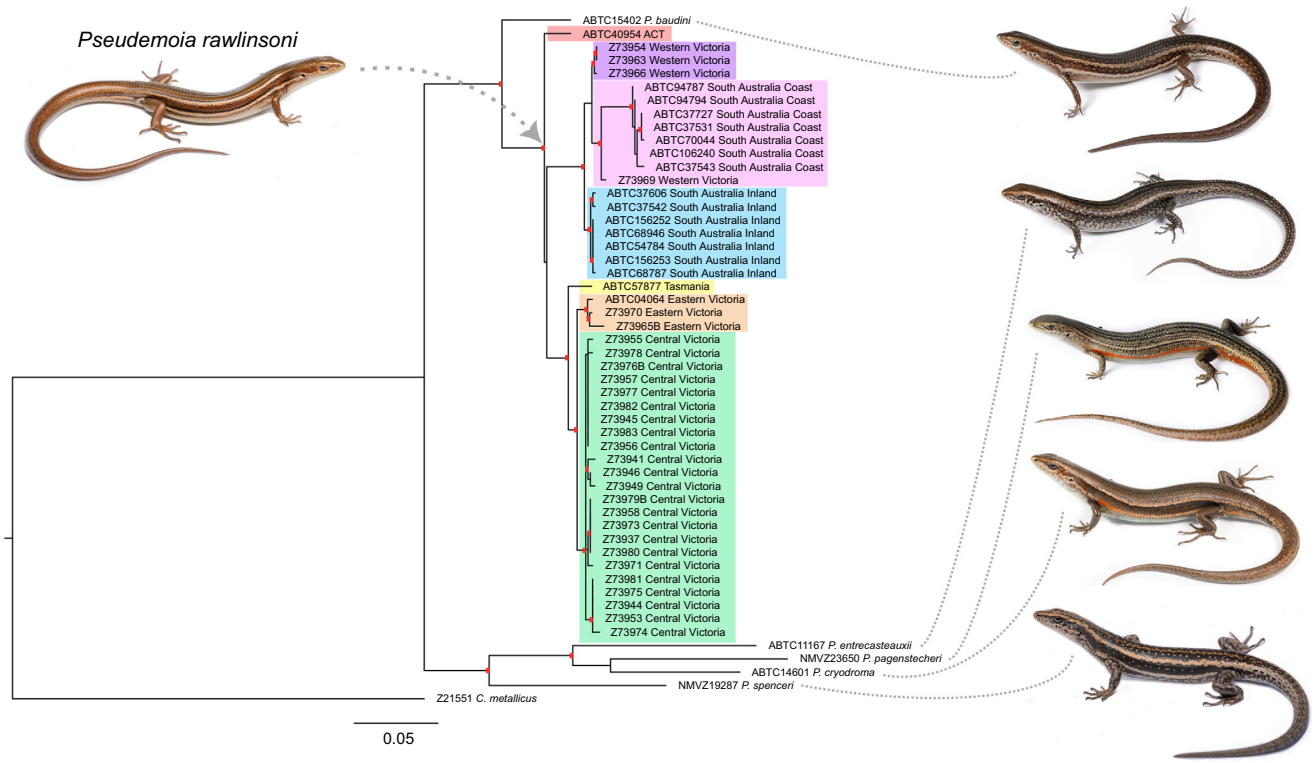

Fig. 3. Maximum likelihood phylogeny for the glossy grass skink (*Pseudemoia rawlinsoni*) based on mitochondrial DNA (794 bp ND4). The red rectangles indicate the branches that are well supported (i.e. posterior probability values over 95, and/or bootstrap values over 70; the precise values are provided in Fig. S3). Seven genetic lineages were determined: SA Inland (blue), SA Coast (pink), Western Victoria (purple), Central Victoria (green), Eastern Victoria (orange), Tasmania (yellow) and Australian Captial Territory (red). Photos by Jules Farquhar.

Table 3. Genetic distances (below diagonal) and standard error (above diagonal) amongst glossy grass skink (*Pseudemoia rawlinsoni*) genetic lineages.

	ACT	EVIC	CVIC	WVIC	Tas.	SAInland	SACoast
ACT	–	0.006	0.006	0.006	0.007	0.006	0.007
EVIC	0.031	–	0.003	0.008	0.005	0.007	0.008
CVIC	0.031	0.011	–	0.007	0.005	0.007	0.008
WVIC	0.037	0.042	0.041	–	0.008	0.004	0.005
Tas.	0.035	0.020	0.022	0.045	–	0.008	0.008
SAInland	0.037	0.039	0.038	0.009	0.043	–	0.006
SACoast	0.046	0.051	0.051	0.021	0.051	0.026	–

Locations are Australian Capital Territory (ACT), Eastern Victoria (EVIC), Central Victoria (CVIC), Western Victoria (WVIC), Tasmania (Tas.), inland South Australia (SAInland), coastal South Australia (SACoast).

We found that sexual dimorphism was evident in the glossy grass skink, with females having larger bodies and interlimb lengths, and males exhibiting larger heads and longer legs. Within squamates, it has been suggested that there is a strong selective pressure for females to have larger body size, and specifically longer interlimb lengths, in order to accommodate more developing eggs and/or young (Cox *et al.* 2003; Scharf and Meiri 2013; Meiri *et al.* 2020). Indeed, this pattern appears to hold in the glossy grass skink as litter size was positively correlated to female body size. Although the mean litter size for the glossy grass skink found in our study was slightly lower (4.6 vs 5.6) than that reported previously for the species by Hutchinson and Donnellan (1988), we found the species to have a broader range of litter sizes than previously reported (1–9 vs 4–9). The larger head size of male glossy grass skinks is concordant with previous reports in other squamates (Cox *et al.* 2003; Scharf and Meiri 2013), including viviparous skinks in south-eastern Australia (Clemann *et al.* 2004; Chapple 2006). Previous hypotheses for the selective advantage of males having larger heads have focused on the ability to grip females during copulation, or the importance of head size in determining the outcome of male–male competition and fights (Vitt and Cooper 1985; Cox *et al.* 2003; Scharf and Meiri 2013). Although it has previously been reported that male glossy grass skinks have longer limbs than females (Hutchinson and Donnellan 1992), the significance of this morphological dimorphism is unknown and warrants further investigation.

Our study provides information that will assist in assessing the glossy grass skink against IUCN Red List criteria. As species respond to changes in their environment at different rates, an estimate of generation length is an important component of most Red List criteria (Mace *et al.* 2008; Bird *et al.* 2020). Generation length, which incorporates information on the age at sexual maturity and lifespan, is important when considering population trends, and determining the capacity of the species to withstand threats or recover from population declines (Isaac *et al.* 2005; Rowe 2008; Bird *et al.* 2020). In addition, knowledge of litter size can provide information on the potential population growth of the species, whilst taking into account the potential for lower fecundity in younger (i.e. smaller) adults (Bird *et al.* 2020; Meiri *et al.* 2020). In regard to the glossy grass skink, it has a longer generation time than many smaller size skinks in south-eastern Australia (Greer 2022), and therefore might take longer to recover from population declines, making it more susceptible to a range of threats (e.g. Senior *et al.* 2021). However, its generation length, and thus intrinsic susceptibility to threats, may be consistent with most other similar-sized viviparous skinks in south-eastern Australia (Wapstra *et al.* 2001; Chapple 2006; Atkins *et al.* 2020).

Our study, combined with the results from Haines *et al.* (2014), indicates that the glossy grass skink is a single widespread, but genetically variable, species. This is because the level of genetic divergence observed in the species was less than, or equivalent to, the level of intraspecific divergence reported for a range of other Australian skink species (Chapple *et al.* 2004, 2005, 2011*a*, 2011*b*; Senior *et al.* 2022). Although some degree of morphological variation was evident within the glossy grass skink, there were no consistent morphological differences among the seven genetic lineages in the species. Interestingly, the location of the seven genetic lineages within the glossy grass skink were largely concordant with the recognised biogeographic boundaries in south-eastern Australia (Chapple *et al.* 2005, 2011*a*, 2011*b*; Bryant and Krosch 2016; Dissanayake *et al.* 2022). Assuming 2% sequence divergence per million years, as used in other Australian skinks (Chapple *et al.* 2005; Haines *et al.* 2014), this would indicate a divergence time of between 0.5 million years ago and 2.6 million years, ago during the Pliocene–Pleistocene boundary (Chapple *et al.* 2005; Dissanayake *et al.* 2022; Senior *et al.* 2022). This period was characterised by climatic fluctuations, and associated fluctuations in sea level (Byrne *et al.* 2011; Chapple *et al.* 2011*a*; Haines *et al.* 2014). The glossy grass skink, and other *Pseudemoia* species, tend to inhabit areas with high precipitation (Hutchinson and Donnellan 1992; Haines *et al.* 2014), and consequently drying due to climatic oscillations may present geographic

boundaries to populations (Byrne *et al.* 2011; Chapple *et al.* 2011*a*; Bryant and Krosch 2016). In particular, an east–west split in Victoria is common in many skink species, and is likely due to historical volcanic activity (Chapple *et al.* 2005; Senior *et al.* 2022). Additionally, alpine populations are often separate from nearby lowland populations due largely to climatic and resource variation (Chapple *et al.* 2005, 2011*a*). Overall, our genetic data might indicate that the glossy grass skink may not disperse across fragmented landscapes, and therefore habitat loss and fragmentation may result in decreased connectivity and genetic diversity within the species (e.g. Crain *et al.* 2008; Dixo *et al.* 2009; Haines *et al.* 2014; Senior *et al.* 2022). However, further work involving more detailed sampling within populations and across the species range, along with additional nuclear loci, would be required to confirm this result.

Conclusions

We have provided a case study for how a targeted, multifaceted research project can be effective at rapidly gathering data that can be used to contribute vital information to the assessment of extinction risk in Data Deficient species. This approach can be broadly applicable to any Data Deficient species, where there are sufficient museum specimens, and tissue samples, available. For the glossy grass skink, we have determined the generation length of the species, providing information that will allow the species to be assessed against Criteria A, C and E on the IUCN Red List (IUCN Standards and Petitions Committee 2022). Our molecular data, combined with recent field-based studies on the species (Farquhar *et al.* 2024), indicates that the species may be susceptible to habitat loss and fragmentation (Criterion B), and provides information (i.e. several distinct genetic lineages) that may be useful for determining the number of subpopulations that are present in the species (Criteria B and C). In a recent related study, we clarified the distribution of the glossy grass skink, used field surveys to determine that the species has disappeared at ~50% of historical sites, and showed that a third of the species' predicted range occurs in cleared agricultural land that is now likely unsuitable for the species (Farquhar *et al.* 2023) (Criteria A, B and C). These findings, combined with the results of the current study, will allow the species to be assessed against IUCN Red List criteria, and will most likely result in the species being listed as Vulnerable under Criterion B.

Supplementary material

Supplementary material is available online.

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Data availability. The data and code associated with this study are provided in the online supplementary material. Our DNA sequence data has been submitted to GenBank under the accession numbers provided in Table 1.

Conflicts of interest. The authors declare that they have no conflicts of interest.

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