Range extension and genetic structure of the narrowly-restricted slider skink, *Lerista rochfordensis* Amey and Couper, 2009 (Reptilia: Scincidae)

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ABSTRACT

Recent field surveys have collected more information on the poorly known species, *Lerista rochfordensis*. Previously known only from one dry rainforest patch of around 2 000 hectares in northern Queensland, the species was discovered in a neighbouring patch, 8 km distant, itself about 1600 hectares in extent. The two populations are separated by cleared grazing land and the Kirk River, an ephemeral tributary of the Burdekin River. Statistically significant but comparatively small genetic and morphological divergence was observed between the two populations, suggesting they should still be considered conspecific under the Evolutionary Species Concept but are undergoing allopatric speciation. ❐ *Lerista rochfordensis, slider skink, Queensland.*

The genus *Lerista* Bell, 1833 is an endemic Australian monophyletic group of sphenomorphine skinks showing varying degrees of adaptation to the subsoil environment. Limb reduction is a prominent feature of their evolution, having occurred independently at least ten times within the lineage (Skinner *et al.* 2008). The genus appears to have undergone an 'explosive radiation' into newly-developed arid environments within the last 13 million years (Kendrick 1988; Skinner *et al.* 2008) and is the second-most speciose reptile genus in Australia with 96 recognised species, exceeded only by another skink genus, *Ctenotus* Storr, 1964 (Cogger 2014).

Lerista rochfordensis was described in 2009 from specimens collected in a dry rainforest patch, Rochford Scrub, in northern Queensland (Amey & Couper 2009). It was diagnosed from all other species of *Lerista* by 'forelimb absent with no groove or other indication, hindlimb 5–8% SVL with a single clawed digit, and five supraciliaries'. The describers considered it to qualify as Vulnerable under the Australian Environment Protection and Biodiversity Conservation Act 1999 (Australian Government 1999) under criteria D2: 'Populations with a very restricted area of occupancy (typically less than 20 km^2) or number of locations (typically 5 or fewer) such that it is prone to the effects of human activities or stochastic events within a very short time period in an uncertain future, and is thus capable of becoming endangered or extinct in a very short time period.' The collection information available at the time indicated that

TABLE 1. Morphological measurements used and their definitions.

1.	Snout–vent length (mm).
2.	Head length (tip of snout to posterior margin of parietals, mm).
3.	Head width (widest point, mm).
4.	Rostral scale length (anteriormost point to tip of projection between nasal scales, mm).
5.	Nasal scale length (anterior to posterior margins, mm).
6.	Length of contact between nasal scales (mm).
7.	Naris width (mm).
8.	Internarial distance (distance between the two nostrils, mm).
9.	Eye–naris distance (anterior margin of eye to posterior margin of naris, mm).
10.	Rostral–frontal distance (posterior median projection of rostral to anterior margin of frontal, mm).
11.	Supraocular width (mm).
12.	Eye width (mm).
13.	Supralabial–ear (distance from posterior edge of last supralabial to anterior margin of ear, mm).
14.	Eye–ear (distance from posterior margin of eye to anterior margin of ear, mm).
15.	Paravertebral scale width (mm).
16.	2nd paravertebral scale width (mm).
17.	Mid-ventral scale width (mm).
18.	Width at midbody (mm).
19.	Hind limb length (mm).
20.	No. midbody scale rows.
21.	No. paravertebrals.
22.	No. supraoculars contacting frontal.
23.	No. enlarged nuchals.
24.	No. supraciliaries.
25.	1st supraciliary contacts 2nd loreal.
26.	1st supraciliary contacts presubocular.
27.	1st supraciliary contacts frontal.
28.	No. preoculars.
29.	No. presuboculars.
30.	No. palpebrals.
31.	No. postoculars.
32.	1st temporal contacts postocular.
33.	1st temporal contacts parietal.
34.	1st temporal contacts 2nd temporal.
35.	No. scales between last supralabial and ear.
36.	No. scales between last infralabial and ear.
37.	No. infralabials.
38.	No. infralabials contacting postmental.
39.	Hind limb length (no. body scales).
40.	No. lamellae under toe.
41.	No. toe supradigitals.

it occurred at two localities, Rochford Scrub and Boori Station, separated by about 20 km. However, further research has shown this to be incorrect; the Boori Station locality is in fact less than 2 km from the other records and still within the Rochford Scrub. The corrected locality gives a known area of occupancy of less than half a square kilometre and, if all of Rochford Scrub is suitable habitat, a possible occupancy area of only about 20 km^2. Rochford Scrub, the only known locality for this species, is not protected except by the efforts of the current lease-holders who graze cattle on the encompassing property. The species thus seems to be very vulnerable to disturbances such as fire, over-grazing and mining.

Surveys of remnant vegetation of Queensland have enabled the mapping of dry rainforests (otherwise known as vine thickets, see Kahn & Lawrie 1987) in northern Queensland (Queensland Department of Science Information Technology and Innovation 2013). These maps show another, similarly-sized patch of dry rainforest, the Barrabas Scrub, in close proximity to Rochford Scrub (Fig. 1). This patch had not been previously surveyed for reptiles. Since dry rainforest appears to be a key habitat for *Lerista* in northern

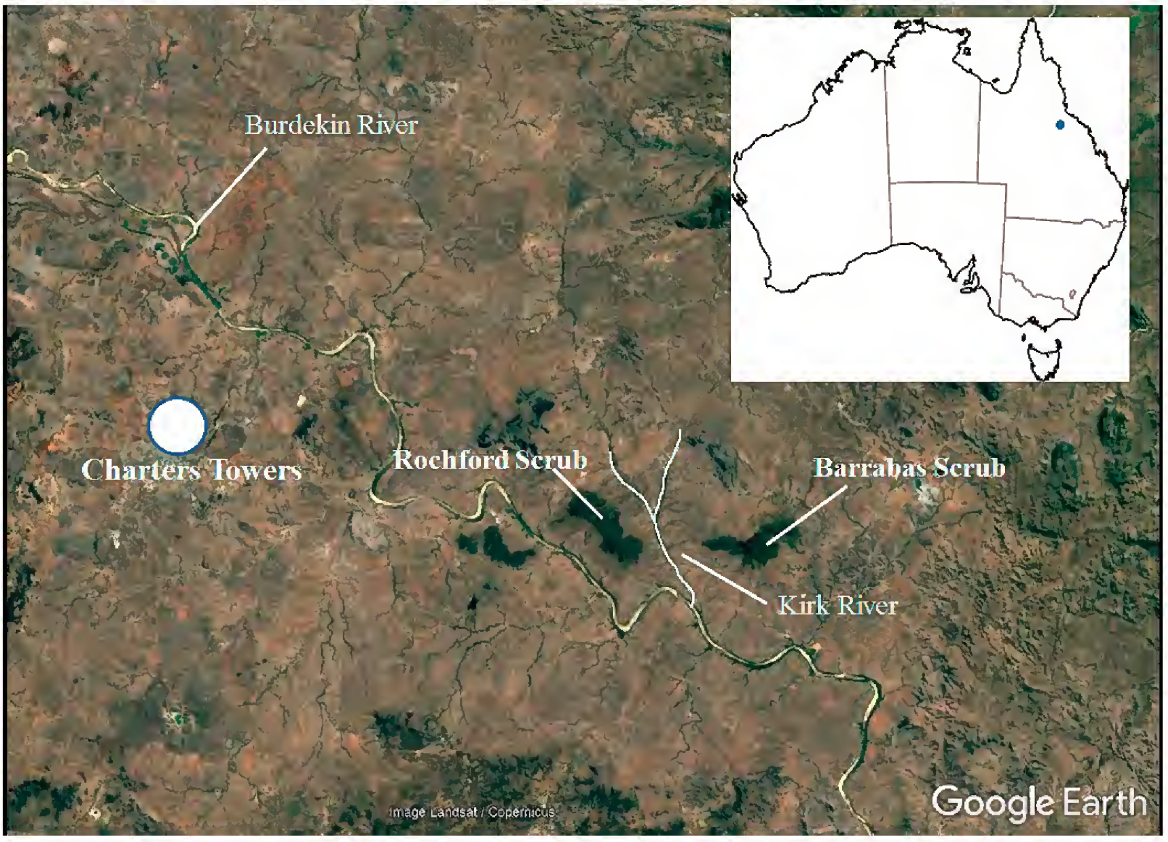

FIG. 1. Map showing Rochford and Barrabas Scrubs, separated by the Kirk River, collection localities for *Lerista rochfordensis*.

Queensland (Amey 2012; Amey & Couper 2009), we targeted this site to discover whether it harboured *Lerista*, and what relationship this population might have to that in Rochford Scrub. The Rochford Scrub population was re-visited to obtain genetic samples which had not been previously collected. As *Lerista* were found in Barrabas Scrub, this allowed us to compare the two populations both genetically and morphologically to assess whether they are best thought of as distinct taxa or as conspecific.

METHODS

Survey. Rochford and Barrabas Scrubs were investigated a few days apart in September, 2014. Both have been mapped as consisting of Regional Ecosystem 11.5.15, defined as 'Semi-evergreen vine thicket on remnant Tertiary surfaces' (Sattler & Williams 1999). Some parts of Rochford Scrub also have a small proportion (30%) of *Acacia* and *Eucalyptus* woodlands (R.E. 11.7.2 and 9.7.2), which are not present at Barrabas. Rochford is approximately 20 km^2 in extent, Barrabas 23 km^2. They are approximately 5 km apart and separated by the Kirk River, an ephemeral tributary of the Burdekin River. Both occur on pastoral leases. All specimens were collected by hand, commonly in friable soil under logs. Five specimens were vouchered from Rochford Scrub and eight from Barrabas. All specimens were lodged at the Queensland Museum.

We tested whether the two populations should be considered as distinct taxa or conspecific under the Evolutionary Species Concept of Simpson (1961) using morphology and genetics.

Morphology. Measurements of preserved voucher material were made as per the methods outlined in Couper *et al.* (2016). Variables used are given in Table 1. Scale definitions follows Lillywhite (2008). Specimens examined are listed in Appendix 1.

Statistical Analysis of Morphological Data. In order to test for morphological differences between the two study populations, we took two approaches. A common approach to classification is to use supervised learning (*sensu* Mohri *et al.* 2012), which aims to find the best way to classify organisms into pre-defined groups, on the basis of several morphological characters. In taxonomy, the most commonly used supervised learning technique is Discriminant Function Analysis (DFA), developed by Fisher (1936), but applied widely since then (see for a review in the context of morphometrics Breitman *et al.* 2013; and Crochet *et al.* 2003 for examples of DFA in lizard taxonomy). A serious problem for DFA is that there are often many more variables than cases (i.e., small N, large p), which makes the application of traditional DFA methods impossible, as these approaches require the inversion of covariance matrices which are then not of full rank (Mitteroecker & Bookstein 2011). Many methods have been proposed to overcome this issue (Sharma & Paliwal 2015). We used a commonly used approach, regularisation, in order to address this small sample size problem. Regularisation methods impose extra constraints on the solution to optimisation problems that can result in shrinkage of parameter estimates (which minimises overfitting) and can allow the construction of sparse solutions such that some parameter estimates may be set to zero, effectively allowing a form of automatic variable selection (Tibishirani 1996). We analysed the forty-one morphological characters using regularised discriminant function analysis (RDA; Friedman 1989). RDA accounts for multicollinearity among variables, and is especially useful in cases where there are more variables than observations, as in our data set. RDA uses a two-parameter regularisation function in order to restrict the outcomes of the analysis to more plausible values. In effect, this regularisation causes the class covariance matrices to be 'shrunk' towards a multiple of the identity matrix, with the multiplier being the mean of the eigenvalues of the class covariance matrix (see Friedman 1989 for details). RDA was conducted using the klaR package for R (R Core Team 2017; Weihs *et al.* 2005). We excluded two specimens due to missing data, resulting in a classification rule based on eight specimens from each locality (Barrabas, Rochford). We first performed a grid search to find the values of the two parameters (λ and γ) that minimised the classification error rate. We then fitted the RDA model to the data using the obtained values for these two parameters, with equal probability priors for the two classes. We calculated posterior probabilities of membership for the two localities and we applied a classification rule that assigned class membership if the probability of membership was greater than 0.5. We computed the Correctness, Accuracy, Ability to Separate and Confidence of the classification, according to the methods of Garczarek (2002) using R package klaR.

Our first approach using DFA seeks to achieve maximum separation of classes based on all variables. Our second approach was to try to reduce the number of variables necessary to provide a more parsimonious classification rule. To do this, we used regularised logistic regression. We used L_1 norm regularisation (i.e., the Lasso, Tibishirani 1996) which allows for simultaneous shrinkage and variable selection, as coefficients for uninformative variables are set to zero. We estimated the regularisation parameter (λ, different from the RDA λ, above) using leave-one-out cross validation, with our optimality criterion being the minimisation of the misclassification error (or equivalently, to maximise Correctness). We performed logistic regression using locality as the response variable (Barrabas = 0, Rochford = 1), with the logit link function. Analysis was performed using the glmnet package for R (Friedman *et al.* 2010). We calculated probabilities of class membership and assigned specimens to their most likely locality, as above. In addition, we predicted the locality for the two Rochford specimens that were deleted from the RDA

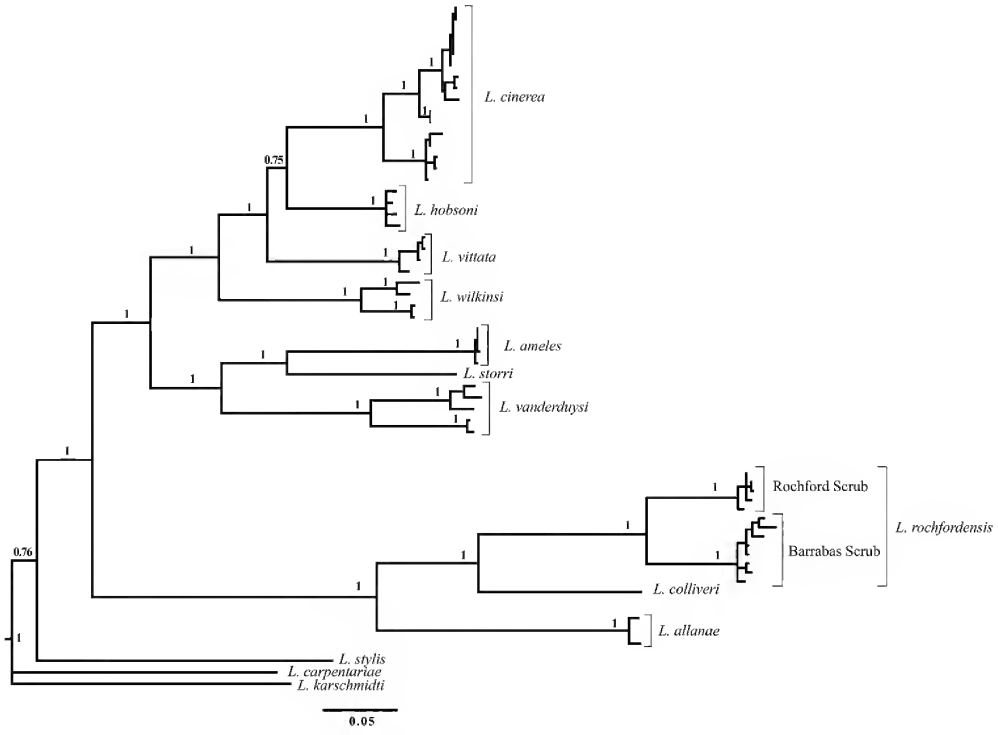

FIG. 2. Majority rule consensus tree of Queensland *Lerista* species and outgroup taxa based on four independent runs of the concatenated data set combining multiple loci (12S, 16S, ND4, flanking tRNAs and nuclear ATPs β). Branches with < 60% support were collapsed and posterior probabilities are indicated above the branches. Scale bar represents 5% of uncorrected sequence divergence.

analysis due to missing data. We also computed Garczarek's (2002) performance measures for the logistic regression classifier using R package klaR.

GENETICS

DNA extraction and sequencing. Tissues from the *Lerista* specimens collected at Rochford (n = 5) and Barrabas (n = 8) Scrubs were sequenced for the same 4 gene regions as used in the Skinner (2007, 2010), Skinner *et al.* (2008), Amey and Worthington Wilmer (2014) and Couper *et al.* (2016) studies of *Lerista* and other *Sphenomorphus*-group scincid lizards. These were mitochondrial DNA (mtDNA) loci 12S and 16S ribosomal rRNA (12S, 16S) and NADH dehydrogenase subunit 4 (ND4) including adjacent transfer tRNA fragments tRNA-His, tRNA-Ser and tRNA-Leu (tRNAs) and the nuclear ATP synthetase-β subunit intron (nucATP). The sequences were then slotted into the existing alignment and phylogeny generated for Couper *et al.* (2016) , which showed that the *L. allanae* group (*L. allanae* (Longman, 1937), *L. colliveri* Couper and Ingram, 1992 and *L. rochfordensis*) as a distinct sister group to the *L. wilkinsi* group taxa.

Total genomic DNA was extracted from all tissues using NucleoSpin Tissue Kits (Macherey-Nagel). The loci 12S, 16S and nucATP were successfully amplified using the primers and annealing conditions as detailed in Skinner (2007; 2010, see Table 1 in either paper). Amplification of the mtDNA ND4 region was obtained using the Arevalo *et al.* (1994) ND4 primers 'ND4' (also in Table 1 of Skinner 2007; Skinner 2010) and 't-Leu' at a 52° C annealing temperature. PCR products were either sequenced directly or gel purified (Macherey-Nagel Gel and PCR

purification kit) and sequencing reactions were carried out according to standard ABI PRISM dye labeled dideoxy terminator sequencing protocols using BigDye Terminator version 3.1. Sequences from the new specimens have been deposited in GenBank nucleotide sequence database and are detailed for each specimen examined in Appendix 2.

Phylogenetic analyses. Chromatographs were checked and all sequences were aligned for each locus using Geneious v 7.1.9 (Kearse *et al.* 2012). Initial alignment of sequences from within a sampling locality was performed using the default settings for the Geneious alignment function (Global Alignment, 93% similarity cost matrix, the highest setting). A consensus alignment of all pre-aligned groups of sequences for each locus was then performed, again implementing the default Geneious multiple alignment function (Kearse *et al.* 2012). All alignments were checked by eye and manual adjustments were made as necessary. Translation of the ND4 region was done to check the appropriate reading frame for codon positions and to identify where the protein coding region ended and the flanking three tRNAs began. The tRNAs were combined into a single dataset due to their short length and functional similarity (Skinner 2007; Skinner *et al.* 2008). The total combined dataset (12S, 16S, ND4, tRNAs and nucATP) for all individuals was manually constructed in Se-Al v 2.0a10 (Rambaut 1996). Prior to phylogenetic analyses, models of sequence evolution/substitution patterns for each of the loci including ND4 first, second and third codon positions were calculated independently and determined by the Akaike information criterion (AIC) in jModeltest v 2.1.3 (Guindon & Gascuel 2003; Posada 2008). Skinner (2010) tested eight different data partitioning strategies and found that a combination of seven partitions (12S, 16S, ND4 first, second and third codon positions, flanking tRNAs and nucATP) provided the best fit to the data. We therefore chose this partitioning strategy for our analyses.

Bayesian phylogenetic analyses were carried out in MrBayes v 3.2.1 (Ronquist *et al.* 2012) and posterior probabilities were calculated using a Markov chain, Monte Carlo (MCMC) sampling approach. Likelihood (lset) and prior (prset) parameters were then set for each of the 7 data partitions within the total concatenated data set (2833 bp). By default MrBayes v 3.2.1 performs two independent runs. We ran the analysis twice so that four independent runs were performed. For each run, starting trees were random and 4 simultaneous Markov chains were run for 5 000 000 generations with trees sampled every 1 000 generations resulting in a total of 20 000 saved trees over the four runs. Burn-in values for each run were set at 1 000 000 generations (1 000 trees) after empirical values of stabilizing likelihoods and the average standard deviation of the split frequencies reached less than 0.01 indicated that convergence of the MCMC chains had been reached. The production of identical consensus trees and their posterior probabilities from each of the MrBayes runs (viewed in *FigTree* v1.4.2, http://tree.bio.ed.ac.uk/software/figtree/) was taken as evidence that the chains had been run for a sufficient number of generations. A final combined majority rule consensus tree from the four independent runs, was generated in PAUP* v 4.b.10 (Swofford 2002) by sequentially importing the four MrBayes tree files (.t files); excluding the first 1 000 trees of each tree file and retaining the previous 4 001 trees in memory (MrBayes starts its first topology at time zero, resulting in 5 001 trees sampled over 5 million generations). The resulting consensus tree was therefore constructed from 16 004 trees. The posterior probabilities on the consensus tree are indicated only where branch support is greater than 0.5 (Posada & Crandall 1998). Inferred relationships were considered strongly supported where indicated by Bayesian posterior probabilities (BPP) of ≥0.95 (Huelsenbeck *et al.* 2001).

RESULTS

Specimens of *Lerista* were obtained at both Rochford and Barrabas Scrubs. Although no rigorous method of estimating population size was applied, the short collecting time (half a day at each site) and small area surveyed suggests good populations are present at both localities. The Barrabas Scrub population

TABLE 2. Parameters and estimates for the Lasso Logistic Regression analysis, forming the classification rule.

Parameter	Estimate
Intercept	16.0514
No. preocular scales	0.0247
No. presubocular scales	-0.4738
No. scales between last supralabial and ear	-1.2416
Hind limb length (no. body scales)	-0.2530
No. lamellae under the toe	-1.1181
No. toe supradigitals	-0.1714

conformed with the diagnosis of *L. rochfordensis* given in its description (Amey & Couper 2009, and see above).

Morphology. Regularised Discriminant Analysis. The grid search for the optimal values of the regularisation parameters that minimise the misclassification error were covariance shrinkage: $\gamma = 0.053$ and regularisation parameter $\lambda = 1$. Note that when $\lambda = 1$, RDA reduces to LDA. The Correctness index was equal to 1; all specimens were classified to their correct locality. The Accuracy and Ability to Separate were both 0.79, indicating that the two classes were well separated by the classification rule. The Confidence in the classification was 0.89, indicating high confidence. Classwise Confidence was 0.87 for Barrabas and 0.91 for Rochford.

Lasso Logistic Regression. The regularisation parameter (λ) that minimised the classification error rate was equal to 0.101. We used this value to fit the Lasso logistic regression model. This resulted in the retention of 6 explanatory variables, with 42.54% of the deviance explained by the model (Table 2). Predicted class membership for all specimens was perfect, i.e., Correctness = 1. Accuracy was 0.191 and Ability to Separate was 0.394, considerably lower than for the RDA analysis. The Confidence index was 0.697. Classwise confidence was 0.682 for Barrabas and 0.710 for Rochford. Confidence was again lower than for the RDA analysis. In addition, the two specimens that were not used to build the logistic regression were assigned to their correct locality. Both were assigned to Rochford, with probabilities 0.829 and 0.808.

Molecular data. The phylogenetic analysis clearly supports *L. rochfordensis* as a monophyletic species (BPP = 1.0), which is split into two strongly supported populations relating to Rochford and Barrabas Scrubs (BPP = 1.0) (Fig. 2). Estimates of uncorrected sequence divergence also support conspecific classification with all levels of sequence divergence among the two populations being well below the average divergence across all loci among all currently sequenced *Lerista* species (Table 3).

DISCUSSION

Analysis of the morphological dataset, showing phenetic similarity, combined with the genetic, suggests that the two populations are distinct but very closely related. With no single character distinguishing them and low sequence divergence, they are best thought of as conspecific populations.

Our morphological analyses suggest that the two populations can be separated using the characters we measured, but with a minimum

TABLE 3. Average sequence divergence for *L. rochfordensis*.

Locus	Within Rochford Scrub	Within Barrabas Scrub	Between Rochford and Barrabas Scrubs	Ave. among all *Lerista* species
12S	0.00%	0.12%	2.46%	8.61%
16S	0.06%	0.05%	1.06%	6.52%
ND4	0.26%	0.43%	3.62%	13.45%
tRNAs	0.21%	0.00%	3.61%	12.37%
nucATP	0.00%	0.08%	0.00%	3.82%

of 6 characters required to correctly distinguish the populations via Lasso logistic regression, although with considerably less confidence than when using the entire morphological data set of 42 characters using RDA. However, there are some caveats. Firstly, our models were trained on the full data set of 8 specimens from each population. It is therefore unsurprising that the models were able to correctly predict class membership of the training data set. The ability to predict population membership for new, out of sample specimens is likely to be lower, although the two specimens that were used for out of sample prediction were assigned correctly with high probability. Nevertheless, our analyses are based on only 18 specimens in total, and our results must be interpreted cautiously. Secondly, our classification rules apply only to the two populations (Barrabas and Rochford) that were available. An analysis of more populations might lead to different classification rules. Also, our classification rules apply to the 41 morphological characters in our data set. There may be other characters that we did not measure that may provide higher confidence in the classification, or better accuracy or ability to separate. This may apply to new single characters or different combinations of multiple characters. Overall, it appears that the two populations have evolved morphological differences which can delimit the two populations, but only with some effort. There is currently no single character that can be used to differentiate the populations.

The biogeographical history of *L. rochfordensis* is as yet unclear. Pre-clearing maps show that Rochford and Barrabas Scrubs were isolated from each other by low open Eucalypt woodland prior to European modification of the landscape (Regional Ecosystem 9.12.1a, Queensland Department of Science Information Technology and Innovation 2018). Our surveys show that the northern Queensland *Lerista* assemblage appears to be dependent on loose, friable soil that they can 'swim' through. In northern Queensland at least, this habitat can be extremely patchy. This may explain the link between some species of *Lerista* and dry rainforest, which is also often correlated with deep, coarse sands (Kahn & Lawrie 1987). The factors underlying the formation and retention of the dry rainforest scrubs in this part of Queensland are poorly understood but likely to be stochastic and idiosyncratic for each scrub (Kahn & Lawrie 1987). Overall, however, evidence indicates that they appeared in north Queensland during relatively recent Holocene time-frames (Fensham 1995). The populations of *Lerista* within Rochford and Barrabas are distinct from each other but the low level of distinctiveness suggests they have only recently diverged. This appears to be an example of the early stages of allopatric speciation in response to vicariance.

When present, *Lerista* can be common but their ability to migrate between patches of suitable habitat is likely to be limited. This can lead to the appearance of healthy populations which are nonetheless naturally fragmented and vulnerable to habitat destruction.

The discovery of a second population of *L. rochfordensis* in another dry rainforest scrub certainly improves the species' prospects of long term survival. Both scrubs, although without legal protection, are managed by sympathetic leaseholders. For example, Barrabas Scrub is fenced to restrict cattle access. The species appears to be quite abundant at both sites, judging by catching effort, although neither has been exhaustively surveyed to determine whether it occurs throughout each scrub. However, there is no cause to regard *L. rochfordensis* as secure and an objective analysis retains its status as Vulnerable, if not a higher category. It is only known from two locations of around 20 km^2 in extent each, which renders it "prone to the effects of human activities or stochastic events within a very short time period in an uncertain future, and is thus capable of becoming endangered or extinct in a very short time period." (Australian Government 1999). The scrubs remain unprotected and reliant on the goodwill of the leaseholders, which they currently enjoy. However, some events are largely beyond leaseholder control, such as fire and, in particular, mining activity. The area has a long history of gold mining and mining has occurred very close to both scrubs. Furthermore, there is significant

divergence between the two populations. A loss of one population, while not signifying extinction of a species, is still a significant loss of biodiversity.

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LITERATURE CITED

Amey, A. 2012. Mount Cooper Striped Skink, *Lerista vittata* Greer, McDonald & Lawrie, 1983. Pp. 232–233. *In,* Curtis, L.K., Dennis, A.J., McDonald, K.R., Kyne, P.M. & Debus, S.J.S. (eds), *Queensland's Threatened Animals* (CSIRO: Collingwood).

Amey, A.P. & Couper, P.J. 2009. A new limb-reduced skink (Scincidae: *Lerista*) from the dry rainforest of north Queensland, Australia. *Zootaxa*, **2173**: 19–30.

Amey, A.P. & Worthington Wilmer, J. 2014. Morphological diversity and genetic structure within *Lerista kalumburu* Storr, 1976 (Squamata: Scincomorpha: Sphenomorphidae)—taxonomic implications. *Zootaxa*, **3821**: 239–252. http://dx.doi.org/10.11646/zootaxa.3821.2.4

Arevalo, E., Davis, S.K. & Sites, J.W. 1994. Mitochondrial DNA sequence divergence and phylogenetic relationships among eight chromosome races of the *Sceloporus grammicus* complex (Phrynosomatidae) in central Mexico. *Systematic Biology*, **43**: 387–418. http://dx.doi.org/10.1093/sysbio/43.3.387

Australian Government 1999. Environment Protection and Biodiversity Conservation Act *C2016C00667*, Canberra, ACT. Available from: https://www.legislation.gov.au/Details/C2016C00667 (Accessed 28 June, 2016).

Bell, T. 1833. Characters of two new genera of reptiles. *Proceedings of the Zoological Society of London*, **1**: 98–99. http://dx.doi.org/10.1111/j.1469-7998.1833.tb06430.x

Breitman, M.F., Morando, M. & Avila, L.J. 2013. Past and present taxonomy of the *Liolaemus lineomaculatus* section (Liolaemidae): Is the morphological arrangement hypothesis valid? *Zoological Journal of the Linnean Society*, **168**: 612–668.

Cogger, H.G. 2014. *Reptiles & Amphibians of Australia* (7th ed.). (CSIRO Publishing: Collingwood).

Couper, P.J., Amey, A.P. & Worthington Wilmer, J. 2016. Cryptic diversity within the narrowly endemic *Lerista wilkinsi* group of north Queensland—two new species (Reptilia: Scincidae). *Zootaxa*, **4162**: 61–91. http://doi.org/10.11646/zootaxa.4162.1.3

Couper, P.J. & Ingram, G.J. 1992. A new species of skink of *Lerista* from Queensland and a re-appraisal of *L. allanae* (Longman). *Memoirs of the Queensland Museum*, **32**: 55–59.

Crochet, P.A., Geneiz, P. & Ineich, I. 2003. A multivariate analysis of the fringe-toed lizards of the *Acanthodactylus scutellatus* group (Squamata: Lacertidae): Systematic and biogeographical implications. *Zoological Journal of the Linnean Society*, **137**: 117–155.

Fensham, R. 1995. Floristics and environmental relations of inland dry rainforest in north Queensland, Australia. *Journal of Biogeography*, **22**: 1047–1053.

Fisher, R.A. 1936. The use of multiple measurements in taxonomic problems. *Annals of Eugenics*, **7**: 179–188. http://dx.doi.org/10.1111/j.1469-1809.1936.tb02137.x

Friedman, J., Hastie, T. & Tibishirani, R. 2010. Regularization paths for generalized linear models via coordinate descent. *Journal of Statistical Software*, **33**: 1–22.

Friedman, J.H. 1989. Regularised discriminant analysis. *Journal of the American Statistical Association*, **84**: 165–175.

Garczarek, U.M. 2002. Classification Rules in Standardized Partition Spaces. Thesis, University of Dortmund, Dortmund.

Guindon, S. & Gascuel, O. 2003. A simple, fast and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology*, **52**: 696–704. http://dx.doi.org/10.1080/10635150390235520

Huelsenbeck, J.P., Ronquist, F., Nielsen, R. & Bollback, J.P. 2001. Bayesian inference of phylogeny and its impact on evolutionary biology. *Science*, **294**: 2310–2314. http://dx.doi.org/10.1126/science.1065889

Kahn, T.P. & Lawrie, B.C. 1987. Vine thickets of the inland Townsville region. Pp. 159–199. *In, The Rainforest Legacy. Australian National Rainforests*

Study (Australian Government Publishing Service: Canberra).

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M.S., S, Buxton, S., et al. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, **28**: 1647–1649. http://dx.doi.org/10.1093/bioinformatics/bts199

Kendrick, P.G. 1988. Evolution in *Lerista*: Electrophoretic evidence for an explosion. *In: Australian Bicentennial Herpetological Conference*. Australian Society of Herpetologists, Queensland Museum, Brisbane.

Lillywhite, H.B. 2008. *Dictionary of Herpetology*. (Krieger Publishing Co: Malabar, Florida).

Longman, H.A. 1937. Herpetological notes. *Memoirs of the Queensland Museum*, **11**: 165–168.

Mitteroecker, P. & Bookstein, F. 2011. Linear discrimination, ordination, and the visualization of selection gradients in modern morphometrics. *Evolutionary Biology*, **38**: 100–114.

Mohri, M., Rostamizadeh, A. & Talwalkar, A. 2012. *Foundations of Machine Learning*. (The MIT Press).

Posada, D. 2008. jModelTest: Phylogenetic model averaging. *Molecular Biology and Evolution*, **25**: 1253–1256. http://dx.doi.org/10.1093/molbev/msn083

Posada, D. & Crandall, K.A. 1998. Modeltest: Testing the model of DNA substitution. *Bioinformatics*, **14**: 817–818. http://dx.doi.org/10.1093/bioinformatics/14.9.817

Queensland Department of Science Information Technology and Innovation 2013. *Copy of the Vegetation Management Act Regional Ecosystem and Remnant Map - Version 6.1. Online RE Map*. Queensland Department of Science Information Technology and Innovation, Brisbane. Accessed on 10 Oct, 2013.

2018. *Copy of the Pre-clearing Regional Ecosystem Map - Version 10.0. Online RE Map*. Queensland Department of Science Information Technology and Innovation, Brisbane. URL: https://environment.ehp.qld.gov.au/map-request/re-broad-veg-group/ Accessed on 6 Feb, 2018.

R Core Team 2017. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. Available from: https://www.R-project.org/.

Rambaut, A. 1996. *Se-A: Sequence Alignment Editor* v2.0a10. Available from: http://evolve.zoo.ox.ac.uk.

Ronquist, F., Teslenko, M., Van Der Mark, P., Ayres, D.L., Darling, A., Hohna, S., et al. 2012. MRBAYES 3.2: Efficient Bayesian phylogenetic inference and model selection across a large model space. *Systematic Biology*, **61**: 539–542. http://dx.doi.org/10.1093/sysbio/sys029

Sattler, P. & Williams, R. 1999. The Conservation of Queensland's Regional Ecosystems. Environmental Protection Agency, Brisbane.

Sharma, A. & Paliwal, K.K. 2015. Linear discriminant analysis for the small sample size problem: an overview. *International Journal of Machine Learning and Cybernetics*, **6**: 443–454.

Simpson, G.G. 1961. *The Principles of Animal Taxonomy*. (Columbia Univeristy Press: New York).

Skinner, A. 2007. Phylogenetic relationships and rate of early diversification of Australian *Sphenomorphus* group scincids (Scincoidea, Squamata). *Biological Journal of the Linnean Society*, **92**: 347–366. http://dx.doi.org/10.1111/j.1095-8312.2007.00843.x

2010. Rate heterogenity, ancestral character state reconstruction and the evolution of limb morphology in *Lerista* (Scincidae, Squamata). *Systematic Biology*, **59**: 723–740. http://dx.doi.org/10.1093/sysbio/syq055

Skinner, A., Lee, M.S.Y. & Hutchinson, M.N. 2008. Rapid and repeated limb loss in a clade of scincid lizards. *BMC Evolutionary Biology*, **8**: 310. http://dx.doi.org/10.1186/1471-2148-8-310

Storr, G.M. 1964. *Ctenotus*, a new generic name for a group of Australian skinks. *Journal of the Royal Society of Western Australia*, **52**: 97–108.

Swofford, D.L. 2002. *PAUP* Phylogenetic Analysis Using Parsimony (*and other methods)* v4. Sinauer Associates.

Tibishirani, R. 1996. Regression shrinkage and selection via the lasso. *Journal of the Royal Statistical Society, Series B (Methodological)*, **58**: 267–288.

Weihs, C., Ligges, U., Luebke, K. & Raabe, N. 2005. klaR analyzing German business cycles. Pp. 335–343. *In,* Baier, D., Decker, R. & Schmist-Thieme, L. (eds), *Data Analysis and Decision Support* (Springer-Verlag: Berlin).

APPENDIX 1

Voucher specimens examined. All material is held at the Queensland Museum. All geographic coordinates use the datum GDA94.

***Lerista rochfordensis* (n = 18). QMJ84790** (Rochford Scrub, CQ 20° 06′ 49″ S, 146° 37′ 03″ E, holotype); **QMJ44385–44386** (Boori Station, border with Amity, CQ, 20° 07′ 24″ S, 146° 38′ 34″ E, paratypes); **QMJ85002** (Rochford Scrub, CQ, 20° 07′ 01″ S, 146° 37′ 49″ E, paratype); **QMJ85007** (Rochford Scrub, CQ, 20° 07′ 05″ S, 146° 37′ 43″ E, paratype); **QMJ93690** (Rochford Scrub, Amity Station, CQ, 20° 06′ 44″ S, 146° 37′ 10″ E); **QMJ93691–93694** (Rochford Scrub, Amity Station, CQ 20° 06′ S, 146° 37′ E); **QMJ93697** (Barrabas Scrub, Kirkton Station, CQ, 20° 09′ 12″ S, 146° 42′ 57″ E); **QMJ93698** (Barrabas Scrub, Kirkton Station, CQ, 20° 09′ S, 146° 43′ E); **QMJ93699** (Barrabas Scrub, Kirkton Station, CQ, 20° 09′ 10″ S, 146° 42′ 56″ E); **QMJ93700–93704** (Barrabas Scrub, Kirkton Station, CQ, 20° 09′ S, 146° 43′ E).

APPENDIX 2

GenBank sequence numbers for material examined in this study.

Species	12S rRNA	16S rRNA	ND4+tRNAs	ATP
Lerista allanae - Capella 1	KU309145	KU309187	KU309272	KU309229
Lerista allanae - Capella 2	KU309146	KU309188	KU309273	KU309230
Lerista ameles - Mt Surprise 1	KU309147	KU309189	KU309274	KU309231
Lerista ameles - Mt Surprise 2	KU309148	KU309190	KU309275	KU309232
Lerista ameles - Mt Surprise 3	KU309149	KU309191	KU309276	KU309233
Lerista ameles - Mt Surprise 4	KU309150	KU309192	KU309277	KU309234
Lerista cinerea - Warrawee Station 1	KU309151	KU309193	KU309278	KU309235
Lerista cinerea - Warrawee Station 2	KU309152	KU309194	KU309279	KU309236
Lerista cinerea - Warrawee Station 3	KU309153	KU309195	KU309280	KU309237
Lerista cinerea - Warrawee Station 4	KU309154	KU309196	KU309281	KU309238
Lerista cinerea - Warrawee Station 5	KU309155	KU309197	KU309282	KU309239
Lerista cinerea - Warrawee Station 6	KU309156	KU309198	KU309283	KU309240
Lerista cinerea - Bletchington Park	KU309157	KU309199	KU309284	KU309241
Lerista cinerea - Gregory Development Rd	KU309158	KU309200	KU309285	KU309242
Lerista cinerea - Rishton Scrub 1	KU309159	KU309201	KU309286	KU309243
Lerista cinerea - Rishton Scrub 2	KU309160	KU309202	KU309287	KU309244
Lerista cinerea - Rishton Scrub 3	KU309161	KU309203	KU309288	KU309245
Lerista cinerea - Sellheim Scrub 1	KU309162	KU309204	KU309289	KU309246
Lerista cinerea - Sellheim Scrub 2	KU309163	KU309205	KU309290	KU309247
Lerista cinerea - Sellheim Scrub 3	KU309164	KU309206	KU309291	KU309248
Lerista cinerea - Sellheim Scrub 4	KU309165	KU309207	KU309292	KU309249
Lerista cinerea - Sellheim Scrub 5	KU309166	KU309208	KU309293	KU309250

Appendix 2 continued …

Species	12S rRNA	16S rRNA	ND4+tRNAs	ATP
Lerista colliveri	KU309167	KU309209	KU309294	KU309251
Lerista hobsoni - Lolworth Homestead 1	KU309168	KU309210	N/A	KU309252
Lerista hobsoni - Lolworth Homestead 2	KU309169	KU309211	KU309295	KU309253
Lerista hobsoni - Lolworth Homestead 3	KU309170	KU309212	KU309296	KU309254
Lerista hobsoni - Pentland	KU309171	KU309213	KU309297	KU309255
Lerista rochfordensis - Barrabas Scrub 1	MF589181	MF589191	MF589212	MF589202
Lerista rochfordensis - Barrabas Scrub 2	MF589182	MF589192	MF589213	MF589203
Lerista rochfordensis - Barrabas Scrub 3	MF589183	MF589193	MF589214	MF589204
Lerista rochfordensis - Barrabas Scrub 4	N/A	MF589194	MF589215	MF589205
Lerista rochfordensis - Barrabas Scrub 5	MF589184	MF589195	MF589216	MF589206
Lerista rochfordensis - Barrabas Scrub 6	MF589185	MF589196	MF589217	N/A
Lerista rochfordensis - Barrabas Scrub 7	MF589186	MF589197	MF589218	MF589207
Lerista rochfordensis - Barrabas Scrub 8	MF589187	MF589198	MF589219	MF589208
Lerista rochfordensis - Rochford Scrub 1	KU309172	KU309214	KU309298	KU309256
Lerista rochfordensis - Rochford Scrub 2	KU309173	KU309215	KU309299	KU309257
Lerista rochfordensis - Rochford Scrub 3	MF589188	MF589199	MF589220	MF589209
Lerista rochfordensis - Rochford Scrub 4	MF589189	MF589200	MF589221	MF589210
Lerista rochfordensis - Rochford Scrub 5	MF589190	MF589201	MF589222	MF589211
Lerista storri - Almaden	KU309174	KU309216	KU309300	KU309258
Lerista vanderduysi - Blackbraes 1	KU309175	KU309217	KU309301	KU309259
Lerista vanderduysi - Blackbraes 2	KU309176	KU309218	KU309302	KU309260
Lerista vanderduysi - Blackbraes 3	KU309177	N/A	N/A	KU309261
Lerista vanderduysi - Glibert Station 1	KU309178	KU309219	N/A	KU309262
Lerista vanderduysi - Glibert Station 2	N/A	KU309220	N/A	KU309263
Lerista vittata - Mt Cooper Station 1	KU309179	KU309221	KU309303	KU309264
Lerista vittata - Mt Cooper Station 2	KU309180	KU309222	KU309304	KU309265
Lerista vittata - Mt Cooper Station 3	KU309181	KU309223	KU309305	KU309266
Lerista vittata - Mt Cooper Station 4	KU309182	KU309224	KU309306	KU309267
Lerista wilkinsi - Torrens Creek 1	KU309183	KU309225	KU309307	KU309268
Lerista wilkinsi - Torrens Creek 2	KU309184	KU309226	KU309308	KU309269
Lerista wilkinsi - Torrens Creek 3	KU309185	KU309227	KU309309	KU309270
Lerista wilkinsi - Torrens Creek 4	KU309186	KU309228	KU309310	KU309271

Appendix 2 continued …

Species	12S rRNA	16S rRNA	ND4+tRNAs	ATP
Outgroups				
Lerista carpentariae	EF672763	EF672834	EF672975	EF672905
Lerista karlschmidti	EF672787	EF672858	EF672999	EF672929
Lerista stylis	EF672811	EF672882	EF373023	EF672952