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Systematic evaluation of molecular genetic, morphological and acoustic variation reveals three species in the *Litoria revelata* complex (Anura: Pelodryadidae)

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Abstract

We used a combination of nuclear and mitochondrial genetic data, body measurements and colouration, and male advertisement calls to analyse the systematic implications of variation in the whirring treefrog *Litoria revelata* complex, which occurs in three allopatric populations—north-eastern New South Wales/south-eastern Queensland, mid-eastern Queensland, and northern Queensland. The three populations each form divergent lineages for both the nuclear (single nucleotide polymorphisms; SNP) and mitochondrial datasets and are diagnosable also on the basis of morphology and advertisement calls. In combination, we use these lines of data to recognise three species: *L. revelata* in north-eastern New South Wales/south-eastern Queensland, *L. eungellensis* **sp. nov.** in mid-eastern Queensland, and the resurrected *L. corbeni* in northern Queensland. We provide a preliminary conservation assessment for each species, with the latter two species being localised to very small upland areas and warranting conservation listing and attention.

Key words: Anura, single nucleotide polymorphisms, SNP, taxonomy, Eungella, Wet Tropics

Introduction

The *Litoria ewingii* Group, *sensu* Tyler & Davies (1978) (Anura: Pelodryadidae) comprises nine small to medium sized frog species, with most species distributed in moist temperate regions of southern and eastern Australia. The constituent species are: *L. calliscelis* (Peters, 1874); *L. ewingii* (Duméril & Bibron, 1841); *L. jervisiensis* (Duméril & Bibron, 1841); *L. littlejohni* White, Whitford & Mahony, 1994; *L. paraewingi* Watson, Loftus-Hills & Littlejohn 1971; *L. revelata* Ingram, Corben & Hosmer, 1982; *L. sibilus* Parkin, Rowley, Elliott-Tate, Mahony, Sumner, Melville & Donnellan, 2024; *L. verreauxii* (Duméril, 1853) and *L. watsoni* Mahony, Moses, Mahony, Lemckert & Donnellan, 2020.

The range of one these species, *L. revelata*, includes three disjunct populations: a southern population from mid-eastern NSW to south-eastern Queensland, a central population in the Clarke Range in mid-eastern Queensland, and a northern population on the Atherton Tablelands in tropical north-eastern Queensland. The two disjunct populations in Queensland have restricted distributions in upland areas (Fig. 1). Ingram *et al.* (1982) compared male advertisement calls of the three populations and did not discern differences of taxonomic note. Anstis (2017) compared larval morphology of the southern and central populations and failed to find differences. Wells & Wellington (1985) raised the northern population to species level, as *Litoria corbeni*, on the basis of its disjunct distribution and alleged differences in male advertisement calls. These differences were based on a misinterpretation of data presented in Ingram *et al.* (1982), and hence subsequent publications did not accept *L. corbeni*.

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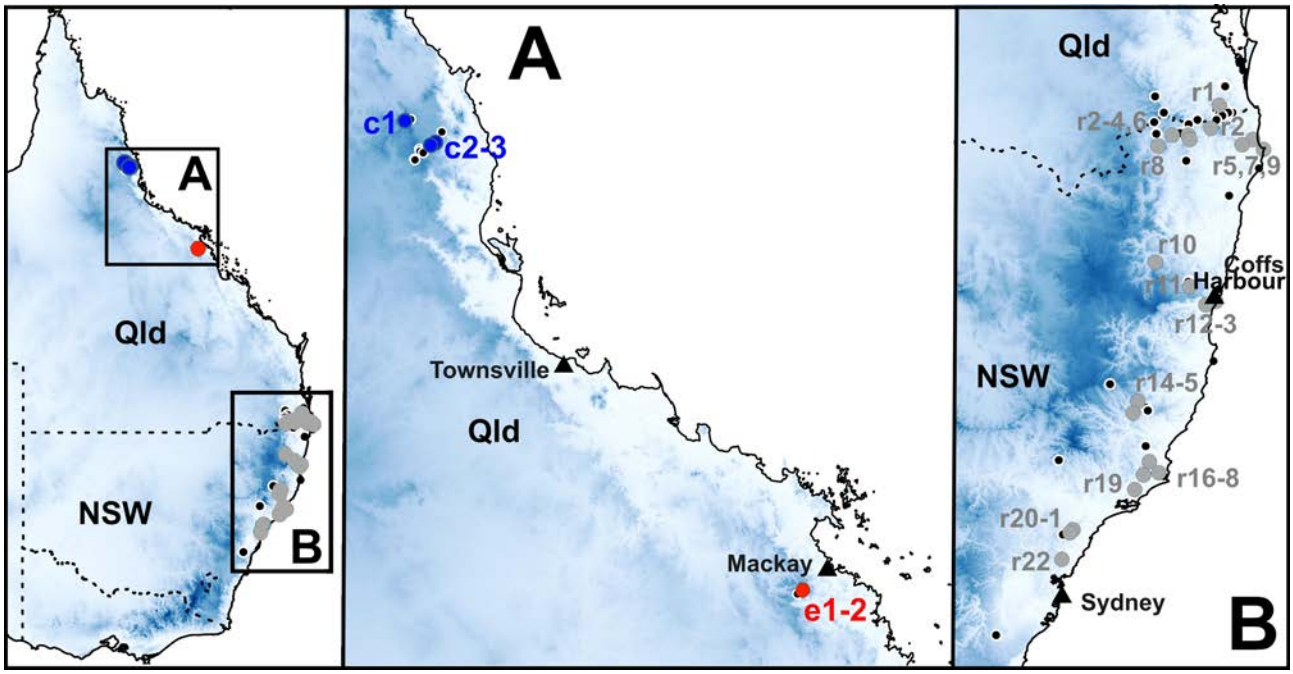

FIGURE 1. Map of eastern Australia showing collection locations and specimen records for the *Litoria revelata* complex, in the states of Queensland (Qld) and New South Wales (NSW). Genetically analysed specimens: blue dots—*L. corbeni*, red dots—*L. eungellensis* **sp. nov.**, grey dots—*L. revelata*. Small black dots are specimen records from the Atlas of Living Australia. See Appendix 1 for location codes. Graded blue background represents the elevational gradient, with darker blue being higher elevations.

To date, phylogeographic analysis of widespread eastern Australian frogs has focused on habitat generalist species including *Limnodynastes peronii* (Duméril & Bibron, 1841) and *Limnodynastes tasmaniensis* Günther, 1858, *Limnodynastes terraereginae* Fry, 1915, *Platyplectrum ornatum* (Gray, 1842), and *Litoria wilcoxii* Günther, 1864 (Schäuble *et al*. 2000, Schäuble & Moritz 2001, Donnellan & Mahony 2004, Parkin *et al.* 2024). Most of these studies, with the exception of *Limnodynastes terraereginae*, did not detect significant divergence across major biogeographic barriers in coastal Queensland, including the Burdekin and St Lawrence Gaps and the Brisbane Valley Barrier (Chapple *et al.* 2011, Bryant & Krosch 2016). However, a stronger effect of these barriers is expected for populations of the *L. revelata* complex because they are habitat specialists restricted to wetter forest, and particularly to rainforest areas in Queensland. Rainforest-associated frogs and reptiles generally show strong patterns of isolation across these historical and current dry barriers (e.g., Rosauer *et al.* 2017). Additionally, the restriction of Queensland populations of this complex to higher elevations adds urgency to resolving their taxonomy, given the predicted impacts of climate change on upland frogs (e.g., Guirguis *et al*. 2023, Luedtke *et al*. 2023).

In order to assess the systematic status of populations of *L. revelata*, we examined variation in mitochondrial and nuclear genetic diversity, morphology, and male advertisement calls from across the range of the species.

Materials and Methods

Supplementary files are available form: https://doi.org/10.6084/m9.figshare.c.7547352.v1

Mitochondrial DNA. DNA was extracted from toe-clips or liver tissue using a Puregene isolation kit (Gentra Systems, Minneapolis, MN) following the manufacturers protocol. Part of the *ND4* gene, the flanking $tRNA^{Ser}$ gene and part of the $tRNA^{His}$ gene (hereafter collectively referred to as the *ND4* gene) were PCR amplified and directly sequenced with the primers 5'-GGT YAC GAG YAA TTA GCA GTT CT-3' and 5'-CAC CTA TGA CTA CCA AAA GCT CAT GTA GAA GC-3', using protocols detailed in Anstis *et al.* (2016). Sequences were aligned using the program Muscle v6.814b (Edgar 2004), implemented in Geneious Pro v8.1.4 (Kearse *et al.* 2012), and GenBank accession numbers are listed in Appendix 1.

For model based phylogenetic inference, we estimated the best substitution model and partition scheme from three data subsets of the 1st, 2nd and 3rd codon positions, with ModelFinder (Kalyaanamoorthy *et al*. 2017) following the Bayes Information Criterion (BIC) criterion. For the maximum likelihood phylogenetic approach, we used IQ-TREE (Nguyen *et al.* 2014) on the IQ-TREE webserver (Trifinopoulos *et al*. 2016). We assessed branch support with 100 standard bootstrap pseudo-replicates (Hoang *et al.* 2017).

Average net genetic distances between species (*dA*) were calculated in MEGA v11 (Tamura *et al.* 2021) as: *d*A = *d*XY–(*d*X + *d*Y)/2, where *d*XY is the average distance between groups X and Y, and *d*X and *d*Y are the within-group means.

Molecular diagnostics. Following the recommendation of Renner (2016), we visually identified diagnostic single nucleotide polymorphisms (SNPs) within the mitochondrial *ND4* gene, using MEGA v11 (Tamura *et al.* 2021). We identified the apomorphic SNPs for each species, using outgroups to assess character state polarity. Sites were numbered from the alignment used for the phylogenetic analysis.

Nuclear SNP data generation. Samples of the nine species of the *Litoria ewingii* Group were submitted for DNA extraction and DArTseq™ 1.0 genotyping at Diversity Arrays Technology PL, Canberra, ACT, Australia. DArTseq™ represents a combination of DArT genome complexity reduction methods and next generation sequencing platforms (Kilian *et al*. 2012). DNA samples were processed in restriction enzyme digestion/ligation reactions using a combination of the *Pst*I/*Sph*I restriction enzymes, and ligated fragments were PCR amplified as described by Kilian *et al*. (2012) and Mahony *et al*. (2020) for single end sequencing for 77 cycles on an Illumina Hiseq2500.

The SNP data and associated metadata were read into a genlight object (Jombart 2008) to facilitate processing with the package dartR (Gruber *et al*. 2018). Only loci with 100% repeatability (reproducibility) were chosen for subsequent analysis. Further filtering was undertaken on the basis of having a call rate >90%, and the locus being present in at least 90% of individuals in the entire data-set or 80% of individuals in *L. revelata* complex-only data-set. We retained only one SNP from each tag at random. Any monomorphic loci arising as a result of the removal of individuals were also deleted. Given the low within-population sample sizes ($n \leq 15$), we did not filter loci for departures from Hardy-Weinberg equilibrium or linkage disequilibrium.

Analysis of the SNP data. Genetic similarity among individuals was visualized using the principal coordinates analysis (PCoA) ordination method, as implemented in the gl.pcoa and gl.pcoa.plot functions of dartR. We used a scree plot of eigenvalues to assess the number of informative PCs to examine, based on the average percentage variation in the original variables explained by the PCs, using the gl.pcoa.scree function in dartR.

We assessed divergence between clusters identified in the PCoA by determining the proportion of loci showing fixed allelic differences between the clusters. Fixed difference at a locus occurs when two populations share no alleles. When many loci are examined and sample sizes are finite, fixed differences will occur through sampling error. We used simulations implemented in dartR (Georges *et al*. 2018) to estimate the expected false positive rate in pairwise comparisons. We used a tloc of 0.05, meaning that SNP allele frequencies of 95/5 and 5/95 percent were regarded as fixed when comparing two populations at a locus.

To identify potential hybrid individuals between genetic groups, we analysed the SNP data with the program NewHybrids (Anderson & Thompson 2002) to identify F_1 and F_2 hybrid individuals and backcross individuals with one of the two parental species. We designated parental reference individuals on the basis of the PCoA. As the NewHybrids software can only utilise 200 loci due to processing limitations, we selected a subset of 200 loci that were most informative in assessing hybridization, namely loci that showed fixed differences between the parental populations, using the gl.nhybrids routine in dartR.

We inferred phylogenetic relationships among the samples using the concatenated SNP data set with two phylogenetic tree building methods suited to SNP data: SVDquartets and maximum likelihood. SVDquartets (Chifman & Kubatko 2014) accounts for differences in the genealogical histories of individual loci and for sequence variability due to both mutational and coalescent variance. Three independent runs of SVDquartets, with sampling of all possible quartets, were conducted in the program PAUP* version 4.0a build 165 (Swofford 2003) to assess topological convergence, each of which included 100 bootstrap replicates.

For the maximum likelihood approach, we used IQ-TREE (Nguyen *et al.* 2014), with the Lewis-type ascertainment bias correction, on the IQ-TREE webserver (Trifinopoulos *et al*. 2016). The ascertainment bias correction considers that no invariant sites are included in the data and helps reduce overestimation of tree lengths (Leaché *et al.* 2015). Heterozygous SNPs were recoded as the appropriate IUPAC ambiguity codes. We estimated the best substitution

model with ModelFinder (Kalyaanamoorthy *et al*. 2017) following the BIC. We assessed branch support with 1000 ultrafast bootstrap pseudo-replicates (Hoang *et al.* 2017).

Morphology. Details of the 183 specimens examined for morphology are listed in Supplementary Table S1. These specimens are held in the Australian Museum (AMS), Queensland Museum (QM) and South Australian Museum (SAMA) collections. Only adult specimens were included in morphometric analyses, and this was determined as follows. Males were included if the animal was calling when collected, nuptial pads were present, or if mature testes were evident on dissection. Females were included only if mature ova were visibly present.

Morphometric characters are described in Table 1. Measurements were taken from the right-hand side of each specimen, where relevant. Twelve measurements (SVL, HW, HL, IOD, IND, ED, TD, ETD, TL, THL, FL, FLL) were taken using digital callipers to the nearest 0.1 mm and two finer measurements (IMT, Fin3DW) were taken under a dissecting microscope with an eyepiece graticule. Summary data are presented as the mean ± SD and range.

For the multivariate analyses, sexes were analysed separately. Potentially confounding variation associated with differing body sizes and allometric growth was minimised by scaling measurements to a standard snout-vent length (using the mean value for each sex) using the following equation from Lleonart *et al.* (2000; p. 88): $y_i^* = y_i(x_o/x_i)^b$, where y_i^* and y_i are, respectively, scaled and measured values of a variable for specimen i, x_o is the standard body size (SVL in this instance) to which measurements are scaled, x_i is the observed body size of specimen I, and b is the mean of the regression coefficients estimated independently for each taxon from logarithmically transformed values of x_i and y_i (see Thorpe 1976, Lleonart *et al.* 2000). We used the R script *GroupStruct*, with the species option, to produce the adjusted mensural data (Chan & Grismer 2022). Multivariate analyses were performed on log transformed scaled measurements.

We used Principal Components Analysis (PCoA), which does not identify groups *a priori*, to explore the pattern of relationships among the specimens for the 14 morphological characters using the R function 'prcomp'. To describe the characters that could identify differences between taxa, and to test classification of individuals to predefined groups, Linear Discriminant Analysis (LDA) was performed on the 14 morphological characters using the 'lda' function from v7.3-40 of the R package MASS (Venables & Ripley 2002), implemented in RStudio v0.98.1028. We chose to use LDA because specimens could be allocated to putative taxa based on the results of the molecular genetic analysis.

TABLE 1. The 14 morphometric characters measured.

Code	Definition	Description
SVL	Snout-vent length	from the tip of the snout to the distal end of the urostyle
HW	Head width	at mid-tympanic level
HL	Head length	between the tip of the snout and the jaw articulation
IOD	Inter-orbital distance	distance between the eyes, anterior edge to anterior edge
IND	Inter-narial distance	distance between the nostrils, interior edge to interior edge
ED	Eye diameter	horizontal eye length (diameter)
TD	Tympanum diameter	greatest tympanum diameter
ETD	Eye-tympanum distance	distance from the anterior margin of the tympanum to the posterior corner of the eye
TL	Tibia length	external distance between knee and ankle with the knee and ankle joints held at right angles
THL	Thigh length	from the urostyle to the knee with the knee and hip joints held at right angles
FL	Foot length:	from the tip of the 4th toe to the proximal end of the inner metatarsal tubercle
IMT	Inner metatarsal tubercle length	greatest length of the inner metatarsal tubercle
FLL	Forearm length	from the flexed elbow to the base of the outer palmar tubercle
Fin3DW	Finger disk width	horizontal diameter of the disk on the 3rd finger

Colour and pattern characters. Within the *Litoria ewingii* Group there is considerable variation in colour and pattern on the concealed areas of the body and limbs. We scored the presence (+) or absence (-) of distinct black spots or blotches that were highly contrasting relative to background colour, and had a well demarked and distinct border, on four body areas illustrated in Figure 2: 1) the axilla, 2) inner thigh and inguinal region (groin), 3) entire concealed surface of the hind side of thigh (posterior thigh), and 4) the underside of the hindlimb. The influence of preservation on these characters was examined by comparing 12 specimens alive and post preservation.

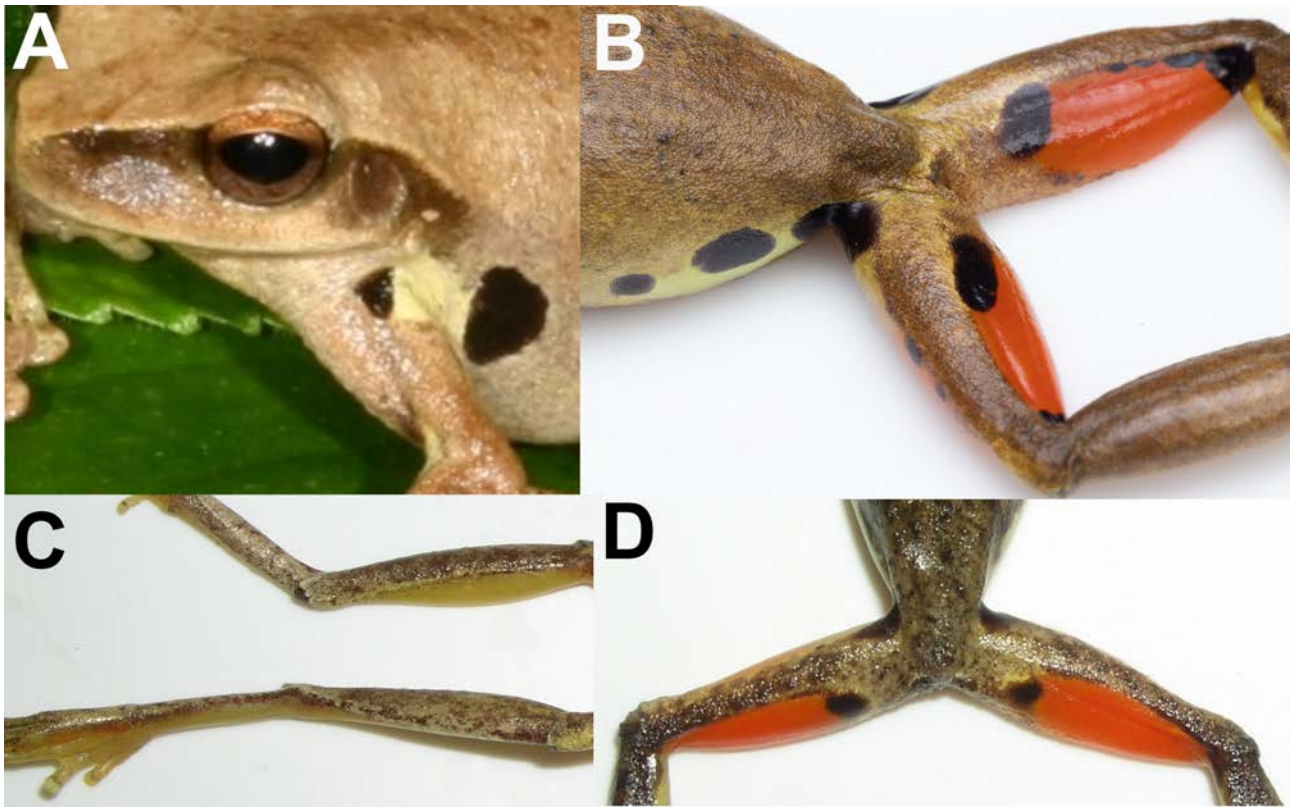

FIGURE 2. An illustration of the variation in colour pattern characters in the *Litoria revelata* complex. **A**) forelimb and axilla (no voucher, Mount William Creek, site e1, Eungella National Park, photo Harry Hines), **B**) inner thigh and inguinal region (no voucher, Eungella, photo Stephen Mahony), **C**) concealed surface of posterior thigh (SAMA R68976, Murrays Scrub, site r4, NSW), and **D**) multiple dark spots and marks on undersurface of hindlimbs (SAMA R68691, from Mt William Ck, site e1, Eungella National Park, Qld).

Advertisement call analysis. We obtained male advertisement calls recorded by LCP, MJM and CH, plus others contributed by colleagues or published on commercial compact discs (e.g. David Stewart, Nature Sound 1992), from published literature sources (Webster *et al.* 2023) and from the national citizen science project FrogID (Rowley *et al*. 2019, www.frogid. net.au) (see Supplementary Table S2 for details of locations and recorders). The latter calls were recorded via the FrogID app on smartphones, and files were made available in .acc format and converted to .wav files (44.1kHz 16 bit) using Switch Sound^{R} file conversion software. Because some calls were recorded in analogue, we converted files using direct line output from a tape player to a digital recorder (Marantz PMD60). Recordings were saved to .wav format at a sampling rate of 44.1 kHz with 16 bits per sample. We analysed these calls in Raven Pro 1.5© http://www.birds.cornell.edu/raven. Audiospectrograms were calculated using a fast-Fourier transform (FFT) of 512 points, 50% overlap, 172 Hz grid-spacing, and Hanning windows.

To describe the structural, temporal, and spectral properties of the sound we use the definitions of Köhler *et al*. (2017). For up to 5 consecutive calls per call recording (= individual), we measured the call duration (time in seconds; s); call repetition rate (calls/s); number of notes per call; note duration (s); note duty cycle (i.e., the note duration/inter-note interval); note repetition rate (i.e., note number divided by duration, as measured from five notes in the centre of the call, as per Köhler *et al*. [2017]); inter-note interval (s); pulse number; pulses rate (i.e., the number of pulses in a note divided by duration, as measured from notes 10 to 15 as per Köhler *et al*. [2017]); call

shape (i.e., the amplitude modulation of notes across the call), reported as call rise time (measured as duration from call start to where highest amplitude is reached, and expressed as a percentage); note rise time (a unitless measure of the shape of amplitude modulation of the pulses within notes, measured from the start of note 10 to the pulse with highest energy, and termed amplitude); dominant frequency of the call (kHz); and secondary frequencies (kHz) at the beginning and end of a series of notes in the middle of the call (notes 10 to 15).

Ambient temperature data was taken for nine of 16 recordings made by authors, and ambient temperatures were reported by Webster *et al*. (2023) for 46 calls of *L. revelata* for which measurements of call duration, number of notes, dominant frequency and note repetition rate were reported. No temperature data is submitted with FrogID calls, so for these recordings we estimated ambient temperature at the time of each recording using the 'bomrang' R (Sparks *et al*. 2017, Sparks *et al*. 2020) and 'chillR' (Luedeling 2019) packages, according to the methods of Mitchell *et al*. (2020). Three FrogID recordings did not return temperature data using these methods.

Some recordings had an associated voucher specimen, but most did not. For those recordings without an associated voucher specimen, we assigned species based upon location (based on the distribution of the three genetic groups). To avoid pseudoreplication, we used an individual (= recording) as the unit of replication, analysing means for each call variable for each individual (Köhler *et al*. 2017). To investigate the relationship between call variables and putative species (based on the genetic data), we performed a non-parametric Kruskal-Wallace (KW) test for each call variable, with species as a factor. To identify group differences, we then conducted pairwise K-W comparisons. To understand whether any of these variables were likely related to ambient temperature, we used a linear regression (we used only the 58 recordings for which temperature was recorded or estimated). We performed all statistical analyses of the advertisement calls in JMP 14.

Results

For clarity, we use the final taxonomic epithets throughout the manuscript rather than use an initial group nomenclature that would be changed to the final epithets in the taxonomy section. This does not mean we assume the separate taxonomic status of the three taxa within the *Litoria revelata* complex, but rather use the results section to test the hypothesis of three species, which are then described in the taxonomy section.

Mitochondrial DNA analysis. The final alignment comprised 787 bp of the 5' end of *ND4* and two flanking tRNAs. Three clades with strong support are consistently found in the phylogenetic analyses of these data: *L. revelata* (locations r1-22), *L. eungellensis* **sp. nov.** (locations e1-2), and *L. corbeni* (locations c1-3) (Figs 1, 3). The analyses also show strong support for a sister-species relationship between the sequences of *L. revelata* and *L. eungellensis* **sp. nov.**, with *L. corbeni* more divergent (Fig. 3). Pairwise average net sequence divergence between *L. revelata* and *L. eungellensis* **sp. nov.** was 3%, compared to 8% between *L. revelata* and *L. corbeni* and 9% between *L. corbeni and L. eungellensis* **sp. nov.** (Table 2). The mtDNA sequence divergence among the three species is of similar magnitude to numerous other sister lineage comparisons among pelodryadids, which range from 4 to 25% (Donnellan *et al*. 2023).

In the 787 bp alignment, *L. revelata* is diagnosed by 23 apomorphic diagnostic nucleotide sites, *L. eungellensis* **sp. nov.** by 23 sites, and *L. corbeni* by 24 sites (Table 3).

SNPs. A total of 142,147 polymorphic SNP loci were scored for 61 individuals of the *L. revelata* complex. After filtering on call rate (indiv. 0.5, loci 0.9) and reproducibility (0.99), the data set comprised 1,709 polymorphic SNP loci, with the proportion of missing data per individual ranging from 1.17% to 16.6% (Supplementary Table S3). In the initial clustering analysis via PCoA, the proportion of explained variance by the first three PC axes was: 1st axis—39.4%; 2nd axis—25.4%, and 3rd axis—6.7%. Three major genetic clusters are apparent in the PCoA: *L. littlejohni/L. watsoni*; the *L. revelata* complex; and a more dispersed group comprising *L. ewingii*/*L. jervisiensis*/*L. verreauxii* group 1/*L. verreauxii* group 2 (Fig. 4A, Supplementary Table S3). Two individuals, ABTC 25436 and 27476, were scattered between the *L. ewingii/L. jervisiensis/L. verreauxii* cluster and the *L. revelata* cluster. Another two individuals, ABTC16984 and 25915, fell between species clusters within the *L. ewingii/L. jervisiensis/L. verreauxii* cluster and may represent hybrids (see below).

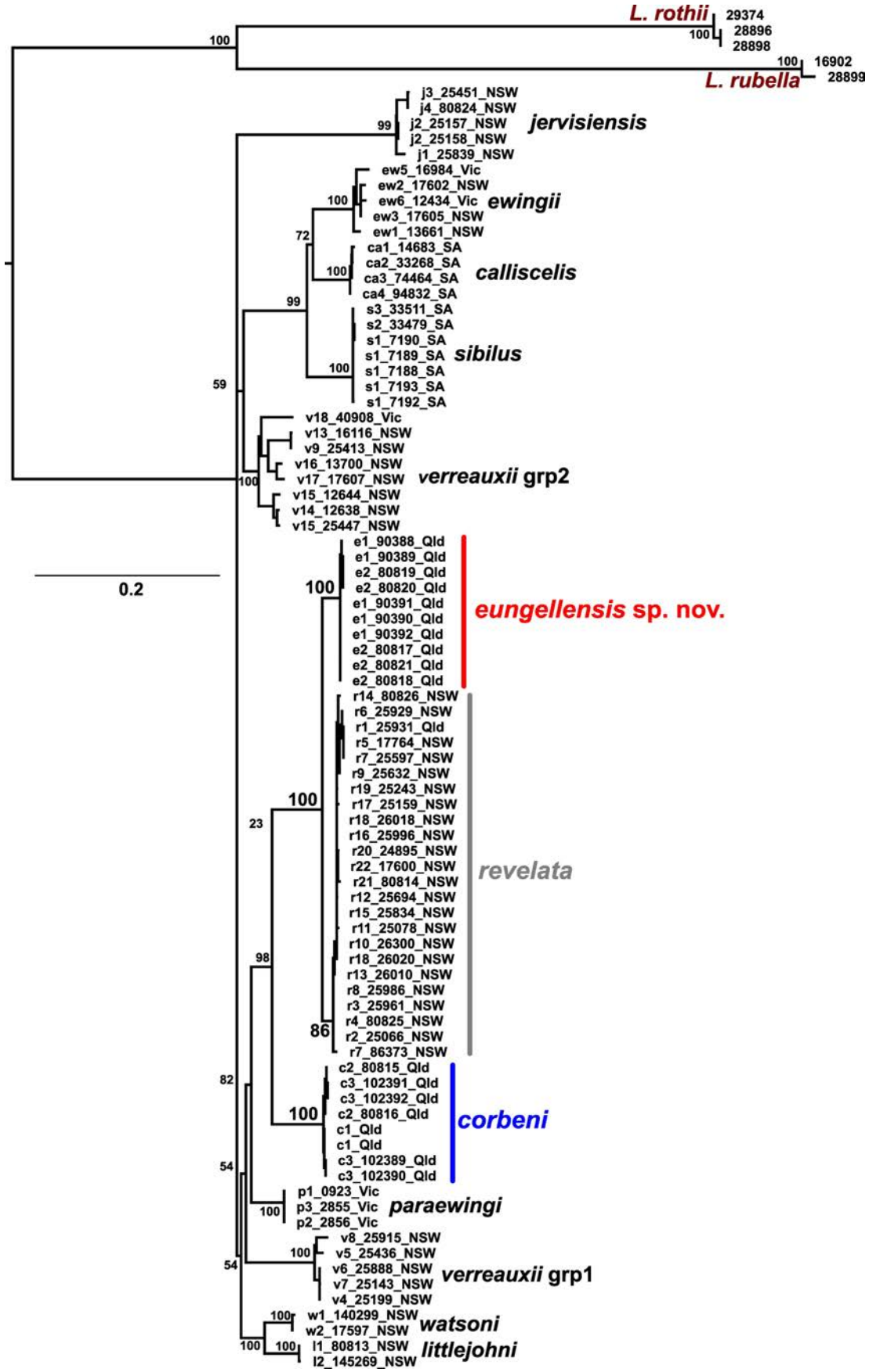

FIGURE 3. Mitochondrial *ND4* maximum likelihood phylogram for the *Litoria ewingii* Group, with bootstrap pseudoreplicate proportions at key nodes. See Appendix 1 for location codes.

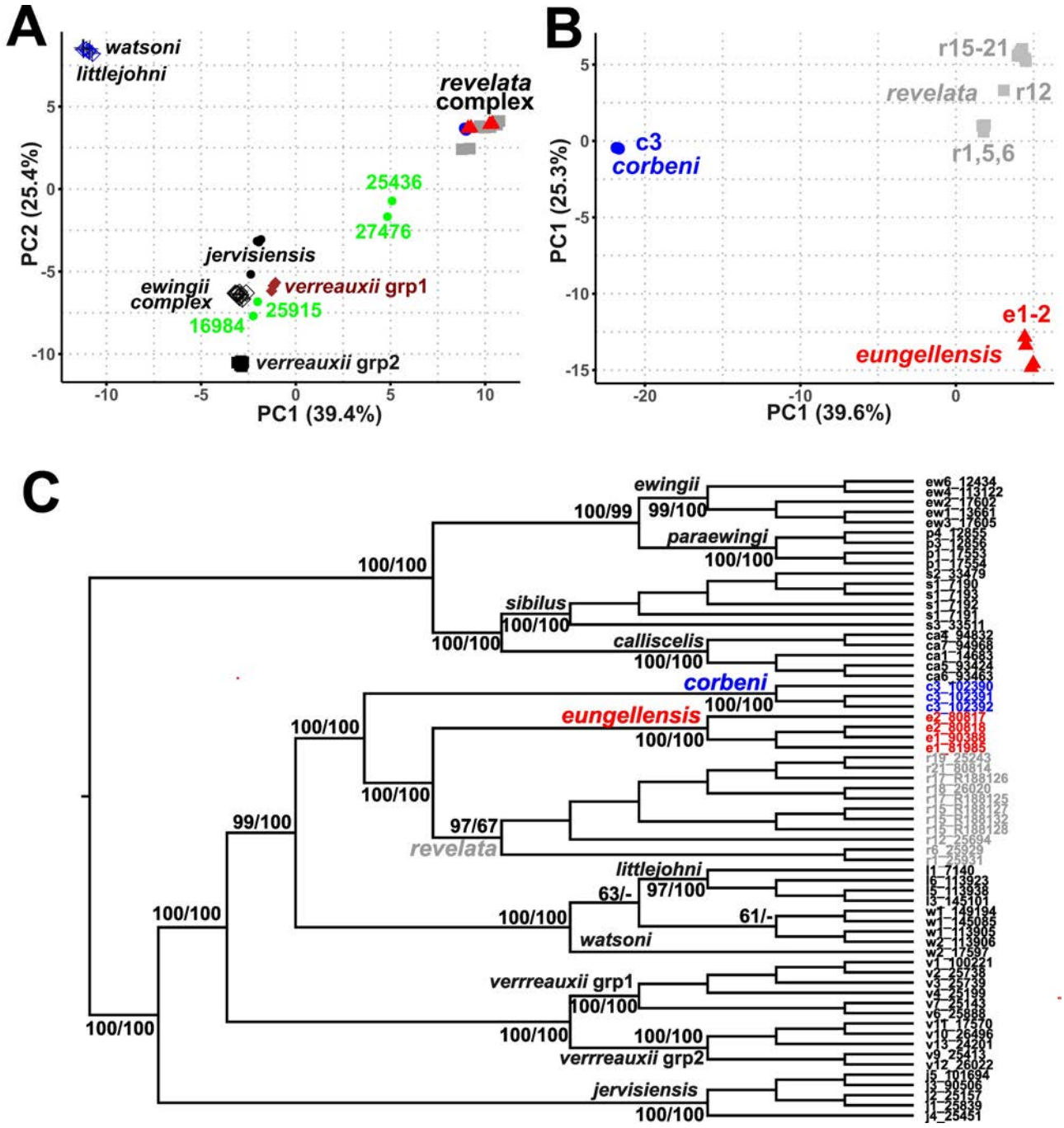

FIGURE 4. Analyses of SNP data for the *Litoria ewingii* Group. **A**) PCoA of SNP data with all species and potential hybrids (green) included. **B**) PCoA of SNP data of *L. revelata* complex only; hybrids not included. For location codes refer to Appendix 1. **C**) SVD Quartets tree, with bootstrap pseudoreplicate proportions indicated at nodes, for SVD Quartets (left) and maximum likelihood (right) analyses. The IQ-TREE is included as Supplementary Fig. S1. Hybrids were not included.

A more detailed analysis was conducted for 20 samples from the *L. revelata* complex cluster. For this analysis, 2,123 loci were retained after filtering on call rate (indiv. 0.5, loci 0.9) and reproducibility (0.99), with the proportion of missing data per individual ranging from 0.9% to 15.8% (Supplementary Table S3). In the PCoA of this dataset, the proportion of variance explained by the first three PC axes was: 1st axis—39.6%; 2nd axis—25.3%, and 3rd axis—6.7%. Three major groups were present in the PCoA: *L. revelata* (north-eastern NSW and south-eastern Qld: locations r1, 5, 6, 12, 15, 17, 18, 19, 21), *L. corbeni* (north-eastern Qld: location c3), and *L. eungellensis* **sp. nov.** (mid-eastern Qld/Eungella: locations e1, 2) (Fig. 4B). The number of loci having fixed differences between

the three taxa ranged from 145 to 892 loci (out of 2,123 loci analysed) (Table 2B). All values were significant after simulation.

TABLE 2. A) Average net genetic distances between taxa of the *Litoria ewingii* Group (*dA*) in the *ND4* alignment. Bold values show distances in the *Litoria revelata* complex.

	1	2	3	4	5	6	7	8	9	10	11	12
1 *eungellensis* **sp. nov.**	-											
2 *revelata*	**0.03**	-										
3 *corbeni*	**0.09**	**0.09**	-									
4 *calliscelis*	0.12	0.12	0.10	-								
5 *sibilus*	0.12	0.12	0.11	0.06								
6 *ewingii*	0.11	0.11	0.10	0.06	0.07	-						
7 *paraewingi*	0.08	0.08	0.08	0.10	0.11	0.10	-					
8 *jervisiensis*	0.14	0.13	0.13	0.13	0.13	0.12	0.12	-				
9 *verreauxii* grp1	0.09	0.09	0.10	0.10	0.11	0.10	0.07	0.12	-			
10 *verreauxii* grp2	0.07	0.07	0.07	0.07	0.08	0.07	0.05	0.10	0.06	-		
11 *littlejohni*	0.08	0.09	0.10	0.11	0.12	0.11	0.08	0.13	0.08	0.06	-	
12 *watsoni*	0.08	0.09	0.09	0.10	0.10	0.09	0.06	0.12	0.08	0.06	0.05	-

B) Numbers of loci showing a fixed difference between taxa of the *Litoria revelata* complex. Upper matrix: numbers of loci showing a fixed difference between taxa, with [number of loci compared]. Lower matrix: numbers of loci expected to show a fixed difference between taxa after simulation. All values were significant after simulation.

	corbeni	*revelata*	*eungellensis* **sp. nov.**
corbeni	-	1019 [2737]	1178 [2153]
revelata	37	-	511 [2769]
eungellensis **sp. nov.**	55	18	-

NewHybrids identified an F_1 hybrid (ABTC16984) between *L. ewingii* and *L. verreauxii* group 2, along with individuals of mixed ancestry between *L. revelata* and *L. verreauxii* group 1 (ABTC17764 [backcross], ABTC25436 [F_2], R188133 [backcross]), between *L. revelata* and *L. verreauxii* group2 (ABTC26476 [F_2]), and between *L. verreauxii* group 1 and *L. verreauxii* group 2 (ABTC25915 [F_2]).

In the phylogenetic analysis based on SVD Quartets, with the individuals of mixed ancestry excluded, the three genetic groups of the *L. revelata* complex are each monophyletic and well-supported, with *L. revelata* in a well-supported sister clade relationship with *L. eungellensis* **sp. nov.** and *L. corbeni* the sister to that clade (Fig. 4C). The maximum likelihood analysis (Supplementary Fig. S1) also recovered the same set of relationships with strong support, as shown by the bootstrap proportions in Figure 4C.

Morphology. Variation among the three taxa in the *L. revelata* complex for 14 metrics and five ratios is summarised in Table 4. In terms of body size, SVL distinguishes female *L. eungellensis* **sp. nov.** from the other two species (Table 4, *L. revelata vs L. corbeni* t-ratio 3.6872, DF 7.86, p=0.0063*, *L. revelata vs L. eungellensis* **sp. nov.** t-ratio -8.5466, DF 7.94, p<0.0001*, *L. corbeni vs L. eungellensis* **sp. nov.** t-ratio 10.3567, DF 7.999, p<0.0001*). In terms of body shape, the PCA did not provide clear separation of the three groups for males (Fig. 5D). In the PCA of males, PC1 explains 26.7% of the overall variation and is dominated by FL, IOD, FLL and TL. PC2 accounts for 10.3% of the variation and is dominated by TD. In the PCA of female shape, female *L. eungellensis* **sp. nov.** were distinguished from the other two taxa (Fig. 5B). In the PCA of females, PC1 explains 49.9% of the overall variation and is dominated by TL, HW and FL. PC2 accounts for 13.8% of the variation and is dominated by TD and IMT.

TABLE 3. Diagnostic sites in the *ND4* alignment for the *Litoria ewingii* Group. The top row shows site number in the *ND4* alignment. Apomorphic sites for the *L. revelata* complex species are indicated in bold.

				1	1	1	2	2	2	2	2	2	2	2	2	3	3	3	3	3	3	3	3	3	3
	3	4	8	0	8	8	1	1	3	3	5	6	7	7	8	0	1	2	3	5	5	7	8	9	9
	2	5	6	1	5	6	2	5	0	9	1	0	6	8	1	2	7	6	8	6	7	4	9	2	5
L. corbeni	C	**C**	T	**G**	T	T	**T**	A	C	A	**G**	T	C	**T**	**A**	T	T	T	A	**T**	**C**	**G**	**C**	T	**C**
L. eungellensis **sp. nov.**	**T**	T	**C**	A	**C**	**C**	C	**G**	**T**	**G**	A	T	**T**	A	G	**C**	**C**	**C**	**G**	A	T	A	T	**C**	T
L. revelata	**T**	T	**C**	A	**C**	**C**	C	**G**	C	A/G	A	**C**	C	G	G	**C**	**C**	**C**	A	A	T	**G**	T	**C**	T
L. calliscelis	C	T	T	A	T	T	C	A	C	A	A	T	C	G	A	T	T	T	A	A	C	A/G	C	T	T
L. ewingii	C	T	T	A	T	T	C	A	C	A	A	T	C	A/G	A	C	T	T	A	A	C	A/G	T	T	T
L. sibilus	C	T	C	A	T	T	C	A	C	G	G	C	C	G	A	T	T	C	A	A	C	A	T	T	T
L. paraewingi	C	T	T	G	T	T	C	A	C	A	A	T	C	A	G	T	T	T	A	A	T	A	T	T	T
L. jervisiensis	C	T	T	A	G/T	T	T	A	T	A	A/G	C	C	A	A	T	T	T	A	A	C	A/G	T	T	C
*L. ver*_grp1	T	T	T	G	C	T	C	A	C	A	A	T	C	T	G	T	T	T	A	A	T	A	T	C/T	T
*L. ver*_grp2	C/T	T	T	A/G	T	T	C/T	A/G	C	A/G	A	T	C	A	G	T	T	T	A	A	C/T	A/G	C/T	C/T	T
L. littlejohni	C	T	T	A	C	T	C	A	C	A	A	T	T	A	G	T	T	T	A	A	T	G	T	T	T
L. watsoni	C	T	T	A	T	T	C	A	C	A	G	T	T	A	G	T	T	T	A	A	T	A	T	T	T

	4	4	4	5	5	5	5	5	5	5	5	5	5	5	6	6	6	6	6	6	6	7	7	7	7
	3	4	4	0	0	0	0	1	1	2	3	3	6	8	0	0	5	6	6	7	9	4	4	5	6
	8	3	6	0	3	4	6	5	8	5	3	6	6	5	5	7	0	3	7	4	2	7	9	5	1
L. corbeni	**T**	**A**	A	A	C	C	C	A	**G**	T	**C**	**C**	**C**	**C**	A	A	**G**	G	C	A	A	**G**	**G**	**C**	**C**
L. eungellensis **sp. nov.**	C	C	A	A	**T**	**T**	**A**	**G**	A	T	T	T	T	T	**T**	A	A	**A**	**T**	**T**	**C**	A	A	T	T
L. revelata	C	C	**G**	**T**	**T**	**T**	**A**	A	A	**C**	T	T	T	T	**T**	**G**	A	G	**T**	**T**	T	A	A	T	T
L. calliscelis	C	C	A	A	C	C	T	A	G	T	T	T	C	T	A	A	A	G	C	G	A	A	A	T	T
L. ewingii	C	C	A	A	C	C	T	A	G	T	T	T	T	T	A	A	A	G	C	A	G	A	A	C	C
L. sibilus	C	C	A	A	C	C	C	A	A	T	T	T	T	T	A	A	A	G	C	A	A	A	A	C	C
L. paraewingi	C	C	A	A	C	C	C	A	A	T	T	T	T	T	A	A	A	G	C	G	A	A	A	T	T
L. jervisiensis	C	A	G	A	C	T	C	A	A	C	T	T	C	T	A	G	A	G	C	T	G	A	A	T	T
*L. ver*_grp1	C	C	A/G	T	C	C	C	G	A	T	T	T	T	T	A	A	A	G	C	A	A	A	A	C	C/T
*L. ver*_grp2	C	C/T	A/G	A	C/T	C	C	A	A	T	T	T	C/T	T	A	A	A	G	C	A	A	A	A/G	T	C/T
L. littlejohni	C	C	G	A	T	C	C	G	A	T	T	T	T	T	A	A	A	G	C	A	T	A	A	T	T
L. watsoni	C	C	A	A	C	C	C	G	A	T	T	T	T	T	A	A	A	G	C	A	A	A	A	T	T

TABLE 4. Summary of data for 14 measurements and five ratios from adult individuals in the *Litoria revelata* complex, presented as mean ± standard deviation and range.

N	*L. corbeni*	*L. eungellensis* **sp. nov.**	*L. revelata*
	31 (23M, 8F)	17 (12M, 5F)	116 (98M, 18F)
SVL (female)	32.1±0.9	38.5±0.9	34.0±1.2
	31.0–33.6	37.3–39.5	32.0–37.1
SVL (male)	27.8±2.1	29.6±1.2	28.2±1.2
	23.7–32.4	28.3–31.8	24.7–31.6
HW	8.7±0.8	9.8±1.5	8.7±0.8
	7.7–10.0	8.5–13.1	7.0–11.2
HL	8.4±0.7	9.3±1.7	8.5±0.9
	7.3–10.6	6.9–12.1	5.6–10.8
IOD	6.1±0.7	6.8±0.7	6.1±0.5
	4.9–7.3	5.8–8.1	4.5–7.5
IND	2.3±0.2	2.5±0.2	2.3±0.3
	2.0–2.8	2.2–2.8	1.3–2.9
ED	3.2±0.3	3.6±0.4	3.5±0.3
	2.7–3.8	3.0–4.5	2.8–4.2
TD	1.6±0.2	1.8±0.3	1.8±0.2
	1.2–2.0	1.3–2.3	1.3–2.4
ETD	1.2±0.3	1.2±0.4	1.2±0.2
	0.1–1.6	0.7–2.1	0.6–1.7
TL	14.9±1.2	17.5±2.6	15.5±1.4
	13.1–17.5	15.0–22.1	13.4–19.5
THL	14.1±1.2	15.9±2.2	14.1±1.5
	12.2–16.1	13.8–20.7	12.2–18.5
FL	13.6±1.3	15.3±1.4	13.6±1.3
	11.0–16.1	13.7–18.4	11.0–17.3
IMT	0.97±0.17	1.14±0.33	1.11±0.21
	0.70–1.30	0.10–1.80	0.30–1.60
FLL	6.75±0.60	7.55±0.89	6.91±0.60
	5.90–8.30	6.60–9.10	5.80–8.80
Fing3DW	1.40±0.20	1.46±0.23	1.31±0.27
	0.90–1.70	1.00–1.90	0.60–2.00
HL/SVL	0.29±0.02	0.29±0.02	0.29±0.02
	0.23–0.34	0.24–0.31	0.20–0.34
HL/HW	0.97±0.07	0.95±0.06	0.98±0.06
	0.81–1.07	0.76–1.03	0.67–1.09
ED/HL	0.39±0.03	0.39±0.03	0.41±0.04
	0.26–0.44	0.33–0.46	0.32–0.52
TD/ED	0.50±0.08	0.51±0.06	0.52±0.06
	0.38–0.70	0.30–0.58	0.38–0.67
TL/SVL	0.52±0.03	0.54±0.02	0.53±0.02
	0.45–0.59	0.52–0.57	0.48–0.58

Plots of the linear discriminant functions (LD) summarising variation in size and shape in females and males are presented in Figures 5A and 5C. For females, 100% of specimens overall were assigned correctly to posterior classification, which reduced to 90% after jack-knifing. Traits with the highest coefficients of linear discrimination were TL, SVL, THL and FLL for LD1 and TL, HW, FLL, and FL for LD2 (Supplementary Table S1). For males, partial separation was achieved with 88% correctly assigned to posterior classification, which was slightly reduced to 83% after jack-knifing. Of the 100 *L. revelata* examined, 92% were classified correctly; 83% of the 12 *L. eungellensis* **sp. nov.** were classified correctly; and 65% of the 23 *L. corbeni* were classified correctly. Traits with the highest coefficients of linear discrimination were THL, FL, ED, HW and TL for LD1 and TL and SVL for LD2 (Supplementary Table S1).

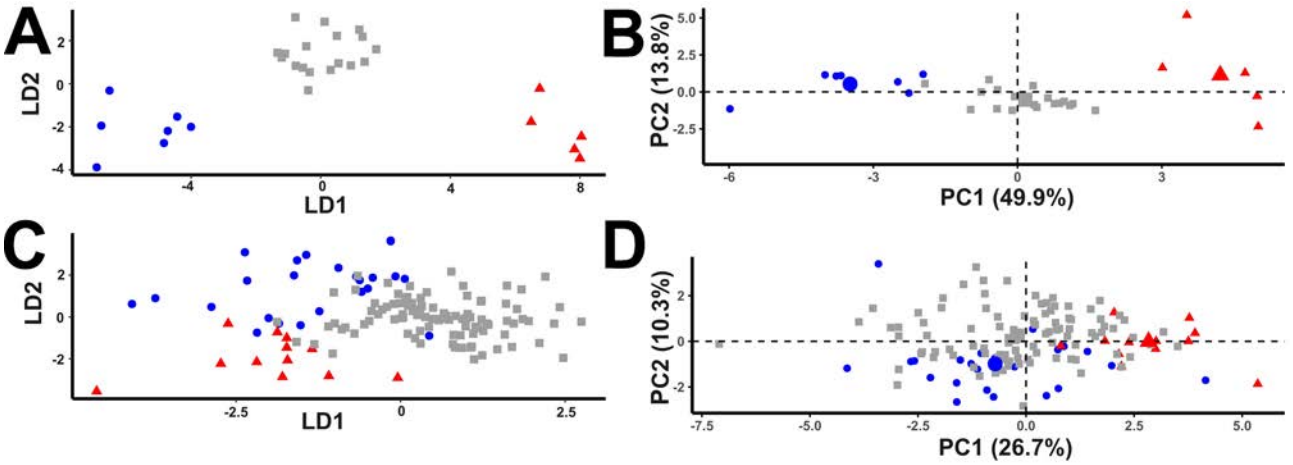

FIGURE 5. LDA and PCA plots for *Litoria revelata* complex for transformed body measurement data. **A**) LDA biplot of females; **B**) PCA biplot of females; **C**) LDA biplot of males; **D**) PCA biplot of males. Large symbols in B and D represent group centroids.

Colour pattern characters. A summary of variation in the frequency of the four colour pattern characters is presented in Table 5 and illustrated in Figure 6. The colour pattern characters across the four body regions differ between the three groups in the *L. revelata* complex. All 10 individuals of *L. eungellensis* **sp. nov.** had black or dark spots or marks on the underside of the hindlimbs, contrasting with their absence in 103 of 111 individuals of *L. revelata* and absence in all *L. corbeni*. The eight individuals of *L. revelata* that had dark spots or marks on the underside of the hindlimb were all from south-eastern Qld/north-eastern NSW (Table 5).

TABLE 5. The frequency of the presence (+) or absence (-) of distinct black spots or blotches located on four body areas of the specimens of *Litoria corbeni*, *L. eungellensis* **sp. nov.** and *L. revelata* from south-eastern Queensland/north-eastern NSW [north of -30.33] (left-hand number) and *L. revelata* from south-eastern New South Wales [south of -31.33] (right-hand number).

taxon	N	axilla	groin	thigh	underside of hindlimb
corbeni	2	+	+	-	-
corbeni	2	-	+	+	-
corbeni	2	-	+	-	-
eungellensis **sp. nov.**	10	+	+	+	+
revelata	6/0	+	+	+	+
revelata	23/0	+	+	+	-
revelata	3/2	+	+	-	-
revelata	1/0	+	-	+	+
revelata	1/0	+	-	+	-
revelata	2/0	+	-	-	-

.....continued on the next page

TABLE 5. (Continued)

taxon	N	axilla	groin	thigh	underside of hindlimb
revelata	1/0	-	+	+	+
revelata	4/1	-	+	+	-
revelata	4/10	-	+	-	-
revelata	3/0	-	-	+	-
revelata	13/37	-	-	-	-

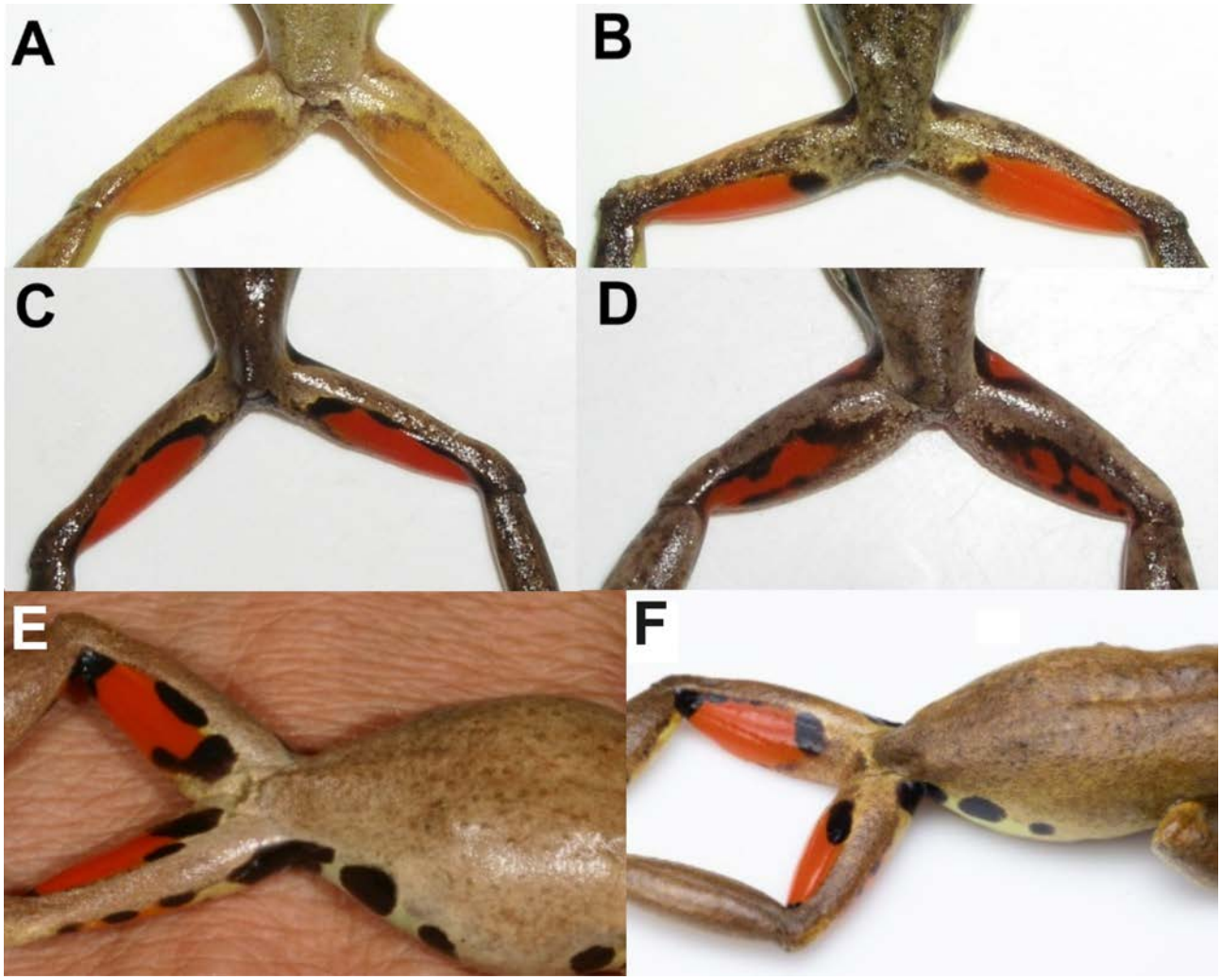

FIGURE 6. Examples of colour pattern character states for the groin and posterior thigh for the *Litoria revelata* complex. **A**) orange without a dark border (SAMA R69002, from Wild Cattle Ck, site r11, NSW); **B**) orange with a distinct dark expanded margin at the anterior and posterior ends (SAMA R68976, from Murrays Scrub, site r4, NSW); **C**) as in B but with a distinct dark border encompassing the orange area (SAMA R68977, from Murrays Scrub, site r4, NSW); **D**) as in C but with the distinct dark border invading the orange area laterally (no voucher, from Watagan State Forest, site r20, NSW); **E**) multiple dark spots and marks in groin and posterior thigh (ABTC81985, from Mt William Creek, site e1, Eungella, Qld, photo Harry Hines); **F**) multiple dark spots and marks in groin, posterior thigh and along hindlimbs (no voucher, Eungella, Qld, photo Stephen Mahony).

Description of advertisement calls. We analysed the male advertisement calls of 79 individuals of *L. revelata,* nine *L. eungellensis* **sp. nov.**, and 15 *L. corbeni* (Table 6). All males were calling in choruses. The three taxa emit calls that are similar in structure, and all produce two distinct vocalisations: (i) the 'whirring' call that is associated with reproduction and fits the definition of an advertisement call according to Köhler *et al.* (2017), and (ii) a shorter

single note call, which is probably an agonistic/territorial call directed at other males (Webster *et al.* 2023). As the shorter call was not recorded for all males, we analysed only the longer advertisement call.

First, we describe the call characteristics for the *L. revelata* complex. In the three species, the advertisement call comprises a series of rapidly repeated notes with a distinct inter-note interval (Fig. 7). Note structure remains the same (pulse amplitude pattern and inter-pulse interval) across the call, except that the number of pulses within each note increases gradually from the commencement of the call until it reaches a stable number after about the first quarter or third of the call. Because note structure is uniform across the call, it is classed as a simple call with note repetition (Kohler *et al.* 2017). In most calls, the average number of notes in a call is above 30 and the call duration is greater than 6 seconds. Males produce calls with a relatively stable mean number of notes, and a relatively constant call repetition rate (Table 6). The amount of energy in a note (relative amplitude of pulses in a note, see Fig. 7C, E, G) increases gradually from the first note to reach a maximum between the middle and two-thirds of the call (Table 6). We define this as the call rise time (see Crocroft & Ryan [1989]). There is a decrease in energy in notes in the last quarter of the call. Frequency modulation occurs both across the call with the first couple of notes with a lower frequency and also within each note where the frequency is modulated gradually from lower to higher frequency (Fig. 7).

TABLE 6. Call data for the *Litoria revelata* complex. N = number of individuals measured, then number of locations. Note duty cycle = note duration/inter-note interval. Values represent means ± SD, and ranges per species. Statistical comparisons of advertisement calls among the taxa used the nonparametric Kruskal-Wallace test with Z scores and P values. [W] indicates data from 45 individuals of *L. revelata* from Wallingat State Forest, NSW from Webster *et al*. (2023) that were included in the analyses. For details of locations, recorder, and temperatures see Supplementary Table S2.

Species	N	Pulse repetition rate (s-1)	Call duration (s)	Note duration (s)	Number of notes in call	Note duty cycle (%)	Dominant frequency (Hz)
L. corbeni	13, 4	130.20±4.50	4.60±0.73	0.15±0.003	27.50±5.80	2.21±0.18	4157±213
		127.30–136.70	3.10–4.90	0.10–0.109	18–33	2.10–2.36	3938–4307
L. eungellensis **sp. nov**.	9, 1	78.12±10.70	7.60±0.29	0.12±0.010	39.30±1.90	2.94±0.37	4211±102
		66.90–86.20	4.40–5.30	0.10–0.13	30–35	2.08–3.12	4125–4313
L. revelata	34 + 45[W], 7	105.30±10.10	5.70±1.30	0.12±0.019	35.30±7.39	2.52±1.26	4403±213
		86.90–127.30	3.30–8.50[W]	0.80–0.18	15–52[W]	1.62–6.02[W]	3962–4996[W]
nonparametric Kruskal-Wallace test							
L. corbeni vs L. revelata		-2.27; 0.0005*	3.76; 0.0318*	2.44; 0.9694	2.31; 0.0035*	1.71; 0.2165	2.71; 0.0037*
L. eungellensis **sp. nov.** *vs L. revelata*		-3.25; <0.0019*	2.02; 0.0100*	0.07; 0.0023*	-1.46; 0.4659	2.33; 0.1706	2.95; 0.0915
L. corbeni vs L. eungellensis **sp. nov.**		-0.50; <0.0009*	1.74; 0.0001*	-3.19; 0.0062*	2.79; 0.0055*	1.17; 0.0218*	1.20; 0.8497

The notes of the call comprise fully amplitude-modulated pulses that are spaced evenly (constant inter-pulse interval, Table 6) and therefore the pulse repetition rate remains reasonably constant across the call (Fig. 7A, B, C; Table 6). At the beginning of the call, the notes comprise an average of 10–12 pulses, and by the middle of the call they average 13–14 pulses. Note duration increases as a direct function of increase in the number of pulses in the notes, until pulse number and note duration are stable in the middle of the call (Fig. 7A, B, C. Table 6). The inter-note interval is consistent across the call (Fig. 7A, B, C. Table 6).

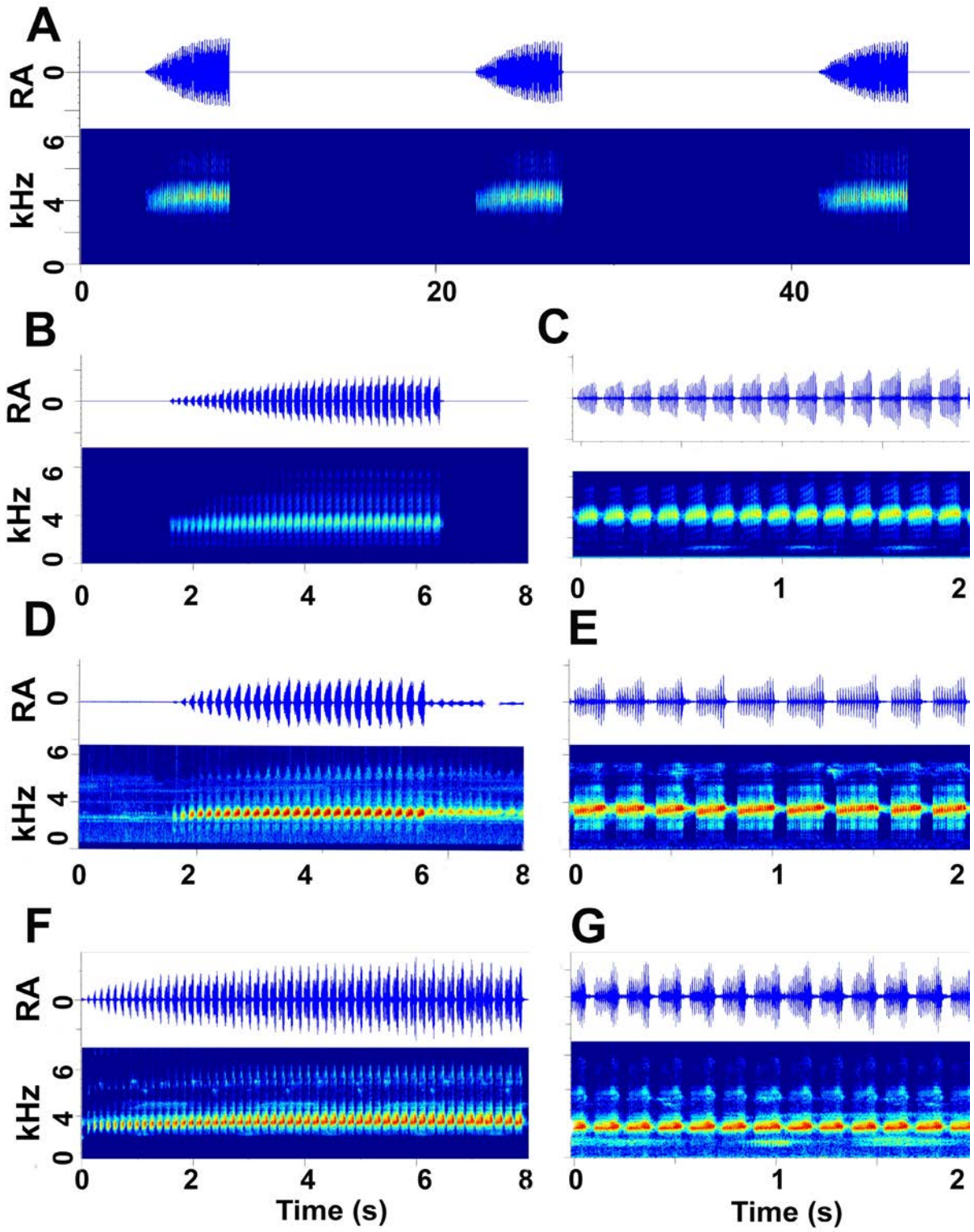

FIGURE 7. Comparative oscillograms and spectrograms of advertisement calls for the *Litoria revelata* complex. *Litoria eungellensis* **sp. nov.** (Eungella, Qld, Stephen Mahony): **A)** three calls over 50 second duration (each call is about 5 seconds duration, with an inter-call interval of about 12 seconds); **B)** single advertisement call over eight seconds duration, to show overall call structure; **C)** middle notes enlarged over a two second duration, to show note and pulse structure. *Litoria corbeni* (Butchers Creek, Atherton Tablelands, Qld, David Stewart: **D)** single advertisement call, over eight seconds duration to show overall call structure; **E)** middle notes enlarged over a two second duration, to show note and pulse structure. *Litoria revelata* (Luke Price, Tooloom Range, NSW): **F)** single advertisement call, over eight seconds duration to show overall call structure; **G)** middle notes enlarged over a two second duration, to show note and pulse structure. Abbreviations: kHz—kilohertz; RA—relative amplitude.

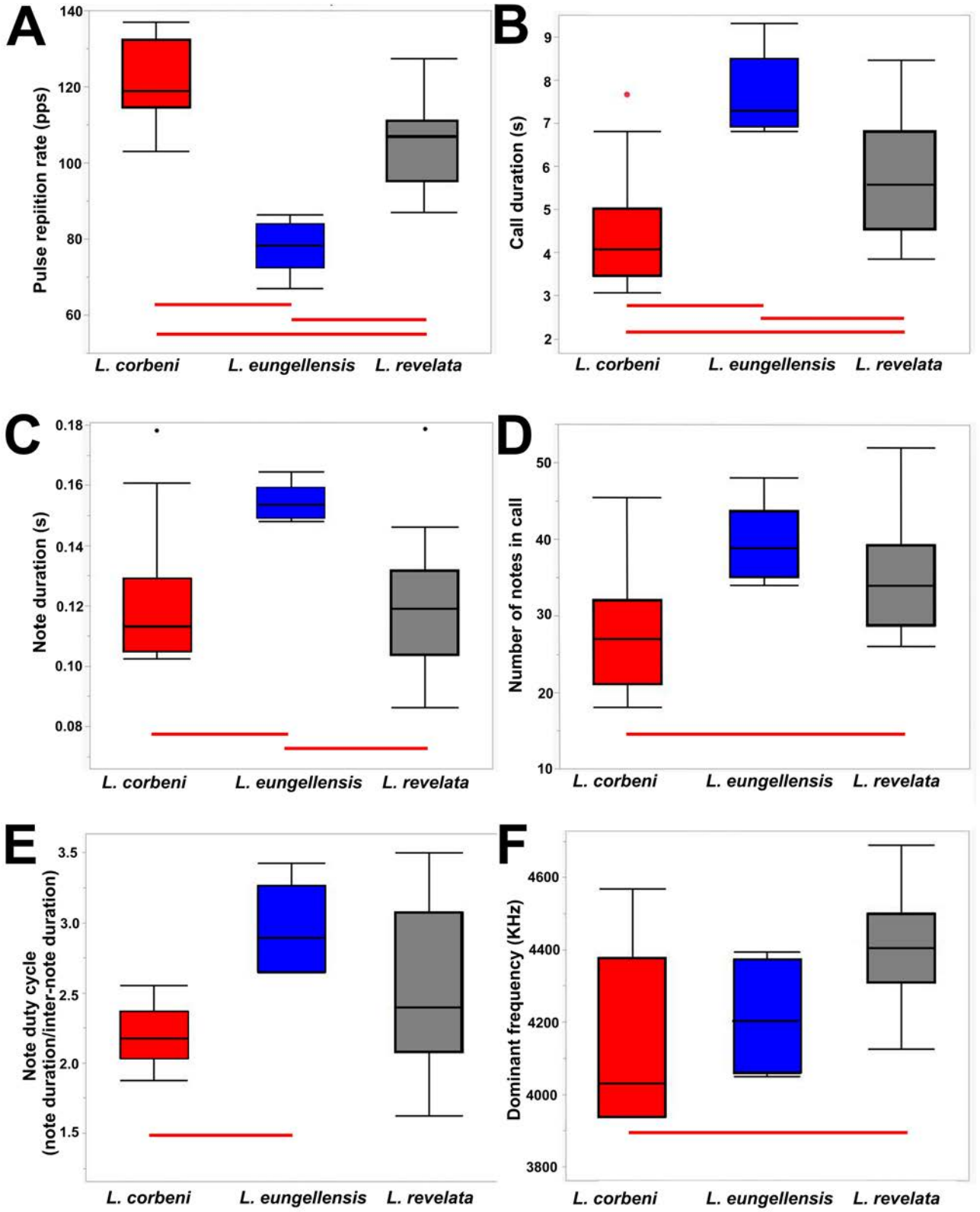

FIGURE 8. Boxplots for selected advertisement call attributes (means ± SE, and range) for the *Litoria revelata* complex: **A**) pulse repetition rate; **B**) call duration (s); **C**) note duration (s); **D**) number of notes in call; **E**) note duty cycle; **F**) dominant frequency (Hz). Red bars at bottom of each plot indicate which pairwise comparisons were significant (see Table 6).

Second, we describe the call characteristics that vary among the species of the *L. revelata* complex. Relative amplitude of the pulses within notes was consistent among individuals within a species but varied among the species. In *L. revelata*, the pulses formed a two-peak pattern (i.e., the amplitude of the waveform rises to a peak followed by a slight or moderate trough before rising again), while in *L. corbeni* and *L. eungellensis* **sp. nov.** they showed a wedged shaped pattern, with the highest energy pulse towards the end of the note (Fig. 7 E, G & C; Table 6). To compare note shape quantitatively, and independent of temperature, we measured the duration from the start of the note to the pulse of peak energy (note rise time; Table 6) and calculated it as a percentage of call length. Spectrograms showed sparse harmonics, with a dominant band (Beeman 1998), so we compare the dominant frequency from notes in the middle of the call and also report higher and lower frequency bands.

Analysis of advertisement calls. Temperature was found to effect temporal attributes of the call: call duration, note duration, note duty cycle and pulse repetition rate. We found a negative linear relationship between temperature and call duration (R^2=0.30, $F_{1,77}$ =33.5; P<0.001), note duration (R^2=0.122, $F_{1,32}$=4.46; P=0.0425), and note duty cycle (R^2=0.167, $F_{1,27}$=5.40; P=0.0279). We found a positive linear relationship between temperature and pulse repetition rate (R^2=0.38, $F_{1,75}$ =46.78; P<0.001). In contrast, temperature did not significantly affect structural attributes: number of notes per call (R^2=0.250, $F_{1,27}$=9.01; P=0.06), number of pulses (R^2=0.019, $F_{1,77}$=0.47; P=0.23), and dominant frequency (R^2=0.024, $F_{1,72}$=1.8; P=0.1840).

Given the similarity in call structure, most metrics overlapped to some degree, but several were significantly different in pairwise comparisons between the species following correction for the effect of temperature (Table 6). The calls of *L. revelata* could be distinguished from those of *L. eungellensis* **sp. nov.** by having a significantly higher pulse repetition rate, a shorter call duration, a longer note duration, and fewer notes (Table 6, Fig. 8A, B, C). The calls of *L. revelata* could be distinguished from those of *L. corbeni* by having a significantly lower pulse repetition rate, a shorter call duration, a larger number of notes per call, and a higher dominant frequency (Table 6, Fig. 8A, B, D, F). The calls of *L. eungellensis* **sp. nov.** could be distinguished from those of *L. corbeni* by having a significantly lower pulse repetition rate, a longer call duration, a longer note duration, and a longer note duty cycle (Table 6, Fig. 8A, B, C, E)

Systematic Implications. The multiple lines of evidence presented above lead us to recognize three species in the *L. revelata* complex. The mtDNA shows that the three major populations are each monophyletic, with divergences between the clades on par with those seen between other recognized *Litoria ewingii* Group species. The SNP data strongly supports this, with three divergent genetic clusters in the SNP-based phylogeny, with relationships that correspond to those of the mtDNA clades. Furthermore, the SNP genetic clusters are defined by numerous fixed differences. Morphological and call traits also differ diagnosably among the three genetic groups.

Taxonomy

Members of the *Litoria ewingii* Group share the following characters: small, medium or large body size (35 mm–68 mm SVL); medium to long legs (TL/SVL 0.42–0.53); medium relative eye size (ED/SVL 0.1–0.12); visible tympanum; vocal sac present; granular nuptial pads; no or reduced finger webbing; minimal or reduced toe webbing; expanded finger and toe discs; toe disc width equal to, or smaller than, finger discs; tadpole oral disc Type 1 (*sensu* Anstis 2017); tadpole body morphology Type 1 (*sensu* Anstis 2017); and small to medium sized, pigmented eggs (Anstis 2017).

Litoria revelata Ingram, Corben and Hosmer, 1982

Southern Whirring Tree Frog
Fig. 9

Holotype: QM J28233, adult male, by original designation. Type locality: O'Reillys, Lamington Plateau, south-eastern Queensland, Australia, -28.23°, 153.13°. Collected by G. J. Ingram, 6 August 1973. Amey & Couper (2022) illustrated dorsal, ventral, and lateral views of the holotype.

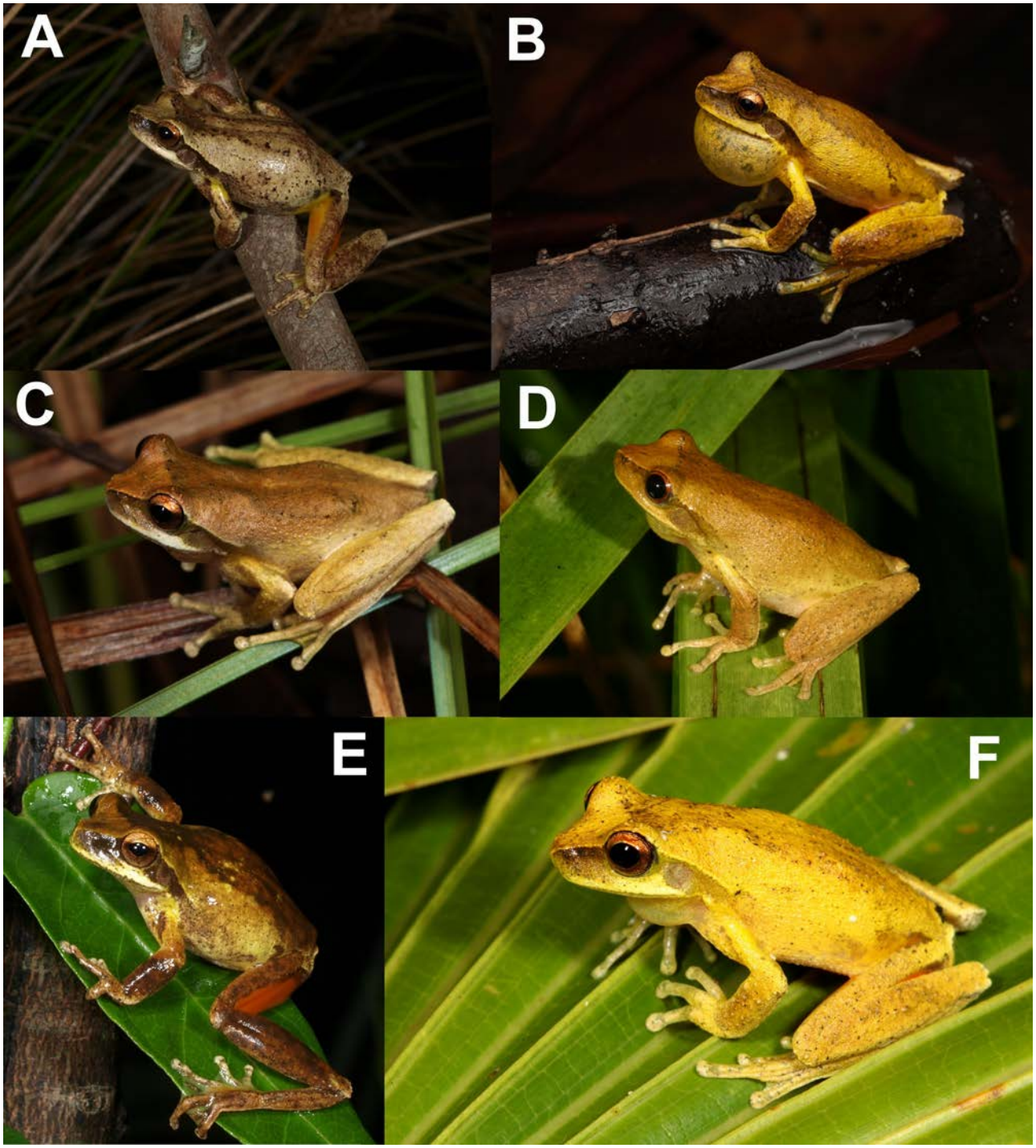

FIGURE 9. *Litoria revelata* in life: **A**) Dorrigo, NSW, photo Stephen Mahony; **B**) Olney State Forest, NSW, photo Stephen Mahony; **C**) Watagan National Park, NSW, photo Stephen Mahony; **D**) O'Reilly's, Lamington Plateau, Qld, QM J92494, photo Harry Hines, **E**) Heaton State Forest, NSW, photo Stephen Mahony; **F**) Wallingat National Park, NSW, photo Stephen Mahony.

Diagnosis. *Litoria revelata* can be diagnosed from *L. littlejohni* and *L. watsoni* by its smaller size (23–37 mm *vs* 43–61 mm and 42–64 mm, respectively), and orange, red or yellow groin and posterior thighs with black spots or patches (*vs* immaculate orange markings on anterior and posterior surfaces of femur and tibia, in groin, and posterior flanks). Can be diagnosed from *L. ewingii*, *L. paraewingi* and *L. verreauxii* by expanded finger and toe terminal discs (*vs* terminal discs similar width or only slightly wider than terminal phalanges). Diagnosed from *L. jervisiensis*

by termination of lateral head stripe near forearm (*vs* terminating along flank), by having dark patches in groin (*vs* absent), and by smaller size (23–37 mm *vs* 37–44 mm). *Litoria revelata* can also be diagnosed from *Litoria ewingii* Group species outside the *L. revelata* complex by a greater mean number of notes in call (31, *vs* 15 in *L. calliscelis*; 9 in *L. ewingii*; 4 in *L. jervisiensis*; 12 in *L. littlejohni*; 6 in *L. paraewingi*; 10 in *L. sibilus*; 23 in *L. verreauxii*; and 12 in *L. watsoni*).

Litoria revelata can be diagnosed from *L. eungellensis* **sp. nov.** by a combination of an absence of black patches or blotches in all of axilla, groin, posterior thighs and underside of the hindlimbs in most individuals (*vs* large prominent black patches and blotches in axilla, groin, posterior thighs and underside of the hindlimb); by smaller females (32.0–37.1 mm *vs* 37.0–39.5 mm); and by having an advertisement call with higher mean pulse repetition rate (105.3 pps, range 86.9–127.3 *vs* 78.1 pps, range 66.9–86.2). *Litoria revelata* can be diagnosed from *L. corbeni* by a lower mean pulse repetition rate (105.3 pps, range 86.9–127.3 *vs* 130.5 pps, range 127.3–136.7). *Litoria revelata* can be diagnosed further from *L. corbeni* and *L. eungellensis* **sp. nov.** by apomorphic states at 30 and 10 nucleotide sites respectively in the *ND4* gene alignment (Table 3).

Measurements of holotype (mm). SVL 26.7, HL 8.9, HW 9.1, TL 14.6, TD 1.4, ED 3.2.

Description. A summary of variation in 14 mensural characters and five ratios is presented in Table 4. Mean SVL: females = 34 ± 1.23, 32–37.1 mm; males = 28.1 ± 1.2, 24.7–31.6 mm.

Snout rounded when viewed from above, blunt in profile. Nostrils more lateral than superior; closer to tip of snout than to eye. Canthus rostralis well defined, straight. Eye relatively large (ED/HL 0.4 ± 0.04, 0.3–0.5); pupil horizontal when constricted (Fig. 9). Tympanum distinct; oval. Head length about equal to head width (HL/HW 0.98 ± 0.06, 0.7–1.1) and approximately one-third of SVL (HL/SVL 0.29 ± 0.02, 0.20–0.32). Tympanum length about equal to or greater than half eye diameter (TD/ED mean 0.5, 0.4–0.7). Vomerine teeth in semi-circular arc from anterior edge of the choanae to midline of palate (assessed on specimen SAMA R14298).

Fingers long, narrow; webbing absent. Subarticular and palmar tubercles prominent. Terminal discs prominent. Fingers in order of length: 3>4>2>1. Hindlimbs moderately long (TL/SVL 0.5 ± 0.02, 0.5–0.6). Toes in order of length: 4>5=3>2=1. Webbing between toes reaches to penultimate phalanges. Subarticular tubercles prominent. Rectangular inner metatarsal tubercle approximately one-quarter length of first toe. Terminal toe discs prominent, smaller than finger discs (Fig. 9).

Narrow dark brown finely granular nuptial pad wraps around dorsal surface of the base of finger I (SAMA R14298–9).

Dorsum finely granular. Upper surface of limbs smooth or finely granular. Chin, undersurfaces of limbs, and lateral aspect of abdomen smooth; remainder of abdomen finely granular.

Colour in life. Dorsal surfaces tan, cream-brown or reddish-brown, continuing onto flanks; middle dorsum has darker shading starting with well demarked margin between eyes, not obvious in some males in nuptial display colour. Some individuals with small to minute dark flecks over dorsal surfaces and flanks. A black or dark or light brown stripe, with lower margin often poorly defined, extends from nostril along canthus rostralis through eye to just past forearm, sometimes incorporating tympanum. Yellow dorsum in males during calling and amplexus. Abdomen white to cream, but males with yellow or orange throat with some dark flecks, particularly around margin. Vocal sac pigmented light cream to yellow (Fig. 9B, C, F). Upper iris bright copper-gold, lower iris brown copper gold (Fig. 9).

Axilla with dark brown or black marks in about half of specimens examined (43% of the 112 specimens), remainder with axilla same colour as flanks or the diffuse eye stripe extends across the tympanum onto axilla. Groin yellow or orange, with 64% of specimens having dark patch in groin (Fig. 6B, C, D), and occasionally smaller dark spots anterior to groin (Fig. 2B). Posterior of thighs uniform orange (Fig. 6A) or with black spot at proximal margin (Fig. 6B), or black dorsal margin (Fig. 6C), or with black pattern spread across thigh (Fig. 6D)a. Eight individuals from the northern end of the range (Yabbra State Forest, Tooloom Range, Lamington National Park, Border Ranges National Park, Richmond Range) have black marks on the underside of the hindlimbs (Supplementary Table S1), otherwise the underside of the hindlimb lacks dark marks (Table 5).

Distribution. Extends from Main Range (Cunningham's Gap) and Mt Tamborine in the north, through other south-eastern Queensland upland areas in the McPherson and Border Ranges and Killarney area, south through coastal ranges and lowlands of northern NSW to the mid NSW coast at Ourimbah State Forest (about halfway between Newcastle and Sydney). Records south of Sydney, at Thirlmere (QM J60187) and in the Wollongong area (Atlas of Living Australia), need confirmation because they sit over 100 km south of all other records (Fig. 1). Some

populations are probably disjunct, particularly in the uplands of south-eastern Queensland and north-eastern NSW. Records are from near sea-level to approximately 900 m a.s.l. Only occurs in mid elevation and upland areas in the north of the range (south-eastern Queensland and far northern NSW) but occurs at low elevations in the rest of the range.

Ecology and reproductive biology. Occupies a wide range of natural and human-modified habitats including: permanent and ephemeral ponds, swamps, dams near forests, and low-flow pools in upland streams in heath, wet or dry sclerophyll forest, and rainforest. In the north of the range occurs predominantly in rainforest, but occupies broader range of habitats in more southerly portions of the range.

Males display dynamic sexual dichromatism during the breeding season (Webster *et al.* 2023). Males change colour during calling and amplexus, from tan, brown, grey, or red-brown to brilliant lemon yellow or yellow-brown over much of the dorsum. Calling has been heard in every month of the year with a slight peak in September and the lowest number in June (FrogID). Anstis (2017) described egg and larval morphology and noted that the sides and venter of tadpoles of *L. revelata* were grey-blue to silver-grey, often with a dull copper sheen (*vs* a bluish sheen in *L. eungellensis* **sp. nov.**; Hero *et al.* 1996).

Conservation status. *Litoria revelata* does not fulfill a threatened species listing based on assessment against IUCN Red List criteria (2012). *Litoria revelata* has an estimated Extent of Occurrence (EOO) of 81,348 km^2 and Area of Occupancy (AOO) of 1,852 km^2. The estimated AOO is less than the 2,000 km^2 threshold for Criterion B2 but the species does not meet any additional conditions of that listing level due to a large number of known 'locations' and a lack of known, inferred or projected decline in extent or quality of habitat, number of subpopulations, or number of mature individuals. However, this is largely due to a lack of data and population monitoring is required across the distribution. The northern, upland populations in south-eastern Queensland and far northern New South Wales are localised and disjunct and may be under threat from climate change; hence they should be a particular focus for monitoring.

Litoria corbeni Wells and Wellington, 1985

Atherton Tablelands Whirring Tree Frog
Fig. 10

Holotype: QM J30116, by original designation, adult male. Type locality: Millaa Millaa Lookout, Atherton, Tableland, north-eastern Queensland, Australia, -17.62º, 145.5678º. Collected by G. J. Ingram on 1 November 1971. Amey & Couper (2022) illustrated dorsal, ventral and lateral views of the holotype.

Diagnosis. *Litoria corbeni* can be diagnosed from *L. littlejohni* and *L. watsoni* by its smaller size (23–33 mm *vs* 43–61 mm and 42–64 mm, respectively, sexes combined). Diagnosed from *L. ewingii*, *L. paraewingi* and *L. verreauxii* by expanded terminal discs on fingers and toes (*vs* terminal discs similar width, or only slightly wider than, terminal phalanges). Diagnosed from *L. jervisiensis* by termination of lateral head stripe near forearm (*vs* terminating along flank), dark patches in groin (*vs* absent), and smaller size (23–33 mm *vs* 37–44 mm, sexes combined). *Litoria corbeni* can be diagnosed further from the following species by its greater mean number of notes in the advertisement call (27.5, range 18–33 *vs* 2.3, range 1–4 in *L. jervisiensis*; 8.8, range 5–16 in *L. littlejohni*; 4, range 3–8 in *L. paraewingi*; 9, range 8–11 in *L. sibilus*; and 6.5, range 3–14 in *L. watsoni*).

Litoria corbeni can be diagnosed from *L. revelata* by a higher pulse repetition rate (130.2 pps ± 4.5, range 127.3–136.7 *vs* 105.3 ± 10.10, range 86.9–127.3) (Table 6). It can be diagnosed from *L. eungellensis* **sp. nov.** by the absence of well-demarked, black spots or blotches along the margins of the lower hindlegs, and by a higher mean pulse repetition rate (130.2 pps ± 4.5, range 127.3–136.7 *vs* 78.1 ± 10.7, range 66.9–86.2) (Table 6). *Litoria corbeni* can also be diagnosed from *L. revelata* and *L. eungellensis* **sp. nov.** by apomorphic states at 30 and 34 nucleotide sites respectively in the *ND4* gene alignment (Table 3).

Measurements of holotype (mm). SVL 30.12, HL 9.72, HW 9.57, TL 17.55, TD 1.41, ED 3.03.

Description. A summary of variation in 14 mensural characters and five ratios is presented in Table 4. Mean SVL: females = 32.1 ± 0.94, 31–33.6 mm; males = 27.8 ± 2.07, 23.7–32.4 mm.

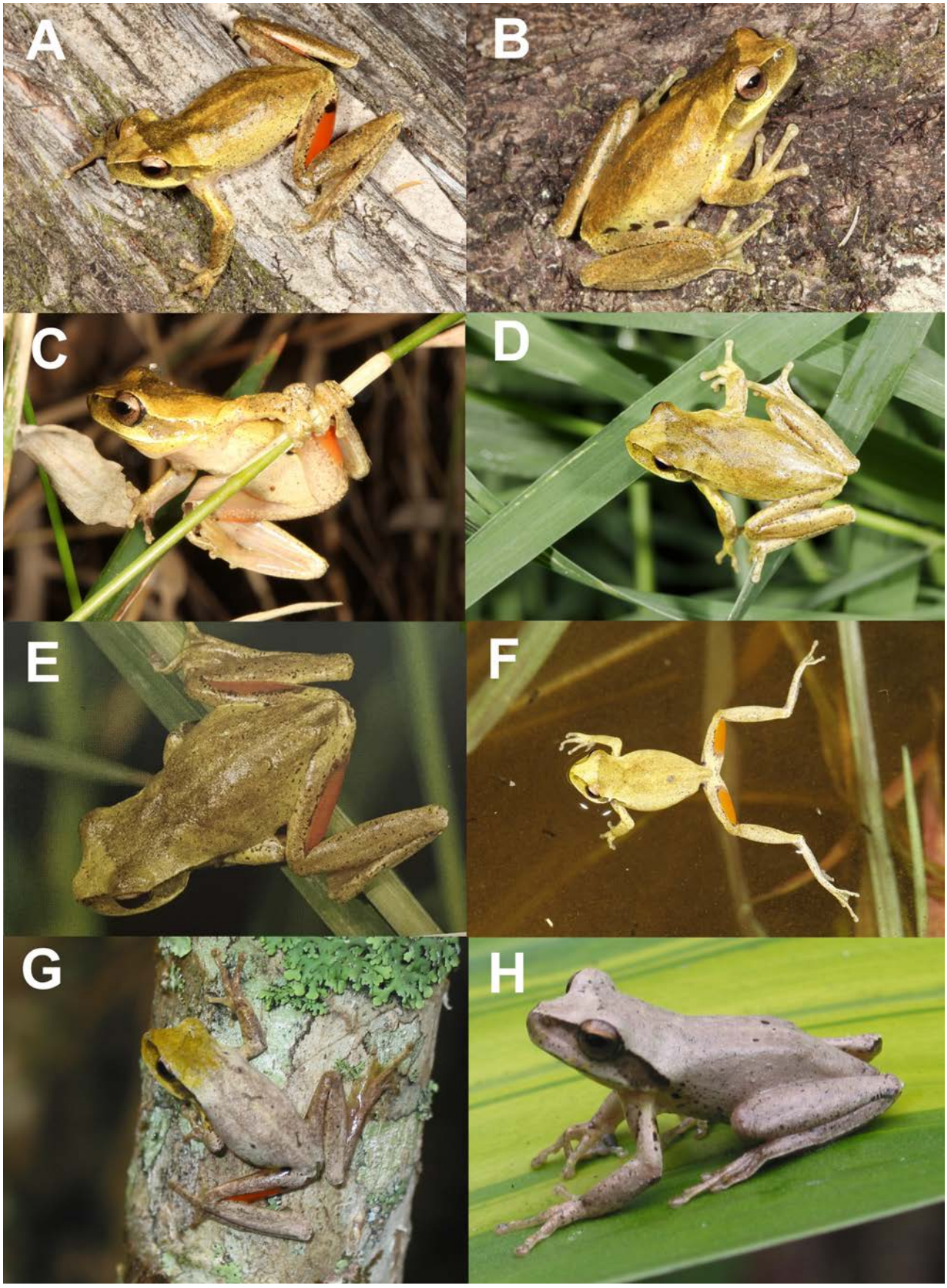

FIGURE 10. *Litoria corbeni* in life: **A–C**) no vouchers, upper Walsh River, Mount Baldy, photos Conrad Hoskin; **D–F**) no vouchers, Millaa Millaa area, photos Mark Sanders; **G–H**) no vouchers, southern Atherton Tableland, photos Luke Price.

Snout; rounded when viewed from above, blunt or rounded in profile. Nostrils more lateral than superior; closer to tip of snout than to eye. Canthus rostralis well-defined, and straight. Head length shorter than, to a bit longer than, head width (HL/HW 0.81–1.07), approximately one-third of SVL (HL/SVL 0.29, 0.23–0.34). Pupil horizontal when constricted (Fig. 10). Eye relatively large (ED/HL 0.4, 0.34–0.44). Tympanum distinct; circular; diameter variable (TD/ED 0.5, 0.38–0.7). Vomerine teeth in short straight rows from anterior edge of choanae to midline of palate (assessed in SAMA R72459).

Fingers long, narrow; webbing absent. Subarticular and palmar tubercles prominent. Terminal discs prominent. Fingers in order of length: 3>4>2>1. Dark brown nuptial pad wraps around upper surface of base of finger I (SAMA R34328–9, R63777, RI72458–9).

Hindlimb length moderate, but variable (TL/SVL 0.5, 0.45–0.6). Toes in order of length: 4>5=3>2>1. Webbing on toes I, II, III, V reaches base of toe disc, and on toe IV to base of the penultimate phalanx. Subarticular tubercles prominent. Rectangular inner metatarsal tubercle approximately one-quarter length of first toe. Terminal discs prominent, but smaller than finger discs.

Dorsum finely granular. Upper surface of limbs smooth or finely granular. Flanks with dense coverage of low tubercles. Chin, abdomen, undersurface of limbs, and lateral aspect of body coarsely granular or granularity confined to lateral margins of abdomen.

Colour in life. Dorsum tan, grey-brown, cream-brown or reddish-brown, continuing onto flanks; in most individuals, middle of dorsum has distinct darker shading that starts with well demarked margin between eyes. Some individuals with small to minute dark flecks over dorsal surfaces and flanks. A dark or light brown stripe, with a lower margin that is often poorly defined, extends from nostril along canthus rostralis through eye to just past forearm, and sometimes incorporates tympanum. Ventral surfaces white to cream, with small dark spots either peripherally or over entire ventral surface (Fig. 10C). Upper iris bright copper-gold, lower iris brown copper-gold Vocal sac pigmented light yellowish cream (Fig. 10C).

Axilla in two of six vouchers examined (33%) with dark marks, remainder with axillae same colour as flanks or covered by diffuse extension of eye stripe. Groin with dark patch (Fig. 10), and occasionally with smaller spots anterior to groin. Posterior of thighs either uniform orange (Fig. 10) or with black spot at proximal margin (Fig. 10). Underside of the hindlimbs lack dark marks (Table 5).

Distribution. Known from elevations between about 700 and 1220 m a.s.l. on the western and southern Atherton Tablelands (Fig. 1). Records form an 'arc' from the Mt Baldy area (near Atherton) on the western Atherton Tableland, to Ravenshoe and Millaa Millaa in the south, to the Topaz area in the south-east (Fig. 1). Most sites on the western and southern Atherton Tableland are above 1000 m a.s.l. but some sites in the Millaa Millaa-Topaz area are lower (down to approximately 700 m a.s.l.).

Ecology and reproductive biology. Known to breed in both disturbed and natural situations where still or slow-moving water is within, or adjacent to, forest. Breeding sites are ponds (including farms dams; e.g., Fig. 11A) and other stationary water bodies (e.g., Mt Hypipamee crater), and slow-flowing pools in streams (e.g., Fig. 11B). Breeding occurs in rainforest and wet sclerophyll forest, and in habitats comprising mosaics of grazing land and rainforest, or wet sclerophyll forest. Most known breeding sites are in agricultural land where the frogs reproduce in farm dams or slow pools along disturbed creeklines, but all breeding sites have some forest in close proximity. Prior to extensive clearing of the southern Atherton Tablelands, breeding sites would have been natural ponds and pools along slow flowing streams through rainforest and wet sclerophyll forest.

Males call from riparian vegetation including reeds, sedges, tall grass, and adjacent bushes and trees. Calling has been heard through most of the year except for May and June (FrogID, CJH observations). Males are bright yellow when calling in the breeding season, whereas the few females encountered in the wild in the breeding season have been a more tan-brownish colour (CJH observations). Other aspects of reproductive biology are not known.

Conservation status. *Litoria corbeni* has a small distribution in an upland area, so warrants conservation assessment against IUCN Red List criteria (2012). The EOO (measured as a minimum convex polygon around all known sites) is about 795 km^2. The actual area occupied within this area (the AOO) would be much smaller, estimated as 76 km^2. All known sites are above 700 m a.s.l. and most are above 1000 m a.s.l., in an area where there is limited suitable habitat over 1100 m a.s.l. and very little over 1200 m a.s.l.. The highest known record is about 1220 m a.s.l. Additionally, even at higher elevations (e.g., > 1000 m a.s.l.), *L. corbeni* is patchy in occurrence, and the reason for the patchiness is not known. Whether the known sites represent one 'location' (a geographically or ecologically distinct area in which a single threatening event can rapidly affect all individuals; IUCN 2012) or many

can only be assessed based on the perceived threat. Fire may be a threat at wet sclerophyll sites on the west of the distribution, but most sites are associated with rainforest and grazing land in cool, wet areas that are unlikely to burn. Most known sites are on private land, where there is limited protection for regrowth rainforest; hence habitat loss and fragmentation through clearing is also a threat. However, the primary perceived threat is climate change, both gradual warming and the extremes of heatwaves and droughts.

Considering climate change as the primary threat, all sites are taken to represent one 'location'. It is hard to assess this species against Criteria A, C, D and E due to limited data, but it can be assessed against Criterion B. Under Criterion B, with an EOO of 795 km^2 and AOO of 76 km^2, *Litoria corbeni* fulfils an Endangered B1/B2 (a, b) listing based on: B1 EOO < 5,000 km^2 (but not < 100 km^2 for Critically Endangered), B2 AOO threshold < 500 km^2 (but not < 10 km^2 for Critically Endangered), (a) number of locations < 5; and (b) decline inferred or projected in (iii) area, extent and/or quality of habitat, (iv) number of subpopulations, and (v) number of mature individuals due to the ongoing effects of climate change.

Conservation of this species should include surveys to find additional breeding sites, monitoring of known breeding sites to assess continued occupancy, and revegetation along gullies throughout its known distribution. The southern Atherton Tablelands, where most populations of *L. corbeni* occur, was once forested but has been extensively cleared. Furthermore, the species occurs in relatively flat areas where it breeds in ponds and slow-moving creeks, which are areas preferred for farming. Most known breeding sites are now in or adjacent to remnant or regrowth rainforest in a matrix of forest patches and cleared gazing land. Although farm dams and gully lines offer breeding habitat, close proximity to forest is vital, so revegetation along gully lines will help to increase the area of rainforest habitat, increase the number of suitable breeding sites, and increase connectivity between them.

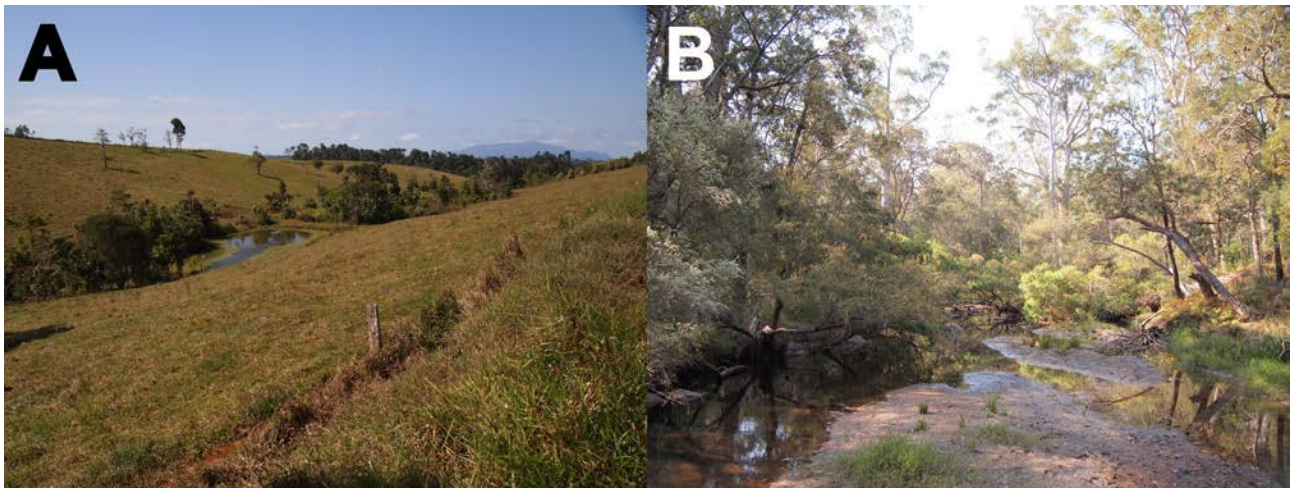

FIGURE 11. *Litoria corbeni* habitat: **A**) Millaa Millaa area, photo Conrad Hoskin; **B**) upper Walsh River, Mt Baldy, Queensland, photo Conrad Hoskin.

***Litoria eungellensis* sp. nov.**
Eungella Whirring Tree Frog
Figs 12, 13, 14

Holotype: QM J35106 (Fig. 12), adult female. Type locality: Thurgood Farm, 18 km from Dalrymple Heights, mid-eastern Queensland, Australia. -21.033º, 148.6º. Collected by G.J. Ingram on 7 December 1978.

Diagnosis. *Litoria eungellensis* **sp. nov.** can be diagnosed from *L. littlejohni* and *L. watsoni* by its smaller size (28–39.5 mm *vs* 43–61 mm and 42–64 mm, respectively), yellow or orange groin, and posterior thighs with black spots or patches (*vs* immaculate orange markings on the anterior and posterior surfaces of the femur and tibia, in the groin, and posterior flanks). Can be distinguished from *L. ewingii*, *L. paraewingi* and *L. verreauxii* by expanded finger and toe discs (*vs* terminal discs similar in width to, or only slightly wider than, the terminal phalanx). Distinguished from *L. jervisiensis* by the termination of the lateral head stripe near the forearm (*vs* terminating along flank), dark patches in groin (*vs* absent), and smaller size (28–39.5 mm *vs* 37–44 mm). *Litoria eungellensis* **sp. nov.** can be diagnosed further from the following species by the greater mean number of notes in the advertisement call (39.3,

range 30–35 *vs* 9, range 4–22 in *L. ewingii*; 2.3, range 1–4 in *L. jervisiensis*; 8.8, range 5–16 in *L. littlejohni*; 4, range 3–8 in *L. paraewingi*; 9, range 8–11 in *L. sibilus*; 16.7, 8–23 in *L. verreauxii*; and 6.5, range 3–14 in *L. watsoni*).

Litoria eungellensis **sp. nov.** can be diagnosed from *L. corbeni* and *L. revelata* by the presence of well-demarked, back spots or patches in all of the axilla, groin, posterior thigh, and underside of the hindlimbs (*vs* variable presence of spots in any of the first three locations, and any spots present being generally smaller and less conspicuous, and absence of dark marks on the underside of the hindlimb) (Table 5). It can be diagnosed further from *L. corbeni* and *L. revelata* by having calls with a lower mean pulse repetition rate (78.1 pps, range 67–86 *vs* 130.5, range 127.3–136.7 for *L. corbeni* and 105.3, range 87–127 for *L. revelata*) (Table 6) and by apomorphic states at 31 and 10 nucleotide sites, respectively, in the *ND4* gene alignment (Table 3).

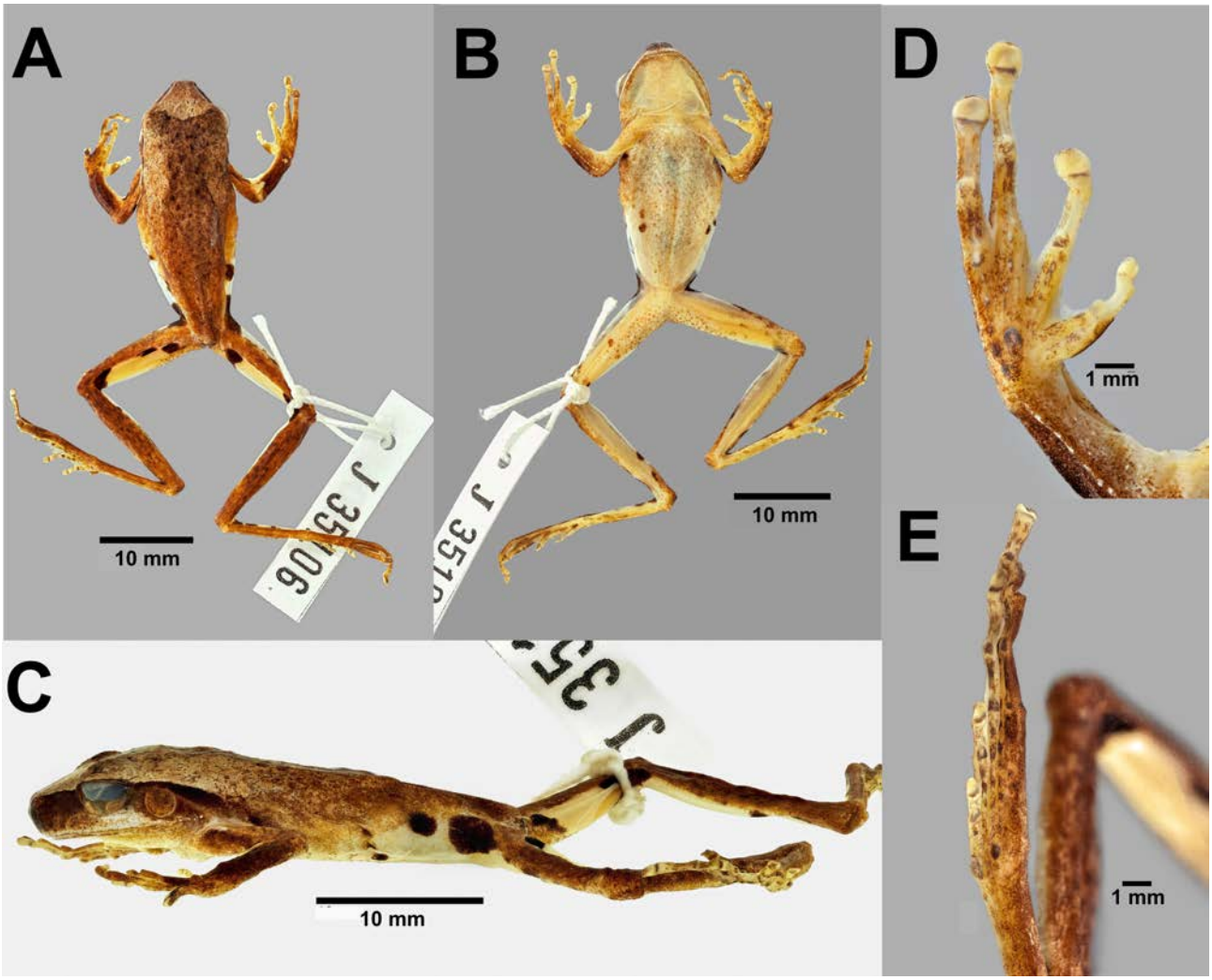

FIGURE 12. Holotype of *Litoria eungellensis* **sp. nov.** QM J35106: **A**) dorsal view, **B**) ventral view, **C**) lateral view, **D**) plantar view of manus, **E**) plantar view of pes (photos Geoff Thompson, Queensland Museum).

Measurements of holotype (mm). SVL 28.8, HL 8.3, HW 8.6, TL 16.3, TD 1.8, ED 3.3 IOD 5.8, IND 2.2, ETD 1.1, THL 14.3, FL 14.2, IMT 1.1, FLL 6.9, Fing3D 1.0.

Description (including holotype; Fig. 12). A summary of variation in 14 mensural characters and five ratios is presented in Table 4. Mean SVL: females = 38.5 ± 0.99, 37.3–39.5, males = 29.6 ± 1.19, 28.3–31.8 mm.

Snout rounded when viewed from above, blunt or rounded in profile. Head length less than, to about equal to, head width (HL/HW 0.95 ± 0.06, 0.76–1.03) and approximately one-third of SVL (HL/SVL 0.29 ± 0.02, 0.24–0.31). Pupil horizontal when constricted (Fig. 13). Nostrils more lateral than superior; closer to tip of snout than to eye. Canthus rostralis well defined, and straight. Eye relatively large (ED/HL 0.4 ± 0.03, 0.33–0.46). Tympanum distinct; circular; diameter about half eye diameter (TD/ED 0.5 ± 0.06, 0.44–0.58). Vomerine teeth in short straight rows from anterior edge of choanae to midline of palate (assessed in SAMA R72462).

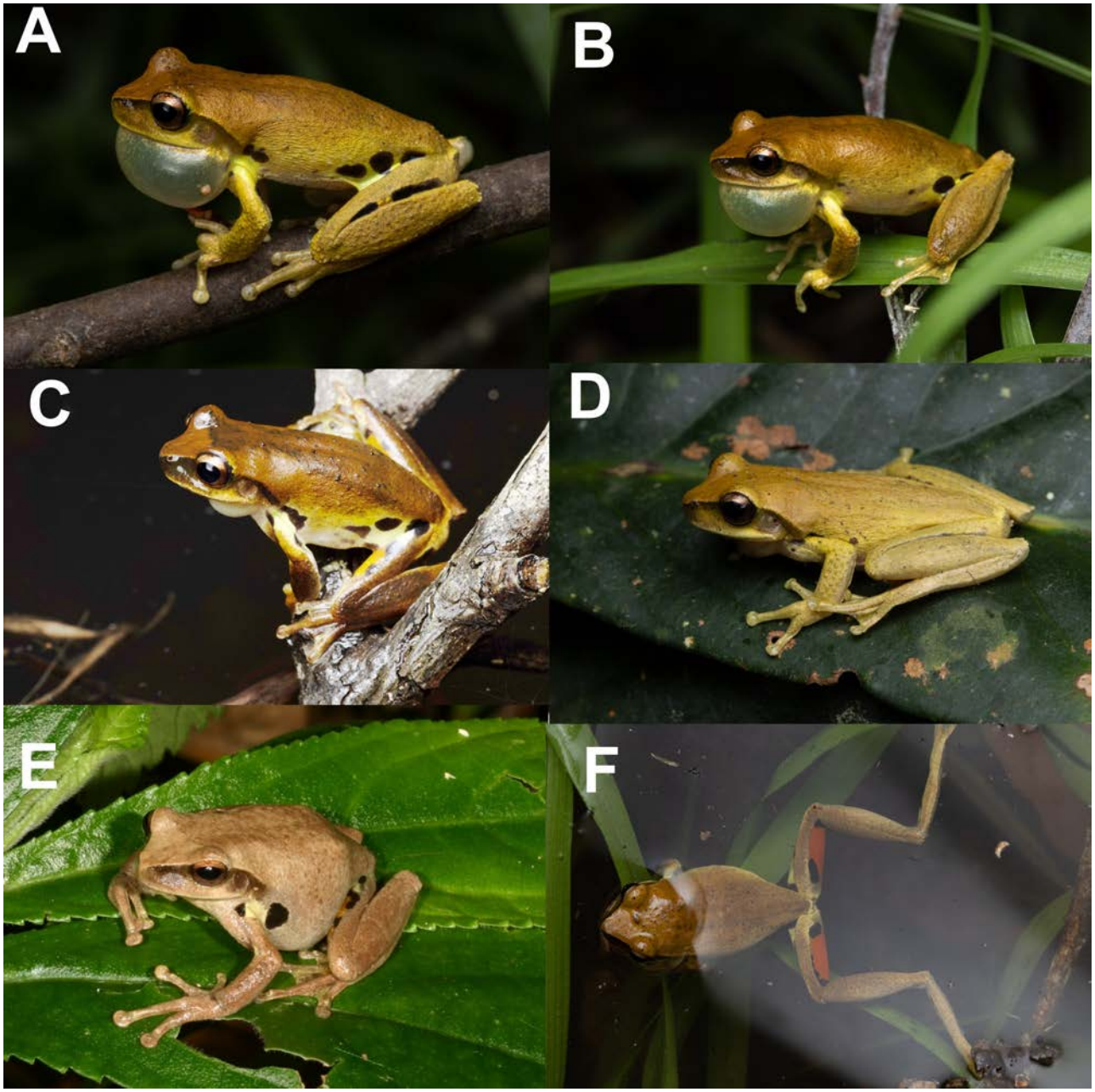

FIGURE 13. *Litoria eungellensis* **sp. nov.** in life: **A–D**) no voucher, Eungella, photos Stephen Mahony; **E**) no voucher, ABTC81985, Eungella, photo Harry Hines; **F**) no voucher, Eungella, photo Stephen Mahony.

Fingers long, narrow; webbing absent. Subarticular tubercles prominent. Terminal discs prominent. Fingers in order of length: 3>4>2>1. Dark brown nuptial pad wraps around inner surface of Finger I from base of finger to base of penultimate phalanx (SAMA R72460–4).

Hindlimbs moderately long (TL/SVL 0.5 ± 0.02, 0.52–0.57). Toes in order of length: 4>5=3>2>1. Webbing on all toes reaches base of penultimate phalanx. Subarticular tubercles prominent. Rectangular inner metatarsal tubercle approximately one-quarter length of first toe. Terminal toe discs not prominently expanded (Fig. 12).

Dorsum finely granular. Upper surface of limbs smooth or finely granular. Flanks with dense coverage of low tubercles. Chin and ventral surfaces of limbs smooth; abdomen finely granular.

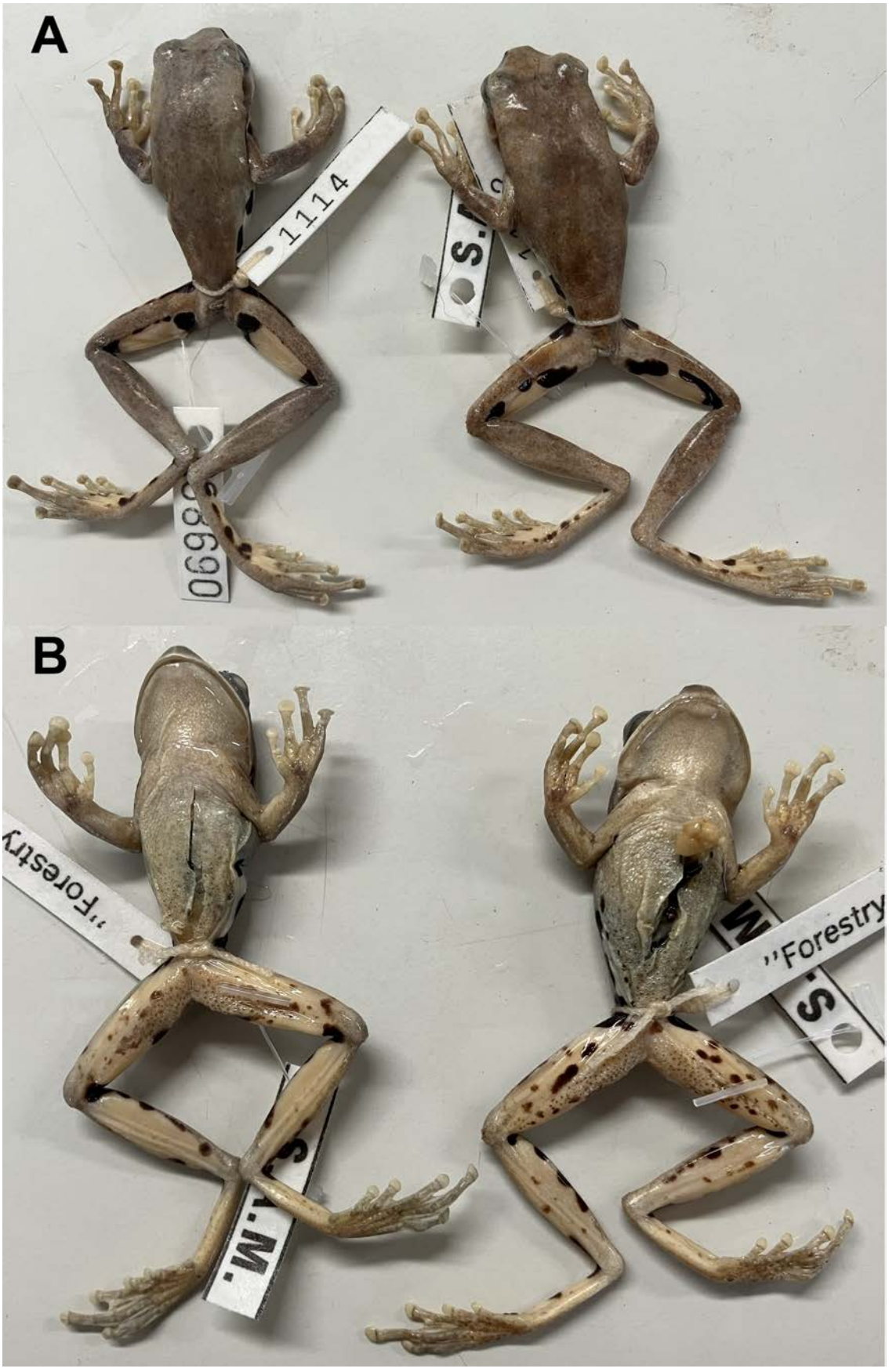

FIGURE 14. *Litoria eungellensis* **sp. nov.** vouchers showing markings on limbs: **A**) dorsal and **B**) ventral views of females SAMA R68690 (left) and SAMA R68688 (right), from Mt William Creek, Eungella National Park.

Colour in life. Dorsal surfaces tan, cream-brown or bronze, continuing onto flanks. Mid-dorsum with darker shading that starts with well-demarcated margin between eyes; this pattern may not be obvious in males in courting colour. Some individuals with small to minute dark flecks over dorsal surfaces and flanks. A black or dark or light brown stripe, with lower margin often poorly defined, extends from nostril along canthus rostralis through eye and over tympanum to just past forearm. Belly white to cream. Inflated vocal sac near transparent with sparse yellow-green pigmentation (Fig. 13A, B). Upper iris bright copper-gold colour, lower iris brown copper-gold.

Axillae have prominent dark marks in all specimens examined. Groin yellow, with dark patch and large dark spots anterior to groin (Figs 13, 14). Posterior of thighs with black spot at proximal and distal margins, or additionally with black dorsal margin, or with black pattern spread across thigh (Figs 6E, F, 13, 14). Variably-shaped black or dark marks present on the underside of the hindlimbs (Table 5, Fig. 14A, B).

Etymology. The species name *eungellensis* refers to the distribution of this species in the 'Eungella' region. According to Kitching (2020) "Eungella is generally supposed to mean 'Land of Clouds'." and "was adopted from local indigenous languages [of the Birri and Wiri people] by the first European residents".

Distribution. Restricted to high elevations (> 880 m a.s.l.) from Pease's lookout, 3km ENE of the Eungella township northeast to Mt David and Mt William in the headwaters of the Cattle Creek North branch, SSW of Mt Dalrymple and the Clarke Range in Eungella National Park and in the west to farm properties west of the Dalrymple Road (Fig. 15). These records fall in the elevational range of about 880–1211 m a.s.l., with most records between 950 and 1000 m a.s.l. It is not known from the fairly large extent of upland rainforest above 1000 m a.s.l. to the north and northwest of the documented historical range, although this may be due to the limited number of sites and frequency of surveys to date in this difficult to access area (Meyer *et al*. (2020).

Extensive surveys at 114 sites through the Eungella region between 2000 and 2015 only found *L. eungellensis* **sp. nov.** between 930–980 m a.s.l. in the small area of the headwaters of Cattle Creek North branch and at nearby farm dams (Meyer *et al.* 2020). Historic records include Pease's lookout (900 m a.s.l.), as an observation (Covacevich & McDonald 1993), and Snake Road (970 m), as a specimen (QM J45838) (Fig. 15), but there have been no records from these areas in recent decades, despite survey effort (Meyer *et al.* 2020).

Two vouchers (NHM 64.7.8.11–12), listed as paratypes in Ingram *et al*. (1982) are from Port Denison (= Bowen), north Queensland. This locality is an anomalous record because it is at sea level, is unsuitable dry sclerophyll habitat, and is more than 100 km north of the Eungella region. We take this locality information to be erroneous and the identity of these specimens needs assessing.

Ecology and reproductive biology. Most occurrence records come from slow-flowing rainforest creeks, where adults have been found sitting in vegetation along streams and eggs and tadpoles have been observed in small pools in bedrock adjacent to the stream (Retallick *et al.* 1997, Retallick 1998, Meyer *et al.* 2020). These small, isolated pools are rare along the streams—for example, breeding habitat was only present along 30 m of a 700 m section of Mt William Creek (Retallick 1998). *Litoria eungellensis* **sp. nov.** also occupies farm dams on small gully lines in pasture adjacent to rainforest habitat west of the Dalrymple Road (Meyer *et al.* 2020). In these situations, adult males call from low vegetation on the margins of the dams. Calling and breeding have been recorded in Spring and early Summer (Retallick *et al.* 1997, Retallick 1998, CJH observations). Anstis (2017) compared the tadpole morphology of *L. revelata* and *L. eungellensis* **sp. nov.** (then denoted as *L. revelata* Eungella) but did not find any differences. Hero *et al.* (1996) described the tadpole in detail, pointing out that a blue sheen was present on the abdomen (*vs* a dull copper sheen on *L. revelata*; Anstis 2017), but the consistency of this trait needs verification.

Conservation status. *Litoria eungellensis* **sp. nov.** has an extremely small spatial and elevational distribution. Rainforest vegetation is present at mid and low elevations at Eungella but all records of the species come from above 900 m, suggesting upland restriction. The highest elevations at Eungella are the summits of Mt David (1200 m), Mt William (1250 m) and Mt Dalrymple (1205 m), and most of the *L. eungellensis* **sp. nov.** records are clustered in creek headwaters between these summits. Below we assess conservation status against IUCN Red List (2012) criteria. The extent of occurrence (EOO, measured as a minimum convex polygon around all records in Fig. 15) is approximately 15 km^2, but most records (including those in Meyer *et al.* 2020) come from an extremely small area of < 1 km^2. These values are well under the EOO specified in Criterion B for Critically Endangered (100 km^2); in fact, the EOO for the currently known sites is less than the area of occupancy (AOO) for Critically Endangered under this criterion (10 km^2). *Litoria eungellensis* **sp. nov.** occurs at a single 'location' because a single threatening event could rapidly affect all individuals (IUCN 2012). Several threats should be considered: fire, clearing, chytridiomycosis, and climate change.

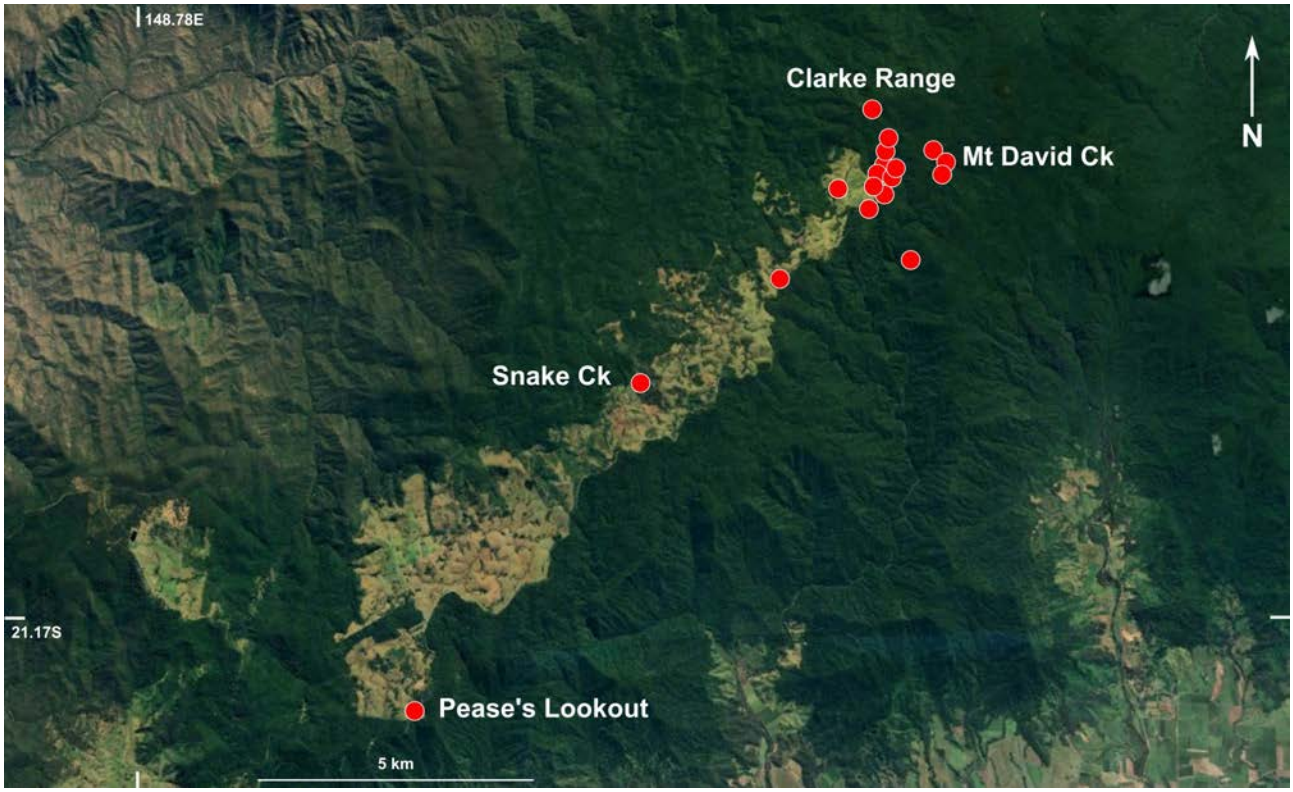

FIGURE 15. Map of records for *Litoria eungellensis* **sp. nov.** from the Atlas of Living Australia (as of September 2024) in the Eungella region, mid-eastern Queensland.

Fire may be a threat given the extensive burning of upland rainforest at Eungella in 2018 (Hines *et al.* 2020). These fires did not burn any habitat known to support *L. eungellensis* **sp. nov.** but did burn similar habitat just to the west, so fire could be considered a future threat. Clearing may be a localised threat to sites on private land (e.g., around dams) because there is limited protection for regrowth rainforest on private land. However, clearing is not deemed a major threat because most habitat is in Eungella National Park. Chytridiomycosis has had severe impacts on some frog species in the Eungella region (e.g., Retallick *et al.* 2004, Meyer *et al.* 2020) but is not known to have caused population declines in *L. eungellensis* **sp. nov.**. It has, however, been detected in the species, with one of 53 individuals tested between 1994 and 1997 showing chytrid infection (by histology) (Retallick *et al.* 2004, Murray *et al*. 2010). Chytridiomycosis could therefore be considered a potential threat, but the species has likely persisted with the disease for decades.

Climate change (both gradual warming and the extremes of heatwaves and droughts) is considered the biggest threat to *L. eungellensis* **sp. nov.**. No population declines are documented to date (but also there has been no monitoring since the 2015) but based on restriction to a very small, high elevation area, this species is a candidate for future impacts. Under Criterion B, *L. eungellensis* **sp. nov.** qualifies for a Critically Endangered B1/B2 (a, b) listing based on: B1 EOO < 100 km^2; B2 AOO < 10 km^2; (a) number of locations < 5 (taken to be 1 for this species); and (b) decline inferred or projected in (iii) area, extent and/or quality of habitat, and (v) number of mature individuals due to the ongoing effects of climate change (considered the primary threat).

Litoria eungellensis **sp. nov.** is in urgent need of conservation attention. Detailed surveys are required to assess continued occupancy of known sites and to find additional breeding sites. Monitoring needs to be established to assess short- and long-term population trends, particularly in regard to potential climate change impacts. Revegetation is required along gully lines and dam margins in the grazing land west of the Dalrymple Road. Any improvement of, or addition to, breeding sites in this area would have significant benefits for the species. An assessment of current amphibian chytrid prevalence and impact on the species is also required.

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APPENDIX 1. *Litoria ewingii* Group specimens examined for molecular genetic analyses. Institution codes: ABTC—Australian Biological Tissue Collection, South Australian Museum; NMV—Museums Victoria; AMS—Australian Museum; SAMA—South Australian Museum; QM—Queensland Museum; ANWC—Australian National Wildlife Collection. State abbreviations: SA—South Australia; VIC—Victoria; NSW—New South Wales; NT—Northern Territory; Qld—Queensland. NP—National Park; SF—State Forest; NR—Nature Reserve; *—GenBank accession numbers.

Species	ABTC	State	Locality	Code	Lat	Long	GenBank*	SNP	Collection	Voucher
calliscelis	14683	SA	Highbury	ca1	-34.830	138.680	OR546026	y		
calliscelis	33268	SA	22 km ESE Mt Compass	ca2	-35.428	138.809	OR546027	-		
calliscelis	74464	SA	1.5 km NE Norton Summit	ca3	-34.912	138.738	OR546028	-		
calliscelis	94832	SA	0.7 km NE Pijanbilli Lodge	ca4	-35.546	138.374	OR546029	y		
calliscelis	93424	SA	1.8 km NNE Caloote	ca5	-34.951	139.272	-	y		
calliscelis	93463	SA	4.6 km ENE Pellaring Flat	ca6	-34.859	139.442	-	y		
calliscelis	94968	SA	Wangoola Homestead	ca7	-35.566	138.367	-	y		
corbeni	-	Qld	Walsh River	c1	-17.2946	145.403		-	No voucher	R1
corbeni	-	Qld	Walsh River	c1	-17.2946	145.403		-	No voucher	R2
corbeni	102389	Qld	Zillie Falls	c3	-17.47	145.65	PP157485	-	QM	J90805
corbeni	102390	Qld	Zillie Falls Road	c3	-17.4667	145.6493	MT497815	y	QM	J90806
corbeni	102391	Qld	Zillie Falls Road	c3	-17.4667	145.6493	MT497816	y		
corbeni	102392	Qld	Zillie Falls Road	c3	-17.4667	145.6493		y		
corbeni	80815	Qld	Millaa Millaa	c2	-17.49	145.61	OR545983	-	SAMA	register
corbeni	80816	Qld	Millaa Millaa	c2	-17.49	145.61	OR545984	-	SAMA	register
eungellensis	81985	Qld	Mt William Creek, Eungella NP	e1	-21.0292	148.6019		y	No voucher	
eungellensis	90388	Qld	Mt William Creek, Eungella NP	e1	-21.0292	148.6019		y	SAMA	R68687
eungellensis	90389	Qld	Mt William Creek, Eungella NP	e1	-21.0275	148.6031		-	SAMA	R68688
eungellensis	90390	Qld	Mt William Creek, Eungella NP	e1	-21.0275	148.6031		-	SAMA	R68689
eungellensis	90391	Qld	Mt William Creek, Eungella NP	e1	-21.0275	148.6031		-	SAMA	R68690

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APPENDIX 1. (Continued)

Species	ABTC	State	Locality	Code	Lat	Long	GenBank*	SNP	Collection	Voucher
eungellensis	90392	Qld	Mt William Creek, Eungella NP	e1	-21.0275	148.6031		-	SAMA	R68691
eungellensis	80817	Qld	Eungella Dalrymple Road	e2	-21.0345	148.5994		y	SAMA	R72464
eungellensis	80818	Qld	Eungella Dalrymple Rpad	e2	-21.0345	148.5994	PP157429	y	SAMA	R72462
eungellensis	80819	Qld	Eungella Dalrymple Road	e2	-21.0345	148.5994		-	SAMA	R72463
eungellensis	80820	Qld	Eungella Dalrymple Road	e2	-21.0345	148.5994		-	SAMA	R72460
eungellensis	80821	Qld	Eungella Dalrymple Road	e2	-21.0345	148.5994		-	SAMA	R72461
ewingii	13661	NSW	Sassafras	ew1	-35.08	150.25		-		
ewingii	17602	NSW	Boydtown	ew2	-37.11	149.88	OR546015	y		
ewingii	17605	NSW	4 km SE Narrabarba	ew3	-37.2744	149.8436	OR546014	y		
ewingii	113122	Vic	Macleods Morass	ew4	-37.8365	147.6226	-	y		
ewingii/ver grp2	16984	Vic	Bessie Belle	ew5	-38.15	141.96	OR545999	-		
ewingii	12434	Vic	3 km E Toura	ew6	-38.6667	146.3667	OR546010	y		
jervisiensis	25839	NSW	Mungo Brush	j1	-32.5417	152.3126	MT497820	y	SAMA	R72495
jervisiensis	25157	NSW	Awabakal NR	j2	-32.99	151.71	PP157369	y		
jervisiensis	25158	NSW	Awabakal NR	j2	-32.99	151.71		-		
jervisiensis	90506	NSW	Kurnell Peninsula	j3	-34.02	151.21	-	y		
jervisiensis	25451	NSW	Darkes Forest	j4	-34.2373	150.9161	MT497819	y	SAMA	R72498
jervisiensis	101694	NSW	Murray's Beach	j5	-35.1294	150.7533	-	y		
jervisiensis	80824	NSW	Kioloa	j6	-35.54	150.38	PP157430	-		
littlejohni	7140	NSW	Watagan SF	l1	-33.01	151.44	-	y		
littlejohni	80813	NSW	Watagan SF	l1	-33.03	151.315	MT497839	-	No voucher	
littlejohni	145269	NSW	Kings Tableland	l2	-33.814	150.38	MT497843	-	No voucher	
littlejohni	145101	NSW	Stokes Creek, Dharawal NP	l3	-34.2217	150.8626	-	y		
littlejohni	113907	NSW	Cataract River catchment, Metropolitan Special Area	l4	-34.29	150.79	-	y		
littlejohni	113938	NSW	Avon River catchment, Upper Nepean SCA	l5	-34.3276	150.6781	-	y		

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APPENDIX 1. (Continued)

Species	ABTC	State	Locality	Code	Lat	Long	GenBank*	SNP	Collection	Voucher
littlejohni	113923	NSW	Lake Cordeaux, Metropolitan Special Area	l6	-34.3668	150.7432	-	y		
paraewingi	17553	NSW	East Albury	p1	-36.08	146.93	-	y		
paraewingi	17554	NSW	East Albury	p1	-36.08	146.93	-	y		
paraewingi	40923	Vic	Lima turnoff on Midland Highway	p2	-36.8393	146.0081	MT497823	-	SAMA	R34675
paraewingi	12856	Vic	6 km W Strathbogie	p3	-36.8486	145.6584	MT497822	y	SAMA	R44066
paraewingi	12855	Vic	7 km N Merton	p4	-36.914	145.7016	MT497821	y	SAMA	R44074
revelata	25931	Qld	Lamington NP	r1	-28.15	153.1155	MT497825	y	SAMA	R72519
revelata	25066	NSW	Border Ranges NP	r2	-28.4142	153.0222		-		
revelata	25961	NSW	Yabbra SF	r3	-28.49	152.58		-	SAMA	R72515
revelata	80825	NSW	Murrays Scrub	r4	-28.49	152.76		-		
revelata/ver grp1	17764	NSW	Mullumbimby	r5	-28.54	153.5		y	SAMA	R45363
revelata	25929	NSW	Richmond Range	r6	-28.5411	152.7781		y	SAMA	R72528
revelata	25597	NSW	Whian Whian SF	r7	-28.6	153.3772		-	SAMA	R72481
revelata	86373	NSW	Whian Whian SF	r7	-28.6	153.3772		-		
revelata	25986	NSW	Tooloom SF	r8	-28.6167	152.4167		-	SAMA	R72523
revelata	25632	NSW	Cinbin Margil Swamp	r9	-28.6553	153.6222		-	SAMA	R72540
revelata	26300	NSW	Chaelundi SF	r10	-29.9438	152.3853	PP157375	-	SAMA	R72537
revelata/ver grp1	25078	NSW	Wild Cattle Creek SF, Dorrigo	r11	-30.2192	152.7786	OR545978	-		
revelata	25694	NSW	Bonville Beach	r12	-30.3913	153.0781		y	SAMA	R72497
revelata	26010	NSW	Pine Creek SF	r13	-30.4313	152.9617	OR545980	-		
revelata	80826	NSW	Maxwell Flat	r14	-31.5269	152.1879	OR545981	-		
revelata/ver grp1	-	NSW	Tapin Tops NP	r15	-31.6187	152.1629	-	y	AMS	R188133
revelata	-	NSW	Dingo Tops Campground, Tapin Tops NP	r15	-31.6657	152.1421	-	y	AMS	R188127
revelata	-	NSW	Dingo Tops Campground, Tapin Tops NP	r15	-31.6657	152.1421	-	y	AMS	R188128
revelata	-	NSW	Dingo Tops Campground, Tapin Tops NP	r15	-31.6657	152.1422	-	y	AMS	R188132

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APPENDIX 1. (Continued)

Species	ABTC	State	Locality	Code	Lat	Long	GenBank*	SNP	Collection	Voucher
revelata	25834	NSW	Dingo Tops Dam	r15	-31.66	152.13		-	SAMA	R72542
revelata	25996	NSW	Coopernook SF	r16	-32.2167	152.3167		-	SAMA	R72532
revelata	25159	NSW	Wallingat SF	r17	-32.3428	152.4247		-		
revelata	-	NSW	Wallingat NP	r17	-31.3418	152.4486	-	y	AMS	R188125
revelata	-	NSW	Wallingat NP	r17	-31.3418	152.4486	-	y	AMS	R188126
revelata	26018	NSW	Bulahdelah SF	r18	-32.3667	152.25		-	SAMA	R72520
revelata	26020	NSW	Bulahdelah SF	r18	-32.3667	152.25		y	SAMA	R72521
revelata/ver grp2	26476	NSW	Bulahdelah	r18	-32.3667	152.25	-	y	SAMA	R72505
revelata	25243	NSW	Nerong SF	r19	-32.5353	152.1501		y	SAMA	R72510
revelata	24895	NSW	Watagan SF	r20	-32.9944	151.4456		-		
revelata	80814	NSW	Gap Creek, Watagan SF	r21	-33.03	151.41		y	SAMA	R68983
revelata	17600	NSW	Ourimbah SF	r22	-33.3333	151.3167	MT497824	-	SAMA	R42610
sibilus	7188	SA	South West River, Kangaroo Island	s1	-35.980	136.870	OR545985	y		
sibilus	7189	SA	South West River, Kangaroo Island	s1	-35.980	136.870	OR545986	y	SAMA	R20183
sibilus	7190	SA	South West River, Kangaroo Island	s1	-35.980	136.870	OR545989	y	SAMA	R20184
sibilus	7192	SA	South West River, Kangaroo Island	s1	-35.980	136.870	OR545987	y	SAMA	R20186
sibilus	7193	SA	South West River, Kangaroo Island	s1	-35.980	136.870	OR545988	y	SAMA	R20187
sibilus	33479	SA	1.5 km NW Rocky River, Flinders Chase NP	s2	-35.940	136.729	OR545990	y	SAMA	R37403
sibilus	33511	SA	Larrikin Lagoon, Flinders Chase NP	s3	-35.835	136.687	OR545991	y	SAMA	R37476
rothii	28896	NT	Black Point	-	-11.15	132.15	MT497813	-	MAGNT	R29049
rothii	28898	NT	Black Point	-	-11.15	132.15	MT497814	-	MAGNT	R26982
rothii	29374	NT	Cape Crawford	-	-16.68	135.72	MT497812	-	MAGNT	R20539
rubella	16902	NT	Borroloola	-	-11.15	132.15	MT497810	-	SAMA	R38511
rubella	28899	NT	Black Point	-	-16.06	136.3	MT497811	-	MAGNT	R29055

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APPENDIX 1. (Continued)

Species	ABTC	State	Locality	Code	Lat	Long	GenBank*	SNP	Collection	Voucher
verreauxii grp1	100221	Qld	Yowgurrabah property, Numinbah Valley	v1	-28.174	153.222	-	y		
verreauxii grp1	25738	NSW	near Bookookorara	v2	-28.76	152.08	-	y		
verreauxii grp1	25739	NSW	Girard SF	v3	-28.93	152.3	-	y		
verreauxii grp1	25199	NSW	Chaelundi picnic area	v4	-29.99	152.4	-	y		
ver grp1/ *revelata*	25436	NSW	Wild Cattle Creek SF	v5	-30.2807	152.7694	-	y		
verreauxii grp1	25888	NSW	Cathedral Rock NP	v6	-30.41	152.25	-	y		
verreauxii grp1	25143	NSW	Styx River camping area	v7	-30.68	152.13	-	y		
ver grp1/*ver* grp2	25915	NSW	Enfield SF	v8	-31.3478	152.0261	-	y		
verreauxii grp2	25413	NSW	Wangat SF	v9	-32.18	151.7		y		
verreauxii grp2	26496	NSW	Mt Royal NP	v10	-32.21	151.31	-	y		
verreauxii grp2	17570	NSW	Wallingat NP	v11	-32.3	152.43	-	y		
verreauxii grp2	26022	NSW	Bulahdelah SF	v12	-32.3667	152.25	-	y		
verreauxii grp2	16116	NSW	Watagan SF	v13	-33.0333	151.3333		-		
verreauxii grp2	24201	NSW	Watagan SF	v13	-33.12	151.17	-	y		
verreauxii grp2	12638	NSW	4 km WSW Burrawang	v14	-34.6056	150.4758		-		
verreauxii grp2	12644	NSW	Penrose SF	v15	-34.621	150.218		-		
verreauxii grp2	25447	NSW	Penrose SF	v15	-34.621	150.218		-		
verreauxii grp2	13700	NSW	Tumbarumba	v16	-35.7751	148.0167		-		
verreauxii grp2	17607	NSW	7 km W Dalgety	v17	-36.5237	148.7626	OR546038	-		
verreauxii grp2	40908	Vic	Dargo High Plains	v18	-37.1	147.15		-		
watsoni	149194	NSW	Gerringong Falls	w1	-34.66	150.648	-	y		
watsoni	145085	NSW	Barren Grounds	w1	-34.69	150.71	-	y		
watsoni	113905	NSW	Parma Creek	w1	-35	150.51	-	y		

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APPENDIX 1. (Continued)

Species	ABTC	State	Locality	Code	Lat	Long	GenBank*	SNP	Collection	Voucher
watsoni	140299	NSW	Parma Creek Nature Reserve	w1	-35.02	150.496	MT497847	-	AMS	R177179
watsoni	17597	NSW	5 km NE Tianjara Falls	w2	-35.1	150.37	MT497848	y	SAMA	R42607
watsoni	113906	NSW	Tianjara Falls	w2	-35.1	150.33	-	y		