



Article

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A new species of *Crinia* (Anura: Myobatrachidae) from the Flinders Ranges, South Australia

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Abstract

We describe, as a new species, the northern Flinders Ranges populations of the myobatrachid frog *Crinia riparia*. It is distinguished from *C. riparia sensu stricto* on the basis of reciprocal monophyly of mitochondrial genes, absence of haplotype sharing in a nuclear gene, fixed differences in allozyme loci and differences in larval oral disc morphology consistent with less adaptation to stream habitats. We were not able to reliably distinguish the taxa on the basis of adult morphology. The geographic range of *C. riparia sensu stricto* is now reduced to a 75 kilometre section of the southern Flinders Ranges from Napperby Gorge in the south to Mt Brown in the north suggesting that an assessment of its conservation status is warranted.

Key words: frog, *Crinia*, conservation, larva, tadpole, mitochondrial DNA, taxonomy

Introduction

The Australian myobatrachid frog genus *Crinia* Tschudi 1838 currently comprises 16 species of small, ground-dwelling frogs including *C. nimba* (*Bryobatrachus nimbus*) Rounsevell *et al.* 1994 (see Read *et al.* 2001), which are distributed widely across the continent and extra-continently in southern New Guinea. Most of the species level diversity in *Crinia* is found in the south-eastern (six species) and south-western (five species) mesic corners of the continent. In the arid zone, *Crinia* is represented by two taxa, *C. deserticola* (Liem & Ingram 1977) a widespread species in north-eastern Australia and by *C. riparia* Littlejohn and Martin 1965 confined to streams in the Flinders Ranges of south central Australia. The streambank froglet, *C. riparia*, appears to be the sister lineage to the widespread south-eastern mesic species *C. signifera* Girard 1853 (Read *et al.* 2001). Their ranges partly overlap in the southern Flinders Ranges where *C. riparia* is found in swift-flowing creeks with rocky substrates and little vegetation while *C. signifera* is found in slow-flowing creeks that are heavily vegetated, with mud or sand substrates (Odendaal & Bull 1982). *Crinia riparia* shows adaptations for life in swift-flowing streams (Littlejohn & Martin 1965). Specifically it attaches its strongly adherent eggs to the undersurface of rocks in streams and the free-living exotrophic larva has a stream-adapted depressed body shape with a large, broad oral disc for improved adherence to rocks (Altig & Johnston 1989).

Three main lines of evidence suggest that *C. riparia* may be composite. First, Symula *et al.* (2008) found two divergent mitochondrial clades within *C. riparia* with an approximate age of divergence of 12MYA estimated from molecular clock analysis. Second, Odendaal *et al.* (1983) found two groups that had fixed allelic differences at 19% of the 26 loci genotyped with multi-locus allozyme electrophoresis and both differed from *C. signifera* at 65% of the 26 loci genotyped. Third, a preliminary comparison of larval morphology from the northern and southern Flinders Ranges in 2004 (Anstis unpubl. data) indicated consistent differences in both larval body form and oral disc features. Although each study included animals from just a small number of localities, all three datasets were concordant in suggesting the presence of two genetically divergent groups that replace each other between Warren Gorge to the north-east of Quorn and Mambray Creek/Melrose to the south of Quorn.

In the present study, we extended the analysis of variation in *C. riparia* to assess the systematic implications of the findings of Symula *et al.* (2008) and Odendaal *et al.* (1983) by surveying mtDNA variation throughout the range of *C. riparia*, including nuclear gene data to establish a 'species' tree of relationships among populations. We also examined more thoroughly the morphological variation in larvae and extended the examination to adult morphology. We determine that '*C. riparia*' comprises a pair of species, one of which we describe herein as new.

Methods

Morphology

Adult specimens were formalin-fixed and preserved in 70% ethanol unless stated. Morphometric measurements (Table 1) of adult specimens followed (Tyler 1968). Measurements were made using a stereo microscope with the aid of an eyepiece micrometer.

TABLE 1. Description of morphometric traits measured in adults and examined or measured in larvae.

Trait	Abbrev.	Description
Adults		
Length of body	SVL	From anterior most point of rostrum to posterior most point of urostyle
Length of head	HL	From anterior most point of rostrum to posterior margin of mandible
Head width	HW	Measured at greatest width
Eye length	EYE	Longest dimension of eye
Eye to rostrum distance	EN	Distance from anterior-most edge of eye to anterior most point of rostrum
Interorbital span	IO	Distance between anterior edge of eyes
Internarial span	IN	Distance between anterior edge of nostrils
Length of arm	ARM	From palm of hand to bent exterior edge of elbow
Length of longest finger	FING	From proximal surface of hand thumb junction to toe tip
Length of tibia	TL	Measured with leg in natural resting position, from knee to tarsus
Length of longest toe	TOE	From heel to toe tip
Larvae		
Total length	TL	From tip of snout to tip of tail
Body length	BL	From tip of snout to end of body
Body depth	BD	Maximum vertical depth of body
Labial tooth row formula	LTRF	after Altig (1970)
Oral disc width	ODW	Width of disc measured at widest point across centre of upper labium, ventral view
First and second anterior tooth rows	A ¹ , A ²	Approximate maximum length (oral disc pinned open flat, but row could not be held completely straight)
Second and third posterior tooth rows	P ² , P ³	Approximate maximum length (oral disc pinned open flat, but row could not be held completely straight)
Length of posterior medial gap in papillae	PG	Measured between last papilla on either side of gap
Snout to spiracle	SS	Distance from snout to posterior edge of spiracle lateral view
Snout to eye	SE	Distance from snout to anterior edge of eye in lateral view
Width of snout	SW	Width of snout measured in ventral view at widest point across middle of oral disc

Images were taken with a Visionary Digital BK plus imaging system with a Canon EOS 5D Mark II 21.1 megapixel camera. Images were produced using Zerene Stacker Zerene Systems LLC software, and cropped and resized in Photoshop CS5.

Selected larvae from both northern and southern populations were anaesthetised in a solution of 0.2% chlorbutol, then photographed with a Nikon D80, 60 mm macro lens and macro flash kit. Measurements of both anaesthetised and additional preserved specimens were obtained with the aid of vernier callipers and a micrometer eye-piece attached to a Wild M5 stereoscopic microscope. Voucher specimens were fixed in 4% buffered formalin and preserved in 70% ethanol. Drawings of preserved specimens were prepared with the aid of a drawing tube attached to the microscope. Larvae were staged according to Gosner (1960). Morphometric traits and aspects of larval anatomy described and analysed are illustrated in Fig. 4. Methods of morphometric measurements of larvae follow Anstis (2002) and Anstis and Tyler (2005) and abbreviations are listed in Table 1.

We used Principal Components Analysis (PCA), which does not identify groups *a priori*, to examine the patterns of relationship among morphological traits (natural log transformed) separately for adults (11 variables) and larvae (6 variables). PCA was performed with the “princomp” routine which is included in the software package R version 2.14.1. The first principal component was interpreted as representing variation in body size and shape and the second and third principal components summarized shape differences.

Collection details of vouchers are presented in Appendix 1B. Abbreviations: ABTC—Australian Biological Tissue Collection, South Australian Museum, Adelaide; SA—South Australia; SAMA—South Australian Museum, Adelaide; QM—Queensland Museum; WA—Western Australia; WAM—Western Australian Museum, Perth.

Molecular genetic analyses

We sequenced a total of 44 frogs for a segment of the mitochondrial genome as follows: 29 *Crinia flindersensis* **sp. nov.** including the holotype, 13 *C. riparia* including typo-typic specimens from Alligator Gorge and two *C. signifera* from the southern Flinders Ranges (Fig. 1, Appendix 1A). Sequences of all other described species of *Crinia* were included along with outgroups selected on the basis of the phylogeny of Read *et al.* (2001). A subset of these individuals was sequenced for a section of the nuclear *RAG1* gene. Details, including GenBank accession numbers, of specimens for which we obtained sequence data, other *Crinia* species and outgroups from Read *et al.* (2001) and Doughty *et al.* (2009) are presented in Appendix 1A.

DNA was extracted using a Puregene™ DNA Isolation Tissue Kit, D-7000A (Gentra Systems) following the manufacturer's instructions. We obtained 654 base pair (bp) mitochondrial DNA sequence comprising 95bp of sequence from the tRNA^{GLN} and tRNA^{MET} genes and 509 bp of the protein coding gene *ND2* by PCR amplification with the light and heavy strand primers: L4221 tRNA^{ILE} 5'-AAG GAC CTC CTT GAT AGG GA-3 and H4980 ND2 5'-ATT TTT CGT AGT TGG GTT TGR TT-3' respectively (Macey *et al.* 1997). We obtained 967 bp of *RAG1* sequence by PCR amplification with forward and reverse primers: Amp-RAG1F 5'-AGC TGC AGY CAR TAC CAY AAR ATG TA-3' and Amp-RAG1R 5'-AAC TCA GCT GCA TTK CCA ATR TCA CA-3' respectively (San Mauro *et al.* 2004). PCR conditions were: 5 µL dilution of template DNA (50-100 ng); 0.2 µL of AmpliTaq Gold DNA polymerase (Perkin Elmer), 4 µL of 25mM MgCl₂, 5 µL of GeneAmp 10 × PCR Gold Buffer (Perkin Elmer), 4 µL of 10mM dNTPs, 2 µL of 0.5 µM of each primer = 4 µL in a total volume of 50 µL. PCR cycling conditions were: one cycle of 94°C for 9 min, 36 cycles of 94°C for 45 sec, 55°C for 45 sec, and 72°C for 1 min, and one cycle of 72°C for 6 min.

BigDye™ cycling conditions were employed to sequence the light strand with the same primer used for PCR amplification. Purification of reaction products was performed using ethanol precipitation. Sequences were edited using SeqEd (version 1.0.3, ABI) and aligned with the MAFFT algorithm implemented in Geneious version 5.5.2. To check for nuclear paralogues all *ND2* protein encoding sequences were translated in Geneious using the standard vertebrate mitochondrial genetic code and examined for unexpected stop or nonsense codons.

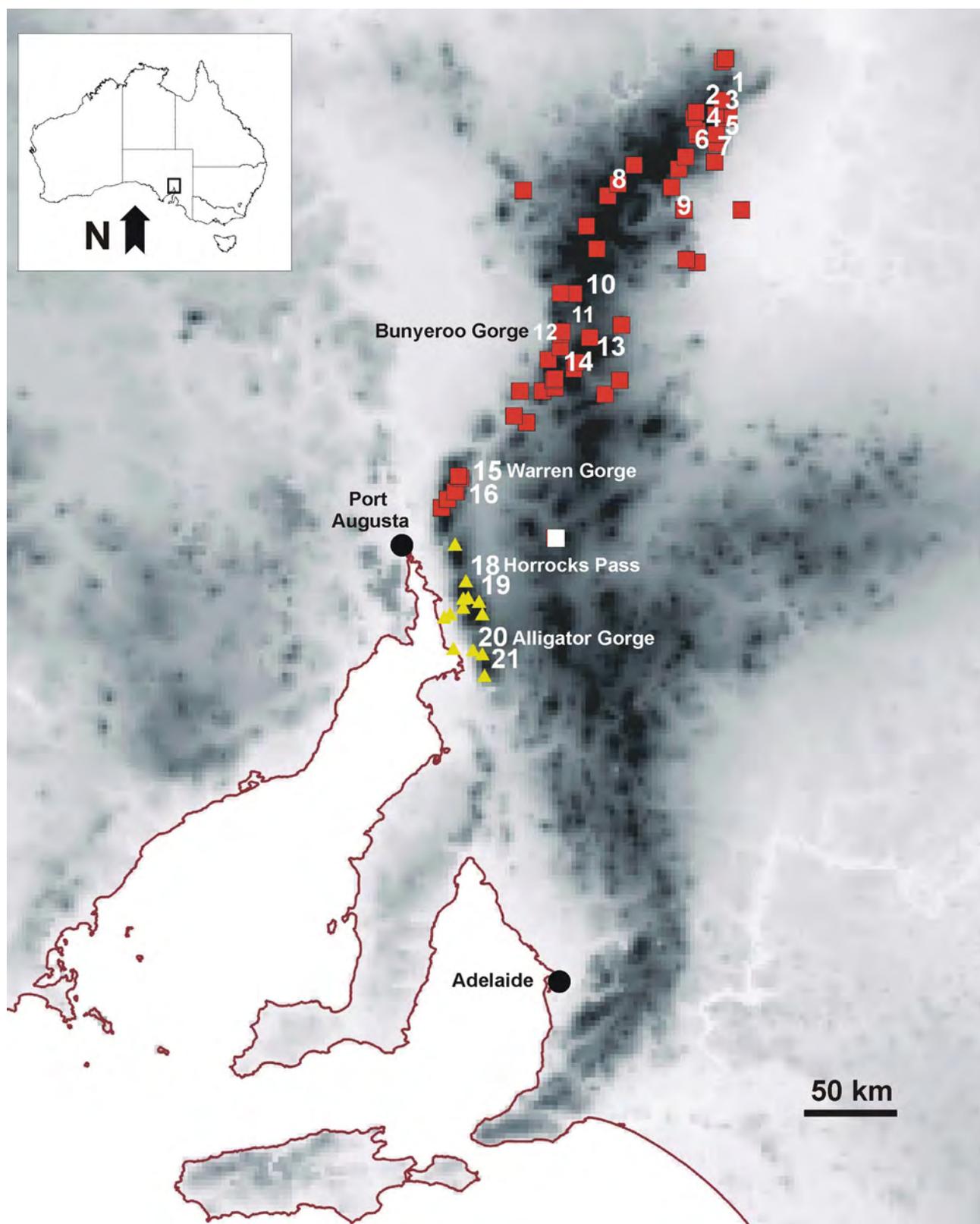


FIGURE 1. Distribution of *Crinia riparia* [green triangles] and *C. flindersensis* sp. nov. [red squares] in the Flinders Ranges, South Australia, showing elevation in grey scale shading. Collection sites of specimens that were used in the molecular genetic analyses are numbered—refer to Appendix 1A for number legend. The type locations for each taxon are also named. The white square denotes the locality, 1.5k SE Bagalowie Homestead, which is discussed in the Remarks section under *C. riparia*.

Phylogenetic analyses

The aligned sequence data were analysed with two phylogenetic methods: Markov-Chain Monte Carlo (MCMC)-Bayesian phylogenetic analyses implemented in MrBayes version 3.1.2 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003) and maximum likelihood (ML) criterion using RAxML using the RAxML Web Server version (Stamatakis *et al.* 2008), which uses the GTR+ Γ model of nucleotide substitution. For the mitochondrial sequence data, we applied four data partitions: 1st, 2nd and 3rd codon positions for the *ND2* gene and the tRNA genes. The robustness of phylogenetic hypotheses was tested with non-parametric bootstrapping from 1000 pseudoreplicates. The program Modeltest version 3.7 (Posada & Crandall 1998, 2001) was used to determine an appropriate model of nucleotide substitution. Four data partitions were applied; the tRNA genes and the three codon positions in *ND2*. Under the Akaike Information Criterion (AIC) a different model of nucleotide substitution was found to be the most suitable for each data partition: HKY+I+G for the tRNA genes, GTR + I + G for the 1st codon position, HKY+I for the 2nd codon position and GTR+G for the 3rd codon position.

Phylogenetic analysis by Bayesian inference was performed in two runs, each with four separate MCMC chains (1 cold) for 1×10^7 generations and sampled every 1000 generations to give a sample of 10,000 trees. Using AWTY (Wilgenbush *et al.* 2004) the cumulative and compare commands were used to assess stationarity. Stationarity was reached by 5×10^6 generations and thus the first 6000 trees were excluded and the remaining 4000 trees, used to derive a strict consensus tree with posterior probabilities of the clades.

Variation among the *RAG1* data was visualised with a median-joining network implemented in Network version 4.610 (Bandelt *et al.* 1999). Nucleotide and haplotype diversity statistics were calculated in DnaSP version 5.10 (Libardo & Rozas 2009). Resolution of heterozygous sites into constituent haplotypes could be achieved by visual inspection as each heterozygous site was unique to that individual. Inter-species net genetic distances (D_A) between mitochondrial sequences were calculated in MEGA version 5.03 (Tamura *et al.* 2011).

Results and Discussion

We use a non-conventional approach to naming groups in the results section to make the paper easier for the reader to follow. We use the final specific epithets throughout the manuscript rather than use an initial group nomenclature that we would change to the final specific epithets in the taxonomy section. Of course we do not assume the separate species status of the two groups within *C. riparia sensu lato* but rather use the results section to test this hypothesis before dealing with the final taxonomy.

Molecular genetic analyses

The aligned dataset comprised 650 bp of *ND2* and 139bp of tRNA nucleotide sequences. Translation of the *ND2* portion of the alignment did not detect nonsense or premature stop codons. A ML tree derived from the RAxML analysis is shown in Fig. 2. We interpreted strong support for a node if the posterior probability (Bayesian analysis) and bootstrap proportions (ML analysis) were above $\geq 95\%$ and $\geq 70\%$ respectively. Well-supported nodes in the phylogenies resulting from the Bayesian and ML analyses showed congruent phylogenetic pattern for all taxa. *ND2* sequences from *C. riparia* fell into two groups—northern and southern with the geographic break between the two falling between Mt Brown and Warren Gorge.

Net divergence (D_A) between the two *C. riparia* clusters was 0.077. Among other *Crinia*, net divergence between sister species ranged from 0.019 [between *C. pseudinsignifera* and *C. subinsignifera*] to 0.109 [between *C. bilingua* and *C. remota*].

Variation among the *RAG1* nucleotide sequences is shown in a median-joining network in Fig. 3. Amongst the *C. riparia* sequences, 11 haplotypes were observed and they fell into two clusters, one containing all of the southern haplotypes (n=4) and the second all of the northern haplotypes (n=7). The *C. signifera* specimen was heterozygous for two haplotypes which were very divergent from any of the *C. riparia* haplotypes.

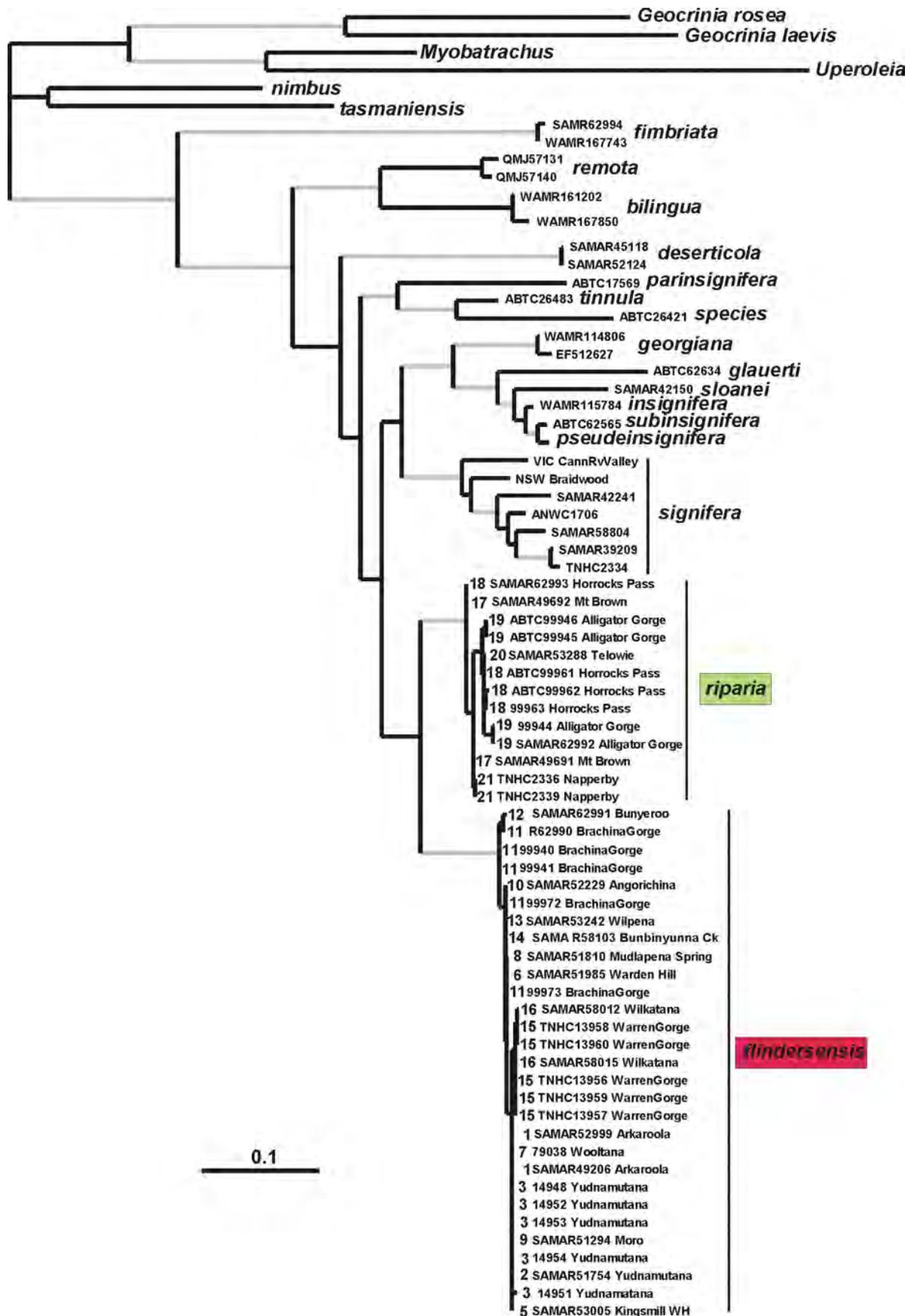


FIGURE 2. A ML tree showing phylogenetic relationships among mitochondrial *ND2* haplotypes for *Crinia*. Branches in grey had Bayesian posterior probabilities ≥ 0.95 and ML bootstrap proportions $\geq 70\%$. Species names and haplotype numbers refer to specimens and collection locations in Appendix 1.

1,3,4,7,8,10,11,14,15,16

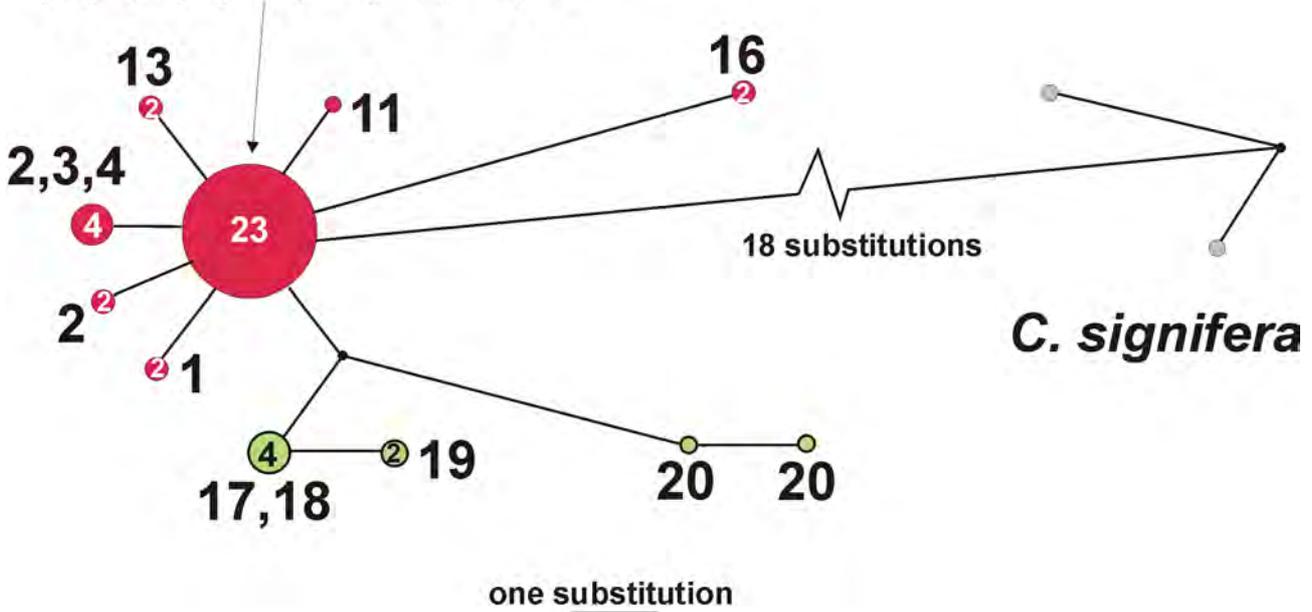


FIGURE 3. Median-joining network of *RAG1* nucleotide haplotypes from *C. flindersensis* **sp. nov.** (red), *C. riparia* (green) and a *C. signifera* (grey). Numbers inside haplotype circles are frequencies and numbers beside are locality labels as per Appendix 1.

Adult morphology

We were not able to identify consistent variation in morphological traits or colour or pattern from a visual inspection of vouchers (*C. flindersensis* **sp. nov.** n=51 and *C. riparia* n=26 see Appendix 1). Details of variation in colour and pattern are presented in the taxonomic accounts below.

The two *Crinia* are very similar in size and proportions. Morphometric variation between *Crinia flindersensis* **sp. nov.** and *C. riparia* in 11 traits is summarised in Table 2. Bivariate plots of the first two principal components (PCs) summarising variation in size and shape of male and female *Crinia* are presented in Fig. 5. Partial separation was achieved in both the male and female PCA, more separation was evident in the male analysis. 3D plots of the first three PCs (not shown) marginally improved separation. For females, the proportion of variance explained by the first three PCs were 0.67, 0.74, and 0.81 respectively. The traits with the highest loadings were: PC1 - TIBIA, TOE, SVL; PC2 - HL and EYE. For males, the proportion of variance explained by the first three PCs were 0.69, 0.1 and 0.05 respectively. The traits with the highest loadings were: PC1 - TIBIA, TOE, SVL and HW; PC2 - IN and HL.

Larval morphology

Detailed descriptions of the larvae of both taxa are presented in the species accounts below. Here, we focus mainly on the traits that show significant differences between the taxa (Table 4).

In the stream-adapted *C. riparia*, the tail has shallow fins (Fig. 6). The dorsal fin arches only slightly or not at all before tapering to a broadly rounded tip. In *C. flindersensis* **sp. nov.** the fins of some larger larvae from Bunyeroo Gorge are moderately arched and the tail tapers slightly to a rounded tip. The larvae are likely to be plastic in body proportions and tail shape in response to changing environmental factors such as the increase in depth or flow rate of water (Vences *et al.* 2002) and samples of *C. riparia* collected on two different occasions (one of which was in a drought and pools were very small), showed variation in body size and fin depth. Specimens developing in overcrowded conditions in very small rock pools without water flow during dry periods were generally small with shallow fins, while those in less crowded situations and deeper pools grew larger with slightly deeper fins. *Crinia riparia* has a slightly longer tail relative to body length than *C. flindersensis* **sp. nov.** (Table 3), which is especially evident in larvae from Horrock's Pass (Fig. 6).

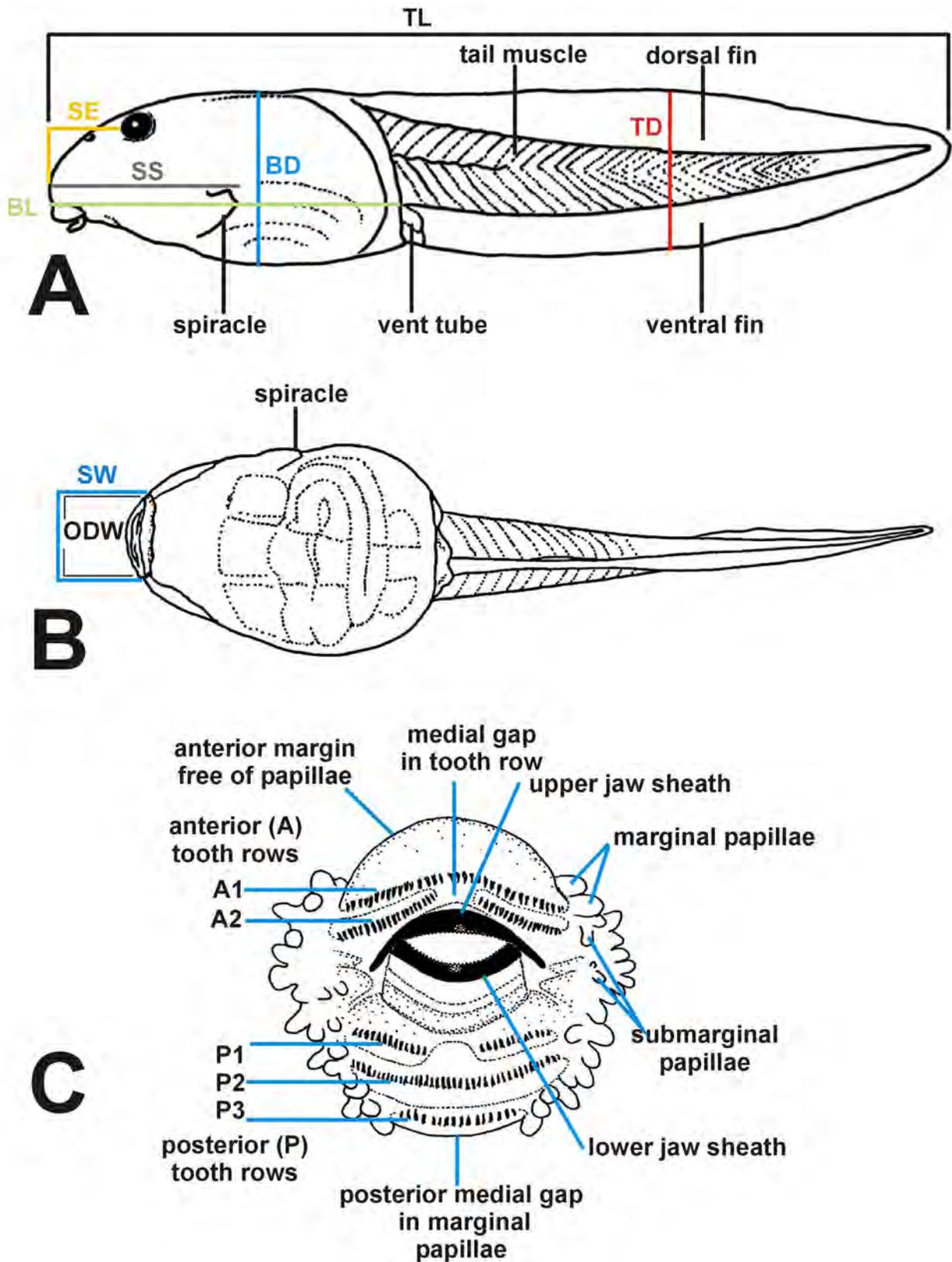


FIGURE 4. Larval traits analysed. **A)** lateral view; **B)** ventral view; **C)** view of oral disc with anterior margin uppermost . Abbreviations are explained in Table 1.

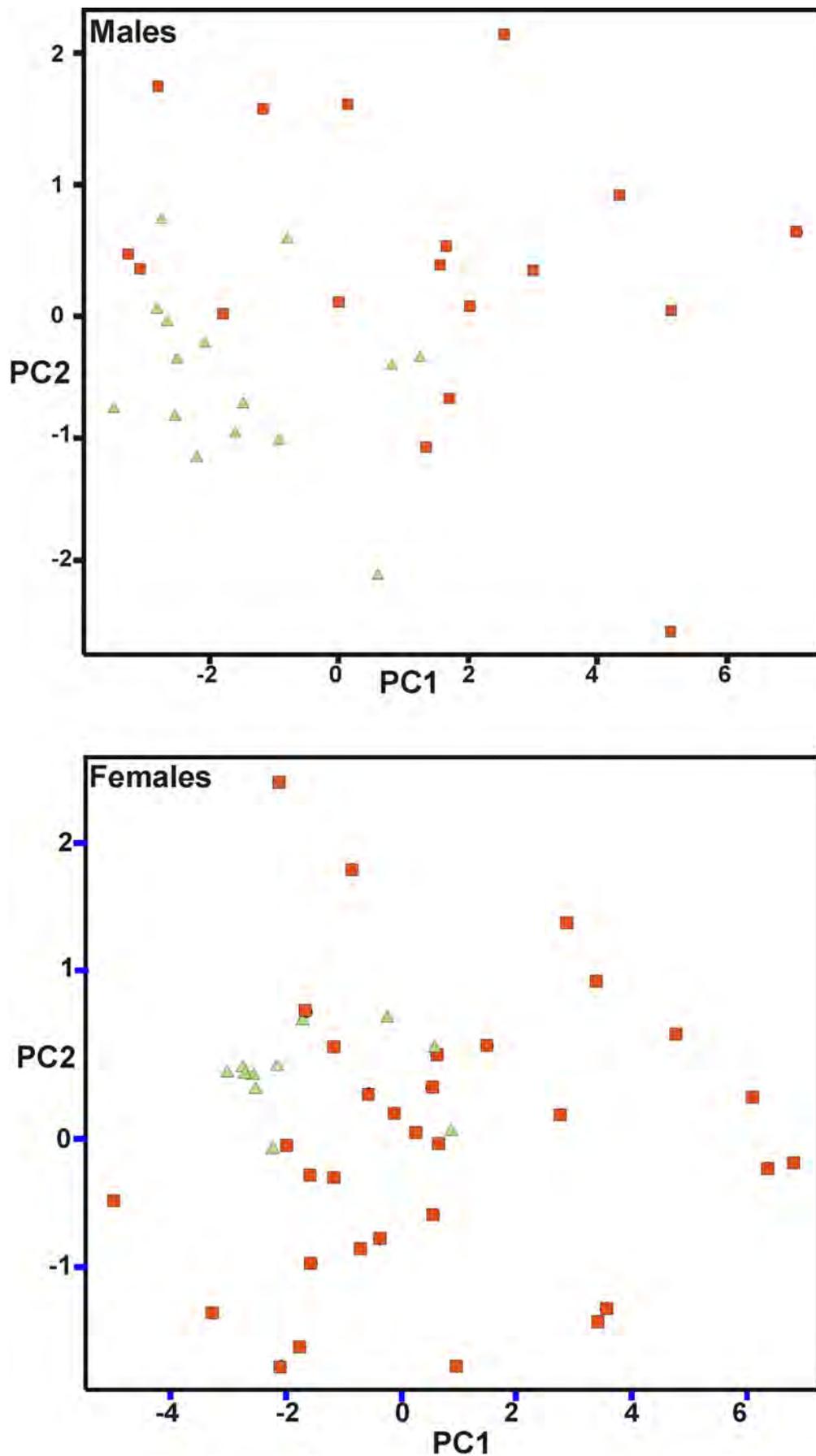


FIGURE 5. Plots of first two principal components (PC1, PC2) derived from PCA of morphometric traits in females and males. *C. riparia*—green triangles, *C. flindersensis* sp. nov.—red squares.

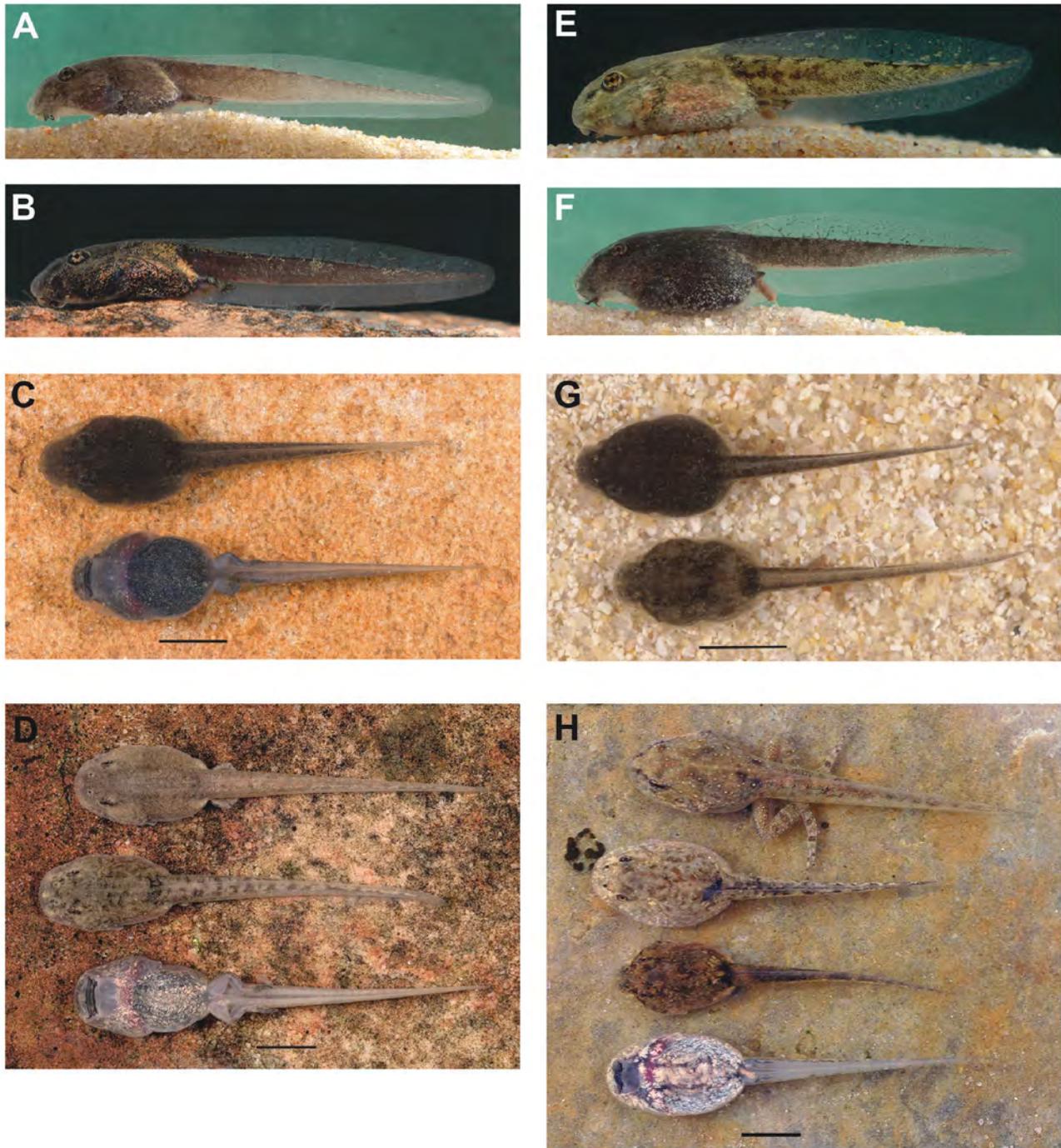


FIGURE 6. Images of larvae in life. Lateral views of *Crinia riparia* **A**) stage 37 (lateral view), Horrock's Pass; **B**) stage 36 (lateral view), Alligator Gorge; **C**) stage 36 (dorsal view) and 37 (ventral view), Alligator Gorge; **D**) stages 39 and 37 (dorsal views) and 39 (ventral view), Horrock's Pass. *Crinia flindersensis* **sp. nov.** **E**) stage 34 (lateral view), Bunyeroo Gorge; **F**) stage 28 (lateral view), Warren Gorge; **G**) stages 30 and 28 (dorsal view), Warren Gorge; **H**) stages 41, 34, 29 (dorsal views) and 35 (ventral views), Bunyeroo Gorge. Scale bar = 5 mm.

Similarly, samples of *C. flindersensis* **sp. nov.** from the more rocky creek in Warren Gorge were darker and more streamlined with shallow fins compared to the more variable, often golden larvae with plump bodies and slightly more-arched fins from the sandy creek (with scattered rocks) in Bunyeroo Gorge. In *C. riparia*, the dorsal and tail muscle pigment is commonly more uniform black or dark rusty-brown (Alligator Gorge), and sometimes golden-brown (Horrock's Pass), occasionally with a few or diffuse patches on body and dorsal tail muscle, whereas in *C. flindersensis* **sp. nov.** the dorsal and tail pigment can be more variable, ranging from bright gold through rusty-brown to black, often with diffuse or more distinct patches or markings in the lighter colour forms.

TABLE 2. Adult measurements. Figures are mean \pm SD (range).

Trait	<i>C. flindersensis</i> sp. nov.		<i>C. riparia</i>	
	female	male	female	male
N	34	18	10	15
SVL	22.3 \pm 2.5 (16.–25.9)	18.7 \pm 1.9 (14.6–21.3)	23.7 \pm 1.5 (20.9–25.4)	20.2 \pm 0.9 (18.5–21.8)
HW	6.4 \pm 0.7 (5.1–8.2)	5.7 \pm 0.7 (4.6–6.9)	7 \pm 0.6 (5.7–7.8)	6.2 \pm 0.4 (5.6–6.9)
HL	6.7 \pm 0.6 (5.2–8)	6.3 \pm 0.9 (4.7–8)	7.4 \pm 5.2 (6.6–8.2)	6.1 \pm 0.5 (5.3–6.9)
EYE	2.5 \pm 0.3 (1.9–3.2)	2.3 \pm 0.2 (1.9–2.7)	2.7 \pm 0.1 (2.5–2.8)	2.5 \pm 0.2 (1.9–2.9)
ARM	5.2 \pm 0.6 (3.9–6.7)	4.7 \pm 0.5 (3.7–5.8)	5.7 \pm 0.2 (5.2–6)	5.3 \pm 0.3 (4.6–5.8)
TL	9.8 \pm 0.8 (8.1–12)	8.7 \pm 0.9 (7.1–10)	10.6 \pm 0.4 (10–11)	9.9 \pm 0.4 (8.9–11)
FING	5.5 \pm 0.6 (4.1–6.7)	4.9 \pm 0.5 (4–5.6)	5.5 \pm 0.5 (4.5–6.3)	5.2 \pm 0.4 (4.4–5.8)
TOE	11.2 \pm 1.3 (8.9–14)	10.3 \pm 1.1 (8–13)	12.1 \pm 0.7 (11–12.9)	11.5 \pm 0.9 (10.3–12.9)
HL/SVL	0.3 \pm 0.03 (0.2–0.4)	0.3 \pm 0.03 (0.3–0.4)	0.3 \pm 0.01 (0.3–0.33)	0.3 \pm 0.02 (0.27–0.35)
HW/HL	0.96 \pm 0.09 (0.8–1.2)	0.91 \pm 0.09 (0.8–1.1)	0.94 \pm 0.08 (0.8–1.1)	1 \pm 0.10 (0.8–1.2)
TL/SVL	0.4 \pm 0.02 (0.4–0.5)	0.47 \pm 0.02 (0.53–0.51)	0.45 \pm 0.03 (0.41–0.49)	0.5 \pm 0.01 (0.47–0.51)

The oral disc provided a more stable feature for comparison despite the variation in body and tail form noted above. Indeed the oral disc features were stable within each taxon over distances of over 100 km (Bunyerroo Gorge to Warren Gorge in *C. flindersensis* **sp. nov.**) and between collections made up to 5 years apart in both taxa (Appendix 1C). Both species have a broad, ventral oral disc which is indented (emarginate) at the sides (Fig. 7). In *C. riparia* the oral disc is somewhat suctorial and adapted to lotic environments (Odendaal & Bull 1980). The oral disc of the new taxon also appears to be somewhat suctorial, but likely to be to less so, given the features described below. In fully grown larvae (stages 34–40), the disc of *C. riparia* is almost as wide as the snout, whereas in *C. flindersensis* **sp. nov.** it is about three-quarters the width of the snout (Bunyerroo Gorge). In all *C. riparia*, there are small crowded marginal papillae around each side of the disc and several submarginal papillae in anterior and posterior labia, whereas in *C. flindersensis* **sp. nov.** the marginal papillae are slightly less numerous and most have no submarginal papillae (three individuals had a very small number). The lack of anterior papillae in either taxon suggests the disc may be less suctorial than larvae of other myobatrachid genera in which papillae completely surround the disc (e.g. *Mixophyes*; Anstis 2002, 2012). Both taxa have two anterior (A) and three posterior (P) tooth rows. In *C. riparia*, however, all tooth rows mostly extend almost to the edges of the disc, whereas in *C. flindersensis* **sp. nov.** only rows A¹, A² and row P² extend almost to the edge. More significantly, in *C. riparia*, there is a wide posterior medial gap in the papillae which is up to half the width of the disc, while in *C. flindersensis* **sp. nov.** the corresponding medial gap is only about one-third the width of the disc (Table 3). In *C. riparia*, P³ is consistently only very slightly shorter than P² and extends well beyond each side of the posterior medial gap in the papillae almost to the edge of the disc, whereas in *C. flindersensis* **sp. nov.**, P³ is about half the length of P² and extends only slightly beyond each side of the narrower posterior medial gap in the papillae.

In *C. riparia*, the oral disc showed some slight variation in A² which was entire in all but five specimens, in which it was medially divided but with no visible gap. In *C. flindersensis* **sp. nov.**, A² usually has a very narrow

gap (fused in two). The labial tooth row formula (LTRF; Altig 1970) for *C. riparia* is 2/3(1), and for *C. flindersensis* 2(2)/3(1).

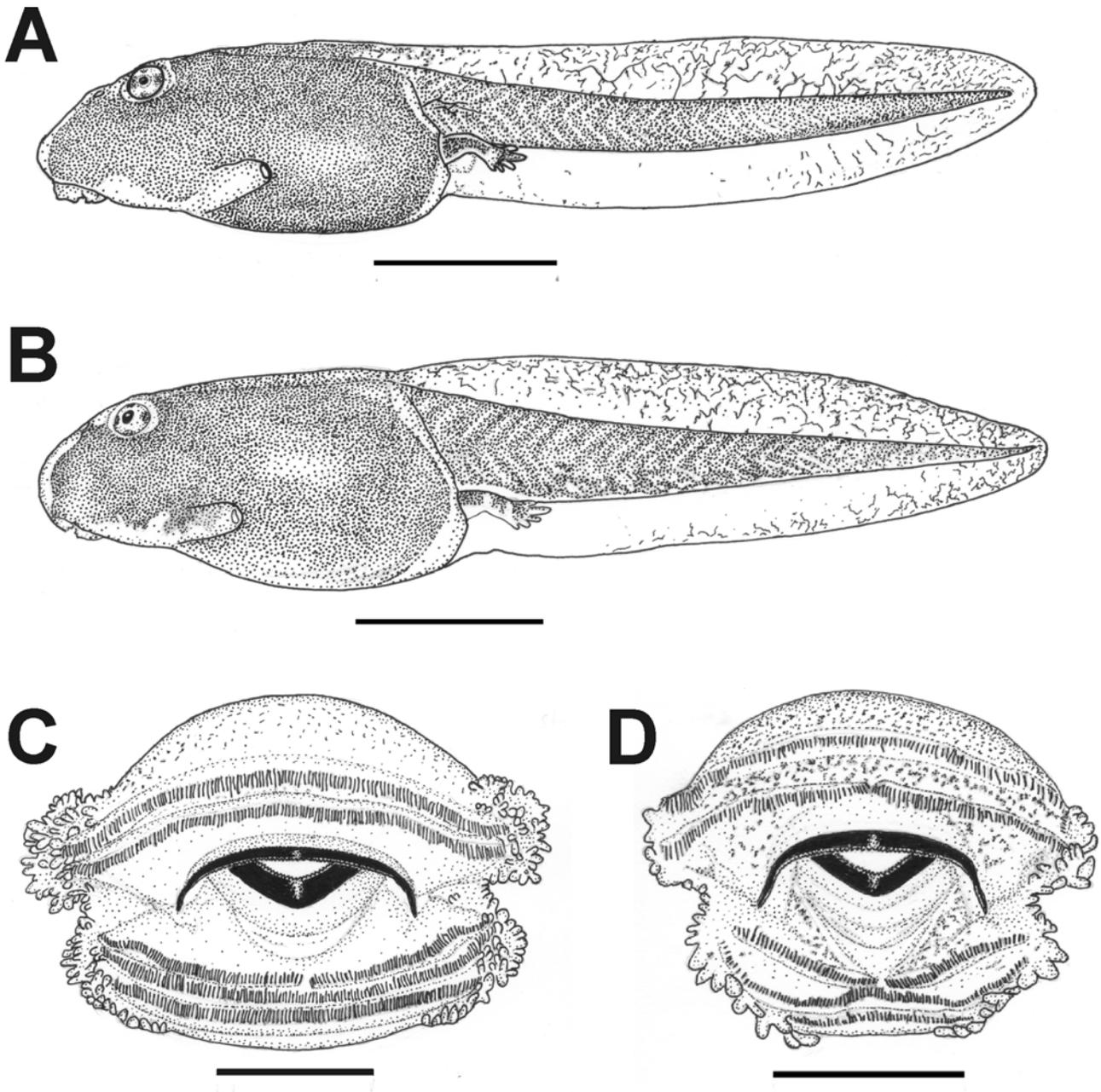


FIGURE 7. Drawings of the lateral views of larvae **A)** *Crinia riparia* stage 37, Alligator Gorge; **B)** *C. flindersensis* stage 37, Bunyerroo Gorge; scale bar = 5 mm. Drawings of the larval oral disc of **C)** *C. riparia* stage 38, Alligator Gorge; **D)** *C. flindersensis* **sp. nov.** stage 36, Bunyerroo Gorge; scale bar = 1 mm.

Bivariate plots of the first two principal components (PCs) summarising variation in size and shape of larval *Crinia* are presented in Fig. 8. Complete separation of larvae of *C. riparia* (n=22) and *C. flindersensis* **sp. nov.** (n=27) was achieved in the PCA. The proportion of variance cumulatively explained by the first two PCs was 95% with PC1 and PC2 explaining 83.95% and 11.94% respectively. The traits with the highest loadings were: PC1—ODW, SW, TL; PC2—BL and PG.

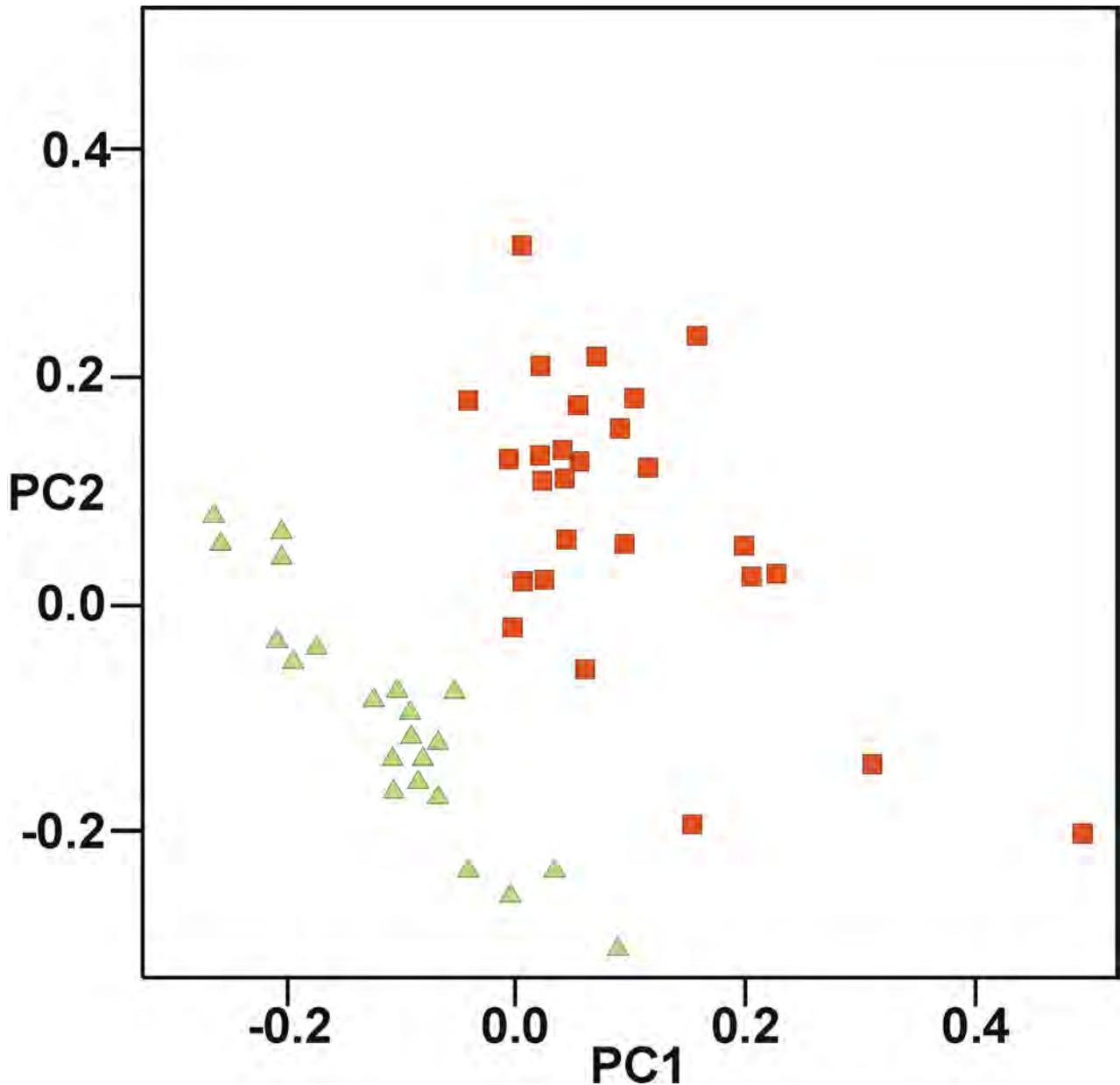


FIGURE 8. Plot of first two principal components (PC1, PC2) derived from PCA of morphometric traits of larvae. *C. riparia*—green triangles, *C. flindersensis sp. nov.*—red squares.

Systematic Conclusions

The combination of reciprocal monophyly of mitochondrial and nuclear loci, differences in larval oral disc morphology consistent with adaptation to lotic versus general stream habitat and evidence of fixed allelic differences at 19% of the 26 loci genotyped with multi-locus allozyme electrophoresis (sampled from Warren Gorge [*C. flindersensis sp. nov.*], Melrose and Telowie Gorge [*C. riparia*]) (Odendaal *et al.* 1983) supports the recognition of two evolutionary species within *C. riparia sensu lato* (Simpson 1951, de Queiroz 1998). Furthermore the nucleotide sequence divergence in mtDNA between the taxa is within the range of values for other well accepted sister taxon pairs of *Crinia*. The absence of discrete diagnostic adult morphological traits distinguishing the two taxa does not deny their separate species status. While we have inferred the ranges of the two taxa based initially on the distribution of mtDNA typed specimens (see Fig.1), noting that our mtDNA sampling includes several locations around the region of shallow allopatry of the two taxa and none of our analyses suggest that the ranges of the taxa overlap.

As the type description of *C. riparia* includes specimens of both *C. riparia sensu stricto* and the new species, we redescribe *C. riparia* before describing the new species.

Taxonomy

Crinia riparia (Littlejohn & Martin 1965)

Southern Flinders Ranges froglet

Figures 6, 9–10.

Holotype. Mature male No. 950163 in the Zoology Department Collection, University of Melbourne collected at Alligator Gorge, 8.5 miles SSW of Wilmington, Flinders Ranges, South Australia between 9 September 1962 and 29 October 1963.

Material examined. See Appendix 1.

Description. Although Littlejohn and Martin (1965) included specimens from within the geographic range of *C. flindersensis* **sp. nov.** in their paper, their description of *C. riparia* is based only on the type series (n=14), which were all collected from Alligator Gorge. Consequently we do not redescribe *C. riparia sensu stricto* herein.

Colour. Using the back pattern polymorphism nomenclature characteristic of some species of the *C. signifera* complex (Main 1957, 1961) the dorsal pattern ranges from ‘lyrate’ to ‘intermediate’ to ‘smooth’ [i.e. unpatterned] (Fig. 9). In a sample of 36 preserved individuals from across the species range, the proportion of individuals with a ‘lyrate’ pattern was 39%, with an ‘intermediate’ pattern 17% and with the ‘smooth’ pattern 17% (27% of specimens were too faded to determine the back pattern). Ventral patterns vary considerably (Fig. 10). In specimens from the southern end of the range, the abdomen has a white background with large highly contrasting black marks and patches, while in the rest of the range the background tends to be cream and the darker marks are smaller, greyer and more numerous.

Mating call structure. Mating calls of a series of males from Alligator Gorge are presented in Littlejohn and Martin (1965).

Larval morphology. Littlejohn and Martin (1965) described the larvae of *C. riparia* from Alligator Gorge but did not have material available beyond stage 28. The larvae we examined from Alligator Gorge showed very little variation in pigment or in the oral disc and matched the description of the type material in pigment and features of the oral disc. Larvae from a creek at Horrock’s Pass were lighter golden-brown in colour (with a few diffuse darker patches on one) after anaesthetisation in warmer water prior to photography. These would likely turn darker in the cold, clear water of the creek.

Larvae from Alligator Gorge (n=18) and Horrock’s Pass (n=3) are described. Maximum length was 38 mm, with a minimum body length of 12.9 mm (stage 36, Horrock’s Pass). The tail is slightly more than twice body length (Table 3). Body small, streamlined, often broadest across gill region and abdomen slightly wider than deep. Snout broadly rounded in dorsal view, elongate and narrowly rounded in profile. Eyes dorsolateral with a dorsal tilt. Iris mostly golden with a gold ring around pupil and a V-shaped projection (umbraculum) in centre of dorsal edge. Nares small, moderately spaced, closer to eyes than tip of snout and open mainly dorsally with a small raised flap on inside rim. Spiracle visible from above, tapers slightly to dorsoposterior opening below horizontal body axis and posterior to midpoint. Vent tube dextral, broad, opens midway up ventral fin and mostly unattached to fin behind.

Dorsum and sides of body (Alligator Gorge) mostly uniform dark brown or black with a diffuse layer of fine copper iridophores (denser laterally) over all but a small darker patch over base of body. Three larvae photographed from Horrock’s Pass were lighter golden-brown, one with small, diffuse darker patches over dorsum and tail muscle (Figure 6A, D). Body wall around sides of head and body transparent in earlier stages, increasingly pigmented with melanophores as larva grows. By stage 41, hind limbs are banded and diffuse darker markings of adult are visible. Small dorsal tubercles present in some. Venter dark over abdomen, clearer anteriorly with fine specks of iridophores increasing during larval growth, but never dense (Alligator Gorge). Larvae from Horrock’s Pass had a more uniform layer of copper sheen over abdomen.

Larvae are known to metamorphose in the field in spring (late October to November), but depending on weather conditions, some may do so in December. Metamorphs are miniatures of adults with variable dorsal

patterns. The undersurface is translucent dark grey with finely scattered whitish granulation. Mean SVL of eight metamorphs from Alligator Gorge is 10.4 mm (9.7–11.4).

Habitat. Odendaal and Bull (1982) describe the habitat of *C. riparia* in detail. The range of *C. riparia* abuts the range of *C. signifera* along a north-south axis of the southern Flinders Ranges with a narrow zone of overlap on the eastern slopes of the ranges or through the middle of the ranges before the creeks flow down the western slopes. Creeks occupied by *C. riparia* tend to have much steeper slopes, swifter water flow and less vegetation than creeks occupied by *C. signifera* in the southern Flinders Ranges.

Larvae are lotic and benthic, hiding among rocks and leaf litter on the substrate where they graze on sediments in gravel and algae on rocks. They often lie on rocks in stream pools or in shallow water at the sides among smaller stones and can swim rapidly undercover if disturbed. With their broad, more elongate and depressed body, wide adherent oral disc and shallow fins, larvae are stream-adapted.



FIGURE 9. Photos in life of *Crinia riparia* from **A,B**) Alligator Gorge, **C**) Horrock's Pass; and *C. flindersensis* **sp. nov.** from **D**) Bunyeroo Gorge (SAMA R62991), **E**) 4.5k NNE Freeling Heights (P. Canty), **F**) Warren Gorge.

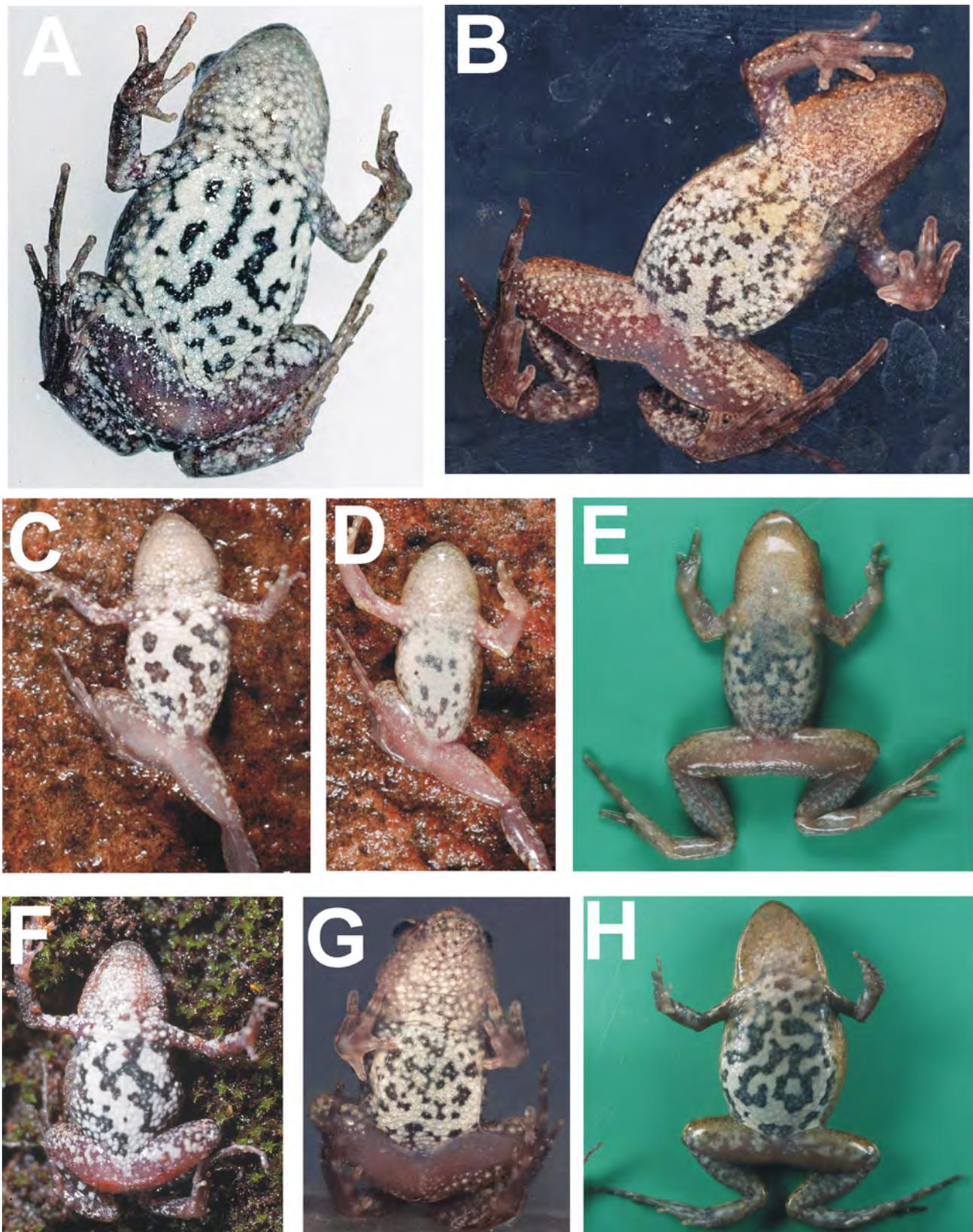


FIGURE 10. Photos of ventral patterns of live *C. riparia* **A**) Mambray Creek (H. Cogger), **B**) Horrock's Pass; *C. flindersensis* **sp. nov.** **C-D**) Warren Gorge, **E**) 11.4km NE Perawilla Spring, **F**) Wilpena Pound (H. Ehmann), **G**) Bunyeroo Gorge, **H**) 0.3km NE Narrina Springs.

Distribution. The Southern Flinders Ranges from Mt Brown Conservation Park 15 km S of Quorn (32° 27' 27"S, 138° 00' 21"E) in the north to Napperby Gorge (33° 09' 11"S, 138° 08' 08"E) in the south.

Remarks. The species identity of a collection (SAMA R13609A-G) from 1.5km south-east Bagalowie Homestead, north-east of Carrieton (32° 19' 12"S, 138° 52' 59"E) collected in May 1973 requires some comment (see Fig. 1). This location is 88km further east than any record of the *C. riparia* complex in the main Flinders Ranges and from an area where either member of the *C. riparia* complex could be present. From an inspection of dorsal and ventral patterns and body size, the specimens clearly do not conform with *C. signifera*, the taxon to which they would intuitively most likely belong. The locality would seem unlikely habitat for a member of the *C. riparia* complex as it is in relatively low relief ranges. We do note however a record of *C. flindersensis* **sp. nov.** (SAMA R64562) about 30km east of the Flinders Ranges on the flood plain of the Big John Creek (30° 41' 08"S, 139° 31' 01"E). In the absence of molecular genetic data or information on larval morphology we are not able ascertain the species identity of the Bagalowie Homestead series.

TABLE 3. Comparative measurements (mm) and ratios of key oral disc and body features for larvae of *C. riparia* and *C. flindersensis* **sp. nov.** Stage = stages of Gosner (1960); N = sample size (*measurements for BL, SE and SE/BL were taken on a smaller sub-set of specimens [number indicated] with better body condition; stage 40 was not included in SE measurements). Values are means with range in parentheses.

Locality	<i>C. riparia</i>			<i>C. flindersensis</i> sp. nov.	
	Alligator Gorge	Alligator Gorge	Horrock's Pass	Warren Gorge	Bunyerroo Gorge
Collection date	Nov. 2004	Oct. 2007	Aug. 2009	Aug. 2009	Nov. 2004
Stage	34–38	37–40	36–38	28–30	28–38
N	15/9*	4/2*	4	5	23/13*
TL	24.9 (20–27.5)	31.3 (30.0–32.7)	36.6 (35.5–38.0)	24.0 (21.0–25.0)	22.7 (11.6–28)
BL	9.09 (7.08–10.6)	11.3 (10.6–11.6)	12.6 (11.8–12.9)	8.8 (7.9–9.5)	9.137 (4.8–10.5)
ODW	3.18 (2.46–3.44)	3.9 (3.7–4.1)	4.2 (3.9–4.4)	2.4 (2.1–2.6)	2.6 (1.3–2.9)
P ³ row	2.25 (1.72–2.46)	2.5 (2.1–2.7)	2.8 (2.5–3.3)	1.3 (0.9–1.6)	1.2 (0.6–1.6)
PG	1.56 (1.31–1.88)	1.8 (1.7–2.0)	1.7 (1.6–1.9)	0.8 (0.7–1.1)	0.9 (0.5–1.1)
SW	3.87(3.11–4.26)	4.9 (4.7–5.3)	4.8 (4.5–5.0)	3.0 (2.6–3.4)	3.44 (2.13–4.1)
BD	4.42 (3.22–4.7) ⁹	6.06 (6–6.11) ²	5.8 (5.6–6.1)	4.2 (4.1–4.5)	5.56 (4.7–6.44) ¹³
SE	2.36 (1.93–2.57) ⁹	3.14 (3.05–3.2) ²	2.9 (2.7–3.1)	2.0 (1.9–2.4)	2.14 (1.7–2.3) ¹³
SE/BL	0.26 (0.23–0.29) ⁹	0.276 (0.275–0.278) ²	0.23 (0.22–0.24)	0.23 (0.22–0.25)	0.22 (0.18–0.23) ¹³
BL/TL	0.37 (0.354–0.39)	0.35 (0.33–0.36)	0.34 (0.33–0.35)	0.37 (0.36–0.38)	0.4 (0.36–0.43)
ODW/SW	0.823 (0.77–0.88)	0.81 (0.79–0.83)	0.87 (0.83–0.91)	0.82 (0.77–0.85)	0.74 (0.61–0.85)
PG/ODW	0.43 (0.23–0.56)	0.46 (0.43–0.49)	0.40 (0.37–0.42)	0.33 (0.25–0.41)	0.33 (0.25–0.41)
P ³ /ODW	0.639 (0.422–0.78)	0.62 (0.55–0.68)	0.67 (0.60–0.75)	0.33 (0.23–0.40)	0.47 (0.34–0.58)

Crinia flindersensis **sp. nov.**

Northern Flinders Ranges froglet

Figures 6,9,10,12

Holotype. SAMA R62991, an adult male collected at Bunyerroo Gorge, Flinders Ranges, Australia (31°25'04"S; 138°33'45"E) on 1 August 2007 by L. Price, S. Donnellan and R. Foster.

Other material examined. See Appendix 1.

Diagnosis. *Crinia flindersensis* **sp. nov.** is placed in *Crinia* by virtue of its molecular genetic phylogenetic placement within the genus and its close adult morphological affinity with *C. riparia*. Adults differ from *C. signifera* and *C. parinsignifera* in the incomplete expression of the back pattern polymorphism, particularly the absence of the ridged morph. Further differs from *C. signifera* in that it has a smooth palm, lacks a tarsal fold, and

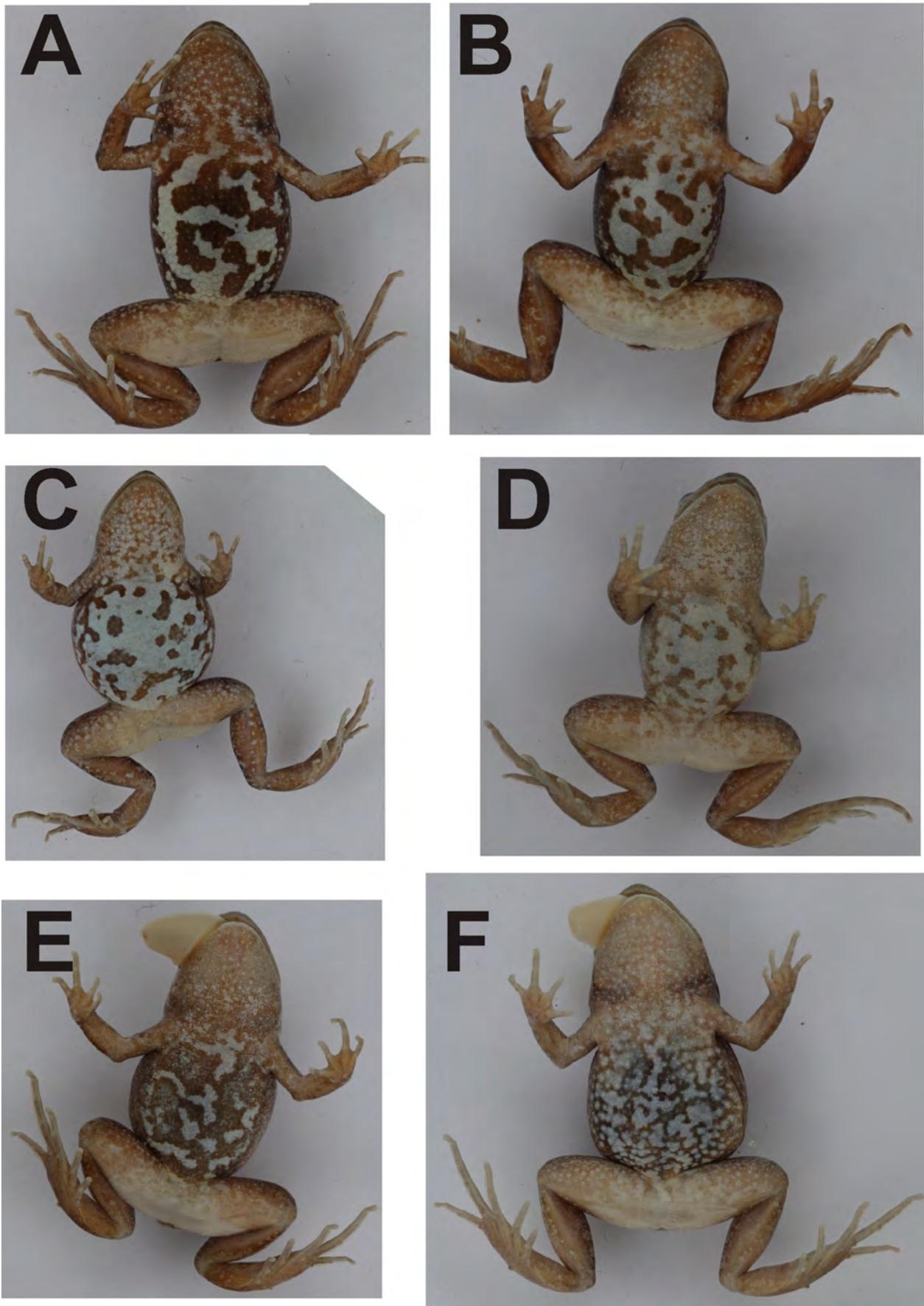


FIGURE 11. Ventral patterns in six individuals of *C. flindersensis* **sp. nov.** from Paralana Hot Springs (SAMA R37994).

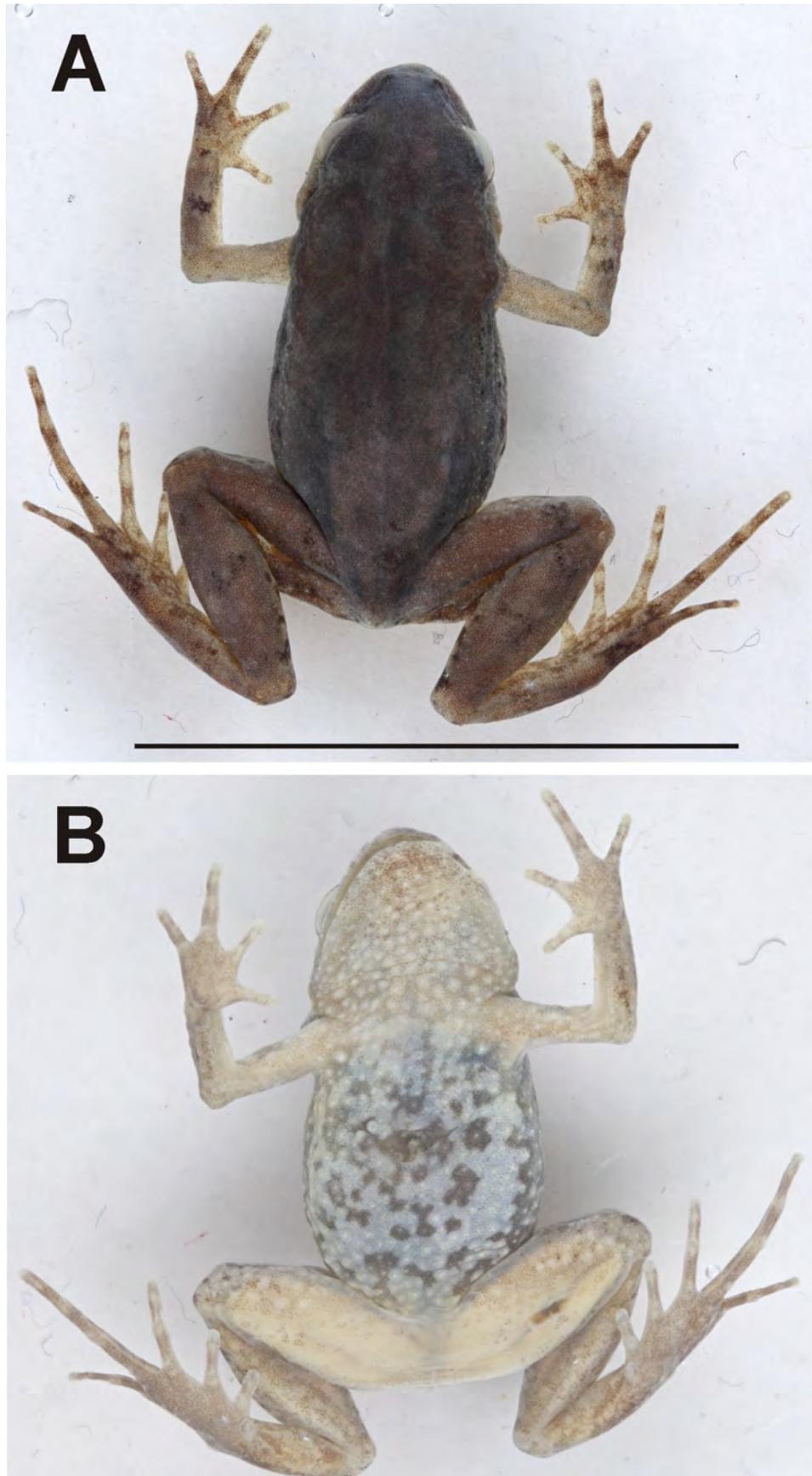


FIGURE 12. A) Dorsal and B) ventral views of the holotype (SAMA R62991) of *C. flindersensis* **sp. nov.** in preservative. Scale bar represents 20mm.

has reduced pigmentation on the throat of breeding males (vs palm markedly tuberculate and tarsal fold well developed in *C. signifera*). Further differs from *C. parinsignifera* in that it has a coarsely mottled ventral pattern with black patches on a white background vs venter finely stippled with dark grey on a white background in *C. parinsignifera*. Not readily distinguished from *C. riparia* on adult external morphology but distinguished by reciprocal monophyly of mitochondrial DNA sequences, fixed allelic difference at the nuclear *RAG1* locus and in the relative size of the larval oral disc, the length of the P¹ and P³ larval tooth rows, and the frequency of occurrence of submarginal papillae on the larval oral disc.

Description of holotype. Small body size (19.6 mm SVL). Snout short and slightly pointed and equal in length to eye; canthus rostralis rounded; loreal region oblique. Tympanum indistinct, lightly tubercular and the same colour as dorsum. Toes and fingers long and without webbing. Finger length: 3>4>2=1. Toe length: 5>3>4>2>1. Outer metatarsal tubercles very small, palmar and plantar tubercles absent. Mild tubercles under fingers, weaker tubercles under toes. Dorsal skin mildly tubercular (in comparison with ventral surfaces), limbs smooth. Throat forearms, and abdomen strongly tubercular whereas ventral surfaces of lower forelimbs and entire hindlimbs smooth.

Colour in preservative. Ventral surface of thighs transparent (muscles visible through skin), throat, forelimbs and lower hindlimbs lightly pigmented with light brown (Fig. 12). Abdomen cream and strongly marked with more than 20 irregularly-shaped dark grey spots and patches. Sharp transition in cream colour of abdomen to dark grey flanks along ventral margin of flanks. Dorsum uniform, dark grey without markings. Dorsal surfaces of forelimbs lighter colour than dorsum but with a few dark grey lateral marks on forearms and digits. Hindlimbs lighter grey than dorsum with a few dark grey lateral marks most prominent on toes. Snout black, but lips light brown.

Variation. Variation in morphometric traits is summarised in Table 2. In a sample of 49 preserved individuals from across the species range, the proportion of individuals with a 'lyrate' dorsal pattern was 43%, with an 'intermediate' pattern 14% and with the 'smooth' pattern 26% (16% of specimens were too faded to determine the back pattern). Variation in dorsal pattern in live individuals is illustrated in Fig. 9.

Ventral patterns vary considerably, with variation within locations being equivalent to that occurring across the entire range of the species (contrast Fig. 10C–H with Fig. 11). The abdomen has a white background with highly contrasting black marks and patches sometimes forming networks, continuing onto the thighs and lower throat in a few individuals.

Mating call structure. Call data are not available at present.

Larval morphology. Larvae at Gosner stages 30–38 from Bunyeroo Gorge (n=23) and at stages 28–30 from Warren Gorge (n=5) are described. Maximum length 35 mm, with a body length of 12.0 mm (stage 42, Bunyeroo Gorge). The tail is slightly less than twice length of body (Table 3). Body small, oval and slightly streamlined, slightly wider than deep across abdomen. Snout rounded in dorsal view and in profile. Eyes dorsolateral. Iris mostly golden, gold ring around pupil and a V-shaped projection (umbraculum) in centre of dorsal edge. Nares small, moderately spaced, and open dorsally with a small raised flap on inside rim. Spiracle just visible from above, tapers slightly and opens dorsoposteriorly below horizontal body axis, posterior to midpoint. Vent tube dextral, short and narrow, opens midway up ventral fin and mostly unattached to fin behind.

Colour of dorsum and sides of body variable (Bunyeroo Gorge), from dense bright gold to dark golden-brown, reddish-brown or black, with diffuse, slightly darker markings in some and a small darker patch over base of body in most. Larvae from Warren Gorge were generally darker brown or rusty-brown to black, with a small darker patch over base of body. Body wall around sides of head transparent in earlier stages, increasingly pigmented as larva grows. By stage 41, hind limbs are banded; diffuse darker markings of adult visible and small tubercles present in some. Venter gradually becomes covered with bright copper-gold clusters increasing to dense, mostly opaque layer by stage 34 (Bunyeroo Gorge), clearer below mouth.

Larvae are known to metamorphose in the field in spring (November), but likely to do so from early spring to early summer. Metamorphs are miniatures of adults with variable dorsal patterns. The belly is translucent dark grey with finely scattered white-tipped granulation. Mean SVL of three metamorphs is 8.5 mm (8.0–9.0).

Key differences between larvae of the three species of *Crinia* from the Flinders Ranges are summarised in Table 4. *C. flindersensis* sp. nov. larvae differ from *C. riparia* in their more oval, often slightly plump body shape with a less elongate snout and slightly more arched fins. They also have a slightly shorter tail relative to body length than *C. riparia* (see Figs 6,7). Their narrower oral disc with its shorter P¹ and P³ tooth rows, absence of submarginal papillae (or only very few in some) and narrower posterior medial gap in papillae, suggest the oral disc is likely to be less suctorial than the larger disc of *C. riparia* with its numerous papillae and very long tooth rows. The two also can differ in ventral pigmentation, which in fully grown *C. riparia* is transparent dark grey

(Alligator Gorge) or with a dull copper sheen (Horrock's Pass), and in *C. flindersensis* **sp. nov.** is bright opaque copper-gold (Bunyeroo Gorge) or with a dull copper sheen (Warren Gorge). Apart from pigmentation, the above differences suggest *C. flindersensis* **sp. nov.** is slightly less adapted to flowing water than occurs in *C. riparia* habitat. *Crinia flindersensis* **sp. nov.** (Bunyeroo Gorge) is more similar in body shape to *C. signifera*, which also has a more oval, plump body with slightly more arched fins than *C. riparia* and similar variable pigmentation, but *C. signifera* has a narrower oral disc than *C. flindersensis* **sp. nov.** with a much shorter P³ row and narrower posterior medial gap in the papillae (Table 4).

TABLE 4. Comparisons of key differences between larvae at Gosner stages 28–38 of the three species of *Crinia* from the Flinders Ranges.

Feature	<i>C. riparia</i>	<i>C. flindersensis</i> sp. nov.	<i>C. signifera</i>
Sides of oral disc	Emarginate	Emarginate	Non-emarginate
Snout shape in profile	Elongate, broadly depressed (streamlined)	Slightly elongate and streamlined	Short, not streamlined
Oral disc width	Greater than $\frac{3}{4}$ width of snout	$\frac{3}{4}$ width of snout	Less than $\frac{1}{2}$ width of snout
Submarginal papillae	Several	Mostly none, very few in some	None
Lateral extent of tooth rows	All tooth rows extend to edge of oral disc	Tooth rows A ¹ , A ² , and P ² extend to edge of oral disc	Tooth rows A ¹ and P ² extend to edge of oral disc
Posterior medial gap in papillae	Up to $\frac{1}{2}$ width of oral disc	Up to $\frac{1}{3}$ width of oral disc	Less than $\frac{1}{3}$ width of oral disc
P ³ tooth row	$\frac{2}{3}$ or greater width of oral disc (almost same length as P ² row)	Up to $\frac{1}{2}$ width of oral disc (about $\frac{1}{2}$ length of P ² row)	Less than $\frac{1}{3}$ width of oral disc (less than $\frac{1}{3}$ length of P ² row)
A ² tooth row	Usually entire	Usually with narrow median gap	Usually with wide median gap
Dorsal pigment	Mostly uniform dark brown or black, golden-brown in some, with or without a few diffuse patches	Variable shades of gold, brown, reddish-brown or black, may have scattered patches	Variable shades of brown, gold, patchy golden-brown, reddish-brown or black. Some with pale mid-dorsal stripe
Ventral pigment	Transparent dark or dull copper sheen	Dull copper sheen (Warren Gorge) or dense, opaque bright copper-gold sheen (Bunyeroo Gorge)	Transparent with or without scattered gold clusters

Habitat. A detailed analysis of the habitat of *C. flindersensis* **sp. nov.** (as “northern allopatry of *R. riparia*”) is presented in Odendaal and Bull (1982) and is summarised here. *Crinia flindersensis* **sp. nov.** occupies rocky creeks with wide beds that generally lack live vegetation in the creek bed or along the banks. These creeks are generally steeper than those occupied by *C. signifera* but not as steep as those occupied by *C. riparia* in the southern Flinders Ranges. The majority of these creeks are shallow, have intermittent flows and are subject to flooding. The creek in Bunyeroo Gorge where larvae were collected, however, did not have steep sides, and had intermittent rocky patches and shallow, slowly flowing pools with scattered clumps of emergent thin-leaved sedges, mainly near the edges of pools.

Larvae are mainly bottom-dwellers, hiding among rocks and leaf litter. They often rest on rocks or sand in creek pools and appear to graze mainly algae and sediments in still or slowly flowing sections, retreating undercover if disturbed. Eggs were found adhering to the undersurfaces of submerged rocks in Bunyeroo Creek and Warren Gorge in a similar manner to *C. riparia*. *Crinia signifera* has quite a different mode of egg deposition, attaching eggs singly or in small clusters to vegetation or twigs in still or slowly flowing pools.

Distribution. The northern Flinders Ranges from Black Springs, Wilkatana Station in the south to Billy Springs in the northern most foothills.

Etymology. Named for the Flinders Ranges, the mountain ranges in central South Australia to which the distribution of the new species is confined.

Acknowledgements

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APPENDIX 1. Specimens examined for molecular genetic analysis (A), adult used for measurements (B) and larval morphology (C). Numbered locations refer to location and sequence labels in Figs 1 and 2. ¹GenBank accession

A)

Taxon	Location	Voucher number	ND2 ¹	RAG1 ¹	Morphology
<i>Crinia riparia</i>	17—Mt Brown CP	SAMA R49691-2	JX473780-1	JX431019	Yes
	18—Horrocks Pass	SAMA R62993	JX473784		Yes
	18—Horrocks Pass	ABTC 99961-3	JX473785, JX473787 JX473792	JX431020	-
	19—Alligator Gorge	SAMA R62992	JX473791	-	Yes
	19—Alligator Gorge	ABTC 99944-6	JX473788- 90	JX431018	-
	20—4.7k NE Telowie	SAMA R53288	JX473786	-	Yes
	21—Napperby Gorge	TNHC 2336	JX473782	-	-
	21—Napperby Gorge	TNHC 2339	JX473783	-	-
<i>C. flindersensis</i>	1—Arkaroola	SAMA R52999	JX473760	-	-
	2—1.8k S Yudnamutana bore	SAMA R51754	JX473751	JX431032-3	-
	3—Yudnamutana	ABTC 14948	JX473783	JX431028	-
	3—Yudnamutana	ABTC 14951-4	JX473761, JX473752-4	J431026-30	-
	4—6k NW Arkaroola Village	SAMA R49206	JX473756	JX431023-4	Yes
	5—Kingsmill Waterhole	SAMA R53005	JX473759	JX431044-5	Yes
	6—4.1k N Warden Hill	SAMA R51985	JX473770	-	-
	7—Munyallina Creek, Wooltana station	ABTC 79083	JX473758	JX431037	-
	8—1k ESE Mudlapena Spring	SAMA R51810	JX473769	JX431031	Yes
	9—Moro Gorge	SAMA R51294	JX473757	-	Yes
	10—Alpana Station	SAMA R52229	JX473772	JX431034	Yes
11—Brachina Gorge	SAMA R62990	JX473778	-	Yes	
11—Brachina Gorge	SAMA R62998-9	JX473776-7	-	Yes	

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APPENDIX 1. a) (Continued)

Taxon	Location	Voucher number	ND2 ¹	RAG1 ¹	Morphology
	11—Brachina Gorge	ABTC 99972-3	JX473771, JX473773	-	-
	12—Bunyerroo Gorge	SAMA R62991	JX473779	-	Yes
	13—3.4k ENE Wilpena homestead	SAMA R53242	JX473774	-	Yes
	14—Bunbinyunna Creek	SAMA R58013	JX473775	JX431042	-
	15 -Warren Gorge	TNHC 13956-60	JX473763-7	JX431038	-
	16—Depot springs, Wilkatana Station	SAMA R58012/ 15	JX473762, JX473768	JX431041/3	-
<i>C. bilingua</i>	Middle Springs, WA	WAM R161202	JX473813	-	-
<i>C. bilingua</i>	Mitchell Plateau, WA	WAM R167850	JX473812	-	-
<i>C. deserticola</i>	Birdsville, Qld	SAMA R45118	JX473815	-	-
<i>C. deserticola</i>	Embarka Swamp, Cooper Creek	SAMA R52124	JX473814	-	-
<i>C. fimbriata</i>	Little Mertens Falls, WA	WAM R167743	JX473816	-	-
<i>C. fimbriata</i>	Little Mertens Falls, WA	SAMA R62994	JX473817	-	-
<i>C. georgiana</i>	Kalgan, WA	-	EF512627	-	-
<i>C. georgiana</i>	10km E Kalamunda, WA	WAM R114806	JX473804	-	-
<i>C. glauerti</i>	5km SE Margaret River, WA	ABTC 62634	JX473805	-	-
<i>C. insignifera</i>	Cardup, WA	WAM R115784	JX473801	-	-
<i>C. nimba</i>	Hartz Mountains, Tas	ABTC 25297	JX473818	-	-
<i>C. parinsignifera</i>	22km E Wagga Wagga, NSW	ABTC 17569	JX473808	-	-
<i>C. pseudinsignifera</i>	Walpole, WA	No voucher	JX473802	-	-
<i>C. remota</i>	20km N Cardwell, Qld	QM J57131	JX473809	-	-
<i>C. remota</i>	Heathlands, Qld	QM J57140	JX473810	-	-
<i>C. signifera</i>	4.4km NNW Narridy	SAMA R58804	JX473795	JX431044-5	-
<i>C. signifera</i>	Napperby Gorge	TNHC 2334	JX473793	-	-
<i>C. signifera</i>	Cann River Valley, Vic	No voucher	JX473799	-	-
<i>C. signifera</i>	Braidwood, NSW	No voucher	JX473817	-	-
<i>C. signifera</i>	1km S Nugent, Tas	SAMA R42241	JX473797	-	-
<i>C. signifera</i>	16km W Penola, SA	SAMA R39209	JX473794	-	-
<i>C. signifera</i>	Kangaroo Island, SA	ANWC A1706	JX473817	-	-
<i>C. signifera</i>	near Guyra, NSW	SAMA R39101	-	AY948939	-
<i>C. sloani</i>	near Albury, NSW	SAMA R42150	JX473803	-	-
<i>C. species</i>	Coffs Harbour area, NSW	ABTC 26421	JX473807	-	-
<i>C. subinsignifera</i>	14km E Mt Hanett, WA	ABTC 62565	JX473800	-	-
<i>C. fimbriata</i>	Little Mertens Falls, WA	SAMA R62994	JX473817	-	-

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APPENDIX 1. a) (Continued)

Taxon	Location	Voucher number	ND2 ¹	RAG1 ¹	Morphology
<i>C. tasmaniensis</i>	Pigsty Ponds, Tas	TMHC 870	JX473819	-	-
<i>C. tinnula</i>	Myall Lakes NP, NSW	ABTC 26483	JX473806	-	-
Outgroups					
<i>Geocrinia rosea</i>	Pemberton, WA	WAM R114841	JX473821	-	-
<i>Geocrinia laevis</i>	Mt Burr, SA	No voucher	JX473820	-	-
<i>Myobatrachus gouldii</i>	Bold Park; Spalding Park, Geraldton, WA	WAM R115075 WAM R116075	JX473822	AY364226	-
<i>Uperoleia laevigata</i>	16km S Crookwell, NSW	SAMA R39846	-	EF107310	-
<i>Uperoleia rugosa</i>	Gunbar, NSW	SAMA R33516	JF263309	-	-

B)

Taxon	Location	Co-ordinates	Females	Males
<i>C. flindersensis</i> sp. nov.	Mt Aroona	-30.583; 138.367	R03318A	
	Warren Gorge	-32.186; 138.014	R13412 B,E,F; R59120, R59122; R62610-3	R59123-4, R62614-7, R62620
	8km NW Hawker	-31.8167; 138.383	R25095-7,9-100	
	Oraparinna	-31.367; 138.7167	R32639	
	Bolla Bollana Waterhole	-30.283; 139.383	R37999	
	Yudnamutana	-30.2; 139.267	R42295-6	R39210-2
	6km NW Arkaroola Village	-30.29056; 139.28472	R49206	
	1.1km ESE Mudlapena Springs	-30.612; 138.81083	R51810	
	0.6km ESE Angorichina Hostel	-31.1294; 138.5625	R52229	
	4.6km NE Freeling Heights	-30.11083; 139.41583	R52997-8	
	Kingsmill Waterhole	-30.33; 139.383	R53004-5	
	3.4km ENE Wilpena HS	-31.49861; 138.64472	R53242	
	Perawilla Springs	-31.669; 138.797	R62527-8	
	Brachina Gorge	-31.3325; 138.57	R62990	
	0.3km NE Narrina Springs	-30.89472; 138.75389	R64542-3	
	15.1km ESE Big John Creek crossing	-30.6856; 139.51694	R64562	
	Buckaringa Gorge	-32.1132; 138.0364		R24302
	Moralana Creek, Blacks Gap	-31.63; 138.53		R26007
	Moro Gorge	-30.6872; 139.21694		R51294
	1.77km S Yudnamutana Bore	-30.167; 139.278		R51753
Bunyeroo Gorge	-31.4178; 138.5625		R62991	
Brachina Gorge	-31.333; 138.57		R62998-9	

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APPENDIX 1. b) (Continued)

Taxon	Location	Co-ordinates	Females	Males
	11.4km NE Perawilla Spring	-31.669; 138.797		R64544
<i>C. riparia</i>	Mt Remarkable Creek	-32.83; 138.15	R05174 A-B	
	Port Germein Gorge	-33.0167; 138	R13611 A, D	
	8.8km E Telowie	-33.04694; 138.151	R53282	
	Mt Remarkable NP, Mambray Ck	-32.85; 137.95	R04268 A-B, R39204	R04268 C-H
	Mt Remarkable NP, Mambray Ck	-32.8; 138.05	R24231-2	R24230
	Mt Brown CP, 13.5km SSW Quorn	-32.46361; 138.0061		R49691-2
	Napperby Gorge	-33.16056; 138.16167		R59128-9, 31-2
	Alligator Gorge	-32.7467; 138.07583		R62992
	Horrocks Pass	-32.658056; 138.065		R62993
<i>incerta sedis</i>	1.5km SE Bagalowie HS, NE Carrieton	-32.43; 138.53	R13609 A	R13609 B-C, E-F

C)

Taxon	Location	Co-ordinates	Registration Number and sample size	Collection Date
<i>C. riparia</i>	Alligator Gorge	-32.7533; 138.0731	SAMA R67294 [n=2], 67295 [n=5], 67296 [n=10]	4 November 2004
	Alligator Gorge	-32.7447; 138.0733	SAMA R67297 [n=4]	20 October 2007
	Horrock's Pass	-32.6119; 138.0148	SAMA R67298 [n=4]	11 August 2009
<i>C. flindersensis</i> sp. nov.	Bunyeroo Gorge	-31.4219; 138.5658	SAMA R67299 [n=23], 67300 [n=2], 67301 [n=5]	4 November 2004
	Warren Gorge	-32.185; 138.0106	SAMA R67302 [n=3], 67303 [n=2]	11 August 2009