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A new species of *Crinia* (Anura: Myobatrachidae) from the high rainfall zone of the northwest Kimberley, Western Australia

Paul Doughty[1], Marion Anstis[2] and Luke C. Price[3]

[1]Department of Terrestrial Zoology, Western Australian Museum, 49 Kew Street, Welshpool, Western Australia 6106, Australia. E-mail: Paul.Doughty@museum.wa.gov.au

[2]26 Wideview Road, Berowra Heights, New South Wales 2082, Australia.
E-mail: Marion.Anstis@studentmail.newcastle.edu.au

[3]University of Adelaide, North Terrace, Adelaide, South Australia 5000, Australia.
E-mail: luke.price@adelaide.edu.au

Abstract – *Crinia* is a large genus of small-bodied myobatrachid frogs that occur throughout most of Australia. They are less diverse in arid regions and northern Australia, and in the Kimberley are currently only represented by *C. bilingua*. Recent exploration of the northwest Kimberley has revealed another species of *Crinia*, here named *Crinia fimbriata* sp. nov. Molecular genetic analyses of mitochondrial nucleotide sequence data indicate the new species is a highly divergent lineage within *Crinia*. Compared to *C. bilingua*, the new species is smaller but with longer legs, has a dorsal ground colour of bluish grey-brown, yellow-brown or red, with distinctive dark brown variegations and the entire dorsal surface is stippled with fine, pale bluish-white tubercles. Males of the new species have wide flanges on the fingers which are not typical of other *Crinia* species. The tadpole is also unlike any other known species of *Crinia* in that it has large jaw sheaths, which may be an adaptation for scraping algae from the rock pools in which it has been found. The male advertisement call has not been recorded. Within the Kimberley region, many species of frogs, reptiles and mammals only occur in the northwest along a narrow high rainfall zone from the Mitchell Plateau to the Prince Regent River Nature Reserve, making this region of especially high conservation value.

Keywords – frog, tropics, conservation, tadpole, mitochondrial DNA, ND2.

INTRODUCTION

Frogs of the family Myobatrachidae Schlegel, 1850 are an ancient lineage of smaller-bodied Gondwanan anurans endemic to Australia and southern New Guinea (the larger-bodied Limnodynastidae Lynch, 1969 are the sister family). The myobatrachids show large phenotypic diversity across the family, especially for burrowing and reproductive modes (Roberts and Watson 1993; Tyler 1994; Tyler and Doughty 2009). However, three speciose genera – *Crinia* Tschudi, 1838, *Pseudophryne* Fitzinger, 1843 and *Uperoleia* Gray, 1841 – show strong conservatism in body form and ecology.

After *Uperoleia*, *Crinia* is the second-largest myobatrachid genus, with 15 species currently recognised (including *C. [Bryobatrachus] nimbus* Rounsevell *et al.*, 1994; see Read *et al.* 2001). Regions with the highest diversity are in southeastern Australia with seven species and southwestern Australia with five species. Most *Crinia* are small (~ 2–4 cm) and conservative in shape and breeding biology. One exception is *C. georgiana* from the southwest with its large body size, red groin and thighs, red or gold eyelids, dimorphism in arm size, polyandry, large egg size, diminutive tadpole and small size at metamorphosis. Because *C. georgiana* is the type species, the genus *Ranidella* Girard, 1853 had often been applied to all other *Crinia* species until recently. The other exceptions to *Crinia* conservatism are *C. riparia* Littlejohn and Martin, 1965 from the Flinders Ranges in South Australia and *C. nimbus* from Tasmania. *Crinia riparia* is a stream-dwelling species that lays its eggs underneath rocks in flowing streams and the tadpole has a stream-adapted body shape with a broader and larger oral disc for improved adherence

to rocks; *C. nimbus* has nidicolous larvae (Altig and Johnston 1989; Mitchell and Swain 1996).

In far northern Australia, *Crinia* is represented by *C. bilingua* Martin, Tyler and Davies, 1980 which occurs from the Kimberley region in Western Australia to northwestern Northern Territory. It is replaced to the east by *C. remota* Tyler and Parker, 1974 (type locality – Morehead in south-eastern Papua New Guinea), which reputedly occurs in Arnhemland and Groote Eylandt in the Northern Territory, and also in northern Queensland (Tyler and Davies 1986; Barker *et al.* 1995). *Crinia bilingua* and *C. remota* are common woodland and savannah species that occur in grassy habitats associated with creeks and ponds, not unlike those of southern species.

Recent collecting expeditions in January 2007 to the Mitchell Plateau and Prince Regent River Nature Reserve in the Kimberley region (Figure 1) have revealed a distinctive new species of *Crinia*. The new taxon is sympatric with *C. bilingua* (see Results), but has only been found in rocky sandstone escarpment platforms in highly dissected mountainous habitat. Here we present molecular and adult and larval morphological analyses on Kimberley *Crinia* and describe the northwest taxon as a new species.

Abbreviations: ABTC – Australian Biological Tissue Collection, Adelaide; SA – South Australia; ANWC – Australian National Wildlife Collection; SAMA – SA Museum, Adelaide; QM – Queensland Museum; WA – Western Australia; WAM – WA Museum.

METHODS

Adult morphology

Morphometric measurements of the 4 adult male specimens of the new taxon were compared with those of 20 adult *C. bilingua* from WAM (Appendix 1). All specimens were formalin-fixed, then preserved in 70% ethanol (unless noted). The *C. bilingua* measured were those collected on the same field trips that specimens of the new taxon were collected, as well as specimens from the central and eastern Kimberley to provide a wider geographical coverage. Table 1 provides definitions of the characters measured. We also calculated the following ratios: HL/SUL, HW/HL and TL/SUL. 'Drink patch' is defined as the skin of the posterior ventral surface and proximal surface of thighs beneath the cloaca. Significance tests of hypotheses were not possible to carry out owing to the small sample size of the type series. Accordingly, we

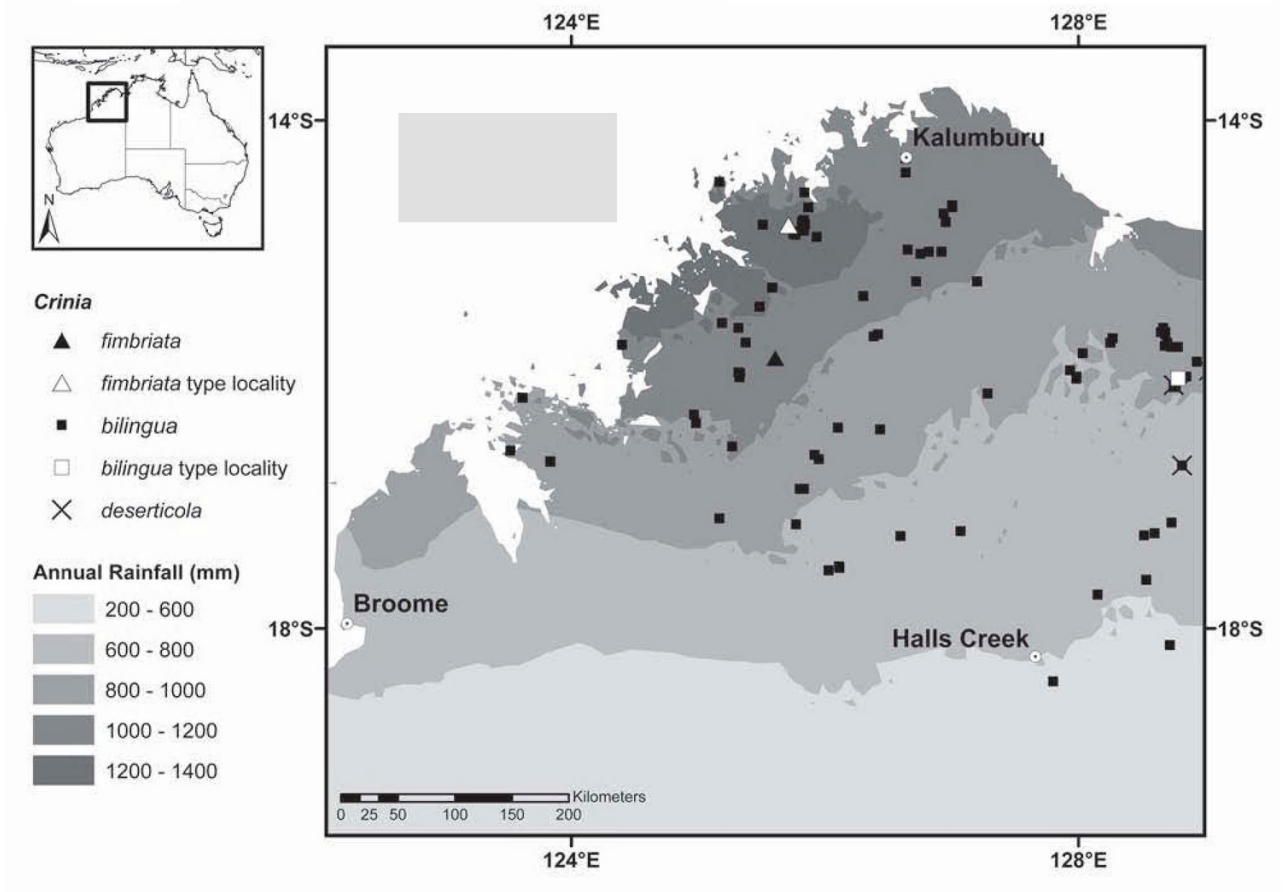

Figure 1. Distribution of *Crinia* species in the Kimberley region, Western Australia, showing annual rainfall.

present the data descriptively and discuss trends qualitatively.

Tadpole morphology and development

Five tadpoles were collected from a small rock pool above Little Mertens Falls, Mitchell Plateau, and one tadpole collected from a small pool next to a creek in the Prince Regent River Nature Reserve. Tadpoles from Mitchell Plateau were at Gosner (1960) stages 36 and 37 when collected and were reared in a 30 cm diameter plastic basin containing stream water to a depth of 12 cm, sand, silt and rocks. One metamorph was raised to adulthood to confirm identity and all other tadpoles were preserved at stages 38–41. Water temperatures ranged from 30–36°C during development. The single specimen from the Prince Regent River region was at stage 41 and preserved on capture. Tadpole descriptions and abbreviations for tadpole morphometric characters are provided in Table 1 and follow Anstis (2002) and Anstis and Tyler (2005). Morphometric measurements were made using vernier callipers and an ocular micrometer, and two tadpoles of *Crinia* sp. nov. and a typical *C. bilingua* tadpole were measured for comparison. Tadpoles were anaesthetised in 1% chlorbutol solution and preserved in 4% buffered formalin. Tadpoles were drawn with the aid of a drawing tube attached to the stereoscopic microscope.

Molecular genetic analyses

Sampling

Species within the genus *Crinia* show remarkably high levels of morphological diversity in both skin colour pattern and texture, even within a single population (Tyler and Doughty 2009). Therefore, it was necessary to confirm whether the morphologically unique *Crinia* specimens represented a unique lineage of *Crinia* or are members of a morphologically variable population of a currently recognised spieces. Assessment of the single female raised from a tadpole was also necessary as this specimen lacked a diagnostic character of the new taxon (i.e. flanged fingers, see below). We performed molecular genetic analyses of mitochondrial nucleotide sequences sampled from all adults from the Mitchell Plateau, but not the male from the Prince Regent River as the sample was too degraded. Additionally, we

Table 1 Characters measured with abbreviations and explanations.

Character	Abbrev.	Explanation of Measurement
A. Adults		
Snout-urostyle length	SUL	From tip of snout to posterior tip of urostyle
Head length	HL	From tip of snout to posterior edge of tympanum
Head width	HW	Width of head at centre of tympani
Eye-naris distance	EN	From anterior corner of eye to posterior edge of naris
Interorbital span	IO	Distance between anterior corners of eyes
Internarial span	IN	Distance between inner edges of nares
Tibia length	TL	Measured with leg in natural resting position, from knee to tarsus
Foot length	FootL	From tip of 4th toe to proximal end of inner metatarsal tubercle
B. Tadpoles		
Total length	TL	From tip of snout to tail tip
Body length	BL	From tip of snout to end of body
Body depth	BD	Maximum height of body
Body width	BW	Widest point of body in dorsal view
Body width at eyes	EBW	Body width at level of eyes in dorsal view
Tail muscle depth	BTM	Depth of tail muscle at base
Tail muscle width	BTMW	Width across tail muscle at base in dorsal view
Tail depth	TD	Measured at midpoint of tail
Dorsal fin depth	DF	Measured at tail depth
Tail muscle depth	TM	Measured at tail depth
Ventral fin depth	VF	Measured at tail depth
Inter-orbital span	IO	Measured in dorsal view
Inter-narial span	IN	Measured in dorsal view
Eye to naris	EN	Measured in dorsal view
Narial diameter	N	Measured in dorsal view
Snout to spiracle	SS	
Snout to naris	SN	
Snout to eye	SE	
Eye diameter	ED	
Oral disc width	ODW	Measured at maximum in ventral view

sampled individuals from the three other northern species of *Crinia*, including individuals near the type localities.

A total of 18 *Crinia* were sequenced in this study: four *Crinia* sp. nov. – the holotype and three paratypes from the type locality, seven *C. bilingua* from near the type locality in WA and across the species' range; four *C. remota* from near the type locality in New Guinea and also Queensland; two *C. deserticola* from near the type locality in Queensland and also SA; and one *C. riparia* from the type locality in SA. Because our goal was to determine conspecificity of both the adult and larval specimens from the type series and ascertain if the type series belongs to a unique lineage within the genus *Crinia*, we selected four outgroups for the analyses. Outgroups were chosen based on the phylogeny of Read *et al.* (2001), which was derived from analysis of combined 12sRNA and ND2 datasets. Details of specimens from which sequence data was obtained, other outgroup taxa and *Crinia* species from Read *et al.* (2001) are presented in Table 2.

DNA extraction, amplification, and sequencing

DNA was extracted using a Puregene™ DNA Isolation Tissue Kit, D–7000A (Gentra Systems) following the manufacturer's instructions. A 655 base pair (bp) mitochondrial DNA fragment comprising 145bp of partial sequence from $tRNA^{ILE}$ (6 bp), $tRNA^{GLN}$ (70 bp), $tRNA^{MET}$ (69 bp) and 510 bp of the protein coding gene ND2 was amplified using the polymerase chain reaction (Saiki *et al.* 1986, 1988). 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 $MgCl_2$, 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. Light and heavy strand primer sequences were: L4221 $tRNA^{ILE}$ 5'-AAGGACCTCCTTGATAGGGA–3 and H4980 ND2 5'-ATTTTTCGTAGTTGGGTTTGRTT–3' respectively (Macey *et al.* 1997).

PCR cycling conditions were: one cycle of 94°C for 9 min, 36 cycles of 94°C for 45 s, 55°C for 45 s, and 72°C for 1 min, and one cycle of 72°C for 6 min. PCR products were assessed for expected PCR product size on 1.5% agarose gel electrophoresis and visualised with ethidium bromide staining and a UV transilluminator before sequencing. PCR products were sent to the commercial sequencing facility Macrogen Inc. (www.macrogen.com) for purification and DNA sequencing. BigDyeTM cycling conditions were employed to sequence the light strand with the same primer used for PCR amplification. Purification of reacted products was performed using ethanol precipitation and sequencing reactions were visualised using automatic sequencer ABI3730XL. Raw sequences were edited using SeqEd (Version 1.0.3, ABI) and aligned by eye using Se-Al (Rambaut 1996) against a subset of homologous myobatrachid sequences from Read *et al.* (2001) that were donated by J. S. Keogh. Sequences from ABTC 99434, WAM R114841 and the sequence from a *M. gouldii* are missing the 5' 145 bp, 114 bp and 114 bp of this fragment, respectively. To check for nuclear paralogues all *ND2* protein encoding sequences were translated in Se-Al using the standard vertebrate mitochondrial genetic code and examined for unexpected stop or nonsense codons.

Phylogenetic analyses

The aligned sequence data were used to explore three methods of phylogenetic analyses: Markov-Chain Monte Carlo (MCMC)-Bayesian phylogenetic analyses implemented in MrBayes v. 3.1.2 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003), maximum parsimony (MP) using PAUP ver. 4.0b5 (Swofford 2000) and maximum likelihood (ML) criterion using Garli (Zwickl 2006).

Two programs were used to determine an appropriate model of nucleotide substitution for Bayesian and ML analyses. For the Bayesian analysis MrModeltest version 2.2 (Nylander 2004) was used and for the ML analysis the Modeltest program version 3.7 (Posada and Crandall 1998, 2001). For Bayesian analysis 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. For ML analysis the most suitable model of nucleotide substitution was found to be TIM + I + G under the AIC.

Phylogenetic analysis by Bayesian inference was performed on the aligned sequences using the appropriate model of nucleotide substitution for each data partition. Analyses were performed in two runs, each with four separate MCMC chains (1 cold) for 1 x 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 x 10^6 generations and thus the first 6,000 trees were excluded and the remaining 4,000 trees, used to derive a 50% majority-rule consensus tree with posterior probabilities of the clades.

The ML analysis was performed with the default parameters in Garli (Zwickl 2006), using the GTR + I + G model and employed a heuristic search strategy. Because it was not possible to

specify the TIM + I + G model, GTR + I + G, a similar model, was selected. The non-parametric bootstrap with 100 pseudoreplicates was used to assess branch support.

For MP analyses all substitutions were weighted equally (see Kluge 1997). A heuristic search strategy was employed, using the random stepwise addition (100 replicates) and tree-bisection-reconnection (TBR) branch swapping options. The non-parametric bootstrap with 1,000 pseudoreplicates was used to assess branch support.

RESULTS

Adult and tadpole morphology

Table 3 summarises the morphological differences between *Crinia* sp. nov. and *C. bilingua*. The two *Crinia* were similar, except *C. bilingua* appears to be slightly larger and *Crinia* sp. nov. has a longer tibia (in both mean and in the TL/SUL ratios). Whereas the fingers of *C. bilingua* share the pointed non-webbed fingers typical of other *Crinia*, males of *Crinia* sp. nov. have unusually wide flanges (Figure 2). Tadpoles of *Crinia* sp. nov. have large, laterally-compressed jaw sheaths which are atypical of other *Crinia* (Watson and Martin 1973; Anstis 2002).

Differences in dorsal pattern and colouration between the two taxa were more apparent (Figure 3). *Crinia bilingua* usually has a light brown or reddish-brown background colour often with a darker rectangular marking on the dorsum and a chevron or Y-shaped mark between the eyes. In contrast, all individuals observed of *Crinia* sp. nov. lack the chevron marking and have either a reddish or bluish hue to a background colour of brown, with a network of minute, pale bluish-white tubercles scattered over the dorsum and limbs.

Molecular genetic analyses

The final dataset comprised 655 bp of aligned *ND2* and tRNA sequences for all currently recognised species in the genus *Crinia* and selected outgroup taxa from the family Myobatrachidae. Of the 655 sites, 339 were constant and 316 characters were variable of which 266 of these were parsimony-informative.

A single majority rule consensus tree derived from the final Bayesian runs is shown in Figure 4. Posterior probabilities (Bayesian analysis) and bootstrap support values (MP and ML analysis) of nodes are indicated on branches if values were above 95% for Bayesian analyses and 60 for MP and ML analyses. Well-supported nodes (> 0.95

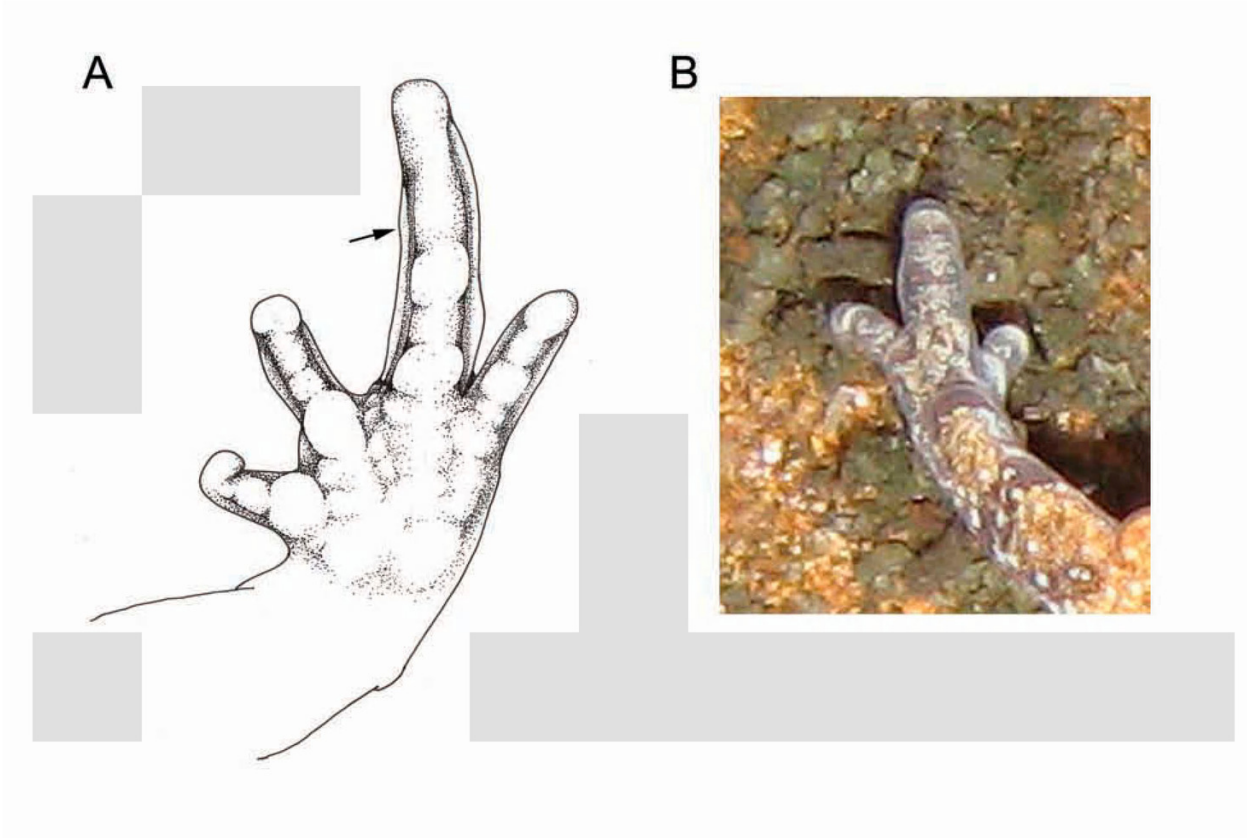

Figure 2 Flanges on the fingers of *Crinia fimbriata* sp. nov. A) diagram of finger with pin holding back flange (WAM R167745); B) close-up of flange of uncollected individual floating in a small pool at the top of Little Merten's Falls, Mitchell Plateau.

Table 2 Details of adult specimens examined and tissues used in this study. Collection location is indicated when known. Where a voucher specimen is available its institution code and specimen number are noted. Museum tissue number refers to the registered tissue sample and institution. mtDNA haplotype numbers are shown for specimens that were sequenced and analysed and correspond to those in Figure 2. Data is sorted by genus, species then museum tissue number and in the absence of the museum tissue number is sorted by specimen number. Tissue numbers marked with an (a) indicate individual frogs from which tissues were sequenced and used in the phylogenetic analyses; those marked with a (b) indicate which individuals were used in the morphometric comparisons; numbers marked with a (c) indicate sequences from Read et. al. (2001) used in phylogenetic analysis which were donated by J. S. Keogh. If the specimen(s) belongs to a type series its type designation is indicated as follows; Holotype (H); and Paratype (P). Institution codes are as follows; Australian Museum, Sydney (AM), Queensland Museum, Brisbane (QM), South Australian Museum, Adelaide (SAM), Australian Biological Tissue Collection, South Australian Museum, Adelaide (ABTC), Australian National Wildlife Collection (ANWC).

Genus	Species	Museum Tissue no.		Voucher no.	Collection Locality	mtDNA Haplotype no.
Crinia	*bilingua*					
		ABTC 12729	a		5km N of Lake Argyle Village, WA	B5
		ABTC 13403	c		Pentecost R., El Questro Stn, WA	B1
		ABTC 17043	a	SAMA R36687	Victoria R., NT	B4
			a	WAM R146019	Kimbolton HS, WA	B6
			ab	WAM R161202	Middle Springs, WA	B7
			ab	WAM R162599	Mount Leake, WA	B2
			a	WAM R162606	Mornington Station, WA	B3
			ab	WAM R167850	Mitchell Plateau, WA	B3
				SAMA R16833	Ivanhoe Crossing, WA	
				SAMA R16834	Mitchell Plateau, WA	
				SAMA R16835–6	Mitchell Plateau, Mining Campsite, WA	
				SAMA R16837–8	1.5km N of Lake Argyle Tourist Village, WA	
				SAMA R16839	Dead Horse Springs, WA	
				SAMA R16840–1	11.5km NE of Lake Argyle Tourist Village, WA	
				SAMA R16842	20km NE of Lake Argyle Tourist Village, WA	
			b	WAM R162541	Grotto Creek, nr Kununurra, WA	
			b	WAM R162579	Mornington Station, WA	
			b	WAM R166143	Doongan Station, WA	
			b	WAM R167851	Mitchell Falls, WA	
			b	WAM R167962	Mount Elizabeth Station, WA	
			b	WAM R167996	Prince Regent River NR, WA	
			b	WAM R168012	Prince Regent River NR, WA	
			b	WAM R168070	Prince Regent River NR, WA	
			b	WAM R168085	Prince Regent River NR, WA	
			b	WAM R168119	Prince Regent River NR, WA	
			b	WAM R168120	Prince Regent River NR, WA	
			b	WAM R168140	Prince Regent River NR, WA	

			b	WAM R168142	Prince Regent River NR, WA	
			b	WAM R168189	Prince Regent River NR, WA	
	deserticola	ABTC 17752	c	SAMA R45118	Birdsville, Qld	D2
		ABTC58875	a	SAMA R52124	Embarka Swamp, Cooper Ck, SA	D1
		ABTC 99434	a		Charleville, Adavale Rd, 6.6km W of Ward R. Rd, Qld	D3
	georgiana	ABTC 62663	c	WAM R114806	Gungin gully, 10km E of Kalamunda, WA	
	glauerti	ABTC 62634	c	WAM R114658	5 km SE of Margaret River, WA	
	insignifera	ABTC 62741	c	WAM R115784	Cardup, WA	
	nimbus	ABTC 25297	c		Harzt Mts, TAS	
	parinsignifera	ABTC 17569	c	SAMA R42202	22 km E of Wagga Wagga, NSW	
	pseudinsignifera		c		Cnr Railway Pde and SW highway, nr Walpole, WA	
	remota	ABTC 58984	a	QM J57140	Heathlands, Qld	R3
		ABTC 58992	a	QM J57131	20km N of Cardwell, Qld	R1
		ABTC 79182	a		Wegamu, Trans-Fly, PNG	R2
		ABTC 79183	a		Wegamu, Trans-Fly, PNG	R4
	riparia		a	SAMA R62992	Alligator Gorge, SA	
	signifera	ABTC 14924	c	SAMA R39209	16 km W of Penola, SA	S4
		ABTC 17180	c	SAMA R42241	1 km S of Nugent, TAS	S3
		ANWC 1706	c		Kangaroo Island, SA	S5
			c		Braidwood, NSW	S2
			c		Cann R. valley, between Cann R. & Noorinbee, VIC	S1
	sloanei	ABTC 17555	c	SAMA R42150	E of Albury, NSW	
	sp.	ABTC 26421	c		Coffs Harbour area, NSW	
	subinsignifera	ABTC 62565	c	WAM R114143	14km E of Mt. Hanett, WA	
	fimbriata sp. nov.		ab/P	SAMA R62994	Little Mertens Falls, Mitchell Plateau, WA	Ss2
		WAM R167743	ab/H	WAM R167743	Little Mertens Falls, Mitchell Plateau, WA	Ss1
		WAM R167744–45	ab/P	WAM R167744–45	Little Mertens Falls, Mitchell Plateau, WA	Ss2
		WAM R163823	b/P	WAM R163823	5.2 km SW Junction of Prince Regent Rv & Pitta Ck, WA	
	tasmaniensis	ABTC 23114	c	TMHC 870	Pigsty Ponds, TAS	
	tinnula	ABTC 26483	c		Mungo Brush Myall Lakes NP, NSW	
Geocrinia	*laevis*		c		Mt. Burr, SA	
	rosea	WAM R114841	c	WAM R114841	Pemberton, WA	
Myobatrachus	*gouldii*	ABTC 63391	c	WAM R115075	Bold Park, Perth, WA	
Uperoleia	*rugosa*	ANWC 1845	c		Shoalwater Bay, Qld	

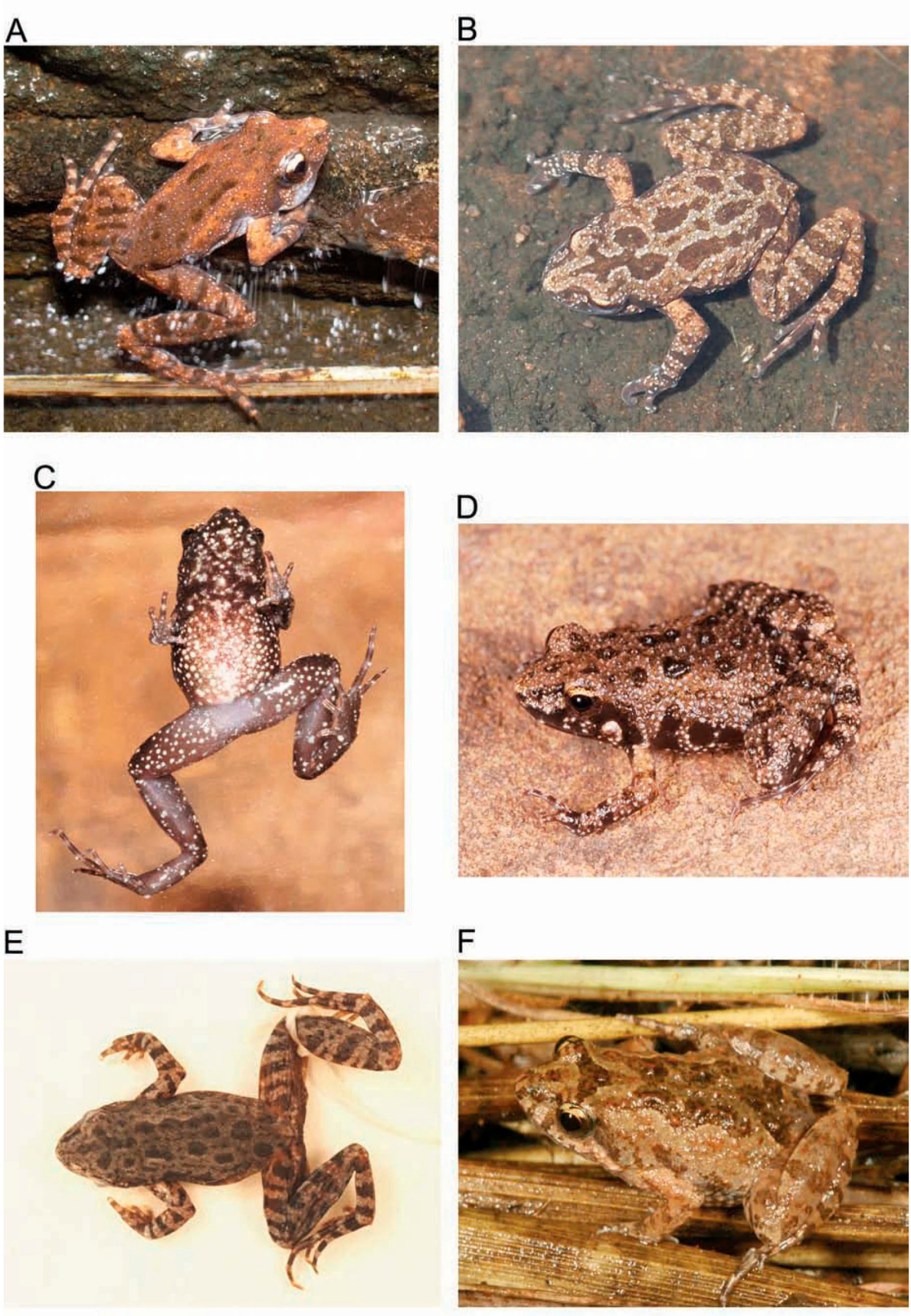

Figure 3 Photos in life of *Crinia* from the Kimberley region, Western Australia. A) *C. fimbriata* sp. nov. from Mitchell Plateau (not collected) (photo J. Francis); B) male *C. fimbriata* sp. nov. from Prince Regent River (WAM R163823)(photo M. Barrett); C,D) ventral and dorsolateral views of captive-reared female *C. fimbriata* sp. nov. (SAMA R62994); E) preserved male holotype (WAM R167743) of *C. fimbriata* sp. nov.; F) male *C. bilingua* from Kununurra.

posterior probability or > 70 bootstrap support values) in the phylogenies resulting from the Bayesian, ML and MP analyses showed congruent phylogenetic pattern for all taxa. *Crinia nimbus* and *C. tasmanienis* form a weakly supported basal clade, sister to all other *Crinia*. The type series (*Crinia* sp. nov.) belongs to a strongly supported, highly divergent lineage which is the sister taxon to the remaining *Crinia*. Within the type series, SAMA R62994, WAM R167744 and WAM R167745 possess the same haplotype (Ss2); WAM R167743 has a unique haplotype (Ss1) differing at four sites in the fragment of ND2 sequenced.

Uncorrected pairwise genetic distance between the *Crinia* sp. nov. lineage and clades of other *Crinia* species ranged between ~16.8% (*C. riparia* and *C. remota*) and ~22% (*C. deserticola* and *C. nimbus*). *Crinia bilingua* and *C. remota* each form well-supported clades which together form a strongly supported clade, sister to the remaining *Crinia* species. Uncorrected pairwise genetic distance between haplotypes from the *C. bilingua* lineage and haplotypes of the *C. remota* lineage ranged from ~12.8% to ~11.3%. *Crinia deserticola* forms a well-supported clade which is grouped with the remaining *Crinia* species by a weakly-supported node. Intra-clade genetic diversity was minimal (0 to ~2%) in *C. bilingua*, *C. deserticola*, *C. remota* and *Crinia* sp. nov. In contrast, intraspecific genetic distances within the *C. signifera* clade were high (~9.4% to ~4.7%), and lineages within this clade show definite phylogeographic pattern. *Crinia parinsignifera*, *C. tinnula* and *Crinia* sp. form a weakly-supported clade, but there is strong support for a subclade comprising *C. tinnula* and *Crinia* sp. There is weak support for *C. riparia* as the sister lineage to a weakly supported clade containing *C. signifera*, *C. georgiana*, *C. glauerti*, *C. sloanei*, *C. insignifera*, *C. pseudinsignifera* and *C. subinsignifera*. However there is strong support for the *C. signifera* clade and moderate support for the clade comprising *C. georgiana*, *C. glauerti*, *C. sloanei*, *C. insignifera*, *C. pseudinsignifera* and *C. subinsignifera*.

Table 3 Morphometric comparisons between *Crinia bilingua* and *Crinia* sp. nov. from the Kimberley. Figures are mean±S.D. (range).

Character:	***Crinia bilingua*** N = 20	***Crinia* sp. nov.** N = 4
SUL	19.1±1.3 (17.5–22.5)	17.0±0.4 (16.5–17.5)
TL	8.3±0.6 (7.2–9.4)	8.8±0.5 (8.1–9.2)
HL	5.2±0.4 (4.7–5.9)	4.5±0.3 (4.2–4.9)
HW	5.8±0.4 (5.1–6.4)	4.5±0.4 (4.7–5.5)
FootL	9.3±0.7 (8.1–10.7)	8.4±1.0 (7.0–9.2)
EN	1.3±0.2 (1.1–1.9)	1.4±0.1 (1.3–1.5)
IN	1.5±0.1 (1.3–1.8)	1.6±0.1 (1.5–1.6)
IO	3.0±0.2 (2.7–3.5)	3.1±0.1 (3.0–3.5)
HL/SVL	0.27±0.01 (0.25–0.30)	0.27±0.02 (0.25–0.29)
HW/HL	1.10±0.06 (1.00–1.30)	1.14±0.04 (1.10–1.20)
TL/SUL	0.43±0.02 (0.39–0.50)	0.52±0.03 (0.48–0.54)

DISCUSSION

Conspecificity and distinctiveness

The molecular results provide strong support for the conspecificity of the type series, which is consistent with the distinctive colour and pattern observed on the adult individuals. Indeed, all genotyped individuals, including the female SAMA R62994, belong to a single divergent mitochondrial DNA lineage which is well supported with a Bayesian posterior probability of 1.00 and MP and ML bootstrap support values of 100. The conspecificity of the female SAMA R62994 also indicates that the distinctive larvae are the juvenile form of the distinctive adults from the type series.

It is reasonable to propose that the *Crinia* sp. nov. lineage represents a divergent population and not an ancestral gene lineage (e.g. Thomaz *et al.* 1996), retained within a population of a sympatric species such as *C. bilingua*. This proposition is supported by the large genetic distances observed between *Crinia* sp. nov. and other lineages of *Crinia*, which implies a substantial period of isolation, and that the range of divergence seen between *Crinia* sp. nov. and the other *Crinia* species exceeds the genetic divergence between described sister species pairs such as *C. bilingua and C. remota*.

The molecular data in conjunction with the distinctive adult and larval morphology provide strong support for the recognition of *Crinia* sp. nov. as a distinct evolutionary species. In particular, the degree of sequence divergence of the *Crinia* sp. nov. lineage relative to currently described sister species pairs in the genus *Crinia* and the monophyly of haplotypes representing the morphologically distinct individuals is compelling evidence in support of species recognition. This diagnosis is consistent with the evolutionary species concept (Simpson 1951; Wiley 1978) and the generalised lineage concept of de Queiroz (1998). Additionally, the flanged fingers of males and the specialised mouthparts of larvae indicate

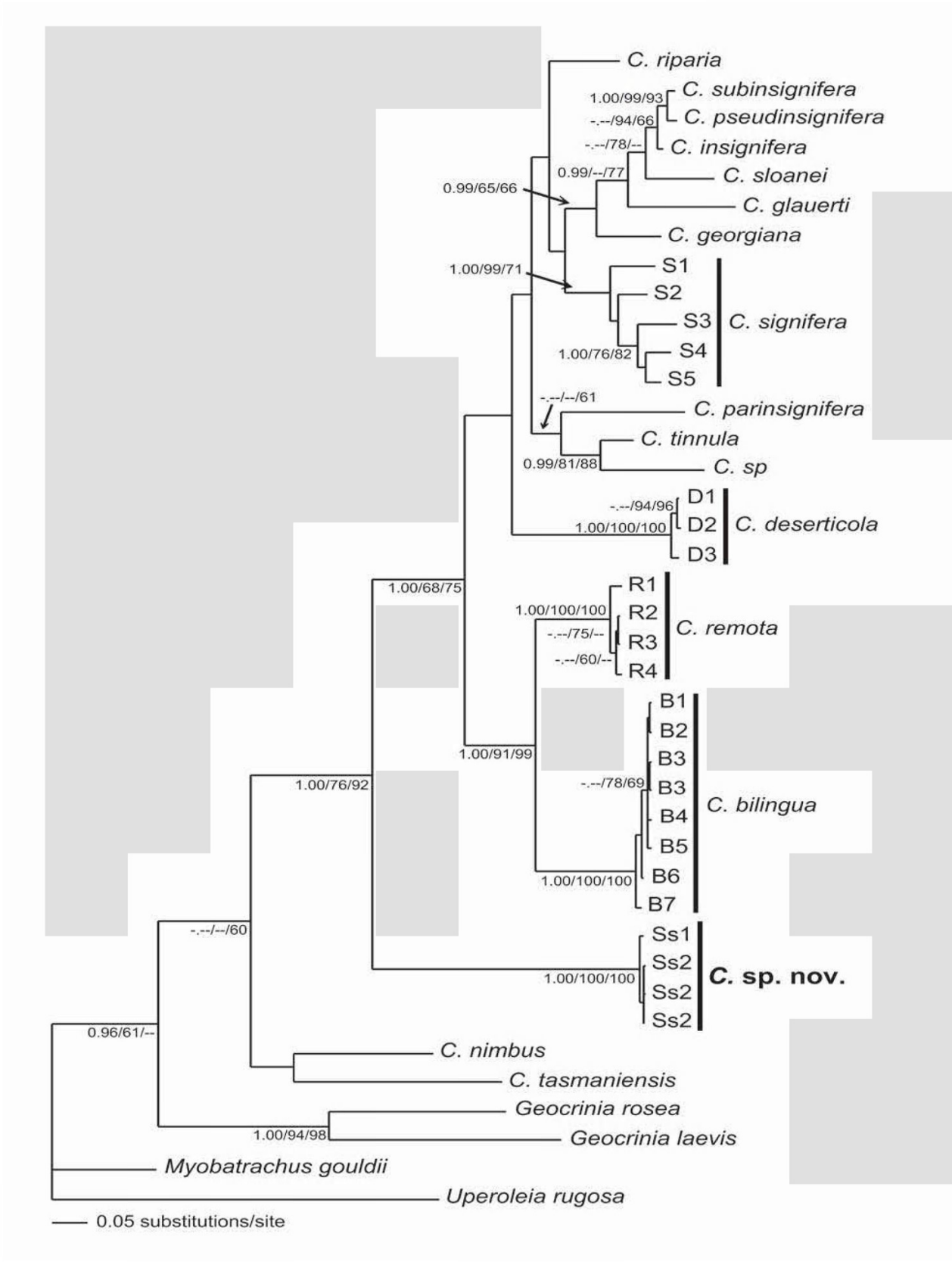

Figure 4 A majority rule consensus tree derived from the final Bayesian runs showing phylogenetic relationships among mitochondrial ND2 haplotypes for *Crinia*. Values at nodes indicate Bayesian posterior probabilities >0.95 and MP and ML bootstrap support values >60, respectively. Haplotype numbers are indicated where more than one haplotype is present in a lineage representing a particular species. Species names and haplotype numbers refer to specimens, tissues and collection locations in Table 2.

a possible change in reproductive behaviour and a high degree of habitat specificity for breeding (i.e. rocky pools), respectively. Although observations of morphological differences such as these are not a direct test of reproductive isolation, they clearly indicate significant adaptive divergence of *Crinia* sp. nov. despite a contemporary sympatric distribution with at least one congeneric species *C. bilingua*. It is therefore logical to suggest these morphological differences indicate reproductive isolation of *Crinia* sp. nov. in spite of the potential for interbreeding with *C. bilingua*. Consequently, species recognition is also supported under the biological species concept (Mayr 1963). Based on the morphological and genetic differences among *Crinia* taxa, we describe the northwest taxon as a new species in the Systematics section below.

Relationships within *Crinia*

Various methods have previously been employed to explore species relationships within the genus *Crinia* (see Read *et al.* 2001 for review). The most comprehensive examination of relationships to date was provided by Read *et al.* (2001). The phylogenetic relationships inferred from analyses in the present study are largely congruent with those of Read *et al.* (2001). However, some relationships depicted by weakly supported nodes in our analyses do conflict with the tree topologies from Read *et al.* (2001). The placement of *C. riparia* as the sister to both *C. signifera* and the clade comprising *C. georgiana*, *C. glauerti*, *C. sloanei*, *C. insignifera*, *C. pseudinsignifera* and *C. subinsignifera* in our analyses is not consistent with the closer relationship of *C. riparia* to *C. signifera* inferred by analyses of Read *et al.* (2001). Additionally, the analyses of Read *et al.* (2001) placed, albeit tentatively, the clade comprising *C. parinsignifera*, *C. tinnula* and *Crinia* sp. as the sister of the clade comprising *C. riparia* and *C. signifera*. We used a smaller fragment of mitochondrial DNA for phylogenetic analysis and as such it is not surprising that phylogenetic relationships inferred by the analyses of Read *et al.* (2001) are different from our own. Interestingly, the only differences in the tree topology between ours and the analyses of Read *et al.* (2001) exist where a shorter fragment of identical sequence was used for all but one of the species in question. Consequently, where support values are weak for the differing node, the tree topology produced by the analyses of Read *et al.* (2001) is a more accurate estimate of phylogenetic relationships. Besides our use of a smaller fragment of mitochondrial DNA we also used different outgroup and in-group specimens and different sample sizes for some species. The use of SAMA R62992 as our representative sample of *C. riparia* instead of ABTC 14948, used in the analyses of Read *et al.* (2001), may also have contributed to the difference in tree topologies.

We do point out that although our analyses included every recognised species of *Crinia*, we elected not to include every recognised divergent lineage within *Crinia*. Recent molecular analyses of 12S and 16S mitochondrial DNA sequences by Symula *et al.* (2008) also included the divergent northern lineage of *C. riparia* to which ABTC 14948 (Read *et al.* 2001) belongs. Likewise, only one of the divergent mitochondrial lineages recognised in *C. georgiana* (see Edwards *et al.* 2007) was included in our analyses. Relationships within *C. signifera* were largely congruent with those of Symula *et al.* (2008), however our analyses lacked representatives from the B1, C2 and C3 mitochondrial lineages/sub-clades.

SYSTEMATICS

Family Myobatrachidae Schlegel, 1850

Genus *Crinia* Tschudi, 1838

Type species

Crinia georgiana Tschudi, 1838, by monotypy.

Diagnosis

Small (1.5–4 cm SUL) ground-dwelling frogs characterised by pointed snout, flattened body shape, small limbs, long unwebbed fingers and toes, toothed upper jaw and long and oval tongue. All species lay pigmented eggs in water, except *C. nimbus* which lays unpigmented eggs in a terrestrial nest and with nidicolous larvae. Genetic data (Read *et al.* 2001; Frost *et al.* 2006) support the monophyly of *Crinia*, including *C. tasmaniensis* + *C. (Bryobatrachus) nimbus* as a basal lineage.

Crinia fimbriata sp. nov.

Kimberley Froglet
Figures 2, 3, 5 and 6

Material examined

Holotype

Australia: *Western Australia*: WAM R167743, an adult male collected at the top of Little Mertens Falls, Mitchell Plateau (14°49′20″S; 125°42′39″E) on 7 January 2007 by L. Price and J. Francis.

Paratypes

Australia: *Western Australia*: WAM R163823 (male), collected 5.2 km southwest of the junction of the Prince Regent River and Pitta Creek (15°52′08″S; 125°36′23.6″E) on 28 January 2007 by M. Barrett, R. Barrett and P. Kendrick; WAM R167744 and WAM R167745 (males) – details as for holotype;

SAMA R62994 (female raised from one of 5 larvae) – Mitchell Plateau (14°49′19.4″S; 125°42′35.2″E) on 11 January 2007 by M. Anstis, J. Francis and J. D. Roberts; WAM R159800–03 (larvae) – details as for SAMA R62994; WAM R159804 (larva) – Prince Regent River Nature Reserve (15°41′S; 125°35′E) on 20 January 2007 by M. Barrett, R. Barrett and P. Kendrick.

Diagnosis

Adults distinguishable from all other *Crinia* in life by a network of minute bluish-white tubercles scattered over dorsum and limbs. Ventral surfaces smooth (except for rugose drink patch) and fingers of males with wide flanges. Tadpoles can be distinguished from all other *Crinia* tadpoles by robust laterally-compressed jaw sheaths, more numerous papillae and an emarginate oral disc.

Description of holotype

Small (17.5 mm SUL) body size with flattened shape and moderately pointed snout. Canthus rostralis slightly rounded, loreal region steep and slightly concave. Tympanum indistinct but tympanic bulge present. Tongue long and narrow, broadly rounded at tip; texture rugose. Fingers unwebbed, but with wide flanges along entire length of digits 1–3, 4th finger with only a narrow flange. Finger length: 3>4>2>1. Outer metacarpal tubercle enlarged; palmar tubercles moderately well-developed, especially on fingers. Metatarsal tubercles small with inner moderately developed; tubercles on plantar surface and toes also small. Toes with a wide flange (constricted near the joints) along entire length of digit; 1st toe with narrow flanges. Toe length: 4>5>=3>2>1. Dorsal skin smooth between prominent, scattered raised tubercles coincident with darker markings (see below). Minute bluish-white tubercles scattered over dorsum and limbs, tending to form irregular networks. Throat and abdomen smooth except for distinct granular tissue in drink patch.

Colour in life a dull blue-grey with dark brown longitudinally-aligned blotches on dorsum. Dark Y-shaped marking between eyes and medial blotch anterior to this on snout. Minute bluish-white-tipped tubercles scattered over dorsal surface and limbs. Upper arms and snout imbued with pale orange. Upper surfaces of limbs with strongly-contrasting dark bars of varying widths. The arms have 5–6 bars, the legs 12–14 bars with banding continuing along digits; thin and thick bands on femur and tibia align when legs folded. Lateral zone dark. Side of head between insertion of arm and upper posterior corner of eye with a dark brown inverted U-shaped arc enclosing a cluster of whitish tubercles. Upper half of iris golden, lower half dark brown. A squarish dark blotch beneath anterior corner of eye, upper lip mostly brown with scattered fine whitish tubercles. Canthus rostralis bordered with thin dark stripe. Tip of snout has a dark bar extending down each side from below each naris. Ventral surfaces dark with extensive lighter markings comprised of diffuse networks of blotches on the throat and chest, strongly contrasting white spots scattered on dark zones (less dense on flanks); an absence of pigment in the centre of the abdomen. Throat dark except for a thin pale medial stripe; white spots on chest at point of arm insertion. Undersides of thighs darker with fine yet distinct white spots; drink patch dark, rest of leg dark with extensive lighter mottling and spots; a central unpigmented patch in groin.

Colour in preservative slate blue-grey with two paravertebral rows of large dark blotches which bear scattered short dark longitudinal lines. In preservative, the fine dorsal tubercles lose their white pigment, and the orange on upper arms and snout is lost.

Variation

The other three male specimens were similar in body size and shape, limb proportions, tubercles and digital flanges to the holotype. The blotches on WAM R163823 are larger and joined together more than the holotype and the 'Y' on the head between the eyes has an anterior- projecting stripe (Figure 3B). On WAM R167745 the 'Y' lacks the stem and appears as a bowed transocular bar. The blotch anterior and below the eye projects forwards in WAM R167745 and is triangular in WAM R167744. There are a similar number of bands on the limbs and digits, although the widths and position of the bands vary moderately. WAM R163823 was preserved in 100% ethanol and has a light ventral surface. The lighter area in the centre of the abdomen is narrower in WAM R167745 and wider in WAM R167744. In WAM R167745 there is an unpigmented zone around the darker glandular drink patch and the white spots are slightly larger on the undersides of the thighs. SAMA R62994, the female raised from a tadpole, lacks the wide digital flanges although the fingers appear very slightly fringed in life (Figure 3C). Colour in life (Figure 3D) is similar to males, with a dorsal colour of reddish brown and the large dark patches, but the marking between the eyes consists of four isolated spots.

Tadpole morphology and development

Tadpoles collected at stages 36–37 developed rapidly and the earliest developmental stage studied was stage 38. A composite description of tadpoles at stages 38–41 is provided. Morphometric measurements of one anaesthetised live tadpole at stage 40 and one preserved tadpole at stage 38 are provided in Appendix 2, together with

measurements of a typical *C. bilingua* tadpole at stage 37 for comparison. Fully grown tadpoles are small, the longest specimen with a total length of 20.5 mm and body length of 8.5 mm (preserved, stage 41).

Body. Body small, rounded and wider than deep across abdomen. Snout rounded in dorsal view and deeply truncate in lateral view, especially by stage 41 (Figures 5 and 6). Eyes dorsolateral with slight anterior tilt (in life). Iris mostly golden, darker at each side; small dark umbraculum present on upper edge. At stage 38, nares small, closer to tip of snout than eyes and open dorsally, tilting more anteriorly by stage 41. Spiracle directed dorsoposteriorly, opens near midpoint of body well below horizontal body axis (almost onto venter in life); stretches higher up body in preserved specimens (Figure 5A). Vent tube dextral (type a; Anstis 2002), broad, opens above edge of ventral fin and mostly unattached to it behind.

Tail. Dorsal fin moderately arched, in a symmetrical arc-shape at stage 41 (Figures 5 and 6), tapers just before rounded tail tip. Ventral fin less arched. Muscle moderate at junction with body, tapers evenly to narrow point.

Oral Disc. Disc is ventral in direction, and emarginate. No anterior papillae, single row of small, crowded marginal papillae around each side and posterior margin with occasional submarginal papillae in some; narrow medial gap in posterior papillae. Two anterior and three posterior tooth rows, narrow or distinct medial gap in A^2, distinct

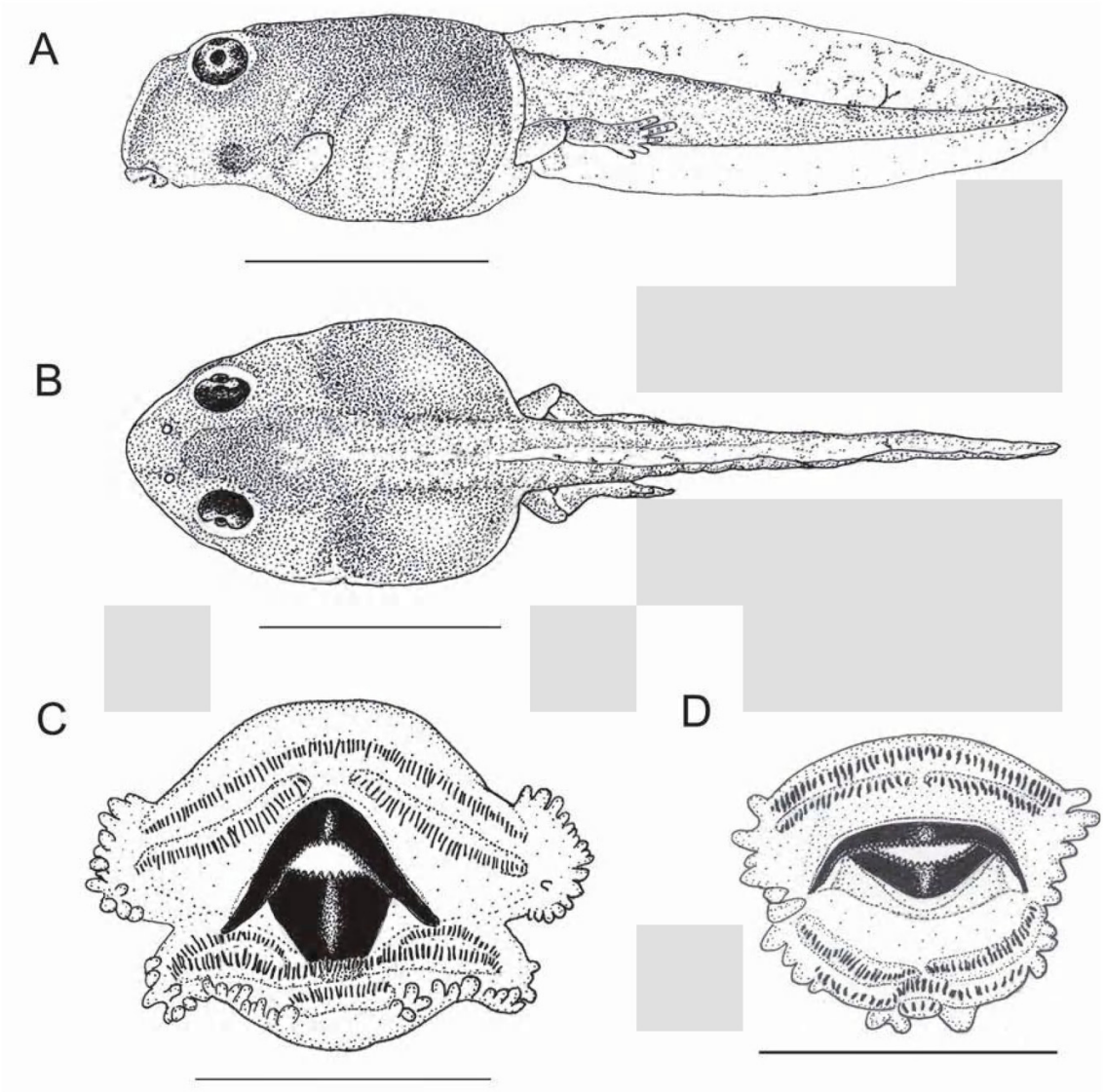

Figure 5. Tadpole and mouthparts of *Crinia fimbriata* sp. nov., and mouthparts of *C. bilingua* from Howard Springs, NT. A, B) *C. fimbriata* sp. nov. stage 38, in lateral and dorsal view, scale bar = 5 mm; C) mouthparts of *C. fimbriata* sp. nov. (SAMA R62994) stage 38, scale bar = 1 mm; D) mouthparts of *C. bilingua* stage 37, scale bar = 1 mm.

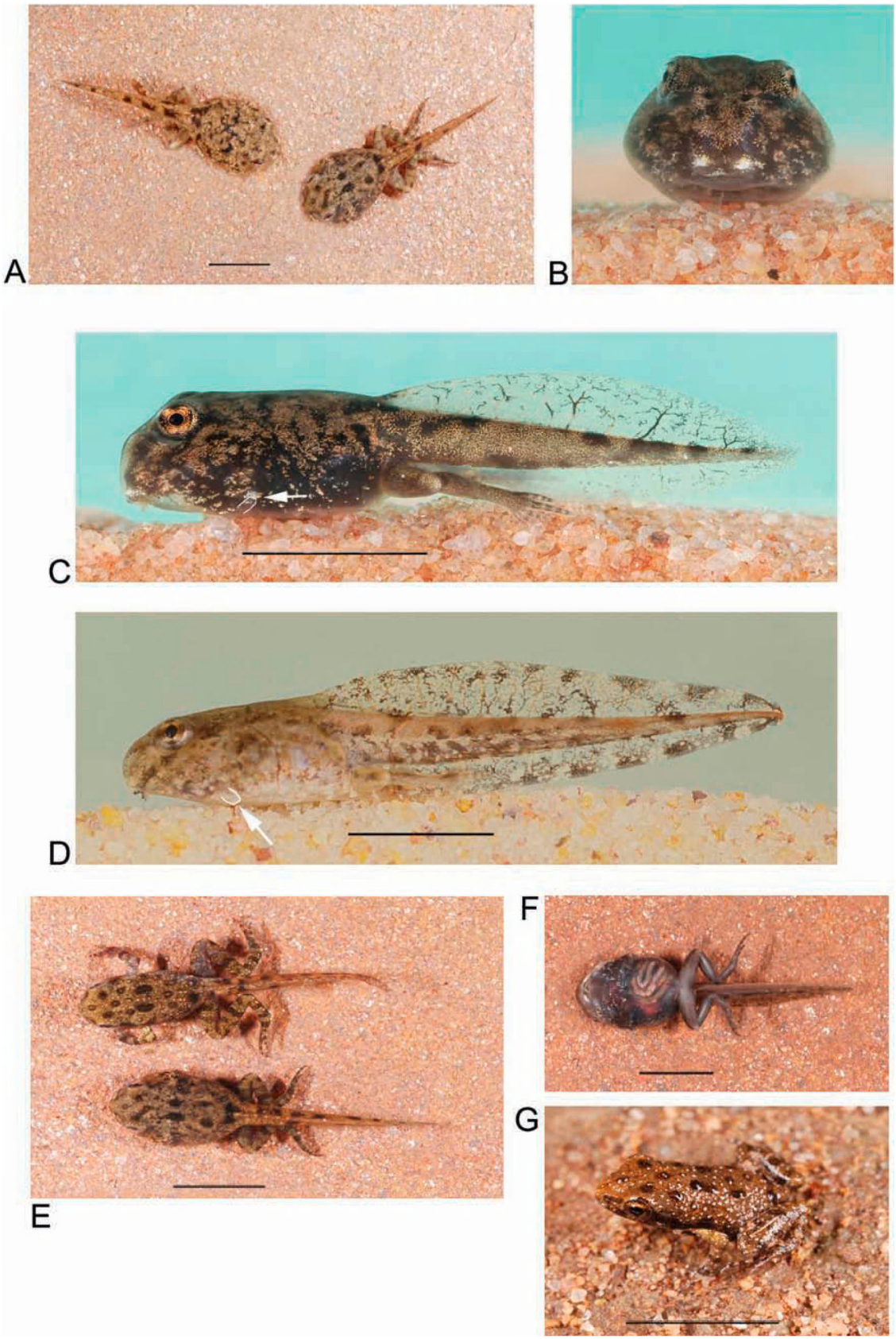

Figure 6 Larval development of *C. fimbriata* and *C. bilingua*. A) *C. fimbriata* stage 40 (left) and stage 41, dorsal view; B) *C. fimbriata* stage 40, anterior view showing pale gold spots on snout; C) *C. fimbriata* stage 41, lateral view, spiracle outlined in white and white arrow indicates opening of spiracle; D) *C. bilingua* stage 39, lateral view, Howard Springs NT, spiracle outlined, white arrow indicates opening of spiracle; E) *C. fimbriata* stage 41, 42, dorsal view; F) *C. fimbriata* stage 41, ventral view; G) stage 46, metamorph. Scale bar = 5 mm.

gap in P^1, P^3 short, equivalent to and just above, medial gap in posterior papillae. Jaw sheaths strongly laterally compressed, upper sheath acutely angled with long straight lateral processes, lower sheath massive and V-shaped (Figure 5C). By stage 41, the keratin on the jaw sheaths is reducing.

Pigmentation in life. Dorsum mostly covered with dull gold layer of iridophores over black layer beneath; irregular small dark patches or prominent spots that show through gaps in gold layer (Figure 6A,C). There is a small dark patch across the base of the body. More advanced tadpoles develop the distinctive broad dark spots often later associated with tubercles in adults (Figure 6E). Sides of body black beneath with scattered gold flecks becoming denser towards dorsum. There is a silver-white spot anteriorly on each side of the snout just above the oral disc (Figure 6B). Ventral surface transparent over gut, which is bordered by melanophores; stippled melanophores over gills and buccal cavity, a few scattered gold flecks over gills, heart, down middle of abdomen and denser on either side of mouth (Figure 6C,F). Dorsal fin pigmented with fine melanophores, some pigmented venation; ventral fin less pigmented, with fine flecks posteriorly. Dense gold layer covers much of dark tail muscle except for some prominent black spots dorsally and a few laterally. The adult pattern becomes more apparent as tadpoles approach metamorphosis.

Pigmentation in preservative. All gold and silver/white pigment is lost and only melanophore pigment remains. The intestines are more visible through the sides and also the venter, which is mostly transparent.

Metamorphosis. Development to metamorphosis is rapid once tadpoles have reached stage 38. One newly metamorphosed froglet (SAM R62994) measured 7.5 mm SUL with essentially adult pigmentation. The dorsum is brown with scattered prominent black round tubercles and numerous minute pale blue tubercles all over the body and limbs; iris golden. The limbs have dark bands and forelimbs are paler brown (Figure 6G).

Comparison with other species

Adults. Crinia fimbriata sp. nov. can be distinguished from sympatric *C. bilingua* by a combination of the network of minute bluish-white tubercles over the dosal surface and limbs, the smooth belly and flanges on the fingers of males. Because of its small size, *C. fimbriata* sp. nov. may be confused with other small syntopic rock-dwelling *Litoria* species (e.g. *L. meiriana*, *L. staccato*), but all potential *Litoria* species have expanded terminal discs, whereas all *Crinia* have no terminal discs.

Tadpoles. Tadpoles are similar to *C. bilingua*, however, they have the most robust and laterally-compressed jaw sheaths of any *Crinia*, the sides of the oral disc are emarginate and marginal papillae are smaller and more numerous. In addition, *C. fimbriata* has a deeper more truncate snout (cf. Figure 6C,D) and a broader, fatter body with a slightly shorter tail relative to body length (body/total length ratio 0.40–0.42 versus 0.36 for *C. bilingua* (see Appendix 2). *Crinia fimbriata* has a spiracle that points dorsoposteriorly in life (Figure 6C), while that of all other northern *Crinia* species points ventrally or ventroposteriorly (Figure 6D). Finally, *C. fimbriata* sp. nov. appears to have much less ventral pigmentation in life than *C. bilingua* (Tyler *et al.* 1983; Anstis, unpublished data).

Habitat

All adult specimens of *C. fimbriata* sp. nov. have been collected from shallow (to 5 cm depth) pools on the top of sandstone rock platforms. At both collection sites, pools were located on the top of large cliffs with running water in creeks and waterfalls close by. The Mitchell Plateau specimens were at the top of Little Mertens Falls and were encountered together while active at night (ca. 2100 h). The pool was near the edge of the rock platform near the forest (ca. 20 m from the river and falls). The Prince Regent River specimen was in a shallow rock pool on the edge of a high rock platform (M. Barrett, pers. comm.). It was collected in the daytime and was sheltering in a crevice when it dived into the pool and remained motionless, even when prodded. In contrast, *C. bilingua* adults were only found associated with temporary flooded ponds and ditches in grassland and woodland, well away from rocky escarpments.

Tadpoles at Mitchell Plateau were found in a temporary, shallow rock pool about 1 x 0.75 m and 4–5 cm deep on top of a dry rocky ledge well above stream level. Water temperature was about 35°C at 1100 hr. The specimen from Prince Regent River Nature Reserve was found in a temporary, shallow pool 1 m in diameter and 5 cm deep (M. Barrett and P. Kendrick, pers. comm.). Tadpoles are benthic and were mostly observed in stationary positions on the sandstone substrate, which was partly covered with some algae, silt and a few leaves.

Etymology

Fimbria is Latin for 'fringed' in reference to fringes on adult males observed in this species. Used as a noun in apposition.

Remarks

Discovery of new tropical species of frogs in northern Australia is still occurring owing to the diverse anuran fauna and the difficulty of travelling to remote areas during the wet season. Recent discoveries include *Uperoleia* species from near Darwin (Young *et al.* 2005) and the northwest

Kimberley (Doughty and Roberts 2008), a rock hylid from the Kimberley (Doughty and Anstis 2007) and a stream-dwelling hylid from north Queensland (Hoskin 2007). All of these species were initially identified by a distinctive call. However, the call of *C. fimbriata* sp. nov. is still unknown. *Crinia fimbriata* sp. nov. was discovered and collected because of their unusual colour and markings. As *C. fimbriata* sp. nov. and *C. bilingua* occur sympatrically, we expect their calls to be sufficiently distinct to enable females to choose males of their own species.

Two features of *C. fimbriata* sp. nov. are unique in *Crinia*. First, the flanges on the fingers of males may have a functional role, but we lack any observations to suggest a function, and the only female has no flanges. Sexual dimorphism in finger flanges is known from *Limnodynastes* Fitzinger, 1843, *Philoria* Spencer, 1901 and *Platyplectrum* Günther, 1863 species, but these are possessed by females to help them make a foam nest of bubbles. Second, the unusually massive and laterally compressed jaw sheaths of *C. fimbriata* sp. nov. tadpoles readily distinguish them from congeners. These are likely to be an adaptation that increases medial rasping pressure of the jaw sheaths, thereby enabling these benthic tadpoles to remove algae embedded in the rock substrate of the pools where they are found. The less benthic tadpoles of *Litoria cavernicola* Tyler and Davies, 1979 were also found in the same pool and, although they have moderately keratinised jaw sheaths, they are not as laterally compressed as in *C. fimbriata* sp. nov.

The existence of a basal lineage containing *C. nimbus* and *C. tasmaniensis* from the southern, temperate latitudes of Tasmania and a second sister lineage comprising *C. fimbriata* sp. nov. from the northern tropical latitudes of the Kimberley represents an interesting and somewhat perplexing phylogenetic pattern. Several alternative historical scenarios may explain the existence of highly divergent mitochondrial lineages with disparate distributions across Australia. One possible explanation is that the ancestral *Crinia* species were once widespread across the Australian continent and the pattern we see now reflects historical vicariance with divergence driven by the aridification of central Australia in the Miocene.

The northwestern Kimberley has high conservation value owing to the large diversity of endemic frog, reptile and mammal species known to occur there. The discovery of *C. fimbriata* sp. nov. along with other frog species from this region in the last two years (Doughty and Anstis 2007; Doughty and Roberts 2008) highlights how little is known of the high rainfall zone of the northwest Kimberley. Although descriptions of new species greatly enhance our appreciation of the high diversity of the region, there is still much to learn about the true distributions, ecology, reproduction and behaviour of the Kimberley fauna. It is essential that the wilderness areas of the northwest Kimberley are preserved to conserve the diversity of organisms that occur there and the ecological processes that have led to the generation of this diversity.

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Appendix 1. *Crinia bilingua* specimens examined (WAM and R prefixes excluded).

Males – 161202, 162477, 162579, 162541, 162599, 166143, 167709, 167851, 168070, 168085, 168140, 168142.

Females – 162478, 167850, 167962, 167996, 168012, 168119, 168120, 168189.

Appendix 2. Tadpole morphometrics (in mm).

Crinia fimbriata sp. nov., stage 38 (preserved): TL 19.1, BL 8.2, BD 4.3, BW 5.6, EBW 4.1, BTM 1.8, BTMW 1.5, TD 3.7, DF 1.5, TM 1.1, VF 1.1, IO 1.6, IN 0.8, EN O.6, N 0.2, SS 4.1, SN O.8, SE 1.6, ED 1.1, ODW 1.6.

Crinia fimbriata sp. nov., stage 40 (anaesthetised): 19.5, 7.9, 3.9, 5.0, 4.5, 1.5, 1.6, 3.5, 1.6, 1.0, 0.9, 1.6, 0.8, 0.6, 0.2, 4.3, 0.6, 1.8, 1.2, 1.6.

Crinia bilingua, stage 37 (anaesthetised): 21.7, 7.9, 4.2, 4.8, 3.9, 2.0, 1.8, 4.0, 1.7, 1.4, 0.9, 1.1, 0.8, 0.5, 0.2, 4.4, 0.7, 1.4, 1.5, 1.2.