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A new species of *Pseudophryne* (Anura: Myobatrachidae) from the central Australian ranges

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Abstract

The myobatrachid frog genus *Pseudophryne* is highly variable in color pattern in eastern Australia where many species are distinguished by distinctive dorsal patterns. In contrast *Pseudophryne* from the western half of the continent are morphologically conservative. Two nominal species are widespread in south-western Australia and north-western South Australia, with one, *P. occidentalis*, being found in semi-arid and arid regions. Using mitochondrial DNA and morphological characters we establish that populations in the ranges of north-western South Australia assigned to *P. occidentalis* are a separate species. The new species comprises one of four major lineages of *Pseudophryne* while *P. occidentalis* falls within another lineage confined to south-western Australia.

Keywords: mitochondrial DNA, frog, Myobatrachidae, *Pseudophryne*, phylogeny, species, systematics

Introduction

The Australopapuan myobatrachid frogs show considerable diversity in morphology and in breeding biology, with some taxa expressing remarkable forms of parental care including gastric brooding in *Rheobatrachus* (Corben *et al.* 1974) and 'hip pocket transport' in *Assa* (Straughan & Lee 1966). Much of this diversity is taxonomically distributed among genera so that often each genus is highly distinctive and recognizable (Littlejohn *et al.* 1993). The obvious morphological differences between genera strongly contrast with the conservative pattern of differentiation within genera. This morphological conservatism is most obvious in two speciose genera of small 'toadlets', *Pseudophryne* (13 species) and *Uperoleia* (27 species). These genera are geographically very widespread and morphologically conservative, although some species of *Pseudophryne* are brilliantly colored, and relatively conservative in male advertisement calls (Catullo *et al.* 2011). Diversity in *Uperoleia* is concentrated primarily in northern Australia from the Pilbara region in the west across the north of the continent and extending into the mesic south-east. The range of *Pseudophryne* overlaps with *Uperoleia* in the north-west Pilbara region and along the eastern seaboard but is exclusive of *Uperoleia* in southern Australia including some of the central desert ranges in north-western South Australia and eastern Western Australia.

An isolated population of *Pseudophryne* was discovered in the north-western ranges of South Australia in 1970 and assigned by Tyler (1972) to *P. occidentalis*, a species which is otherwise distributed in south-western Australia (Fig. 1). Accumulation of new voucher specimens with accompanying images in life, and of frozen tissues from the Everard and Musgrave Ranges has provided the opportunity to comprehensively assess relationships of the *Pseudophryne* from the central ranges with congeners utilising molecular genetic methods and more detailed morphological comparisons. The molecular genetic data demonstrate that the central ranges population is a long-isolated lineage within *Pseudophryne* and we herein describe it as a distinct species.

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TABLE 1. Museum registration numbers, GenBank accession numbers and locality data for taxa examined. * - indicates a type location. NSW—New South Wales, SA—South Australia, Qld—Queensland, Vic—Victoria, WA—Western Australia.

Taxon	Code		Voucher	GenBank Acc	Location
australis	1	ABTC1246	AMSR133189	JX430942	Narara, NSW
	2	-	AMSR155209	JX430943	Royal National Park, NSW
bibronii	1	ABTC12661	SAMAR40871	JX430944	32km N Abercrombie River, NSW
	2	ABTC13609	No voucher	JX430945	Norton Summit, Adelaide
	3	ABTC16465	SAMAR39894	JX430946	Moockra Tower, SA
	4	ABTC80751	SAMAR59996	JX430947	Kangaroo Island, SA
	5	ABTC58289	SAMAR45983	JX430948	2.8km WSW Duck Island HS, SA
	6	ABTC57314	SAMAR39942	JX430949	Beetaloo Reservoir, SA
coriacea	1	ABTC3837	SAMAR33655	JX430950	23km E Emuvale, Qld
	2	ABTC25496	No voucher	JX430951	Mann River Nature Reserve, NSW
corroboree		ABTC80957	ANWCA1855	JX430952	Toolong Plain, Snowy Mtns, NSW
covacevichae		ABTC16795	SAMAR39938	JX430953	Ravenshoe, Qld
dendyi		ABTC40918	No voucher	JX430954	Dargo High Plains, Vic
douglasi	1	ABTC80336	WAMR135088	JX430955	Kotka Gorge, WA
	2	ABTC80337	WAMR125741	JX430956	East of Python Pool, WA
	3–4	ABTC80519–20	WAMR102442/4	JX430957-8	Kookhabinna Gorge, WA
	5	WAMR154909	WAMR154909	JX430959	Cape Range, WA
guentheri	1	ABTC15894	SAMAR41988	JX430960	Quairading, WA
	2	ABTC80338	WAMR116499	JX430961	Nanson, WA
	3	ABTC80339	WAMR113460	JX430962	Moorine Rock, WA
	4	ABTC80340	WAMR140460	JX430963	Cataby, WA
	5	ABTC80341	WAMR116531	JX430964	Depot Hill, WA
	6	ABTC80342	WAMR113476	JX430965	Wyalkatchem, WA
major	1	ABTC15777	No voucher	JX430966	Barmera, Qld
	2	ABTC16482	SAMAR39902	JX430967	4km SE Gayndah, Qld
robinsoni **sp.nov.**	1–6	ABTC17427–8, 17444–7	SAMAR38413–8	JX430997-02	Victory Well, Everard Ranges, SA
	7–10	ABTC73450	SAMAR54630–2	JX431004-6	5.3km ESE Mt Illbillee, SA
	11–14	ABTC79969–72	SAMAR59119	JX431007-10	Alalka Ck Gorge, 14km N Pukatja, SA
	15	ABTC121843	SAMAR64657	JX431011	7.1k WNW Amata, SA
occidentalis	1,17	ABTC79973, 82045	WAMR112859	JX430968, JX430984	Ora Banda, WA
	2	ABTC79974	WAMR113468	JX430969	Moorine Rock, WA
	3	ABTC79975	WAMR113763	JX430970	11km SW Bulla Bulling, WA
	4	ABTC79976	WAMR113765	JX430971	Coolgardie, WA
	5	ABTC79979	WAMR113779	JX430972	W side of Coolgardie, WA
	6	ABTC79980	WAMR129874	JX430973	40km NE Kalgoorlie, WA
	7	ABTC79983	WAMR135202	JX430974	Dundas Rock, WA
	8	ABTC79984	WAMR141258	JX430975	near Ora Banda, WA
	9	ABTC79987	WAMR152924	JX430976	Koolyanobbing area, WA

.....continued on next page

TABLE 1. (Continued)

Taxon	Code		Voucher	GenBank Acc	Location
	10	ABTC80343	WAMR115906	JX430977	Lake Cronin, WA
	11	ABTC15574	SAMAR39870	JX430978	near Balladonia, WA
	12	ABTC80344	WAMR136411	JX430979	Dundas, WA
	13	ABTC80346	WAMR113469	JX430980	Yellowdine, WA
	14–5	ABTC15882–3	SAMAR40346–7	JX430981-2	Balladonia, WA
	16	ABTC82043	WAMR135202	JX430983	Norseman, WA
	18	ABTC79981	WAMR132638	JX430985	Koolanooka Spring, WA
	19	ABTC79982	WAMR132696	JX430986	Wurrah Rock, WA
	20	ABTC79985	WAMR141358	JX430987	Wogarno Hill, WA
	21	ABTC80345	WAMR140403	JX430988	Gullamilyaroo Well, WA
	22	ABTC80348	WAMR132613	JX430989	Camel Soak, 39km E Perenjori, WA
	23–4	ABTC82581–2	SAMAR59029–30	JX430990-1	Mt Gibson Station, WA
	25, 27–8	ABTC79978, 80347	AMSR113774–6	JX430992/ 94-5	66km N Leonora, WA
	26	ABTC79986	WAMR145282	JX430993	Hill 50 Mine, Mount Magnet, WA
	29	ABTC81113	WAMR151412	Appendix 2	Bulga Downs Station, WA
	30	ABTC81111	WAMR115171	Appendix 2	9.9km S Mt West, WA
pengilleyi		ABTC80962	ANWCA01922	JX430996	Brindabella Range, NSW
raveni	1	ABTC80489	No voucher	JX431012	Wongai State Forest, Qld
	2	ABTC51255	No voucher	JX431013	Crediton, Qld
semimarmorata		ABTC79706	No voucher	JX431014	Rosedale, Vic
Metacrinia nichollsi			SAMAR40296	JX431015	Manjimup SF, WA
Myobatrachus gouldii			WAMR116075	JX431016	Spalding Park, Geraldton, WA
Uperoleia laevigata			SAMAR42629	JX431017	Ourimbah State Forest, NSW

Materials and methods

Specimens examined. Tissues from 71 Pseudophryne were available for molecular genetic analyses (Table 1). We included two individuals from each nominate taxon of Pseudophryne from eastern Australia for comparative purposes. An array of related taxa were used as outgroups. The monotypic Metacrinia and Myobatrachus were included because a recent mtDNA based phylogeny (Read et al. 2001) showed that these genera are the closest relatives of Pseudophryne. Uperoleia was included as a more distant outgroup. Institutional codes used are: ABTC: Australian Biological Tissue Collection, South Australian Museum, Adelaide; AMS: Australian Museum, Sydney; ANWC: Australian National Wildlife Collection, CSIRO, Lyneham; SAMA: South Australian Museum, Adelaide; and WAM: Western Australian Museum, Perth. Distributional data were compiled from voucher records in the South and Western Australian Museums as of April 2012 and October 2009 respectively.

Morphometrics. Specimens were scored for a range of colour, pattern and external skin, limb and digit characters that are known to vary among *Pseudophryne* (Tyler *et al*. 1984). Sex was determined by visual inspection of gonads. Measurements of the following parameters were taken either with callipers or an eye piece graticule under a stereomicroscope to the nearest mm: SV—snout to vent length, HW—head width measured at the posterior edge of the mandible (i.e. at widest point of the head) , EN—eye to naris, EY—eye diameter,

IN—internarial distance, IO—interorbital distance, HAND—proximal edge of palm to the tip of the third (longest) finger, F1—first finger length, F3—third finger length, ARM—forearm length from outer flexure of wrist to outer margin of flexed elbow, TL—tibia length, FOOT—from the distal base of the inner metatarsal tubercle to the tip of the third (longest) toe, T2—second toe length, T3—third toe length**,** IM—diameter of inner metatarsal tubercule, OM - diameter of outer metatarsal tubercule. We used Principal Components Analysis (PCA), which does not identify groups *a priori*, to examine the patterns of relationship among 16 morphological traits (natural log transformed) with the "princomp" routine implemented in the software package R v2.14.1. The first principal component (PC) was interpreted as representing variation in body size and shape and the second and third PCs summarized shape differences. Images were taken with a Canon EO5D Mark II digital camera with a 100 mm L series macro lens and processed with Zerene Stacker software.

Molecular genetic methods and phylogenetic analyses. DNA was extracted from frozen or alcohol preserved toe or liver tissue using a Puregene DNA isolation kit (Gentra Systems, Minneapolis, MN) following the manufacturers protocol for DNA purification from solid tissue. PCR was used to amplify the entire 1364 bp of the mitochondrion NADH dehydrogenase subunit 4 (*ND4*) gene and part of *ND4L* and *tRNA^{His}* in two overlapping fragments for each DNA sample using primers LM449, LM450, HM358 and HM438 (Table 2). We also sequenced a shorter 136 bp diagnostic fragment of the *ND4* gene from several museum vouchers that had not been preserved optimally for DNA analysis using primers LM622 and HM623 (Table 2). Each PCR was carried out in a volume of 25 ml with a final concentration of 1X GeneAmp PCR Gold buffer, 2–4 mM $MgCl_2$, 200 M of each dNTP, 0.2 mM of each primer and 0.5 U of AmpliTaq Gold DNA polymerase (Applied Biosystems, Foster City, CA). Amplifications consisted of an initial denaturation step of 94°C for 9 min, followed by 34 cycles of PCR with the following temperature profile: denaturation at 94°C for 45 s, annealing at 55°C for 45 s, and extension at 72°C for 1 min, with an additional final extension at 72°C for 6 min. The double-stranded amplification products were visualised on 1.5% agarose gels and purified using an UltraClean PCR clean-up DNA purification kit (Mo Bio Laboratories Inc., CA) before cycle-sequencing using the BigDye Terminator v3.1 cycle-sequencing kit (Applied Biosystems).The cycling protocol consisted of 25 cycles of denaturation at 96°C for 30 s, annealing at 50°C for 15 s, and extension at 60°C for 4 min. All samples were sequenced on an Applied Biosystems 3700 DNA sequencer. Sequences were aligned with MAFFT v6.814b (Katoh *et al*. 2005) implemented in Geneious Pro v5.5.2.

TABLE 2. Names, locations and sequences of primers used for PCR and sequencing.

Primer name	3′position	Primer sequence 5′ to 3′
HM358	*tRNA^{His}*	AGAGTCACAGACTAGGGTTT
HM438	*ND4*	GGATTTTAGGTCTGTYTGTCG
LM449	*ND4L*	TACGGCTCAGAYAAYCTAAAYTC
LM450	*ND4*	ACTYCCCAAAGCWCACGTAGA
LM622	*ND4*	CCYTAYTTYCTTATYGATGAAAT
HM623	*ND4*	AATARAATRAGGTTRTTTGCGA

Bayes factors were used to assess all possible alternative partitioning strategies for four data subsets—1st, 2nd, and 3rd codon positions and the tRNA in PartitionFinder v1.0.0 (Lanfear *et al*. 2012). The Akaike information criterion (AIC) and Bayes Information Criterion (BIC) were used to assess the best fit partition strategy and nucleotide substitution model for each data subset in the selected partition strategy. Sequences were analysed phylogenetically using Bayesian and maximum likelihood methods. Bayesian analysis was conducted using MrBayes v3.1.2 (Ronquist & Huelsenbeck 2003). The analysis was run with model parameters unlinked using default priors for two million generations with two independent runs and two chains sampling every 1000 generations. The first 25% of sampled trees were discarded as burn-in and convergence was assessed by examining effective sample sizes (ESS values), split frequencies of clades across runs and likelihood plots through time in TRACER v1.4.1 (Rambaut & Drummond 2007). Partitioned maximum likelihood (ML) analysis was performed using RAxML v7.1.0 (Stamatakis 2006). The GTR+g substitution model was used as recommended (Stamatakis 2006) to perform 200 independent ML searches with 1000 non-parametric bootstrap replicates.

Net average sequence divergence between lineages (d_A) was calculated in MEGA v5 (Tamura *et al.* 2011) as: $d_A = d_{XY} - (d_X + d_Y)/2$, where, d_{XY} is the average distance between groups X and Y, and d_X and d_Y are the within-group mean.

Results and Discussion

Below, we use the final specific epithets throughout the manuscript rather than use an initial group nomenclature that we would then change to the final specific epithets in the taxonomy section. We did not assume the separate species status of groups, but rather used the results section to test this hypothesis before presenting the final taxonomy.

Molecular genetic analyses. The final alignment comprised 1384bp for all except three samples. For r15 we obtained 827bp and for o29–30 we obtained 136bp from the short amplicon (Appendix 2). The most suitable partitioning scheme found with PartitionFinder included four data subsets (with the substitution model selected for each)—1st *ND4* codon position (HKY+G), 2nd *ND4* codon position (HKY+G), 3rd *ND4* codon position (TIM+G) and the $tRNA^{His}$ (F81).

In the phylogenetic analysis, the *Pseudophryne ND4* sequences fell into four major clades: 1) Western Australian *P. occidentalis* + *P. guentheri*; 2) *P. robinsoni* **sp. nov.**; 3) *P. douglasi*, and 4) all of the eastern Australian species of *Pseudophryne* (Fig. 2). Monophyly of each of the first three clades is well supported, while monophyly of the fourth lacks strong support. Genetic distances between the major clades are high. Uncorrected net sequence divergence (d_A) between the first three major clades ranged from 11.6% to 14.8%.

Diversity within the *P. robinsoni* **sp. nov.** clade is low relative to the other clades that have similar levels of within taxon sampling (Fig. 2) and it is geographically structured (Fig.1). Two well supported sub-clades are evident within *P. robinsoni* **sp. nov.**, with one confined to the Everard Ranges (r1–10) and the other to the Musgrave Ranges (r11–15). In contrast, diversity within the *P. douglasi* clade is of a level seen between some sister species in the eastern *Pseudophryne* clade, e.g. between *P. raveni* and *P. coriacea*. More striking is the level of diversity within the Western Australian *P. occidentalis* - *P. guentheri* clade, wherein *P. occidentalis* falls into two sub-clades with one of these the sister to *P. guentheri*. As with *P. douglasi*, diversity within each of the *P. occidentalis* sub-clades is high and of an order similar to divergence between sister species in the eastern *Pseudophryne* clade. Of particular note is the individual from 9 km S Mt West in east-central Western Australia (o30) that is allied with *P. occidentalis* from the eastern part of the species distribution (o25–29) but separated from them by at least 700 km. As we were only able to obtain a short *ND4* fragment from individuals o29 and o30, we also conducted a phylogenetic analysis in which the sequence alignment was restricted to the shorter fragment length for all individuals. In this analysis (not shown), o29 was placed in the sub-clade including o25-28, while o30 was placed one node more basally than its position in the larger sequence dataset analysis as sister to the major western *P. occidentalis-guentheri* clade.

Morphological comparisons. Morphometric variation among *P. guentheri*, *P. occidentalis* and *P. robinsoni* **sp. nov.** is summarized in Table 3. Bivariate plots of the first two PCs summarizing variation in size and shape of males and females are presented in Fig. 3. Partial separation of all three taxa was achieved in both the male and female PCAs. While more separation was evident in the female analysis despite fewer females being available. For females, the proportion of variance explained by the first three PCs were 0.50, 0.67 and 0.74 cumulatively. The traits with the highest loadings were: PC1—SV, ARM, HAND, EN; PC2—F3, T2, EYE, T3. For males, the proportion of variance explained by the first three PCs were 0.47, 0.68, and 0.76 cumulatively. The traits with the highest loadings were: PC1—SV, HAND, EYE, F3; PC2—T2, T3, OM, FOOT, IM.

Pseudophryne robinsoni **sp. nov.** differs from all eastern species of *Pseudophryne* by the absence of a post-femoral gland. It further differs from *P. australis*, *P. coriacea*, *P. corroboree*, *P. pengilleyi* and *P. raveni* by the absence of either a complete red crown, or red-brown dorsum or yellow/black stripes; from *P. dendyi* by the absence of yellow on the elbows or urostyle; from *P. semimarmorata* by absence of red to yellow on the ventral surfaces of legs and inguinal and gular regions.

Pseudophryne robinsoni **sp. nov.** differs qualitatively from all western species of *Pseudophryne* in two aspects of their external morphology – size of the metatarsal tubercules and ventral patterns. In *P. guentheri* the inner and outer metatarsal tubercules are equal in size and large, in *P. occidentalis* both tubercules are noticeably smaller but

still equal in size and in *P. douglasi* the outer tubercule is either absent or small and the inner tubercule is large (Main 1964). In contrast both tubercules in *P. robinsoni* **sp. nov.** are very small and usually unequal in size (unequal in 14 individuals, equal in three) (Fig. 4).

TABLE 3. Morphological measurements of adult *Pseudophryne* specimens, Figures are mean ± SD (range).

Trait	*P. robinsoni* **sp. nov.**		*P. guentheri*		*P. occidentalis*	
Sex	F	M	F	M	F	M
N	4	13	11	14	19	16
SV	27±1.8	25.3±1.3	33.38±3.83	28.99±4.17	30.04±2.65	26.26±3.49
	25.2–29.4	23.8–27.3	26.2–38	23.5–35.5	25–35.5	23.4–36.7
HW	8.5±0.4	7.9±0.54	12.35±1.29	11.49±1.4	9.99±1.14	8.88±0.85
	8.1–9	7–8.9	10.5–14.4	8.9–13.5	8.2–12.7	7.8–11
EN	1.65±0.17	1.81±0.2	2.3±0.39	2.16±0.26	1.78±0.19	1.66±0.15
	1.5–1.8	1.5–2.07	1.7–3	1.8–2.6	1.5–2.1	1.5–2
EY	2.1±0.2	2.2±0.2	3.14±0.44	2.66±0.43	2.17±0.17	2.15±0.24
	1.9–2.3	1.9–2.6	2.3–4	1.6–3	2–2.6	1.7–2.5
IN	1.8±0.3	1.6±0.33	2.29±0.39	1.97±0.40	1.67±0.16	1.55±0.24
	1.6–2.2	1.44–2	1.8–3.0	1.2–2.9	1.4–1.9	1.2–1.9
HAND	5.53±0.26	5.27±0.46	6.5±0.57	5.8±0.82	6.01±0.53	5.4±0.5
	5.3–5.9	4.6–6.15	5.5–7.4	4.5–7	5.2–7	5-6.5
F1	1.37±0.25	1.36±0.21	1.72±0.25	1.52±0.32	1.64±0.24	1.39±0.11
	1–1.5	1–1.84	1.4–2.1	1.1–2	1.2–2	1.2–1.6
F3	3.2±0.18	2.92±0.28	3.22±0.47	3.05±0.49	3.19±0.25	2.85±0.31
	3–3.4	2.5–3.48	2.5–4	2.3–3.8	2.8–3.7	2.4–3.4
ARM	5.5±0.4	5.5±0.4	7.15±0.87	5.97±0.67	6.18±0.5	5.5±0.73
	5–6	5–6.4	5.5–8.2	5–7	5.5–7	4–7
TL	8.53±1.08	8.76±0.52	8.86±1.16	8.16±1.09	8.61±0.69	7.59±0.71
	7–9.5	8.2–9.92	7–10.5	6.5–10	7.5–10	6–8.8
FOOT	10.1±0.63	9.58±0.66	9.83±0.92	8.67±1.24	9.18±0.72	8.13±0.83
	9.5–11	8.5–11	8.5–11	7–10.5	8–10.5	7–10
T2	2.03±0.13	2.03±0.31	1.95±0.27	1.75±0.36	1.99±0.21	1.85±0.2
	1.9–2.2	1.7–2.99	1.5–2.5	1.1–2.3	1.7–2.4	1.5–2.3
T3	3.2±0.36	3.17±0.41	3.18±0.27	2.76±0.53	3.06±0.31	2.85±0.29
	2.8–3.5	2.6–4.25	2.8–3.5	2–3.6	2.5–3.5	2.4–3.4
IM	0.45±0.1	0.55±0.12	1.21±0.23	1.1±0.18	0.78±0.16	0.68±0.17
	0.3–0.5	0.4–0.8	0.9–1.6	0.9–1.5	0.4–1	0.5–1
OM	0.55±0.06	0.7±0.17	1.44±0.29	1.28±0.24	1.1±0.15	1±0.09
	0.5–0.6	0.4–0.9	1–2	1–1.7	0.9–1.5	0.8–1.2

The ventral pattern in *P. guentheri* comprises small black marks on a white background, in *P. occidentalis* it comprises large black marks on a white background, in *P. douglasi* it comprises slaty grey or fine black patterning on a white background. In contrast, in *P. robinsoni* **sp. nov.** the ventral pattern comprises large white marks on a black background (Fig. 5).

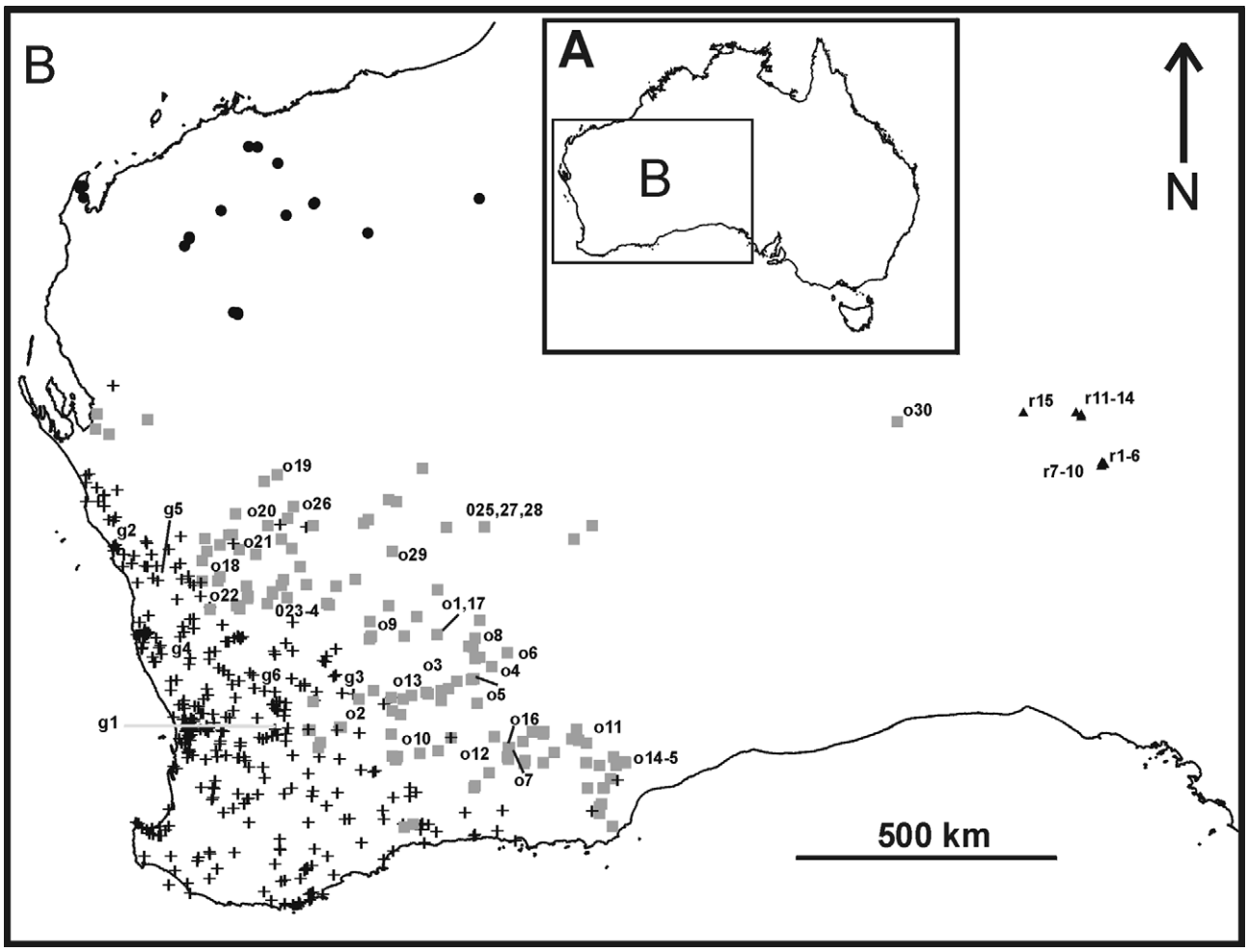

FIGURE 1. Map of western Australia showing species ranges and collection locations of *Pseudophryne*. See the Appendix for code explanations. *Pseudophryne douglasi* = black circles, *P. guentheri* = cross, *P. occidentalis* = gray square, *P. robinsoni* **sp. nov.** = black triangle.

Taxonomic treatment. The populations of *Pseudophryne* from the Musgrave and Everard Ranges in north-western South Australia were nominally attributed to *P. occidentalis* by Tyler (1972). Although allopatric with western populations of *P. occidentalis*, they are clearly not genetically closely related to that species or indeed to any other species of *Pseudophryne*. Accordingly they are worthy of recognition as a distinct species given the deep evolutionary divergence from all other *Pseudophryne*. *Pseudophryne robinsoni* **sp**. **nov**. is also morphologically diagnosable from all other *Pseudophryne* on the basis of at least two morphological traits (metatarsal tubercles and ventral pattern).

The status of *Pseudophryne* from western locations in the central ranges, i.e. Bell Rock and Tomkinson Ranges remains unresolved. Three adult specimens (WAM R115170-2) were collected by David Pearson in 1994 from 9 km S of Mt West in the Bell Rock Range [26.3038S, 128.7967E]. The short *ND4* sequence that we obtained from one of these (WAM R115171, o30) is placed with one of the western *P. occidentalis* sub-clades (Fig. 2) but is nevertheless quite divergent from this sub-clade and may represent another taxon. However, as the specimens are faded and the molecular genetic data are based on one short sequence, we consider the evidence insufficient to determine their affinities at present, preferring to refer them to *P. occidentalis*.

In 2005, John Read photographed an adult *Pseudophryne* at Ninuku Spring, near Kalka in the Tomkinson Ranges, about 36 km E of Mt West in the Bell Rock Range, WA and about 200 km W of the Amata record of *P. robinsoni* **sp. nov**. This individual resembled *P. robinsoni* **sp. nov.** in its ventral pattern, but given its geographic proximity to the Bell Rock Range population and the genetic affinities of the latter, we await genetic conformation of its affinities. The Mann Ranges, which lie between the Musgrave and Tomkinson Ranges, are yet to be surveyed for *Pseudophryne*.

The species status of the two divergent *P. occidentalis* sub-clades in the western *P. occidentalis-guentheri* major clade is not resolved here and requires further molecular genetic and more detailed morphological analyses.

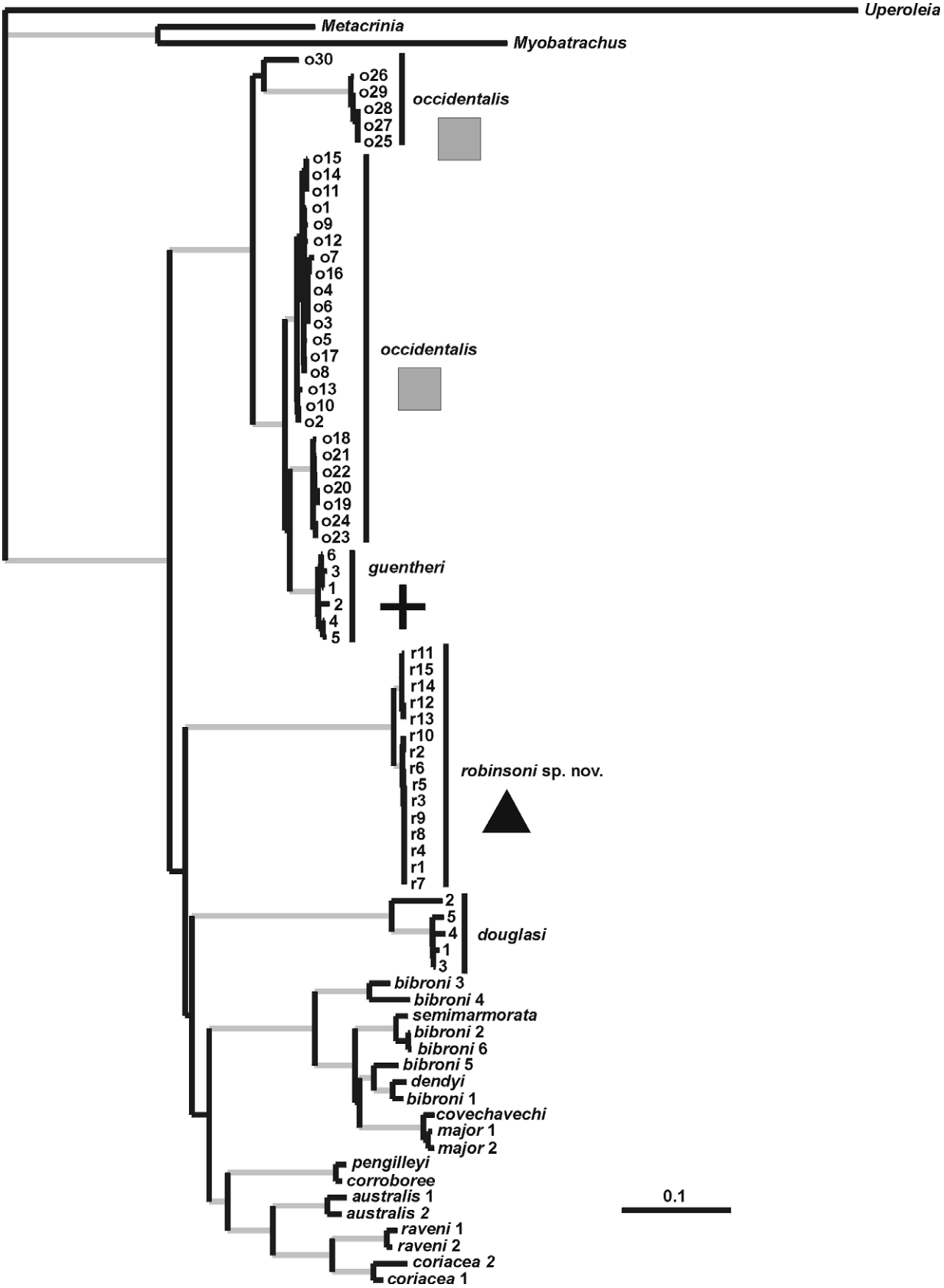

FIGURE 2. Maximum likelihood inference phylogram of evolutionary relationships of *Pseudophryne* mitochondrial *ND4* sequences. Branches in gray indicate that Bayesian posterior probabilities were ≥0.95 and ML bootstrap proportions were ≥70%. Symbols and numbers refer to locations and taxa on maps (Figs 1, 7) and Table 1.

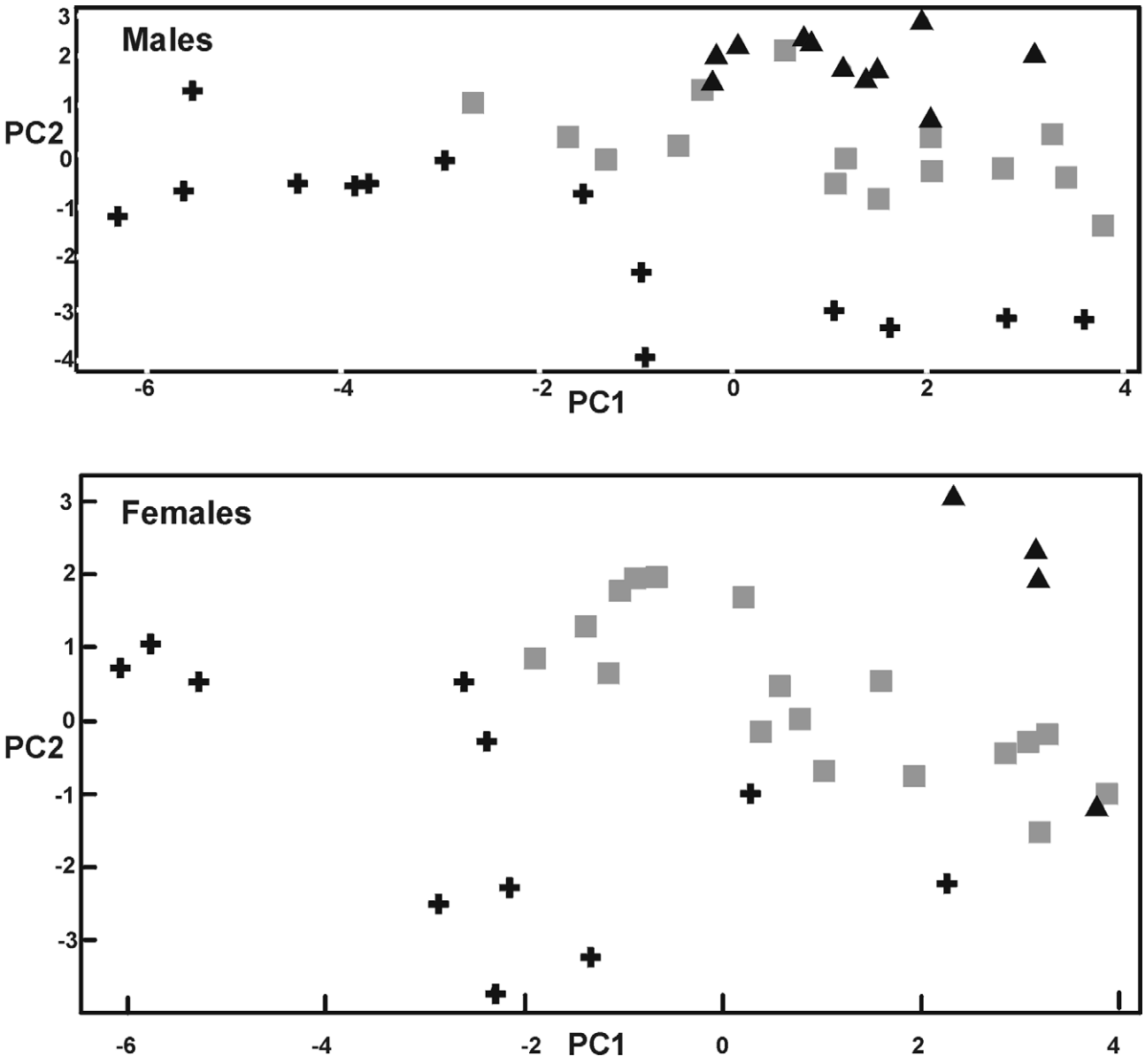

FIGURE 3. PCA plots of the first two PCs for males and females. *Pseudophryne guentheri* = cross, *P. occidentalis* = gray square, *P. robinsoni* **sp. nov.** = black triangle.

Systematics

Pseudophryne Fitzinger, L.J. 1843. Systema Reptilium. Vienna: Braümüller u. Seidel vi 106 pp.

Type species: *Phryniscus australis* Duméril & Bibron, 1841 by original designation (as *Phrynisc. australis* (non Gray); = *Pseudophryne semimarmorata* Lucas 1892, see Parker H.W. (1940).

Pseudophryne robinsoni **sp. nov.**

Central Ranges Toadlet
(Figs 4–6)

Holotype. SAMA R58751, an adult female collected from Alalka Creek gorge, 14.1km N Pukatja Homeland, Musgrave Ranges, South Australia (26.164S, 132.113E) on 19 March 2004 by P. Lang and P. Canty.

Other material examined. See Appendix.

Diagnosis. The new species is clearly assignable to *Pseudophryne* based on the molecular genetic data presented above. *Pseudophryne robinsoni* **sp. nov.** differs from all eastern species of *Pseudophryne* by the absence of a post-femoral gland. It further differs from *P. australis*, *P. coriacea*, *P. corroboree*, *P. pengilleyi* and *P. raveni* by the absence of either a complete red crown, or red-brown dorsum or yellow/black stripes; from *P. dendyi* by the absence of yellow on the elbows or urostyle; from *P. semimarmorata* by absence of red to yellow on the ventral surfaces of legs and inguinal and gular regions.

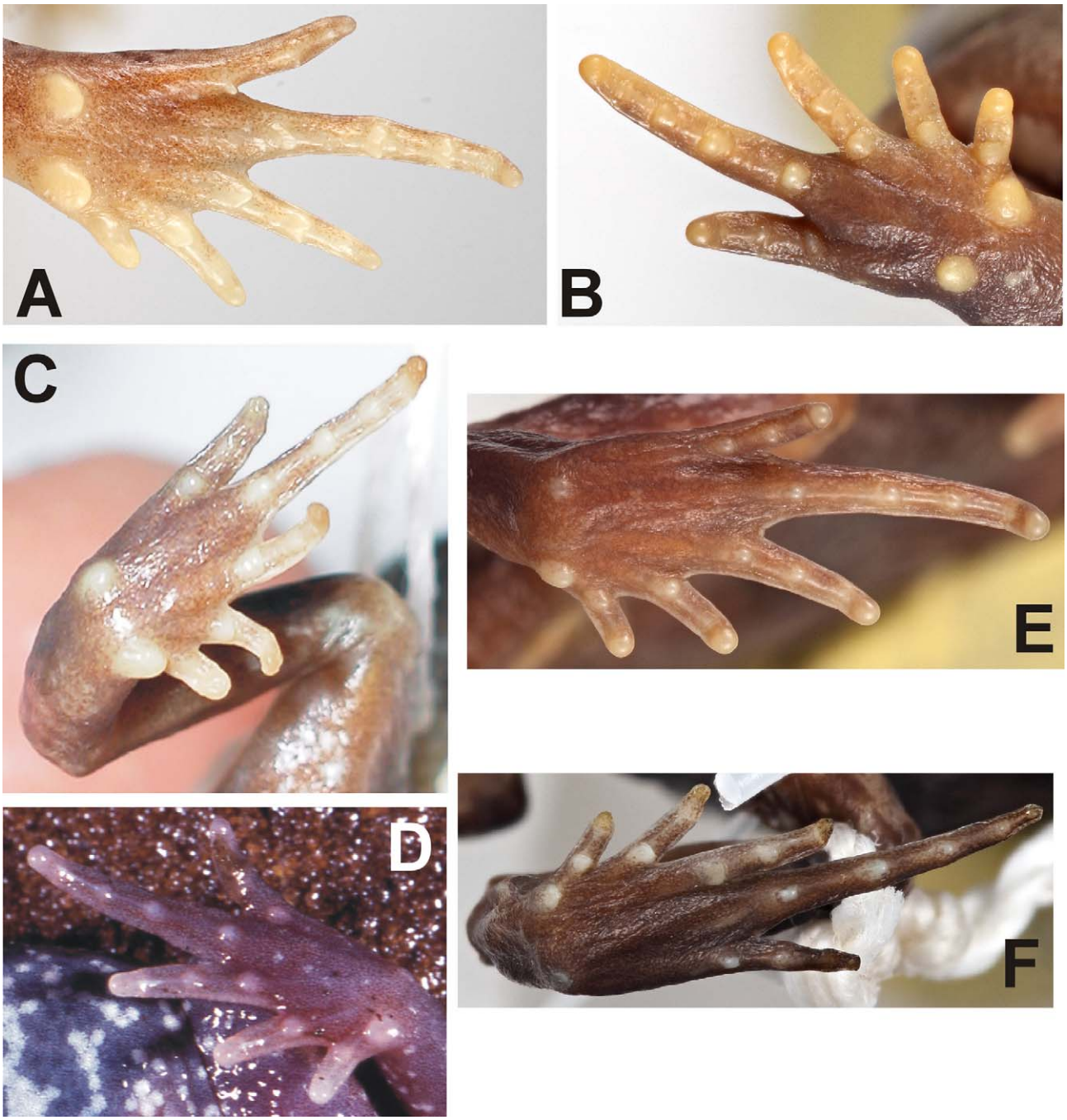

FIGURE 4. Images of plantar view of feet of **A)** *Pseudophryne guentheri* (SAMA R13876) from Moore River, WA; **B)** *P. occidentalis* (SAMA R14399) from 5.6 km SW Jerandilla Tank, Balladonia Station, WA; **C)** *P. occidentalis* (no voucher) from Leonora, WA; **D)** *P. douglasi* no locality data; **E)** *P. robinsoni* **sp. nov.** (SAMA R25101) from 2 km E Victory Well, Everard Ranges, SA; and **F)** *P. robinsoni* **sp. nov.** (SAMA R58751) from 13.9km N Pukatja, Musgrave Ranges, SA; showing variation in size of the metatarsal tubercules.

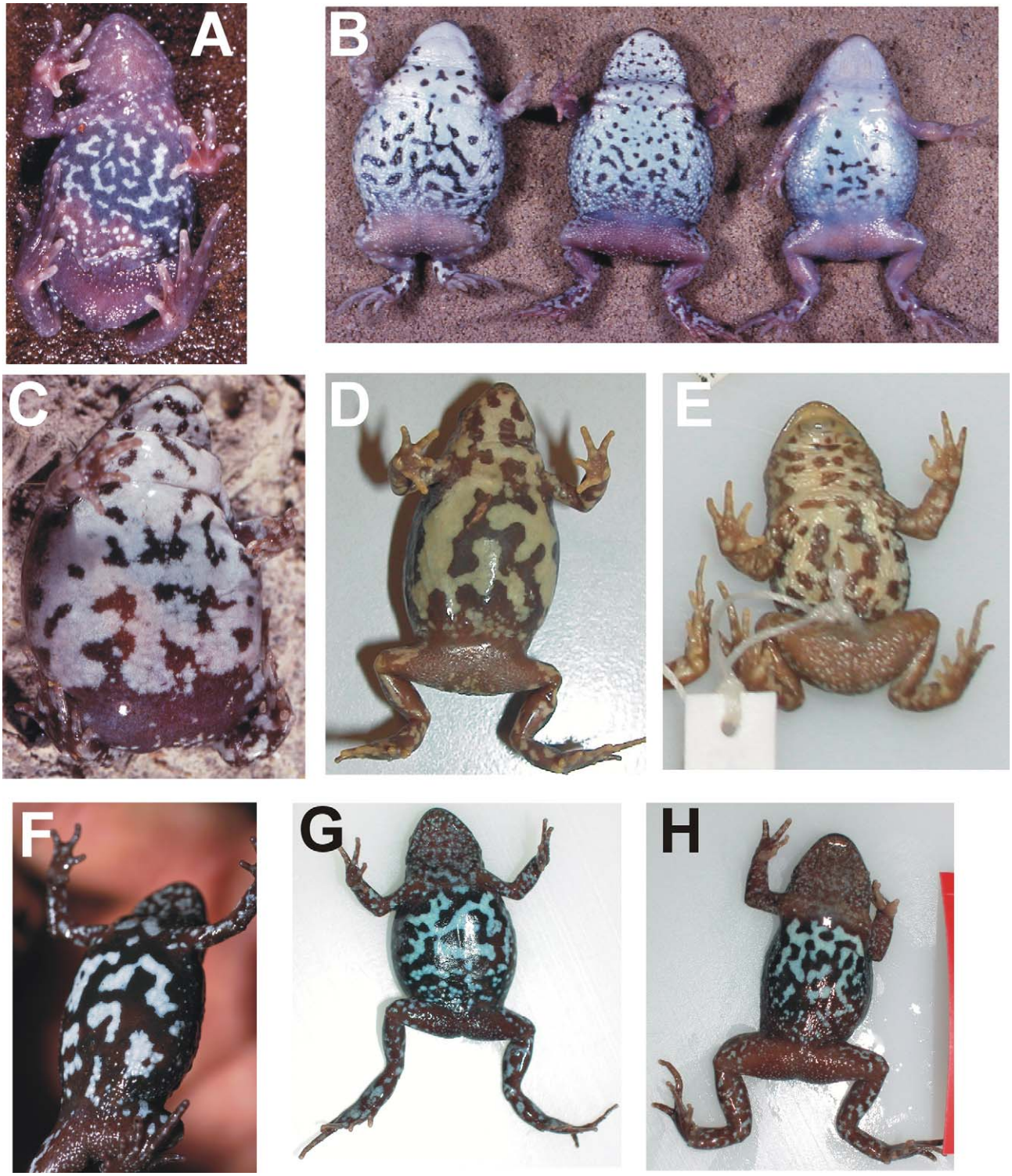

FIGURE 5. Images of ventral patterns of **A)** *P. douglasi* no locality data (in life); **B)** *P. guentheri* York to Quairading Road, WA (in life); **C)** *P. occidentalis* from Paynes Find, WA (in life); **D)** *P. occidentalis* SAMA R14399 from 5.6 km SW Jerandilla Tank, Balladonia Station, WA (preserved); **E)** *P. occidentalis* (no voucher) from Leonora, WA (preserved); and **F)** *P. robinsoni* **sp. nov.** SAMA R54632 from 5.3 km ESE Mount Illbillee, Everard Ranges, SA (in life); **G)** *P. robinsoni* **sp. nov.** SAMA R58751 from Alalka Creek Gorge, Musgrave Ranges, SA (in life); **H)** *P. robinsoni* **sp. nov.** SAMA R59119 from Alalka Creek Gorge, Musgrave Ranges, SA (in life).

Pseudophryne robinsoni **sp. nov.** differs from the western species of *Pseudophryne* in the size of the metatarsal tubercules and ventral patterns. In *P. guentheri* the inner and outer metatarsal tubercules are equal in size and large, in *P. occidentalis* both tubercules are noticeably smaller but still equal in size and in *P. douglasi* the outer tubercule

is either absent or small and the inner tubercule is large (Main 1964), while in *P. robinsoni* **sp. nov.** both tubercules are very small and usually unequal in size. The ventral pattern in *P. guentheri* comprises small black marks on a white background, in *P. occidentalis* it comprises large black marks on a white background, in *P. douglasi* it comprises slaty grey or fine black patterning on a white background, while in *P. robinsoni* **sp. nov.** it comprises large white marks on a black background.

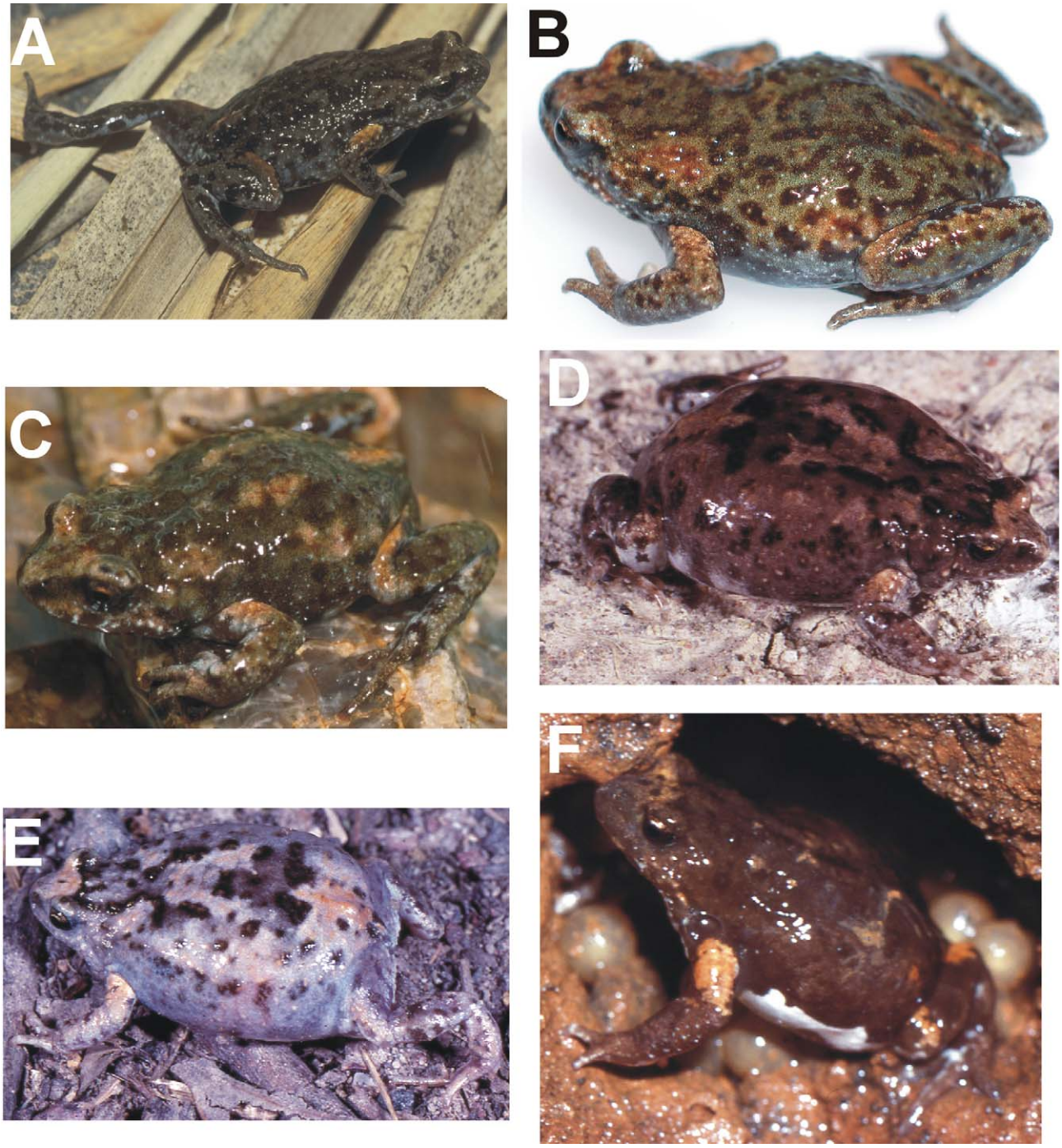

FIGURE 6. Images in life of *P. robinsoni* **sp. nov.** A) Holotype (SAMA R58751) from the gorge of Alalka Creek, eastern side of Musgrave Ranges; B) SAMA R59119 from the gorge of Alalka Creek; C) from 5.3km ESE Mt Illbillee, Everard Ranges; and *P. occidentalis* D,E) from Paynes Find, WA; F) from 93 km W Balladonia, WA. Photo R. Knowles.

Description of holotype. Head longer than broad, its length ~1/3 SV. The snout slightly prominent, rounded when viewed from above and in profile. The nares located at the dorso-lateral margin of the snout, their distance from the end of the snout less than that from the eye. EN approximately equivalent to IN (EN/IN = 1.13). The canthus rostralis defined poorly and straight. The eye moderately sized and prominent, its diameter approximately

equivalent to EN. The tympanum absent. Vomerine teeth absent. The tongue elongate, its diameter approximately one third of the gape of the mouth.

Fingers short and cylindrical, lacking lateral fringes and interdigital webbing; in decreasing order of length 3 > 4 = 2 > 1. The subarticular and palmar tubercules large and prominent. Hindlegs short—TL 35% of SV. Toes short, slightly compressed and lack lateral fringes; length 4 > 3 > 5 > 2 > 1. Subarticular tubercules moderately large and prominent. Interdigital webbing absent. Inner metatarsal tubercle small and rounded, outer tubercle small, rounded and poorly developed.

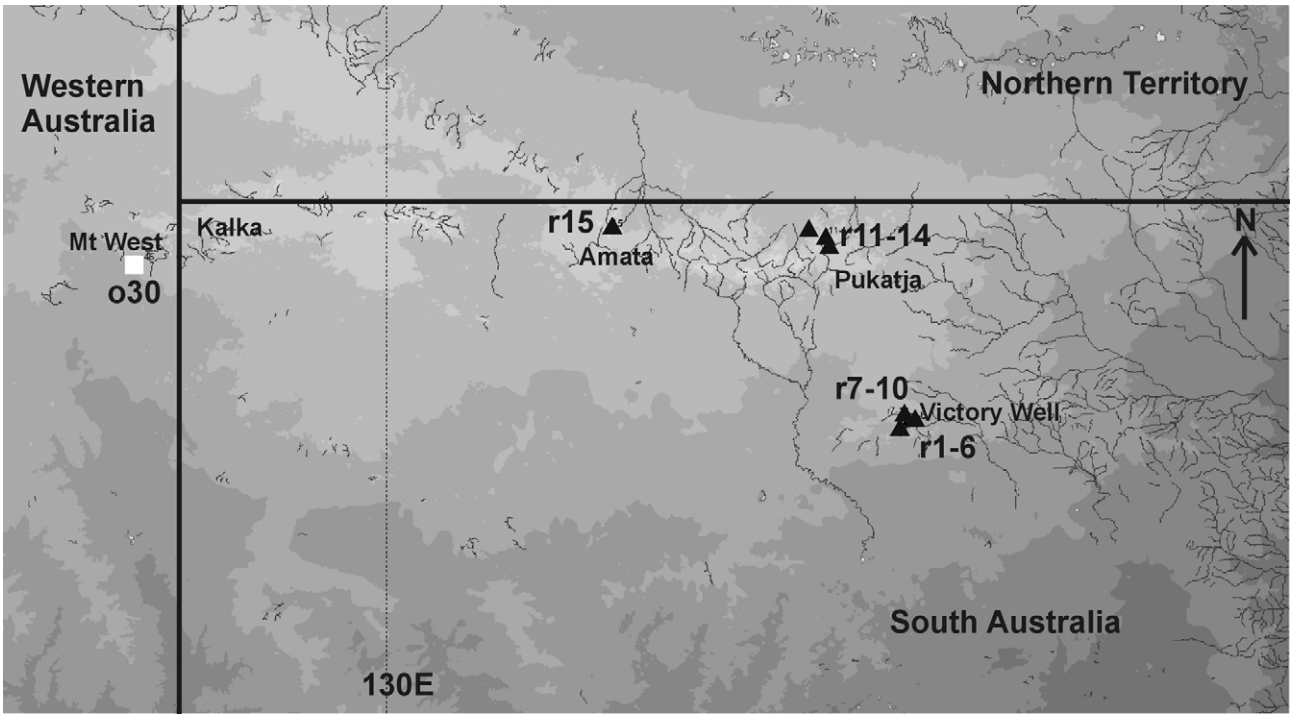

FIGURE 7. The distribution of *Pseudophryne* in central Australia. Gray tone background represents a digital elevation model with higher elevations represented by lighter tones; drainages are also indicated. Thick lines are state borders. White square = *P. occidentalis*, black triangle = *P. robinsoni* **sp. nov.**

Dorsal surface tubercular with some longitudinal ridges discernable. Supratympanic fold absent. Ventral surfaces smooth except for the under surface of thighs and groin, which are coarsely granular. Post-femoral glands absent.

Dimensions of holotype (in mm). SV—25.2, HW—8.7, EN—1.8, EY—1.9, IN—1.6, IO—3.1, HAND—5.5, F1—1.5, F3—3.4, ARM—5.5, TL—9, FOOT—11, T2—2.2, T3—3.5, IM—0.5, OM—0.6.

Color. Color in life of *P. robinsoni* **sp. nov.** is shown in Fig. 6 alongside images of *P. occidentalis* for comparison. Light brown crown patch of low contrast with darker patterning on dorsum. Dorsum marked with dark, tending to black, irregular short dark lines or patches on khaki to light brown background. Very narrow orange-brown urosytle stripe present, about 10% of SV in length. Orange-brown patch on upper surface of upper arm just reaching elbow and along full length of upper surface of thigh. Abdomen mottled with large white irregular patches on black background (Fig. 5). Pattern on gular region very fine white spots on dark brown and black background. Groin area with fine white spots on dark brown background, ventral surfaces of legs and arms similar to abdominal pattern but on dark brown background.

Variation. Variation in 15 morphometric traits is summarised in Table 2. Pseudophryne robinsoni **sp. nov.** appears to be smaller than P. guentheri and P. occidentalis as evidenced by the contribution of SV to PC1 in separating the three taxa in the bivariate PC plots.

Variation in dorsal pattern is shown in Fig. 6. Dorsum variably marked with dark, tending to black, irregular short dark lines or patches on khaki to light brown background. Light colored low contrast crown patch variably present. Width of orange-brown urosytle stripe varies but length is typically about 10% of SV in length. Orange-brown patch on upper arm and thigh variably present. Variation in the shape and extent of white abdominal marks is shown in Fig. 5. Pattern on gular region either a continuation of abdominal pattern or very fine white spots on black background. Groin area plain dark brown or with fine white spots on dark brown background, ventral surfaces of legs and arms either with a continuation of the abdominal pattern or fine white spots on dark brown background.

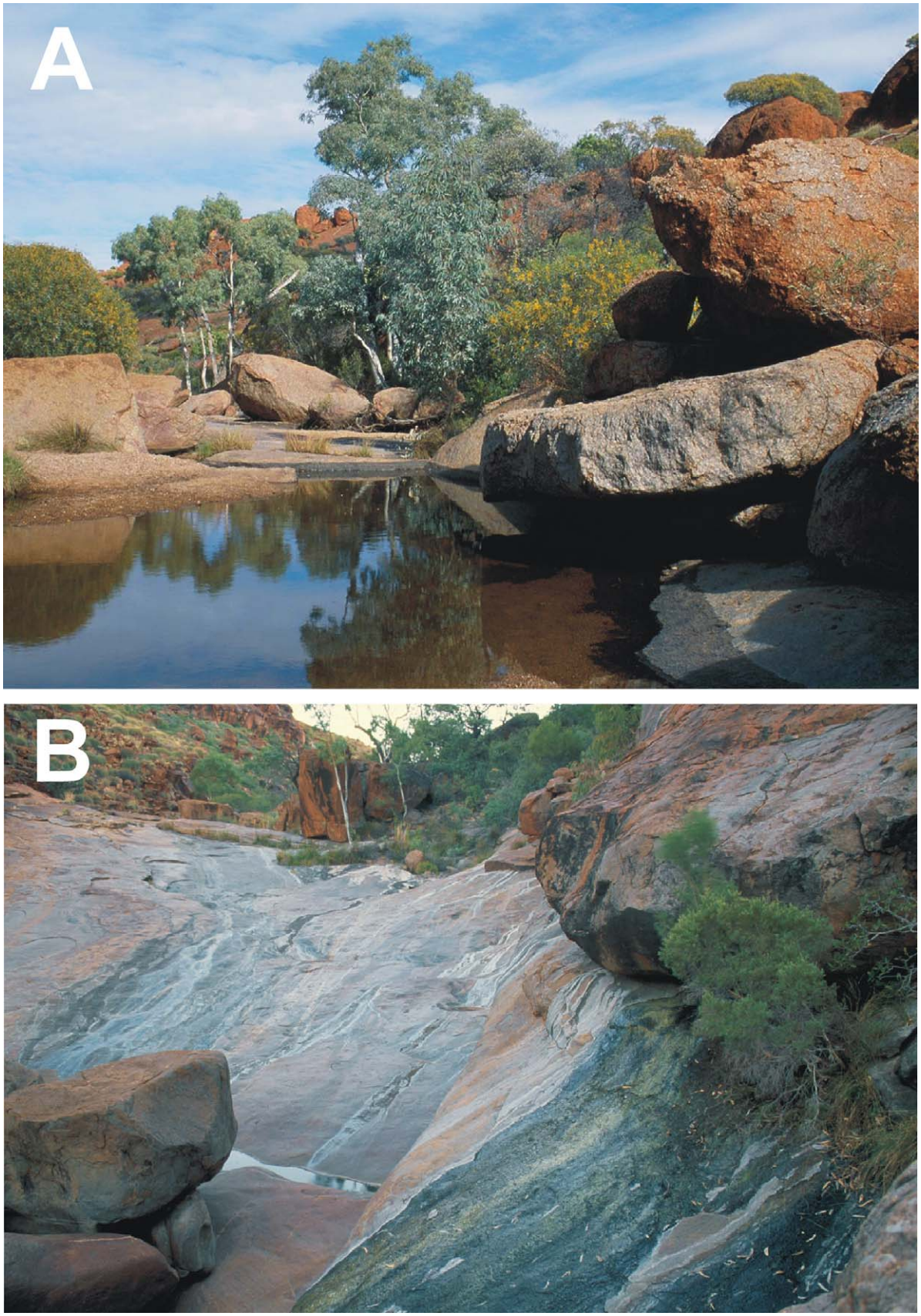

FIGURE 8. Habitat of *Pseudophryne robinsoni* **sp. nov**. **A)** The main waterhole at Victory Well Creek, Everard Ranges in May 2000 when specimens were collected in water under loose rocks. Photo P. Lang. **B)** Gorge of Alalka Creek, 13.8 km N Pukatja, eastern side of Musgrave Ranges. A specimen was captured in damp mud and litter under the *Cyperus vaginatus* sedge growing at the base of the *Melaleuca* on the right. Photo P. Canty.

Reproductive Biology. The male advertisement call is not known. Testes are darkly pigmented.

Etymology. Named for Dr Tony Robinson, formerly of the South Australian Department for the Environment and Natural Resources who was chiefly responsible for the initiation and sustained management of a state wide biodiversity survey conducted over 25 years from the early 1980's.

Distribution, habitat and status. Known from the Musgrave Ranges and a limited number of locations close to Mt Illbillee in the Everard Ranges of north-western South Australia (Fig. 7).

During the SA Department for the Environment and Natural Resources' Biological Survey in May 2000, *Pseudophryne* were collected in water under loose rocks in the main waterhole at Victory Well Creek, Everard Ranges. In the eastern Musgrave Ranges in March 2004, frogs were collected around spring-fed pools on damp soil under either collapsed sedge (*Cyperus vaginatus*) growing in rock crevices below the spring seepage or dead bulrush (*Typha domingensis*) stems below stands of dense bulrushes growing on the margin of the pool (Fig. 8). In the western Musgrave Ranges, at the 7.1 km WNW of Amata locality, *Pseudophryne* were calling from horizontal crevices just above the water line (John Read pers. obs.).

The Musgrave Ranges population is represented by six specimens collected between 1973 and 2009 in the months of March, June and September, while specimens from the Everard Ranges have been collected over a 30 year period from 1970 to 2000 in the months of May, September, October and November. Robinson *et al*. (2003) found the species to be common in rock holes in the Everard Ranges area during the Anangu Pitjantjatjara lands biological survey though 1991–2001. They recommended a broader survey of suitable habitat through the region to establish the range of the species, as the surveys had not targeted the rocky ranges for logistical reasons.

Conservation status. We recommend that *P. robinsoni* **sp. nov.** be considered in the data deficient category since much of the north-western range country of South Australia and eastern central western Australia remains unsurveyed for amphibians. Potential threats to highly fragmented and restricted populations in the region include damage to riparian habitats by introduced species such as camels and donkeys and draw down of ground water impacting on spring water persistence by mining operations.

Acknowledgments

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APPENDIX 1. Specimens examined morphologically

Collection	Registration Number	Locality	Latitude (dec)	Longitude (dec)
P. guentheri				
WAM	R141999	17 km NNE Tammin	–31.5008	117.5478
WAM	R141934	2km W Nembudding	–31.1969	117.5475
WAM	R156764	Bandalup Hill	–33.5886	120.3300
WAM	R134359, R136542/5	near Beacon	–30.6333	118.4833
WAM	R137478–9, R141000	Eneabba	–29.8169	115.2669
WAM	R134391/9, R136549	Jouerdine Nature Reserve	–30.6333	118.4167
WAM	R141908–10	Manmanning	–30.8538	117.0967
WAM	R134366/8	Mollerin Lake	–30.5466	117.5500
WAM	R136476–7	Stirling Range NP	–34.8500	118.4167
WAM	R141769	Yorkrakine Rock Nature Reserve	–31.4180	117.5156
WAM	R134885	Buntine Nature Reserve	–29.9666	116.5667
WAM	R144003	East Nugadong Nature Reserve	–30.2000	116.8919
WAM	R141775	Jerdacuttup	–33.6000	120.1833
WAM	R135107	Mundijong	–32.3000	115.9833
WAM	R141711	Waddi Farms, near Badgingarra	–30.5100	115.5031
WAM	R141799	Ledge Point	–35.0166	118.0000
P. occidentalis				
WAM	R158303–5	44 km SSE Wooramel Roadhouse	–26.1666	114.3333
WAM	R126996–7	8 km WNW Maggie Hayes Hill	–32.2500	120.5000
WAM	R132613/5/7/9	39 km E Perenjori	–29.7022	116.3717
WAM	R135216	Dundas Rock	–32.3666	121.7500
WAM	R157848/52/54/56	Eyre Highway	–32.4675	123.1681
WAM	R132638, R132640–2	Koolanooka Spring	–29.1869	116.6867
WAM	R115906/8	Lake Cronin	–32.3833	119.7500
WAM	R157801	Lochada Station	–29.1075	116.5478
WAM	R141258	near Ora Banda	–30.3666	121.0667
WAM	R157800	Boiada Hill	–29.1886	116.5164
WAM	R136405	near Norseman	–32.2000	121.7833
WAM	R165720	Ora Banda	–30.3666	121.0500
WAM	R113780–1, R113787	W Coolgardie	–30.9500	121.1333
WAM	R120079, R120081–2	Wurala Homestead	–28.4166	116.2833
WAM	R113435–6, R113544	Yellowdine	–31.2917	119.6501

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APPENDIX 1. (Continued)

Collection	Registration Number	Locality	Latitude (dec)	Longitude (dec)
P. robinsoni **sp. nov.**				
SAMA	R11738–9, R11741–2, R38413–4, R38416–8	Victory Well, Everard Ranges	–27.06	132.5166667
SAMA	R25101	2 km E Victory Well, Everard Ranges	–27.06	132.5333333
SAMA	R13276B/D	1.5 miles SE Mount Illbillee, Everard Ranges	–27.08	133.4833333
SAMA	R54630, R54632–3	5.3 km ESE Mount Illbillee, Everard Ranges	–27.05066667	132.5052778
SAMA	R58751	13.9km N Pukatja, Musgrave Ranges	–26.1606	132.1186
SAMA	R59119	Alalka Creek Gorge, Musgrave Ranges	–26.19805556	132.1263889
SAMA	R64657	7.1 km WNW Amata, Musgrave Ranges	–26.125	131.0803

APPENDIX 2. Short *ND4* sequences

WAMR151412
TAATTCTCACCTGCTGATTAACGCCTTTAACACTTCTAGCAAGCCAAAGTAAAATCTCTAAAGAACCA
GTATCGCGACAACGCTCCTATATCTTCTCCATTCTATTTCTACAAATAACTACTATCCTTGCCTTCCT
WAMR115171
TAATCCTCACCTGCTGATTAACGCCTCTAACACTTCTAGSAAGCCAAAGTAAAATTTCTAAAGAACCA
ATATCACGGCAACGCTCCTATATCTTCTCTATTCTATTTCTACAAATAACTACTGTCCKTGCTTTTCT