Extended descriptions of Geocrinia vitellina and Geocrinia alba (Anura: Myobatrachidae) from south-western Australia, with comments on the status of G. lutea

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Abstract

Both *Geocrinia vitellina* and *G. alba* have direct development of the eggs. Electrophoretic studies indicate that they are genetically distinct. The species differ in ventral colour and male call, and have restricted ranges in the Margaret River to Augusta region. They are related to *G. rosea* and *G. lutea* but are distinguished by differences in colour and male call and there is significant genetic differentiation. The status of *G. lutea* (Main) as a genuine species is confirmed: this species differs consistently from *G. rosea* in ventral colour and is genetically distinct.

Introduction

Geocrinia (Blake, 1973) was erected for smooth-bellied species of frogs previously included in Crinia. The species referred to Geocrinia shared a smooth ventral skin, terrestrial egg deposition and direct development or partial terrestrial egg development followed by an aquatic tadpole as well as a number of osteological features. Three species from south-western Western Australia were included in Geocrinia: G. leai (Fletcher, 1898), G. rosea (Harrison, 1927) and G. lutea (Main, 1963). Tyler et al. (1984) treated G. lutea as a synonym of G. rosea.

Two new species of *Geocrinia* from two sets of geographically isolated populations in south-west W.A. were described by two of us (W-J. and R.) in 1989 in a short note drawing attention to their urgent need for conservation. Here we give detailed taxonomic descriptions of them and also comment on and resurrect *G. lutea. G. vitellina* and *G. alba* were based on unspecified syntypes. Here we designate lectotypes and enunciate the former syntypic series. We also specify type localities. In this study we have followed the evolutionary species concept of Simpson (1961) expanded by Wiley (e.g. Wiley, 1981). Under this definition isolated populations are considered species if they are diagnosably distinct, and it is thought likely that they will have separate and divergent evolutionary futures. The latter decision is clearly a value judgement taken in the context of what is known about comparable sympatric species. The biological species concept cannot be applied in this case as neither *G. vitellina* nor *G. alba* is sympatric with closely related forms.

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Geocrinia from south-western Australia

Materials and Methods

Frogs were collected during a broader survey of geographic variation in Geocrinia from W.A. All specimens preserved are deposited in the Western Australian Museum and all register numbers are for the W.A. Museum collection. Call structure analysis was based on techniques described by Mahony & Roberts (1986). Pulse rates were averaged over the whole call for five calls per male. Pulse characteristics and dominant frequencies were averaged from three values for three different calls. Genetic comparisons between taxa were made using electrophoretic techniques described by Barendse (1984); details of enzyme systems etc. surveyed will be given by Barendse, Roberts and Wardell-Johnson (in prep.). Genetic differences between taxa are given as Nei distances (D) or Identities (I, Nei 1978) and % fixed differences (Richardson et al. 1986). We measured 11 morphometric variables: naris-snout (NS, anterior margin of naris to line projecting laterally from tip of snout measured with head viewed perpendicularly from side), snout overhang (SO/H, tip of snout to distal end of mandible), internarial span (between inner margins of nares), eye-naris (EN, anterior corner of eye to posterior margin of naris), eye length (EL, anterior to posterior corner of eye), interorbital distance (ID, between anterior corners of eyes) (all measured with a calibrated micrometer eyepiece in a binocular microscope), head length (HL, diagonal measure from tip of snout to posterior end of quadratojugal), head width (HW, between tips of quadratojugals), tibia length (T, measured with leg flexed), leg length (L, cloaca to tip of longest toe) and snout-vent length (SV, all measured with dial calipers). Except for the lectotypes, measurements were done on live animals to avoid distortion caused by preservation (Lee 1982). In addition we noted details of colours in life and pattern features on the dorsal and ventral surfaces.

Systematics

Geocrinia vitellina Wardell-Johnson and Roberts, 1989

Figures 1, 2 and 3

Syntypes

Twelve males, R86472-76, 2 Dec. 1982, G. Wardell-Johnson & J.D. Roberts; R86477-82, 16 Dec. 1982, G. Wardell-Johnson & J.D. Roberts; R94467, 15 Nov. 1985, G. Wardell-Johnson and J.D. Roberts; and one female, R86483, 13 Nov. 1982, G. Wardell-Johnson: all collected at the intersection of Spearwood Creek and Denny Road, 20.4 km ESE of Witchcliffe, W.A. (115°19'E, 34°4'S).

Selection of lectotype

Specimen R94467 is here selected to be the lectotype; the remainder become paralectotypes.

Diagnosis

Small frog (adult males 21.0-24.3 mm, female 18.0 mm SV), light brown to grey, mating call a series of 9-15 pulses, ventral surface bright yellow. Distinguished from related species by its call and ventral colouration.

Description of lectotype

Head slopes from eyes to snout in profile. Snout rounded and overhanging lower jaw slightly. Canthus rostralis slightly concave, loreal region flattened and sloping laterally

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from a line joining eye to naris. Nostrils directed laterally and forwards. Head slightly longer than broad (HL/HW 1.18). Eyes small, not prominent (EL 2.3 mm, EL/HL 0.24). Vomerine teeth in short, medially-separated series behind choanae, not extending laterally beyond inner edge of the choanae. Maxillary teeth present. Tongue narrow, an elongate ellipse. Nostril about equidistant between eye and snout (EN/NS 1.1). Fingers short, 3>4>2>1. Toes short and unwebbed, 4>3>5>2>1. Fingers and toes approximately circular in transverse section. Limbs short and muscular (T/SV 0.41). Ventral skin smooth. Dorsal colour in preservative light brown with darker spots that almost form flattened warts. Dorsal surface has widely-spaced pores, generally opening in the centre of a raised darker spot. Brown spots are aligned as two parallel rows of larger spots extending from eyes to cloaca. An irregular trans-orbital bar is formed by amalgamating spots. Between the major rows of spots along the back there is an indistinct mid-dorsal row of smaller spots and the entire dorsal surface is covered in finer, irregularly spaced brown spots. Loreal region dark brown with distinct edge line

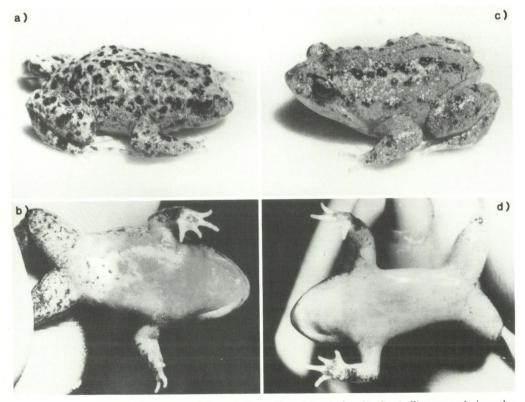


Figure 1 Lectotypes photographed live. a) G. vitellina, lateral view b) G. vitellina ventral view, the darker area extending from the mandibular margin to about half-way between the arms and legs is coloured yellow-orange in life c) G. alba lateral view d) G. alba ventral view, generally white without the yellow-orange area in G. vitellina.

extending from snout through nostril to anterior corner of eye. Indistinct series of spots extends from posterior corner of eye as a row along the flank. Dorsal surface of arms and legs covered in irregularly-spaced, fine and larger brown spots. Ventral surface appears clear white in preservative flecked with very fine darker spots on posterior one-third of venter between insertion of arms and the legs. Area of darker pigment, edged by lighter line, surrounds cloaca and extends to legs for third of distance to knee. When viewed posteriorly legs are marked by a broad-based triangle, with the vertex on the cloaca. Plantar surfaces dark brown but toes white. Ventral surface of legs bears very fine brown spots. Colour of dorsal surface in life was similar but light brown changed to grey if kept on light background colours. Ventral surface brilliant orange-yellow (the colour of egg yolk) from mandible to about two thirds of way back from insertion of arms to cloaca.

Measurements of lectotype (mm): SV 21.0, HW 8.3, HL 9.8, IN 1.8, NS 1.2, SO/H 0.5, EN 1.3, EL 2.3, ID 3.9, T 8.6, L 27.9.

Variation

Paralectotypes agree generally with lectotype. In some, dorsal rows of spots are clearer than in lectotype; in others they are more obscure. In six specimens triangle of darker pigment surrounds cloaca and extends down onto legs, as in lectotype, but in remainder, back of femur is uniformly dark. Extent of yellow on venter varies but always extends beyond insertion of arms. In some specimens area of yellow broken up by fine white flecks along posterior margin. Background colour of dorsal surface of female paralectotype light grey to almost blue on flank, rather than light brown.

Mating Call

Calls were recorded from 15 males on three dates: 2 & 16.12.1982 and 6.11.1984. Eight of these were collected and are paralectotypes (R86472-79). Call consists of a discrete

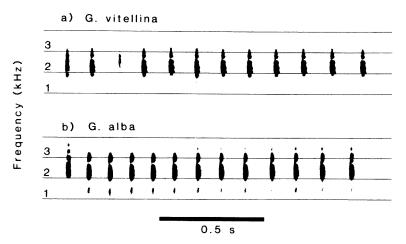


Figure 2 Sound spectrogrammes for a) G. vitellina (soil temperature 14.7°C) b) G. alba (soil temperature 15.4°C.) Both were made with a 45 Hz bandpass filter.

train of 9-15 pulses with pulses repeated just slowly enough to resolve by ear. Dominant frequency is low. Call is repeated irregularly. Details of call structure are given in Table 1 and a representative sound spectrogram is given in Figure 2.

Table 1 Call structure analyses for G, vitellina and G. alba.
(a) temperature-independent variables, values given are mean ± SE
(b) regression equations relating pulse rate and soil temperature. SEb is the standard error of the slope.

Species	Pulse number	Dominant frequency (kHz)	Pulse duration (ms)	Sample size
vitellina	11.1 ± .46	2.152 ± .04	7.00 ± .41	15
alba	15.1 ± .49	$2.430 \pm .04$	5.20 ± .15	19*
(b) Regres	ssions of pulse rate on soil	temperature.		
vitellina	Pulses.s ⁻¹ = .6609Temp. -2.4793 , SEb = .193			
alba	$Pulses.s^{-1} = .5992Temp 0.1913, SEb = .116$			

All mean comparisons are significantly different, $F_{1/33} = 33.5$, $F_{1+32} = 24.5$, $F_{1+32} = 20.4$ for pulse number, dominant frequency and pulse duration respectively: all, p < .001. Both regressions are significant: *alba*, $t_{18} = 5.2$, p < .001, temp. range $13.9^{\circ} - 17.5^{\circ}$ C; *vitellina*, $t_{13} = 3.4$, p < .01, temp. range $14.4^{\circ} - 18.0^{\circ}$ C. Slopes of regression lines do not differ significantly, $t_{31} = 0.023$, n.s., but elevations are significantly different, $F_{1/31} = 34.7$, p < .001.

*n = 20 for pulse number and regression analyses for alba.

Distribution

This species is known only from the section of Spearwood Creek extending from Denny Road to the Blackwood River, and from small seepages on the northern bank of the Blackwood River to the west of the mouth of Spearwood Creek (Figure 3). It is most abundant in seepages on the eastern side of Spearwood Creek and is rare over the flat bottom of the creek valley. It has not been heard at any of 45 sites on creeks visited within a 10 km radius of the type locality. The creeks where *G. vitellina* was not found vary from rocky to wide flat-bottomed creeks similar to Spearwood Creek. However, Spearwood Creek is in an area of major topographic relief. Other flat-bottomed creeks are at sites where topographic relief is poor. The occurrence at Spearwood Creek may reflect very localised suitable conditions.

Breeding Biology

Males call from small depressions in clay under dense vegetation cover. Egg masses often were found close to calling males, and are typical of frogs of the *G. rosea* group: i.e. deposited in small depressions, eggs hatch and the tadpoles develop in a jelly mass with no free swimming or feeding stage. Metamorphlings, reared in the laboratory from three clutches of eggs, have been preserved (R86484-502).

Comparison with other species

G. vitellina differs from G. rosea and G. lutea in lacking black colouration on the sub-mandibular skin in males, and in other aspects of ventral and dorsal colouration and

pattern: rows of spots do not occur on the dorsal surface of either G. rosea or G. lutea. The ventral surface of G. rosea varies in colour, but always bears a trace of a luminous pink pigment (Harrison 1927, Wardell-Johnson & Roberts, in prep.). The ventral surface of G. lutea is light yellow to fawn with an irregular wash of rusty-orange (Main, 1963; Wardell-Johnson & Roberts, in prep.). There is also a difference in call: calling is a continuous "tk... tk..." in G. rosea and G. lutea (Main, 1963, 1965). G. vitellina differs from G. leai in ventral colouration, dorsal pattern and male call: calls of G. leai comprise an introductory and repeated, pulsed note (J.D.R., unpublished data) and in breeding biology; eggs of G. leai are deposited close to water, hatch and then the tadpole drops into the water (Main, 1965). G. vitellina is distinguished from G. alba (see below) by ventral colour and details of male call (Table 1). G. vitellina is distinguished from Crinia species, that it might be confused with, by the smooth ventral skin (granular in Crinia).

Etymology

From the latin, vitellinus, egg-yolk yellow, with reference to the distinctive ventral colouration.

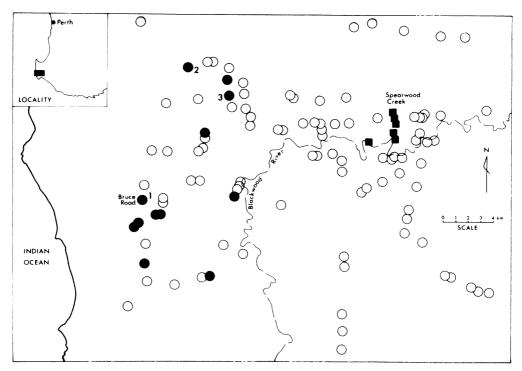


Figure 3 Distribution of *G. vitellina* (solid squares) and *G. alba* (solid circles). The type localities are named. For *G. alba* sites numbered 2 and 3 are sites where paratypes were collected, other sites are localities where only calls have been heard. Open circles are sites where neither species has been heard calling on nights when males were calling at other sites.

Geocrinia alba Wardell-Johnson and Roberts, 1989. Figures 1, 2 and 3

Syntypes

Fourteen males, R94457, R95955, R95957-62, R95964-69 and a single female R95956, 4 Nov. 1984, J.D. Roberts & D. Hebbert, all collected on Bruce Road, 11.5 km S of Witchcliffe, W.A. (115°10'E 34°3'S), 1 male, R94466, 15 Nov. 1985 by G. Wardell-Johnson & J.D. Roberts, same locality. 2 males R95971-72, 7 Nov. 1984, Brooks Road, 7.3 km ESE of Witchcliffe, J.D. Roberts & D. Hebbert; 1 male, 95970, Davis Road, 3.5 km ESE of Witchcliffe, 7 Nov. 1984, J.D. Roberts & D. Hebbert.

Selection of lectotype

Specimen R94466 is here selected to be lectotype; the remainder become paralectotypes.

Diagnosis

Small (adult males 20.1-24.2 mm, female 16.6 mm SV), light brown to grey, mating call a series of 11-18 pulses, ventral surface white or with a very faint yellow wash in both sexes. Distinguished from related species by its call and ventral colouration.

Description of lectotype

Head slopes from eyes to snout in profile. Snout rounded, projecting slightly and marginally overhanging lower jaw. Canthus rostralis slightly concave, loreal region flattened and sloping laterally. Nostrils directed laterally and forwards. Head slightly broader than long (HL/HW 0.96). Eyes small, not prominent (EL 2.4 mm, EL/HL 0.30). Vomerine teeth in short, medially-separated series behind choanae, do not extend laterally beyond inner edge of choanae. Maxillary teeth present. Tongue narrow, an elongate ellipse. Nostril closer to eye than to snout (EN/NS 0.72). Fingers short, 3>4>2>1. Toes short, unwebbed, 4>3>5>2>1. Fingers and toes approximately circular in transverse section. Limbs short and muscular (T/SV 0.40). A small, flattened inner metatarsal tubercle, barely obvious. No sub-articular tubercles. Ventral skin smooth. Dorsal surface bears widely-spaced pores, generally opening in centre of a raised darker spot. In preservative, dorsal colour light brown. Two rows of large raised spots extend from eves to cloaca and form a trans-orbital bar. Parallel, lateral and adjacent to the row of large spots, a line of finer spots giving the impression of a double line. A line of fine dark spots almost forming a continuous mid-dorsal stripe extends from the cloaca about two thirds of the distance to the eyes. Loreal region dark with distinct line of spots continuous from eye along flank. A triangular, darker region with vertex on cloaca and extending to back of thigh. Ventral surface white with irregular darker flecks and fine spots on underside of the legs. Plantar surfaces dark brown but toes white. In life, colours similar. A photograph of the lectotype is given in Figure 1. The photograph of G. alba in Wardell-Johnson & Roberts (1989) is of G. lutea not G. alba.

Measurements of lectotype (mm): SV 21.5, HW 8.1, HL 7.8, NS 1.7, SO/ H 0.4, IN 1.7, EN 1.2, EL 2.4, ID 3.4, T 8.7, L 28.9.

Variation

Paralectotypes agree generally with lectotype except in details of colouration. In many specimens ventral surface washed with a very pale, almost indistinct yellow. Edge of submandibular skin often had a grey-brown wash. Dorsal pattern sometimes forms a

series of distinct, well-defined raised, large spots. In all paralectotypes there was an approximately triangular darker region with vertex on cloaca and extending onto back of thigh: usually edged by a fine line of luminous pink characteristic of ventral surface of *G. rosea.* On dorsal side of arm insertions, many frogs had patch of luminous pink. In specimen from Davis Road (R95970) dorsal pattern a clearly defined series of dark brown stripes (replacing spots seen in frogs from Bruce Road) overlain and edged by pink or yellow-green markings. Two specimens from Brooks Road (R95971-2) approached the form of dorsal pattern described for the Davis Road specimen.

Mating Call

Call consists of a discrete train of 11-18 pulses repeated rapidly — almost too fast to resolve accurately by ear. Dominant frequency is low. Call is repeated irregularly. Details of call structure are given in Table 1 and a representative sound spectrogram is given in Figure 2.

Distribution

Specimens have only been collected at three localities (Figure 3). However, calls have been heard at another nine localities in the Witchcliffe-Karridale area and we presume that these 12 sites define the range of this species. The range is larger than for *G. vitellina* but is still very restricted. *G. alba* does not occur in all creek systems in this area (Figure 3).

Breeding Biology

Males call from small depressions in clay under dense vegetation cover. Egg masses were often associated with calling males and are typical of frogs of the G. rosea group: i.e. they are deposited in small depressions, eggs hatch and the tadpoles develop in a jelly mass with no free swimming or feeding stage.

Comparison with other species

Differs from G. rosea, G. lutea and G. leai and Crinia species in the ways described above for G. vitellina except for the difference in colour. Differs from G. vitellina by ventral colour (white or white with a faint yellow wash, not white with brilliant yellow) and details of male call (higher pulse repetition rates, higher dominant frequencies, shorter pulse durations and higher pulse numbers, Table 1). Dorsal pattern also distinguishes this species from G. vitellina: the spots along the back are larger in G. alba and there are less fine spots making the dorsal rows of spots more obvious.

Etymology

From the latin albus, white. Named for the ventral colour.

Additional Data

Genetic Differences

G. alba and G. vitellina are genetically distinct. Based on electrophoretic analysis of variation at 12 enzyme and four general protein loci, the Nei D value is 0.194 (I = 0.823) with 12.5% fixed differences. Details of genetic differences will be given by Barendse,

Roberts and Wardell-Johnson (in prep.). These two new species are also very different from *G. rosea* and *G. lutea* with D values of 0.582 or greater and fixed differences greater than or equal to 25%.

Morphological Differentiation: ratios

In our analyses of morphological variation only data from the male paralectotypes were used. We investigated a total of 33 possible ratios of the 11 morphometric variables measured: none of the ratios can reliably discriminate these two new species or discriminate them from *G. lutea* and *G. rosea*. Multivariate analyses (canonical variates, discriminant analysis) of these data sets do not reliably discriminate the four species either: these analyses will be presented in detail by Wardell-Johnson and Roberts (in prep.).

Taxonomic Status of G. lutea

We examined 37 live specimens of G. rosea (R86517-524, The Colonels, Warren River; R86540-41, Power Road, 3 km S of Pemberton; R86542-554, Tramway Trail, 5.5 km NW of Pemberton; and R86458-571, Pine Creek Road, 27 km WSW of Manjimup) and 27 live specimens of G. lutea (R86503-516, Meredith Road, 10 km NW of Walpole; and R86527-39, Angove Road, 4 km NW of Walpole). The colour differences from G. rosea reported by Main (1963) are confirmed. We have recorded male calls and find little evidence of overall call differentiation between G. lutea and G. rosea (Roberts and Wardell-Johnson; in prep.). However, some males of G. lutea call at much higher rates than others. In a sample of calls from five males recorded at Angove Road the range of call repetition rates was 3.57s⁻¹ to 7.57s⁻¹ with a temperature range of only 0.4°C. The lower values are typical of G. rosea. The variation in calling rate is probably a consequence of interactions between males; and therefore variations in chorus density may account for Main's (1963) claim of higher calling rates in G. lutea. These two taxa are also genetically distinct: Nei's D is 0.404 (1 = 0.668) with 12.5% fixed differences based on the same number of electrophoretically determined enzyme and protein phenotypes as discussed above. These levels of differentiation are characteristic of species level differentiation (Thorpe 1982). We regard G. lutea as a valid species. The synonomy with G. rosea suggested by Tyler et al. (1984) is not justified but is understandable given the faded nature of the holotype of G. lutea. A photograph of G. lutea (labelled G. alba) is given in Wardell-Johnson and Roberts (1989).

Discussion

The recognition of G. vitellina and G. alba is justified by the clear-cut differences in ventral colour and the less obvious but significant difference in male call. These characters are also the most useful in species identification as there is little evidence of other morphological differentiation. The recognition of these two new species is further supported by the genetic data which indicate a significant level of divergence between them.

Similar levels of differentiation in colour and genotype exist between G. rosea and G. lutea and there are also incipient call differences between these two species.

The level of differentiation of the two western (*G. alba* and *G. vitellina*) from the two eastern (*G. rosea* and *G. lutea*) isolates is high in colour and call and as measured by electrophoresis. The genetic differences are, however, below the levels characteristic of generic differentiation (Thorpe 1982). Detailed data on the differentiation of these four *Geocrinia* species, and discussion of the implications of these data for speciation processes and biogeography in south-western Australia will be given by Barendse, Roberts and Wardell-Johnson (in prep.: genetic data), Roberts and Wardell-Johnson (in prep.: male call) and Wardell-Johnson and Roberts (in prep.: colour, morphology and distribution).

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References

- Blake, A.J.D. (1973). Taxonomy and relationships of myobatrachine frogs (Leptodactylidae): a numerical approach. Aust. J. Zool. 21: 119-149.
- Barendse, W. (1984). Speciation in the genus *Crinia* (Anura: Myobatrachidae) in southern Australia: a phylogenetic analysis of allozyme data supporting endemic speciation in southwestern Australia. *Evolution* **38**: 1238-1250.
- Barendse, W., Roberts, J.D. and Wardell-Johnson, G. (in prep.) Genetic differentiation between isolated populations of *Geocrinia* (Anura: Myobatrachidae) from southwestern Australia.
- Fletcher, J.J. (1898). Contributions to a more exact knowledge of the geographical distribution of Australian Batrachia. 5. Proc. Linn. Soc. N.S.W. 22: 660-684.
- Harrison, L. (1927). Notes on some Western Australian frogs, with descriptions of two new species. *Rec. Aust. Mus.* 15: 277-287.
- Lee, J.C. (1982). Accuracy and precision in anuran morphometrics: artifacts of preservation. Syst. Zool. 31: 266-281.
- Mahony, M.J. and Roberts, J.D. (1986). Two new species of desert burrowing frogs of the genus Neobatrachus (Anura: Myobatrachidae) from Western Australia. Rec. West. Aust. Mus. 13: 155-170.
- Main, A.R. (1963). A new species of *Crinia* (Anura: Leptodactylidae) from National Park, Nornalup. *West.* Aust. Nat. 8: 143-144.

Main, A.R. (1965). Frogs of Southern Western Australia. W.A. Naturalist's Club, Perth.

- Nei, M. (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89: 583-590.
- Richardson, B.J., Baverstock, P.R. and Adams, M. (1986). Allozyme Electrophoresis. Academic Press, Sydney.
- Roberts, J. D. and Wardell-Johnson, G. (in prep.). Call structure differences between isolated populations of *Geocrinia* (Anura: Myobatrachidae) from south-western Australia.

Simpson, G.G. (1961). Principles of Animal Taxonomy, Columbia University Press, New York.

Thorpe, J.P. (1982). The molecular clock hypothesis: biochemical evolution, genetic differentiation and systematics. Ann. Rev. Ecol. Syst. 13: 139-168.

Tyler, M.J., Smith, L.A. and Johnstone, R.E. (1984). Frogs of Western Australia, W.A. Museum, Perth. Wardell-Johnson, G. and Roberts, J.D. (in prep.). Distribution and morphological variation in frogs of the Geocrinia rosea group from south-western Australia.

Wardell-Johnson, G. and Roberts J.D. (1989) "Endangered" Landscope 5(1): 17. Wiley, E.O. (1981). Phylogenetics, Wiley, New York.