

Direct development in two Myobatrachid Frogs, *Arenophryne rotunda* Tyler and *Myobatrachus gouldii* Gray, from Western Australia

Marion Anstis¹, J. Dale Roberts², and Ronald Altig³

¹26 Wideview Rd., Berowra Heights, NSW 2082, Australia. Email: frogpole@tpg.com.au

²School of Animal Biology (M092), University of Western Australia,
35 Stirling Highway, Crawley, Western Australia 6009 Australia

³Department of Biological Sciences, Mississippi State University, Mississippi State, MS 39762 USA

Abstract – The closely related Western Australian myobatrachid frogs *Arenophryne rotunda* and *Myobatrachus gouldii* deposit eggs in burrows that are dug by the adults in moist sand. Embryonic development requires up to two months and is completed entirely within the jelly capsule. The developmental stages of these two taxa are described and compared with those of the South American direct developing leptodactylid frog *Eleutherodactylus coqui*.

Key words: Australia, direct development, embryo, endotrophic, myobatrachid

INTRODUCTION

The frogs *Arenophryne rotunda* and *Myobatrachus gouldii* (Myobatrachidae) are widely distributed in semi-arid and arid regions of southwestern Australia (Tyler *et al.* 2000). Both species are forwards burrowers that oviposit deep underground in moist sand where embryos undergo direct development, an endotrophic breeding mode in which all embryonic development through to a froglet takes place within the jelly layers of the egg (Altig and Johnson 1989). *Arenophryne rotunda* calls from July–November (austral winter to spring). Pairs of males and gravid females not in amplexus have been found together in November at a mean depth of 45 cm, and in February and April (late summer to autumn) at mean depths of 75–78 cm, but eggs were only found in April (Roberts 1984). *Myobatrachus gouldii* calls from September–February (spring to late summer); a male and female burrow together, not in amplexus, into deep, moist sand where they appear to remain together until autumn when they deposit eggs at depths of 80–115 cm (Roberts 1981, 1984). Tyler's (1976a) suggestion of a close relationship between these two species and with *Metacrinia* was supported by Maxson and Roberts (1985), Read *et al.* (2001) and the recent analysis by Frost *et al.* (2006).

Direct development in amphibians has evolved in at least seventeen genera from nine families of anurans worldwide (Thibaudeau and Altig 1999). Although the life histories of a number of these species have been described, especially for the genus *Eleutherodactylus* (e.g. Gitlin 1944; Jameson

1950; Wake 1978; Townsend and Stewart 1985), there are no available descriptions of the Australian species which include the myobatrachid genera *Arenophryne*, *Myobatrachus* and *Metacrinia* and the microhylid genera *Austrochaperina* and *Cophixalus*.

The South American leptodactylid genus *Eleutherodactylus* consists of several direct developing species and the field staging system developed for *E. coqui* by Townsend and Stewart (1985) is the most comprehensive system available for this breeding mode. We describe some preserved embryonic material in the Western Australian Museum of *A. rotunda* and *M. gouldii* and compare them to *E. coqui* (see Table 3 and Discussion). Brief comparisons to Australian direct developing microhylids and also to species from other Australian endotrophic guilds including the nidicolous, paraviviparous and exoviviparous species are made where relevant. These are not direct developers because they have a hatched tadpole stage (*sensu* Altig and Johnston 1989), but have some similar characteristics to *A. rotunda* and *M. gouldii* in early stages.

MATERIALS AND METHODS

Fifteen embryos of *A. rotunda* from four clutches collected near Shark Bay, WA and reared in the laboratory in April 1981 by J. D. Roberts, were preserved at irregular intervals in Tyler's fixative (Tyler 1962) and transferred to 70% ethanol when accessioned into the West Australian Museum: WAM R97047-50, 97053, 97057, R97059-60 (see

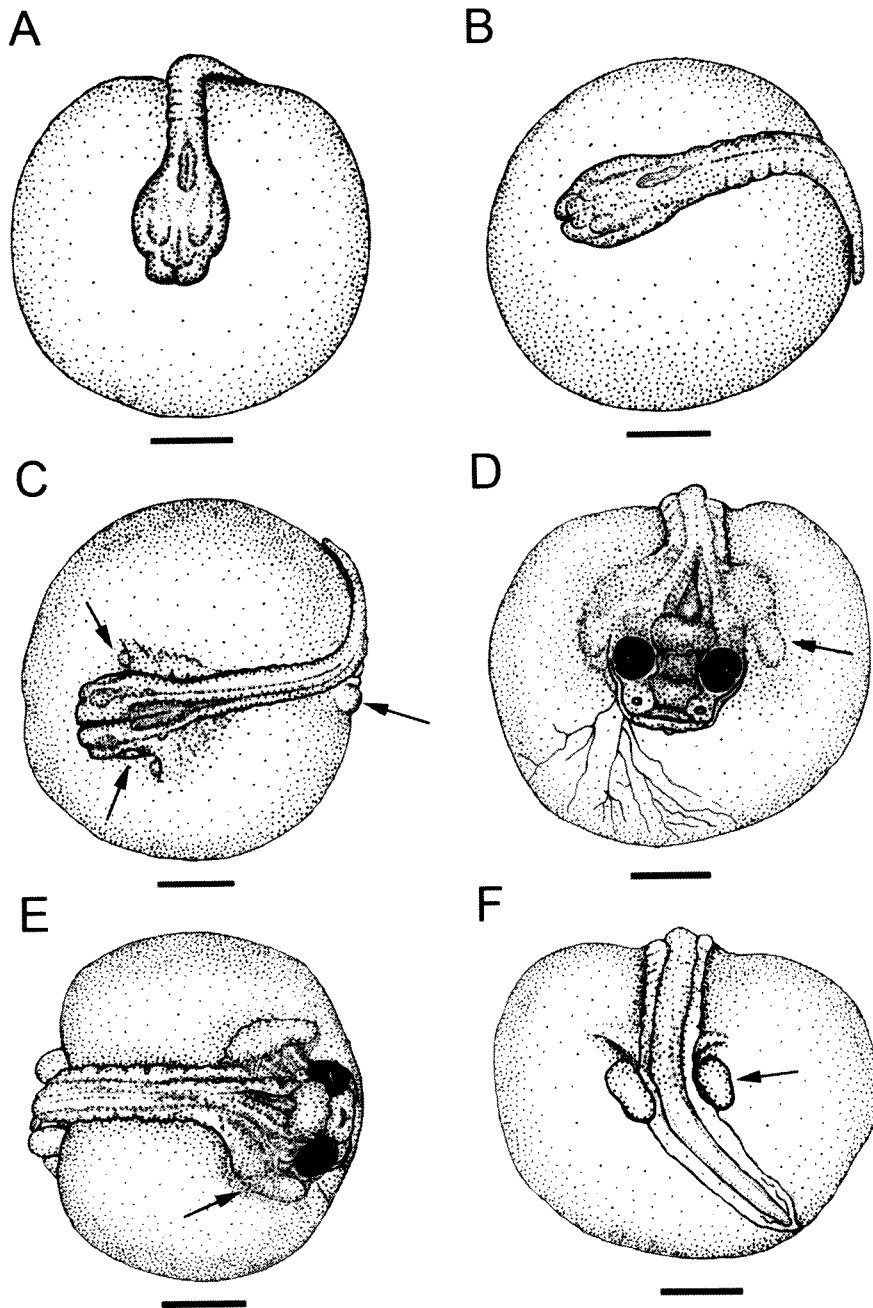


Figure 1 Stages 3, 4 and 6 (Townsend and Stewart, 1985) of *Arenophryne rotunda*. A and B = stage 3, anterior and lateral view; C = stage 4, dorsal view; D, E and F = stage 6, anterior, dorsal and posterior views. Scale bar represents 1 mm. Arrows indicate features highlighted in bold in Table 1.

Appendix 1). Nine embryos up to stage 13 of Townsend and Stewart (T&S; 1985) from one clutch of *M. gouldii* were collected 15 km north-east of Perth, WA, then reared and preserved at irregular intervals: WAM R97036-40. Six individuals just prior to hatching and recently hatched from four marked nests in the field were preserved after being excavated in April 1982: WAM R97041-42, 97044-45 (see Appendix 2). All embryos were reared in total darkness at ambient room temperatures in the laboratory which were lowered slightly (approximately 17–20°C) to better simulate cooling conditions at the nest sites in the field.

Measurements were taken with an ocular micrometer attached to a Wild M5 stereoscopic microscope and drawings were prepared with the aid of a camera lucida. The photograph (Figure 4F) was taken with a Nikon D70 digital SLR camera and 60 mm micro lens. Embryos were staged using the system of Townsend and Stewart (1985) which was devised for the direct developing leptodactylid *E. coqui*, with additional references to toe development based on the staging table for aquatic larvae of Gosner (1960). For the sake of completeness, descriptive observations on egg clutches provided for *A. rotunda* and *M. gouldii* by

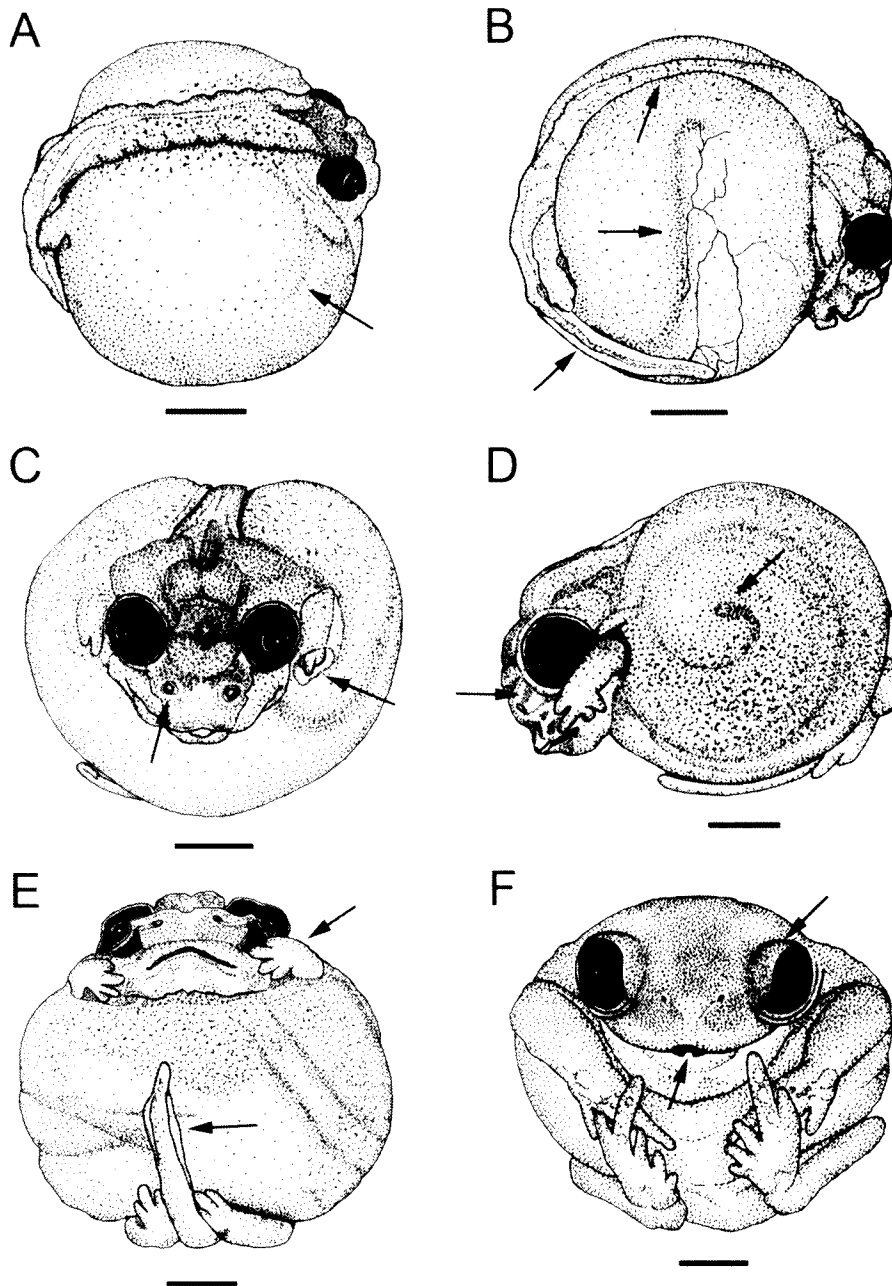


Figure 2 Stages 6, 7, 9, and 15 (Townsend and Stewart, 1985) of *Arenophryne rotunda*. A = stage 6, dorsolateral view; B and C = stage 7, lateral and anterior views; D and E = stage 9, lateral and ventral views; and F = stage 15, just prior to hatching, ventral view. Scale bar represents 1 mm. Arrows indicate features highlighted in bold in Table 1.

Roberts (1984 and 1981, respectively) are summarised prior to the descriptions for each species, with additional notes on development (Roberts, unpubl. data). Embryos in stages 1, 2, 3-7, 9-11, 13 and 15 are described and most stages are illustrated (Figures 1-4). Brief observations were made on live embryos during early cell division. The partial deterioration of the youngest preserved embryos of *A. rotunda* (stages 1 and 2), and specimens of *M. gouldii* at stages 11 and 13, limited their descriptions.

Results

The two species have various morphological

characteristics in common. Both have a generally similar parallel progression through the developmental stages described in Tables 1 and 2. Measurements of embryos for each species are given in the Appendices and Table 3 summarises key differences between the Australian species and *E. coqui*.

Development of *Arenophryne rotunda*

Clutch sizes of fertilised eggs ranged from 4-11 (mean 7, $n = 5$). Ovarian development commences in spring (late August), but ovum maturation is not completed until late summer. Three females collected in February 1981 contained 8, 8 and 4 pale

Table 1 Development of *Arenophryne rotunda*. T&S = Townsend and Stewart (1985) stages. Figure numbers in parenthesis. Features indicated by arrows in Figures 1 and 2 are highlighted in bold.

T&S	Head and Body	Tail	Limbs	Eyes	Mouth	Pigmentation
3 (Fig. 1A,B)	<ul style="list-style-type: none"> - head region small and narrow - narrow neural tube raised and groove closed, myotomes indistinct - yolk sac large and round - slight gill arch bulges - small indent in yolk in region of vent 	<ul style="list-style-type: none"> - tail bud short, narrow, adpressed around base of yolk, tip pointed - dorsal fin a shallow ridge 	<ul style="list-style-type: none"> - none 	<ul style="list-style-type: none"> - optic bulges small, indistinct, unpigmented 	<ul style="list-style-type: none"> - stomodaeum a small central pit in narrow crevice beneath top of snout, broad anterior bulge on either side 	<ul style="list-style-type: none"> - unpigmented
4 (Fig. 1C)	<ul style="list-style-type: none"> - head region slightly broader, adpressed against yolk - narrow cleft separates indistinct narial regions - no external gills or adhesive organs - small vent opening - some blood vessels present 	<ul style="list-style-type: none"> - tail bud narrow, longer, wraps over limb buds and vent to one side around yolk - fin ridges just visible 	<ul style="list-style-type: none"> - small round external hind limb buds - small round forelimb buds beneath fold of operculum that extends from side of head across to yolk 	<ul style="list-style-type: none"> - upper crescent of optic bulges slightly pigmented 	<ul style="list-style-type: none"> - mouth slit open, about one-third head width 	<ul style="list-style-type: none"> - unpigmented
6 (Fig. 1D-F, 2A)	<ul style="list-style-type: none"> - head broader, slightly raised above yolk; snout short, broad, blunt - vertebral region broader and thicker - no spiracle develops - nares perforated - blood vessels anteriorly over yolk - early gut development begins – faint arc in yolk anteriorly 	<ul style="list-style-type: none"> - fins low, slightly vascular near tip - muscle narrow - tip rounded to acuminate 	<ul style="list-style-type: none"> - forelimb buds longer, alongside head beneath operculum - hind limb buds longer, as in Gosner stages 30-31, slight knee constrictions and foot paddle 	<ul style="list-style-type: none"> - mostly pigmented, slight choroid fissure in lower half of iris - eye diameter 0.7 mm 	<ul style="list-style-type: none"> - mouth opening about one-third head width - upper and lower jaws further developed 	<ul style="list-style-type: none"> - some fine melanophores stippled over brain, vertebral region and onto yolk - tail unpigmented

7 (Fig. 2B-D)	<ul style="list-style-type: none"> - body broadens slightly - vertebral region flatter and recessed within yolk (Fig. 2C) - no external gills develop - internal flap inside naris - first gut wall in each side of yolk; vitelline blood vessels increased - groove down either side of vertebral region 	<ul style="list-style-type: none"> - tail longer, tightly adpressed around yolk, extends to about midway around yolk - fins low 	<ul style="list-style-type: none"> - limbs lengthen, slight indent on each side of foot paddle as for Gosner stage 33 - left forelimb partly protrudes through operculum (Fig. 2C) 	<ul style="list-style-type: none"> - diameter noticeably increased to 1.14 mm - fully pigmented, choroid fissure closed 	<ul style="list-style-type: none"> - mouth widens further 	<ul style="list-style-type: none"> - melanophores denser over head, vertebral region and down sides of yolk
9 (Fig. 2D, E)	<ul style="list-style-type: none"> - head well defined and prominent, snout broadly truncate, chin raised above yolk - vertebral myotomes more defined - nares oval, diameter 0.1 mm, directed anteroventrally, internal flap more distinct; lacrimal groove from naris to eye (Fig. 2D) - vent bordered by narrow rim - thick, spiral yolk-filled coil in gut 	<ul style="list-style-type: none"> - tail remains narrow, extends two-thirds around yolk - tip narrow 	<ul style="list-style-type: none"> - forelimbs completely erupted through operculum - hind limbs longer with distinct knee joints, toes with 5 digits discernable, as for Gosner stage 36 	<ul style="list-style-type: none"> - eyes lateral, prominent, diameter 1.3 mm 	<ul style="list-style-type: none"> - mouth about half head width - small, conical structure begins to project upwards on inside centre of lower lip, corresponding notch above in upper lip 	<ul style="list-style-type: none"> - entire dorsum and sides of yolk uniform light brown - tail unpigmented
15 (Fig. 2F)	<ul style="list-style-type: none"> - head and body fully defined - intestinal coil thick, yolk-filled, partly visible through epidermis, not yet differentiated into digestive system of adult 	<ul style="list-style-type: none"> - fully resorbed 	<ul style="list-style-type: none"> - limbs fully developed, forearms more robust than hindlimbs 	<ul style="list-style-type: none"> - eyes large, eyelids and nictitating membrane formed 	<ul style="list-style-type: none"> - mouth extends to below each eye - conical structure fully formed, fits into notch in upper lip (now deeper posteriorly) when mouth closes 	<ul style="list-style-type: none"> - entire dorsum uniform brown, including limbs (above and beneath)

Table 2 Development of *Myobatrachus gouldii*. T&S = Townsend and Stewart (1985) stages. Figure numbers in parenthesis. Features indicated by arrows in Figures 1 and 2 are highlighted in bold.

T&S	Head and Body	Tail	Limbs	Eyes	Mouth	Pigmentation
5 (Fig. 3A-C)	<ul style="list-style-type: none"> - head and snout fairly broad, adpressed against yolk - vertebral region narrow, raised above yolk - yolk large and round, some blood vessels anteriorly - gill arch bulges, no external gills - no adhesive organs - nares perforated, widely spaced - small medial vent beneath base of tail, rim around opening 	<ul style="list-style-type: none"> - well advanced, extends almost halfway around yolk - fins low at base of body, broaden towards tip - tip broadly rounded, vascular 	<ul style="list-style-type: none"> - small, round to ovoid external hind limb buds - small round forelimb buds mostly covered by opercular fold extending from each side of head (Fig. 3B) 	<ul style="list-style-type: none"> - iris mostly pigmented around outer third - narrow choroid fissure 	<ul style="list-style-type: none"> - mouth slit open, one-third head width 	<ul style="list-style-type: none"> - few fine melanophores scattered over brain and vertebral region to base of body
6 (Fig. 3D,E)	<ul style="list-style-type: none"> - head larger, broader, chin slightly raised above yolk - snout short, broad, truncate - vertebral region broad, recessed into yolk - nares deeper, no spiracle - yolk slightly expanded, first gut coil begins - vitelline blood vessels increased 	<ul style="list-style-type: none"> - tail as for stage 5 in single specimen observed 	<ul style="list-style-type: none"> - hind limb buds broad, longer, as in Gosner stage 30 - forelimb buds longer, beneath operculum alongside head 	<ul style="list-style-type: none"> - almost fully pigmented - choroid fissure narrow - diameter 0.8 mm 	<ul style="list-style-type: none"> - mouth opening slightly more than one-third head width - upper and lower jaws defined 	<ul style="list-style-type: none"> - dorsal pigment increased over head, vertebral region and partly onto yolk - tail unpigmented
9 (Fig. 3F, 4A)	<ul style="list-style-type: none"> - head broad, frog-like - snout short, broad and truncate, directed anteroventrally - body slightly broader - vertebral region broader, slightly raised, shallow groove down either side and down centre, myotomes visible (Fig. 3F) - nares oval, anterior, rim present, internal flap extends downwards - thick spiral intestinal coil 	<ul style="list-style-type: none"> - tail full length, extends to beneath head - fins broader towards tip - muscle broad at base, flat against body, begins to taper from mid-length to tip 	<ul style="list-style-type: none"> - hind limbs adpressed ventrally around yolk, 5 toe digits as for Gosner stage 35 - forelimbs longer, alongside head, fingers visible beneath operculum (Fig. 4A) 	<ul style="list-style-type: none"> - eyes lateral - choroid fissure closed - diameter 1.2 mm 	<ul style="list-style-type: none"> - small, conical structure (visible when mouth opened), begins to project upwards from inside centre of lower lip; small corresponding notch above in upper lip 	<ul style="list-style-type: none"> - dorsum uniform brown over brain, vertebral region; less dense over yolk to midway down sides of body - limbs partly pigmented - tail unpigmented

10 (Fig. 4B,C)	<ul style="list-style-type: none"> - head broad, well defined, more frog-like - diameter of nares 0.16 mm, distinct lacrimal groove from each naris to each eye - thick spiral coil in gut, visible through epidermis 	<ul style="list-style-type: none"> - tail extends to forearm in one specimen - muscle slightly translucent down centre of posterior half 	<ul style="list-style-type: none"> - hind limbs longer, toes as for Gosner stage 37, shorter than fingers - both forearms completely erupted through skin, robust 	<ul style="list-style-type: none"> - eyes prominent - diameter 1.2 mm 	<ul style="list-style-type: none"> - mouth opening about half head width - conical structure further developed, nonkeratinised 	<ul style="list-style-type: none"> - entire dorsum uniform brown, darker over head and vertebral region - sides of body lightly pigmented to partway across venter - limbs lightly pigmented, mainly over upper half - tail unpigmented
11	<ul style="list-style-type: none"> - specimen dead prior to preservation, misshapen - vertebral myotomes quite distinct 	<ul style="list-style-type: none"> - tail almost full length of body 	<ul style="list-style-type: none"> - toes as for Gosner stage 38, to about half length at hatching 			<ul style="list-style-type: none"> - dorsum uniform brown, lighter brown over limbs and sides - mid-ventral region remains unpigmented
13 (Fig. 4D)	<ul style="list-style-type: none"> - head broadens further - snout very short and blunt - body appears fully formed, but dead prior to preservation, partly misshapen 	<ul style="list-style-type: none"> - tail regresses to about midway around side of body - muscular ridges clearly defined - tip still broadly rounded 	<ul style="list-style-type: none"> - limbs fully developed, similar to about Gosner stage 43 - subarticular tubercles present 			<ul style="list-style-type: none"> - dorsum uniform dark brown, slightly lighter over limbs and sides of body - throat and chest stippled with brown ventrally - creamy yellow of yolk visible ventrally - tail unpigmented but for a few dark specks anteriorly over muscle
15 (Fig. 4E, F)	<ul style="list-style-type: none"> - head and body fully defined as a miniature of adult - intestinal coil still yolk-filled, partly visible through epidermis, not yet differentiated into digestive system of adult - nares very small, diameter 0.08 mm, opening further obscured by internal flap 	<ul style="list-style-type: none"> - tail fully resorbed 	<ul style="list-style-type: none"> - limbs fully developed - forearms more robust than hindlimbs 	<ul style="list-style-type: none"> - like those of adult, eyelid, nictitating membrane present 	<ul style="list-style-type: none"> - mouth extends to below each eye, tongue visible - conical structure fits into notch in upper lip (now deeper posteriorly) when mouth closes 	<ul style="list-style-type: none"> - entire dorsum and limbs uniform dark brown - entire venter and undersurface of limbs stippled brown, darker over throat - soles of feet darker

Table 3 Differences between available preserved stages of *A. rotunda* and *M. gouldii* and those of similar live stages for *E. coqui*. As no observations of behaviour or ECD (endolymphatic calcium deposits, visible in life) were available for the Australian genera, these are not included here for *E. coqui*. Stages prior to stage 4 and features which are the same for each are excluded. T&S = Townsend and Stewart stages (1985).

T&S	<i>Eleutherodactylus</i>	<i>Arenophryne</i> and <i>Myobatrachus</i>
4	<ul style="list-style-type: none"> • eye bulges distinct • gill arches present, but no gills present • tail bud first apparent 	<ul style="list-style-type: none"> • eye bulges discernable • slight gill arches • tail bud elongates enough to bend around yolk to one side
5	<ul style="list-style-type: none"> • forelimbs round to ovoid, external • eyes prominent, unpigmented • gill buds first appear from gill arches, gill circulation • tail bud elongates enough to bend, small thin fin 	<ul style="list-style-type: none"> • forelimb buds beneath operculum • eyes partly pigmented • indistinct gill arches, no gills • tail long (especially <i>Myobatrachus</i>), wraps around yolk, fins well developed and vascular (<i>Myobatrachus</i>),
6	<ul style="list-style-type: none"> • forelimbs develop externally • eye distinct from rest of head, pupil clear • gills well developed • tail over one-half final length, small, membranous fin • widely scattered melanophores over dorsum 	<ul style="list-style-type: none"> • forelimbs develop beneath operculum • eye pigment well developed, slight choroid fissure • no external gills develop • fins low, poorly developed, slightly vascular (<i>Arenophryne</i>), or well developed and more vascular (<i>Myobatrachus</i>) • some fine melanophore stippling over brain and vertebral region
7	<ul style="list-style-type: none"> • hind limbs with obvious knee joints, foot paddles first evident • elbow visible on forelimbs • tail $\frac{2}{3}$ final length, fin almost full size, vascular 	<ul style="list-style-type: none"> • foot develops up to 3 early toe nubs • one forelimb begins to break through operculum (<i>Arenophryne</i>) • tail at full length, fins remain low (<i>Arenophryne</i>) • beginning of first gut wall within yolk
9	<ul style="list-style-type: none"> • limbs elongate, digits on hands and feet • tail $\frac{2}{3}$ full length with full size fin • pigmentation expands to about midway down yolk 	<ul style="list-style-type: none"> • forelimbs fully erupted in <i>Arenophryne</i>; still beneath operculum alongside head in <i>Myobatrachus</i>; toe digits similar to Gosner stages 35-36 • tail full length in both • pigmentation expands around sides and partly over venter • beginning of small, conical projection on inside centre of lower lip, small notch in centre of upper lip
10	<ul style="list-style-type: none"> • toes to $\frac{1}{3}$ of hatching length • pigmentation dense on dorsum, less on head 	<ul style="list-style-type: none"> • forelimbs now erupted in <i>Myobatrachus</i>; toes all individually separate and a bit longer, similar to Gosner stage 37 • pigmentation uniform over entire dorsum and sides, denser over head and vertebral region
11-12	<ul style="list-style-type: none"> • tail full length with full fin • egg tooth first develops on upper lip by late stage 12 	<ul style="list-style-type: none"> • tail at full length from stage 9 • nonkeratinised conical projection on inside lower lip
13	<ul style="list-style-type: none"> • toes full length, toe pads first evident • egg tooth develops keratin • yolk reserve still large 	<ul style="list-style-type: none"> • limbs well advanced, subarticular tubercles develop on hands and feet, no toe pads • no egg tooth, nonkeratinised conical projection fits into deeper notch in upper lip when mouth closes • intestinal development now obscured by pigment
15	<ul style="list-style-type: none"> • tail remnant half or less of full length at hatching 	<ul style="list-style-type: none"> • no tail remnant prior to hatching

yellow, mature ovarian eggs with a mean diameter of 4 mm. An adult male found on 1 April 1981 was sitting on a clutch of seven eggs buried in sand at a depth of 80 cm and soil temperature in the nest site at 0845 hr was 25°C. It was not possible to determine if there was a burrow leading to the frog and eggs, or a chamber around them, as the sand

caved in during excavation. The eggs in stage 1 had a mean capsule diameter of 5.5 mm (5.0–6.0 mm) and initially adhered together in a cluster by means of the sticky outer surface of each capsule. As they became covered in sand, individual capsules separated. Another clutch at the same depth was unattended by an adult (Roberts 1984).

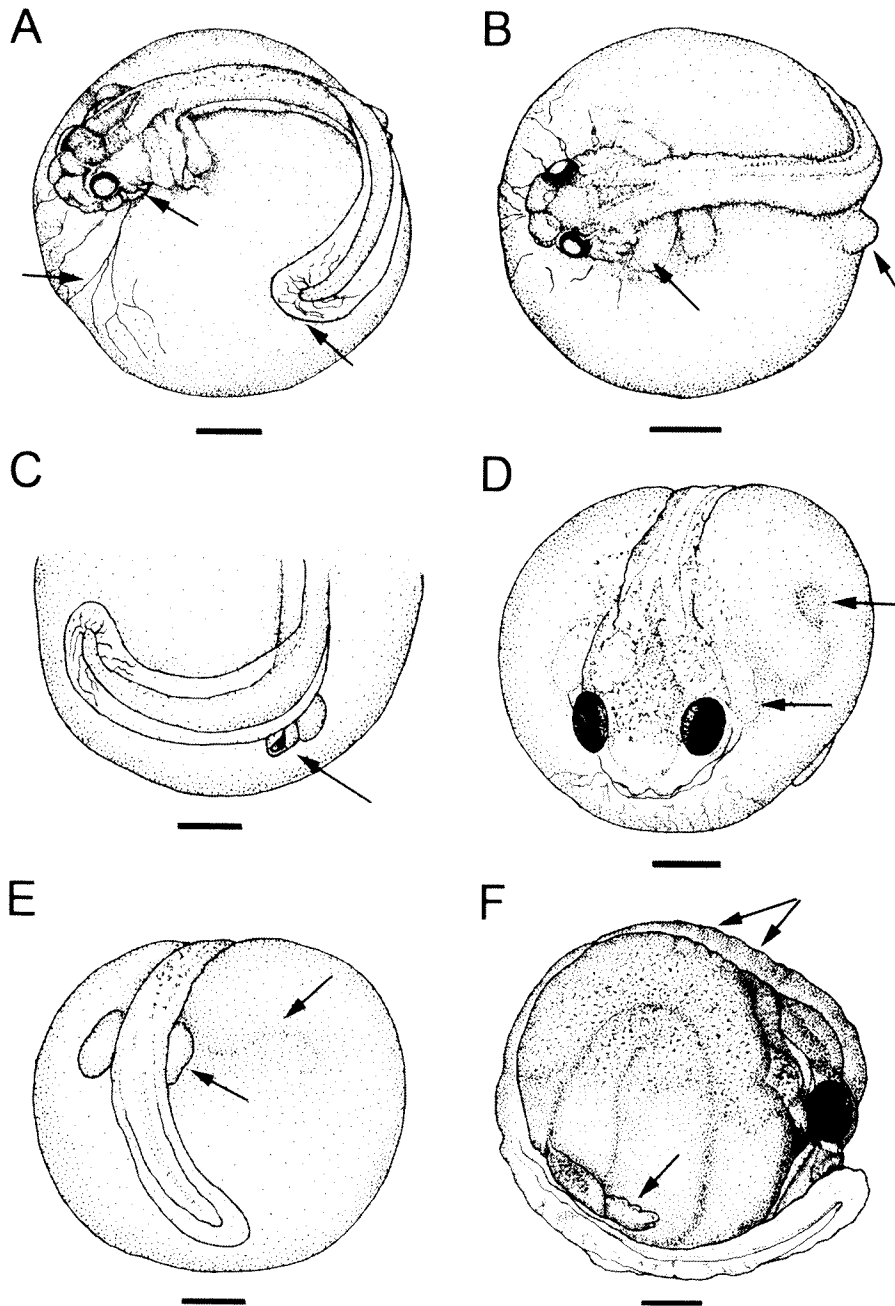


Figure 3 Stages 5, 6, and 9 (Townsend and Stewart, 1985) of *Myobatrachus gouldii*. A = stage 5, dorsal view; B and C = stage 5, dorsal and partial posterior views (line indicates forelimb beneath operculum in B and vent in C); D and E = stage 6, anterior and posterior views; and F = stage 9, lateral view. Scale bar represents 1 mm. Arrows indicate features highlighted in bold in Table 2.

Embryos at stages 1, 2-4, 6, 7, 9 and 15 are described in Table 1, and illustrated in Figures 1 and 2. Embryos are unpigmented during stages 1-4. Live embryos were not easily studied due to fine sand over the capsules. A pair of frogs collected on 1 April 1981, laid 11 eggs (clutch 4) some time between 1-3 April and cleavage furrows were observed during early to mid-cleavage on 4 April. Gastrulation and blastopore formation seemed typical of those described for aquatic tadpoles (Gosner 1960) and the dorsal lip was a distinct indentation. Estimating 2 April as the approximate

date eggs were laid, late gastrula was reached after about 5 days and the neural plate began to form (stage 2) after about 10 days. Stage 5 was reached after about 16 days (none preserved) and stage 6 after 24 days.

Hatching and embryonic life span

Well formed froglets from clutch 4 were observed twitching within the capsules from about 45 days after the eggs were laid, and after about 50 days some were unhatched and adpressed tightly against the capsule wall with no yolk remaining. The last

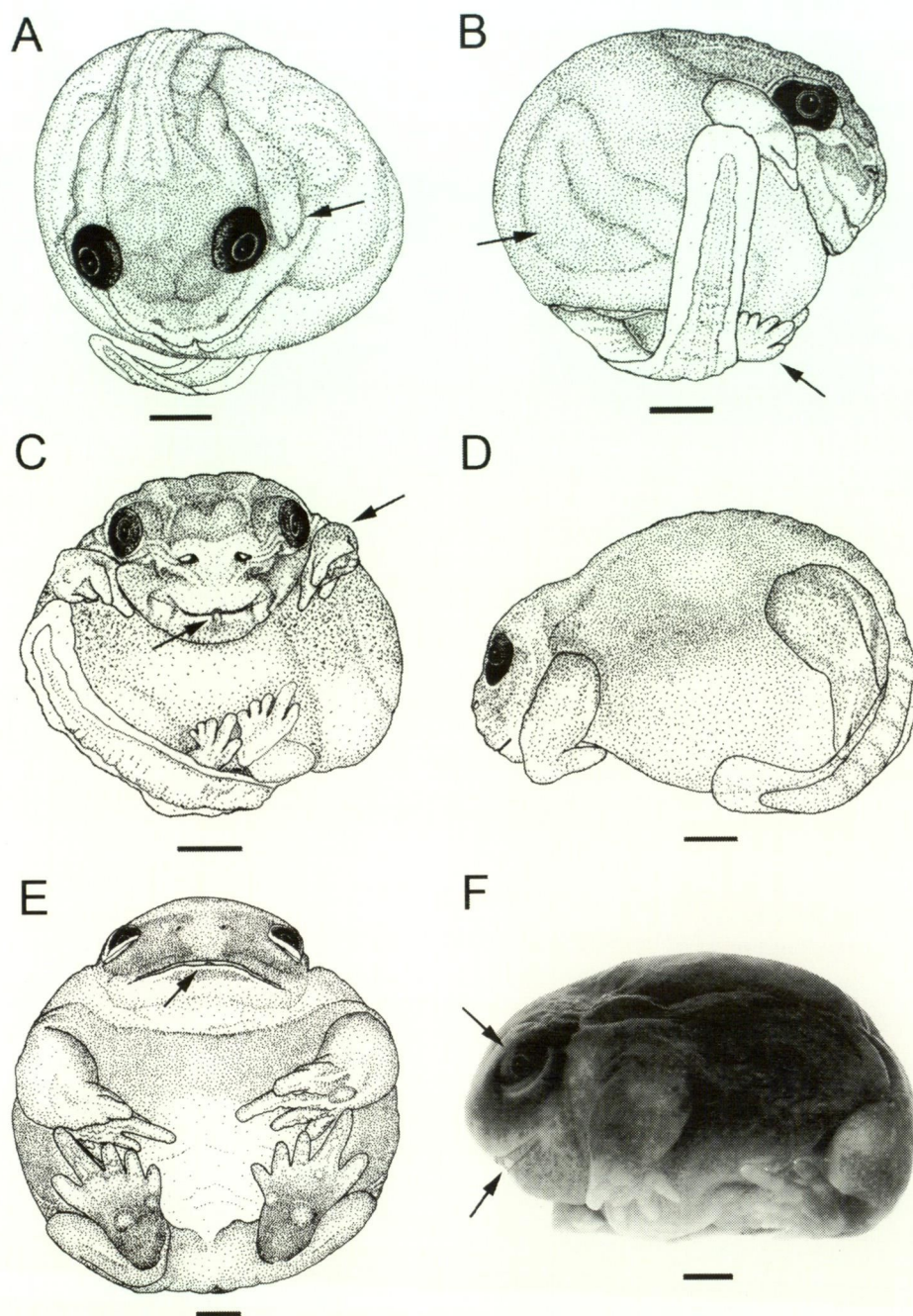


Figure 4 Stages 9, 10, 13 and 15 (Townsend and Stewart, 1985) of *Myobatrachus gouldii*. A = stage 9, anterior view, forelimb beneath operculum; B and C = stage 10, lateral and ventral views; D = stage 13, lateral view; and E and F = stage 15, ventral view and photograph of lateral view, just prior to hatching. Scale bar represents 1 mm. Arrows indicate features highlighted in bold in Table 2.

froglet hatched (yolk present in intestinal loops) on 12 June 1981, about 64 days after the eggs were laid (SV = 10.4 mm, weight 0.22 g). On 5 June, one froglet from another clutch which had been collected at stage 1 (dorsal lip to mid-gastrula) on 1 April, was found beginning to hatch with one hind limb extended through the capsule wall. When removed and washed to remove sand, the remaining jelly layers came free and the froglet became active and soon began to burrow (SV = 9.9 mm, weight 0.24 g). There was a distinct middorsal stripe and the remnant yolk mass was quite large.

Minimum embryonic life span for this individual was about 65 days (estimating four days from fertilisation to mid-gastrula). Three recent hatchlings 1–2 weeks old measured 11.2–11.4 mm (mean 11.3 mm) and all weighed 0.25 g.

Development of *Myobatrachus gouldii*

Clutch sizes of fertilised and ovarian eggs ranged from 9–38 (mean 25, $n = 5$; Roberts 1981). The mean diameter of 28 ovarian eggs from a female caught in February 1979 is 5.1 mm, and mean diameters of 26, 38 and 9 ovarian eggs from three females collected

in November and December 1979 are 5.0, 5.3 and 4.9 mm, respectively (Roberts 1981). The mean capsule diameter of 23 live embryos from one clutch at early cell division collected in February 1979 is 7.4 mm (± 0.5 SD). The live embryos are creamy white, and the surface of the capsules are sticky to the touch, fairly tough and covered with fine sand. They soon become like firm, round balls to touch. Early cell division in live embryos at stage 1 is similar to that described for *Heleioporus eyrei* at Gosner stage 4 (Packer 1966) with four, dorsal micromeres and two perpendicular, incomplete cleavage furrows at the vegetal pole. Embryos at the earliest preserved stages available at stage 5 (T&S) have small limb buds, but unlike *E. coqui* at this stage, the eyes are partially pigmented (see Discussion).

Stages 5, 6, 9, 10, 11, 13 and 15 are described in Table 2, and illustrated in Figures 3 and 4.

Hatchlings

Hatching was not observed. Five fully formed froglets just prior to and just after hatching measured 8.9–10.8 mm (mean 10.1), were exact miniatures of the adult in form and pigmentation and began to burrow soon after hatching.

In summary, *A. rotunda* and *M. gouldii* share the following characteristics: large, unpigmented ova encapsulated with a thin outer layer (that becomes fairly tough) and an inner jelly layer, no external gills or adhesive organs, early limb bud development prior to any optic pigmentation, no spiracle and forelimbs covered by the operculum until at least stage 7. Eyes develop pigment gradually from stage 5 and increase noticeably in diameter during stages 6–8. The neural tube is initially raised above the large yolk (stages 4–5), gradually flattens and broadens from about stage 6 onward, then as vertebrae develop, the vertebral column appears as a broad, thickened ridge. The vent tube begins to develop from about stage 4, the gut gradually develops from initial divisions in the yolk at stage 6, into a thick intestinal coil by stage 9, and a small internal flap develops inside each naris from about stage 9.

The mouth begins as a small stomodaeal pit at stage 4, then becomes a simple slit that gradually widens with jaw development and never develops the oral mouthparts of a tadpole. During stage 9, a small flexible conical structure (visible when the mouth is opened) begins to project upwards from the inside centre of the lower lip and inserts into a corresponding notch centred in the upper lip; this projection becomes more defined in subsequent stages and the inner margin of the notch deepens posteriorly. These structures remain in the adult and are also present in the Australian microhylids (Anstis unpublished observations). Frog-like features of the head develop from as early as stage 9.

DISCUSSION

Comparative development

Although not all stages were available for the two species, an adequate comparative understanding of their development can be gained from the existing material, because in those stages where direct comparison was possible, similarities were quite evident and differences were minor. No pairs of adults for either species were observed in amplexus and the mode of fertilisation could not be determined, but as eggs are laid in sand, internal fertilisation could be advantageous. The relatively large size of the ova and the small clutch sizes are also characteristic of direct developing species (e.g., 2.0–10.0 mm and clutch sizes of 1–94; Thibaudeau and Altig 1999). Based on the similarity in early cell division noted here between *H. eyrei* and *M. gouldii*, it is likely that cleavage is holoblastic, but more live material needs to be studied to verify this. The tough external capsules may protect developing embryos but do not prevent desiccation in *M. gouldii* (Roberts 1981). Death by desiccation may be a result for embryos of both species if normal winter rains are delayed.

Arenophryne rotunda has a shorter tail with low fins and a narrower muscle and much narrower tail tip than *M. gouldii*. *Myobatrachus gouldii* has a long tail with more prominent fins that provide a greater degree of vascularisation, a broad muscle, broadly rounded tip and the tail is well advanced by stage 5 (Figure 3A, Table 3). Pigmentation is generally less dense in *A. rotunda* during stages 4–9. The forelimbs emerge through the epidermis during stage 7 for *A. rotunda* and about stage 10 for *M. gouldii*.

Arenophryne rotunda and *M. gouldii* have forward burrowing behaviour (Tyler *et al.* 1980; Main *et al.* 1959; Lindgren and Main 1961), and the minute flap in the narial canal which persists in adults, possibly prevents sand particles being lodged in the nostrils during burrowing.

Hatching

Hatching in these species has not been fully observed, but in *A. rotunda*, one embryo pushed a hind limb through the capsule wall at the onset of hatching. In a description of the hatching process of the microhylid *Cophixalus darlingtoni* from Papua New Guinea, Tyler (1976b) observed that prior to hatching, the embryo used only abrupt, outstretched movements of the arms and legs to split the capsule. From the one observation of the *A. rotunda* hatchling, it appears that the hatching process in *A. rotunda*, and probably *M. gouldii*, is similarly precipitated by abrupt movements of the limbs, since the outer layer of the jelly capsule is dry and tough and the embryos already have quite robust forelimbs.

The turgidity of the egg jellies of direct developers would seem to require the use of an egg tooth during hatching, and embryos of species of *Eleutherodactylus* are known to poke at the inside of the egg capsule with the keratinised egg tooth on the upper lip (Townsend and Stewart, 1985; Duellman and Trueb, 1986). In *A. rotunda* and *M. gouldii*, however, there is no egg tooth, only the small, nonkeratinised conical projection described.

Comparisons with *Eleutherodactylus coqui*

The two Australian myobatrachids differ from *E. coqui* in that they deposit eggs in subterranean sites, they do not develop an egg tooth, the initial development of the forelimbs is internal prior to stage 7 or 10 (exposed from stage 4 in *E. coqui*), the tail is more advanced in development by stage 5 (*M. gouldii*) and there are no external gills. Apart from the differences noted above and those in Table 3, they have a generally similar developmental life history to *E. coqui*, but it has not been possible to adequately compare aspects of gut, mouth and eye development (choroid fissure), vitelline circulation and behaviour.

Comparisons with other Australian myobatrachids and microhylids

Arenophryne rotunda and *Myobatrachus gouldii* share key features typical of direct development as defined by Altig and Johnston (1989) including the lack of mouthparts and a spiracle. The absence of a spiracle and mouthparts are also typical of other Australian endotrophic guilds including *Assa darlingtoni* and *Bryobatrachus nimbus* (Anstis 2002). The paraviviparous genus *Rheobatrachus* and the nidicolous species of *Geocrinia*, however, have a vestigial spiracle and much reduced mouthparts, including a few very small lateral marginal papillae and nonkeratinized jaw ridges (Anstis unpublished observations; Watson and Martin 1973; Tyler and Davies 1983). Adhesive glands are absent in *A. rotunda* and *M. gouldii* and in *Spicospina flammocaerulea* from southwestern Australia, a species with aquatic development in which the hatchlings are fully supported within thick algae mats (Dziminski and Anstis 2004), negating the need for adhesive glands.

Exposed forelimb bud development throughout embryonic stages is found in the microhylid genus *Cophixalus* (Tyler 1976b; Anstis unpublished observations) and also in the earlier stages of *Phyloria*, which has terrestrial, nidicolous larvae. In at least three species of *Phyloria* (*P. sphagnicolus*, *P. kundagungan* and *P. loveridgei*), all four limb buds are initially exposed from about Gosner stage 20, but the forelimbs are soon covered by the operculum and continue development internally during larval stages,

breaking through the operculum at Gosner stage 42 (Anstis 1981; De Bavay 1993; Ingram and Corben 1975; Anstis 2002).

Further studies on the Australian direct developing genera are required to improve our understanding of their morphology, physiology and general biology, including mode of fertilisation, embryonic behaviour, life span and the hatching process, so that adequate future comparisons can be made with other direct developing genera.

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REFERENCES

- Altig, R. and Johnston, G.F. (1989). Guilds of anuran larvae: relationships among developmental modes, morphologies, and habitats. *Herpetological Monographs* 3: 81–109.
- Anstis, M. (1981). Breeding biology and range extension for the New South Wales frog *Kyarranus sphagnicolus* (Anura: Leptodactylidae). *Australian Journal of Herpetology* 1: 1–9.
- Anstis, M. (2002). *Tadpoles of south-eastern Australia: a guide with keys*. Reed New Holland, Sydney, Australia.
- De Bavay, J. M. (1993). The developmental stages of the sphagnum frog, *Kyarranus sphagnicolus* Moore (Anura: Myobatrachidae). *Australian Journal of Zoology* 41: 275–93.
- Duellman, W.E. and Trueb, L. (1986). *Biology of Amphibians*. McGraw-Hill Book Company, New York.
- Dziminski, M. A. and Anstis, M. (2004). Embryonic and larval development of the sunset frog, *Spicospina flammocaerulea* (Anura: Myobatrachidae), from southwestern Australia. *Copeia* 2004: 893–899.
- Frost, D.R., Grant, T., Faivovich, J., Bain, R., Haas, A., Haddad, C.F.B., de Sá, R.O., Donnellan, S.C., Raxworthy, C.J., Wilkinson, M., Channing, A., Campbell, J.A., Blotto, B.L., Moler, P., Drewes, R.C., Nussbaum, R.A., Lynch, J.D., Green, D. and Wheeler, W.C. (2006). The amphibian tree of life. *Bulletin of American Museum of Natural History*. 297: 1–370.
- Gitlin, D. 1944. The development of *Eleutherodactylus portoricensis*. *Copeia* 1944: 91–98.
- Gosner, K. L. (1960). A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* 16: 183–190.
- Ingram, G. J. and Corben, C.J. 1975. A new species of *Kyarranus* (Anura: Leptodactylidae) from Queensland, Australia. *Memoirs of the Queensland Museum*. 17: 335–339.

- Jameson, D. L. 1950. The development of *Eleutherodactylus latrans*. *Copeia* 1950: 44-46.
- Lindgren, E., and Main, A.R. (1961). Natural history notes from Jigalong, IV: Frogs. *West Australian Naturalist* 7: 193-195.
- Main, A. R., Littlejohn, M.J. and Lee, A.K. (1959). Ecology of Australian frogs, p. 396-411. In *Biogeography and Ecology in Australia*, eds A. Keast, R. L. Crocker and C. S. Christian. Junk: The Hague.
- Maxson, L. R. and Roberts, J.D. (1985). An immunological analysis of the phylogenetic relationships between two enigmatic frogs, *M. gouldii* and *A. rotunda*. *Journal of Zoology (London)* 207: 289-300.
- Packer, W. C. (1966). Embryonic and larval development of *Heleioporus eyrei* (Amphibia: Leptodactylidae). *Copeia* 1966: 92-97.
- Read, K., Keogh, J.S., Scott, I.A.W., Roberts, J.D. and Doughty, P. (2001). Molecular phylogeny of the Australian frog genera *Crinia* and *Geocrinia* and allied taxa (Anura: Myobatrachidae). *Molecular Phylogenetics and Evolution* 21: 294-308.
- Roberts, J. D. (1981). Terrestrial breeding in the Australian leptodactylid frog *Myobatrachus gouldii* (Gray). *Australian Wildlife Research* 8: 451-62.
- Roberts, J. D. (1984). Terrestrial egg deposition and direct development in *Arenophryne rotunda* Tyler, a myobatrachid frog from coastal sand dunes at Shark Bay, W.A. *Australian Wildlife Research* 11: 191-200.
- Thibaudeau, G., and Altig, R. (1999). Endotrophic Anurans, p. 170-188. In *Tadpoles: the Biology of Anuran Larvae*, eds R. W. McDiarmid and R. Altig. University of Chicago Press, Chicago.
- Townsend, D. S. and Stewart, M.M. (1985). Direct development in *Eleutherodactylus coqui* (Anura: Leptodactylidae): a staging table. *Copeia* 1985: 423-436.
- Tyler, M. J. (1962). On the preservation of anuran tadpoles. *Australian Journal of Science* 25: 222.
- Tyler, M. J. (1976a). A new genus and two new species of leptodactylid frogs from Western Australia. *Records of the Western Australian Museum* 4: 45-52.
- Tyler, M. J. (1976b). *Frogs*. Australian Naturalist Library. Collins, Australia.
- Tyler, M. J., Roberts, J.D. and Davies, M. (1980). Field observations on *Arenophryne rotunda* Tyler, a leptodactylid frog of coastal sandhills. *Australian Wildlife Research* 7: 295-304.
- Tyler, M. J., Smith, L.A. and Johnstone, R.E. (2000). *Frogs of Western Australia*. Western Australian Museum, Perth.
- Wake, M. H. 1978. The reproductive biology of *Eleutherodactylus jasperi* (Amphibia, Anura, Leptodactylidae), with comments on the evolution of live-bearing systems. *Journal of Herpetology* 12(2): 121-133.
- Watson, G. F., and Martin, A. A. (1973). Life history, larval morphology and relationships of Australian leptodactylid frogs. *Transactions of the Royal Society of South Australia* 97: 33-45.

APPENDIX 1

Collection and preservation dates (day/month/1981), stage (Townsend and Stewart 1985), and embryo dimensions (mm, diameters to stage 9, snout-vent length for stage 15) of *Arenophryne rotunda*. WAM = West Australian Museum. N = 1 in each case, see footnote.

Clutch	WAM	Coll.	Pres.	Stage	Dimensions
1	R97056	1/4	1/4	1	5.0
2	R97054	1/4	1/4	1	4.5x4.4
4	R97046	3/4	10/4	2	5.0
4	R97047	3/4	16/4	3	5.0x4.7
1	R97057	1/4	10/4	4	5.2x5.0
4	R97048	3/4	24/4	6 ¹	5.0x4.2
3	R97049	1/4	16/4	6	5.1x4.5
3	R97050	1/4	24/4	7	4.8x4.6
4	R97052	1/4	15/5	7	5.7x4.7
1	R97059	1/4	24/4	9	5.6x5.5
3	R97053	1/4	22/5	15	6.6 SVL
1	R97060	1/4	1/5	15	4.8 SVL

¹ external egg diameter of one individual = 6.1 mm.

APPENDIX 2

Collection and preservation dates, stage (Townsend and Stewart 1985), and embryo dimensions (mm, diameters to stage 10, snout-vent length for stages 13-15) of *Myobatrachus gouldii*. WAM = West Australian Museum. N = 1 in each case, except R97037 = 4, range in parenthesis.

Clutch	WAM	Coll.	Pres.	Stage	Dimensions
1	R97036	4/3/81	4/3/81	5	5.7x5.6
1	R97036	4/3/81	4/3/81	5	5.7x5.7
1	R97037	4/3/81	13/3/81	6	5.1x5.5 (4.8-5.4x5.2-5.8)
1	R97038	4/3/81	27/3/81	9	6.4x6.2
1	R97040	4/3/81	16/4/81	9	6.6x6.4
1	R97039	4/3/81	3/4/81	10	6.1x5.8
2	R97041	14/4/82	14/4/82	13	8.5 SVL
3	R97042	14/4/82	14/4/82	15	10.8 SVL ¹
3	R97043	23/4/82	23/4/82	15	10.5 SVL
5	R97044	7/4/81	7/4/81	15	8.9 SVL
5	R97044	7/4/81	7/4/81	15	10.0 SVL ²
4	R97945	14/4/81	14/4/81	15	10.1 SVL

¹ Egg dimensions = 11.3 x 10.8 mm

² Egg dimensions = 11.3 x 10.5 mm