DOI: 10.18195/issn.0312-3162.25(4).2010.399-440
Records of the Western Australian Museum **25**: 399–440 (2010).

A comparative study of divergent embryonic and larval development in the Australian frog genus *Geocrinia* (Anura: Myobatrachidae)

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Abstract - Embryonic and larval development of the seven *Geocrinia* species across Australia are described and compared. This Australian myobatrachid genus includes three species with terrestrial embryonic development followed by aquatic exotrophic larval development and four species with entirely terrestrial and endotrophic development. Comparisons are made among species within the terrestrial/exotrophic group and the endotrophic group, and between the two breeding modes of each different species-group. Morphological differences are noted between northern and southeast coastal Western Australian populations of *G. leai* tadpoles. The *G. rosea* group shares some similarities with the other Australian endotrophic species in the genus *Philoria* and *Crinia nimbus*.

INTRODUCTION

About 38 species of anurans from 22 genera and 7 families worldwide are known to have nidicolous endotrophic larvae, and if endotrophy occurs in a genus, usually all species in that genus are of that developmental guild (Thibaudeau and Altig 1999). These authors listed some known exceptions, including *Gastrotheca* (one endotrophic and one exotrophic guild), *Mantidactylus* (one endotrophic and several exotrophic guilds) and *Megophrys* (one endotrophic and one exotrophic guild). The Australian myobatrachid genus *Geocrinia* also includes two developmental guilds as defined by Altig and Johnston (1989), with three terrestrial/exotrophic species and four terrestrial/endotrophic species.

The family Myobatrachidae in Australia has a great variety of breeding modes representative of various guilds (Altig and Johnston 1989) from the entirely aquatic (e.g. *Uperoleia, Mixophyes, Taudactylus, Notaden* and most *Crinia*) to the terrestrial/aquatic (e.g. *Pseudophryne* and *Geocrinia laevis* group), nidicolous (*Philoria, Crinia nimbus, Geocrinia rosea* group), the exoviviparous *Assa*, paraviviparous *Rheobatrachus* and the three closely-related direct developers *Arenophryne*, *Myobatrachus* and *Metacrinia* (Roberts 1993; Anstis *et al.* 2007; Anstis 2008).

Across southern Australia and Tasmania, there are seven species of frogs currently assigned to the myobatrachid genus *Geocrinia*. Three species, including *G. victoriana* and *G. laevis* found in the southeast (Littlejohn and Martin 1964; Watson and Martin 1973) and *G. leai* from southwestern Australia (Main 1957, 1965), have terrestrial embryonic development and exotrophic (aquatic, feeding) larval development. The remaining four allopatric species in southwestern Australia (*G. alba*, *G. lutea*, *G. rosea* and *G. vitellina*) belong to the *G. rosea* species-group (Wardell-Johnson and Roberts 1993; Roberts 1993) and have terrestrial endotrophic (non-feeding) embryonic and larval development (Main 1957; Roberts *et al.* 1990; Roberts 1993). All seven species of adult frogs are small, ranging from 19–33 mm snout-vent length (SVL) and are generally similar in morphology (Littlejohn and Martin 1964; Driscoll 1997).

Read *et al.* (2001) presented a mitochondrial gene tree for the myobatrachids as then defined with an emphasis on *Crinia* and *Geocrinia*. They included all species of *Geocrinia* except *G. lutea*, and their data divided *Geocrinia* into two strongly supported lineages: (a) *G. leai*, *G. victoriana* and *G. laevis* (with *G. leai* somewhat divergent) and (b) *G. rosea*, *G. alba* and *G. vitellina* (*G. lutea* is presumed to belong in this lineage based on other characters). Edwards (2007) found that *G. leai* consists of three distinct lineages which show no morphological differences as adults and occur in: (i) the northern Darling escarpment, (ii) the southeast coast and (iii) the southern coastal regions of southwestern Australia. Comparisons of *G. leai* tadpoles from populations (i) and (iii) are included here. To facilitate descriptions, *G. leai*, *G. laevis* and *G. victoriana* (i.e. lineage 'a' of Read *et al.* 2001) are referred to as the *G. laevis* group (terrestrial/exotrophic) and the other four species as the *G. rosea* group (terrestrial/endotrophic).

These two groups are also clearly separated by their breeding biology, call structure (Littlejohn and Watson 1974; Roberts *et al.* 1990; Roberts and Wardell-Johnson 1995) and larval life history (Main 1957, 1965; Littlejohn and Martin 1964; Roberts *et al.* 1990; Roberts 1993). Anstis (2008) included the *G. rosea* group in the nidicolous endotrophic category of Altig and Johnston (1989) because the larvae of this group remain in a terrestrial nest, hatch from the eggs as free-moving tadpoles and metamorphose without feeding, unlike direct developers, which do not have a tadpole stage and differ significantly in other aspects of their physiological development (Altig and Johnston 1989; Thibaudeau and Altig 1999; Callery *et al.* 2001).

Various descriptions of the embryonic and larval development of the two eastern exotrophic species have been published (Littlejohn and Martin 1964; Martin 1967; Littlejohn *et al.* 1971; Watson and Martin 1973; Martin and Littlejohn 1982; Gollmann and Gollmann 1991a,b, 1992a,b, 1993, 1994, 1995, 1996a,b; Anstis 2002). For the southwestern species, there are studies including some developmental descriptions of *G. leai* and *G. rosea* by Main (1957, 1965) and of *G. vitellina* by Mitchell (2001).

This paper reviews the known breeding biology of all *Geocrinia* species based on the published literature and original observations. It then provides comprehensive descriptions of embryos and larvae with a developmental staging system for the *G. rosea* group, and enables detailed comparisons between individual species and between each group of the seven *Geocrinia* species during their embryonic and larval development. It should enhance our comparative knowledge of the developmental life history and morphology of *Geocrinia* embryos and larvae and facilitate a better understanding and interpretation of the evolution of these divergent cases of embryogenesis.

A summary is provided of key differences in the early development of the *G. laevis* group and the *G. rosea* group as compared to Gosner (1960) stages 18–26, and shows the main characters of limb, oral, optic and opercular development that are not reconcilable with the Gosner staging system during early development (see Appendix 3). Comparisons of the *G. rosea* group with tadpoles of the only other Australian nidicolous species, *Crinia nimbus* and the genus *Philoria*, are summarised in the Discussion.

REVIEW OF *GEOCRINIA* BREEDING BIOLOGY

This section reviews the known literature on *Geocrinia* breeding biology (as cited), supplemented with original observations while in the field during the study.

Terrestrial/exotrophic species: *Geocrinia laevis* group

Geocrinia leai

This species occurs mainly in forest habitats of southwestern Australia from the Darling Scarp east of Perth along the southwest coastal region to southern forests and Cape Leeuwin, and east to Albany (Main 1957). Dorsal colour is variable, but most have a broad brown to black band (which may be divided) on a lighter background. Ventral colour is dull, translucent greenish-yellow (Figure 1A,B). Males usually call from April to late October (Main 1957) from hidden shady sites on land within or beside dry creek beds, above existing water in ponds or swamps, or in other areas that will be flooded later. Frogs are mostly found in leaf litter, beneath or within clumps of sedges or grass, or under logs in moist areas beside creeks, swamps, ponds or dry creek beds later to be flooded during the winter wet period.

Eggs are laid on land from April to late October (autumn to spring), usually prior to rain, and are found in moist leaf litter, beneath matted reeds, under logs or attached to living vegetation. After the eggs have been laid, the male frog remains in the vicinity and resumes calling. For this reason, clutches of more than one female at different developmental stages may be found in the nesting territory of the same male. Eggs of a single clutch adhere together (Figure 3C). Several other clutches contained 52–96 eggs (Main 1957). Hatching begins from 15 days after the eggs are laid (Main 1957). Metamorphosis occurs in spring, usually from October (Main 1957). The duration of larval development from eggs reared to metamorphosis in the laboratory was 149–174 days (Main 1957).

Geocrinia laevis

This species occurs in Tasmania, southwestern Victoria including the Grampians, and across to Mount Burr in southern coastal South Australia (Woodruff and Tyler 1968). Adults have a brown dorsum, often with a darker bifurcated patch less obvious in some, but dorsal pattern is variable (Littlejohn and Martin 1964). Ventral surface of males from Garvoc is white with dark flecks and a dull yellow throat and in females it is all white with darker flecks and patches (Figure 1C-E).

In Tasmania calling begins in autumn from late February to April (Littlejohn and Martin 1964) and mostly from about mid-March to mid-May in southwestern Victoria, depending on the weather (Littlejohn and Watson 1973; Harrison and Littlejohn 1985). In peak periods males call day and night while hidden beneath vegetation in matted grasses, sedges or leaf litter and at the base of tussocks within or beside low-lying areas

Figure 1 Adults of the *Geocrinia laevis* complex. A-B = *G. leai* male, dorsal and ventral views, Walpole WA; C-D = *G. laevis* male dorsal and ventral views; E = *G. laevis* spent female ventral view, Garvoc, Vic; F-H = *G. victoriana* male, two dorsal views showing unusual colour variants from Carlisle River, Vic (photos Ron Anstis) and ventral view.

Table 1 Material and localities for embryos and larvae of the genus *Geocrinia*. N = number of clutches. Stage = Gosner (1960) for *G. leai*, *G. laevis* and *G. victoriana*. For the four members of the *G. rosea* group, the stages are as given in Table 2. All clutches were studied live, except for the preserved clutch of *G. rosea* at stages 18–19*.

Species	Locality/State	N	Stage
G. leai (northern)	Near Harvey, WA	2	9
	Kangaroo Gully, Darling Scarp, WA	3	9, 18, 20
G. leai (southern)	Frankland River near Walpole, WA	3	17, 19, 20
	Nornalup, WA	1	12
G. laevis	Garvoc, Vic	3	2–3
G. victoriana	Carlisle River, Vic	2	17, 19
G. alba	Forest Grove, 13 km SE Withcliffe, WA	2	23, 28
G. lutea	4 km NW Walpole, WA	3	25, 26
G. rosea	Pine Rd, Giblett block, near Pemberton, WA	3	23
G. rosea	Near Pemberton, WA	1	*18–19
G. vitellina	Spearwood Creek near Witchcliffe, WA	2	18, 28

that will later be flooded. Many make nest sites on sloping banks above more permanent ponds. Egg clutches are laid in shallow excavated depressions and usually a male (and sometimes a female if the eggs have been recently laid) is nearby. Littlejohn and Martin (1964) reported that six egg clutches contained a mean of 111 eggs (76–147). Hatching can be delayed for up to four months if conditions are not suitable (Littlejohn and Martin 1964).

Geocrinia victoriana

The range of this species is fragmented across Victoria with populations in eastern and central Victoria and the eastern end of the southwest region, where a narrow hybrid zone with *G. laevis* occurs (Littlejohn *et al.* 1971; Littlejohn and Watson 1985). Dorsal colour is variable, but many adults are brown or grey with or without a darker interorbital patch which continues and bifurcates posteriorly, tapering to a point down each side of the vertebral region. Dorsal colour is especially variable in the Otway district of the southwest (Littlejohn and Martin 1964), some bearing striking pale markings akin to lichen formations (Figure 1F,G). Males call from mid-March to November, depending on region and climate conditions. Calling sites are similar to those of *G. laevis* (Littlejohn and Martin 1964). Several males often call in a polyphonic chorus, each in response to others.

A detailed description of the oviposition process is presented by Littlejohn and Martin (1964), and eggs are usually laid in autumn (March to May). Egg clutches may be attached on land to reeds, in moss, in or under tussocks, in small hollows or at the edge of small puddles (Gollmann and Gollmann 1996b). The mean number of eggs in 20 egg masses from Kinglake West (Littlejohn and Martin 1964) was 121 (90–162). Early development has been well documented by Littlejohn and Martin (1964), Martin and Cooper (1972) and Gollmann and Gollmann (1991a). The mean diameters of ova prior to stage 10 vary from 3.1 mm at Kinglake West (Littlejohn and Martin 1964) to 1.9 mm (at Gellibrand; Gollmann and Gollmann 1996b), the latter nearer to the hybrid zone with *G. laevis*. Twenty capsules from Kinglake West had a mean external diameter of 6.2 mm (5.7–7.2; Littlejohn and Martin 1964). Gollmann and Gollmannn (1992b) reported that hatching time varied greatly among clutches, some hatching 2–4 days after flooding and others over an extended period of time. Tadpoles have been described by Littlejohn and Martin (1964), Gollmann and Gollmann (1995) and Anstis (2002).

Endotrophic species: *Geocrinia rosea* group

Geocrinia rosea

This species occurs within an area of about 1885.7 km^2 in southwestern Australia (Wardell-Johnson and Roberts 1993) around Warren River valley, Pemberton and Dombakup (Main 1957, 1965). Driscoll and Roberts (2008) found two separate genetic populations of *G. rosea*, the northern populations occurring in association with catchments of the Donnelly and Warren Rivers and Dombakup Brook, the southeastern populations in the Shannon and Gardner River catchments,

and a very narrow hybrid zone between northern and southeastern populations. Frogs commonly inhabit riparian seepage sites at stream headwaters, streams in minor valleys and terraces in major valleys (Wardell-Johnson and Roberts 1993) in Karri forests (*Eucalyptus diversicolor*), where they secrete themselves in muddy depressions beneath vegetation or leaf litter, beneath dense thickets beside small creek-lines, or sometimes in rotting logs away from creeks. Soil at breeding sites of all four species in this group is highly acid, normally does not dry out or get flooded, and consists mainly of moist fine and coarse sand, and organic peaty matter (Wardell-Johnson and Roberts 1993).

Adults are dark reddish-brown at night, usually with a darker bifurcated patch that is more obvious during daytime, and small scattered dorsal tubercles. Ventral colour is rose pink, and calling males have a black throat (Figure 2E,F). Males call from late winter to early summer (August to December; Roberts *et al.* 1990).

Eggs are laid singly on land within a small moist depression in peaty soil beneath vegetation, or in rotting logs, mainly in spring during September and October. Three nests collected near Pemberton in the vicinity of a calling male in an area of 0.5 m^2 contained 11, 12 and 13 embryos all at the same stage (Table 1). A further series of 30 nests from Pemberton contained from 26–32 embryos (Main 1957). Main (1956) states that larval duration in the field for *G. rosea* may be over 60 days.

Geocrinia lutea

This species is restricted to an area of about 148.2 km^2 in the south coast of Western Australia around Walpole and Nornalup (Wardell-Johnson and Roberts 1993). Frogs occur in seepage areas along the Deep River catchment area beside small creeks and swampy areas in heathland and forest, where they are hidden in moist sphagnum moss or peaty-mud depressions beneath or within clumps of vegetation.

Adults are dark brown at night with a slightly darker bifurcated patch and dorsal tubercles, and lighter brown by day. Ventral colour is white with a yellow wash, and males have a black throat (Figure 2C,D). Males call from July to December (Roberts *et al.* 1990; Roberts and Wardell-Johnson 1995).

Eggs are laid singly in a small frog-sized moist depression in peaty soil beneath vegetation or in sphagnum moss. Three nests collected in October near Walpole contained 11, 12 and 18 embryos at stages 25–26. Two were found near each other in a spherical well within a thick clump of sphagnum moss beside a small flowing creek. The third was uncovered beneath vegetation in a small excavated hollow in peaty mud beside a creek. Ova were not observed, but from observations of embryos prior to hatching which had large pale yolk sacs similar to other species of this group (Figure 11E), it is likely eggs are large and unpigmented.

Geocrinia alba

This species is restricted to about 56 discrete populations within an area of about 130 km^2 of remaining, partly cleared forest habitat in southwestern Australia in the Witchcliffe to Karridale region (Roberts *et al.* 1990, 1999; Wardell-Johnson and Roberts 1993; Conroy 2001). Frogs occur in seepage areas beside small creeks in forest where they are hidden in depressions in peaty soil beneath clumps of vegetation.

Adults are dark brown at night, usually with a slightly darker bifurcated patch and prominent dorsal tubercles. The ventral surface is all white in both sexes (Figure 2A,B). Males call from August to early December (Roberts *et al.* 1990; Roberts and Wardell-Johnson 1995; Conroy 2001).

Eggs are laid singly in a small moist depression about 2–3 cm diameter in sandy, peaty soil beneath vegetation beside small creeks. Clutch counts of 230 nests range from 1–19 (mean 11; Conroy 2001). Larval life span of various clutches ranged from about 28–98 days (most longer than 60 days) and clutches laid earlier in the season generally take longer to reach metamorphosis, probably due to cooler temperatures (Driscoll 1996).

Geocrinia vitellina

This species is currently known to occur in only six populations in an area of about 6 km^2 of suitable remaining forest in southwestern Australia in the Blackwood River (Spearwood Creek) region (Roberts *et al.* 1999; Tyler *et al.* 2000; Mitchell 2001). Frogs occur in seepage areas mainly on the eastern slopes of Spearwood Creek, where they are hidden in depressions in peaty soil beneath mats of vegetation (Tyler *et al.* 2000; Conroy 2001; Mitchell 2001).

Adults are dark brown at night with a slightly darker bifurcated patch and prominent dorsal tubercles. By day they may be quite pale with prominent darker tubercles. Ventral colour is rich egg yellow, usually with a whitish area over the lower abdomen, and mottled brown and white over underside of the hind limbs (Figure 2G,H). Males call from late winter to early summer and breeding is known to occur from late August to early December (Roberts *et al.* 1990; Conroy 2001).

Eggs are laid singly in 1–2 layers within the nest in a small muddy depression 18.2–29.1 mm maximum diameter (mean 23 mm) and 7.5–20.4 mm maximum depth (mean 13.8 mm; Mitchell 2001) in peaty soil beneath mats of clumping vegetation or dead grass. Clutch counts of 191

Figure 2 Adult males of the *Geocrinia rosea* complex showing contrasting ventral colour. Localities are given in Table 1. A-B = *G. alba*; C-D = *G. lutea*; E-F = *G. rosea*; G-H = *G. vitellina*.

nests range from 3–18 (mean 11; Conroy 2001) and another 23 clutches (Mitchell 2001) range from 5–15 (mean 10). The mean diameter of 42 large, unpigmented ova from eight clutches was 2.8 mm (2.6–3.1; Mitchell 2001). A large, thick, dense jelly capsule surrounds the vitelline membrane. A capsule at stage 18 has a very thin, adhesive external membrane that envelops each capsule and can be torn with a pin and separated from the dense jelly beneath. The mean diameters of 15 capsules from three clutches of *G. vitellina* was 9.1 mm (8.2–10.2; Mitchell 2001). The jelly capsule remains discrete and broadly rounded in form until the tail of each embryo begins to lengthen (Figure 11E), after which the jelly gradually expands then begins to break down prior to tadpoles hatching, and becomes more viscous and liquefied (Mitchell 2001). This consistency is then maintained during the rest of development and enables oxygen transfer and movement of tadpoles within the nest basin. Hatching occurred at a controlled temperature of 15°C when embryos were at stages 23–25, 19–26 days after the eggs are laid, and the mean total length of 15 hatchlings was 11.0 mm (9.5–13.0; Mitchell (2001). Larval duration was 86–87 days in embryos raised at 15°C (Mitchell 2001).

METHODS

Material and measurements

Material and localities are listed in Table 1. No live material was available earlier than stages 18 for *G. vitellina* and *G. rosea*, stage 24 for *G. lutea*, stage 23 for *G. alba*, stage 9 for *G. leai*, stage 2 for *G. laevis* and stage 17 for *G. victoriana.* Material will be lodged in the Western Australian Museum and Museum Victoria at the conclusion of further studies.

Measurements of live and preserved embryos and tadpoles were taken to the nearest 0.1 mm using an ocular micrometer attached to a Wild M5 stereoscopic microscope. Tadpole descriptive terminology follows Anstis (2002), and the labial tooth row formula (LTRF) of Altig (1970) is used in oral disc descriptions. Descriptions of pigmentation of the tail refer to skin colour only. SVL of metamorphs was measured in ventral view. For the embryos and tadpoles of *G. leai* and *G. laevis,* a more detailed description is provided, and for *G. victoriana*, only additional material and revised descriptions with reference to previous studies listed above are included. Specimens at various stages were anaesthetised in 1% chlorbutol solution prior to photography and measurement, then preserved in 4% buffered formalin (Tyler 1962). Photographs of live specimens were taken with a Nikon D80 and 60 mm macro lens. For photography, it was necessary to anaesthetise and immerse the terrestrial tadpoles of the *G. rosea* group in water for lateral views, and because of the similarity between these species, photographs of only two species are provided in Figure 12. Drawings of various stages of preserved specimens were made with the aid of a drawing tube attached to the microscope. Specimens of *G. rosea* used for Figure 13 were stained with 1% Toluine Blue.

Staging

Any developmental descriptions of the nidicolous *G. rosea* group of tadpoles are difficult because their development cannot be aligned with the universally accepted staging table devised for aquatic tadpoles by Gosner (1960) beyond stage 19 (Mitchell 2001). De Bavay (1993) presented a comprehensive description and staging table for the nidicolous species *Philoria sphagnicola*. Mitchell (2001) adapted some of the early De Bavay stages (19–26) to better apply them to the early development of *G. vitellina* and used the De Bavay stages (27–37) for the rest of the development to metamorphosis. However, she noted difficulties with this system because it uses the development and disappearance of keratinised jaw sheaths for some stages and these never develop in the *G. rosea* group. As *Philoria* embryos differ from the *G. rosea* group in a number of significant ways, I present a staging table (Appendix 1) from stages 18–40 (metamorphosis complete) that specifically targets the *G. rosea* group and incorporates some adaptations of stages 19–26 from Mitchell (2001). In this table, Gosner (1960) stages (indicative of hind limb development only) are given in parenthesis to assist comparison with the Gosner limb development stages in the exotrophic *Geocrinia* species. For the exotrophic species, stages in Appendix 2 are those of Gosner (1960) and incorporate features for stages 20–26 of Gollmann and Gollmann (1991a).

While most features of the three exotrophic species of the *G. laevis* group correspond adequately with Gosner stages from stage 26 to metamorphosis, synchronisation differences occur during stages 21–25 as a result of the slower development of mouthparts and gut and the precocious development of the hind limb buds. Stages 20–26 of Gollmann and Gollmann (1991a) devised to suit these early development stages of the eastern *G. laevis* and *G. victoriana* are incorporated here for *G. leai* as well (Appendix 2).

Rearing

Embryos were maintained in a mobile caravan and vehicle over a period of three and four weeks (southwestern species) or one week (southeastern species), before being transported to NSW. As a result, temperature could not be controlled during development.

Figure 3 Habitat and comparative sample stages of embryonic development in life of the *Geocrinia laevis* group. Scale bar represents 1 mm. A = habitat of *G. victoriana*, pond at Carlisle River, Vic; B = non-hydrated egg clutch of *G. leai* on land beneath dead reed stems on sloping bank; C = hydrated egg clutch of *G. leai* beside pond, Nornalup, WA; D = embryos of *G. leai* at stage 19 tightly coiled within vitelline capsule; E = *G. laevis* at stage 21 (left) compared with *G. victoriana* at stage 20 to show size difference, which persisted during development; F = 'twin' *G. victoriana* embryos at stage 21 in individual vitelline capsules, arrow indicates single outer membrane; G = *G. leai* at stage 25 prior to hatching; H = *G. leai* at stages 24–25, arrow indicates one just hatching; I = *G. laevis* stage 26 showing tooth rows and spiral gut, arrow indicates reduced adhesive glands; J = *G. leai* hatchlings, stage 25 from Nornalup, showing gold iridophores and bright gold tip of tail muscle.

Table 2 Timeline in hours and minutes for the development of a single clutch of *Geocrinia laevis* from stage 2 until the first 7 embryos hatched. Timing begins when tadpoles were first collected at stage 2. Actual time from oviposition to stage 2 is unknown, but is likely to be no more than about 4–5 h. Times represent only the first embryos to enter each stage, as individual differences in developmental rate were observed. Stages represent those given in Table 1. Temperature range: 8–22°C.

Time (h/min)	Stage
0	2
4.24	6–7
25.42	9
45.15	12
68.56	14
92.06	17
104.09	18
169.51	19
190.15	20
217.42	21
246.00	22
289.42	23
361	24
389	25
554.30	26
582.30	26 (hatched)

Terrestrial/exotrophic species: Geocrinia laevis *group*

During the terrestrial phase, embryos were placed in sealed containers (18 x 12 x 6 cm high) and reared on moist leaf litter, moss or on the vegetation to which they were originally attached when collected. Each clutch was lightly covered with damp paper towel and sprayed briefly each day with rain water. Various stages of development were photographed in and out of water. Moisture was increased from stage 24, when tadpoles were more active within the capsules. From stage 25 (*G. leai*) and stage 26 (*G. laevis* and *G. victoriana*), embryos were placed in containers with water depth to at least half capsule diameter to initiate hatching. Some were laid in very shallow water when at stages 22–23 to determine if earlier hatching was possible.

Hatched tadpoles were reared in plastic dishes (40 cm diameter, opaque sides) containing rain water to a depth of 15 cm, washed river sand, sediments, leaf litter and rocks. Dishes were placed outdoors under translucent overhead cover, with access to morning sun. In addition to available algae and sediments in the containers, tadpoles were fed three times per week on small amounts of finely crushed algae discs and appeared to maintain good condition and steady growth.

Endotrophic species: Geocrinia rosea *group*

Each nest was excavated from the substrate to a radius of about 5 cm around the nest, including a depth of about 6 cm of soil or moss which was retained around the nest for support and moisture. The nest and surrounding substrate was placed in a circular plastic container (diameter 11 cm, depth 9 cm), and covered with a perforated lid. A dampened piece of fine gauze material was placed beneath the lid to aid moisture retention.

Embryos were observed twice a day. Feeding was not required and the thick peat or sphagnum moss substrate maintained moisture in the container throughout development.

RESULTS

Early development for the three exotrophic species is essentially similar and a general description for all three from stages 17–26 is given in Appendix 2. For the *G. rosea* group, larval development is similar among the four species so a composite description is given below and a staging system presented in Table 2. Observations on the breeding biology and larval descriptions of the aquatic tadpole phase are given for each species.

Geocrinia leai

Eggs (Table 3, Figure 3)

Females lay all eggs in a single clutch, or deposit the clutch in multiple smaller clusters. One female in captivity laid her clutch in two clusters about 3 cm apart, with 37 eggs in one cluster and 50 in the other. Five clutches from Kangaroo Gully and near Harvey ranged from 38–87 eggs. Only in the case of the 87 eggs laid by one captive pair is it known that these were the total number laid by one female.

Embryos prior to stage 9 were not observed. The animal pole is dark brown to black and vegetal pole is white. Two jelly layers surround the vitelline membrane, the outer of which is enclosed in a thin, strongly adhesive membrane that joins each capsule to other capsules and the supporting substrate material. Diameters of individual layers of a single hydrated preserved egg at stage 9 (from outermost membrane to vitelline membrane) were 5.8, 5.3, 3.9 and 2.3 mm.

Table 3 Measurements of live ova of the *G. laevis* group. N = sample size, Stage = Gosner (1960). Capsules are non-hydrated except for * which indicates hydrated capsules. Northern and southern coastal *G. leai* are indicated (see Table 1). Separate measurements are given for the three *G. laevis* clutches.

Species	N	Stage	Ovum	Capsule
G. leai (northern)	18	9	2.0 (1.9–2.1)	2.7 (2.4–3.1)
G. leai (southern)	9	12	1.5 (1.5–1.6)	6.0 (5.8–6.3)*
G. laevis clutch 1	16	9	1.7 (1.6–1.8)	
G. laevis clutch 2	11	7	1.5 (1.5–1.6)	2.7 (2.6–2.9)
G. laevis clutch 3	8	5	1.6 (1.5–1.6)	2.7 (2.3–3.2)
G. victoriana	25	9	2.2 (2.1–2.4)	3.0 (2.6–3.3)
G. victoriana	7	23		4.2 (4.0–4.5)

Table 4 Measurements of total length (TL) and body length (BL) of hatchlings of the *G. laevis* group. N = sample size, Stage as in Gosner (see Appendix 2). Those marked '*' hatched earlier than others from the same clutch. Northern and southern coastal populations of *G. leai* are indicated (see Table 1).

Species	N	Stage	TL	BL
G. leai (northern)*	10	23, 24	7.7 (6.9–8.1)	
G. leai (northern)	10	25, 26	9.7 (8.9–10.5)	3.1 (2.9–3.4)
G. leai (southern)	9	25	8.7 (8.4–9.3)	2.8 (2.7–3.0)
G. leai (southern)	11	26	9.6 (8.9–10.6)	3.3 (2.9–3.5)
*G. laevis**	30	26	10.0 (9.0–10.9)	3.2 (2.7–3.5)
G. laevis	27, (1)	26, (27)	9.6 (8.5–12.5)	3.2 (2.8–3.5)
*G. victoriana**	9	23	9.8 (9.7–10.0)	3.3 (3.2–3.4)
G. victoriana	16	26	11.9 (11.4–12.6)	4.0 (3.9–4.2)
G. victoriana	14	27	12.7 (12.4–13.4)	4.2 (3.9–4.5)

Hatchlings (Table 4, Figure 3)

A clutch from Nornalup began hatching at stage 25, 21 days after the eggs were laid. Hatching stages and times were variable, but most embryos were actively writhing within the capsule by stages 24–25, when the jelly capsule began to expand further and gradually break down. Most tadpoles then wriggled out of the jelly during stages 25–26. Two samples of embryos at stages 21–22 and 23–24 hatched readily when placed in shallow water, but immersion did not trigger hatching in all embryos at these earlier stages.

Hatchlings have lateral, golden eyes and a cylindrical body, with the posterior tooth rows not yet fully complete. They are brown dorsally, with numerous gold iridophores over body and dorsal tail muscle (Figure 3J). The gut has about three thick yolk-filled loops, but is not yet fully developed in length (Figure 3I). The yolk supply gradually diminishes in unhatched tadpoles, and very late hatchlings are less vigorous.

Tadpoles (Figures 4A, 5)

The largest tadpole found had a total length of 37.0 mm with a body length of 11.5 mm (stage 41, Walpole). The following general description is based mainly on a typical tadpole at stage 37, with relevant ontogenetic and geographical comparisons for southern coastal and northern populations (see Table 5 for larval measurements).

Body: Small, cylindrical to plump (southern coastal; Figure 5A) or more oval (northern; Figure 5B) and slightly wider than deep across abdomen; slightly broader across gill region in southern coastal compared with northern tadpoles. Snout broadly rounded and slightly more elongate in dorsal view in northern tadpoles than in southern coastal tadpoles, rounded in lateral view. Eyes quite close to tip of snout and lateral with slight dorsal tilt after hatching to about stage 27 (Figure 3J). As the body grows, the eyes are either lateral to near lateral in southern coastal tadpoles or slightly dorsolateral in northern tadpoles (Figure 5A,B). Iris mostly copper-gold, gold ring around pupil and darker at each side. Nares moderately spaced, open dorsoanteriorly (mainly dorsally in earlier stages) and equidistant between snout and eyes. Spiracle visible from above, opens dorsoposteriorly (posteriorly at stages 25–26) on or just below

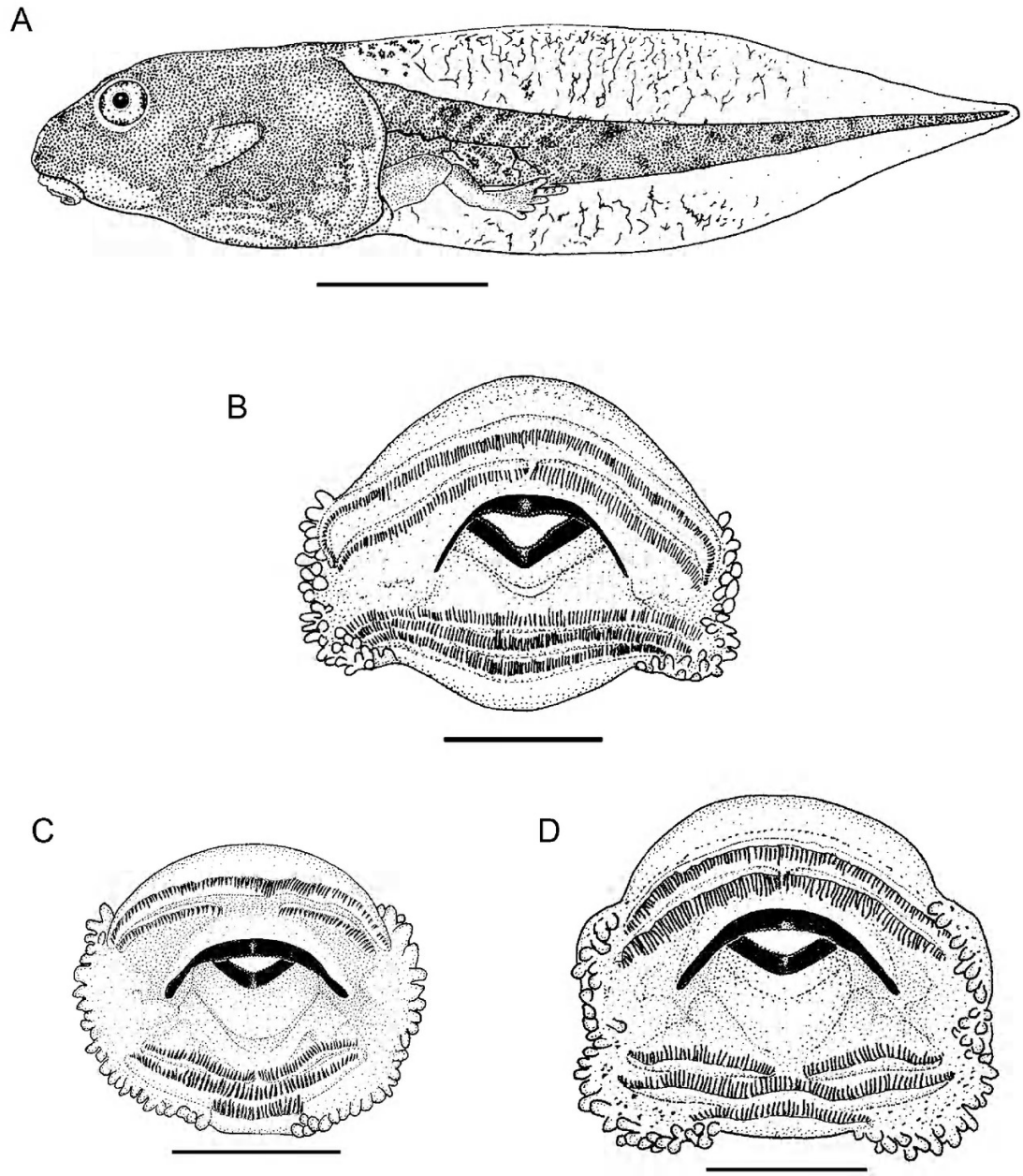

Figure 4 Preserved tadpoles and oral discs of the *G. laevis* group. Scale bar in A represents 5 mm, scale bars in B-D represent 1 mm. A = Tadpole of *Geocrinia leai* at stage 38 in lateral view; B = oral disc of *Geocrinia leai* at stage 38 from Walpole (southern): note length of P^3 tooth row and posterior medial gap in papillae; C = oral disc of *G. laevis* from Grampians, Vic. (Anstis 2002); D = oral disc of *G. victoriana* from Warburton, Vic. (Anstis 2002).

horizontal body axis, posterior to midpoint of body; outer edge of opening flares laterally and inner edge is unattached to body. Vent tube dextral (type (a); Anstis 2002), short, opens partway up ventral fin, mostly unattached to fin behind. A tiny hind limb bud is first visible at stage 25.

Tail: Fins moderately arched. Dorsal fin begins just onto body, arches to about midpoint and tapers to a rounded tip in northern specimens or a more elongate to narrowly rounded tip in southern coastal specimens. Ventral fin less arched, of similar depth along length before tapering. Muscle shallow to moderate, tapers to narrow point.

Pigmentation: Dorsum of hatchlings at stages 25–26 brown or dark brown with fine gold iridophores over body and tail, often forming a gold stripe down each side of head and body and merging to single stripe at base of body and down dorsal surface of tail muscle (Figure 3J). Body wall mostly transparent around snout. Sides of body with gold specks over abdomen. Venter mostly transparent with a few copper-gold flecks.

By stage 31 and beyond, some tadpoles retain dull gold dorsal stripes down each side of the darker vertebral region, and others become more uniformly dark brown, rusty-brown or silvery-grey,

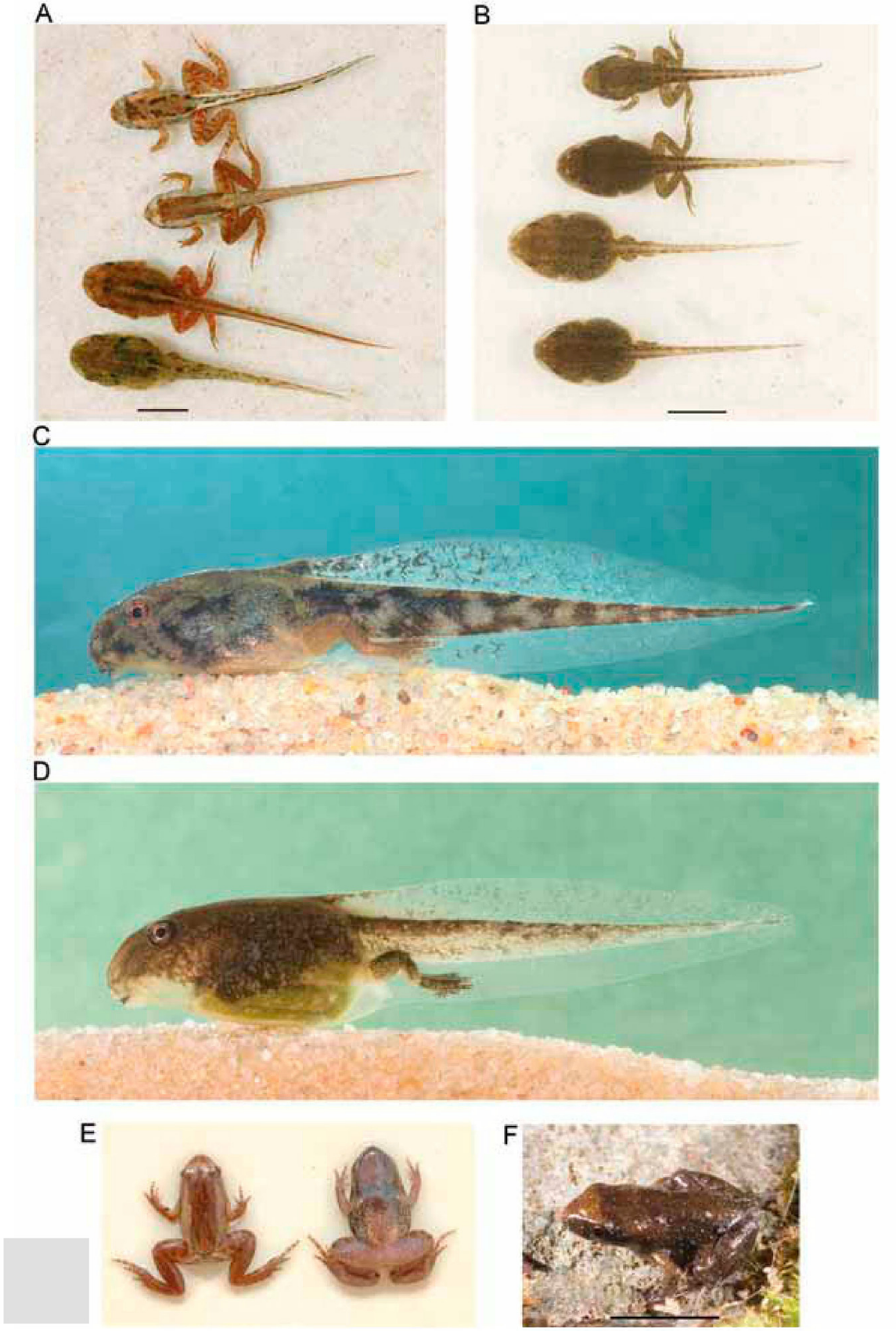

Figure 5 Tadpoles of *Geocrinia leai* from northern and southern populations in life showing differences in body form and pigmentation. Scale bar represents 5 mm. A = (from top down, dorsal view) stage 42 and 42 showing colour variation, stage 41 and stage 37 from southern site near Walpole; B = stage 42, 41, 37 and stage 35 from northern site, Kangaroo Gully, Darling Scarp; C = stage 40 lateral view, southern site near Walpole; D = stage 38 lateral view, northern site Kangaroo Gully; E = stage 46 dorsal and ventral views, Walpole; F = stage 46, Kangaroo Gully.

often with dark flecks or patches. Snout often more translucent anterior to eyes, especially in southern coastal tadpoles. Darker dorsal pigment beneath often shows through in patches where iridophore pigment does not form a complete cover. Rows of fine gold lateral lines visible. Sides vary from a fine gold or copper layer over dark beneath to broader silvery-grey patches (dark between); opaque copper sheen extends from below spiracle to venter where it covers most of abdomen by stage 27 apart from a band of melanophore stippling down the middle. By stage 37, copper sheen is denser with the band of stippled melanophores down the middle from heart to mid-abdomen; mostly clearer anteriorly except sides of gill region, where a dark layer beneath is mostly covered with silver-gold iridophores. Northern specimens do not appear to vary as much in pigmentation and most observed were more uniform dark brown with less obvious lighter dorsal stripes (Figure 5B).

Fins clear with dense melanophore flecks and reticulations mainly on dorsal fin, and finer ones posteriorly on ventral fin; small gold clusters scattered over both fins. Tail muscle usually uniform dark brown to black with variable small to broad gold or silvery patches or mottling, or a more continuous layer of silver, gold or rusty brown.

Oral Disc (Figure 4B): Oral disc ventral, almost as wide as snout, slightly emarginate (mainly in northern individuals). No papillae around anterior margin, wide medial gap in posterior papillae. The posterior medial gap in papillae is almost half the width of the oral disc for the southern coastal specimens (mean ratio of posterior gap width to oral disc width = 0.49), whereas for northern specimens the gap is just over one-third the width of the oral disc (mean ratio = 0.38; Table 7). There is one row of marginal papillae laterally and around posterior corners of the disc, with from none to two additional rows of submarginal papillae, often more numerous at each side of the lower labium. Most specimens from Kangaroo Gully (northern) had no submarginal papillae, a few had one submarginal row on the lower labium only. All southern coastal specimens (Walpole and Nornalup) had submarginal papillae in one or two irregular rows on the lower labium and one row on each side of the anterior labium.

Two anterior and three posterior tooth rows, A^2 with a narrow medial gap; $P^{1,2,3}$ usually all entire (P^1 occasionally with very narrow gap) and of similar length; P^3 slightly shorter than P^2 and extends beyond the width of the posterior medial gap in papillae on each side. P^3 row consistently more than half the width of the oral disc (P^3/ODW = 0.6) in northern tadpoles to almost three-quarters the width of the oral disc (P^3/ODW = 0.7) in southern coastal tadpoles (Table 6). Jaw sheaths slender, upper quite broadly arched with long sides (Figure 4B), lower sheath slightly more heavily keratinised than upper. LTRF = 2(2)/3.

Larval duration and metamorphosis

Tadpoles metamorphose in the field and in the laboratory in late October. Twelve metamorphs at stage 46 from Walpole had a mean SVL of 11.0 (9.6–12.8 mm) and showed the typical colour patterns of the adult with a broad dorsal band (Figure 5E). Some were reddish-brown overall, but most were yellow-brown. The underside was mostly transparent with numerous small whitish specks including limbs. Northern specimens were often dark brown with a lighter area anterior to eyes and scattered fine bluish tubercles (Figure 5F).

Geocrinia laevis

Eggs (Table 3, Figure 6)

Three clutches were collected near Garvoc, Victoria on 1 May 2008, each from a small depression in damp, matted dead grasses beneath leaf litter or low growing surface vegetation within a dry swamp area. They contained 150, 168 and 183 eggs, respectively. Rain had occurred in the area during the previous two days and brief heavy rain (25 mm) had fallen two weeks prior. One clutch approaching stage 2 was found at 1330 hrs within a slightly excavated nest site, with a calling male and a spent female nearby. Each clutch formed a sticky rope or chain of tightly packed adherent eggs (Figure 6A). This entire clutch measured 4.2 cm long and 1 cm wide. Another clutch laid by a second pair was also at stage 2 and in two clusters but otherwise similar to the first. Under normal seasonal conditions after heavier, more prolonged rain in early winter, this area fills with shallow water and breeding ideally takes place some weeks before the pond fills.

During early cleavage (stages 3–7), the animal pole was dark brown and the vegetal pole was white (Figure 6B,C). Cleavage follows normal Gosner stages. From stage 10, the vegetal pole was light grey-brown. Stages 17–26 (to hatching) are described in Appendix 2, with reference to Figures 6 and 7, and Table 2 provides a developmental sequence for one clutch of *G. laevis* from stage 2 to first hatching at stage 26.

The capsule is small and firmly spherical while eggs are developing out of water prior to hatching, and non-immersed capsules maintain a similar diameter throughout most of embryonic development. There are two jelly layers around each embryo, the outer covered with a very thin adhesive membrane that attaches each egg to adjacent ones and to substrate material. From stage 23 onwards the growing embryo is very tightly

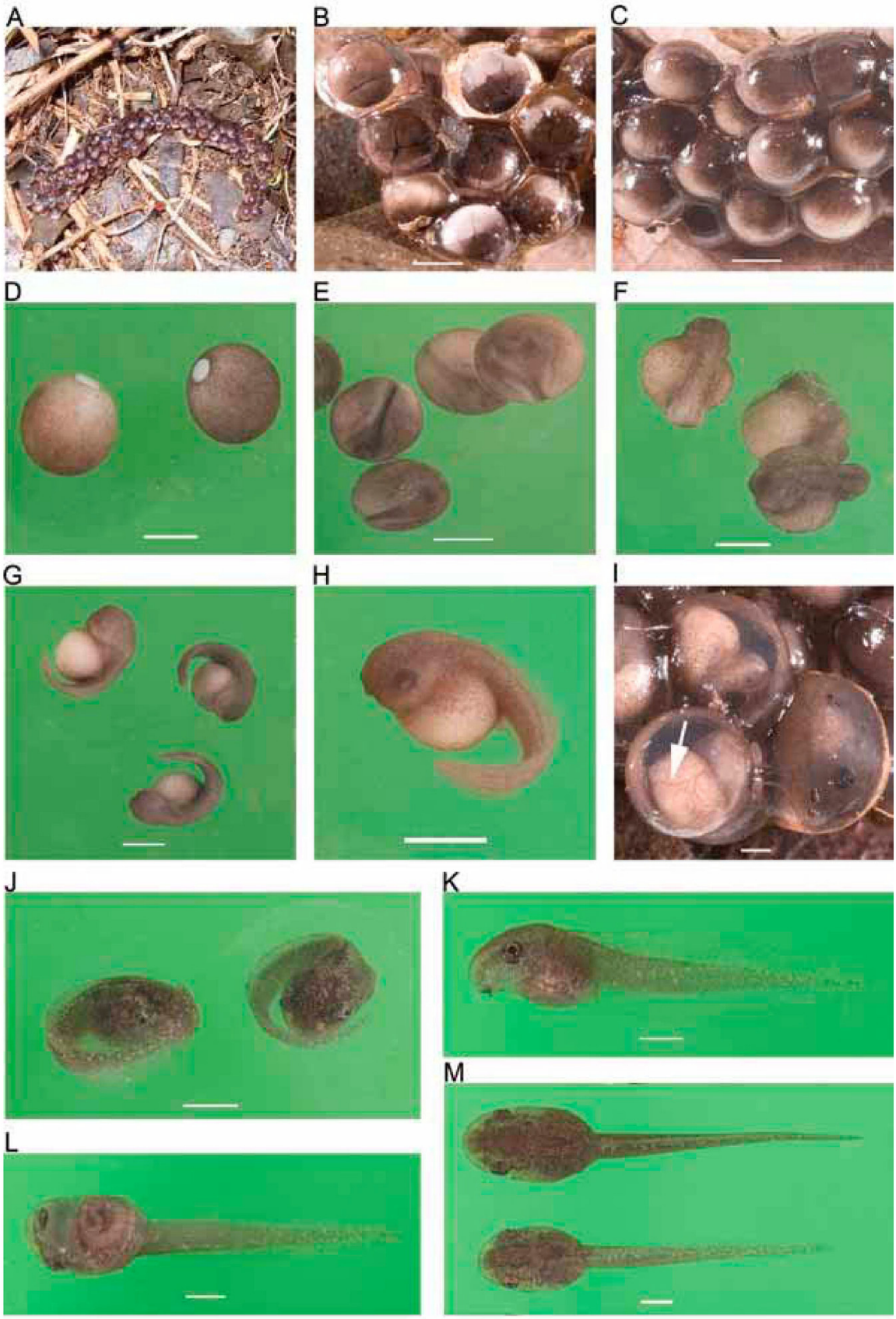

Figure 6 Sample stages of embryonic development in life of *Geocrinia laevis* and *G. victoriana* up to hatching. Scale bar represents 1 mm. A-L = *G. laevis.* A = egg clutch in nest site beneath surface leaf litter. B = stages 5–6; C = stage 9; D = stage 12; E = stage 14; F = stage 17; G = stage 19; H = stage 21; I = stage 23, arrow to vitelline blood vessels; J = stage 25; K-L = stage 26 hatched. M = hatched *G. victoriana* at stage 26 (above) compared with hatched *G. laevis* at stage 26 (below).

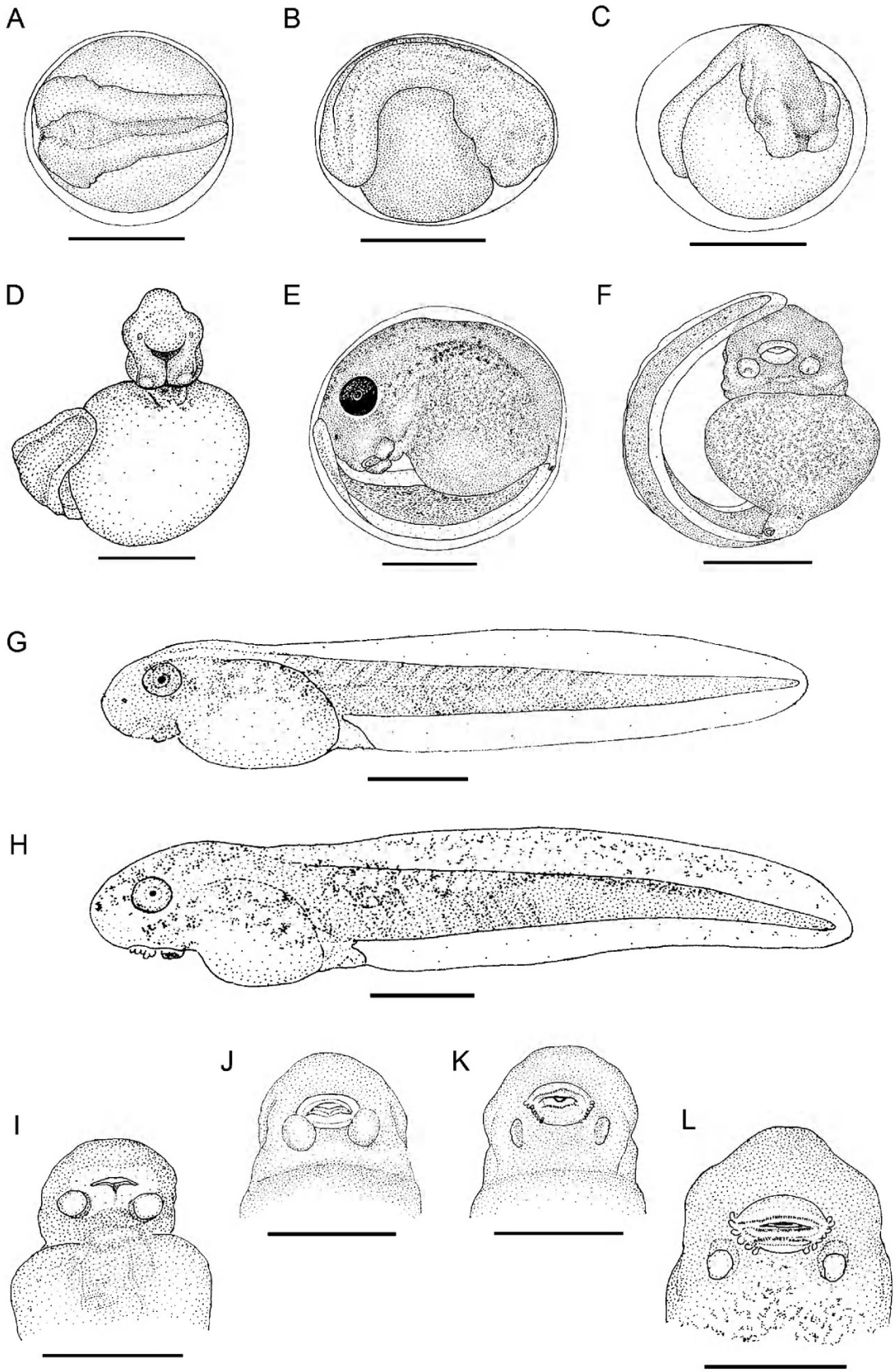

Figure 7 Sample preserved stages of embryonic development of the *Geocrinia laevis* group prior to hatching. Scale bar represents 1 mm. Stages are those of Gosner (1960) with those of Gollmann and Gollmann (1991) incorporated for stages 21–26 (Appendix 2). A-C = *G. laevis* stage 14 dorsal, stage 17 lateral, stage 17 anterior views; D = *G. victoriana* stage 19; E-F = *G. laevis* stage 22 lateral and ventral views; G = *G. leai* stage 23 lateral view, iris now golden; H = *G. leai* stage 24 lateral view; I-L = development of oral disc, ventral view for *G. laevis* stage 21, *G. laevis* stage 23, *G. leai* stage 24 and *G. victoriana* stage 25.

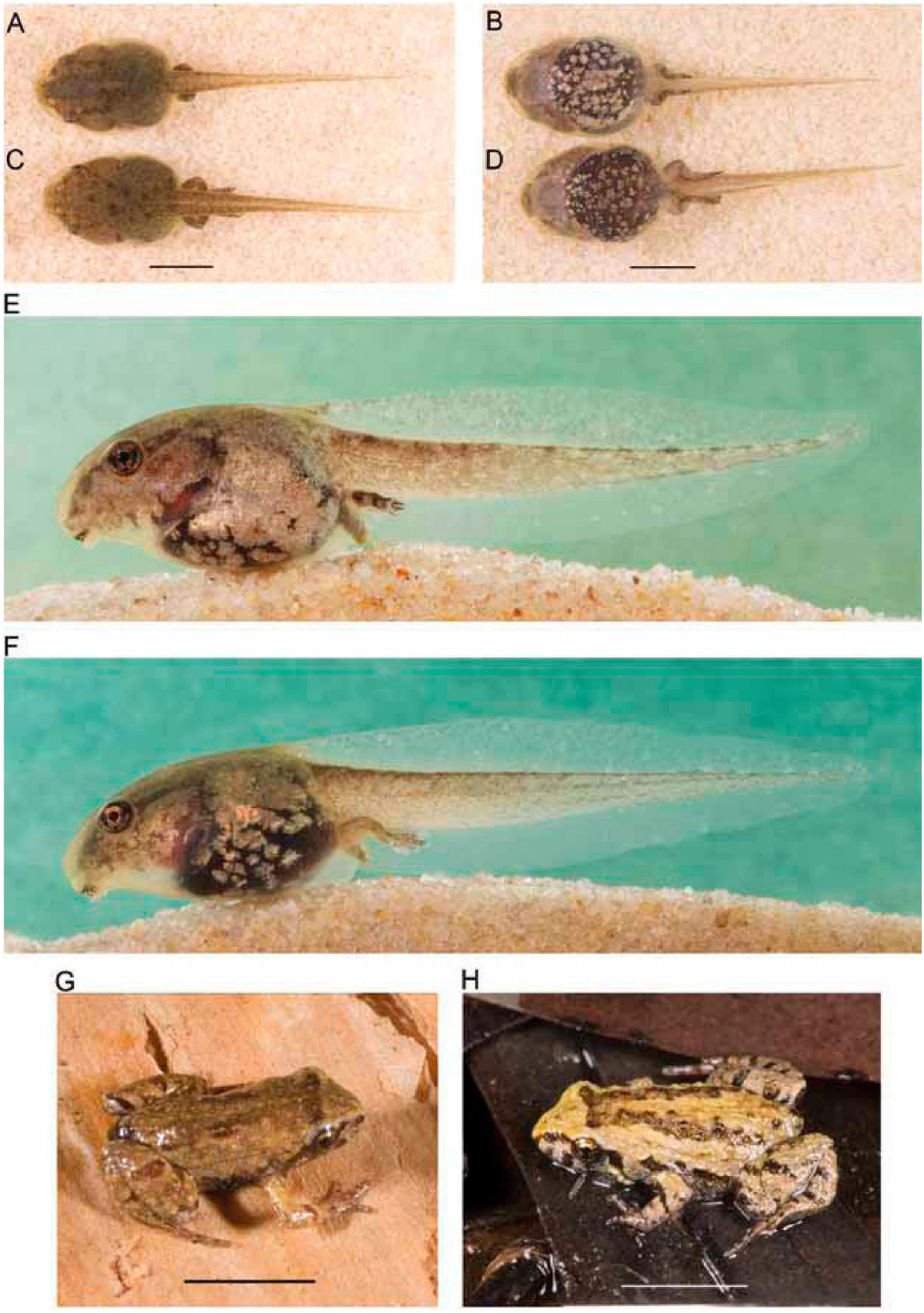

Figure 8 Tadpoles of *G. laevis* and *G. victoriana* in life. Scale bar represents 5 mm. *G. laevis* are from Garvoc and *G. victoriana* from Carlisle River, south-western Vic. A-B = *G. laevis* stage 37 dorsal and ventral view; C-D = *G. victoriana* stage 38 dorsal and ventral view; E = *G. laevis* stage 34 lateral view; F = *G. victoriana* stage 37 lateral view; G = *G. victoriana* stage 46; H = *G. laevis* stage 46.

coiled within the small non-hydrated capsule (Figure 6I,J). If immersed in water, the jelly layers expand (the inner layer is poorly defined and difficult to see). Diameters of individual layers of a single hydrated preserved egg at stage 7 (from outermost membrane) are 3.0, 2.7 and 2.2 mm (ovum 1.6 mm).

Hatchlings

When larvae were at stage 26, each of the three clutches was partly submerged in water. Within 12 h after immersion in water, the first few tadpoles from three clutches hatched at stage 26, 26–27 days after the eggs were laid. Hatching was then staggered over a further 25–47 days until the last one hatched 72 days after the eggs were laid. Those still unhatched remained at a similar size and stage or grew slightly within the jelly capsule, and a few did not hatch until stage 27.

No external gills were present during development. Hatchlings at stage 26 (Figure 6K-M – bottom tadpole) had fully developed mouthparts and gut and began feeding soon after they entered water. They were translucent brown with gold iridophores over the dorsum, sides of body, dorsal fin and tail muscle and the eyes were dense gold. The dorsal fin and posterior end of the ventral fin were lightly pigmented with melanophores. The ventral surface was dark over the abdomen with a few iridophore clusters and the anterior half was unpigmented.

Tadpoles (Figure 8A,B,E)

The largest tadpole raised in captivity had a total length of 29.8 mm and body length of 11.0 mm (stage 37; Table 5).

Body: Small, cylindrical to fairly plump, wider than deep across abdomen. Snout broadly rounded in dorsal view, rounded in lateral view. Eyes lateral (may appear more dorsolateral in preserved specimens) and set quite close to snout, iris mostly golden, darker rim at sides. Nares moderately spaced, about equidistant between eyes and snout and open anterodorsally. Spiracle visible from above, outer edge of opening flares laterally and inner edge is unattached to body; opens posteriorly or posterodorsally on horizontal body axis, posterior to midpoint of body. Vent tube dextral, inferior corner of opening just above edge of ventral fin in life (may shrink further in preserved specimens).

Tail: Fins shallow to moderate and similar in shape. Dorsal fin begins near end of body, arches slightly to near midpoint of tail and tapers evenly to narrowly rounded tip. Ventral fin slightly less arched. Muscle moderate and tapers to a narrow point.

At stage 26, hatchlings mostly had melanophore flecks and a few gold flecks over dorsal fin, a few melanophores near end of ventral fin, and melanophore stippling all over muscle. Melanophore flecks gradually increased over dorsal fin from stage 27 onwards and by stage 36, most had pigmented venation and numerous dendritic melanophores over the dorsal fin while the ventral fin remained clear with very little pigmentation. By stage 37, the tail muscle was mostly brown with gold patches dorsally and some scattered gold clusters laterally.

Pigmentation: Dorsum dark brown macroscopically (layer of fine gold iridophores over black beneath) at stages 27 and 28, sides of body with copper clusters over black at stage 27 that gradually increased in area to cover entire sides by stage 30 in most tadpoles. Clearer body wall was visible around head and sides in dorsal view, with a few small melanophore flecks. By stage 34, a continuous dark dorsolateral stripe was usually present from naris to eye then behind each eye, with a thin dark border around brain region and behind gill regions in some tadpoles. Nares were surrounded by gold, and tadpoles of some clutches had a weakly defined dull gold middorsal stripe. The black dorsal layer beneath was gradually obscured during later stages as fine iridophores increased over most of body wall, and the gold middorsal stripe became less apparent.

By stage 37, some tadpoles had scattered diffuse darker spots over dorsum. Venter was dark over abdomen from stage 26 with a few gold flecks, anterior half transparent. During stages 27–30, copper clusters became larger over abdomen ventrally until, in many, they almost covered the abdomen by stage 33, with a few dark gaps between. A dark layer with fine gold iridophores above covered each side of gill region ventrally.

Oral disc (Figure 4C): Oral disc ventral, not emarginate, mean width 2.4 mm (2.1–2.6) mm. No papillae around anterior margin, posterior medial gap in mostly single row of marginal papillae, occasionally two rows on posterior corners of lower labium. Some have a few submarginal papillae anteriorly. Two upper and three lower tooth rows; A^2 with a distinct or narrow medial gap, P^1 with or without a narrow medial gap – tadpoles from the same clutch appear to be the same in this respect (gap or no gap). P^3 is the shortest row, usually slightly less than one-third the width of the disc and about the same width as the posterior medial gap in papillae. Jaw sheaths slender; upper jaw sheath broadly arched. LTRF = 2(2)/3(1) or 2(2)/3.

Larval duration and metamorphosis

Tadpoles from eggs laid on 1 May 2008 metamorphosed from 28 September to 11 October 2008, giving a larval duration from eggs to

metamorphosis in captivity of 150–163 days. Metamorphosis occurs in spring from late September to early November (this study; Littlejohn and Martin 1964), after autumn hatchlings have over-wintered. Eighteen metamorphs from Garvoc at stages 45 and 46 had a mean body length of 9.3 (8.4–10.1) mm. They resembled the adult and were brown or yellow-brown with a darker bifurcated dorsal patch that bridged the eyes before dividing posteriorly on either side of the vertebral region (Figure 8H). Many had a row of small pale tubercles down the middle of each side of this patch, and some had a coppery tinge over the dorsum. The venter was dark grey finely suffused with white.

Geocrinia victoriana

Eggs

The ovum is pigmented; animal pole dark brown, vegetal pole white. Embryos at Carlisle River were found on 30 April 2008, attached to dead matted reed stems and hidden beneath overhanging reed clumps on a sloping bank about 20 cm from the edge of a permanent dam (Figure 3A). Both clutches at stages 17 and 23 were found within the same nest site near a calling male. Non-hydrated capsules are smaller (Figure 3B), and expand when hydrated. The jelly capsule is comprised of two main layers of approximately equal thickness surrounded by a thin adhesive outer membrane that adheres each capsule to adjacent ones and to substrate material. In one clutch from Carlisle River, three 'twin' pairs from the same clutch were observed, in which a single outer membrane enclosed the entire jelly layers of two adjoining embryos (Figure 3F). The first jelly layer just beneath the outer membrane expands readily in water; the inner layer is difficult to detect visually in live embryos without staining.

Hatchlings

The first tadpoles to hatch from one clutch did so at stage 26, 24 h after initial immersion in water (27 days after the eggs were laid) and the final two tadpoles (also at stage 26) hatched 22 and 27 days after immersion (48 and 53 days after the eggs were laid). Some hatchlings were larger and at stage 27. A second clutch began hatching at stage 23 after first partial immersion in water one day after collection, then hatching was staggered thereafter over 14 days. Hatchlings were generally larger than those of *G. laevis* (Table 4), but otherwise similar (Figure 6M).

Tadpoles (Figure 8, Table 5)

Tadpoles are essentially similar to those of *G. laevis* and *G. leai*, but can be distinguished from *G. laevis* by the form of the vent tube in life. In *G. victoriana* it is shorter, broader and opens higher up the ventral fin than in *G. laevis* or *G. leai* tadpoles. This character may be less reliable in preserved specimens, in which the vent tube may shrink slightly, but only in *G. laevis* is the inferior corner of the opening normally attached as low as just above the edge of the ventral fin.

Oral Disc (Figure 4B-D, Tables 6, 7): *G. victoriana* had a mean oral disc width of 3.0 mm and *G. laevis* and *G. leai* had a mean oral disc width of 2.4 mm. Other differences in the oral disc among the three species of the *G. laevis* group include:

- oral disc slightly emarginate (*G. victoriana* and *G. leai*); not emarginate (*G. laevis*)
- width of posterior gap in papillae greatest in *G. leai*: mean ratio of width of posterior medial gap in papillae to width of oral disc (PG/ODW) in species order from greatest to smallest: southern coastal *G. leai* (0.49), northern *G. leai* (0.38); *G. victoriana* (0.31), *G. laevis* (0.29)
- P^3 tooth row longest in *G. leai*: mean ratio of length of P^3 tooth row to oral disc width (P^3/ODW) in order of species from longest to shortest: southern coastal *G. leai* (0.7), northern *G. leai* (0.6); *G. victoriana* (0.43) and *G. laevis* (0.38)
- mostly two rows posterior papillae and 1–2 anteriorly (*G. victoriana*; Figure 4D); mostly one row posterior papillae (northern *G. leai*) with no submarginal papillae (occasional specimen with one row posterior submarginal papillae on lower labium only), southern coastal *G. leai* with 2–3 rows posterior papillae and two rows on anterior labium (Figure 4B); mostly single row of papillae around disc, occasionally a few submarginal papillae on posterior corners (*G. laevis*).

Larval duration and metamorphosis

Tadpoles from embryos at stage 17 on 30 April 2008 metamorphosed from 26 September 2008 to mid October. Adding about four days for development from fertilisation to stage 17 (similar to *G. laevis*), the larval life span for this clutch in captivity ranged from about 150–178 days. Metamorphosis occurs in spring (Littlejohn and Martin 1974; this study), after autumn hatchlings have overwintered. Eleven metamorphs from Carlisle River at stages 45 and 46 had a mean body length of 10.2 (9.7–11.1) mm. They resembled the adult and were mostly brown with a darker brown bifurcated dorsal patch which bridged the eyes before dividing posteriorly on either side of the vertebral region. There were numerous minute white tubercles scattered all over the dorsal surfaces and dark bands across the limbs (Figure 8G). Many had a row of small pale tubercles down the middle of each side of this patch, and some had a coppery tinge over the dorsum. The venter was mostly dark grey suffused with white.

Table 5 Measurements (in mm) of *Geocrinia leai*, *G. victoriana* and *G. laevis* tadpoles from post hatching stage 27 to stage 46 (Gosner 1960). St = stage; N = no. of specimens; TL = total length; BL = body length.

	Geocrinia leai			*Geocrinia laevis*			*Geocrinia victoriana*		
St	N	TL	BL	N	TL	BL	N	TL	BL
27	9	19.8 (17.2–21.0)	7.7 (7.2–8.2)	3	15.5 (14.2–17.3)	5.5 (5.3–7.6)	14	12.7 (11.9–13.4)	4.2 (3.9–4.6)
28	2	20.0, 20.0	7.4, 8.2	1	17.7	6.7	1	20.0	7.9
29	0			3	20.5 (19.3–22.0)	7.9 (7.1–8.2)	3	20.6 (19.3–21.5)	8.0 (7.7–8.3)
30	2	22.0, 22.0	8.4, 8.5	1	20.0	8.1	3	22.0 (21.0–22.7)	8.7 (8.7–8.8)
31	4	20.9 (20.0–22.5)	8.5 (8.1–9.2)	3	20.8 (19.4–22.0)	8.0 (7.7–8.5)	2	23.8, 24.0	8.9, 9.2
32	3	20.1 (17.1–23.0)	8.2 (7.6–8.9)	2	21.0, 21.8	8.2, 8.7	2	22.0, 22.2	8.2, 8.2
33	5	20.7 (19.6–21.2)	7.9 (7.6–8.2)	5	23.0 (22.5–23.5)	9.0 (8.5–9.5)	1	26.0	9.7
34	2	21.5, 22.5	8.2, 8.9	6	25.9 (23.8–29.0)	9.6 (9.1–10.3)	1	26.5	10.0
35	6	23.0 (22.0–24.0)	8.8 (8.5–9.0)	6	26.0 (24.0–29.0)	10.1 (9.7–10.8)	4	27.6 (25.4–29.0)	10.0 (9.5–10.5)
36	4	24.4 (24.0–25.5)	9.5 (9.3–9.4)	3	24.8 (23.2–25.8)	9.5 (9.0–10.1)	7	28.3 (27.0–29.5)	10.2 (10.0–10.6)
37	12	25.8 (23.0–26.6)	9.9 (9.0–10.5)	4	28.3 (27.0–29.0)	10.4 (10.1–10.9)	11	30.8 (28.0–36.0)	10.7 (9.5–11.4)
38	2	23.2, 25.5	9.0, 9.5	1	26.5	10.3	8	30.1 (28.3–37.4)	10.7 (10.0–12.4)
39	3	26.8 (24.5–30.5)	9.7 (9.5–10.0)	2	27.0, 27.0	9.2, 10.14	3	30.2 (30.0–30.6)	11.1 (11.1–11.2)
40	3	30.2 (30.0–30.5)	9.4 (9.3–9.7)	2	30.0, 30.3	10.1, 10.3	2	29.5, 30.6	10.6, 11.6
41	5	35.4 (33.0–37.0)	10.5 (9.5–11.5)	0			1	30.4	11.4
42	0			2	25.0, 28.0	8.5, 10.0	0		
46	12		11.0 (9.6–12.8)	18		9.3 (8.4–10.1)	13		9.5 (6.7–11.3)

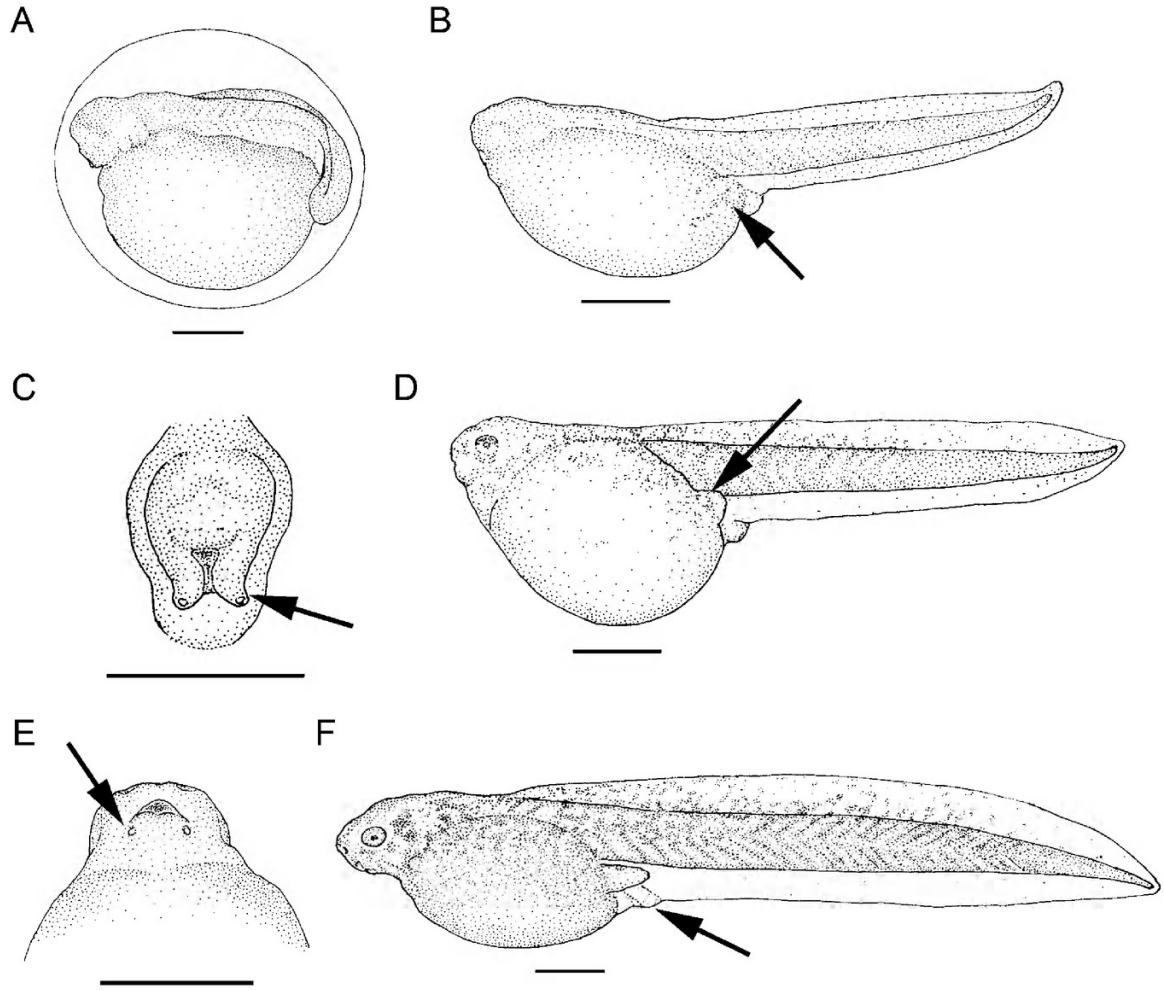

Figure 9 Sample preserved stages of the embryonic development of *Geocrinia rosea* group: *G. vitellina* (A), *G. rosea* (B-F). Scale bar represents 1 mm. A = stage 18, lateral view; B = stage 19, arrow indicates initial hind limb bud bulge; C = anterior view of head, stage 19 showing stomodaeal pit, arrow indicates adhesive glands at full development; D = stage 20, lateral view, arrow indicates hind limb bud; E = stage 22 ventral view, arrow indicates remnant adhesive glands beneath arc-shaped mouth slit; F = hatched tadpole at stage 24, lateral, arrow indicates vent tube. Note increase in melanophores and length of tail.

Geocrinia rosea group (Figures 9–12)

Development of all four members of the *G. rosea* group is entirely terrestrial. During development tadpoles are nourished by yolk stored in the gut and although they never feed, they have small vestigial mouthparts. As larval development for all four species within the *G. rosea* group is very similar, a generalised composite description is given here. Minor differences between tadpoles of each species are noted. Refer to Appendix 1 for descriptions of developmental stages as defined in this study. Specific details of early development where known for each species are provided in the reviews of breeding biology section above.

Embryonic development

Early development from stages 7–19 followed Gosner (1960). A composite description of development for all four species from stage 18 to metamorphosis is provided here, with reference to stages used in Appendix 1.

Hatchlings

Hatching occurred during stages 22–24 when hind limb buds were equivalent to about Gosner stages 28–30. When hatched, the embryo straightened out and could move around within the now liquefied jelly medium (Figure 11F).

The body of hatchlings was very small and pear-shaped in dorsal view with a short narrow head, large yolk-filled abdomen, no spiracle or external gills and a small mouth opening (Figure 9E,F). Embryos were pale grey or grey-brown with a lighter yolk (Figure 11F). The eyes were black with a few fine iridophores. The vent tube opening was just visible on the edge of the ventral fin and

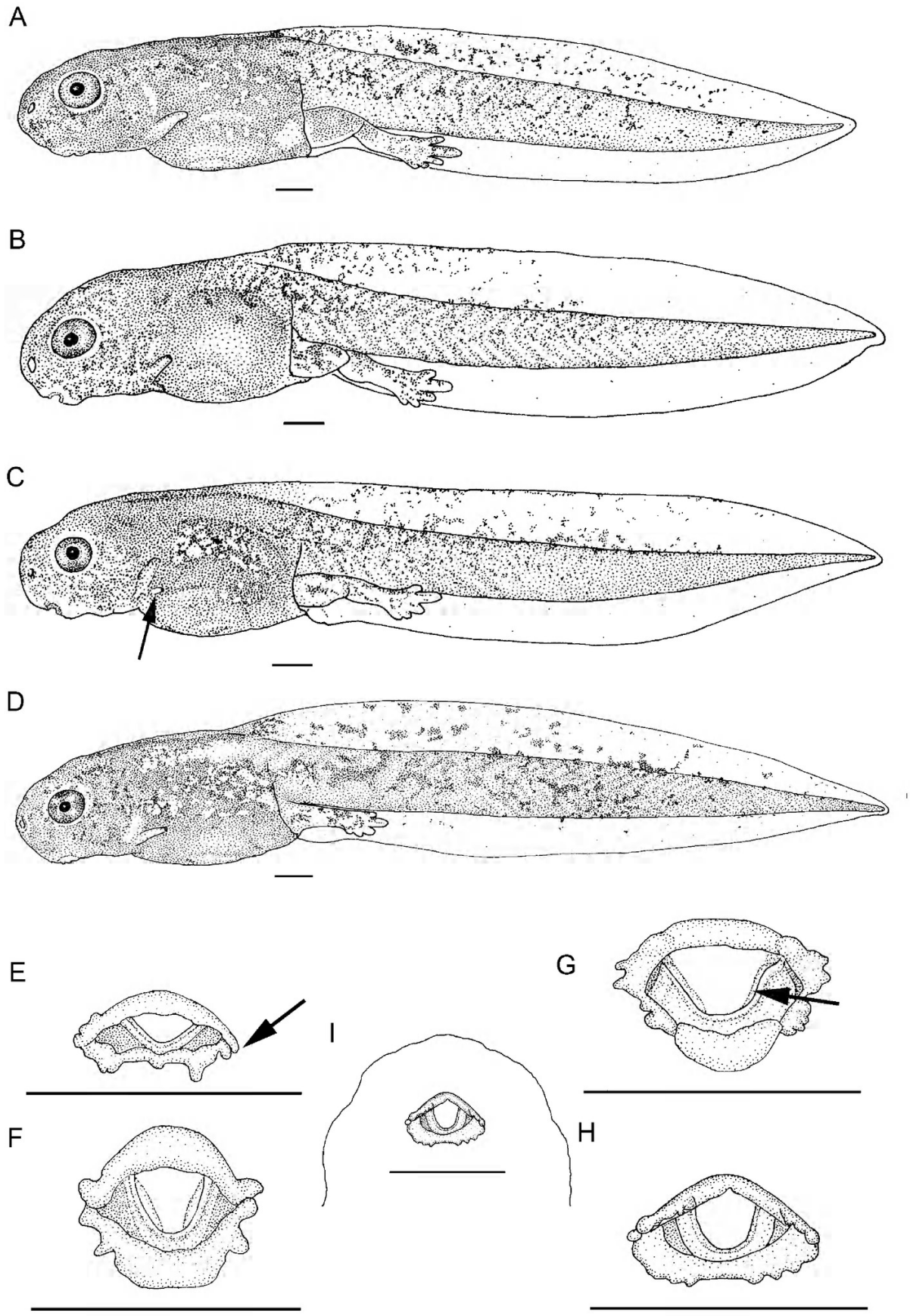

Figure 10 Preserved tadpoles and oral discs of the *Geocrinia rosea* group. Scale bar represents 1 mm. See Appendix 1 for stages. A = *G. alba* stage 31; B = *G. lutea* stage 31; C = *G. rosea* stage 31, arrow indicates spiracle; D = *G. vitellina* stage 30; E-H = oral discs of *G. alba* (arrow indicates papillae), *G. lutea*, *G. rosea* (arrow indicates lower jaw) and *G. vitellina*; I = head outline of *G. vitellina* in ventral view showing size of oral disc relative to head width.

Table 6 Ratio comparisons of oral disc features for the *G. laevis* group. Stages after Gosner (1960), N = sample size, PG/ODW = ratio medial gap in posterior papillae (PG) to oral disc width (ODW); P^3/ODW = ratio third (lowest) posterior tooth row (P^3) to oral disc width. Mean with range in parenthesis. Comparisons of northern and southern coastal population samples are given for *G. leai.*

Species	Stages	N	PG/ODW	P^3/ODW
G. laevis	31–39	15	0.29 (0.20–0.35)	0.38 (0.27–0.49)
G. victoriana	30–39	13	0.31 (0.22–0.41)	0.43 (0.34–0.49)
G. leai (north)	35–38	6	0.38 (0.33–0.44)	0.60 (0.59–0.66)
G. leai (south)	32–38	6	0.49 (0.44–0.57)	0.70 (0.62–0.75)

Table 7 Sample measurements (mm). Stages are those of Gosner (1960), N = number, ODW = oral disc width, P^3 row = third posterior tooth row, Post. Gap = medial gap in posterior papillae. Mean with range in parenthesis. Comparisons of northern and southern coastal population samples are given for *G. leai.*

Species	Stages	N	ODW	P^3 row	Post. Gap
G. laevis	30–38	15	2.4 (1.85–2.62)	0.8 (0.57–1.02)	0.7 (0.57–0.82)
G. victoriana	30–38	13	3.0 (2.57–3.28)	1.4 (1.1–1.6)	0.9 (0.65–1.14)
G. leai (north)	35–38	6	2.3 (2.21–2.46)	1.4 (1.31–1.47)	0.9 (0.82–1.06)
G. leai (south)	32–38	6	2.4 (2.13–2.70)	1.6 (1.47–1.96)	1.07 (0.98–1.55)

deflected slightly to the right. Adhesive glands were barely visible (Figure 9E). Forelimb buds developed internally. Five hatchlings of *G. rosea* at stages 22–24 had a mean total length of 12.4 (10.5–12.9) mm, with a mean body length of 4.2 (3.7–4.3) mm.

Live hatchlings of *G. alba* and *G. lutea* were not observed, but are likely to be similar to others of this group, based on recently hatched tadpoles collected at stage 24. Embryos of *G. lutea* at stage 20 prior to hatching were pale brown with a cream yolk sac (Figure 11E), small hind limb buds and showed the first signs of vitelline and tail fin circulation. The jelly capsules were greatly expanded.

Tadpoles

A composite description of tadpoles of *G. rosea, G. lutea, G. alba* and *G. vitellina* is provided here and Appendix 3 shows how developmental stages differ from Gosner and from the *G. laevis* group. Measurements of available material are provided in Table 8.

Body: On hatching at stages 23–24, the head was noticeably narrower than the yolk in dorsal view, but gradually broadened until it was almost as wide as the body by stage 29, when tadpoles were very slightly wider than deep across the abdomen. At stage 29, the snout was broadly rounded in dorsal and lateral views; eyes lateral, iris stippled with copper-gold, copper ring around pupil; nares widely spaced, open anteriorly right on edge of snout, diameter almost 0.2 mm.

The spiracle appeared vestigial and was directed dorsoposteriorly or posterodorsally with an indistinct opening just below the horizontal body axis posterior to the midpoint of the body. The spiracle was very small (0.1–0.4 mm long) and much reduced or undetectable in *G. rosea* (Figure 10C), but a little more defined in the other three species, especially *G. alba* and *G. vitellina* (Figure 10A-D). First visible from stage 27, it became a very small, narrow tube by about stage 28, reached full size during stages 30-31 and closed by about stages 33–34.

The vent tube initially formed as a deep groove within a bulge beneath the tail bud during stages 18–19; by stages 23–24 it became a narrow, mainly medial tube with a minute opening that extended slightly below ventral fin edge or deflected very slightly to the right of fin edge. By stage 29 it opened dextrally just inside edge of fin in most tadpoles and by stage 31 it opened from partway to midway up right side of fin. Hind limbs developed

Table 8 Measurements (mm) for the *Geocrinia rosea* complex. St = stages as presented in Appendix 1 (Gosner 1960 hind limb stage equivalent shown for each stage in parenthesis); N = number; TL = total length; BL = body length. Mean with range in parenthesis.

	Geocrinia alba			*Geocrinia lutea*			*Geocrinia rosea*			*Geocrinia vitellina*		
St	N	TL	BL	N	TL	BL	N	TL	BL	N	TL	BL
21 (27)							1	10.9	3.7			
22 (28)							1	10.5	3.7	1	11.8	3.9
23 (29)							2	11.6, 12.9	3.9, 4.3			
24 (30)							2	12.4, 12.9	4.1, 4.3			
25 (31)				2	12.3, 13.4	4.2, 4.3						
26 (32)				1	13.5	4.1	3	13.2 (12.7–13.9)	4.3 (4.2–4.3)			
27 (33)							2	13.5, 14.5	4.7, 4.7	1	15.6	4.9
28 (34)				2	13.4, 14.7	4.5, 4.7	2	14.7, 14.8	4.7, 4.8	1	15.8	4.8
29 (35)				1	13.2	4.5						
30 (36)	1	15.4	5.3	1	14.5	4.7	10	13.8 (13.0–14.2)	4.6 (4.5–5.0)	1	16.3	5.3
31 (37)	1	16.3	5.6	3	15.1 (14.8–15.6)	5.0 (5.0–5.0)	6	15.0 (14.1–15.5)	4.9 (4.8–5.1)	2	16.1, 17.4	4.9, 5.4
32 (38)				3	15.5 (15.5–15.6)	5.0 (5.0–5.0)						
33 (39)				1	13.8	5.0	3	14.7 (14.2–15.3)	5.0 (4.8–5.3)			
34 (40)	1	16.6	5.5	1	14.0	4.7	3	14.7 (14.5–14.8)	4.9 (4.8–5.0)	1	18.2	5.5
35 (41)				2	15.5, 15.5	5.0, 5.0	1	15.5	4.8			
36 (42)				1	14.0	4.7	5	14.8 (14.7–15.0)	4.9 (4.8–5.0)			
37 (43)				1	14.8	5.2	1	14.3	4.8			
38 (44)				1	11.3	5.5						
39 (45)	1		6.1	6		5.5 (5.6–5.6)	7		5.5 (5.3–5.6)	3		6.1 (5.9–6.2)
40 (46)	3		6.2 (5.9–6.4)				4		5.8 (5.6–6.1)	2		6.3, 6.4

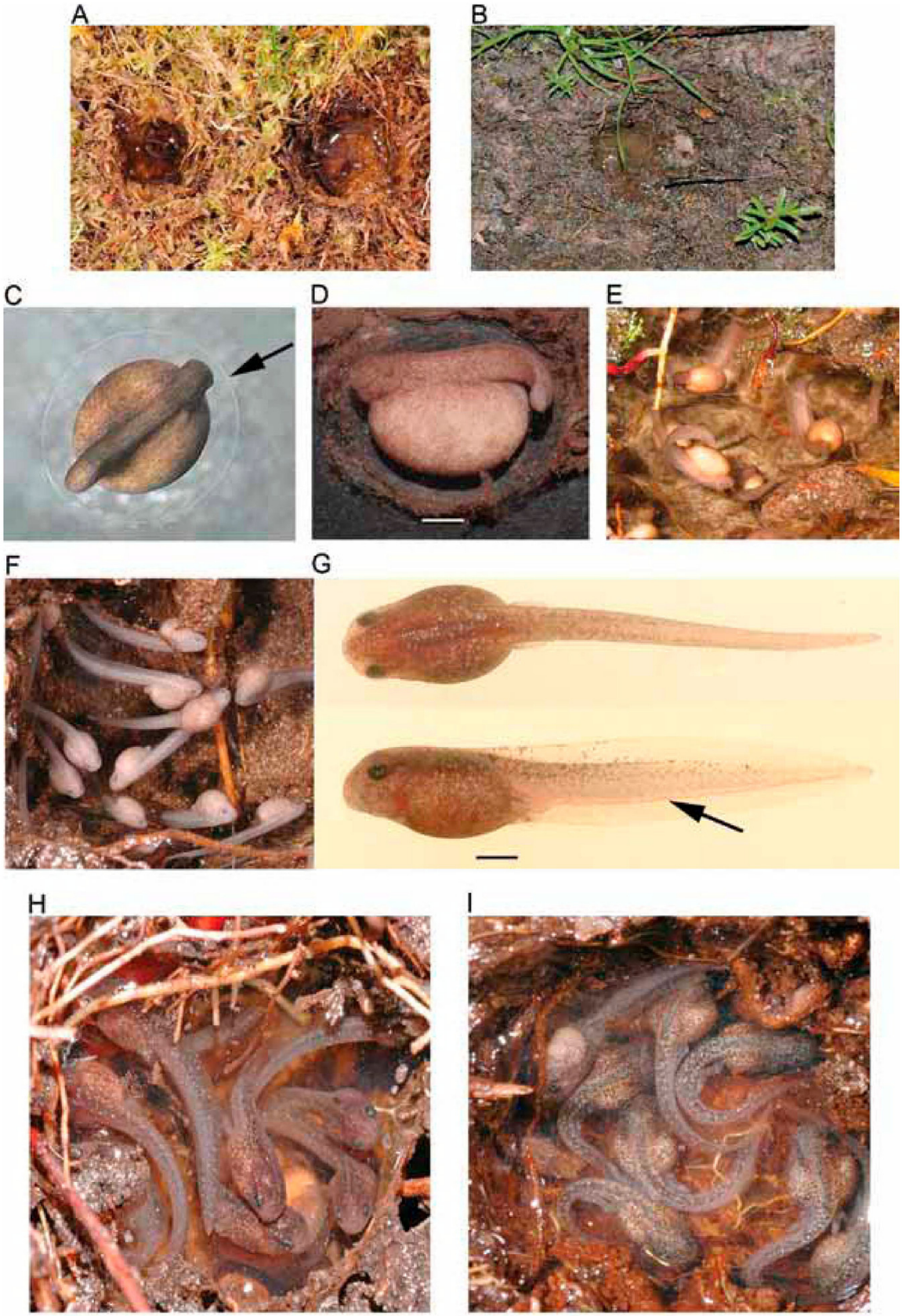

Figure 11 Sample of preserved stages of embryonic and larval development of live *Geocrinia rosea* group from stages 17–30 (see Appendix 1). Scale bar represents 1 mm. A = two nests of *G. lutea* tadpoles (stage 25) in sphagnum moss; B = nest of *G. rosea* in peaty sand; C = *G. vitellina* embryo stage 17 dorsal view, arrow indicates vitelline membrane, note large yolk (photo N. Mitchell); D = *G. vitellina* stage 18 lateral view; E = *G. lutea* about stages 20–21 in large jelly capsules prior to hatching in nest (photo M. Dziminski); F = *G. rosea* hatched tadpoles at stage 22 in liquefied jelly within nest; G = *G. lutea* stage 27, dorsal and lateral views, arrow indicates major blood vessel beneath tail muscle, note blue specks on dorsum; H = *G. lutea* stage 25 in nest; I = *G. rosea* stage 31 in nest.

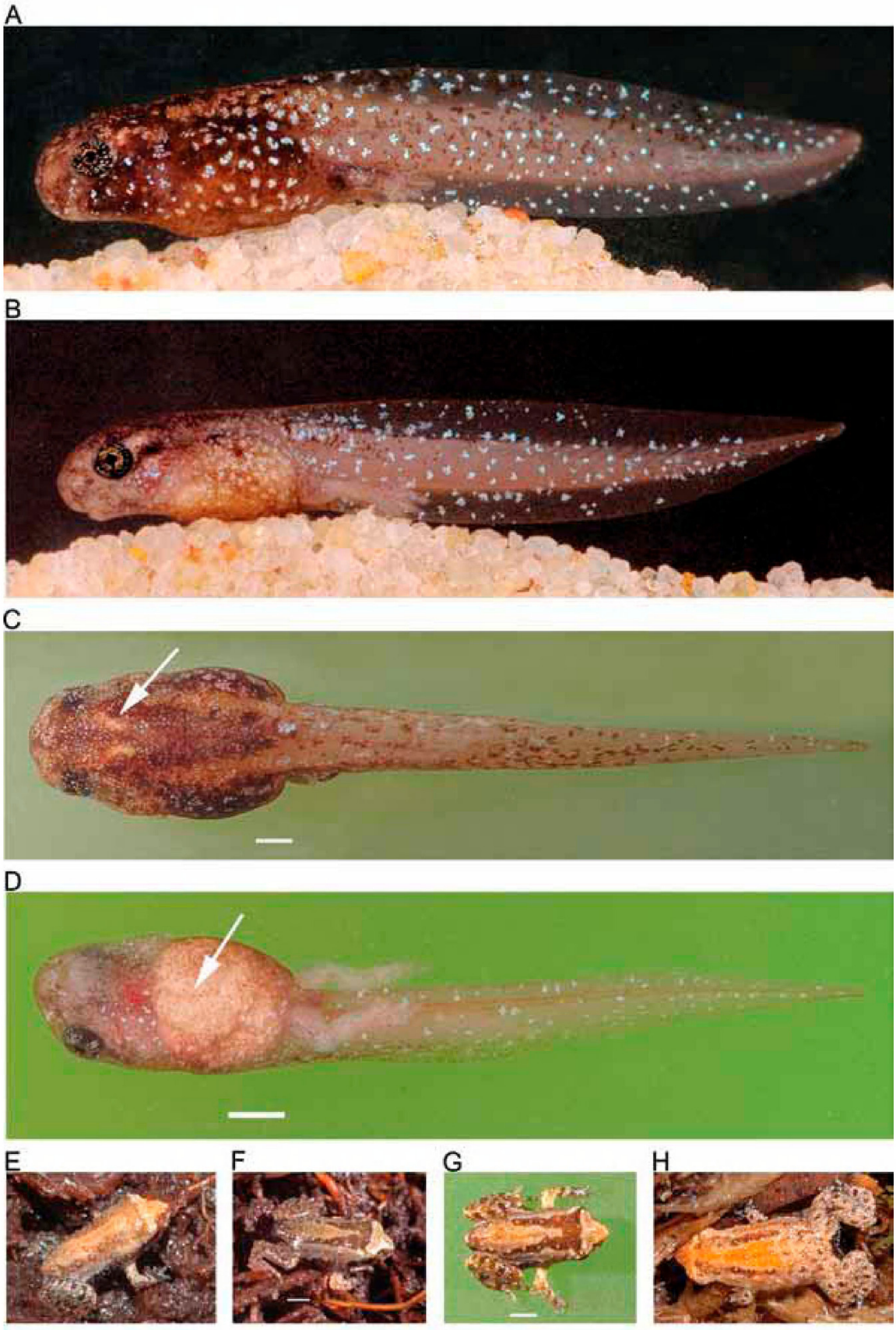

Figure 12 Selections of live tadpoles and metamorphs of the *Geocrinia rosea* group. Scale bar = 1 mm. ECD = endolymphatic calcium deposits. Stages as in Appendix 1. A = *G. alba* stage 30 lateral view; B = *G. lutea* stage 31 lateral view; C = *G. vitellina* stage 30 dorsal view, arrow indicates ECD; D = *G. lutea* stage 31 ventral view, arrow indicates thick loops in yolk-filled gut; E = *G. vitellina* stage 40; F = *G. lutea* stage 39; G = *G. rosea* stage 40, note fine pale blue tubercles; H = *G. vitellina* 3 weeks after metamorphosis showing yellow dorsal colour.

externally through stages that were similar to those of Gosner stage 26–41 (Appendix 1) and forelimbs developed internally, as in aquatic tadpoles.

Tail: Fins shallow or slightly more arched, especially in *G. alba* and *G. vitellina*. Dorsal fin begins just onto body, arches slightly and tapers gradually towards narrowly rounded tip, which may curve up slightly. Ventral fin less arched, of similar depth along length before tapering posteriorly. Muscle moderate, tapers to narrow point.

Pigmentation: While some subtle differences in pigmentation among species were observed during larval development (noted separately below), all four species have the following common features: dorsum and sides of body of tadpoles at stages 28–29 mostly brown or grey-brown (lighter translucent brown or patchy in some). Fine, iridescent silver-blue specks first visible over dorsum from stages 22–23, and more distinct blue spots gradually developed laterally over body and tail, becoming more defined and prominent during stages 26–31, especially so in *G. alba* and *G. vitellina*. Venter dusky brown or more translucent, transparent window below mouth; silver-blue spots around each side, scattered over entire venter by stage 33.

Entire tail increasingly covered with prominent, distinct iridescent blue spots during stages 24–32 (Figure 12A,B). Numerous melanophore flecks gradually developed over dorsal fin and muscle by stage 30. Muscle usually darker dorsally, and light golden or grey-brown laterally, with variable density of melanophore flecks. Many larvae developed diffuse patches of melanophores over sides of abdomen before becoming fully pigmented by about stage 32. Larvae in some clutches were less heavily pigmented dorsally over the yolk until about stage 32. *G. alba* and *G. vitellina* tended to have more prominent blue specks over body and tail. Endolymphatic calcium deposits were first visible at stage 24 as a small white patch on each side of head, extending into a white V-shape behind brain by stage 27.

Oral Disc: Similar in all, with some slight individual differences in papillae (Figure 10E-H). Disc very small, ventral and vestigial. No papillae around anterior margin. A few small, irregular papillae around sides and/or posterior margin, barely evident in some. No tooth rows or tooth row ridges. Small, non-keratinised jaw ridges, upper mostly hidden, but gradually becomes more visible anteriorly by stage 37 (Figure 13A). Mouth opening widens from stage 31. Development of the jaws and flexible conical projection at the point of the mentomeckelian cartilage is shown in Figure 13.

Larval duration and metamorphosis

Froglets of all four species began to climb to the top of the nest as the tail was resorbing, but readily returned into remnant jelly in the nest basin if disturbed. Metamorphs of all species have the following pigmentation in common: dorsum beige, grey-brown or yellow-beige with a dark brown bifurcated patch and numerous minute pale blue tubercles over the entire body (Figure 12E-G). Belly dark brown with silver specks. For each species, metamorphosis is known to occur as follows:

G. rosea – From November to December (late spring to summer). Tadpoles at stage 23 on 10 October began to metamorphose from 21 November after 42 days, so the total larval duration for these embryos from egg to metamorphosis is likely to have been at least 60 days (less for clutches laid in warmer temperatures later in spring). Five metamorphs ranged from 5.6–6.2 (mean 5.7) mm.

G. alba – From October to early December. Four metamorphs ranged from 5.9–6.4 (mean 6.2) mm.

G. lutea – From November to December (late spring to summer). Tadpoles at stage 31 on 22 October began to metamorphose on 26 November after 35 days. The total larval duration for this species is likely to be at least 46 days (longer for clutches laid in cooler temperatures of early spring). Seven metamorphs ranged from 5.5–5.6 (mean 5.6) mm.

G. vitellina – From October and November (Driscoll 1997; Conroy 2001). Four metamorphs from one clutch ranged from 5.9–6.3 (mean 6.1) mm. A few weeks after metamorphosis, juveniles can become quite yellow (Figure 12H).

DISCUSSION

This study has documented embryonic and larval development for all species of *Geocrinia* and enables a comparison between the two divergent terrestrial/exotrophic and endotrophic breeding modes within the genus. Additional comparisons are made here between the developmental breeding modes of *Geocrinia* and those of other Australian frogs with similar breeding modes, concluding with a discussion on evolutionary trends.

Comparisons between the two species-groups

The two species-groups of *Geocrinia* are highly divergent in their embryonic and larval development and are adapted to different life histories, as summarized below

Exotrophic species: Geocrinia laevis *group*

1. Terrestrial development

The terrestrial/exotrophic larvae can remain on land during less favourable times for up to four months during the winter (Martin and Cooper 1972), which is more than half the total period of their larval development. Some features of

development that are suited to their initial terrestrial development within the egg are noted below.

Larger yolk volume in amphibians corroborates with a longer time taken for development from ovum to hatching stage (Bradford 1990). The mean ovum diameter of the three species of the *G. laevis* group (2.0 mm, range 1.5–3.1 mm) is smaller than that of the *G. rosea* group (2.9 mm, range 2.4–3.5 mm), but larger than the diameter of most Australian aquatic developing ova which most commonly range from 1.0–1.6 mm and hatch in a period of 2–8 days (Anstis 2002; Anstis unpublished). Although members of the *G. laevis* group eventually hatch as aquatic larvae, adequate yolk supplies are necessary for sustenance over what may be an even longer non-feeding terrestrial period than for the *G. rosea* group. This may also indicate that the composition of the yolk may be of a greater density than would be required in ova of aquatic developers which hatch much more quickly (Bradford 1990; Thibaudeau and Altig 1999). Clutch sizes of 52–183 are consistently larger for the *G. laevis* group than for the *G. rosea* group (1–32 eggs per clutch).

Oxygen requirements in the terrestrial phase of development for globular egg masses which lack air spaces between eggs must be met by diffusion (Mitchell and Seymour 2003). A possible adaptation of the egg clutches of the *G. laevis* group to terrestrial development may include the form of the clutch, as eggs are normally stretched out in long, narrow 'ropes' only about 2 or 3 eggs across (Figure 6A), which would enhance diffusion of gases. In addition, the jelly capsules of non-hydrated fresh eggs are very thin and close to the ovum (Figure 6C), further facilitating gas diffusion. When the jelly capsules hydrate in moist situations and become turgid (Figure 3C), respiratory competition between embryos may be reduced (Seymour 1999). As the vitelline membrane gradually expands during embryonic growth and movement after stages 18–19 and the jelly layers decrease in thickness (Figure 6I), the effective surface area and oxygen uptake through the capsule would likely increase in line with greater oxygen demands of the embryo (as shown for the terrestrial eggs of *Pseudophryne bibronii*; Seymour and Bradford 1987).

During drier periods, the vitelline membrane and jelly layers shrink and tightly confine the growing embryo (e.g. to stage 26 in *G. laevis*, Figure 6J), and movement is restricted. This restriction may stabilize or reduce rates of metabolism of embryos during periods of suspended development, when a relatively low metabolic rate would be necessary if the embryo is to survive (Bradford and Seymour 1985).

Adhesive glands were prominent from stage 18 and long-lasting, persisting during stage 26 (Figure 3I) when most tadpoles first enter the aquatic phase.

Earlier hatchlings of the *G. laevis* group may have an advantage over very late hatchlings because they have more yolk remaining in the gut. The few latest hatchlings in which development was arrested for 9–10 weeks after oviposition had almost no yolk, lacked vigour when hatched and either did not survive early aquatic life or developed much more slowly than earlier hatchlings from the same clutch.

Tadpoles do not begin to develop hind limbs until Gosner stage 23 at the earliest, and not before stage 25 or 26 in most individuals. Limb development does not normally proceed further than stages 26–27 and can be suspended at these stages prior to hatching, contrasting with suspended hatching in the terrestrial/exotrophic species of *Pseudophryne*, in which limb buds can develop as far as stage 36 prior to hatching (Thumm and Mahony 2002 for *P. australis*).

2. Aquatic development

Newly hatched tadpoles of *G. victoriana* are slightly larger than those of *G. laevis*, also reported by Gollmann and Gollmann (1994), and similarly slightly larger than *G. leai*. Hatched tadpoles of *G. victoriana* showed reductions in dry mass, total length, tail fin length and fin height when reared out of water, compared with those reared in hydrated situations which grew larger Andrewartha *et al.* (2008). The few embryos of the *G. laevis* group that hatched later than all others in the present study showed less foraging vigour, developed more slowly post-hatching, and metamorphosed last, supporting the possible fitness benefits of earlier (and larger) hatchlings as suggested by the above authors.

During their aquatic period, the *G. laevis* group tadpoles feed and grow much larger than larvae of the *G. rosea* group (Tables 5, 8) and during stage 26 have feeding mouthparts, a long coiled gut for digestion (e.g. early stage 26, Figure 6L), a well developed, fully functional spiracle and vent tube and other features typical of lentic, benthic aquatic tadpoles (Altig and Johnston 1989). Slight differences in the vent tube of the aquatic tadpoles are noted here between *G. laevis* and *G. victoriana*, while that of *G. leai* is more similar in shape, size and direction to *G. laevis*.

Limb development proceeds slowly during cooler winter periods (June to August) after hatching, and some tadpoles can double their size between stages 26 and 29 (e.g. *G. laevis*, Table 5).

Of the three species in the *G. laevis* group, the oral disc is widest in *G. victoriana* (Table 7). In a comparative study of *G. victoriana* and *G. laevis* tadpoles, Gollmann and Gollmann (1995) found a similar result (mean width of the oral disc for *G.*

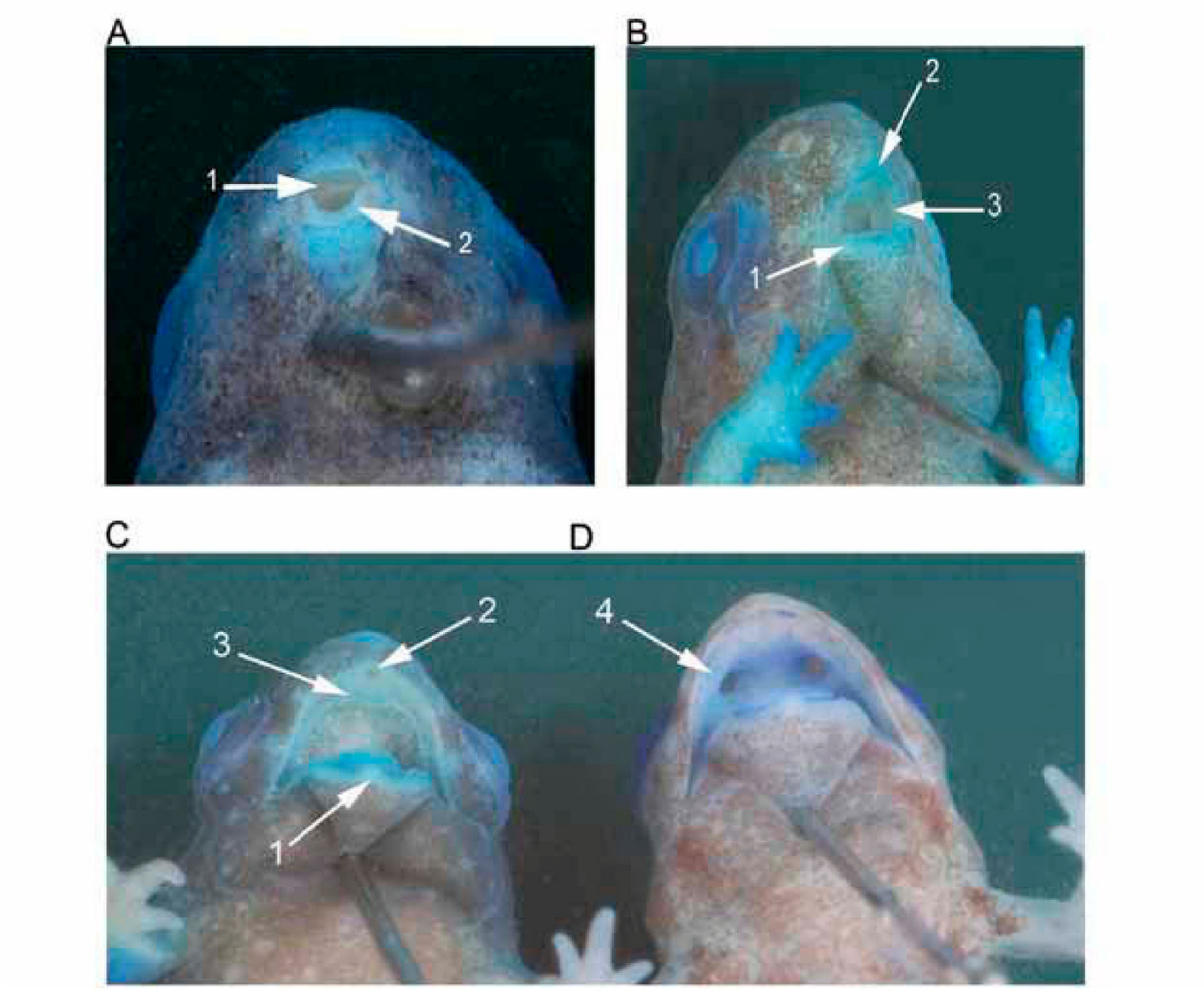

Figure 13 Oral development (stained) in later stages of *Geocrinia rosea* larvae (stages as in Appendix 1). A = stage 31, arrows indicate upper jaw (1), lower jaw and lower lip over jaw (2); B = stage 36, arrows indicate lower lip over jaw (1), upper lip with medial notch beneath (2) and upper jaw (3); C = stage 39, arrows indicate conical projection at point of mentomeckelian cartilage (1), corresponding notch in upper jaw (2), formation of hard palate behind upper jaw (3); D = stage 40, arrow indicates choanae (4).

victoriana 2.13 mm, and 1.94 mm for *G. laevis*). The width of the posterior medial gap and the length of the P^3 tooth row however, are significantly wider in *G. leai* than both the eastern species (Table 7). The presence or absence of a medial gap in tooth row P^1 may be a genetic polymorphism, as in the three clutches of *G. laevis* studied, the gap was not present in all tadpoles examined from two clutches but present in all tadpoles of a third clutch. The presence of this gap was found to be equally common in *G. laevis* and *G. victoriana* (Gollmann and Gollmann 1995).

Although there are no conspicuous morphological differences between the adults of *G. leai* across its known range (Edwards 2007), preliminary observations indicate differences in the mouthparts of tadpoles between the northern and southern coastal populations. Specimens from the southern coastal populations have more submarginal papillae (northern ones have none or only a few), a wider medial gap in the posterior papillae and a longer P^3 tooth row relative to the width of the oral disc than northern tadpoles (Table 7). Larger samples of tadpoles from all three lineages of Edwards (2007) need to be measured to determine the consistency of these observations.

Features of the oral disc of tadpoles of the three species in this group are quite similar and appear to reflect homology. Compared to the southeastern Australian species, southern coastal populations of *G. leai* in Western Australia are more like *G. victoriana* in the configuration of oral papillae, and northern *G. leai* are more like *G. laevis.* All samples of *G. leai* and *G. victoriana* have an emarginate oral disc, while *G. laevis* does not.

Endotrophic species: Geocrinia rosea *group*

The endotrophic species do not feed and can survive on yolk supplies for over three months.

Terrestrial development has resulted in the following modifications away from the typical morphology of exotrophic aquatic larvae.

The egg is macrolecithal (Main 1957) and clutch sizes are small (1–32), typical of endotrophic species (Thibaudeau and Altig 1999). The development of the gut is much slower in the *G. rosea* group than in the *G. laevis* group, and does not start to differentiate until stage 27, when the hind limb bud is at Gosner stage 33. Although the gut of the tadpole gradually forms into a thick, yolk-filled spiral (Figure 12D), it never develops into the long, thin multi-coiled spiral of aquatic tadpoles that actively feed.

The large jelly capsule (diameter to about 1 cm) is a likely adaptation to entirely terrestrial development because its greater volume when liquefied at the time of hatching provides a suitable, lasting medium for an extended period of terrestrial larval development in the nest. The very thick jelly layer surrounding pre-hatched embryos would not impede oxygen diffusion if the perivitelline space is large enough (Seymour 1999), and as the embryo grows and extends the tail, the vitelline membrane expands.

Hind limb buds develop precociously, unlike the much later limb development in the exotrophic species. Earlier limb development may slightly hinder swimming speed and maneuverability in the exotrophic species, but would not be a problem for the non-swimming terrestrial species confined to the small space in the nest.

Tadpoles have non-keratinised, non-feeding mouthparts which are very small and apparently vestigial, showing minimal development other than non-keratinised jaw sheaths, labia and a few diminutive papillae in most individuals (Figure 10E-I;13A).

The spiracle is generally reduced in diameter and seems vestigial. *Geocrinia vitellina* and *G. alba* appear to have a slightly longer, more defined spiracle, while that of *G. lutea* and especially *G. rosea* is smaller (Figure 10B,C). In some *G. rosea* tadpoles a spiracle could not be found at developmental stages when it was present in others. The spiracle develops later (from stage 27 = Gosner limb bud stage 34), atrophies sooner than in aquatic tadpoles and is absent by stage 34 (Gosner stage 40).

The adhesive glands are much reduced (Figure 9E), and the poorly defined vent tube appears vestigial and is narrow with a minute dextral aperture.

The development of the conical projection at the point of the mentomeckelian cartilage and the corresponding notch in the centre of the upper jaw (see Figure 13 and Appendix 1) is also described in the development of the direct developers *Arenophryne rotunda* and *Myobatrachus gouldii* (Anstis *et al.* 2007) and for *Metacrinia nichollsi* (Anstis 2008). Similarly, the development of the endolymphatic calcium deposits (also described in the above studies) is paralleled in the *G. rosea* group, and is more obvious in endotrophic development than in exotrophic development.

Similarities between tadpoles of the two *Geocrinia* species-groups

Despite the differences discussed above, there are several morphological similarities between tadpoles of the two species-groups within *Geocrinia*. Apart from slight exceptions noted below, the eyes of most larvae are lateral in life. Gollmann and Gollmann (1994) reported some slight geographical variation in the eye position of *G. victoriana* tadpoles, and tadpoles of *G. leai* in the current study also show slight variation in eye position between northern and southern coastal populations (Figure 5). In dorsal view, the body shape of live members of both the *G. laevis* and *G. rosea* groups is similar (after about stage 29 in the latter) and in lateral view, the tail shape is generally similar. The vent tube is dextral in all species, although it appears to be vestigial and non-functional in the *G. rosea* group.

Developmental timing

All species of the *G. laevis* group breed in autumn and larvae overwinter during development in southeastern and southwestern Australia. In this study, it was not possible to control temperature during field work and in transit. However, temperature does not affect the sequencing of the developmental stages, and Bachmann (1969) concluded that the relative timing of all visible developmental processes in amphibians is the same within the normal temperature range experienced during development for any given species. Table 2 shows that the time taken for tadpoles of *G. laevis* to develop completely keratinised mouthparts from the appearance of the first to the last keratinised tooth row and fully keratinised jaw sheaths was a minimum of 11 days, which is almost half the total minimum time taken for tadpoles to develop from stage 2 to hatching at stage 26 (Table 2). This is a much longer period for this part of development than is taken by embryos that develop in water, and the greatest period of time elapsed between stage 25 (four tooth rows present) and stage 26 (five tooth rows, P^3 was the final row). In some species of aquatic *Crinia* embryos, such as *C. georgiana* and *Spicospina* embryos of southwestern Australia, development of mouthparts is slower (Dziminski and Anstis 2004; Anstis, unpublished data), but never as slow as in exotrophic *Geocrinia*. Incubation time is longer in terrestrial-breeding amphibians and appears to be a consequence of selection

for hatchlings that are large and emerge at an advanced stages of development (Bradford 1990).

Comparisons between the *Geocrinia rosea* group and other Australian taxa with nidicolous development

In amphibians, the adoption of a terrestrial life style is broadly correlated with a trend towards direct development (Duellman and Trueb 1986). Altig and Crother (2006) suggest that the multiple occurrences of nidicoly (across many independent lineages), represent independent truncations of normal development. They further assert that nidicolous development proceeds by patterns similar to typical (exotrophic) larvae. The observations reported here among members of the *G. rosea* group add support to this contention because they represent an example of an independent occurrence of nidicoly among Australian myobatrachids, and although tadpoles of this group are diminutive and non-feeding, they still retain some similar morphological characteristics to their exotrophic relatives in the *G. laevis* group.

Key features of direct developers include a large unpigmented egg, the lack of a free-swimming hatched larval stage, no feeding mouthparts, no spiracle, a precocial neural tube anatomy, a different mode of abdominal wall formation, and development to a froglet entirely within the jelly layers (Altig and Johnston 1989; Elinson and Fang 1998; Schlosser 2003; Altig and Crother 2006). The characteristics of oral disc and spiracle are of particular comparative interest in tadpoles of the nidicolous Australian species in relation to a possible trend towards direct development. *Philoria* tadpoles have some features of feeding mouthparts such as keratinised jaw sheaths, papillae and a functional spiracle (de Bavay 1993; Anstis 2002). Tadpoles of the *G. rosea* group diverge from *Philoria* in that they have small non-keratinised jaw sheaths, only a few remnant papillae and a small, narrow spiracle with a reduced opening. The spiracle is even smaller or absent in *G. rosea*. While the specimens of *G. rosea* showed some variability, the spiracle (if present) was more reduced than in any other species of this group, which may be an indication of a possible further trend towards direct development within this group. More material of live *G. rosea* tadpoles from northern and southeastern populations needs to be examined to determine the characteristics of the spiracle and its degree of functionality.

Crinia is the only myobatrachid genus other than *Geocrinia* that has divergent breeding modes. All species are entirely aquatic except for *Crinia nimbus*. Unlike *Philoria* and the *G. rosea* group, tadpoles of *C. nimbus* and the exoviviparous *Assa* have no spiracle or oral papillae (Anstis 2002), but otherwise have a similar life cycle to those of the *G. rosea* group (Rounsevell *et al.* 1994; Mitchell and Swain 1996) and *Philoria* (Watson and Martin 1973; Anstis 1981; de Bavay 1993). The absence of a spiracle and any remnant papillae or mouthparts of the feeding tadpole in *C. nimbus* and *Assa* are similar to direct developers and thus further divergent from *Philoria* and the *G. rosea* group.

ACKNOWLEDGEMENTS

A grant for field work assistance during 2007–08 from The Australian Biological Resources Study is gratefully acknowledged. The University of Newcastle is sincerely acknowledged for the award of a scholarship for 2009–2010, and I thank my supervisor Michael Mahony for advice and assistance. The Department of Environment and Conservation, Western Australia and the Department of Sustainability and Environment of Victoria are sincerely thanked for the issue of scientific permits allowing collection of specimens for this study. Thanks are also due to Dale Roberts and Martin Dziminski of the University of Western Australia and Don Driscoll for help with localities. Martin Dziminski also assisted with field work and provided some preserved tadpole samples of *G. rosea* and *G. lutea* and a photograph (Figure 11E). The Museum Victoria and the Western Australian Museum are acknowledged for the loan of specimens. I thank Nicola Mitchell for the use of a photograph (Figure 11C), Ron Anstis for providing the photographs for Figures 1F and 1G and Alcoa of Australia for publication costs. Ronn Altig, Michael Mahony, Hal Cogger and Paul Doughty provided helpful suggestions on the manuscript.

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Manuscript received 1 May 2009; accepted 13 November 2009

APPENDIX 1

Development of *Geocrinia rosea* group. Stages 18–19 (after Gosner, 1960), stages 20–26 with reference to Mitchell (2001). Limb bud descriptions included from stage (st) 21 follow Gosner (1960) = (G). ECD = endolymphatic calcium deposits. Key characters in bold.

Stage	Developmental characters
17, 18 (Figure 9A, 11C-D)	• **optic bulges small, indistinct** • **three visceral arch bulges** • **small stomodaeal cleft between mandibular arch bulges** • **head short and narrow, adpressed against yolk** • neural tube raised above bulbous yolk • pronephric bulges visible • narrow slit in vent tube bulge • hatching gland ridge begins to develop across top of snout • tail bud curves around yolk to one side, myotomes visible • slight fin ridges • trunk and tail muscle dark brown, yolk creamy brown
19 (Figure 9B)	• **heartbeat may be observed** • **rudimentary hind limb buds just visible** • **head narrow, longer than stage 18, elevates from yolk, projects straight out from body** • **optic bulges slightly more discernable** • **narrow stomodaeal pit** • **tail extends widely out to one side from yolk (within expanded jelly capsule), fins very shallow, opaque, about one-quarter depth of muscle** • **small adhesive glands on tips of mandibular arch bulges** • visceral arches indistinct • hatching gland present
20 (Figure 9D)	• **hind limb buds = st 26 (G)** • **eyes more defined, lightly pigmented dorsally, pupil just discernable** • **mouth opening diamond-shaped** • **tail lengthens, fins low, translucent, circulation begins in some** • **vitelline circulation first becomes visible as a pink flush over yolk** • **narial pits visible** • **small, faintly pigmented adhesive glands at base of mandibular arch bulges** • trunk and tail muscle slightly lighter grey-brown, distinct melanophores specks begin to appear over dorsum, anterior half of tail muscle and dorsal fin
21	• **hind limb buds triangular = st 27 (G)** • **iris darker around upper half, fading into grey below, pupil visible** • **mouth deepens and widens into arc-shape** • **fins mostly clear, circulation visible in most tadpoles** • **narial pits deepen** • **melanophore flecks more distinct over dorsum and yolk**

Stage	Developmental characters
	• head short, much narrower than very broad yolk • vent tube medial, protrudes below ventral fin, opening perforated • increased melanophores on dorsal fin
22 (Figure 11F)	• **hind limb buds = st 28 (G)** • **eyes small (diameter 0.3 mm), iris mostly dark grey, lower medial area lighter grey** • **mouth deepens and widens slightly into arc shape, labia begin to form, slight medial groove in lower labial region** • **remnant adhesive glands very small, unpigmented, well below each corner of mouth** • **nares partly perforated** • **pale blue specks scattered over dorsum** • hatching gland still partly visible • head broadens slightly (dorsal view), more smoothly contoured • tail slightly longer, fins more developed, mostly clear, tip narrowly pointed • fine melanophores extend halfway down sides of yolk • hatching first begins in some clutches
23	• **hind limb buds longer = st 29 (G), may protrude laterally** • **iris black apart from narrow choroid fissure, first gold iridophores appear** • **lower jaw partly visible (white, no keratin), vertical medial groove in lower labium** • **adhesive glands barely visible or gone** • **long blood vessel extends down each fin above and below muscle** • some pale blue specks extend laterally, a few on tail • body grey-brown over dorsum, slightly lighter over yolk • melanophores extend broadly over body, increase over tail muscle and dorsal fin • nares well perforated, widely spaced, directed anteriorly beneath remnant hatching gland • blood vessels visible over yolk under surface melanophores • most hatched
24 (Figure 9F)	• **hind limb buds = st 30 (G)** • **small round forelimb bud visible beneath body wall** • **ECD (endolymphatic calcium deposits) visible as small white patch on each side of vertebral region just behind brain** • **mouth a broader slit, slight labial ridges, medial groove in lower labium fused** • **network of blood vessels well developed over yolk** • nares well perforated, widely spaced, directed anteriorly • melanophores increase over tail and now cover yolk • gold iridophores increase over black iris, very narrow choroid fissure
25 (Figure 11H)	• **hind limb buds = st 31 (G), may protrude laterally** • **iris completely black beneath increased gold iridophores, choroid fissure closed** • **flattened labial ridges border mouth** • **silver-blue spots more prominent over sides and on fins**

25 (cont) (Figure 11H)	• **eyes lateral, diameter increases slightly (0.4 mm), cornea protrudes slightly beyond side of head** • head slightly longer and broader, still a little narrower than yolk • vent tube opening minute, deflects slightly to right just below edge of fin • network of blood vessels over yolk • anterior half of dorsal fin same height as tail muscle in most • melanophores increase over dorsum and head, dorsal tail muscle and dorsal fin; abdomen mottled grey-brown and cream in some, uniform brown in others • hatching complete
26	• **hind limb bud = st 32 (G), slight constriction at knee, foot paddle broadens – blood vessels visible** • **eye diameter increases (0.6 mm)** • **diminutive labial papillae first evident in most** • head broadens slightly • dorsal fin arches slightly to almost same height as muscle at midpoint, tail tip narrows to point • vent tube opening almost flush with edge of fin • melanophores denser over dorsal tail muscle and dorsal fin anteriorly • patches of melanophores over limb bud • venter dark grey-brown, prominent silver-blue specks around sides
27 (Figure 11G)	• **hind limb bud = stage 33 (G)** • **diminutive spiracle gradually develops in many** • **first gut loop begins to develop** • **ECD extend and converge posteriorly into V-shape behind brain** • body wall more translucent • silver-blue spots evenly spaced all over tail and sides of body • increased layer of melanophores over dorsum, less posteriorly on either side of vertebral region in some • venter slightly clearer medially over abdomen and below mouth, scattered melanophores and silver-blue spots anteriorly and around sides
28	• **hind limb = st 34 (G)** • **very narrow, white upper jaw-like structure, mostly hidden further inside buccal cavity** • **gut loop more defined** • **spiracle first open in some** • head broadens further • dorsal fin slightly arched in many, prominent melanophores over dorsal fin and most of muscle
29	• **hind limb = st 35 (G)** • **body more elongate** • **gut mostly obscured by pigment** • vent tube opening now just inside right edge of ventral fin • nares open anteriorly on edge of snout • fine iridophores over darker brown dorsum, broader clusters posteriorly in some • dull gold patches along length of dorsal tail muscle

Stage	Developmental characters
30 (Figure 10D, 12A)	• **hind limb = st 36 (G), longer, toes partly pigmented** • **forelimbs partly visible** • **oral papillae slightly more defined** • **gut loop in thick spiral formation** • **spiracle fully developed, open in most, often not visible or much less defined in *G. rosea*** • **body shape fully developed, elongate, more cylindrical in most - head almost as wide as abdomen in dorsal view, snout broadly rounded** • eye diameter increases (0.8 mm), thin copper ring around pupil • fins fully developed, dorsal fin arches then tapers evenly over length • brilliant silver–blue specks and larger spots at full development all over body and tail, dorsum dark brown, paler down either side of vertebral region in *G. alba* and *G. vitellina* (prior to development of bifurcated patch)
31 (Figure 10A-C, 11I 12B,D, 13A)	• **hind limbs = st 37 (G), longer with distinct knee joints and separate toes** • **forelimbs visible beneath operculum** • **upper jaw mostly visible as a white 'shelf' further back inside buccal cavity** • **ECD extend laterally to each eye, mostly obscured by pigment** • melanophores form mostly continuous stripe down dorsal tail muscle on either side of fin • hind limbs mostly dark with fine blue specks • dorsal pigment mostly uniform dark brown beneath iridophores, paler over abdomen or down either side of vertebral region in tadpoles of some clutches • yolk-filled gut loops clearly visible • vent tube narrow, dextral, opens just inside edge of fin (or partway up in some)
32 (Figure 12C)	• **hind limb = st 38 (G), pigment increases on toes** • **forelimb bulges prominent** • **snout becomes slightly pointed** • **tadpoles reach maximum length** • forelimb bulges prominent • metatarsal tubercle indistinct
33	• **hind limb = st 39 (G)** • **forelimb bulges prominent (dorsal view)** • **upper jaw more visible anteriorly** • **slight swelling begins to develop in centre of lower jaw in some**
34	• **hind limb = st 40 (G), pigment increased over foot** • **spiracle shrinks, not present in some** • **small conical flexible projection begins to develop in centre of lower jaw (mentomeckelian cartilage), less distinct in some** • yolk appears to diminish slightly • eye diameter increases (0.9 mm) • eyelids begin to develop • silver-blue specks begin to increase over dark venter • hind limbs mostly dark with fine iridophores all over

35	• **hind limb = st 41 (G), fully formed** • **forelimb bulges larger, hole in operculum as first forearm prepares to emerge** • **mouth broadens transversely, anterior edge of upper jaw deepens (vertically) and now appears to be aligned just posterior to lower jaw** • darker brown bifurcated patch develops from between eyes to base of body • a few paler tubercles develop in row down each side of bifurcated patch
36 (Figure 13B)	• **forelimbs emerge, limbs = st 42 (G)** • **fins shrink slightly** • **upper jaw more prominent and easily visible, papillae gone** • **conical projection at point of mentomeckelian cartilage more defined in most, corresponding notch forms in centre of upper jaw** • **eyes more prominent, nictitating membrane begins to develop** • bifurcated dorsal patch more distinct • silver-blue dots increase over dorsum crowning small scattered tubercles, especially over dorsal patch
37	• **limbs fully developed = st 43 (G)** • **tail tip begins to resorb** • copper pigment over iris • thick spiral yolk-filled gut loop still visible • diffuse darker bands and silver iridophores and dots over hind limbs, underside of thighs dusky brown with silver specks; forearms silvery-brown above, whitish beneath • ECD fused • silver-blue dots more concentrated over darker dorsal patch, dorsum lighter brown or beige around this • silver-blue iridophore specks over most of dusky brown venter, especially throat
38	• **body = st 44 (G)** • **tail half to two-thirds resorbed** • **mouth widens to a point in line with midway across diameter of eye, conical projection on lower lip fits into small hollow or notch in centre of upper jaw** • as above • small dorsal tubercles more numerous and prominent
39 (Figure 13C)	• **body = st 45 (G)** • **nictitating membrane present** • **tail stub** • **froglet eyes fully formed** • each corner of mouth extends around head to a point in line with posterior edge of eye • dorsum yellow-brown, beige or brown, with darker brown bifurcated patch and numerous silver-blue tubercles and spots • venter dusky brown with scattered silver-blue specks
40 (Figure 12E-H, 13D)	• **body = st 46 (G), tail fully resorbed** • **internal nares (choanae) develop**

APPENDIX 2

Early development of the exotrophic *Geocrinia* species, *G. leai*, *G. laevis* and *G. victoriana*. Key diagnostic features for each stage are highlighted in bold. Stages 17–19 equivalent to Gosner (1960), stages of Gollmann and Gollmann (1991a) incorporated in stages 20–26.

Stage	Developmental features
17 (Figure 6F, 7B,C)	• **head truncate, broad, adpressed against yolk** • **optic bulges indistinct** • **three faint visceral arches** • **slight stomodaeal depression** • **mandibular arch bulges visible, narrow groove between** • neural tube raised above bulbous yolk • pronephric bulges visible • small vent tube bulge • tail bud almost as broad as head region, flattened against yolk then gradually curves to one side • trunk and tail muscle dark brown, yolk brown (creamy grey in *G. laevis*)
18	• **cleft beneath head as it elevates slightly from yolk** • **optic bulges more discernable** • **shallow stomodaeal pit** • **adhesive glands first develop on bottom of now prominent mandibular arch bulges, pigmented groove adjoins glands beneath** • **first muscular response late in this stage** • visceral arches slightly more defined • narial pits just visible • tail bud extends partly round yolk to one side, myotomes visible • slight dorsal and ventral fin ridges
19 (Figure 6G, 7D)	• **head lengthens slightly, arched above brain, snout begins to form** • **optic region more defined, unpigmented** • **stomodaeal pit widens and deepens** • **adhesive glands more distinct** • **tail lengthens around body, fins very low** • small slit in vent tube bulge • trunk and tail muscle grey-brown, yolk dusky brown or grey-brown (paler in *G. laevis*)
20	• **head larger, higher off yolk (if jelly is hydrated)** • **no external gills** • **optic bulges larger, slightly dusky, pupil faintly visible** • **stomodaeal pit deepens further** • **adhesive glands broaden (now not joined by ventral groove)** • operculum begins to expand • narial pits small, pigmented, slightly indented • rim forms around vent tube opening • tail longer, coiled round body to tip of snout, fins slightly more developed and opaque dusky

21 (Figure 6H, 7I)	• **head larger, still narrower than yolk** • **vitelline circulation begins** • **eye darker around upper edge to upper half, first few iridophores appear on iris** • **mouth forms a wider, narrow arc-shaped slit, labia begin to form (slight medial groove in lower labium), edge of lower jaw first visible (not keratinised)** • **adhesive glands well developed at each side of lower labium (centre pigmented in *G. laevis*)** • **first distinct melanophore flecks appear over dorsum and tail** • **tail longer, fins further developed, translucent dusky brown, circulation begins in some** • nares perforated • vent tube begins to develop with small opening on edge of ventral fin • yolk often paler than earlier stages
22 (Figure 7E,F)	• **head lengthens, yolk still wider** • **vitelline circulation more obvious** • **iris mostly black apart from narrow choroid fissure, gold iridophores increase on upper half, pupil milky grey** • **jaw ridges more obvious** • **adhesive glands fully developed, prominent operculum expands further, small slit on edge (left side)** • fins well formed, tail tip rounded, gradually clearing, circulation present in most • melanophores increase over dorsal surface, yolk and tail muscle • few gold iridophore flecks over dorsum • hatching can occur in some from this stage to stage 25, if eggs are flooded
23 (Figure 6I, 7G,J)	• **head broadens, yolk elongates (less broad)** • **labia well defined around mouth (medial groove in lower labium fused), tooth row ridges develop but no labial teeth** • **iris entirely black, gold iridophores increase, gap in gold at base, choroid fissure closes** • **spiracle begins to develop from slit in operculum** • **body wall begins to clear** • **vent tube has small opening on edge of ventral fin** • small hind limb buds begin to develop late in stage in some • nares open anteriorly on front of snout • faint shadow ventrally in yolk – indicates beginning of gut development, vitelline blood vessels visible • melanophores denser dorsally and ventrally over gut, entire tail muscle, dorsal fin and posterior one-third of ventral fin; gold iridophore flecks increase over body, dorsal tail muscle and a few on fins • short gold stripe from each eye to vertebral region, fine iridophores down either side of vertebral region and over yolk in many *G. leai*
24 (Figure 7H,K)	• **first keratinised labial teeth develop on A^1 and P^1 tooth row ridges, A^2 and P^2 start to become faintly keratinised in some, edges of jaws keratinised, papillae begin to develop** • **first thick gut loop develops** • **head almost as wide as gut, body more cylindrical, snout broadly rounded** • **eyes lateral, quite close to tip of snout, iris mostly covered with iridophores (dense gold in *G. laevis*)** • **adhesive glands reduce slightly, positioned below mouth on either side**

Stage	Developmental features
24 (cont.) (Figure 7H, K)	• spiracle almost full length • vent tube opening deflects slightly to right on edge of ventral fin *(G. laevis)* • head slightly longer and broader, still a little narrower than yolk
25 (Figure 7L)	• **all tooth rows keratinised except P^3, development of all papillae complete** • **hind limb buds first visible in some that had not developed these earlier** • **vent tube narrow, dextral, small, opens just above edge of fin (*G. laevis*), shorter and opens partway up ventral fin in *G. leai* and *G. victoriana*** • **two or three gut loops in most** • **spiracle full length, partly open** • **body wall clearer all over** • nares prominent, bordered by gold around lateral edge • increased iridophores over all of iris, gold ring around pupil with narrow black strip devoid of gold at bottom • gold iridophores increase over body, a few ventrally over gut in some • melanophores denser, increase over ventral surface of gut • tail fins deeper, melanophore and iridophore flecks increase over dorsal fin in many, more uniform over tail muscle
26 (Figure 6K-M)	• **P^3 develops keratinised labial teeth** • **hind limb bud visible in all (stage 26, Gosner)** • **hatching occurs in most** • **eyes larger and prominent, dense bright gold over iris in most, lower gap in gold ring around pupil closes** • **spiracle fully developed** • **tail lengthens further, coils right round body to other side of head in non-hydrated capsules** • **gut loops in thin spiral, four or five loops in most** • nares widely spaced, directed anterodorsally • dorsum dark or lighter brown with fine gold iridophores, brighter clusters over gills, sides of body and a few over gut ventrally in many – see individual species variations in text • venter clear, unpigmented anterior half, stippled melanophores and a few iridophore flecks over abdomen

APPENDIX 3

Comparison of selected key characters from the early developmental stages 18–26 of Gosner (1960) for aquatic embryos, with equivalent stages of the two *Geocrinia* species groups, showing how the terrestrial embryos of exotrophic *Geocrinia* species *G. leai*, *G. laevis* and *G. victoriana* and the endotrophic *G. rosea* group differ from Gosner stages (st = stage). NB, characters which are similar in all are not included.

St (G)	Selected Key Characters - Gosner	Exotrophic *Geocrinia laevis* group	Endotrophic *Geocrinia rosea* group
18	• adhesive glands joined beneath by crescent-shaped ridge • yolk small	• adhesive glands joined beneath by straight dark groove • yolk large	• no adjoining ridge beneath • yolk very large
19	• external gills begin to develop • crescent-shaped ridge gone in most, adhesive glands further developed • no external limb buds	• no external gills • straight groove gone in most, adhesive glands further developed • no external limb buds	• no external gills • small adhesive glands form on tips of mandibular arches • rudimentary hind limb buds begin to form
20	• gill circulation begins • optic bulge unpigmented • tail fins opaque, no circulation • N/A • hatching begins in many species	• N/A • optic bulge slightly dusky, pupil just discernable • tail fins dusky to opaque, circulation begins in some • N/A • N/A	• N/A • optic bulge unpigmented, pupil just discernable • tail fins begin to clear slightly, circulation begins • circulation first visible over yolk as pink flush • N/A
21	• no hind limb buds • cornea transparent in some, iris partly pigmented • external gills lengthen • adhesive glands fully developed • labial ridges begin to form • vitelline circulation not visible	• no hind limb buds • cornea not transparent, iris mostly pigmented except lower middle • N/A • adhesive glands not fully developed • labial ridges begin to form • vitelline circulation begins	• hind limb buds = st 26 (G) • cornea not developed, optic bulges faintly pigmented • N/A • small adhesive glands faintly pigmented • no labial ridges, mouth opening broadens • vitelline circulation more obvious
22	• no hind limb buds • tail fins transparent, circulation begins • iris well pigmented, narrow choroid fissure • N/A • labia broaden, jaw ridges visible • operculum begins to expand • initial gut development begins in some	• no hind limb buds • tail fins clearing, circulation begins in some • choroid fissure narrows, closes in some • N/A • jaw ridges visible • N/A • no gut development	• hind limb buds = st 27 (G) • tail fins transparent, circulation distinct • iris darker around upper half, fading into grey below, pupil visible • first iridescent pale blue flecks • mouth deepens and widens slightly into arc shape • N/A • no gut development

St (G)	Selected Key Characters - Gosner	Exotrophic *Geocrinia laevis* group	Endotrophic *Geocrinia rosea* group
23	• no hind limb buds	• hind limb bud early stage 26 in some	• hind limb buds = st 28 (G)
	• iris mostly fully pigmented, narrow choroid fissure, gold iridophores appear	• iris black, choroid fissure closes in most, first gold iridophores appear	• most of iris dark grey, lower medial choroid fissure lighter grey
	• jaw ridges formed (non-keratinised)	• labia and jaw ridges formed (non-keratinised)	• mouth slightly wider, labia begin to form
	• operculum expands further, partly covers external gills	• N/A	• N/A
	• adhesive glands prominent	• adhesive glands prominent	• remnant adhesive glands very small or gone
	• spiracle begins to form in some	• spiracle begins to form	• no spiracle yet present
	• hatching continues in some	• hatching begins in some	• hatching begins in some
24	• no hind limb buds	• hind limb buds = stage 26 (G) some	• hind limb buds longer = st 29 (G), may protrude laterally
	• operculum closes on right side	• N/A	• N/A
	• choroid fissure closed in most, iridophores increase over iris	• choroid fissure closed, iridophores increase over iris	• narrow choroid fissure, first gold iridophores begin to develop on top half of iris
	• adhesive glands begin to reduce in most	• adhesive glands full size	• adhesive glands gone
	• keratin on edges of jaw sheaths, tooth row ridges begin to form	• A and P^1 tooth rows present, jaws keratinised on edge	• non-keratinised lower jaw partly visible, no tooth rows
25	• no hind limbs buds in most species	• hind limb buds = stage 26	• hind limb buds = st 30 (G), slight constriction beneath knee region, small forelimb bud visible beneath body wall
	• operculum closes over gills on left side	• N/A	• N/A
	• jaw sheaths and tooth rows fully keratinised in most	• A^2 and P^2 tooth rows keratinised	• mouth a small broad slit, slight labial ridges form
	• spiracle formed and open	• spiracle formed and open	• no spiracle yet present
	• N/A	• N/A	• ECD visible as small white patch on each side of vertebral region just behind brain
	• tadpole begins to feed	• some hatch and begin to feed	• most hatched
26	• hind limb bud first apparent in most	• hind limb bud stage 26–27 (G)	• hind limb buds = st 31 (G)
	• N/A	• P^3 tooth row keratinised	• no tooth rows develop
	• tadpoles actively feeding and growing	• most tadpoles hatch, feeding begins	• hatching complete, tadpoles do not feed