Early developmental biology of *Platymantis vitiana* including supportive evidence of structural specialization unique to the Ceratobatrachidae

E. J. Narayan^{1,*}, J-M. Hero¹, K.S. Christi², C.G. Morley³

^{1*} Environmental Futures Centre, School of Environment, Griffith University, Gold Coast campus, QLD 4222, Australia.

² Division of Biological Sciences, School of Biological and Chemical Sciences, Faculty of Science, Technology and Environment, University of the South Pacific (USP), Fiji Islands.

³ Department of Conservation, Kauri Coast Office, 150 Colville Road , RD 7, Dargaville, New Zealand.

Correspondence Edward Narayan, E-mail: Environmental Futures Centre, School of Environment, Griffith University, Gold Coast campus, QLD 4222, Australia. Tel: +61 (0) 755527784; Fax: +61 (0) 755528067; Email: edward_nryn@yahoo.com

Short Title Direct embryonic development in *Platymantis vitiana*

Abstract

Direct embryonic development belongs to one of six unique developmental guilds within the endotrophic anurans. Few studies have been conducted on the embryonic development of direct developers. Herein, we present a unique form of embryonic development for direct developers from the genus *Platymantis* (Family Ceratobatrachidae). We incubated fertile eggs (n = 2 egg clutches; 40 eggs per clutch) of the endangered Fijian ground frog (*P. vitiana*) under controlled laboratory conditions (25°C and 100% relative humidity). Embryonic development (fertilization to hatching) took on average 29 days. Several unique embryonic structures were recorded, including the presence of very large eggs (8.5 mm diameter inclusive of egg-jelly and yolk, with the largest yolk diameter (6.0 mm) recorded for the genus *Platymantis*), the complete loss of the usual larval mouthparts, egg-tooth, gill buds and gills. Embryonic structural specialization included large abdominal sacs with blood capillaries which are likely the main medium of gas and waste exchange in *P. vitiana*. We provide a novel 10-stage staging system of embryonic development for *P. vitiana* which may also be useful for staging other members of the *Platymantis* genus. Our study contributes to existing knowledge on the developmental biology of the little studied direct developing endotrophic anurans.

Keywords Direct development; embryo; *Platymantis*; endotroph; anuran; respiration

Introduction

Tropical ecosystems support a high diversity of terrestrial anurans. One of the major steps in the evolution of terrestriality in tropical and temperate anuran species is the deposition of eggs out of water. Terrestrial egg deposition may have evolved in response to pond-drying, competition, fresh flooding and predation in large bodies of water (Magnusson & Hero, 1991). The diverse terrestrial embryonic developmental forms are recognized as endotrophic anurans, which obtain their entire developmental energy from parental sources, most commonly the vitellogenic yolk (Altig & Johnston, 1989). There are also intermediaries between terrestrial egg deposition involving delayed tadpole hatching, and complete direct development. The role of phenotypic plasticity in the evolution of terrestrial reproduction for the selection of various types of embryonic development is discussed by Werner (1986). The characterization of a taxon as endotrophic is often based on the presence of a small clutch of large, usually non-pigmented ovarian eggs. Direct development is the most common embryonic developmental form within endotrophic anurans (Altig & Johnston, 1989). Direct developing embryos complete a modified development via oogenic energy sources in a site other than the reproductive tract of the mother and hatch as a froglet (Altig & Johnston, 1989). Direct development has evolved independently in many anuran families, including the Leptodactylidae, Microhylidae, Ceratobatrachidae and Rhacophoridae (Thibaudeau & Altig, 1999). Direct development exemplifies a key adaptation and probably explains the overwhelmingly successful radiation of amphibians as one of the most species-rich group of extant terrestrial vertebrate. For example, there are 600 species in the direct developing genus *Eleutherodactylus* and a diverse

monophyletic radiation in the family Microhylidae, with more than 170 species in 20 genera of Australo-Papuan microhylids (Bickford, 2004). Despite this high species diversity and common occurrence of direct development, the developmental data on these endotrophic anurans are generally lacking.

The early study of the leptodactylid (*Eleutherodactylus nubicola*) by Lynn (1942) provided the most in-depth anatomical details of an endotroph. To date, the only complete staging table for any endotroph is based upon *Eleutherodactylus coqui* (Townsend & Stewart, 1985). Salthe & Mecham (1974) suggested that direct development of *Eleutherodactylus* may represent the most advanced type of endotrophy, although this idea may only reflect the paucity of data for other direct developing groups. Hence, the focus of the current study is on the embryonic development of the little studied genus of *Platymantis* (Ceratobatrachidae).

The *Platymantis* genus is endemic to the Philippines, Papua New Guinea, Palau, the Moluccas (eastern Indonesia), New Britain and Admiralty Island, Solomon Islands, and the Fiji Islands. The genus contains 71 species, of which there are 40 species yet to be described (Brown, 1997). Embryonic development of only four *Platymantis* species has been described to date (Alcala, 1962; Lamotte & Lescure, 1977; Gibbons & Guinea, 1983).

The aims of the current study were to provide the first account of the main features and complete stages of embryonic development of the endangered Fijian ground frog (*Platymantis vitiana*). We provide illustrations of the stages of development of live embryos of this species observed under high magnification. We also discuss the developmental characteristics of *P. vitiana* embryos in comparison with *E. coqui*

(Townsend & Stewart, 1985) and provide further supporting evidence for the embryonic structures unique to Ceratobatrachidae as observed earlier by Alcalá (1962) in three other *Platymantis* species.

Material and Methods

Study species

The genus *Platymantis* represents the eastern-most distribution of frogs in the South-west Pacific (Brown, 1997). The Fijian ground frog (*P. vitiana*) is endemic to Fiji Islands. *Platymantis vitiana* has an annual reproductive cycle and breeding occurs around the Fijian wet season from August to January (Narayan *et al.*, 2010). The population density (Morrison *et al.*, 2004), anecdotal records of the ecology (Gorham, 1971; Ryan, 1984) and key causes of *in-situ* population declines (Morley *et al.*, 2004) have been documented. However, no work on the embryonic development of *P. vitiana* has been published.

Field site

Field surveys for nesting areas of the Fijian ground frogs were conducted on Viwa Island (a 60 ha island located 900 m east off the coast of mainland, Viti Levu, Fiji). Surveys were conducted every month (av. 15 days per month) within the peak breeding season from January 2006 until December 2007 (Narayan *et al.*, 2008). Egg clutches were removed (n = 2 clutches; 40 eggs per clutch) from the nesting areas in the wild and incubated under controlled laboratory conditions at the University of the South Pacific,

Suva, for the study of the embryonic development. Descriptions of nesting areas (Narayan *et al.*, 2008) and captive husbandry (Narayan *et al.* 2009) of *P. vitiana* are available.

Laboratory egg incubation

Eggs of *P. vitiana* (n = 2 clutches; 40 eggs per clutch) were incubated in the laboratory under constant temperature of 25°C and relative humidity of 100% similar to the physical conditions generally required for incubation of terrestrial amphibian eggs as described by Elinson *et al.* (1990). To record the embryonic stages, eggs were transferred into a Petri dish (total area of 35 mm x 35 mm; depth of 12 mm) and partially filled with de-ionized water to maintain moisture level. Microscopic examinations were carried out using an Olympus SZX12 stereoscopic microscope mounted with an Olympus DP12 digital camera. The length of the embryo was measured using digital callipers (± 0.01 mm).

Stages of development

The early stages of egg cell divisions of Fijian ground frogs (c.f. stage 1 for *Eleutherodactylus coqui*, Townsend & Stewart, 1985) were not observed because freshly oviposited eggs were not collected from the wild. The staging in this study used the first ever *P. vitiana* egg clutches reported in the wild (Narayan *et al.*, 2008). The stages were recorded by comparing with a modified nomenclature for Fijian tree frog in Gibbons & Guinea (1983) and staging scheme for *E. coqui* (Townsend & Stewart, 1985). The morphological changes in the embryonic structures were recorded throughout the embryonic development.

Results

Staging table for *Platymantis vitiana*

Embryonic development of *P. vitiana* was divided into 10 main stages based upon readily discernible changes in major aspects of morphology. The number of days between each developmental stage and the major features of each stage are summarized in Table 1, and compared with developmental stages of *E. coqui*. Further details on the main structures of *P. vitiana* embryos are provided in the following section.

Egg. (Fig 1A) — Creamy unpigmented (average diameter = 8.5 mm, n = 40) with a transparent outer membrane and inner membrane. The embryo lies on top of the egg yolk. Each egg has an outer, thin thread-like cuticle attaching it to neighboring eggs in the clutch (n = 40 eggs). There are two jelly layers in total (1 and 2). The first jelly layer is 1.0 mm thick and bounded by the inner membrane and outer membrane. The second jelly layer is 0.5 mm thick, adjacent to the vitelline membrane. The vitelline space surrounds the egg yolk and is 0.5 mm in width. The overall distance between the outer membrane and the outer periphery of the egg yolk is approximately 2 mm. Mean wet mass of egg was 0.29 ± 0.02 g (n = 40).

Egg yolk. (Fig 1A-F)— A large, creamy egg yolk (average diameter = 6.5 mm, n = 40) is present throughout the embryonic developmental stages and reduces to ‘leaf-like’ in shape as development proceeds. The position of the embryo (above and below the egg

yolk) changes as development proceeds. Initially, the lighter embryo is positioned above the heavier egg yolk during stages 1-4 of development. As the embryo grows, it becomes heavier than the egg yolk and drifts below the egg yolk during stages 5-6. Thereafter, majority of the egg volume is occupied by the voluminous abdominal sac (stages 7-9) and this keeps the lighter embryo on top until hatching.

Eyes. (Fig 1B-F) — Two large eyes are distinctive. Their position is evident as large anterior bulges in the cephalic region by stage 3. Also, the iris shows pigment and darkens progressively until black in stage 6. During stage 7, the pupil begins to darken and the iris lightens. By stage 9, the pupil is dark and the iris attains the adult colour characteristics with a mixture of golden and bronze-brown.

Body. (Fig 1E-F) — The body of the embryo takes the shape of a froglet during stage 7 and represents a complete froglet by stage 9 prior to hatching. The dorsal abdomen of the frog is highly pigmented and is golden brown in colour. The ventral portion of the abdomen is dominated by the remains of egg yolk, and the skin pigmentation covers the ventral abdominal area at the periphery.

Limbs. (Fig 1B-F)— Limb buds first appear in stage 2 as rounded swellings lateral to, and slightly separated from, the body. The buds increase in size and join the trunk in stage 3. The hind limb buds are slightly larger than the fore limb buds. Both the front and hind limb buds are roundish in appearance early in stage 4, becoming more oblong as this stage progresses. Elbow and knee joints appear as constrictions during stage 5 and are

quite evident in stage 6. Toes appear on the anterior and posterior limbs during stage 7. The anterior limbs have four toes each while the posterior limbs have five toes. The second toe on both the anterior and the posterior limb is longer than other toes. Limbs and toes elongate from stages 7 to 9 at which point, the limbs reach full length, relative to their length at the hatching. Toe pads appear in stage 8.

Tail. (Fig 1B-F) — The tail occupies one-third of the embryonic body at stage 3 (embryo head to tail tip length = 11 mm). The tail fin is not prominent. The tail undergoes rhythmic lateral contractions which help re-orientate the embryo to its original dorsal position when stimulated by either movement or strong illumination. The tail is reduced in stage 8 and completely reabsorbed during stage 9 of embryonic development.

Endolymphatic calcium deposits (ECDs). (Fig 1B-C) — Two symmetrical cream coloured neural grooves, situated posterior to the eyes appear during stage 3. During late stage 3 and early stage 4, the ECDs extend possibly forming a vertebral column across the head to vent region. There are nine vertebral segments (vertebrae) in total. As development progresses, the vertebral segments are covered by the dorsal muscle and skin of the embryo.

Abdominal sacs. (Fig 1B-F) — A large fluid filled, vascularised abdominal sac with prominent blood capillaries is evident from stage 3 and prominent until stage 10 at hatching. The abdominal sac is partitioned into two portions, each occupying the embryonic body and covering the thin ‘leaf-like’ egg yolk across the head-vent axis. The

embryo sits above the abdominal sac during the later stages of development (stages 8-9). The supply of blood capillaries around the abdominal sac is highest ($n = 20-30$) between stages 6 to 8 but the majority of the blood capillaries get reabsorbed within the egg yolk at stage 9 just before hatching. The remaining blood capillaries are visible in the underbelly abdomen (remaining egg yolk) of a newly hatched froglet (see Fig. 3).

Heart and blood capillaries. (Fig 1C-F) — The minute heart is visible in the upper thoracic region of the embryo during stage 4 and it becomes prominent during stages 5-8. Two prominent blood capillaries (reddish orange) originate near the heart and extend along the abdominal sac. Several finer blood capillaries converge near the heart across the abdominal sacs. As development proceeds (stages 2-9), the blood capillaries surrounding the abdominal sacs reduces in number (Fig. 2).

Hatching. — During the hatching at late stage 9, each froglet shows rapid ‘kicking’ movement of both anterior and posterior limbs to break itself out of the abdominal sac. A newly hatched froglet (Fig. 2) has dark brown skin colouration and it appears slimy. The average wet body mass of a newly hatched froglet is $0.1 \text{ g} \pm 0.02$ ($n = 5$).

Froglet. — Two characteristic features of newly hatched *P. vitiana* froglet are a pattern of brown warts on the dorsal head region and distinctive blackish brown stripes evenly marked on the dorsal areas of both the anterior and posterior limbs. Remains of the egg yolk (length = 3 mm) are still present at the periphery of the lower abdomen of each newly hatched froglet up to 5 days (Fig. 3). Embryonic development required an

incubation period of approximately 29 days at constant temperature of 25°C and relative humidity of 100%.

[Figure 1.0 here]

1 [Figure 2.0 here]

2

3

4

5

6

7

8

9

10

11

12

13 [Figure 3.0 here]

14

15

16

17

18

19

20

21

22

23

24

25 [Table 1.0 here]

26

27

28

29

30

31

32

33

34

35

Discussion

The stages of embryonic development of the endangered Fijian ground frog (*Platymantis vitiana*) are markedly different from the well studied direct developing species *Eleutherodactylus coqui* (Leptodactylidae family, see detailed comparison in Table 1.0). The complete loss of the usual larval mouthparts, egg-tooth, gill buds and gills were features different from *E. coqui*. Our study describes the embryonic development of *P. vitiana* under controlled laboratory conditions and two main embryonic structural features unique to the genus *Platymantis* (Ceratobrachidae). These embryonic structural features include the presence of thin tail and vascularised abdominal sacs (Table 1.0; Fig 1). Alcalá (1962) reported similar structural features in Philippine direct developers of the genus *Platymantis* including *P. guentheri*, *P. hazeli* and *P. meyeri*. Interestingly, abdominal sacs were also reported in *Ceratobatrachus guentheri* (Ranidae family) by Thibaudeau & Altig (1999) however they were described as “wrinkles in the lateral body wall that developed as the yolk reserve was depleted”, suggesting it is unlikely to have a respiratory function. The enlarged vascularised abdominal sacs reported herein, support the earlier suggestion by Alcalá (1962) that the abdominal sac serves as a respiratory surface for the genus *Platymantis*. Oxygen is most likely carried via the blood capillaries between the abdominal sacs, the heart and the respiratory tissues of the *Platymantis* embryo during development. It is already known that amphibian embryos are essentially aerobic and that they must exchange gases with their environment during development (Duellman & Trueb, 1994). Furthermore, as the development proceeds and the metabolic activity of the embryo increases, the reduction in blood capillaries surrounding the abdominal sacs (Fig 2) is likely to be associated with the ‘need’ to reduce the chance of absorbing toxic wastes through the abdominal fluid in the

61 abdominal sac. In embryos of *E. coqui*, excess fluid in the egg-jelly causes a lethal
62 syndrome known as edema (Yun & Elinson, 2008). We hypothesize that the reduction
63 in blood capillaries and anoxic state of the abdominal fluid could also provide a
64 ‘trigger’ for hatching in *P. vitiana*. Hypoxia restricts their development rate of the
65 embryos and can ultimately kill them (Seymour *et al.* 1991). The vitelline membrane
66 and jelly capsule are key structures responsible for oxygen supply through water
67 uptake from the nesting areas. Changes in the dynamics of the vitelline space due to
68 excessive flooding can affect the oxygen supply to the embryos. For example, in the
69 terrestrial laying Australian frogs of the genus *Pseudophryne*, hatching is reported to
70 be triggered by anoxia caused by flooding of the nests (Seymour *et al.* 1991). From
71 an evolutionary perspective, it is interesting that the vascularised abdominal sacs
72 described for the genus *Platymantis* presents a similar vascularised structure as the
73 allantois in the amniotic egg, which is also known to function in gas exchange and
74 waste removal (Stewart, 1977; Gauthier *et al.*, 1988). Future investigations on the
75 oxygen content of the abdominal fluid and respiration rates of the *Platymantis*
76 embryos could be useful to support this hypothesis.

77 Respiratory specializations in embryos of other direct developing anuran
78 species, such as the enlarged vacularised tail in *E. coqui* (Townsend & Stewart, 1985)
79 The embryo of the microhylid, *Phrynomantis robusta* (Duellman & Trueb, 1994),
80 also have a tail that is greatly expanded and pressed against the vitelline membrane to
81 provide a respiratory surface. Gills and other associated structures such as operculum
82 play a key respiratory function in other direct developing species. Most of the species
83 within the genus *Eleutherodactylus* (*E. geuntheri*, *E. nasutus*) have no external gills,
84 while *E. johnstonei* has a residual gill arch but no gill filaments. The operculum
85 functions in respiration in addition to the tail in *E. coqui* (Townsend & Stewart, 1985)

while, *E. inoptatus* and *E. portoricensis*, whose tails do not reach the same size as in the other species, have external gills (Lamotte & Lescure, 1977). The simplification of the aortic arches and the development of the much vascularised tail fill a respiratory function in *E. coqui* (Lamotte & Lescure, 1977). In contrast, the tail was reduced and reabsorbed as development proceeded in *P. vitiana*. Bell gills, derived from the brachial arches, serve a respiratory function in the Andean Marsupial frog (*Gastrotheca riobambae*) of the family Hemiphractidae (del Pino & Escobar, 1981).

Another key observation is that embryos of *P. vitiana* did not develop an egg tooth, as found in *E. coqui* (ownsend & Stewart 1985; Hanken *et al.*, 1997). Hatching (breaking of the jelly layers) in *P. vitiana* is most likely assisted by the observed rapid movements of anterior and posterior limbs of the embryo. Froglets of *P. vitiana* required an incubation period of approximately 29 days to hatching at a constant temperature of 25°C and 100% relative humidity. Eggs of *P. vitiana* had a basic structure of frog eggs as proposed by Altig & McDiarmid (2007), an egg yolk (ovum) with a vitelline membrane surrounded by jelly layers 1 and 2 of oviductally produced materials. Eggs of *P. vitiana* were similar in morphology to those observed in other *Platymantis* species reported by Alcala (1962). Furthermore, the eggs of *P. vitiana* recorded the largest clutch size (av. 40 eggs) and largest yolk diameter (av. 6 mm) for the genus *Platymantis*; compared to *P. guentheri* (av. 8 eggs; av. yolk diameter 3 mm), *P. hazelae* (av. 7 eggs; av. yolk diameter 3.40 mm) and *P. meyeri* (av. 27 eggs; av. yolk diameter 3.30 mm) [Alcala, 1962]. For the much smaller sized *P. vitiensis* (adult frogs are three times smaller than *P. vitiana*) eggs of a similar size (8 mm) have been reported however they have smaller clutch sizes of between 18-20 eggs (Ryan, 1984). It is suggested that the actual size of the egg jelly could vary between species since for many species of anuran amphibians, the egg jelly layers will swell

enormously based upon exposure to moisture (Duellman & Trueb, 1994), which would greatly change measurements of egg thickness. As with other *Platymantis* species, newly hatched froglets of *P. vitiana* did not feed for at least five days as the froglets continued to consume the remaining substantial yolk deposit (Fig. 3).

In summary, the *Platymantis* species (Ceratobatrachidae) have highly vascularised, thin walled lateral abdominal sacs – a unique development structure, as demonstrated in the embryos of *P. vitiana* (and observed in three other species by Alcala 1962). Our study supports the hypothesis that the vascularised abdominal sacs are a structural specialization for respiration, and is likely to play an important role in gas and waste exchange, similar to the allantois in the amniotic egg (Stewart, 1977; Gauthier *et al.*, 1988). Thus, this may be a unique physiological feature of the genus *Platymantis* as developmental descriptions for other direct developers have different adaptations for respiration (enlarged vascularised tails or gills and operculum). Overall, the developmental dynamics and evolutionary significance of the early respiratory system of direct developers, especially the genus *Platymantis* should be further investigated. Our study has presented a new staging table and details of the structural specializations of the little studied genus *Platymantis*.

Acknowledgements

We would like to thank Richard P. Elinson for a critical review of this manuscript and for providing key literature support. We also thank the indigenous community of Fiji (Viwa Islands) for donating *P. vitiana* specimens for this study and the captive breeding facility at the University of the South Pacific, Fiji for access to the lab and animals. Funding for this research was provided by the Australia Pacific Science Foundation (Grant number: 07/6).

136 **References**

- 137 Alcala, A.C. 1962. Breeding behaviour and early development of frogs of Negros,
138 Philippine Islands. *Copeia* **4**, 679–726.
- 139 Alcala, A.C. & Brown, W.C. (1982). Reproductive biology of some species of
140 *Philautus* (Rhacophoridae) and other Philippine anurans, Kalikasan. *Philipp. J. Biol.*
141 **11**, 203–226. Altig, R. & Johnston, G.F. (1989). Guilds of anuran larvae:
142 Relationships among developmental modes, morphologies, and habitats. *Herpetol.*
143 *Monogr.* **3**, 81–109.
- 144 Altig, R. & McDiarmid, R.W. (2007). Morphological diversity and evolution of egg and
145 clutch structure in amphibians. *Herp Monog.* **21**, 1–32.
- 146 Bickford, D.P. (2004). Differential parental care behaviors of arboreal and terrestrial
147 microhylid frogs from Papua New Guinea. *Behav. Ecol. Sociobiol.* **55**, 402–409.
- 148 Brown, W.C. (1997). Biogeography of amphibians in the Islands of the South-west
149 Pacific. *Proc. Cal. Acad. Sci.* **50**, 21–28.
- 150 del Pino, E.M. & Escobar, B. (1981). Embryonic Stages of *Gastrotheca riobambae*
151 (Fowler) during maternal incubation and comparison of development with that of
152 other egg-brooding hyliid frogs. *J. Morphol.* **167**, 277–295.
- 153 Duellman, W.E. & Trueb, D. (1994). *Biology of amphibians*. McGraw-Hill, New York.
- 154 Elinson, R.P., Del Pino, E.M., Townsend, D.S., Cuesta, F.C. & Eichorn, P. (1990). A
155 practical guide to development of terrestrial breeding frogs. *Biol. Bull.* **179**, 163–177.
- 156 Gauthier, J., Kluge, A.G. & Rowe, T. (1988). The early evolution of the amniota. In *The*
157 *phylogeny and classification of the tetrapods*: 103–155. Benton, M.J. (Ed.). Vol 1:
158 amphibians, reptiles, birds. Oxford: Clarendon Press.
- 159 Gibbons, R.H. & Guinea, M.L. (1983). Observations on development of the Fijian tree
160 frog, *Platymantis vitiensis*. *Herpetofauna* **14**, 83–86.

161 Gorham, S.W. (1971). Field identification of Fiji frogs. *Fiji Agricultural Journal*. **33**,
162 31–33.

163 Hanken, J., Jennings, D.H. & Olsson, L. (1997). Mechanistic basis of life-history
164 evolution in anuran amphibians: direct development. *Am. Zool.* **37**, 160–171.

165 Lamotte, M. & Lescure, J. (1977). Adaptive tendencies toward liberation from the
166 aquatic environment in the anuran amphibians. *Terre et la Vie*. **2**, 225–312.

167 Lynn, W.G. (1942). The embryology of *Eleutherodactylus nubicola*, an anuran which
168 has no tadpole stage. *Carnegie Inst. Washington Contrib. Embryol.* **30**, 27–62.

169 Magnusson, W.E. & Hero, J.M. (1991). Predation and the evolution of complex
170 oviposition behavior in Amazon rainforest frogs. *Oecologia* **86**, 310–318.

171 Morley, C.G., Ambrose, M. & Hero, J.M. (2004). *Eradicating cane toads (Bufo*
172 *marinus) and Pacific rats (Rattus exulans) from Viwa Island, Fiji*. Feasibility study
173 for the Invasive Species Specialist Group, IUCN University of the South Pacific.

174 Morrison, C., Naikatini, A., Thomas, N., Rounds, I., Thaman, B. & Naikula, J. (2004).
175 Rediscovery of an endangered frog *Platymantis vitiana*, on mainland Fiji:
176 Implications for conservation and management. *Pac. Cons. Biol.* **10**, 237–240.

177 Narayan, E., Molinia, F., Christi, K., Morley, C. & Cockrem, J. (2010). Annual cycles
178 of urinary reproductive steroid concentrations in the endangered *Platymantis vitiana*.
179 *Gen. Comp. Endocrinol.* **166**, 172–179.

180 Narayan, E., Christi, K. & Morley, C. (2009). Captive propagation of the endangered
181 native Fijian Frog *Platymantis vitiana*: Implications for *ex situ* conservation and
182 management. *Pac. Cons. Biol.* **15**, 47–55.

183 Narayan, E., Christi, K. & Morley, C. (2008). Ecology and reproduction of endangered
184 *P. vitiana Platymantis vitiana* (Anura, Ranidae). *South Pac. J. Nat. Sci.* **26**, 28–32.

185 Ryan, P.A. (1984). Fiji Amphibia. *Domodomo* **11**, 87–98.

186 Salthe, S.N. & Mecham, J.S. (1974). Reproductive and courtship patterns. In
 187 *Physiology of the Amphibia*: 309–351. Lofts, B. (Ed). New York: Academic Press.

188 Stewart J.R. (1997). Morphology and evolution of the egg of oviparous amniotes. In
 189 *Amniote origins-completing the transition to land*: 291–326. Sumida, S. & Martin, K.
 190 (Eds). London: Academic Press.

191 Seymour, R.S., Geiser, F. & Bradford, D.F. (1991). Metabolic cost of development in
 192 terrestrial frog eggs (*Pseudophryne bibronii*). *Physiological Zoology*. **64**, 688–696.

193 Thibaudeau, G. & Altig, R. (1999). Endotrophic anurans: development and evolution.
 194 In *Tadpoles. Biology of anuran larvae*: 170–188. McDiarmid, R.W. & Altig, R (Eds).
 195 London: The University of Chicago Press, Ltd.

196 Townsend, D.S. & Stewart, M.M. (1985). Direct development in *Eleutherodactylus*
 197 *coqui* (Anura: Leptodactylidae): a staging table. *Copeia* **1985**, 423–436.

198 Werner, E.E. (1986). Amphibian metamorphosis: Growth rate, predation risk, and the
 199 optimal size at transformation. *American Naturalist*. **128**, 319–341.

200 Yun, L.S. & Elinson, R.P. (2008). Abnormalities of forelimb and pronephros in a direct
 201 developing frog suggest a retinoic acid deficiency. *Appl. Herpetol.* **5**, 33–46.

202

203

204

205

206

207

208

209

210

Figure Captions

Figure 1

Digital micrographs of *P. vitiana* embryos. **A: Stage 2**, OM – outer membrane, IM – inner membrane, V – vitelline membrane, VS – vitelline space, H – head, ALB – anterior limb bud, PLB – posterior limb bud, T – tail, 1 – first jelly layer, 2 – second jelly layer, B – blood vessels; **B: Stage 3**, AS – abdominal sac, ECD – endolymphatic calcium deposits, E – eye; **C: Stage 4**, VS – vertebral segments, P – pigmentation; **D: Stage 5**, H – heart, IV – invagination; **E: Stage 7**, AL – anterior limb, AT – anterior toes, M – mouth, PL – posterior limb, PP – posterior toes, S – snout; **F: Stage 8**. Scale = 1 mm.

Figure 2

Hand drawings of *P. vitiana* embryos emphasizing the abdominal sac (AS). A) Stage 3, egg yolk large, abdominal sac first appears with minute blood capillaries. B) Stage 7, egg yolk reduces and abdominal sac enlarges with prominent blood capillaries. C) Stage 9, dorsal view prior to hatching; tail reabsorbed, egg yolk greatly reduced ‘leaf-like’, vascularised abdominal sac prominent but without major blood capillaries (reabsorbed). Scale = 1 mm.

Figure 3

Newly hatched *P. vitiana* froglet (SVL = 8 mm) [Stage 10] showing remains of egg yolk (Y), skin pigmentation (P) and blood capillaries (B).

Table Captions

Table 1

The major diagnostic features of *P. vitiana* embryo and comparison to direct developer *E. coqui* (Townsend and Stewart, 1985). Length (days) for each stage of embryonic development of *P. vitiana* is provided in parenthesis.