

DIRECT DEVELOPMENT IN *PHILAUTUS* *GLANDULOSUS* (ANURA: RHACOPHORIDAE)

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Variation in reproductive pattern is a characteristic feature of lower vertebrates, and it is most pronounced among amphibians. Many species exhibit prolonged larval developmental stages that may be adapted to aquatic, semi-aquatic, arboreal, or even terrestrial conditions. However, members of a few frog families exhibit direct development without a tadpole stage (Salthe and Mecham 1974; Elinson and Fang 1999). The Western Ghats mountain range of India harbours 123 known species of amphibians, and among these 24 species of Philautinae (Family Rhacophoridae) are reported (Daniels 1992). Of these, nine have been recorded from the locality of the present study (Krishnamurthy and Katre 1993; Krishnamurthy and Hussain 2000). In spite of India's rich amphibian diversity, information regarding developmental patterns has been restricted to only a few common species that develop via tadpoles. Direct development has been recorded only for *Philautus variabilis* from Dharwad, at the eastern extreme of the Western Ghats (Patil and Kanamadi 1997). In this study, we report direct development in *P. glandulosus* Jerdon 1875 for the first time.

MATERIALS AND METHODS

During the monsoon of every year (June–September) individuals of *P. glandulosus* were sighted in the dense, moist evergreen forest of the central Western Ghats. On 12 August 2000 at 1900 h, after a brief monsoon shower, amplexus of *P. glandulosus* was observed following repeated mating calls at a height of 1.7 m above the ground in a bush in Kerekatte forest, Kudremukh National Park (13°10'–13°26' N, 75°05'–75°10' E). Amplexus was axillary (Fig. 1), with the male smaller than the female (21 vs 25 mm SVL, respectively). After 6.5 h (01.30 h on 13 August) eggs were laid on a wet leaf

After spawning, the amplexant pair separated and the parents remained near the spawn. The leaf containing the egg mass was carefully transferred along with the parents to a large aerated container and transported to the laboratory. In the laboratory, eggs and parents were maintained in a terrarium of dimensions 45 x 22.5 x 22.5 cm under temperature and humidity conditions simulating those in the frogs' habitat (Table 1). The time of oviposition was considered for all tabulation of developmental events.

RESULTS

A clutch of 14 eggs in a single layer occupied an area of 140 mm². Each egg was unpigmented, cream-colored, large, and covered by a thick jelly coat (Fig. 2A). Egg size varied slightly (diameter of egg including jelly coat 4.9 ± 0.9 mm; diameter of egg excluding jelly coat 2.2 ± 0.1 mm; $n = 14$). The gray crescent was observed 24 h after oviposition. Thereafter, cleavage, gastrulation, and neural plate and fold formation occurred within 48 h. Subsequently, the embryo elongated and organs appeared in the form of buds. These buds differentiated within 75–78 h (Fig. 2B). Following the formation of tail and head, pigmentation appeared (around 92 h), initially on the middorsal line. Membranous



Figure 1. Amplexing pair of *Philautus glandulosus* from central Western Ghats. Photo by SVK

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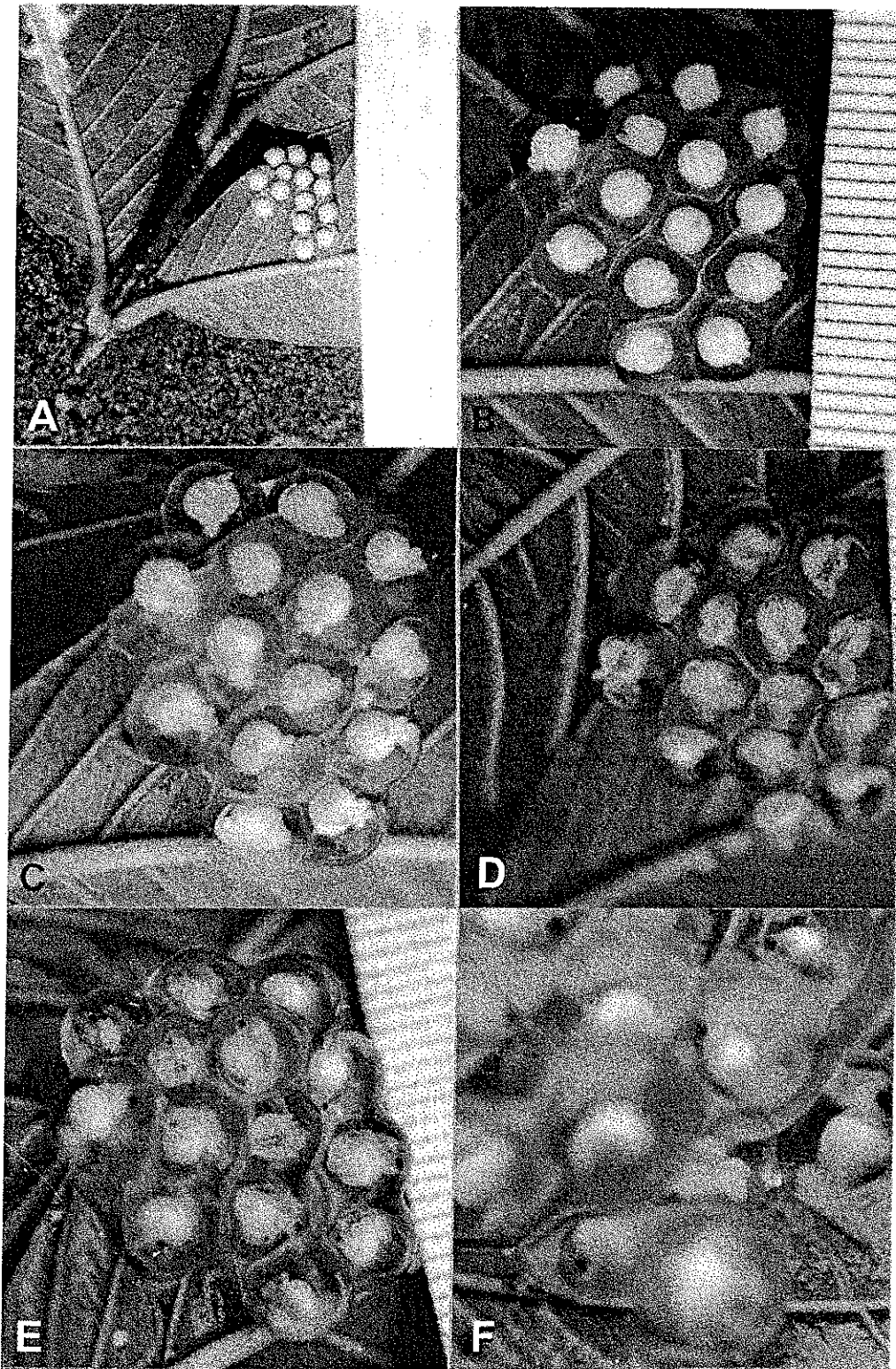
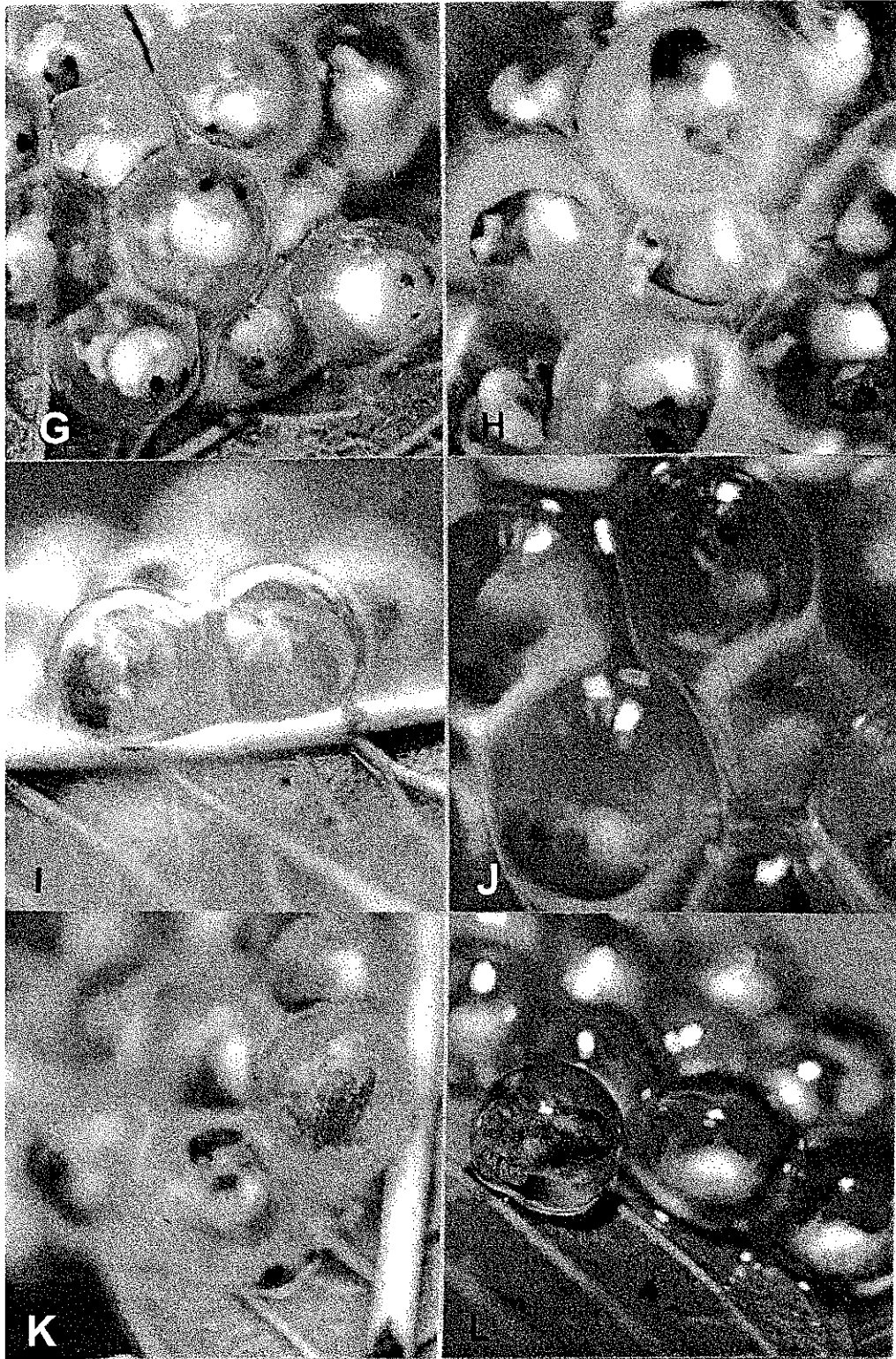


Figure 2. Stages of direct development in *Philautus glandulosus* from the Western Ghats, India. (A) Unpigmented creamy eggs immediately after spawning. Embryos undergoing development are pictured on Day 3 (B), Day 5 (C) with limb buds and a tail present, Day 6 (D) with an eye prominently visible,



Day 7 (E), Day 10 (F), Day 11 (G), Day 13 (H) with the lower jaw clearly visible, and Day 14 (I) with clearly differentiated digits. A froglet with tail (J) and pre-hatching juveniles (K, L) are the final stages of direct development in this species.

TABLE 1. Abiotic parameters at the breeding site of *Philautus glandulosus* and conditions in the laboratory terrarium. Values in the parenthesis denote the ranges of the respective variables.

Parameters	Natural Habitat* (n = 24)	Laboratory Terrarium (n = 23)
Air temperature (°C)	22.5 ± 0.3 (22.3–23.0)	23.3 ± 0.9 (22.0–25.5)
Humidity (%)	81.8 ± 3.2 (77.8–87.0)	73.8 ± 3.7 (71.2–86.0)
Light Intensity (lux)	1561.9 ± 778.8 (249.6–2240.0)	1400.8 ± 107.6 (160.0–2280.0)

*Pooled data from the previous observations on breeding of the same species from the same locality during monsoon (duration 3 mo)

gills appeared a short while later and disappeared by Day 5 (Fig. 2C). After 120 h melanophores increased in number and extended to all regions of the embryo, including the yolk surface.

On Day 5 (Fig. 2D) pigmentation and formation of the head progressed further, with a remarkable triangular black spot appearing at the interorbital space, accompanied by clear differentiation of eyes. Within another 24 h (140–147 h) the head widened and the anterior region of the body, excluding forelimb buds, became clearly differentiated, with a noticeable heartbeat and an elongated tail (Fig. 2E). By this time, the external gills had totally disappeared. Pigmentation had deepened further and started extending downward to the ventral side, along with differentiation of the lower jaw; the yolk had become restricted to the abdomen (Fig. 2F; 220 h). The hindlimb buds elongated and further recognizable differentiation of digits began, though these lacked pigmentation. Following this, there is a visual decrease in yolk and an increase in pigmentation (Fig. 2G; 250 h). Within the next 48 h, the embryo acquired the shape of a juvenile frog with prominent forelimb buds, an elongated tail, and adult dorsal coloration (Fig. 2H; 13 d old embryo). Subsequent to this, with the deepening of pigmentation and further reduction of yolk (Fig. 2I), the forelimb bud elongated, while digits on the hindlimb appeared like buds. By Day 14 the mouth had differentiated into upper and lower lips (Fig. 2J), and differentiation of pigmented digits was complete. From Day 18 onward, the tail became

reduced considerably and the froglet acquired juvenile features (Fig. 2K, L) including a small, reduced tail bud and clear cross bars on the limb. By Day 22 all juveniles had hatched and they looked similar to that of the adult except for their size.

The events of the direct development in *P. glandulosus* are summarized in Table 2. The size of hatched juveniles was only one-fifth of the parents (SVL juveniles 4.6 ± 0.6 mm; parents 22.0 ± 2.2 mm) and juveniles exhibited similar morphological patterns as the adults. Rate of development varied somewhat in each egg. The entire developmental process can be clearly categorized into a neural plate stage, a tail and limb bud formation stage, an external gill stage, commencement of pigmentation, differentiation of eye and other organs, differentiation of forelimb and reduction in tail length, and a pre-hatching stage. These events could be clearly observed through the transparent jelly coat.

DISCUSSION

Direct development has been studied in various groups of amphibians and pattern, duration, and staging vary widely among different species (Lutz 1947, Goin 1959, Townsend and Stewart 1985, Patil and Kanamadi 1997). Direct development in the genus *Philautus* has been studied in detail only in *P. variabilis* (Patil and Kanamadi 1997), although preliminary reports (Daniels 1992) have suggested that direct development is more frequent in this diverse genus. Compared to *P. variabilis*, direct development in *P. glandulosus* has several distinct characteristics. *Philautus glandulosus* lays small clutches of 12–18 eggs (pers. obs.) whereas *P. variabilis* egg clutches contain 52–64 eggs (Patil and Kanamadi 1997). Although the developmental processes in both species appear similar, in *P. glandulosus* it is prolonged and hatching takes place after 20–22 d compared to only 12 d in *P. variabilis*. Another remarkable difference in the development of *P. glandulosus* and *P. variabilis* is their gills, which are lamellar in *P. glandulosus* and exist for about 24 h, whereas they are highly reduced in *P. variabilis* and persist for a longer duration (nearly 72 h). The female of *P. variabilis* is said to exhibit parental care by collecting eggs beneath her abdomen and by aggressively attacking intruding males (Patil and Kanamadi 1997). In our study, *P. glandulosus* did not exhibit any aggressive behavior but was found near the egg clutch.

TABLE 2. Summary of the developmental process of *Philautus glandulosus* from the Western Ghats, India. Each stage covers 2 d, with exception of stage 10 (4 d)

Stage	Prominent Developmental Process
1	Gray crescent, gastrula, neural plate and folds
2	Elongation of embryo, appearance of limb bud, head and gills
3	Disappearance of gills, differentiation of head, prominent pigmentation, clear differentiation of eye
4	Widening of head, elongation of limb bud and tail, pigmentation spreads downwards to yolk in the belly region
5	Heart beat, differentiation of mouth, differentiation of digits begin in the hindlimb
6	Elongation of tail, differentiation of digits, eyes prominent and protruded
7	Reduction in yolk, pigmentation extends to mid ventral line on the yolk, hind limb clearly differentiated, tail length starts regression
8	Forelimb clearly differentiated, yolk greatly reduced, reduction in tail length, 'small juvenile with tail', movement of eyelid
9	Juvenile within jelly coat, clear adult body coloration, 'juvenile with reduced tail'
10	Hatching into juveniles

The habitats of adults of *P. glandulosus* and *P. variabilis* are similar but whereas the microhabitat of *P. variabilis* is more often on forest floor that is covered with moist leaf litter and low bushes, *P. glandulosus* is almost exclusively bush-dwelling or found in the lower to mid canopy. *Philautus glandulosus* are generally more active during the monsoon season than *P. variabilis*. Although both species have the same breeding season, microhabitats of breeding sites vary considerably. *Philautus variabilis* was observed to lay eggs on the moist leaf litter or sometimes on leaves of low-level bushes. In contrast, *P. glandulosus* lays eggs on leaves of bushes higher off the ground (height range 1.5–3.0 m; pers. obs.). In *P. glandulosus* fewer eggs requiring a longer time for complete development were produced. In contrast, *P. variabilis* lay many eggs that have shorter developmental time. Both species prefer breeding during the monsoon season (pers. obs.) and undergo direct development in a short time compared to ranoids of the region; this might indicate strong adaptation exhibited by both species. Examination of these differences present avenues for further study.

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