

Larval external morphology and development in *Feihyla kajau* (Dring, 1983) (Amphibia: Anura: Rhacophoridae)

Indraneil Das¹, Hairi Hedeir¹, Yong Min Pui¹, Stefan T. Hertwig^{2,3} & Alexander Haas⁴

Abstract. The external morphology and development of the tadpoles of *Feihyla kajau* (Dring, 1983) from Kubah National Park, Sarawak, East Malaysia (Borneo), are described. The species produce small clutches of 7–10 (mean $8.60 \pm \text{SE } 0.51$) eggs within a mass of jelly-like substance that is stuck to leaves near standing bodies of water, such as stagnant pools and swamps. The tadpoles reach 26.7 mm in total length, their body shapes subglobose to ovoid in dorsal view; eyes positioned dorsolaterally; spiracle sinistral; oral disk anteroventral; marginal papillation of oral disk with broad gap on upper lip and no gap on lower lip; oral disk marginal papillae arranged in single row; labial ridges with uniserial keratodont rows; and Labial Tooth Row Formula $4(2-4)/3$. Morphological changes during development are documented according to the staging table of Gosner (1960). Development was recorded from Stage 26 until Stage 42. Time taken to reach Stage 42 was 110 days. It is shown that developmental time and stages have a pronounced nonlinear relationship; stages are only ordinal. Ex situ conditions required to raise the tadpoles of the species are described, which include water temperature, food and condition of growing provided.

Key words. Tadpole, growth, description, staging, metamorphosis, *Feihyla kajau*, Malaysia, Borneo

INTRODUCTION

Feihyla kajau (Dring, 1983) is a small (SVL to 22.6 mm; Fig. 1) rhacophorid (Amphibia: Anura: Rhacophoridae) frog, originally described as *Rhacophorus kajau* from Gunung Mulu (250 m asl), Sarawak, East Malaysia (Borneo) by Dring (1983), and relocated to *Feihyla* by Hertwig et al. (2013). This species was subsequently reported from other parts of Sarawak, Sabah and from Brunei Darussalam, from primary forests at lower altitudes to submontane limits (20–1,200 m asl) (Inger & Stuebing, 2005; Das, 2007).

Members of the Rhacophoridae are arboreal, at least on Borneo. Tadpoles of the family Rhacophoridae are free swimming, active feeders (Inger, 1985; Inger & Stuebing, 2005), with the exception of most known members of the genus *Philautus* (see Hertwig et al., 2011). Many build foam nests for oviposition in or close to tree holes (*R. harrisoni*, *Theلودerma* spp.) or on leaves or twigs overhanging bodies of flowing waters (*R. gauni*, *R. cyanopunctatus*) or (*Polypedates*

leucomystax, *R. borneensis*) (Inger, 1985). The foam nests provide a moist environment where embryos can develop without drying out and are safe from aquatic predators. However, *Feihyla kajau* does not produce foam nests, but lays jelly egg masses on leaves overhanging stagnant pools and swamps. Lack of a foam nest in *F. kajau* is considered a secondary loss in the context of rhacophorid phylogeny (Hertwig et al., 2013), and foam-nesting itself appears to have evolved once (Grosjean et al., 2008; Wilkinson et al., 2002), or convergently, at least twice (Meegaskumbura et al., 2015).

Information on Bornean tadpoles is sparse (Das & Haas, 2005). A brief description of the tadpoles of *Feihyla kajau* was provided by Dring (1983), based on a single Stage 25 tadpole. During our field work, we realised that Dring's description need to be complemented by additional features for unequivocal determination of its tadpoles in the field, especially features of live colouration and size at growth climax. In this paper, we describe the larval morphology and development of *F. kajau*. Specific objectives of this study are:

1. To provide a suite of character states to identify the larvae of *F. kajau* in the field and of museum specimens,
2. To determine the growth and differentiation of *F. kajau* on the basis of a generalized staging table (that of Gosner, 1960), including notes on morphological characteristics in each stage.
3. To contribute information on basic conditions to raise the larvae of *F. kajau* in captivity.

¹Institute of Biodiversity and Environmental Conservation, Universiti Malaysia Sarawak, 94300, Kota Samarahan, Sarawak, Malaysia. Email: idas@ibec.unimas.my. (*corresponding author)

²Naturhistorisches Museum der Burgergemeinde Bern, Bernastrasse 15, CH-3005 Bern, Switzerland.

³Institute of Ecology and Evolution, University of Bern, Baltzerstrasse 6, CH-3012 Bern, Switzerland.

⁴Center for Natural History, Universität Hamburg, Martin-Luther-King-Platz 3, 20146 Hamburg, Germany.

Field sampling was conducted at Kubah National Park, Sarawak, between September to December 2006 and eggs of *F. kajau* were obtained in December 2006, which coincided with heavy rainfall.

MATERIAL AND METHODS

Study site. Kubah National Park (headquarters at 01°36'39"N, 110°11'32"E; datum: WGS84) is located ca. 22 km north-west of Kuching, Sarawak State, East Malaysia, on north-western Borneo, and includes the sandstone ridge of the Gunung Serapi massif, which consists of fairly steep terrain. Altitudinal range of the Park is 20–777 m above sea level. Red-yellow podzolic soils cover most of the rocks, and another common soil found on exposed ridges upward from about 300 m above sea level, is peat soil (Hazebroek & Morshidi, 2000). Kubah is largely covered with mixed dipterocarp forest and on ridge tops grows a different kind of forest, the “ridge top forest”, where the soil is peaty; tree heights are reduced, is more slender and have smaller crowns. *Kerangas* forest is formed on slopes of sandstone bedding surfaces, mainly on the western flanks of Gunung Serapi and its north-west ridge.

Sampling details. Three sampling trips for this study were conducted at Kubah National Park: 29 September–1 October 2006, 17–19 November 2006 and 17–20 December 2006. The sampling areas included the main trail from the Kubah chalet (122 m asl) to the Rayu Trail (290 m asl). Sampling was conducted between 1900–2300 hr. Five clutches of egg were collected from leaves surrounding spots where adults were sighted. Eggs, along with some leaves, were placed in plastic containers and brought back to the laboratory. The humidity and temperature of the collection area were recorded.

Laboratory work. Husbandry: The eggs, that were wild-collected from the surface of the leaves, were placed in plastic cups of diameter 65 mm that were filled with dechlorinated water. Larvae hatching from eggs dropped into the cups that had a liquid volume of ca. 180 ml. The larvae were raised in the plastic cups, in which the three quarters of the water was changed every two days, to reduce build-up of nitrogenous compounds. In order to minimise the stress levels of larvae, the macrophyte, *Ipomoea aquatica* (a commonly available vegetable) was introduced to provide places of concealment. The larvae were fed on chicken egg yolk once every two days. Daily morphological observations and monitoring of larvae were performed using compound and stereoscopic microscope. The total length and body length of the respective larva was measured by using Mitutoyo™ vernier callipers and by temporarily putting the larva on a glass slide. Larvae were handled gently, and care taken not to place them outside water for over two minutes.

Molecular work. During these procedures, specimens were removed as vouchers for certain stages. Tadpole vouchers were stored in a 4% buffered formalin solution,



Fig. 1. Adult *Feihyla kajau* from Kubah National Park, Sarawak.

with a dilution of 1:9 of 36% formaldehyde stock solution. All voucher specimens of tadpoles and adults have been accessed with the museum of the Institute of Biodiversity and Environmental Conservation, Universiti Malaysia Sarawak (UNIMAS 8264; 8522; 8642 and 8852) and Biozentrum Grindel und Zoologisches Museum, Universität Hamburg (ZMH A13410, ZMH A13452, ZMH A13466, ZMH A10821 and ZMH A10822).

Partial sequences of the mitochondrial 16S rRNA gene were used to check for conspecificity of the Kubah population to that from Gunung Mulu, Sarawak (the type locality). Total genomic DNA was extracted from macerated tail muscle samples using Wizard_SV Genomic DNA Purification System (Promega, Switzerland), according to the manufacturer's protocols. The following primers were used for PCR amplification: 16SC (forward) 5'-GTRGGCCTAAAAGCAGCCAC-3', 16SD (reverse) 5'-CTCCGGTCTGAACTCAGATCACG TAG-3' (Vences et al., 2005). 25 µl PCR reaction volume was used containing 1 µl DNA, 1 µl of each primer (20 pmol µl⁻¹ (20µM), 1.5 µl MgCl₂ Magnesium chloride, 12.5 µl GoTaq_Hot Start Green Master Mix (Promega) and 8 µl ddH₂O (Promega). The cycling conditions for amplification were: denaturation at 94°C for 2:00 min; 35 cycles at 94°C for 0:30 min, 48°C or 50°C for 0:30 min, and 72°C for 1:00 min; then one final extension cycle at 72°C for 5:00 min, stopped at 4°C. All PCRs were performed on a Techne TC-512 thermo-cycler. PCR products were excised from agarose gels and cleaned using the Wizard® SV Gel and PCR Clean-UP System (Promega). Sequencing was done in both directions at LGC Genomics (Berlin, Germany) using the same primers mentioned above. Sequence editing and management was performed in Geneious Pro 8.1.6 (Drummond et al., 2011). We compared our tadpole sample from Kubah (ZMH A12577, GenBank accession number KT382330) with sequences of adult *Feihyla kajau* from Kubah (GenBank accession number KC961088, NMBE1057090) and Mulu (GenBank accession number JN377362, NMBE1056500). The sequences were aligned and compared using Geneious Pro and the implemented MAFFT plug-in with the E-INS-i mode and standard parameters set.

Table 1. Number of eggs of *Feihyla kajau* hatched. EC– Clutches of eggs.

	Total Number of Eggs	Number of Eggs Hatched
EC 1	9	5
EC 2	7	1
EC 3	9	8
EC 4	10	3
EC 5	8	4
Mean ± SE	8.60 ± 0.51	4.20 ± 1.16

RESULTS AND DISCUSSION

Genetic assignment. The sequences of the obtained 750 bp of 16S of the tadpole and adult samples from Kubah are identical. Uncorrected genetic p-distance between the samples from Kubah and the Mulu sample is 0.67%, corresponding to five substitutions. We consider the tadpole sample as conspecific with the morphologically determined adult specimens of *Feihyla kajau* from Kubah and Mulu (type locality of this species), the observed low genetic distance is interpreted as intraspecific variation.

Clutch size and type. Eggs of *Feihyla kajau* were obtained on 18 December 2006, between 1900–2100 h. A total of five clutches were collected at the site (off the Summit Trail, close to Park Headquarters of Kubah National Park, Matang Range, western Sarawak), where adults were observed during a previous sampling trip (on 19 November 2006). The range of eggs numbers was 7–10 per clutch (mean 8.60 ± SE 0.51; Table 1).

The species differs from all known Bornean species of the family Rhacophoridae in producing eggs within a mass of jelly-like substance that are stuck to leaves of plants growing on the edge of standing waterbodies. Confamilial species on the island produce eggs within foam nests that are deposited on leaves, tree trunks or in tree hollows. The genus *Feihyla* has a mostly Indo-Chinese distribution (Frost, 2015), the four extra-Bornean species (including *F. fuhua* Fei et al., 2010; *F. hansenae* [Cochran, 1927]; *F. palpebralis* [Smith, 1924] and *F. vittata* [Boulenger, 1887]) known from southern China, north-eastern India, Myanmar, Thailand and Vietnam. Life histories, especially breeding biology of all except three of these species remain unknown, making comparisons difficult. Fei et al. (2010) reported *F. vittata* to produce eggs on leaves in a jelly coating as did Rognes (2015) for *F. hansenae*, and Sheridan and Ocock (2008) mentioned eggs being produced in a “gelatinous mass” similar to the condition we here report for *F. kajau*.

Larval rearing. Five larvae of *Feihyla kajau* were collected on 29 September 2006 and brought back to the laboratory; however all died within 10 days. There was no information available at that time on the nutritional requirements of the species for rearing under controlled conditions. Subsequently, the water (as well as lab ambient) temperature was maintained



Fig. 2. Tadpole of *Feihyla kajau* from Kubah National Park (UH 518). Top: lateral view; lower left: ventral view showing transparency of ventral skin (gills and heart red, gut coils clearly visible); lower right: portrait in lateral view showing details of iris coloration, naris size and position, and head pigmentation.

at around 27°C and pH at 7.2. Larvae were offered ad libitum commercial fish flakes, leaves of the Chinese cabbage, *Brassica rapa*, yeast and chicken egg yolk; only egg yolk was accepted.

Larval description. Colour in life: Viewed in daylight, the tadpoles appear brown dorsally. On the trunk region, especially at the trunk-tail transition, there is a darker brown saddle from flank to flank. In close-up view this saddle-like pattern is less distinct. Colour variation in our sample was noted. Dorsum of body with brown background colour, with groups of lighter bronze pigment cells overlaying on the trunk region and scattered lighter pigmentation on forehead region. On flanks, dark pigmentation decreases towards centre. Clusters of bronze pigmentation are present at the gill region and small groups along the flanks and cheeks. More ventrally, larger, silvery shining iridophore cells are present in the trunk region. In ventral view, the gular, gill and abdominal parts, as well as the oral disk, are transparent. Gills, heart and gut coils are clearly visible. Some iridophores may be present below the gill region. On the tail, fairly dense spindle-shaped melanocytes give the base of the tail an overall brown ground colour. Groups of round, larger melanocytes form mottling predominantly on the muscular part of the tail, but also extending onto the dorsal fin; the ventral fin is clear.

The iris is golden in the upper and lower sector around the pupil, with a copper-red stripe running across. The pupil is separated from the scleral part of the eye by a narrow black ring. The scleral part of the eye is densely covered with silvery to golden (depending on light conditions) shiny pigments. The blood vessels of the tail muscles are inconspicuous. Dorsal and lateral images of a live tadpole are given in Fig. 2.

Colour in preservation (4% formalin): The iris and the sclera are black. The skin of the tadpoles is mostly transparent in preservation. The brown saddle-like dark bands across the trunk region, characteristic of living specimens, are visible

Table 2. Snout-vent length (SVL) of tadpoles of *Feihyla kajau*, according to stage, at the beginning of each stage. In mm.

Stage	N	Minimum	Maximum	Mean ± SE
Min SVL 26	3	6.53	6.98	6.78 .13
Min SVL 27	3	6.61	7.35	6.99 .21
Min SVL 28	3	6.90	7.08	6.98 .05
Min SVL 29	3	7.02	7.13	7.067 .03
Min SVL 30	3	7.12	7.57	7.32 .13
Min SVL 31	3	7.19	7.68	7.51 .16
Min SVL 32	3	7.66	7.96	7.77 .10
Min SVL 33	3	7.65	7.81	7.74 .05
Min SVL 34	3	8.01	8.28	8.14 .08
Min SVL 35	3	8.23	8.44	8.35 .06
Min SVL 36	3	8.34	8.77	8.55 .12
Min SVL 37	3	8.52	9.15	8.82 .18
Min SVL 38	3	9.14	9.16	9.15 .01
Min SVL 39	3	9.17	9.26	9.22 .03
Min SVL 40	3	9.20	9.27	9.24 .02
Min SVL 41	3	9.20	9.26	9.23 .02
Min SVL 42	2	9.23	9.35	9.29 .06

but less pronounced. Blotches on the tail lighten, but their melanocytes are visible.

External morphology. *Feihyla kajau* has a moderately large tadpole for a small-sized rhacophorid, reaching up to 26.7 mm in total length as a larva (Tables 2, 3). The body shape is subglobose to ovoid in dorsal view, wider than deep. The snout is relatively narrow and rounded in dorsal and lateral views. The eyes are positioned dorsolaterally, the cornea failing to project beyond the body contour. The widely spaced external nares are closer to the snout than to the eyes in lateral view. The spiracle is sinistral. The spiracular tube is fused to the body. The spiracle is directed posterolaterally, opening ventral to the longitudinal body axis in lateral view. The oral disk is anteroventral (Fig. 3). The marginal papillation of the oral disk has a broad gap on the upper lip and no gap on the lower lip. Lateral oral disk indentations are present. The oral disk marginal papillae are arranged in a single row, but submarginal papillae are located in lateral areas of the upper and lower lips, thus, double-row papillation is present in these areas. Marginal papillae are moderately long and terminate in blunt tips. The labial ridges bear uniserial keratodont rows. The Labial Tooth Row Formula (LTRF) is 4(2–4)/3.

The dorsal tail fin begins at the body-tail junction. The tail fins are arched and taper posteriorly into a subacute tip. Dorsal and ventral fin are approximately of the same height. The maximum height of the tail is in the middle third of the tail length. The anal siphon is located at a dextral position, at the base of the ventral tail fin. The height of the muscular tail at the base of the tail is clearly less than body height.

Syntopic species. In the field, we found the larvae of *Feihyla kajau* associated with other rhacophorids such as *Rhacophorus pardalis* and *Polypedates macrotis*, as well as species of the *Limnonectes kuhlii* group and *Abavorana*



Fig. 3. Schematic representation of mouthparts of *Feihyla kajau* tadpole (after ZMH A13779; Stage 35)

luctuosa. Tadpoles of *F. kajau* are smaller than *R. pardalis* and *P. macrotis* larvae, and much smaller than *A. luctuosa*, at comparable stage and do not have the lateral eyes of *P. macrotis* and most other *Polypedates* species. Larvae of *F. kajau* are most likely confused with larvae of *Limnonectes* species due to size, dorsolaterally positioned eyes, and relatively globose body shape. *Feihyla kajau* larvae however, can be identified in the field reliably by a combination of these characters: red horizontal stripe on golden iris, dorsolateral eyes, brown saddle-like band across trunk and labial tooth row formula 4(2–4)/3.

Growth. Tables 2 & 3 present data on snout-vent length (SVL) and tail length (TL). Minimum mean measurements were taken on the first day of each stage; maximum mean measurements were taken on the last day of each stage. The size of late stage *F. kajau* tadpoles were relatively small compared to other tadpoles of the same family on Borneo (Table 4), and at 23.1–25.4 mm, rather similar to that of *Feihyla vittata* (Fei et al., 2010: 442: total length: 24 mm). Pool duration may influence larval size (Morey, 1998). The tadpoles in the present study were obtained

Table 3. Tail length of tadpoles of *Feihyla kajau* according to stage, at the beginning of each stage. In mm.

Stage	N	Minimum	Maximum	Mean ± SE
Min tail L 26	3	14.54	14.79	14.68 .074
Min tail L 27	3	14.01	14.83	14.31 .26
Min tail L 28	3	14.28	14.86	14.64 .18
Min tail L 29	3	12.98	14.70	13.99 .52
Min tail L 30	3	12.88	14.97	14.17 .65
Min tail L 31	3	14.73	15.16	14.93 .12
Min tail L 32	3	14.59	15.66	15.00 .33
Min tail L 33	3	13.65	15.65	14.77 .59
Min tail L 34	3	14.33	16.97	15.40 .80
Min tail L 35	3	13.35	18.22	15.45 1.45
Min tail L 36	3	13.94	18.15	15.72 1.26
Min tail L 37	3	15.79	17.63	16.51 .57
Min tail L 38	3	15.26	17.41	16.53 .65
Min tail L 39	3	16.03	17.39	16.49 .45
Min tail L 40	3	15.14	17.16	16.22 .59
Min tail L 41	3	13.99	16.35	15.55 .78
Min tail L 42	2	11.67	14.99	13.33 1.66

Table 4. Comparison of total length (in mm) of *Feihyla kajau* larvae and those of other members of the family Rhacophoridae from Borneo. Abbreviations: F.k. = *Feihyla kajau*, R.a. = *Rhacophorus angulirostris*, K.ap. = *Kurixalus appendiculatus*, R.c. = *Rhacophorus cyanopunctatus*, R.d. = *Rhacophorus dulitensis*, R.g. = *Rhacophorus gauni*, R.h. = *Rhacophorus harrissoni*, R.n. = *Rhacophorus nigropalmatus* and R.p. = *Rhacophorus pardalis*. R. sp. is an unallocated tadpole (Inger, 1985). Sources: Inger (1985); Inger & Tan (1990) and Leong (2004).

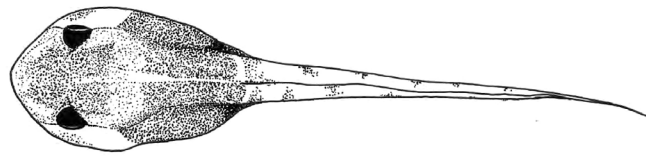
Stages	F.k	R.a	K.ap	R.c	R.d	R.g	R.h	R.n	R.p	R. sp
35	24	–	18.7– 20.7	–	42.7	–	–	–	–	–
37	25.7	–	25.7– 26.9	28.2	–	–	–	–	43.2	–
38	25.6	–	–	–	–	–	–	–	–	37.3
39	25.4	35.3	25.5	–	–	–	–	50	–	–
41	23.1	44.5	23.9– 26.5	–	–	35.4	41.3	–	–	–

from small puddles (of depth 10–12 cm) that presumably are ephemeral, relative to larger bodies of stagnant water. Larvae from bigger, longer lasting pools tended to be larger at metamorphosis than larvae from shorter duration pools (Loman, 2002), and confamilial species, such as members of the genera *Polypedates* and *Rhacophorus* are associated with deeper standing water bodies.

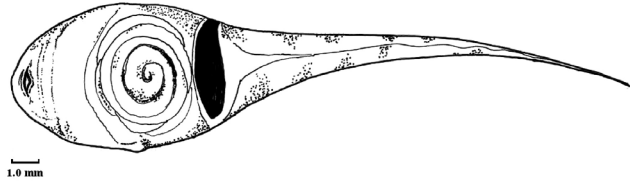
Here, we describe the morphological changes of *Feihyla kajau* during metamorphosis. While morphological traits in tadpoles during Stages 26–30 were identical to those documented by Gosner (1960), a significant difference is the hind limb bud of *F. kajau* was observed to become progressively narrower, forming a waist-line shape, at the end of the hind limb bud. Stages 31–35 of *F. kajau* undergo the morphological change, as described by Gosner (1960), in which the stages were marked by conspicuous appearance of indentations at the limb bud. However, a difference is noted in the shape of the limb buds of *F. kajau* tadpoles

compared to the tadpoles in Gosner (1960: *Hyla versicolor*), as in the former, by Stage 36, the toe discs become visible. Toe pads of arboreal frogs are though primarily adaptive to help adhere to vertical surfaces (Emerson & Diehl, 1980).

Stages succeeding Gosner's Stage 40 are associated with significant changes associated with metamorphosis. Total length begins to diminish, the effect of resorption of the tail, and the larval mouth parts begin to disintegrate. Nonetheless, coupling of metamorphic events may not be consistent across anuran amphibians and are adaptive for relative timing changes in metamorphosis, and it is possible that some species may retain a functional tail until the forelimbs are developed to support locomotion (Downie et al., 2004). The length of the tail starts to reduce at Stage 40 and beyond (Fig. 4). During Stage 41, the hand is visible on ventral aspect, and by Stage 42, the interphalange joints are well-formed and can be observed clearly.

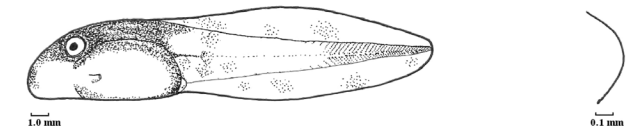


Dorsal

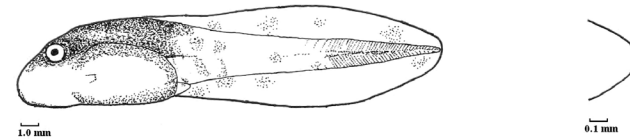


Ventral

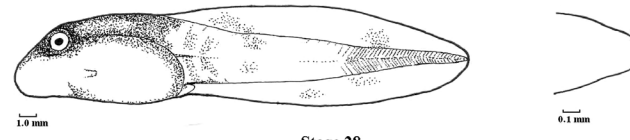
Fig. 4. Dorsal and ventral views of *Feihyla kajau* tadpole (EC 1; Stage 26).



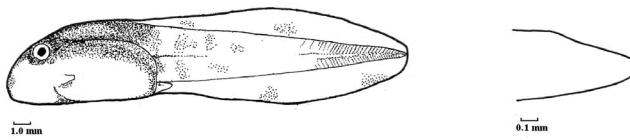
Stage 26



Stage 27

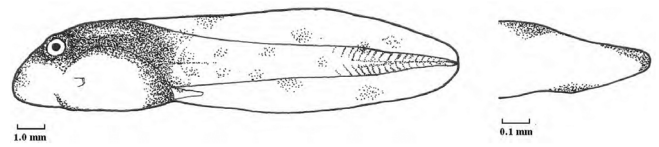


Stage 28

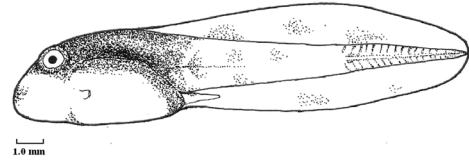


Stage 29

Fig. 5. Morphological changes of *Feihyla kajau* tadpoles from Stages 26 to 29.

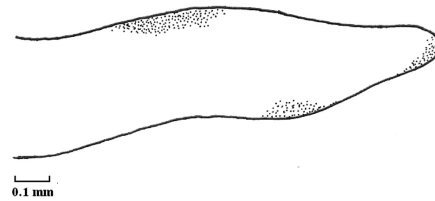
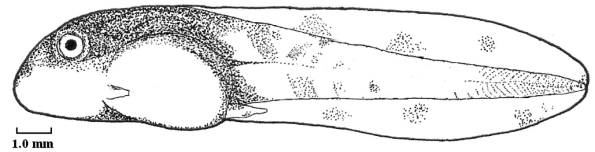


Stage 30

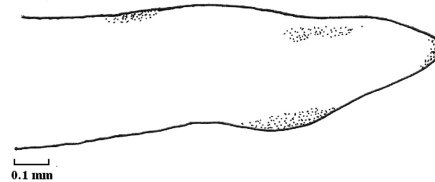
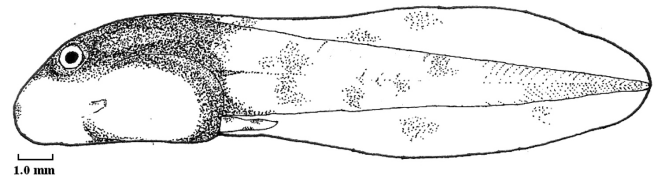


Stage 31

Fig. 6. Morphological changes of *Feihyla kajau* tadpoles from Stages 30 to 31.



Stage 32



Stage 33

Fig. 7. Morphological changes of *Feihyla kajau* tadpoles from Stages 32 to 33.

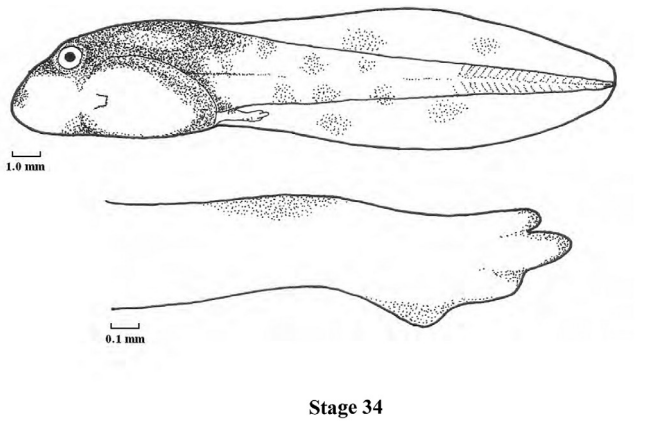


Fig. 8. Morphological changes of *Feihyla kajau* tadpoles from Stages 34 to 35.

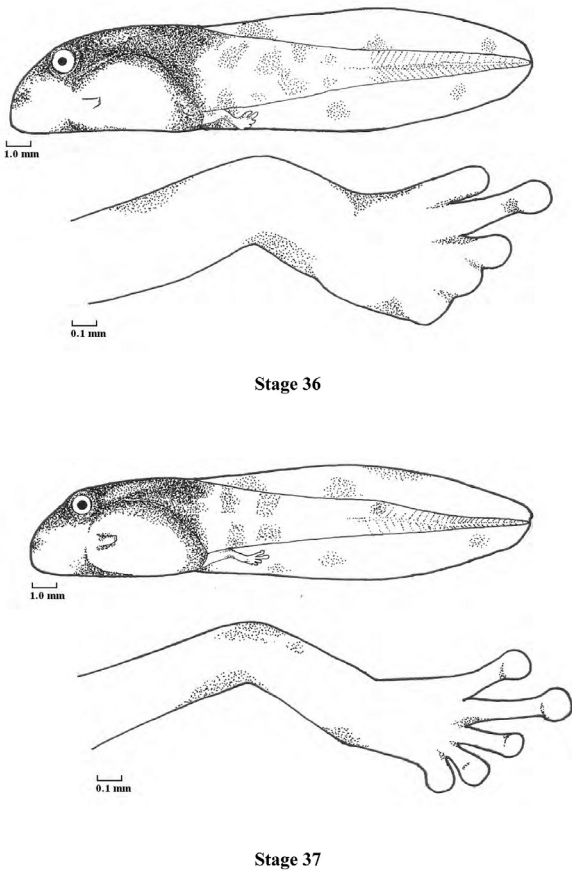


Fig. 9. Morphological changes in *Feihyla kajau* tadpoles from Stages 36 to 37.

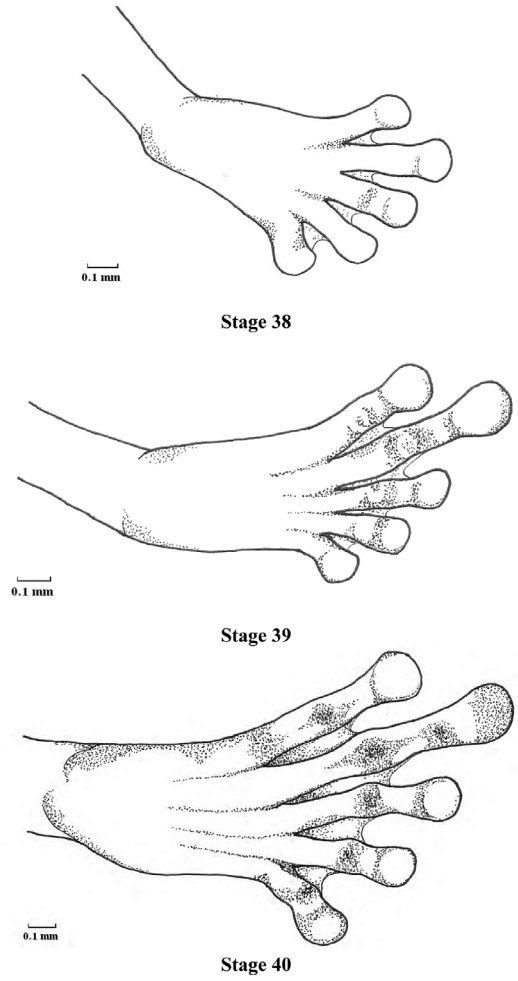


Fig. 10. Morphological changes in right pes of *Feihyla kajau* tadpoles from Stages 38 to 40.

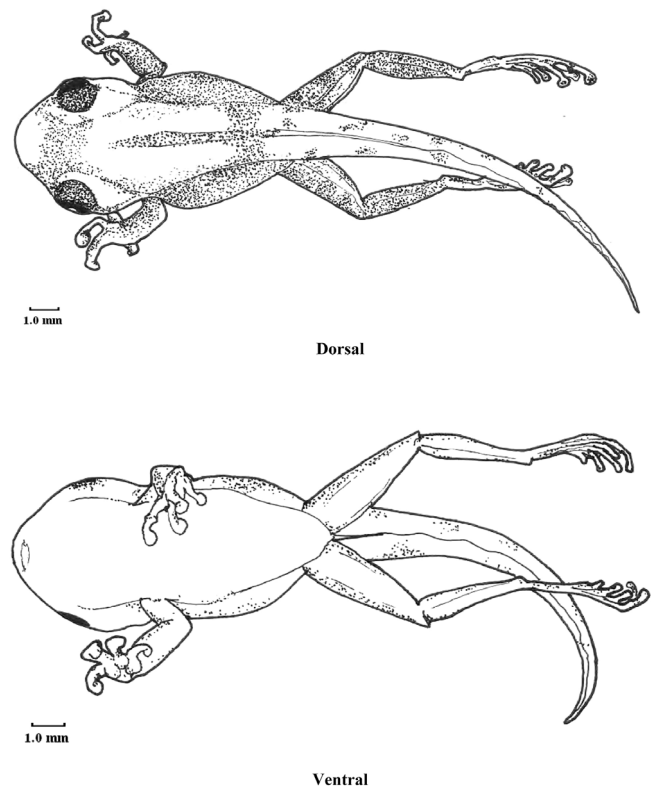


Fig. 11. Morphological changes of *Feihyla kajau* tadpole at Stage 41 in dorsal (top) and ventral (bottom) views.

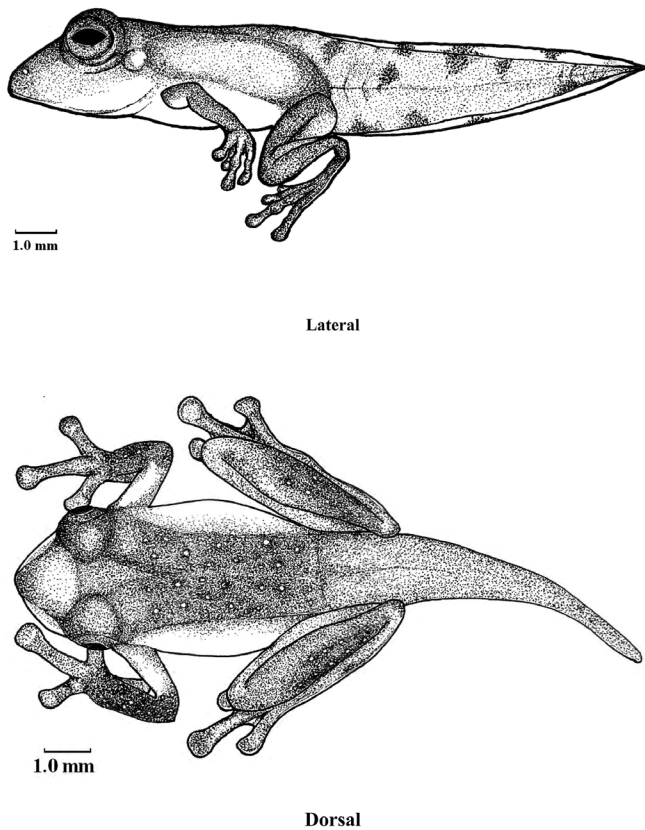


Fig. 12. Morphological changes of *Feihyla kajau* tadpole at Stage 42 in lateral (top) and dorsal (bottom) views.

Development time. A total of 21 of the 43 eggs from five clutches of *Feihyla kajau* collected in this study hatched, but only one individual survived to day 111 in the laboratory, revealing that the tadpoles may take up to 110 days post-hatching to reach Stage 42.

The time interval between Stages 26–29 is short, as these involve only the extension of limb bud. In other words, there is no development of other more complex morphological trait during these stages. Furthermore, the findings show that among the three tadpoles that have survived till Stage 41, the time intervals between the metamorphosis stages differed between individuals. Downie et al. (2004) suggest that the body condition of tadpoles may be a factor involved in the variability of metamorphic duration. Pandian & Marian (1985) stated that metamorphosis being an energetically demanding process, some tadpoles may be better placed to undertake it than others, owing to the circumstances under which they grew (Pandian & Marian, 1985).

Four factors may explain the differences in development rates between *Feihyla kajau* and other species of amphibians. Two factors that have been suggested by Downie et al. (2004) are the developmental constraints and predation level. First, the time taken to completion is likely to be dependent on the amount of tissue to be transformed. A positive correlation between metamorphic duration and tadpole size is thus predicted, both between and within species. Secondly, predation pressure is important in selecting for shorter metamorphic duration (Wassersug & Sperry, 1977). Longer durations are expected in species subject to low predation

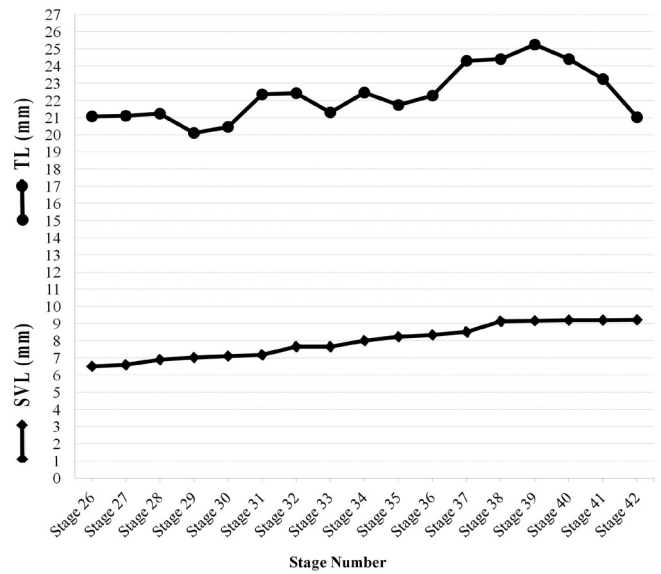


Fig. 13. Relationship between snout-vent length and tail length between Stages 26 and 42 in *Feihyla kajau*.

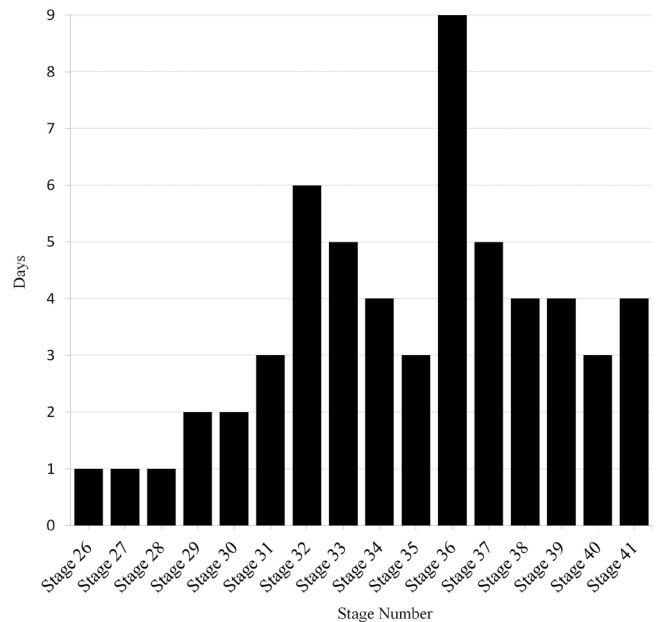


Fig. 14. Mean number of days in each developmental stage (between Stages 26 and 41) in *Feihyla kajau*.

pressure. The tadpoles in this experiment were obviously not subjected to predation pressure, and metamorphoses may thus have been slower compared to conspecific tadpoles in its natural habitat, where there are possibilities of the presence of predators. The presence of predator will increase growth rates of tadpoles, to permit metamorphs to leave waterbodies quicker. Furthermore, in the presence of predators, tadpoles are less active, in order to avoid detection. Thus, more energy is diverted for development than used for predator avoidance. The third factor that may have contributed to slow metamorphosis rate of tadpoles in captivity is difference in available food resources and therefore, nutrient intake. Tadpoles in this experiment were only fed on egg yolk compared to the presumably more diverse food dietary resources these tadpoles have in the wild. Development is faster in ponds rich in algae (Loman,

Table 5. Mean number of days under each stage in tadpoles of *Feihyla kajau*.

	N	Minimum	Maximum	Mean ± SE	
N days stage 26	3	1	1	1.00	.000
N days stage 27	3	1	2	1.33	.333
N days stage 28	3	1	2	1.67	.333
N days stage 29	3	2	2	2.00	.000
N days stage 30	3	2	6	4.00	1.155
N days stage 31	3	3	12	7.00	2.646
N days stage 32	3	6	6	6.00	.000
N days stage 33	3	5	5	5.00	.000
N days stage 34	3	4	10	7.33	1.764
N days stage 35	3	3	8	5.00	1.528
N days stage 36	3	9	10	9.67	.333
N days stage 37	3	5	9	7.00	1.155
N days stage 38	3	4	4	4.00	.000
N days stage 39	3	4	4	4.00	.000
N days stage 40	3	3	4	3.33	.333
N days stage 41	2	4	4	4.00	.000

2002). Another factor suspected to be associated with slow rate of metamorphosis is stress imposed on tadpoles when measurements were taken. In this study, tadpoles took up to 110 days to reach Stage 42 (Table 5).

Mortality. In our experiment, mortality rates at Stage 26 and earlier were high. Tadpoles were susceptible to physical damage, and injuries during these stages caused death. This is in contrast to the tadpoles in the later stage (Stage 36 onwards) that appeared to survive similar injury, such as on the tails and on the toe pads. Of 43 larvae collected in this study, only one survived to day 111. A few factors may be linked to larval mortality: unintentional injury inflicted on the tadpoles during measurements; stress via dehydration upon removal from water for measurements and high rearing density (seven tadpoles in a single cup died simultaneously, presumably due to high population density and nitrogenous product accumulation). Thus, to increase tadpole survival rate, the densities need to be lower (from our experience), the maximum number of tadpoles is two individuals per cup of capacity 180 ml. Furthermore, maintenance of water condition (for example, intervals of water changes too short or too long) can be sources of stress and may increase mortality. More laboratory experiments and field observations are required to understand the ecology of larval growth and metamorphosis in this species.

ACKNOWLEDGEMENTS

For the permission and providing of facilities to conduct field work at the Kubah National Park, we thank Datuk Cheong Ek Choon and Sapuan Ahmad, Directors and Controllers of National Parks and Nature Reserves, Sarawak Forest Department (Permit number NCCD.907.4.4.Jld12-170). We are also grateful to the staff of Kubah National Park, in particular Mohidin Rajuli, Park Manager and Suziani binti Sulaiman, Conservation Officer. Funding for field work was provided by a grant from Volkswagen-Stiftung,

Germany (grant number: 1/79 405), entitled: 'Inventory and biodiversity of the frog fauna of East Malaysia (Borneo) with emphasis on their larval forms (Amphibia: Anura). Additional support came from NRGs/1087/2013(01). Pang Sing Tyan helped translate Chinese literature. We thank Mohd Tajuddin Abdullah, Head of Department of Zoology, for laboratory space and Andrew Alek Tuen, Director, Institute of Biodiversity and Environmental Conservation, for other facilities to conduct this research. Finally, we are grateful to Ulmar Grafe and an anonymous reviewer for comments on the manuscript.

LITERATURE CITED

- Boulenger GA (1887) An account of the batrachians obtained in Burma by M.L. Fea of the Genoa Civic Museum. *Annali del Museo Civico di Storia Naturale di Genova*, Serie 2, 5: 418–424.
- Cochran DM (1927) New reptiles and batrachians collected by Dr. Hugh M. Smith in Siam. *Proceedings of the Biological Society of Washington*, 40: 179–192.
- Das I (2007) *Amphibians and Reptiles of Brunei*. Natural History Publications (Borneo) Sdn. Bhd., Kota Kinabalu, 200 pp.
- Das I & Haas A (2005) Sources of larval identities for amphibians from Borneo. *Herpetological Review*, 36: 375–382.
- Downie JR, Bryce R & Smith J (2004) Metamorphic duration: an under-studied variable in frog life histories. *Biological Journal of the Linnean Society*, 83: 261–272.
- Dring J (1983) Some new frogs from Sarawak. *Amphibia-Reptilia*, 4: 103–115.
- Drummond AJ, Ashton B, Buxton S, Cheung M, Cooper A, Duran C, Field M, Heled J, Kearsse M, Markowitz S, Moir R, Stones-Havas S, Sturrock S, Thierer T & Wilson A (2011) Geneious v6.0.5 <http://www.geneious.com>.
- Duellman WE & Trueb LR (1986) *Biology of Amphibians*. McGraw-Hill Book Company, New York, 670 pp.
- Emerson SB & Diehl D (1980) Toe pad morphology and mechanisms of sticking in frogs. *Biological Journal of Linnean Society*, 13: 199–216.
- Fei L, Ye C & Jiang J (2010) *Colored Atlas of Chinese Amphibians*. Sichuan Publishing Group, Sichuan Publishing House of Science & Technology, Chengdu, 522 pp.

- Frost DR (2015) Amphibian Species of the World: An Online Reference. Version 6.0. Electronic Database. American Museum of Natural History, New York. Available from: <http://research.amnh.org/vz/herpetology/amphibia/index.php> (Accessed 26 June 2015).
- Gosner KL (1960) A simplified table for staging anurans embryos and larvae with notes on their identification. *Herpetologica*, 16: 183–190.
- Grosjean S, Delorme M, Dubois A & Ohler A (2008) Evolution of reproduction in the Rhacophoridae (Amphibia, Anura). *Journal of Zoological Systematics and Evolutionary Research*, 46: 169–176.
- Hazebroek H & Morshidi AKA (2000) National Parks of Sarawak. Natural History Publications (Borneo), Kota Kinabalu. xii + 503 pp.
- Hertwig S, Das I, Schweizer M, Brown RM & Haas A (2011) Phylogenetic relationships of the *Rhacophorus everetti*-group and implications for the evolution of reproductive modes in *Philautus* (Amphibia: Anura: Rhacophoridae). *Zoologica Scripta*, 41: 29–46.
- Hertwig ST, Schweizer M, Das I & Haas A (2013) Diversification in a biodiversity hotspot- the evolution of Southeast Asian rhacophorid tree frogs on Borneo (Amphibia: Anura: Rhacophoridae). *Molecular Phylogenetics and Evolution*, 68: 567–581.
- Inger RF (1985) Tadpoles of the forested regions of Borneo. *Fieldiana Zoology new series*, 26: 1–89.
- Inger RF (1986) Diets of tadpoles living in a Bornean rain forest. *Alytes*, 5: 153–164.
- Inger RF & Tan FL (1990) Recently discovered and newly assigned frog larvae (Ranidae and Rhacophoridae) from Borneo. *Raffles Bulletin of Zoology*, 38: 3–9.
- Inger RF & Stuebing RB (2005) A Field Guide to the Frogs of Borneo. Second Edition. Natural History Publications (Borneo) Sdn. Bhd., Kota Kinabalu, iv + 201 pp.
- Leong TM (2004) Larval descriptions of some poorly known tadpoles from Peninsular Malaysia (Amphibia: Anura). *The Raffles Bulletin of Zoology*, 52: 609–620.
- Loman J (2002) Temperature, genetic and hydroperiod effects on metamorphosis of brown frogs *Rana arvalis* and *Rana temporaria* in the field. *Journal of Zoology London*, 258: 115–129.
- Meegaskumbura M, Senevirathne G, Biju SD, Garg S, Meegaskumbura S, Pethiyagoda R, Hanken J & Schneider CJ (2015) Patterns of reproductive-mode evolution in Old World tree frogs (Anura, Rhacophoridae). *Zoologica Scripta*, 44(5): 509–552.
- Morey SR (1998) Pool duration influences age and body mass at metamorphosis in the western spadefoot toad: implications for vernal pool conservation. In: Witham CW, Bauder ET, Belk D, Ferren WR, Jr. & Ornduff R (eds.) *Ecology, Conservation, and Management of Vernal Pool Ecosystems*. Proceedings of a 1996 Conference. California Native Plant Society, Sacramento, CA. Pp. 86–91.
- Pandian TJ & Marian MP (1985) Time and energy costs of metamorphosis in the Indian bullfrog *Rana tigrina*. *Copeia*, 1985: 653–662.
- Rognes K (2015). Revision of the frog fly genus *Caiusa* Surcouf, 1920 (Diptera, Calliphoridae), with a note on the identity of *Plinthomyia emimelania* Rondani, 1875. *Zootaxa*, 3952: 1–80.
- Sheridan JA & Ocock JF (2008) Parental care in *Chiromantis hansenae* (Anura: Rhacophoridae). *Copeia*, 2008: 733–736.
- Smith MA (1924) New tree-frogs from Indo-China and the Malay Peninsula. *Proceedings of the Zoological Society of London*, 1924: 225–234.
- Wassersug RJ & Sperry DG (1977) The relationship of locomotion to differential predation on *Pseudacris triseriata* (Anura: Hylidae). *Ecology*, 58: 830–839.
- Wilkinson JA, Drewes RC & Tatum OL (2002) A molecular phylogenetic analysis of the family Rhacophoridae with an emphasis on the Asian and African genera. *Molecular Phylogenetics and Evolution*, 24: 265–273.