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Description and development of the tadpole of *Rhacophorus feae* (Anura; Rhacophoridae)

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The genus *Rhacophorus* Kuhl & Van Hasselt is currently known to contain 92 species of frogs (Frost 2018), distributed across south and south-east Asia. *Rhacophorus feae* Boulenger is a large member of this genus and has a seemingly expansive range been recorded from southern Yunnan in China, the Karen hills in Myanmar, northern Thailand, northern Laos and northern Vietnam (Chanard *et al*. 1999; Orlov *et al*. 2002; Nguyen *et al*. 2005; Stuart 2005; Yang 2008). It is currently included in the intrageneric *R. dennysi* group (Dubois 1986) along with *R. duboisi* Ohler, Marquis, Swan & Grosjean , *R. dennysi* Blanford, *R. dugritei* (David), *R. minimus* Rao, Wilkinson & Liu, *R. hungfuensis* Liu & Hu, *R. dorsoviridis* Bourret, *R. nigropunctatus* Liu, Hu & Yang, and *R. smaragdinus* (Blyth).

Rhacophorus feae is a foam-nesting frog (Wilkinson *et al*. 2002) and in captivity, females are known to deposit at least 400 eggs (Bagaturov & Bagaturova 2012). Eggs are distributed throughout a whitish, foamy secretion (Altig & McDiarmid 2007; Li *et al*. 2012). The tadpoles of this species have not yet been formally described. Tadpole identification is essential for many research purposes including distribution surveys and habitat inventories (Haas *et al.* 2012). *Rhacophorus* tadpoles are generally difficult to differentiate from congeners in the field, which hinders specific determination (Grosjean & Inthara 2016). Excepting some extremely specialised forms such as *R. vampyrus* Rowley, Le, Thi, Stuart & Hoang (Rowley *et al*. 2012), the general pattern of body morphology in *Rhacophorus* tadpoles is broadly similar, especially bearing in mind the multiple members of the genus which may occupy the same breeding sites across a species' range. Important characters used to separate *Rhacophorus* tadpoles are medial gaps in tooth rows and interruptions in lower papillae (e.g., Hendrix *et al.* 2007; Grosjean & Inthara 2016). To generalise, tadpoles of the *R. dennysi* group tend to have an ovoid and slightly depressed body shape, blunt and rounded snout, ventrally-positioned oral disc and a moderate to long tail ending in a rounded tip (Inger 1966, 1985; Chou & Lin 1997; Haas *et al.* 2012; Vassilieva *et al.* 2016). Herein we describe the larvae of *R. feae* and provide a detailed account of larval development, with important features compared to those of other potentially sympatric species in the genus *Rhacophorus* for which larval descriptions are available.

A mixed-sex adult group of eight *R. feae* is maintained at the Zoological Society of London (ZSL) London Zoo. These frogs were acquired from a zoological garden in Russia. The Russian founding stock were acquired from different sources, but all animals are thought to have originated from Sa Pa, northern Vietnam, a known locality for *R. feae* (Ohler *et al.* 2000; Orlov *et al*. 2001). The animals in this study can be confidently assigned to *R. feae* due to the large size attained by these specimens (ca. 14 cm SVL in adult females; Bagaturov & Bagaturova 2012) and the green iris, a feature not seen in any other species in the genus (Ostroshabov *et al*. 2013). The larvae described herein were bred naturally in captivity and, due to the large number of tadpoles produced (ca. 200 individuals), batches of the developing tadpoles were euthanised on population management grounds as part of routine management for this species. Specimens were euthanised in buffered Tricaine Methanesulfontae (MS-222) and fixed in 10% formalin; this was reviewed and approved by the Zoological Society of London under the project reference ZDZ90.

Egg masses hatched 5 days after being produced. *Rhacophorus feae* tadpoles were reared in three individual 50 x 30 x 30 cm clear glass aquaria with biological filtration (air stream sponge filters; Boyu, China) and live emergent plants (*Scindapsus aureus*) to process nitrogenous waste. Tadpoles were reared in water with an alkalinity of 125 mg/L and a water conditioner (Tap Water Conditioner, API, USA) which was added to remove chloramine and heavy metal pollutants. Commercially available tannin (Tropol, JBL, Germany) was added at a dose of 0.15 ml per L of water to help keep pH constant at 7.2 and to inhibit bacterial and fungal growth. No supplementary lighting other than ambient room lighting was provided. Tadpoles were fed twice daily on a homogenised mixture of commercially-available freeze-dried aquatic invertebrates, fish flake and pellets, *Spirulina* algae and cuttlefish bone. Water changes were performed in response to water quality. Water temperature ranged from 19.0–22.0 ºC. For the duration of rearing, stocking density was 10–70 tadpoles per 45 L tank of water, with densities reducing over time with mortality (natural and artificial for population management) and eventual metamorphosis, which occurred between 52 and 71 days post hatching.

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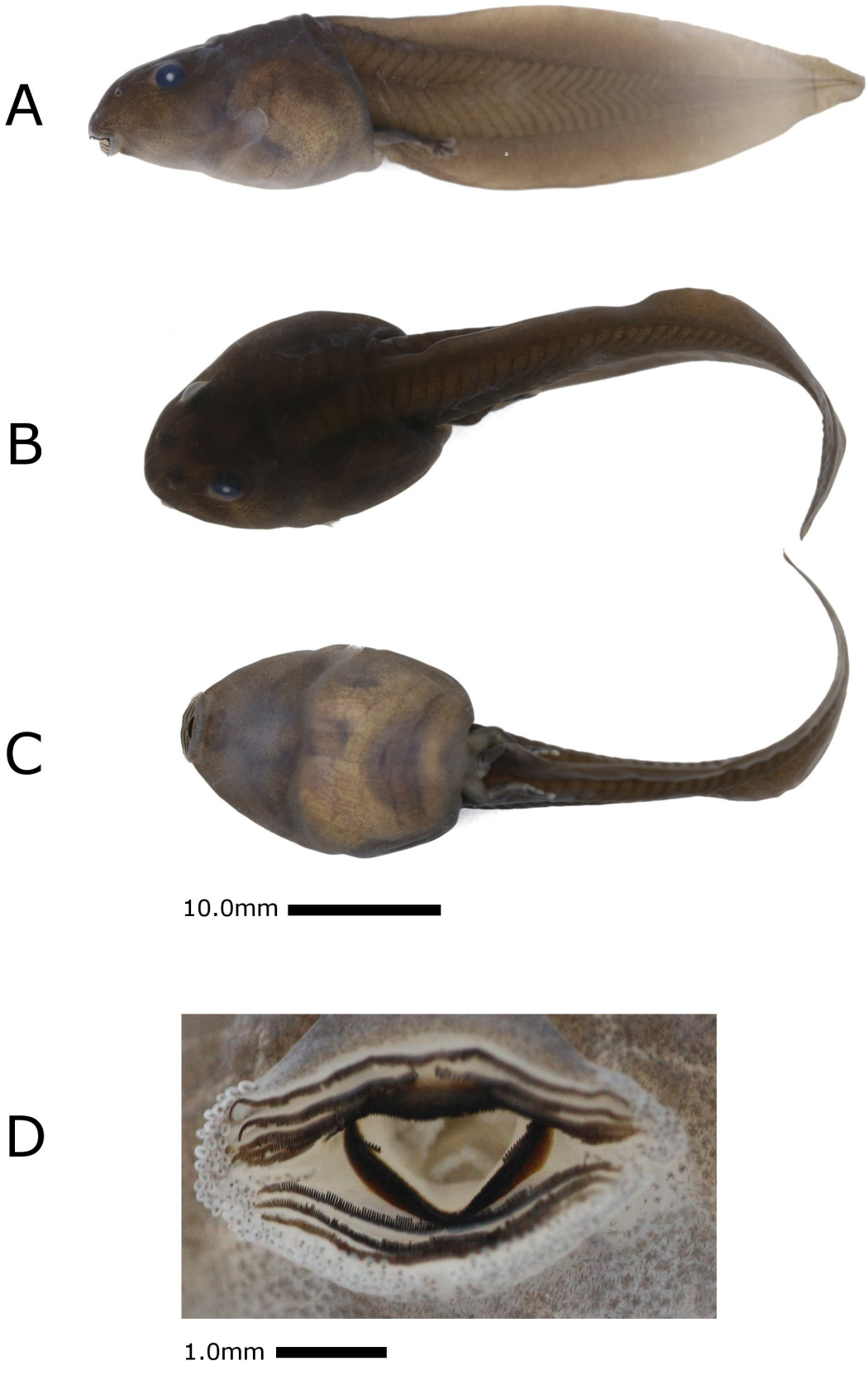

FIGURE 1. *Rhacophorus feae* tadpole (BMNH 2018.5513) at Gosner Stage 36. (A) Lateral view, (B) ventral view, (C) dorsal view, (D) oral disc.

Tadpole specimens were retained from culled stock five times over a period of 46 days. A table of measurements of a series of 24 tadpoles, Gosner Stages 24–41 (Gosner 1960) is provided (Table 1). The specimens are deposited at the Natural History Museum, London (NHM UK; BMNH 2018.5511—BMNH 2018.5534. Measurements of morphology follow nomenclature of Altig & McDiarmid (1999) and Grosjean (2001): BL body length; TL total length; TAL tail length; IN internarial distance; IP interorbital distance, measured between centers of pupils; BW maximum body width; BH maximum body height; TBW tail base width; TH maximum tail height; SVL snout-vent length; SSP snout-spiracle length; UF maximum upper tail fin height; LF maximum lower tail fin height; RN rostro-narial distance; NP naro-pupilar distance; ED horizontal eye diameter; and ODW horizontal oral disc width. Due to the small size of specimens and delicate nature of their tissues, measurements were taken digitally from photographs of the tadpoles next to a measuring scale. Morphometric measurements were taken in ImageJ (Schindelin *et al.* 2012). It is worth noting that several specimens (BMNH 2018.5526; BMNH 2018.5524; BMNH 2018.5515; BMNH 2018.5531; BMNH 2018.5529) did not appear to progress from Gosner Stage 25, however specimens attained a larger size (3.7–4.4 times total length) than many other larvae at Gosner Stage 25; the cause for this is unclear and beyond the scope of the present work. Colour change was recorded using photographs of the live animals' dorsal surface against a standard colour chart to provide a visual reference.

Tadpole: Description based on individuals between Gosner Stages 32 and 37 (N = 7). A series of photographs are presented in Fig. 1 to show the form of a single individual at Gosner Stage 36 (BMNH 2018.5513). Body shape ovoid and longer than wide (BW/BL = 0.67), body slightly depressed (BH/BW = 0.86); neuromasts arranged in lines running anterior-posterior clearly visible over the body of the specimen, snout blunt and rounded in dorsal and lateral views; nares positioned on dorsal aspect of body, forward and outward-pointing, nares closer to eyes than snout (RN/NP = 0.65); eyes positioned on lateral aspects of body, eyes moderately close together compared to body width (IP/BW = 0.52); spiracle sinistral, protuberant, posteriorly-directed and without specific ornamentation; tail long relative to body length (TAL/BL = 1.86), maximum tail height slightly exceeds maximum body height (TH/BH = 1.02), tail base muscular (TBW/BW = 0.36), tail tip slightly elongated and rounded in lateral view, upper and lower tail fins of almost equal maximal height (UF/LF = 1.03) and tail fins reach maximum height around second third towards tip of tail, upper and lower fins do not extend onto trunk, tail musculature tapering posteriorly to tail tip; myomeres prominent; vent tube positioned medially with opening at base of tail, level with edge of fin; oral disc ventral, of moderate size (ODW/BW = 0.34), terminal, upper and lower labia protruding slightly laterally, lower labium directed further forward than upper labium; jaw sheaths of moderate size and coloured black; upper jaw sheaths lined with 5 rows of keratinised labial teeth with a medial gap in anterior tooth rows 2–5 with three uninterrupted tooth rows on the lower jaw, giving a labial tooth row formula of 5(2–5)/3; marginal papillae arranged in two rows on lateral and ventral aspect of oral disc; a short ventral gap appears in the mental area.

In life, general colouration of tadpoles between Gosner Stages 32–37 is dark brown dorsally, fading to grey with greenish hues on the ventral surface; outermost half of both upper and lower tail fins as well as spiracle protuberance lacking melanophores, rendering them translucent; eyes largely black with golden speckling throughout iris. In preservative, dorsal coloration is slightly darker brown whereas the ventral surface appears slightly lighter grey; both upper and lower tail fins as well as tail musculature are whitish; grey-white marginal papillae; iris of the eye is black while the pupil is bright white.

Full tadpole descriptions of species of the *R. dennysi* group, and of sympatric species belonging to other intrageneric groups are scarce. Only the following species are available for, at least, partial comparison: *R. annamensis* Smith, *R. bipunctatus* Ahl, *R. dennysi*, *R. kio* Ohler & Delorme, *R. minimus*, *R. nigropunctatus*, *R. orlovi* Ziegler & Köhler, *R. rhodopus* Liu & Hu, *R. smaragdinus*. We summarize the key morphological differences between captive-bred *R. feae* and those species with which confusion in the field or during larval study is likely (Table 2). Main differences between *R. feae* and congeners in the *R. dennysi* group include a mental gap in papillae of lower labium (absent in *R. annamensis, R. kio, R. orlovi* and *R. rhodopus*), however specimens of *R. nigropunctatus* (Yang 2008) of unknown Gosner stage have a clear mental gap in papilla of lower labium (Grosjean & Inthara 2016). Unfortunately, the current published partial description of *R. nigropunctatus* does not allow us to differentiate *R. feae* tadpoles from tadpoles of this species. Tadpoles of *R. kio, R. minimus*, *R. rhodopus* and *R. smaragdinus* have similar-sized eyes to *R. feae,* whereas *R. annamensis* and *R. orlovi* have a significantly smaller eye diameter. Tadpoles of *R. feae* differ from *R. dennysi* by having a labial tooth row formula 5(2–5)/3 [5(2–4)/3(1)] and from *R. minimus* and *R. smaragdinus* by having uninterrupted lower tooth rows.

TABLE 1. Morphometric measurements (in mm) of *Rhacophorus feae* tadpole specimens examined in this study. TL Total length; BL body length; TAL tail length; BW maximum body width; BH maximum body height; TH maximum tail height; TBW tail base width; SVL snout-vent length; SSP snout-spiracle length; UF maximum upper tail fin height; LF maximum lower tail fin height; IN internarial distance; IP interorbital distance; RN rostro-narial distance; NP naro-pupilar distance; ED eye diameter; ODW oral disc width. Means and ranges are included.

Gosner Stage	24 (*N* = 3)	25 (*N* = 6)	27 (*N* = 2)	28 (*N* = 2)	32 (*N* = 1)	34 (*N* = 2)	36 (*N* = 2)	37 (*N* = 2)	40 (*N* = 2)	41 (*n* = 2)
TL	12.22 (11.77–12.61)	48.01 (14.89–65.55)	35.74 (22.28–38.10)	38.15 (37.83–38.44)	57.78	50.78 (49.60–51.95)	59.47 (56.73–62.20)	60.63 (59.10–26.15)	58.06 (57.40–58.71)	63.79 (62.55–65.03)
BL	5.05 (4.73–5.48)	16.95 (5.12–22.07)	13.59 (13.04–14.13)	13.99 (13.83–14.15)	19.25	18.26 (17.68–18.83)	20.52 (19.49–21.54)	20.42 (19.28–21.56)	17.73 (17.61–17.84)	20.36 (19.97–20.74)
TAL	7.22 (6.45–7.88)	31.06 (9.77–43.48)	22.16 (20.34–23.97)	9.00 (8.84–9.16)	34.53	32.52 (31.92–33.12)	38.95 (37.24–40.66)	40.21 (39.82–40.59)	40.33 (39.56–41.10)	43.44 (42.58–44.29)
BW	3.21 (3.11–3.40)	11.63 (3.42–15.53)	8.94 (8.40–9.47)	7.94 (7.71–8.17)	12.37	12.48 (12.20–12.75)	13.77 (13.31–14.23)	14.07 (13.64–14.50)	12.32 (11.97–12.67)	15.00 (14.84–15.15)
BH	2.81 (2.27–3.47)	9.90 (3.25–12.49)	7.83 (7.31–8.34)	8.17 (8.09–8.24)	10.99	10.84 (10.53–11.15)	11.71 (11.68–11.74)	11.74 (11.35–12.13)	10.09 (10.07–10.11)	11.80 (11.53–12.06)
TH	3.18 (2.95–3.43)	10.38 (2.98–13.74)	8.18 (7.70–8.66)	8.17 (8.09–8.24)	10.07	11.38 (11.68–11.74)	12.18 (11.90–12.46)	12.35 (12.00–12.69)	11.76 (11.42–12.10)	12.84 (12.40–13.27)
TBW	0.92 (0.85–1.00)	4.17 (0.92–6.30)	2.18 (2.08–2.28)	2.45 (2.27–2.63)	4.28	4.41 (4.12–4.69)	4.94 (4.90–4.97)	5.42 (5.26–5.57)	5.03 (4.98–5.08)	5.74 (5.46–6.02)
SVL	5.33 (5.19–5.56)	17.84 (5.31–24.98)	13.88 (13.12–14.64)	14.39 (14.26–14.51)	20.58	18.41 (17.95–18.86)	21.58 (20.14–23.01)	22.14 (21.45–22.82)	20.81 (20.55–21.07)	23.38 (22.68–24.07)
SSP	-	10.27 (3.39–13.35)	7.73 (7.57–7.89)	8.41 (8.36–8.45)	11.62	11.29 (11.07–11.51)	12.67 (12.45–12.86)	12.13 (11.05–13.20)	11.87 (11.50–12.24)	12.62 (12.16–13.07)
UF	0.97 (0.86–1.11)	3.41 (0.94–4.68)	2.83 (2.71–2.95)	2.89 (2.88–2.89)	3.24	3.92 (3.82–3.93)	4.23 (4.06–4.39)	4.04 (3.93–4.15)	4.09 (3.95–4.22)	4.34 (4.34–4.34)
LF	0.93 (0.87–1.01)	3.45 (0.99–4.48)	2.77 (2.53–3.00)	2.88 (2.64–3.11)	3.15	3.61 (3.39–3.82)	4.08 (4.06–4.10)	4.07 (3.95–4.18)	3.99 (3.95–4.22)	4.01 (3.97–4.05)
IN	1.29 (1.20–1.36)	3.22 (1.40–3.86)	2.95 (2.94–2.95)	2.96 (2.91–3.00)	3.43	3.53 (3.49–3.56)	3.67 (3.59–3.74)	3.68 (3.42–3.93)	3.33 (3.13–3.52)	3.24 (3.21–3.27)
IP	2.12 (1.89–2.30)	5.97 (1.93–7.98)	4.64 (4.35–4.92)	5.15 (2.05–5.25)	6.46	6.61 (6.48–6.73)	7.00 (6.98–7.01)	7.11 (7.02–7.19)	7.21 (7.19–7.22)	7.60 (7.56–7.63)
RN	0.90 (0.71–1.22)	1.91 (0.80–2.25)	1.91 (1.84–1.97)	1.84 (1.76–1.92)	2.41	2.07 (2.02–2.12)	2.33 (2.25–2.40)	2.17 (1.91–2.42)	1.82 (1.72–1.92)	1.83 (1.81–1.85)
NP	1.01 (0.99–1.03)	3.13 (1.10–4.46)	2.39 (2.30–2.48)	2.50 (2.43–2.56)	3.55	3.29 (3.28–3.30)	3.56 (3.50–3.62)	3.45 (3.29–3.60)	3.55 (3.47–3.63)	4.03 (3.98–4.08)
ED	0.65 (0.62–0.69)	1.64 (0.56–2.52)	1.09 (1.06–1.11)	1.26 (1.25–1.26)	1.63	1.72 (1.66–1.77)	2.11 (2.08–2.14)	2.06 (1.88–2.24)	2.22 (2.13–2.31)	2.44 (2.40–2.48)
ODW	1.23 (1.16–1.33)	3.85 (1.31–4.76)	3.67 (3.60–3.74)	3.24 (3.21–3.27)	4.48	4.23 (4.03–4.42)	4.55 (4.49–4.61)	4.42 (4.01–4.83)	4.25 (4.12–4.37)	4.63 (4.46–4.79)

TABLE 2. Morphometric comparisons of *Rhacophorus feae* tadpole with those of sympatric species and other members of the *R. dennysi* gr. for which data are available.

Species	Gosner Stage	Mental gap	Mean ED/BL	LTRF	Eye position dorsal/lateral	Mean IP/BW	Mean TAL/BL	Mean TL (mm)	Mean TH/TAL	Reference
R. feae (*N* =2)	32–37	Present	0.10	5(2-5)/3	More lateral than dorsal	0.52	1.86	57.17	0.31	This study
R. annamensis (*N* =4)	41	Absent	0.17	7(3-7)/3	Laterally, directed slightly anteriorly	0.65	2.12	41.22	0.25	Hendrix *et al*. 2007
R. dennysi (*N* not reported)	Not reported	Not reported	Not reported	5(2-4)/3(1)	Not reported	Not reported	Not reported	Not reported	Not reported	Hendrix *et al*. 2007
R. kio (*N* = 9)	36	Absent	0.083	Not reported	Dorsolaterally with anterodorsal direction	0.65	1.51	53.9	0.27	Grosjean & Inthara, 2016
R. minimus (*N = 1*)	35	Present (narrow)	0.1	5(2-5)/3(1)	Dorsal	0.37	1.77	25.5	0.24	Rao *et al*. 2006
R. orlovi (*N* = 1)	40	Absent	0.16	5(2-4)/3(1)	Dorsolaterally positioned	0.63	2.04	24.5	0.18	Wildenhues *et al*. 2011
R. rhodopus (*N* = 8)	36	Absent	0.081	6(2-6)/3(1)	More dorsal than lateral	0.49	1.48	45.7	0.27	Grosjean & Inthara 2016
R. smaragdinus (*N* = 1)	35	Present (small)	0.12	5(2-5)/3(1)	Dorsolaterally	0.63	1.99	39.3	Not reported	Wildenhues *et al*. 2010; Ohler & Deuti 2018
R. bipunctatus (*N* not reported)	30–37	Absent	Not reported	Not reported	Not Reported	Not reported	Not reported	Not reported	Not reported	Inger *et al*. 1999, in Grosjean & Inthara 2016
R. nigropunctatus (*N* not reported)	Not reported	Present	Not reported	4(2-4)/3(1)	Not reported	Not reported	Not reported	Not reported	Not reported	Liu, Hu & Yang 1962, in Grosjean & Inthara 2016

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