

Rhacophorus vampyrus (Anura: Rhacophoridae) Reproductive Biology: A New Type of Oophagous Tadpole in Asian Treefrogs

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ABSTRACT.—We report on the reproductive biology of *Rhacophorus vampyrus* (Rhacophoridae, Anura) from the Lang Bian Plateau in southern Vietnam. Unlike most other members of the genus *Rhacophorus*, *R. vampyrus* is a phytotelm breeder and reproduces in water-filled tree hollows. The species shows a unique tadpole mouthpart morphology not reported for any other anurans: presence of a specific serrated horny arch on the upper jaw and two large, fang-like horny teeth on the lower jaw. This, together with the presence of a large extensible stomach and relatively short digestive tract length, indicates an adaptation toward macrophagous feeding. We observed two clutch types in *R. vampyrus*: fertilized eggs included in a typical foam nest and unfertilized eggs, apparently having a trophic function, enveloped by dense mucous. The digestive tracts of all tadpoles examined contained up to 40 trophic eggs and showed no remains of any other food. Keratinous mouthparts undergo considerable remodeling during tadpole development presuming the change of feeding mode in ontogeny. We speculate that these findings indicate that the tadpole of *R. vampyrus* is an obligatorily and highly specialized oophagous type with unique mouthpart structures. The advanced form of parental care, including the maternal provisioning of unfertilized eggs, is reported for the first time for the genus *Rhacophorus* and is the second documented record for the Rhacophoridae.

The Vampire Treefrog, *Rhacophorus vampyrus*, is a newly described species of Asian treefrog (Rhacophoridae) inhabiting montane evergreen forests of the Lang Bian Plateau (Rowley et al., 2010). It was also reported from neighboring mountain systems of southern Vietnam (Orlov et al., 2012). More recently, Rowley et al. (2012) provided a preliminary description of *R. vampyrus* tadpole. This species is distinct from the majority of its congeners by its reproduction in water-filled tree hollows, which is observed rarely among *Rhacophorus* species, and a peculiar larval morphology. Unlike all previously known *Rhacophorus* tadpoles, which are characterized by the presence of a horny beak and several keratinized denticle rows on the upper and lower labia (Inger, 1985; Chou and Lin, 1997; Haas et al., 2012), larval *R. vampyrus* possess a highly specialized oral disc armed with a pair of large horny hooks on the lower jaw, which are considered to be an adaptation to oophagy (Rowley et al., 2012). However, the precise functional role and the homology of these unique oral structures remain unclear. The data on reproductive biology of the species, including the presence and mode of parental provisioning, are also insufficient. Herein we describe *R. vampyrus* breeding sites, its egg and tadpole morphology, and the development of the unique larval keratinous mouthparts; report the presence of an advanced form of parental care including the maternal provisioning of unfertilized eggs; and discuss diverse aspects of larval ecology in this species.

MATERIALS AND METHODS

Fieldwork in the Bidoup—NuiBa National Park (Lam Dong Province, Vietnam; 12°11'N; 108°41'E) took place 5–14 July 2011 at the onset of the rainy season. Air temperature varied from 18 to 25°C, with rain every 2–3 days. Breeding habitats of *R. vampyrus* were observed at three locations: in the vicinity of the Giang Ly Ranger Station in the Da Nhim River Valley, Bidoup, and Hon Giao Mountains. All locations were within 15 km of

each other. The breeding sites were located at 1,460–1,820 m a.s.l. in primary montane mixed evergreen forests.

We observed 13 water-filled tree hollows with egg clutches, tadpoles, or adult individuals of *R. vampyrus*. We measured tree diameter at the hollow and the height of the hollow above ground. Water, eggs, and larvae were removed from the hollow and the volume of water was measured. We recorded the clutch type (fertilized/unfertilized, presence/absence of foam nest), egg and tadpole number and stage, and number and sex of adults (Table 1).

Three fertilized clutches were removed from tree hollows (3, 9, and 11) and reared at the forest station in small plastic aquaria under natural temperature and light conditions. Aquaria were filled with 150 ml of rainwater changed every second day. Tadpoles from these clutches were fixed several days after moving from the nest into the water. Unfertilized eggs were removed from the hollows and fixed in 10% formalin for counting and measuring. Twelve eggs from fertilized clutches from two hollows (9 and 11) were removed from the foam nest for fixation and measurement. Thirty eggs from two fertilized and two unfertilized clutches were measured with outer capsule soon after fixation. Tadpoles were photographed, fixed in 10% formalin, counted, measured, and staged with the use of Gosner's simplified table (Gosner, 1960). All measurements were taken to the nearest 0.01 mm with the use of a digital caliper. Voucher series of *R. vampyrus* tadpoles were deposited in the Zoological Museum of the Lomonosov Moscow State University (ZMMU NAP-02472, NAP-03713, NAP-03715, NAP-03719).

Thirty formalin-fixed tadpoles were used for morphological examination. Morphometrics followed Altig (2007): total length (TL), body length (BL), tail length (TaL), maximal body width (BW), maximal body height (BH), maximal tail height (TH), snout-vent length (SVL), snout-spiracle length (SSp), maximal upper fin height (UF), maximal lower fin height (LF), eye diameter (ED), narial aperture diameter (ND), and oral disk width (ODW). We also measured SVL of newly metamorphosed juveniles. Larval mouthparts were examined on the tadpoles of stages 24–42. We used a LEICA EZ4 dissecting stereo

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TABLE 1. Breeding sites of *Rhacophorus vampyrus* in the Bidoup–Nui Ba National Park examined in the study.

Number	Breeding tree hollow			Clutch	Larvae and metamorphs (stages)						Adult frogs
	Height above ground (m)	Trunk diameter (cm)	Water volume (ml)		24–30	31–39	40–41	42–43	44–46	Total	
1	0.8	17	150	Unfertilized	12	10	5	3	1	31	–
2	1.3	17	200	–	–	–	1	1	1	3	–
3	0.7	12	70	Fertilized	–	–	–	–	–	9	–
4	1.4	6	55	–	5	1	1	–	–	7	–
5	0.8	9	170	–	7	5	2	2	3	19	–
6	1.0	20	25	Unfertilized	–	–	1	–	–	1	–
7	1.2	25	90	Unfertilized	–	–	–	–	–	–	–
8	1.0	10	75	–	17	24	–	–	–	41	–
9	1.3	12	250	Fertilized	–	–	–	–	–	5	–
10	0.7	13	70	–	–	7	19	–	–	26	–
11	0.9	15	300	Fertilized	–	–	–	–	–	–	1 male
12	0.6	15	80	–	4	16	25	–	–	45	–
13	0.6	15	300	–	–	–	–	–	–	–	1 male, 1 female

microscope (Germany) with digital photo attachment for gross morphology and an MBS-10 compound light microscope (LZOS, Russia) for examining mouth denticles from stage 24–27 larvae. Original drawings are by A. B. Vassilieva based on a series of microphotographs. Photographs of tadpoles in life were taken by E. A. Galoyan with Nikon D90 camera and Tamron 90/2.8 macro lens.

Stomach and intestine contents were studied in 20 tadpoles of stages 28–40. We removed the digestive tract from seven formalin-fixed specimens of stages 36–38 and compared its length with that of formalin-fixed tadpoles of *Rhacophorus calcaneus*, *Rhacophorus annamensis*, *Polypedates leucomystax*, and *Theloderma stellatum*.

RESULTS

Breeding Sites.—All tree hollows used by *R. vampyrus* were situated in the trunks of broadleaf evergreen trees of small or medium diameter (mean [\pm SD] 14.3 \pm 4.9 cm) and located at 0.6–1.4 m above ground (mean [\pm SD] 0.9 \pm 0.28 m, Table 1). All hollows were filled with water (water volume (mean [\pm SD] 141.1 \pm 95.1 ml), Table 1) and often contained a layer of brown detritus as substrate.

Two kinds of egg clutches were found. Fertilized clutches ($N = 3$, Table 1) were found only in hollows without tadpoles. Eggs were enveloped in a dense creamy-white foam nest, attached above waterline, to the wall or edge of the tree hole (Fig. 1). Diameter of a freshly deposited foam nest (hollow 9) was ca. 3.5 cm. This clutch contained 82 eggs; 6–7 of which showed signs of cleavage 3 days after deposition. The relatively dense and resilient foam nest gradually liquefied after several days and slid down into the water, where the hatched larvae continued their development.

Unfertilized clutches or their remains were found in three hollows, two of which also contained tadpoles at various developmental stages (Table 1). A freshly deposited unfertilized clutch was found in the water in hollow 1 (Fig. 2A). The eggs formed two distinct masses (presumably originating from two separate oviducts) enveloped in a transparent, highly viscous mucous. The number of eggs in this clutch was about 250, excluding those consumed by tadpoles (see below). They showed no signs of cleavage during the following 5 days. Two other unfertilized clutches from hollows 6 and 7 consisted

of mucous remains and single eggs (four and seven eggs, respectively, Table 1).

There were no visible differences among fertilized and unfertilized eggs in their size or structure. Eggs were white, with a very thin transparent outer capsule. Diameter varied from 0.70 to 1.29 mm with mean (\pm SD) 1.10 \pm 0.18 mm ($N = 30$).

The foam nest in hollow 11 was presumably deposited at night before it was collected the following day. Several eggs began to cleave; however, eggs stopped developing and subsequently died.

Tadpoles from the clutch found in hollow 9 slid into the water on the sixth day after the nest was collected. All five larvae left the nest at stage 24. Approximately 20 unfertilized eggs were left in the clutch after the tadpoles hatched. After 3 days the hatched tadpoles were fixed in 10% formalin at stage 25, with TL 7.5–7.6 mm.



FIG. 1. Foam nest of *Rhacophorus vampyrus* in a tree hollow.

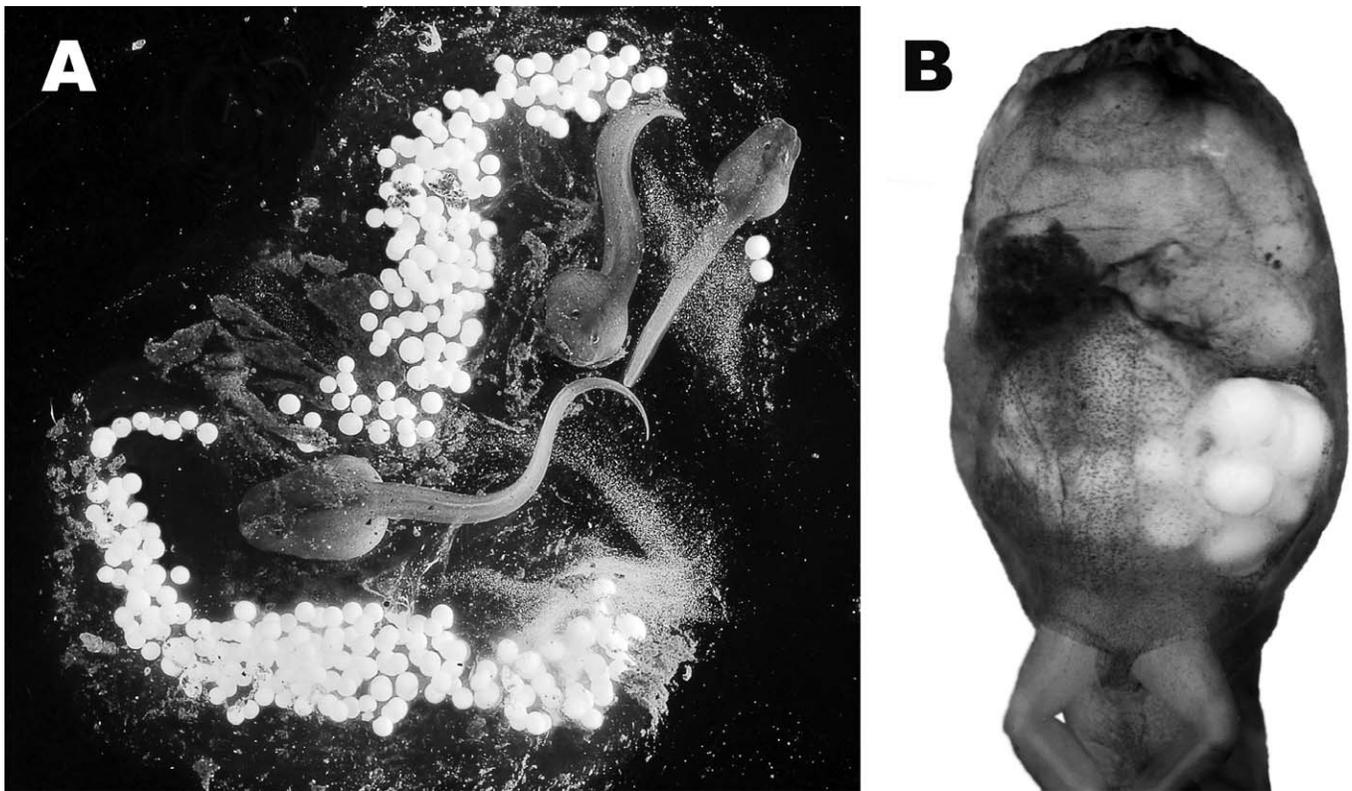


FIG. 2. Feeding of *Rhacophorus vampyrus* tadpoles. (A) Tadpoles on the unfertilized clutch, and (B) tadpole with stomach full of eggs.

Tadpoles in hollow 3 had already hatched, but remained in the foam nest at the time of clutch collection. Tadpoles left the nest 2 days later at stage 26, and after 10 days were preserved. All of them were still at stage 26, but differed significantly in size (TL 8.5–13.0 mm) and the degree of pigmentation (from completely white to light brown with whitish tails).

Tadpoles of different stages were recorded in eight tree hollows (Table 1). Young metamorphs of stages 43–45, found in three hollows (1, 2, and 5), had mean body size of 15.1 mm (SVL 14.4–16.1 mm, $N = 5$).

Tadpole Description.—Description of the larval gross morphology was based on a single specimen of stage 37 (NAP-02472, TL = 35.6 mm; Fig. 3A–D, Table 2).

Body somewhat elliptical, larger than wide (BW/BL = 0.68), bluntly truncated on rostral end, and slightly flattened dorsoventrally. Head remarkably sculptured, nostrils projecting dorsolaterally, internarial protuberance and upper edge of orbits prominent. Eyes relatively small (ED/BL = 0.15), situated dorsally, pupils oriented rostrrolaterally. Spiracle sinistral, tubular, located on ventrolateral surface at mid body. Narial apertures small, oval, oriented caudolaterally, protuberant, without any specific ornamentation. Rows of neuromasts visible only on rostral and ventrolateral surfaces of head.

Tail long (TaL/BL = 3.1), compressed laterally, tail height slightly exceeding body height (TH/BH = 1.2); tail muscular part well developed, tail base wide, exhibiting prominent myomeres; tail tip acuminate, without terminal filament. Upper and lower tail fins relatively low, reaching maximum height in second third of tail; upper tail fin not extended onto trunk and rising somewhat distally from tail base; upper tail fin slightly higher than lower one. Vent tube medial, its opening at base of lower tail fin.

General coloration uniformly dark brown; narrow margins of tail fins lacking pigment. Keratinized mouth elements black; eyes black with golden speckling on iris.

Mouth terminal; upper labium overhanging lower one and bearing two ventrally directed long, flexible hook-like outgrowths, slightly curved posteriorly and framing the oral aperture (Fig. 4A,B). Upper jaw armed with solid horny arch of nine pointed denticles, sharply curving toward the mouth; lower jaw bearing two large (ca. 0.35 mm in length), slightly separated, curved rostroventrally fang-like teeth; any other keratinized structures absent. Two slightly rounded, rostrally oriented protuberances located laterally to “fangs.”

Variations.—Morphological variation during larval development concerned tadpole size and body proportions (Table 2), spiracle shape, coloration, and mouthparts structure.

Minimal size was recorded at hatching (stages 24–25; TL = 7.5 mm), maximal at stage 41 (TL = 41.6 mm). Tail-length to body-length ratio was 2.8 on average (TaL/BL: 2.4–3.1) and was maximal on stages 25–37.

In tadpoles of stages 25–36 spiracle aperture was covered with a thin skin flap; in tadpoles of more advanced stages this flap often fused, forming a short tube.

Tadpoles of earliest stages (25–26) differed from more advanced larvae by paler coloration. They were whitish or grayish, with scattered chromatophores present only on the dorsal body surface. At stages 27–30 the body acquired dense dark pigmentation earlier than the tail did, so tadpoles at these stages had brown bodies and white tails. Metamorphs had yellowish coloration.

Tadpole mouthpart morphology varied considerably depending on the developmental stage. In early stages (24–25) the horny arch on the upper jaw contained 14–15 small conical serrations, slightly curved toward the mouth. Fang-like teeth on

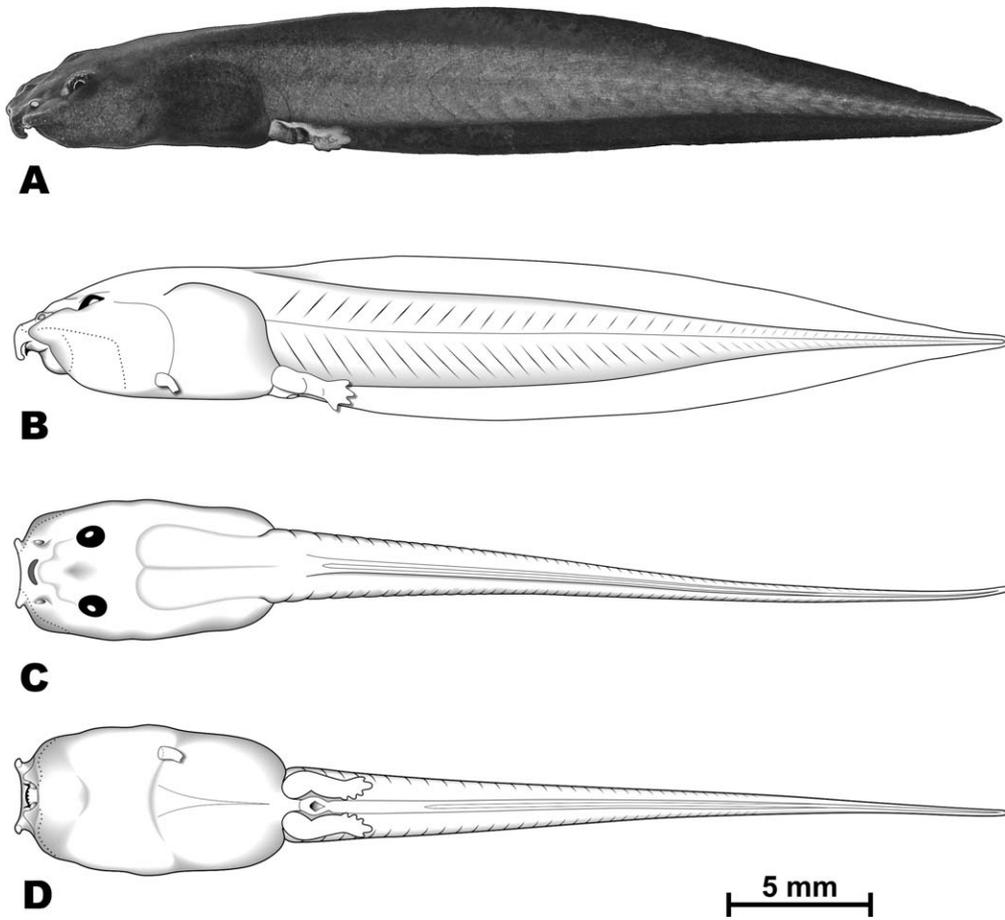


FIG. 3. *Rhacophorus vampyrus* tadpole external morphology (stage 37, NAP-02472). (A) Coloration, (B) lateral view, (C) dorsal view, and (D) ventral view.

the lower jaw were absent. In their place were two rounded, keratinized elements bearing short, flattened crests with two, occasionally three serrations oriented upwards; their shape varied slightly among specimens (Fig. 5A).

These early larval mouthparts were replaced during stages 25–26. The horny arch on the upper jaw and paired serrated crests on the lower jaw disappeared and new structures were formed instead. In a tadpole in later stage 25, the horny element on the upper jaw was totally absent; the lower jaw possessed two fang primordia in form of small conical denticles. In four tadpoles (stage 26) between two and seven large, single, widely spaced denticles were seen on the upper jaw; they had a wide base and were sharply curved backwards; the lower-jaw fangs

were enlarged (Fig. 5B). The arrangement and size of the upper-jaw denticles in one tadpole (late stage 26) indicated that they likely were not formed simultaneously, because the new primordia appeared between other already well-developed teeth (Fig. 5C). During stage 27 the number of upper-jaw denticles reached 9–11. Their bases were enlarged and fused, forming a unique serrated arch. Fangs obtained their definitive shape (Fig. 5D). Larval mouthparts disappeared at stages 41–42, when the intense metamorphic transformation of the head took place and the mouth progressively widened.

Digestive System.—Digestive tract included well-defined stomach with thin, extensible walls, folded when stomach is empty. Filled stomach occupied the entire ventral cavity, and

TABLE 2. Main morphometric parameters (in millimeters) of *Rhacophorus vampyrus* tadpoles of different developmental stages (for character abbreviations see Materials and Methods section).

Stage	TL	BL	TaL	BW	BH	TH	SVL	SSp	UF	LF	ED	ND	ODW
25	15.6	3.8	11.8	2.2	1.8	2.0	4.7	2.2	0.6	0.5	0.2	0.2	1.3
27	16.2	4.5	11.7	2.4	1.9	2.1	5.1	2.4	0.5	0.4	0.3	0.2	1.4
28	23.5	5.8	17.8	3.4	2.2	2.9	6.8	3.5	0.8	0.8	0.4	0.2	2.0
34	29.3	7.3	21.9	5.1	4.2	4.5	8.6	4.2	1.1	0.9	0.9	0.3	2.0
36	36.2	9.2	27.1	5.4	4.7	5.7	10.8	5.5	1.4	1.2	1.1	0.3	2.2
37	35.6	8.7	26.9	5.9	5.1	6.1	9.9	5.3	1.5	1.1	1.3	0.3	2.4
38	39.9	10.7	29.2	7.4	5.9	7.5	12.0	6.7	1.9	1.9	1.5	0.3	2.5
39	39.0	10.4	28.6	6.9	5.8	6.8	11.0	6.5	1.8	1.6	1.6	0.3	2.6
40	36.9	10.3	26.5	6.9	5.5	6.4	11.4	6.8	1.5	1.5	1.6	0.3	2.3
41	41.6	12.2	29.5	8.5	7.1	8.1	12.5	8.2	2.1	1.9	1.8	0.3	2.8

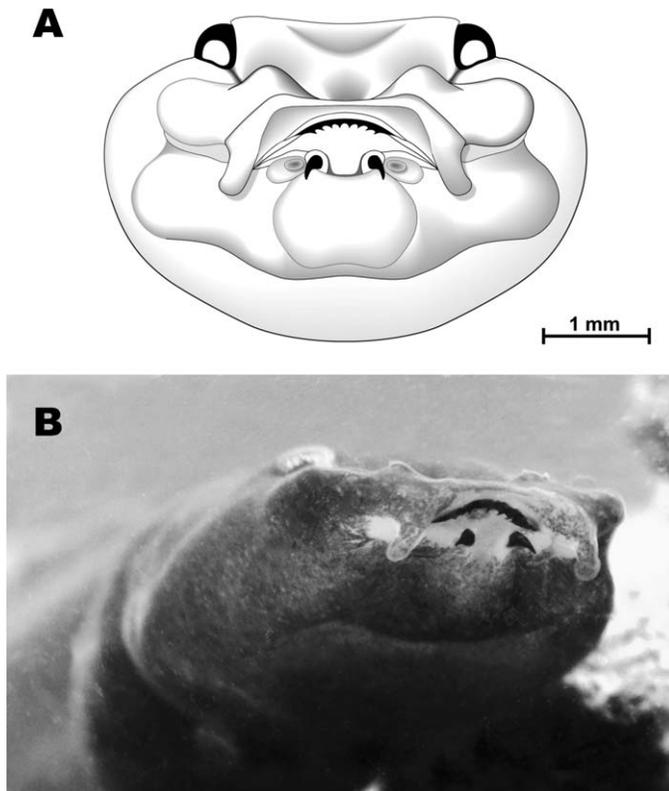


FIG. 4. Facial view of the *Rhacophorus vampyrus* tadpole. (A) Schematic drawing and (B) in life.

larvae appeared swollen (Fig. 2A). Intestine was arranged in wide loops not forming a spiral; its length varied in fixed specimens (BL 10.1–12.0 mm) from 28 to 40 mm and depended on gut fullness, but did not extend body length more than 3.5 times (Table 3).

Stomach contents of tadpoles from all hollows (excluding 4) contained 12 to 42 eggs (Fig. 2B). Most eggs were undamaged, apart from minor deformation caused by the dense packing. Gut content of 18 specimens consisted of a whitish homogeneous substance, presumably partly digested eggs. No other alimentary materials were recorded in the tadpole digestive tracts, except occasional grains of sand and detritus in the posterior part of the gut. Two tadpoles from hollow 4 had empty, collapsed stomachs; only the terminal portion of the gut contained some detritus and sand particles.

DISCUSSION

Old World treefrogs (Rhacophoridae) are a large group of mostly arboreal species, which typically reproduce outside of bodies of water, laying eggs on the ground near the edge of water or attaching clutches to branches or leaves above the surface of water, thereby allowing tadpoles access to water immediately after hatching. Some rhacophorids, however, reproduce in water-filled tree hollows, where tadpoles develop until metamorphosis is complete. Such reproductive biology is typical for the genus *Theloderma*, a widespread Southeast Asian genus of forest-dwelling rhacophorids (Orlov et al., 2010). Among the genus *Rhacophorus* such breeding biology is extremely rare. However, it was reported for the Bornean species *Rhacophorus harrissoni* (Inger, 1966; Malkmus and Dehling, 2008) and for *Rhacophorus georgii* from Sulawesi

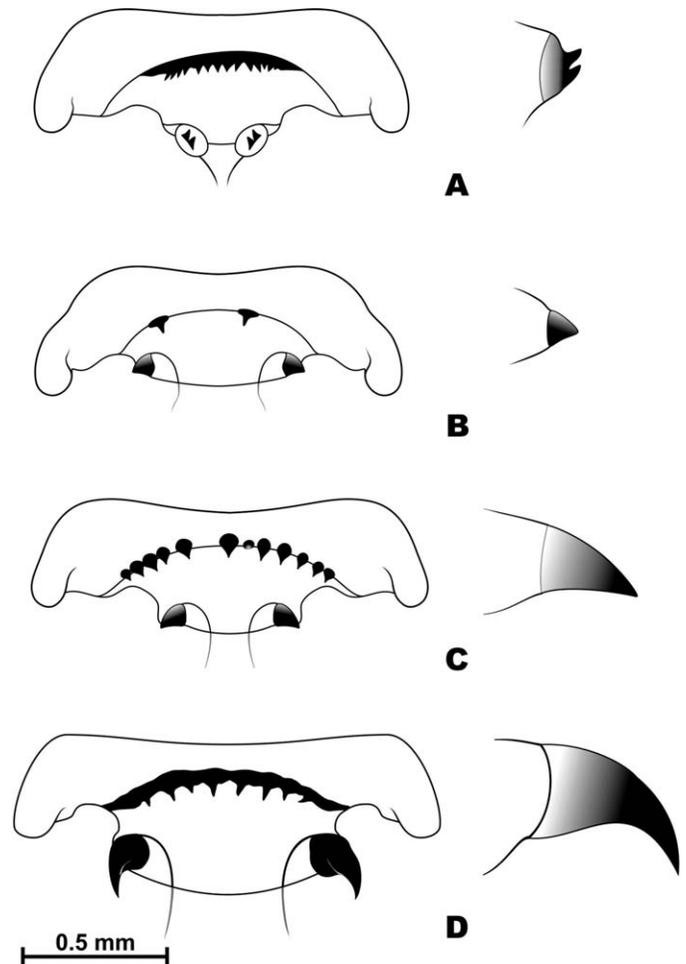


FIG. 5. Mouthparts of *Rhacophorus vampyrus* tadpoles (series NAP-03719) of 25–27 developmental stages (left, facial view) and lower-jaw fangs (right, side view). (A) Stage 25, (B) early stage 26, (C) late stage 26, and (D) stage 27.

(Gillespie et al., 2007). Among *Rhacophorus* species found in mainland Southeast Asia, *Rhacophorus vampyrus* is the only species known that breeds and rears its tadpoles in tree hollows.

Many frog species of the families Hylidae, Dendrobatidae, Mantellidae, and Rhacophoridae are known as phytotelm breeders, i.e., they use the available water held by plants (such as empty bamboo internodes, tree hollows, epiphytes) for reproduction. Compared to breeding in larger bodies of water, phytotelm breeding has many advantages, such as better protection of tadpoles from predators and less intense intraspecific competition; the main disadvantage is that in phytotelm breeders tadpole survivorship is strictly limited by food availability (Caldwell and Araújo, 2004). Tadpoles developing in small bodies of water (e.g., limestone caverns) or in nests located outside of water (as in some Leptodactylidae and Hylidae frogs) are faced with the same problem (Wells, 2007). One possible solution is the use of conspecific eggs as a food source for tadpoles. The strategy where the newly hatched tadpoles feed on unfertilized eggs of the same clutch, as in *Leptodactylus labyrinthicus* (Leptodactylidae), is likely the most primitive type of oophagy in larval amphibians (Prado et al., 2005). Tadpoles of some species eat fertilized eggs or developing embryos from later clutches laid in the same tree hollow, as reported for the hylids *Phrynohyas resinifictrix* (Schiesari et al.,

TABLE 3. Morphometric parameters of some rhacophorid eggs and larvae. Mean digestive tract length (DTL) relative to body length (BL) with ranges in parentheses (stages 28–38) and egg diameter (measured on freshly laid eggs or early cleavage stages, in millimeters) are given.

Species	<i>Rhacophorus vampyrus</i>	<i>Rhacophorus calcaneus</i>	<i>Rhacophorus annamensis</i>	<i>Polypedates leucomystax</i>	<i>Theloderma stellatum</i>
DTL/BL	3.1 (2.8–3.5) (N = 7)	9.7 (9.2–10.2) (N = 3)	10.7 (10.0–11.1) (N = 3)	9.2 (8.0–10.4) (N = 5)	9.8 (9.6–10.0) (N = 2)
Egg diameter	1.1 (0.7–1.3) (N = 30)	4.2 (4.0–4.3) (N = 5)	3.2 (3.1–3.3) (N = 11)	1.99 (1.9–2.1) (N = 10)	2.5–3.0 Orlov et al. (2011)

2003) or *Aplastodiscus perviridis* (Haddad et al., 2005). The next stage of this strategy is parental provisioning, where the parents deliberately visit the body of water with developing larvae and lay additional eggs to feed their offspring. This form of parental care has been reported for at least six families of anurans (Lehtinen and Nussbaum, 2003). In some species, only females visit the offspring, whereas in others, both males and females take part in parental provisioning. In latter cases tadpoles feed on fertilized eggs and/or developing embryos, for example, in the hylid *Osteocephalus oophagus* (Jungfer and Weygoldt, 1999) or the dendrobatid *Ranitomeya vanzolinii* (formerly *Dendrobates vanzolinii*) (Caldwell and de Oliveira, 1999). The most specialized strategy is feeding the offspring with truly trophic unfertilized eggs (Perry and Roitberg, 2006). In this case the female visits the nest site alone and lays unfertilized eggs only to provide the larvae with a food source. In some species, such maternal provisioning is a facultative food supplement because larvae normally feed on detritus or invertebrates, as in *Mantella laevigata*, Mantellidae (Heying, 2001). In other species, unfertilized conspecific eggs are the only food source for the tadpoles, and maternal provisioning is required for normal development and metamorphosis, as is the case in the dendrobatid *Oophaga pumilio* (formerly *Dendrobates pumilio*) (Brust, 1993), the hylid *Osteopilus brunneus* (Lannoo et al., 1987), and the leptodactylid *Leptodactylus fallax* (Gibson and Buley, 2004). Among rhacophorid frogs oophagous tadpoles feeding obligatorily on unfertilized eggs were to date reported only for one species, *Kurixalus eiffingeri* (formerly *Chirixalus eiffingeri*), from Taiwan and the southernmost Ryukyu Islands (Ueda, 1986; Kam et al., 1996), which reproduces in water-filled bamboo stems. A similar type of maternal care was assumed for a closely related *Kurixalus* cf. *carinensis* (formerly *Philautus* sp. cf. *carinensis*) from Thailand and Indochina; however, the biology of this species remains insufficiently studied (Wassersug et al., 1981).

Our data on *R. vampyrus* reproductive biology suggest that this species exhibits an evolutionarily advanced strategy of parental care with maternal provisioning and highly specialized, obligatorily oophagous tadpoles. This assumption is supported by different aspects of the reproductive biology of *R. vampyrus*. First, it is concordant with the presence of two clutch types. The fertilized clutches in *R. vampyrus* are laid in foamy nests, as is typical for other *Rhacophorus* species (Liem, 1970; Grosjean et al., 2008). The egg clutches found in the tree hollows with developing larvae apparently have a trophic function. Absence of cleavage indicated all such clutches were unfertilized. In rhacophorids, foam nests are formed during amplexus by either the male or both partners by whipping the mucous with the hindlimbs (Coe, 1964; Wells, 2007). The fact that trophic clutches were covered with mucous envelopes but not with foam indicates that female *R. vampyrus* lay these clutches likely without male participation.

The assumption that *R. vampyrus* larvae feed on eggs is supported by the observations of Rowley et al. (2012) and, in

our study, by the presence of intact or partly digested eggs in the stomachs of larvae from all studied hollows except 4, where tadpoles had empty digestive tracts and were evidently starving. Moreover, in the digestive systems of all tadpoles examined we never observed the remains of any other food items, such as chitinous parts of invertebrates or plant material. In some tadpoles detritus particles and grains of sand were found in the rectal part of the intestine, but these were most likely consumed passively. Thus, it appears that the only food source of *R. vampyrus* larvae are unfertilized conspecific egg clutches.

The larval digestive system morphology of *R. vampyrus* tadpoles also indicates a high level of specialization to obligatory oophagy. It is known that carnivorous tadpoles tend to have shorter digestive tracts than herbivorous ones (Altig and Kelly, 1974). It was shown that oophagous tadpoles have comparatively shorter digestive tracts than their nonoophagous sister taxa and possess large stomachs, which indicate an adaptation to intermittent macrophagy (Lannoo et al., 1987; Liang et al., 2002). Our data show that *R. vampyrus* tadpoles have notably shorter digestive tracts than herbivorous or detritophagous tadpoles of other studied rhacophorids, including the tree-hole breeding species *Theloderma stellatum* (Table 3).

A remarkable feature of *R. vampyrus* is a comparatively small egg size for a medium-sized *Rhacophorus*—just 1.1 ± 0.18 mm on average. Other sympatric *Rhacophorus* species of similar body size have significantly larger eggs (Table 3).

Several authors point out that frogs with obligatorily oophagous tadpoles tend to have comparatively smaller eggs than in closely related species with typical reproductive biology (Wassersug et al., 1981; Jungfer and Weygoldt, 1999; Summers et al., 2007). This trend may be explained as a direct adaptation to oophagy because smaller eggs are more easily consumed by tadpoles (Jungfer and Weygoldt, 1999).

The peculiar mouthpart morphology of *R. vampyrus* tadpoles also seems to be an adaptation to obligatory oophagy. The serrated arch on the upper jaw; large, fang-like denticles on the lower jaw; and the hook-like protuberances on the sides of the mouth are unique among all known anuran tadpoles, including rhacophorids, which otherwise show little variation in mouthpart morphology (Inger, 1985; Chou and Lin, 1997).

In other obligatorily oophagous tadpoles a trend indicating a reduction of the number of labial denticle rows was reported (Lannoo et al., 1987; Chou and Lin, 1997; Rödel, 1998; Jungfer and Weygoldt, 1999). In all known *Rhacophorus* species the oral disc of tadpoles always bears several rows of labial denticles, but these are completely absent in tadpoles of *R. vampyrus*. Homology of keratinized mouthparts found in *R. vampyrus* is not completely understood. The serrated horny arch on the upper jaw is believed to be homologous with the upper sheath of other anuran larvae (Rowley et al., 2012); however, the developmental pattern of this structure contradicts this assumption. In other tadpoles, both upper- and lower-jaw sheaths are

formed as whole units, which are keratinized beginning at the medial part of the each sheath, with later keratinization spreading laterally and basally (Kaung, 1975; Thibaudeau and Altig, 1988). In early *R. vampyrus* larvae, we observed that a row of separated denticles is formed on the upper jaw initially, which subsequently fuses to the entire arch-shaped element at the bases (Fig. 5B–D). Moreover, new denticle primordia can form between already well-developed denticles (Fig. 5C). Thus, the arch is formed not in a lateral direction as in other anurans, but by means of intercalary formation of new elements.

Keratinized fangs on the lower jaw also have a complex development, with their morphology being changed significantly. Recently hatched larvae lack the horny fangs, but possess a pair of bicuspid ridges with denticles orientated upwards, which are later replaced with conical denticles orientated downwards. We assume that the transformation of the lower-jaw mouthparts may be connected with the changes in feeding mechanism during larval development and growth. Oophagous tadpoles in various groups of Anura at early stages with small larval body size break the egg capsule and consume the yolk, whereas larger tadpoles swallow and digest the whole egg without damaging the capsule (Ueda, 1986; Brust, 1993; Jungfer and Weygoldt, 1999). We have not observed such behavior in *R. vampyrus*; however, it is evident that the larvae at early stages cannot swallow the whole egg because their small body size, whereas tadpoles at later stages had entire eggs in their digestive tracts. In spite of the assumption made by Rowley et al. (2012), we do not believe that tadpoles can grow large enough on their own yolk reserves (considering the small egg size in *R. vampyrus*) to start feeding on whole undamaged eggs. Thus, at early developmental stages larvae have to feed by extracting the egg content from the capsule until they are able to swallow the egg whole. This change of the feeding mode in the ontogeny is likely supported by the observed remodeling of all keratinous mouthparts at early larval stages.

Understanding the homology of the horny fangs, presumably derived from labial denticles (Rowley et al., 2012), and the keratinized arch of the upper jaw in *R. vampyrus* tadpoles, requires detailed histological and morphological studies. However, it is noteworthy that the observed morphology of the mouthparts in larval *R. vampyrus* is only suited for macrophagy and does not allow the tadpoles to feed on resources other than eggs. The large keratinized fangs on the lower jaw pointing forward and the denticles of the upper jaw curved backwards cannot be used for scraping or detritus grinding. We assume that these structures, together with the hook-like protuberances at the sides of the mouth, are used mainly to retrieve the trophic eggs from the dense resilient mucous. In addition, the reduction of upper and lower oral disc labia in *R. vampyrus* may enhance the extreme widening of tadpole gape (Rowley et al., 2012).

Body proportions in *R. vampyrus* larvae are also quite unusual for rhacophorids: in this species tadpoles have a comparatively long tail which is about three times longer than the body length (Table 2). In typical pond-type tadpoles of other *Rhacophorus* species the tail length is much shorter and is not more than twice the body length (Inger, 1985; Chou and Lin, 1997). Some authors report longer tails for tadpoles of obligatorily oophagous anuran species (Wassersug et al., 1981; Lannoo et al., 1987; Gibson and Buley, 2004). Most likely, the longer tail is an adaptation for swimming in liquid with greater viscosity because of significant amounts of mucous brought in by the female along with the trophic eggs (Lannoo et al., 1987).

It appears that maternal provisioning with trophic eggs allows large numbers of larvae to complete development in the limited space of small bodies of water. *Rhacophorus vampyrus* demonstrates high density of tadpoles up to 45 per nest (Table 2). By comparison, tree-hole breeding *Theleodermis* species, which do not feed their progeny, have smaller clutches of less than 10 eggs (Wassersug et al., 1981; Orlov et al., 2010). Contrarily, *K. eiffingeri* providing maternal care can rear up to several dozen tadpoles in a bamboo trunk with a small volume of water (Ueda, 1986; Kam et al., 1996). Thus, the high density of *R. vampyrus* tadpoles in the various tree hollows also indicates that larval development is completed with the use of energetic resources obtained from parental feeding.

The observed high variability in stage and body size of tadpoles in one tree hollow may be explained in several ways. First, as was shown in *K. eiffingeri*, stratification of tadpoles into size groups may be a result of a density effect: tadpoles that initially developed faster than their siblings have an advantage in competition for food resources. The higher the tadpole density, the greater the variability in time to complete metamorphosis and thus the difference in body size (Kam et al., 1998; Chen et al., 2001; Kam et al., 2001). Our observations on early development of the tadpoles from hollow 3 suggest that the stratification in body size within the larvae from one clutch appears very quickly—within the first 10 days size differentiation reaches more than 30%, and can increase even further. However, the presence of tadpoles of all developmental stages (from the earliest larvae to metamorphosing frogs) (e.g., hollows 1 and 5) cannot be explained exclusively by the effect of tadpole density. In *K. eiffingeri* new fertilized clutches can be laid in the hollow with already developing tadpoles, which causes the presence of several larval generations in one breeding nest (Kam et al., 2001; Chen et al., 2001). This phenomenon could be found in *R. vampyrus* as well.

In conclusion, we may confidently state that *R. vampyrus* is a tree-hollow breeding frog with extremely specialized tadpole morphology and advanced form of parental care that includes the obligatory feeding of larvae with unfertilized eggs. The morphology of the tadpoles of *R. vampyrus* is unique among anurans. Details of the reproductive biology of *R. vampyrus*, such as maternal provisioning behavior and the possible role of males in parental care, remain unclear and should be studied further.

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LITERATURE CITED

- ALTIG, R. 2007. A primer for the morphology of anuran tadpoles. *Herpetological Conservation and Biology* 2:71–74.
- ALTIG, R., AND J. P. KELLY. 1974. Indices of feeding in anuran tadpoles as indicated by gut characteristics. *Herpetologica* 30:200–203.
- BRUST, D. G. 1993. Maternal brood care by *Dendrobates pumilio*: a frog that feeds its young. *Journal of Herpetology* 27:96–98.
- CALDWELL, J. P., AND M. C. ARAÚJO. 2004. Historical and ecological factors influence survivorship in two clades of phytotelm-breeding frogs (Anura: Bufonidae, Dendrobatidae). In R. M. Lehtinen (ed.), *Ecology and Evolution of Phytotelm Breeding Anurans*, Miscellaneous Publications V. 193, pp. 11–21. Museum of Zoology, University of Michigan, Ann Arbor, MI.
- CALDWELL, J. P., AND V. R. L. DE OLIVEIRA. 1999. Determinants of biparental care in the spotted poison frog, *Dendrobates vanzolinii* (Anura: Dendrobatidae). *Copeia* 1999:565–575.
- CHEN, Y.-H., Y.-J. SU, Y.-S. LIN, AND Y.-C. KAM. 2001. Inter- and intraclutch competition among oophagous tadpoles of the Taiwanese tree frog, *Chirixalus eiffingeri* (Anura: Rhacophoridae). *Herpetologica* 57:438–448.
- CHOU, W.-H., AND J.-Y. LIN. 1997. Tadpoles of Taiwan. Special Publication National Museum of Natural Science 7:1–98.
- COE, M. J. 1964. Observations of the ecology and breeding biology of the genus *Chiromantis*. *Journal of Zoology* 172:13–34.
- GIBSON, R. C., AND K. R. BULEY. 2004. Maternal care and obligatory oophagy in *Leptodactylus fallax*: A new reproductive mode in frogs. *Copeia* 2004:128–135.
- GILLESPIE, G. R., M. ANSTIS, S. D. HOWARD, AND D. LOCKIE. 2007. Description of the tadpole of the Rhacophorid frog *Rhacophorus georgii* Roux (Rhacophoridae) from Sulawesi, Indonesia. *Journal of Herpetology* 41:150–153.
- GOSNER, K. L. 1960. A simplified table for staging anuran embryos and larvae. *Herpetologica* 16:183–190.
- GROSJEAN, S., M. DELORME, A. DUBOIS, AND A. OHLER. 2008. Evolution of reproduction in the Rhacophoridae (Amphibia, Anura). *Journal of Zoological Systematics and Evolutionary Research* 46:169–176.
- HAAS, A., S. T. HERTWIG, W. KRINGS, E. BRASKAMP, J. M. DEHLING, P. Y. MIN, A. JANKOWSKI, M. SCHWEIZER, AND I. DAS. 2012. Description of three *Rhacophorus* tadpoles (Lissamphibia: Anura: Rhacophoridae) from Sarawak, Malaysia (Borneo). *Zootaxa* 3328:1–19.
- HADDAD, C. F. B., J. FAIVOVICH, AND P. C. A. GARCIA. 2005. The specialized reproductive mode of the tree frog *Aplastodiscus perviridis* (Anura: Hylidae). *Amphibia-Reptilia* 26:87–92.
- HEYING, H. E. 2001. Social and reproductive behaviour in the Madagascar poison frog, *Mantella laevisgata*, with comparisons to the dendrobatids. *Animal Behaviour* 61:567–577.
- INGER, R. F. 1966. The systematics and zoogeography of the Amphibia of Borneo. *Fieldiana Zoology* 52:1–402.
- INGER, R. F. 1985. Tadpoles of the forested regions of Borneo. *Fieldiana Zoology* 26:1–89.
- JUNGFER, K.-H., AND P. WEYGOLDT. 1999. Biparental care in the tadpole-feeding Amazonian treefrog *Osteocephalus oophagus*. *Amphibia-Reptilia* 20:235–249.
- KAM, Y.-C., Z.-S. CHUANG, AND C.-F. YEN. 1996. Reproduction, oviposition-site selection, and tadpole oophagy of an arboreal nester, *Chirixalus eiffingeri* (Rhacophoridae), from Taiwan. *Journal of Herpetology* 30:52–59.
- KAM, Y.-C., C.-F. LIN, Y.-S. LIN, AND Y.-F. TSAI. 1998. Density effects of oophagous tadpoles of *Chirixalus eiffingeri* (Anura: Rhacophoridae): importance of maternal brood care. *Herpetologica* 54:425–433.
- KAM, Y.-C., Y.-J. SU, J.-L. LIU, AND Y.-S. LIN. 2001. Intraspecific interactions among oophagous tadpoles (*Chirixalus eiffingeri*: Rhacophoridae) living in bamboo stumps in Taiwan. *Journal of Zoology* 255:519–524.
- KAUNG, H.-L. C. 1975. Development of beaks of *Rana pipiens* larvae. *The Anatomical Record* 182: 401–414.
- LANNOO, M. J., D. S. TOWNSEND, AND R. J. WASSERSUG. 1987. Larval life in the leaves: arboreal tadpole types, with special attention to the morphology, ecology, and behavior of the oophagous *Osteopilus brunneus* (Hylidae) larvae. *Fieldiana Zoology* 38:1–31.
- LEHTINEN, R. M., AND R. A. NUSSBAUM. 2003. Parental care: a phylogenetic perspective. In B. G. M. Jamieson (ed.), *Reproductive Biology and Phylogeny of Anura*, pp. 343–386. Science Publishers, Enfield, NH.
- LIANG, M.-F., C.-H. HUANG, AND Y.-C. KAM. 2002. Effects of intermittent feeding on the growth of oophagous (*Chirixalus eiffingeri*) and herbivorous (*Chirixalus idiotocous*) tadpoles from Taiwan. *Journal of Zoology* 256:207–213.
- LIEM, S. S. 1970. The morphology, systematics, and evolution of the Old World treefrogs (Rhacophoridae and Hyperoliidae). *Fieldiana Zoology* 57:1–145.
- MALKMUS, R., AND J. M. DEHLING. 2008. Anuran amphibians of Borneo as phytotelm-breeders—a synopsis. *Herpetozoa* 20:165–172.
- ORLOV, N. L., S. A. RYABOV, N. B. ANANIEVA, AND A. A. EVSYUNIN. 2010. Asian Treefrogs of Genus *Theloderma* Tschudi, 1838 (Amphibia: Anura: Rhacophoridae: Rhacophorinae). Russian Academy of Sciences, Zoological Institute, Saint Petersburg, Russia.
- ORLOV, N. L., N. A. POYARKOV, A. B. VASSILIEVA, N. B. ANANIEVA, TH. T. NGUYEN, N. S. NGUYEN, AND P. GEISSLER. 2012. Taxonomic notes on Rhacophorid frogs (Rhacophorinae: Rhacophoridae: Anura) of southern part of Annamite Mountains (Truong Son, Vietnam), with description of three new species. *Russian Journal of Herpetology* 19: 23–64.
- PERRY, J. C., AND B. D. ROITBERG. 2006. Trophic egg laying: hypotheses and tests. *Oikos* 112:708–717.
- PRADO, C. P. A., L. F. TOLEDO, J. ZINA, AND C. F. B. HADDAD. 2005. Trophic eggs in the foam nests of *Leptodactylus labyrinthicus* (Anura, Leptodactylidae): an experimental approach. *The Herpetological Journal* 15:279–284.
- RÖDEL, M. O. 1998. A reproductive mode so far unknown in African ranids: *Phrynobatrachus guineensis* Guibé and Lamotte, 1961 breeds in tree holes. *Herpetozoa* 11:19–26.
- ROWLEY, J. L., L. T. T. DUONG, T. T. A. DAO, B. L. STUART, AND H. D. HUY. 2010. A new tree frog of the genus *Rhacophorus* (Anura: Rhacophoridae) from southern Vietnam. *Zootaxa* 2727:45–55.
- ROWLEY, J. L., T. T. A. DAO, L. T. T. DUONG, H. D. HUY, AND R. ALTIG. 2012. The strangest tadpole: the oophagous, tree-hole dwelling tadpole of *Rhacophorus vampyrus* (Anura: Rhacophoridae) from Vietnam. *Journal of Natural History* 46:2969–2978.
- SCHIESARI, L., M. GORDO, AND W. HÖDL. 2003. Tree holes as calling, breeding, and developmental sites for the Amazonian canopy frog, *Phrynohyas resinifictrix* (Hylidae). *Copeia* 2003:263–272.
- SUMMERS, K., C. S. MCKEON, H. HEYING, J. HALL, AND W. PATRICK. 2007. Social and environmental influences on egg size evolution in frogs. *Journal of Zoology* 271:225–232.
- THIBAudeau, D. G., AND R. ALTIG. 1988. Sequence of ontogenetic development and atrophy of the oral apparatus of six anuran tadpoles. *Journal of Morphology* 197:63–69.
- UEDA, H. 1986. Reproduction of *Chirixalus eiffingeri* (Boettger). Scientific Reports of Laboratory of Amphibian Biology, Hiroshima University 8:109–116.
- WASSERSUG, R. J., K. J. FROGNER, AND R. F. INGER. 1981. Adaptations for life in tree holes by rhacophorid tadpoles from Thailand. *Journal of Herpetology* 15:41–52.
- WELLS, K. 2007. *The Ecology and Behavior of Amphibians*. The University of Chicago Press, Chicago.

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