

First record of *Amolops cremnobatus* from Thanh Hoa Province, Vietnam, including an extended tadpole description and the first larval staging for *Amolops*

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Abstract. We record *Amolops cremnobatus* INGER & KOTTELAT, 1998 for the first time from Thanh Hoa Province, north-central Vietnam, which also represents the northernmost record of this species. Specimens of *A. cremnobatus* were found at three limestone karst forest sites within the Pu Hu Nature Reserve at altitudes between 277 and 526 m a.s.l. Adults and larval stages matched the diagnostic morphological characters of the original description of *A. cremnobatus*, such as small size, tarsal gland present, vomerine teeth present, tympanum distinct, nuptial pads distinctly developed in males, and tadpoles with upper jaw sheath divided and labial teeth row formula 10(5–10)/6(1). Besides natural history notes for both developmental stages and adults, we present an extended larval description based on a tadpole at GOSNER (1960) stage 31. The tadpole belongs to the exotrophic, lotic, gastromyzophorus larval type after MCDIARMID & ALTIG (1999) and possesses the typical characteristics of a fast-water stream dweller, i.e., a large abdominal sucker disc, thick tail musculature, and reduced fins. In addition, the first larval staging for the genus *Amolops* in general is provided in this paper, based on the species *A. cremnobatus*.

Key words. Amphibia, Anura, *Amolops*, *A. cremnobatus*, new record, tadpole description, larval staging, natural history, north-central Vietnam.

Introduction

The Southeast Asian ranid genus *Amolops* COPE, 1865 currently comprises 47 described species (FROST 2013). Due to their obligatory preference for strong-current mountain streams with steep-faced rocks, all members of the genus *Amolops* share distinct characters such as enlarged finger and toe discs in adults and an abdominal sucker disc formed by a rim of rough epidermis in the gastromyzophorus tadpoles, which otherwise can only be found in few amphibian genera, such as *Atelopus* and *Huia* (MCDIARMID & ALTIG, 1999). The systematics of *Amolops* remain unresolved as yet (MATSUI et al. 2006, YANG 1991).

Amolops cremnobatus was recently described from Nam Phao River, Khammouan Province, Laos, based on a sample of syntopically occurring adults and larvae (INGER & KOTTELAT 1998). Although the species is morphologically similar to *A. ricketti*, MATSUI et al. (2006) demonstrated that

these species were genetically different. *Amolops cremnobatus* is a stream-breeding species that lives on boulders and rock faces along stream cascades and waterfalls in evergreen forest (INGER & KOTTELAT 1998, STUART 1999). Only a few populations of *A. cremnobatus* are so far known from Laos and Vietnam (NGUYEN et al. 2009). The species has been reported to occur at three localities in north-central Laos, and in three provinces in north-central Vietnam, namely Ha Tinh, Quang Binh, and Nghe An (FROST 2013). Due to its small distribution range (Fig. 1), *A. cremnobatus* is categorised as Near Threatened. Additionally, because the species is restricted to streams and hence particularly vulnerable to habitat degradation, *A. cremnobatus* is close to qualifying for Vulnerable (IUCN 2013).

During our recent field research in the Pu Hu Nature Reserve (Fig. 2), Thanh Hoa Province, in north-central Vietnam, a population of *Amolops* was discovered that matched the diagnostic morphological characters of

A. cremnobatus provided by INGER & KOTTELAT (1998), such as small size, tarsal gland present, vomerine teeth present, tympanum distinct, nuptial pads distinctly developed in males, and tadpoles with upper jaw sheath divided and labial teeth row formula $10(5-10)/6(1)$. Thus, we herein report *A. cremnobatus* based on a series of tadpoles and adults from three different reproduction sites for the first time from Thanh Hoa Province, which also represents the northernmost record of the species. In addition, we provide an extended larval description including photographs and drawings based on a tadpole in GOSNER (1960) stage 31, complementing the brief description provided by INGER & KOTTELAT (1998), together with natural history notes of both developmental stages and adults. Finally, we

document for the first time the larval development in the genus *Amolops* based on the species *A. cremnobatus*.

Material and methods

Voucher specimens were collected by ANIKA DOGRA and CUONG THE PHAM by hand or with small landing nets from 20 May to 01 July 2012, between 7:00 and 10:00 h p.m., in the vicinity of Pu Hu Mountain in the Pu Hu Nature Reserve, Thanh Hoa Province, north-central Vietnam. Collected individuals were photographed alive with a digital single-lens reflex camera (Canon, EOS 600D, EF-S 15–55 mm), and subsequently anaesthetised and euthanised with gaseous ethylene acetate. Frogs were fixed in 40% ethanol for four to eight hours, labelled with field numbers, and subsequently preserved in 70% ethanol. Tadpoles were photographed alive (Canon, EOS 600D 18 mpix, EF100 mm f/2.8 macro USM) and preserved in vials containing 4% formalin solution.

All collected specimens were subsequently deposited in the collections of the Zoologisches Forschungsmuseum Alexander Koenig (ZFMK), Bonn, Germany, and the Institute of Ecology and Biological Resources (IEBR), Hanoi, Vietnam.

Identification of adults and tadpoles followed INGER & KOTTELAT (1998). Morphometric measurements of the preserved specimens were taken with digital callipers to the nearest 0.01 mm (BurgWächter, PS7215). Larval staging



Figure 1. Map showing the distribution of *Amolops cremnobatus* according to FROST (2013), including the type locality after INGER & KOTTELAT (1998) in Khammouan Province, Laos (marked with black dot) and our first record from Thanh Hoa Province (red dot).

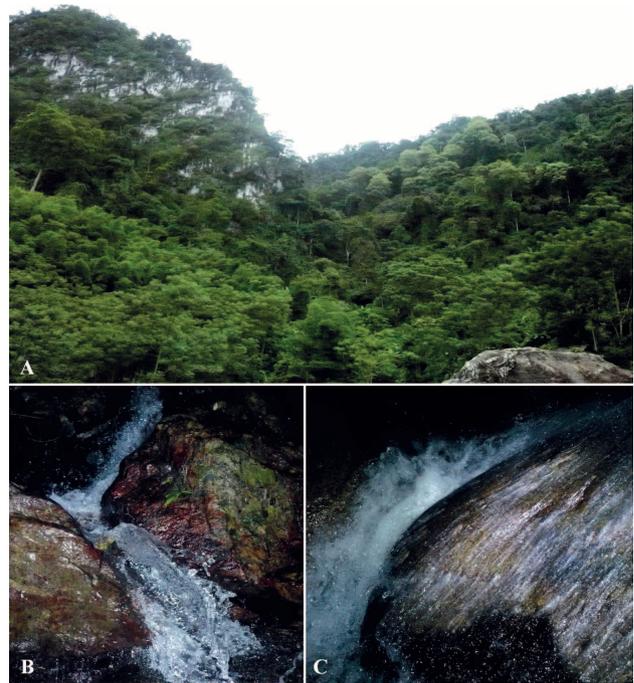


Figure 2. Natural habitat of *Amolops cremnobatus*. A) Limestone karst forest in the vicinity of En Village in the Pu Hu Nature Reserve; B+C) rocks in strong-current cascading streams, the typical microhabitat of *A. cremnobatus*.

was effected based on both collected preserved individuals and photographs taken in the habitat; developmental staging of larvae and terminology for exotrophic tadpoles follow GOSNER (1960), based on *Bufo* (now *Incilius*) *valliceps*, as presented in MCDIARMID & ALTIG (1999). Accordingly, eggs to stage 19 are specified as ‘embryos’; specimens at stages 20–25 are termed ‘hatchlings’; stages 31–40 are referred to as ‘larvae’; and froglets at stages 42 and 45 are classified as ‘metamorphs’. Our classification and specification of the larval labial teeth row formula follow MCDIARMID & ALTIG (1999).

Every voucher specimen in the collection was directly collected from its habitat during field surveys. Thus, certain developmental stages were not available for examination and are therefore not included in our description.

Abbreviations for adult morphological character states are as follows: SVL: Snout–vent length, measured from tip of snout to end of urostyle; ED: Maximum horizontal diameter of eye; SN: Snout–nostril distance, measured from tip of snout to anterior margin of nostril; SNL: Snout length, measured from tip of snout to anterior margin of eye; TYD: Horizontal tympanum diameter; TIB: Tibia length, measured from anterior circumference of the knee to the posterior circumference of the tibio-tarsal articulation; FD: first digit length (from tip to insertion); SD: second digit length (from tip to insertion); FDDW: first digit disc width; TTDW: third toe disc width.

Abbreviations of larval morphological character states are as follows: BL: maximum body length; BW: body width at the middle of the body; ED: horizontal eye diameter; RED: rostrum–eye distance, from tip of snout to anterior margin of eye; RND: rostrum–nostril distance, from tip of snout to nostril; TMW: width of caudal musculature at the base; BH: maximum body height; SS: snout–spiracle distance; TMH: height of caudal musculature at base; TAL: tail length; UF: maximum height of upper tail fin; MTH: maximum tail height; ODW: oral disc width; SDL: length of sucker disc from tip of snout to posterior edge of disc; IND: internarial distance, between centres of nares; IP: interpupilar distance, between pupil centres; TL: total length; LTRF: labial tooth row formula.

Abbreviations for water parameters are as follows: NO₃: Nitrate; NO₂: Nitrite; GH: general hardness; KH: carbonate hardness/ total alkalinity; pH: per hydrogen.

Collected adults: IEBR A.2013.106 ♂: Phu Son (20°28′715″ N, 104°55′748″ E), 298 m a.s.l., collected on 20 May 2012 at 7:04 h p.m.; IEBR A.2013.107 ♀: Phu Son (20°28′780″ N, 104°55′845″ E), 277 m a.s.l., collected on 22 May 2012 at 7:08 h p.m.; ZFMK 95588 ♂: En (20°33′307″ N, 104°58′269″ E), 425 m a.s.l., collected on 11 June 2012 at 7:25 h p.m.; ZFMK 95589 ♂, ZFMK 95590 ♀: Yen (20°20′378″ N; 104°52′551″ E), 388 m a.s.l., collected on 25 June 2012 at 9:14 h p.m.; ZFMK 95591 ♀, ZFMK 95592 ♀, ZFMK 95593 ♂: Yen (20°28′024″ N, 104°50′464″ E), 402 m a.s.l., collected on 28 June 2012 at 8:08 h p.m.

Collected larval series and subadults: ZFMK 95596 GOSNER stages 1–24: En (20°33′272″ N, 104°58′213″ E), 463 m a.s.l., collected on 11 June 2012 at 07:45 h p.m.; IEBR

A.2013.108 GOSNER stages 25–40: Yen (20°28′024″ N, 104°50′464″ E), 402 m a.s.l., collected on 28 June 2012 at 8:09 h p.m.; ZFMK 95594 and 95595 froglets at stage 42 and 45: Yen (20°28′352″ N, 104°51′641″ E), 526 m a.s.l., collected on 01 July 2012 at 9:46 h p.m.

Results

Adult morphology

The morphology of adults from the Pu Hu Nature Reserve, Thanh Hoa Province, north-central Vietnam, matched well the characters provided in the original description of *Amolops cremnobatus* by INGER & KOTTELAT (1998): A small-sized *Amolops* species; snout–vent length in males 29.85–33.42 mm (n = 3, mean = 31.85 mm), in females 36.06–40.06 mm (n = 5, mean = 37.68 mm); snout depressed and weakly projecting; canthus rostralis sharp; loreal region concave and vertical; nostril slightly closer to tip of snout than to eye (SN/SNL = 0.38); snout–nostril distance shorter than diameter of eye (ED/SN = 1.76); head very broad; body slender; vomerine teeth present in small oval groups between choanae; tympanum distinct; toes fully webbed to the wide discs; hind limbs long (TIB/SVL =



Figure 3. *Amolops cremnobatus* from the Pu Hu Nature Reserve. A) The male specimen IEBR A.2013.106 in dorsal view; B) the female specimen ZFMK 95592 and the male specimen ZFMK 95593 in axial amplexus.

0.63); first digit shorter than second ($FD/SD = 0.91$); fingers and toes with enlarged discs bearing circummarginal grooves; discs of toes smaller than discs of fingers ($FDDW/TDDW = 1.93$); disc of first finger wider than tympanum diameter ($FDDW/TYD = 1.74$); thin inner metatarsal tubercle, outer metatarsal tubercle only slightly visible or absent; tarsal gland distinct; dorsal skin coarse, with small, white, spinose tubercles; ventral skin smooth; dorsolateral fold very distinct; males with whitish, spiny, nuptial pad on dorsal and medial surface of pollex, gular pouches absent.

Colour in life: dorsum dark olive with green reticulated pattern (Fig. 3); dorsal surfaces of limbs with light green transverse bars; short greenish bars present along dorso-lateral fold; tubercles on limbs and flanks whitish; venter cream with some slightly greyish spots on throat.

Larval morphology

The description is based on a larva at stage 31 from the tadpole series IEBR A.2013.108 (Fig. 4). The tadpole belongs to the exotrophic, lotic, gastromyzophorus larval type after MCDIARMID & ALTIG (1999). It possesses the typical characteristics of a fast-water stream dweller: large abdominal sucker disc, thick tail musculature and reduced fins. The tadpole series was identified as representative of the species *Amolops cremnobatus* based on the following: 1) tadpoles were collected in the immediate vicinity of adult *A. cremnobatus*; 2) no other *Amolops* species was recorded from within the Pu Hu Nature Reserve; 3) larval morphology agrees with the tadpole description of *A. cremnobatus* provided by INGER & KOTTELAT (1998), and the larva possesses the distinctive characteristics of this species, in particular a divided upper jaw sheath and a high number of labial teeth rows: $10(5-10)/6(1)$.

Dorsal view: body shape oval and elongated ($BW/BL = 0.48$), snout long, broad, rounded ($RED/BL = 0.42$); eyes moderately large ($ED/BL = 0.12$), positioned dorsally and

directed laterally; dorsal musculature well developed; nares small, round, with a tiny dorsal protuberance, positioned dorsolaterally and directed laterally; nares much closer to eyes than to snout ($RND/RED = 0.73$); internarial distance smaller than interorbital distance ($IND/IP = 0.54$); longitudinal constriction lateral to dorsal muscle cords; caudal musculature of moderate strength at base ($TMW/BW = 0.56$); sinistral spiracle slightly visible in dorsal view.

Lateral view: body strongly flattened and depressed ($BH/BW = 0.62$), with a convex structure; maximum body height situated posterior to eyes; spiracle at second half of body ($SS/BL = 0.57$), below longitudinal axis, inner wall present as a slight ridge, not attached to body wall, opening round and in dorsoposterior direction; tail long and pointed ($TAL/BL = 1.81$, $TAL/TL = 0.64$); myosepts visible, especially in first third of the tail; caudal musculature well developed ($TMH/MTH = 0.8$); fins thin and slightly convex ($UF/MTH = 0.16$), almost parallel to caudal musculature; origin of lower fin distal to origin of upper fin; vent tube dextral, slightly visible in lateral view, opening round; foot paddles slightly visible; upper edge of oral apparatus slightly visible in lateral view.

Oral apparatus: oral disc ventrally positioned, as wide as the body ($ODW/BW = 1.04$, $ODW/BL = 0.5$); margins free, slightly emarginated on both ventrolateral sides; dorsal gap moderately wide; upper and lower labia with very short, rounded transparent papillae; submarginal papillae absent; black jaw sheaths strongly keratinised, both with serrations, upper jaw sheath M-shaped, divided; labial tooth row formula: $10(5-10)/6(1)$; first denticle row on margin of upper labium; adoral with a large abdominal sucker that almost reaches the base of caudal musculature ($SDL 0.69$ of BL).

Colour in life: dorsum and tail uniformly dark with numerous, shiny reddish brown to yellowish spots; fins transparent, dark, with some irregular lighter spots; venter and ventral part of tail greyish white, not pigmented; oral apparatus and sucker disc not pigmented.

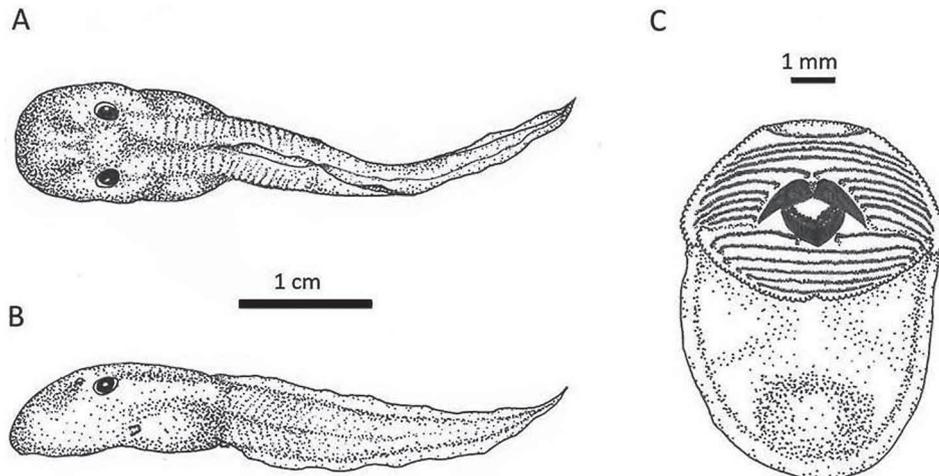


Figure 4. Drawing of the larva from the series IEBR A.2013.108 of *Amolops cremnobatus* from the Pu Hu Nature Reserve at developmental stage 31. A) Dorsal view; B) lateral view; C) oral apparatus (Drawings by A. DOGRA).

Larval staging

Eggs of the same clutch were at the same developmental stage. The separated ovum at stages 5–9 was non-pigmented, enclosed with a thick transparent layer, and about 3 mm in diameter (Fig. 5A). Another collected clutch was referable to stage 14 based on the distinctly developing neural fold (Fig. 5B). A large yellowish yolk reserve was visible ventrally to the embryo at stage 19. The characteristic larval shape about to form, with slightly differentiated body parts like a somewhat flattened tail with marginally fins discernible; small gill buds started to develop (Fig. 5C). It was only at stage 20 that an elongated tail with slightly visible myosepts was detected. External gills were distinct, and slightly bulging eyes were discernible but still non-pigmented (Fig. 5D). The opening of the mouth could be identified. At stages 21–22, the hatchlings were still surrounded by the thick jelly layer. The yolk reserve was still visible, but the hatchling had noticeably gained in body size and thus started to coil up inside the membrane. On the dorsum, two muscle cords appeared. Pigmentation of the eyes had started, and the cornea was distinct. The tail was distinctly

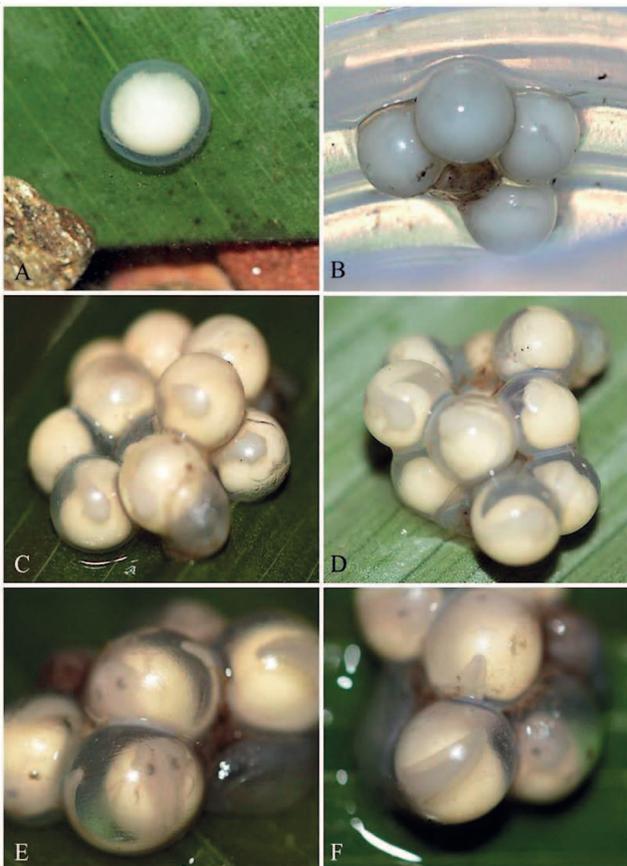


Figure 5. Embryos of *Amolops cremnobatus*. A) Isolated egg at stages 5–9; B) embryos at stage 14; C) embryos at stage 19; D) embryos in stage 20; E) embryos at stages 21–22; F) view of caudal region of an embryo at stages 21–22 with distinctly discernible, developed fins.

pointed, with distinctly transparent fins. Pigmentation of the body had begun, and the opening of the mouth was obvious (Figs 5E, F).

Transition to a mobile tadpole started at stage 24. Hatchlings were no longer enclosed by the jelly layer, but still stuck together in clusters on rock surfaces through the gelatinous, sticky layer, which made separating them during collecting very difficult. At this stage, the hatchlings had a length of about 9.47 mm. The yolk reserve had almost atrophied, the vent tube was discernible, and the nares were distinct and the operculum closed. Additionally, the oral apparatus had started forming, and the labia could be differentiated. At stage 25, the greyish body pig-

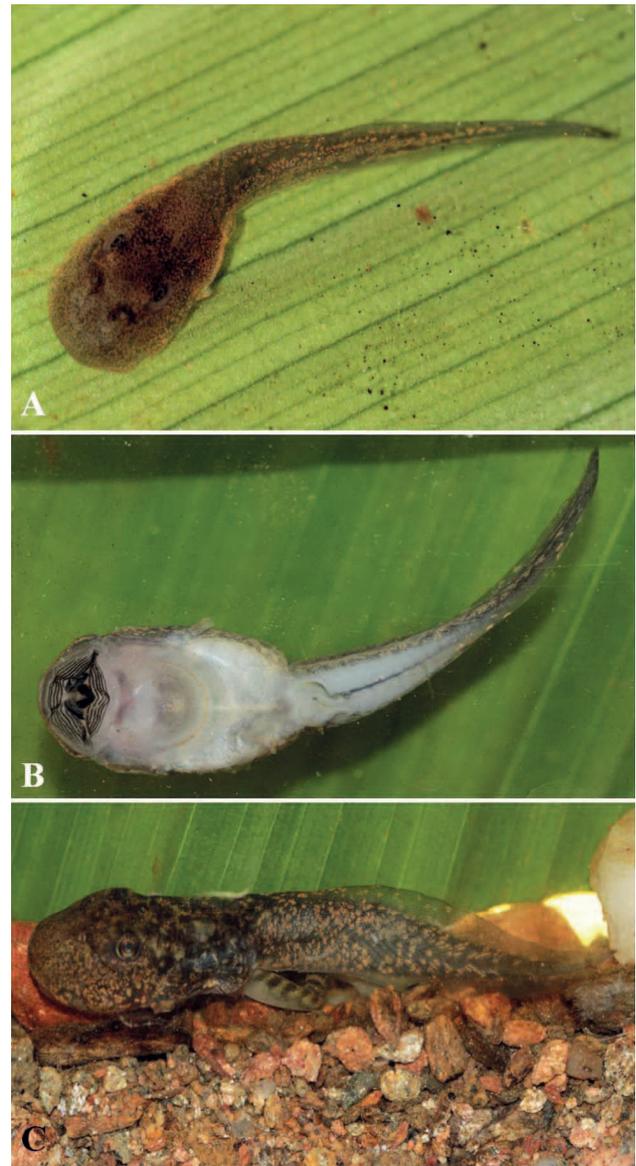


Figure 6. Hatchling and larvae of *Amolops cremnobatus*. A) Hatchling at stage 25 in dorsal view; B) larva at stage 34 in ventral view with well-developed oral disc and abdominal sucker; C) larva at stage 35 in dorsolateral view.

Table 1. Developmental stages of embryos and hatchlings of *Amolops cremnobatus* from the Pu Hu Nature Reserve, Thanh Hoa Province, Vietnam. TL – total length.

	Stage	Diagnostic feature	TL [mm]	Developmental stage
Embryos	5–9	irregular cleavage	–	morulation and blastulation
	14	neural fold	–	neurulation
	19	large yolk sac; gill buds; no eyes visible; tail flattened; fin only very slightly visible; body parts slightly differentiated	–	elongation
Hatchlings	20	tail elongated; eyes slightly bulging, but transparent; external gills; fins slightly visible; myosepts slightly visible; mouth opening discernible	–	
	21–22	body size increased; tail pointed; eyes slightly pigmented, cornea visible; fins more transparent; initiation of body pigmentation; mouth opening discernible	–	
	24	vent tube visible; yolk sac almost atrophied; head separated; myosepts visible; operculum closing; nares distinct; body pigmentation denser; oral apparatus develops; labia differentiated	9.47	transition to active, mobile tadpole
	25	intestine forming; vent tube distinct; body pigmentation dense; spiracle forming on the left; oral apparatus with sucker disc developed; yolk sac entirely atrophied; gelatinous layer completely vanished; body size increased; body depressed	12.24–25.40	

Table 2. Developmental stages of larvae and metamorphs of *Amolops cremnobatus* from the Pu Hu Nature Reserve, Thanh Hoa Province, Vietnam. TL – total length.

	Stage	Diagnostic features	TL [mm]	Developmental stage
Larvae	31	body flattened; foot paddle developed, some pigmentation on base	42.96	transition to active, mobile tadpole
	32	indentation between toes 5 and 4; pigmentation on limb basis increasing	43.74	
	34	indentation between toes 2 and 3; pigmentation on hind limbs extends to tibio-tarsal articulation	45.65	
	35	indentation between toes 1 and 2; pigmentation on toes	47.06	
	37	toes well developed, fully separated; tail atrophying; colouration on hind limbs discernible as barred pattern	43.38	
	40	foot tubercles present; inner metatarsal tubercle visible; typical colour pattern on hind limbs distinct; light spots at posterior corner of eyes marking beginning of dorso-lateral fold	38.94	
Metamorphs	42	fore limbs emerged; oral apparatus still distinct, but lower denticle rows atrophying; vent tube has vanished; froglet body shape; typical body colour pattern well developed; eyes bulging, laterally positioned	36.92	
	45	tail stub; mouth posterior to eye, but oral apparatus still atrophying; snout slightly pointed; lower eyelid distinct	23.13	

mentation densified. The sinistral spiracle and intestinal coils were visible. Furthermore, the oral apparatus with the abdominal sucker disc was distinct, and jaw sheaths as well as tooth rows were discernible.

Hatchlings with entirely atrophied yolk reserves and a dense brownish pigmentation all over the dorsal surface of body (Fig. 6A) were found congregating in strong-current cascades, adhering to the rock surface with their fully developed oral suckers. While the sticky layer had by now vanished from the hatchling body, hardened leftovers remained on the rock wall. The depressed tadpoles were very difficult to detach from the wet rock, because of their strong adherence by means of their abdominal sucker disc. Although these hatchlings had more than doubled their body

size to about 25.40 mm, we still classified them as hatchlings at stage 25, based on the missing limb buds, which denotes the beginning of the larval stage only at stage 26.

At stage 31, the larva had reached a total length of 42.96 mm. The whitish foot paddle began to develop and was already slightly pigmented at the basis. An indentation between toes 5 and 4 was discernible at stage 32, as well as increased pigmentation on the limb base. At stage 34, the larva reached a total length of 45.65 mm (Fig. 6B). Pigmentation now extended to the tibiotarsal articulation, and the indentation between toes 2 and 3 was visible. With a total length of 47.06 mm, a larva at stage 35 was the largest individual collected (Fig. 6C). Its pigmentation extended to the toes, and an indentation between toes 1 and 2 was rec-

ognizable. Furthermore, the brownish colouration of the body was intermixed with darker spots. The tail already atrophied at stage 37, thus the total length of the larva was reduced to 43.38 mm. At this stage, all toes were well developed and separated, and additionally, the colouration on the hind limbs was discernible as a barred pattern, as is typical for adult individuals. At stage 40, the foot tubercles were present, and the inner metatarsal tubercle was visible as well. The vent tube was only visible as remnants. The typical pattern of the hind limbs was distinct, and light spots at the posterior corner of the eyes marked the beginning of the dorsolateral fold. During the entire larval stadium, the oral disc with the sucker disc remained unaltered and functional. All observed larvae adhered with their abdominal sucker to wet rock surfaces.

Also in the metamorph at stage 42, the oral apparatus was still distinct, but the lower denticle rows started to atrophy. The fore limbs emerged, and the typical froglet body shape developed (TL 36.92 mm). The vent tube had

completely vanished. The iridescent pigmentation of the eyes was well developed. The eyes were bulging and shifted towards a more lateral position. Additionally, the typical body colour pattern of the adults was well developed, and light spots were identified along the dorsolateral fold (Fig. 7A, B). The metamorph at stage 45 is the most developed subadult individual in our collection. With a total length of 23.13 mm, it only had a tail stub left. Its mouth was already discernible and the snout slightly pointed, but the oral apparatus with the sucker disc was still distinct (Figs 7C, D). Furthermore, the lower eyelid was developed.

Natural history

Adults and larvae were found from 20 May through 01 July 2012, between 7:04 and 9:46 h p.m., at altitudes from 277 to 526 m a.s.l. Recorded temperatures were 25 to 27.6°C, and relative humidity ranged from 74 to 89%. Water parameters



Figure 7. Froglets (ZFMK 95594, 95595) of *Amolops cremnobatus* from the Pu Hu Nature Reserve. A+B) Metamorph at stage 42 in lateral and ventral views; C+D) metamorph at stage 45 in dorsolateral and ventrolateral views.

of the torrents were measured as NO_3^- : 0; NO_2^- : 0; GH: 4.2; KH: 4.5; pH: 6.

Frogs and froglets were observed perched at high densities on moist leaves of small bushes about 0.5–5 m above small, fast-flowing, cascading streams or torrents, or on wet rocks in strong-current streams (Fig. 8A). Frogs and froglets were only observed within approximately 2 m distance from cascading streams. At the end of June, some adults, e.g., the female ZFMK 95592 and the male ZFMK 95593 were observed in axillary amplexus (Fig. 8B), sitting amongst several egg clutches and larvae on the wet surface of a rock in a cascading stream. We did not notice any mat-



Figure 8. Breeding habitat of *Amolops cremnobatus* in the Pu Hu Nature Reserve. A) Adult individual in a torrent; B) mating pair in axillary amplexus on a wet rock surface next to a cascading stream.

ing calls, which could be due to the incessant noise from the strong-current stream, though.

Egg clutches, embryos, congregations of hatchlings and larvae were often found together at one breeding site (Fig. 9) between 11 and 28 June 2012. At the end of June, egg clutches, hatchlings, larvae, freshly metamorphosed individuals, and mating adults were observed on big rocks in a torrent with small cascades (Fig. 8B). Egg clutches were about 5–10 cm in diameter, consisting of about 10–30 eggs, encased with a thick, transparent, gelatinous layer, which also secured the clutch to the rock. Oviposition sites were always restricted to wet rock surfaces in cascading streams (Fig. 9A), where the egg clutches were constantly washed over with strong-current water. The tadpoles stuck together in groups on the washed-over rocks within cascading streams, adhering to the surfaces with their abdominal sucker discs. On 1 July 2012, metamorphs were observed among several adult mating pairs, as well as embryos, hatchlings, and larvae. They adhered with their abdominal suckers to a wet rock surface in a strong cascading torrent, scaling the rocks upstream through the cascades.

In addition to the mentioned breeding localities (in En and Yen villages) in the Pu Hu Nature Reserve, we identified five more sites with several mating pairs and larvae within the surrounding of Yen Village in late June and early July. All of the sighted embryos, hatchlings and tadpoles were restricted to wet rock surfaces in strong torrents. Their dark brownish pigmentation rendered the tadpoles perfectly camouflaged on the substrate (Fig. 10A). Remnants of the sticky gelatinous egg layer were discovered on the rocks in the cascading streams on several occasions (Fig. 10B).



Figure 9. Breeding habitat of *Amolops cremnobatus* in the Pu Hu Nature Reserve. A) Egg clutches deposited directly on a rock wall in a strong-current cascading stream; B) congregation of hatchlings at stage 24 on a rock in a torrent.

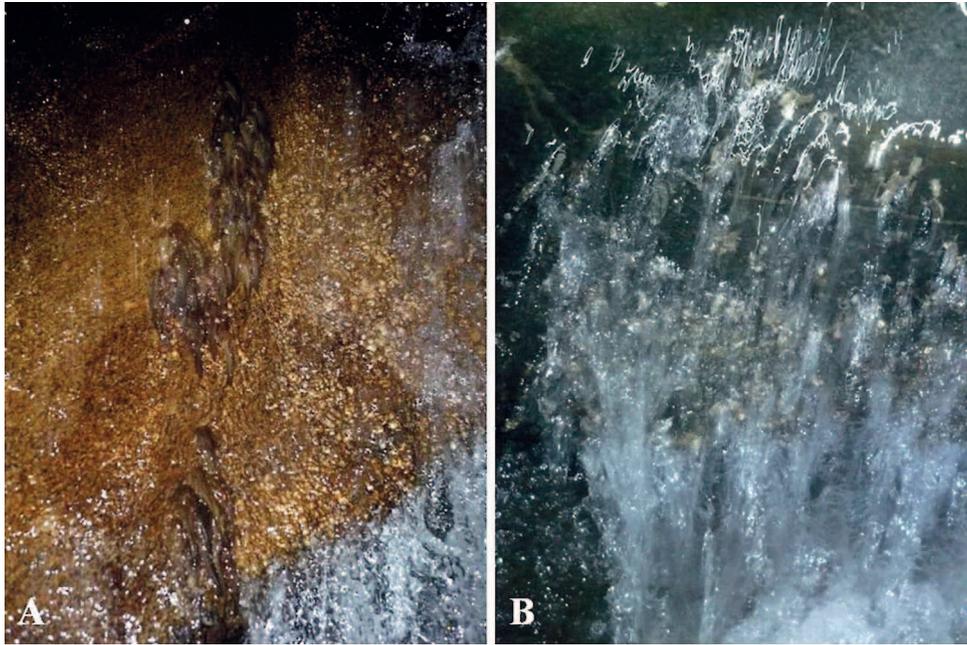


Figure 10. Breeding habitat of *Amolops cremnobatus* in the Pu Hu Nature Reserve. A) Congregation of hatchlings and larvae at stages 25 and 31–35 on a wet rock surface in a cascading stream; B) remnants of the sticky, gelatinous layer, which surrounded the larvae on a very strongly washed-over rock.

Discussion

Although our field research in the Pu Hu Nature Reserve began in May of 2012, when some adult *Amolops cremnobatus* could already be observed in the natural habitat, egg clutches and early developmental stages (larvae at stages 1–24) were not found before June of 2012. Larvae at more advanced stages and metamorphs (stages 25–45) were only found at the end of June and in early July. This may point to seasonally restricted mating and breeding activity of *A. cremnobatus*. After the end of the dry season, precipitation and temperatures begin to increase in the Pu Hu Nature Reserve around the middle of March. The average temperature during field excursions in July of 2012 was 28.1°C between 5:00 and 7:00 h p.m., which is approximately 2°C above that recorded during field excursions in May and June (26.4°C). Also, the measured average relative humidity was > 4% higher in July of 2012 (88.3% between 5:00 p.m. and 7:00 h p.m.) than in May and June of 2012 (83.8%). INGER & KOTTELAT (1998) found eggs and larvae of *A. cremnobatus* in Laos in the middle of March, when temperatures and precipitation likewise begin to increase in Khammouan Province.

The larval development largely corresponded to the staging system provided by GOSNER (1960). Nonetheless, we observed some specific deviations at some larval stages of *A. cremnobatus* compared to generalized tadpoles. Like in other tadpoles of torrent-dwelling genera, e.g., in *Ansonia* and *Meristogenys* (NODZENSKI & INGER 1990), the atrophication process of the oral apparatus is delayed and commences only at stage 42 (stage 41 in GOSNER's scheme).

The denticles on the lower labium gradually decrease in size, whereas most generalized larvae completely lose their beaks and denticle rows at this stage. During stages 42 to 45, the atrophication process of the oral apparatus proceeds only slowly in *A. cremnobatus*. At stage 45, the metamorphs have lost all denticles and jaw sheaths, but the characteristic abdominal sucker will still be present. As an adaptation to the gastromyzophorus mode of life, the larvae and metamorphs of *A. cremnobatus* cling with their abdominal sucker disc to rocks in the strong current of waterfalls. Hence, compared with generalized larvae, the oral disc, including the abdominal sucker, remains functional for longer in *A. cremnobatus*, similar to *Ansonia* and *Meristogenys* tadpoles (NODZENSKI & INGER, 1990). The reduced tailfins of larval *A. cremnobatus* are another characteristic adaptive feature of tadpoles adapted to living in strong currents. The ability to cling to heavily washed-over rocks even during the metamorphic climax and to only switch to a terrestrial habitat at post-metamorphic stage might offer a predation risk advantage (NODZENSKI & INGER 1990). The pigmentation of the tadpoles presents an optimal mimicry of the rock surfaces, which probably makes them difficult to detect for potential predators. With their dorsal colour pattern, the adults are well camouflaged in rocky environment interspersed with bushes and branches as well, and with their enlarged finger and toe discs, they are perfectly adapted to a life as torrent frogs, enabling them to securely climb on wet rock surfaces in cascading streams. Besides nuptial pads being present only in males (INGER & KOTTELAT 1998), we also observed a distinct sexual size dimorphism in *A. cremnobatus*.

With our first record of *A. cremnobatus* from the Pu Hu Nature Reserve, we could extend the known distribution from Khammouan Province in Laos, over the adjacent Vietnamese provinces of Quang Binh and Ha Tinh, as well as Nghe An, northwards to Thanh Hoa Province. Although the number of individuals sighted in the Pu Hu Nature Reserve was remarkably large, all observations were restricted to a certain microhabitat, namely fast-current rocky cascade forest streams. The species is apparently able to tolerate some degree of habitat disturbance (INGER & KOTTELAT 1998, STUART 1999). However, *A. cremnobatus* might be threatened by habitat degradation or even loss due to logging and infrastructure development, but is particularly sensitive to changes in water quality and flow. According to IUCN (2013), the total population of *A. cremnobatus* has a decreasing trend. Thus, although the species is sporadically abundant within its small range, its habitat requirements as stream-breeding species places the species, which is currently categorised as Near Threatened, close to qualifying for Vulnerable (IUCN 2013).

Declining populations of species with special microhabitat requirements, such as *A. cremnobatus*, primarily need improved in situ conservation (i.e., habitat protection measures). However, facing populations or even species in danger of extinction, conservation breeding programs can be an important supportive tool. During the Amphibian Ark Amphibian Conservation Needs Assessment for the Indochinese amphibian species in Hanoi in March of 2012, the species of the genus *Amolops* were, besides other torrent-dwelling anurans, recommended to be classified as husbandry-analogue (i.e., poorly or unknown genera in terms of captive husbandry/reproductive biology). With their special adaptations to a gastromyzophorus mode of life, *Amolops* representatives might be difficult to keep and propagate in captivity (compare GAWOR et al. 2012), however, which would be prerequisite to conservation breeding for endangered taxa if such measure should be recommended. Extensive outdoor terraria with emulated cascade habitats were set up in the Me Linh Station for Biodiversity in northern Vietnam in May of 2013 (ZIEGLER et al. 2013) for breeding and development research programmes for husbandry analogues. Hence, with our observations on mating, larval development, habitat requirements and breeding ecology in general, basic data for potential *Amolops* husbandry and *ex situ* propagation are here provided.

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References

- FROST, D. R. (2013): Amphibian Species of the World: an Online Reference. Version 5.6. – American Museum of Natural History, New York, USA, available at <http://research.amnh.org/herpetology/amphibia/index.html>, accessed 16 November 2013.
- GAWOR, A., A. RAUHAUS, D. KARBE, K. VAN DER STRAETEN, S. LÖTTERS & T. ZIEGLER (2012): Is there a chance for conservation breeding? Ex situ management, reproduction, and early life stages of the Harlequin toad *Atelopus flavescens* Duméril & Bibron, 1841 (Amphibia: Anura: Bufonidae). – *Amphibian and Reptile Conservation*, **5**: 29–44.
- GOSNER, K. L. (1960): A simplified table for staging anuran embryos and larvae with notes on identification. – *Herpetologica*, **16**: 183–190.
- INGER, R. F. & M. KOTTELAT (1998): A new species of ranid frog from Laos. – *Raffles Bulletin of Zoology*, **46**: 29–34.
- IUCN (2013). IUCN Red List of Threatened Species. Version 2013.2. – Available at www.iucnredlist.org, accessed 20 November 2013.
- MATSUI, M., SHIMADA, T., LIU, W. Z., MARYATI, M., KHONSUE, W. & N. ORLOV (2006): Phylogenetic relationships of Oriental torrent frogs in the genus *Amolops* and its allies (Amphibia, Anura, Ranidae). – *Molecular phylogenetics and evolution*, **38**: 659–666.
- MCDIARMID, R. W. & R. ALTIG (eds) (1999): Tadpoles: the biology of anuran larvae. – University of Chicago Press.
- NGUYEN, V. S., C. T. HO & T. Q. NGUYEN (2009): Herpetofauna of Vietnam. – Edition Chimaira, Frankfurt am Main, 768 pp.
- NODZENSKI, E. & R.F. INGER (1990): Uncoupling of related structural changes in metamorphosing torrent-dwelling tadpoles. – *Copeia*, 1047–1054.
- STUART, B. L. (1999): Amphibians and reptiles. – *Wildlife in Lao PDR*, 43–67.
- YANG, D. T. (1991): Phylogenetic systematics of the *Amolops* group of ranid frogs of southeastern Asia and the Greater Sunda Islands. – *Fieldiana. Zoology*, **63**: 1–42.
- ZIEGLER, T., A. RAUHAUS, D. KARBE, NGUYEN, T. Q., PHAM, C. T. & H. D. PHUONG (2013): New amphibian keeping and breeding facilities created at the Me Linh Station for Biodiversity, northern Vietnam. – *AArk Newsletter* **23**: 14–15.