The first record of *Amolops himalayanus* (Anura: Ranidae) from Bhutan.

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ABSTRACT - During a series of surveys carried out in areas of broadleaf forest in the eastern Himalayas in early spring, *Amolops himalayanus* was identified for the first time in Bhutan. Information is provided on the ecology, habitat, reproduction, and geographical distribution of the species together with notes on the water chemistry of the collection site. A brief synopsis is given of the morphological differences between *A. himalayanus* and *A. formosus*, with which latter species *A. himalayanus* is often confused. *A. himalayanus* has been reported from seven localities in northern India and Nepal but, of these, only the original description of the taxon from Darjeeling was based on incontrovertible data.

INTRODUCTION

T he 49 species of Cascade frogs belonging to the genus *Amolops* occur from Nepal and northern India to western and southern China and south to Malaysia (Frost, 2015). In Bhutan, *A. mantzorum* has been recorded at Choetenkora in Trashiyangtse District (Wangyal, 2013), *A. marmoratus* at Sershong in Sarpang District (Das & Palden, 2000), and *A.* cf. *monticola* at Ririchu in Wangdue Phodrang District (Wangyal & Gurung, 2012). Wangyal (2014) predicted the occurrence of *A. himalayanus* (and *A. formosus*) in Bhutan on the basis that the country was suitable for these two species both geographically and climatically.

During a series of amphibian surveys undertaken in Trashigang District in March and April 2015 by a team from the University of Bhutan's Sherubtse College, eight *A. himalayanus* (four males and four females) were identified in a stream near Khaling.

This is the first record of *A*. *himalayanus* from Bhutan and the only distinct, published collection of the taxon since its original description by Boulenger (1888).

SYSTEMATIC REVIEW

Amolops himalayanus (Boulenger, 1888)

Rana himalayana Boulenger, 1888: 507. "Darjeeling", India. Common names: Himalaya Cascade frog; Himalaya Sucker frog; Himalayan frog.

Variation

A. himalayanus was considered by Dubois (1974) to be a subspecies of *A. formosus* (Günther, 1876) but the taxon *himalayanus* was returned to specific level by Yang (1991: 12) based on diagnostic differences between the two forms (Frost, 2015).



Figure 1. Delineation of the seven terrestrial ecoregions occurring in Bhutan. The conservation status of the ecoregions is represented by the following abbreviations: RS/I – relatively stable/intact; V – vulnerable; CR/EN – critical/endangered (www. worldwildlife.org (2)). Localities: 1 – Jere Chhu; 2 – Khaling stream; 3 – Bodidrang stream.

MATERIALS AND METHODS

Study Area

Bhutan lies within the Himalaya Hotspot as defined by Conservation International (www.cepf.net) and Khaling is located in the Eastern Himalayan Broadleaf and Conifer Forests (Global 200 ecoregion no. 67) and, more precisely, in the Eastern Himalayan broadleaf forests (Fig. 1), which are outstanding globally for both species richness and endemism (www.worldwildlife.org (1)).

As a part of a Rufford Small Grant project, a team comprising five members surveyed Jere Chhu (*Chhu* = river, water), on 28 and 29 March, 5 April and 19 April 2015 from 7.00 pm to 10.00 pm. Jere Chhu is located on the outskirts of Khaling town in Trashigang District, eastern Bhutan (Fig. 1).

Further observations of individuals considered to be *A. himalayanus* (Fig. 2E and F) were made in Trashigang District at Bodidrang stream (27° 17′20.33′′ N, 91° 30′56.28′′E) on 19 July 2014, 28 February 2015 and 22 May 2015 and at Khaling stream (27° 11′26.18′′N, 91°36′09.40′′E) on 29 June 2015. Bodidrang stream lies 12.1 km north-west of Khaling town while Khaling stream, into which Jere Chhu flows, is located 1.7 km south of the town (Fig. 1).

Specimens and measurements

Eight individual *A. himalayanus* were collected and photographed *in situ* using a Nikon D5100 camera with an AF-S Micro Nikkor 40 mm lens.

External measurements of the eight individuals were taken to the nearest 0.01 mm using Vernier callipers. The measurements, which are listed in Table 1, are defined as follows: SVL- snout to vent length; HL-head length; HW – head width; and TL- tibia length. Sex was determined by size and the nuptial pad on first finger following Boulenger (1920).

Six of the specimens were released and two (one male and one female) were sacrificed humanely in accordance with the U.K. Government's Code of Practice issued under Section 21 of the Animals (Scientific Procedures) Act 1986 (www.gov.uk (1)). The two frogs were placed individually into secure plastic containers containing cotton wool impregnated with chloroform. Upon proof of permanent cessation of circulation, the specimens were preserved in 70% ethanol and deposited in the scientific collection at Sherubtse College Museum, Kanglung, Trashigang District, Bhutan. The two specimens were sacrificed specifically for the following reasons:

1. To permit the taxonomic identity of the species to be determined with certainty under laboratory conditions.

2. To provide material for the scientific reference collection at Sherubtse College, which the lead author is trying to establish as a centre of excellence for the study and conservation of amphibians in Bhutan (there is no such centre in Bhutan at present). This reference material is invaluable in enabling students to identify the species in the field and for comparative purposes during the course of research into the protection of the species, particularly with regard to differentiating *A. himalayanus* from similar taxa (e.g. *A. formosus*).

Six water samples were taken from Jere Chhu from 9.00 am to 10.00 am on 29 March at 100 m intervals between the collection site and Jigme Sherubling Higher Secondary School. Results were obtained using a G.R.E.E.N. (Global Rivers Environmental Education Network) water quality monitoring kit. Nitrate and phosphate levels and the pH balance of the water were measured as follows:

Nitrate test – 5 ml of stream water were transferred to a plastic bottle, in which one Nitrate Wide Range CTA TesTab was dissolved. After five minutes, the resulting water colour was compared with the G.R.E.E.N. nitrate colour chart supplied and the corresponding level (in parts per million (ppm)) was recorded.

Phosphate test – 10 ml of stream water were transferred to a plastic bottle, in which one Phosphate TesTab was

Table 1. Measurements (in mm) of A. himalayanus from JereChhu.

Field no.	Accession no.	SVL	HL	HW	TL	Sex
TND003	_	72.8	24.6	26.0	45.0	8
TND004	_	74.1	26.8	27.3	48.6	8
TND005	_	73.0	24.7	25.4	47.1	8
TND012	SC-AMP-012	76.7	24.0	25.0	46.8	8
TND001	_	83.4	28.3	28.6	51.0	4
TND002	_	80.5	26.4	26.8	46.0	4
TND008	-	89.0	27.0	27.6	52.7	9
TND011	SC-AMP-011	89.0	26.0	31.0	53.0	9

dissolved. After five minutes, the resulting water colour was compared with the G.R.E.E.N. phosphate colour chart supplied and the corresponding level (in ppm) was recorded.

pH test – 10 ml of stream water were transferred to a plastic bottle, in which one pH Wide Range TesTab was introduced. When the Testab had dissolved fully, the resulting water colour was compared with the G.R.E.E.N. pH colour chart supplied and the corresponding level recorded.

Air temperature was taken at 7.45 pm on 28 March and water temperature was taken at approximately 9 pm on 28 and 29 March 2015. Water and air temperatures were recorded in degrees Celsius (°C).

RESULTS

New material

1 $\stackrel{\circ}{\circ}$ (adult), SC-AMP-012; 1 $\stackrel{\circ}{\leftarrow}$ (adult), SC-AMP-011; Jere Chhu (27° 12'21.90" N, 91° 36'12.20" E), Khaling, Trashigang District, Bhutan, 2,073 m above mean sea level, 28 March 2015, collected by T. Nidup, N.T. Lepcha, Penjor, D. Gyeltshen, and S. Dorji.

Diagnosis and description

A. himalayanus can be identified by its large size and a combination of characters, the most significant of which are horny spicules on the side of the head and body and in a cluster at the base of the forelimbs; the lack of grooves on the first finger; dark banding on the limbs; and an olive skin colour with consistent brown patches on the dorsum. Measurements of females are greater than males (Table 1). The morphological characters of specimens from Jere Chhu agree in all respects with the description of the taxon given by Boulenger (1888) with the exception of the SVL values of SC-AMP-011 and TND008 (Table 1), which are 89.0 mm. Boulenger (1888) gives a simple SVL value of 80.0 mm for the four specimens he examined, a figure he revised upwards to 83.0 mm subsequently (Boulenger, 1920: 220). The difference of 6.0 mm between Boulenger's maximum SVL value and that of SC-AMP-011 and TND008 could be explained by shrinkage of the Darjeeling specimens during their (presumably lengthy) retention in preserving fluid between collection and examination.

In his study of the phylogenetic systematics of the Amolops Group of ranid frogs of south-eastern Asia



Figure 2. A – Jere Chhu showing discarded plastic containers. **B** – Amplectant *A. himalayanus* in Jere Chhu. Scale = 50 mm. **C** – Male *A. himalayanus* next to an egg clutch beneath a rock in Jere Chhu. Scale = 50 mm. **D** – Egg clutch of *A. himalayanus*. Scale = 25 mm. **E** – *A.* cf. *himalayanus* observed at Bodidrang stream. Scale = 50 mm. **F** – Juvenile *A.* cf. *himalayanus* observed at Khaling stream. Scale = 50 mm.

and the Greater Sunda Islands, Yang (1991) gives a maximum length of 88.0 mm. The author does not refer to individual specimens but does list those institutions whose collection of specimens he examined, which includes the British Museum of Natural History (now the Natural History Museum (N.H.M.)), where the type series of *A. himalayanus* is held (Boulenger, 1890). It would not seem possible for Yang to state a measurement of 88.0 mm were that measurement not of Boulenger's holotype or one of the type series. Our own measurement in 2015 of the SVL of the single female *himalayanus* in the collections of the N.H.M. was 78.75 mm, a value that corresponds, conversely, more with the SVL measurements given in Boulenger (1888).

Ecology and habitat

At Jere Chhu, *A. himalayanus* was recorded sitting on rocks, at the water's margin, and in the stream's free-flowing waters. The stream was heavily polluted, the same caused principally by the discharge of effluents and waste water directly into the watercourse from the kitchens of Jigme Sherubling Higher Secondary School. Tangible pollutants comprised plastics, papers, and cloths (Fig 2A). Measurements taken at the stream indicated a mean water temperature of 16 °C, an air temperature of 18.9 °C, a nitrate level of 5 ppm, a phosphate level of 4 ppm, and a pH value of 8.

At the collection site, the stream is bordered on either side by cultivated fields supporting maize and potato crops. Other than algae, watercress (*Nasturtium officinale*) was the sole aquatic plant noted in the stream, itself. Floral species in the surrounding area included the Himalayan Coral tree (*Erythrina arborescens*), the Yellow Himalayan raspberry (*Rubus ellipticus*), and the herb, Indian madder (*Rubia manjith*).

Individuals considered to be A. himalayanus were

found in intact forest at both Bodidrang stream and Khaling stream. At both locations they were observed above ground sitting on the stems and leaves of the invasive plant species, *Eupatorium adenophorum* (Fig. 2 E,F).

Reproduction

Between ten and fifteen *A. himalayanus* were found in Jere Chhu on each of the three study visits. Amplectant individuals were recorded on 5 April (Fig. 2B). A single *A. himalayanus* that was consistent in size with a male was observed beneath a rock in the middle of the stream next to an egg clutch (Fig. 2C), which appeared to be attached to the underside of the rock. The upper section of the clutch was above the water line while the lower section was either resting on the water's surface or was partially submerged (Fig. 2D). The flow of the freshwater stream was constant. The diameter of individual eggs within the clutch was estimated to be 5 mm or less.

Tadpoles and juvenile *A. himalayanus* also were present. At Bodidrang stream, tadpoles were evident on 22 May and a single tadpole was collected by hand and released back into the stream after its dorsolateral and ventral surfaces had been photographed (Fig. 3A,B). Clearly visible in Fig. 3B is the ventral sucker, which is present in the tadpoles of very few amphibian genera but which is a principal character of the genus *Amolops* (see Pham et al. 2015).

On 19 April, no mating couples were found and no egg clutches were apparent. Individuals were observed sitting on rocks and at the water's edge.

DISCUSSION

Outside Bhutan, *A. himalayanus* is known currently only from Indian records (Fig. 4), which comprise the type series of four specimens collected between 1872 and 1887 at Darjeeling in West Bengal (Boulenger, 1888), two of

which were referred initially by Boulenger (1882: 70) to *Rana formosa* (Günther, 1876) [= *A. formosus*] (see Boulenger, 1888: 508); unverifiable documentation of the species' presence in four districts of Himachal Pradesh (Mehta, 2005); a field observation in northern Sikkim in 2012 (Barkha, 2015); and an entry on the collections database of the Field Museum of Natural History recording a single preserved specimen, also from Sikkim. A report of the species' occurrence in Nepal (Kripalani, 1961) is likely to be based on a tadpole of another *Amolops* species (see Schleich et al., 2002; Frost, 2015).

Mehta (2005) reports the presence of *A. himalayanus* in the districts of Kangra, Kullu, Shimla, and Sirmour in the Indian state of Himachal Pradesh but does not give verifiable details (either of individual specimens or of localities). The need to substantiate Mehta's locality records of the species was identified by Frost (2015). In addition, the identification key appearing in Mehta (2005: 270-272) would seem to be of questionable accuracy as some of the characters of specimens Mehta regards as *A. himalayanus* disagree with the description of the taxon by Boulenger (1888, 1920), for example the relationship of head width to head length, relative finger length, and the degree of prominence both of the tympanum and of subarticular and inner metatarsal tubercles.

A solitary photograph of a ranid frog that, visually, compares favourably with *A. himalayanus* was posted on the India Biodiversity Portal website by Barkha (2015). The image was taken in May 2012 at approximately 27° 49'25"N, 88° 33'23"E, a position north of Chungthang in the Indian state of Sikkim. No measurements, description, or scale accompanied the photograph and the taxonomic identity of the frog cannot be verified.

The Field Museum of Natural History in Chicago records a single specimen of *A. himalayanus* (FMNH 15806) on its collections database (www.fieldmuseum. org). The record of the preserved specimen, which was collected by Herbert Stevens in 1931, is not accompanied by any data other than the collection locality of "Sikkim, India".

Kripalani (1961) reported the occurrence of a tadpole of *A. himalayanus* (as *Staurois himalayana*) from Chagna in Nepal, the same having been collected during the course of the Indian Cho-Oyu Expedition in 1958. Schleich et al. (2002: 80) doubted this record as the tadpole was found in the same habitat as an adult *A. marmoratus* while Frost (2015) states that the tadpole is "likely assignable to *A. formosus.*"

Kripalani refers to the Nepalese locality as Chagna and, elsewhere in the paper, as Changa. In a list of the collecting stations visited in Nepal by the 1958 Indian Cho-Oyu Expediton, Datta (1961) refers to Chagma. The true locality is most probably Chyangma, which lies at the approximate co-ordinates of the collecting station (27° 40'N, 86° 18'E) given by Datta (1961).

The collection of *A. himalayanus* in Bhutan is the only published record based on actual material since the original description of the taxon by Boulenger in 1888 although the species may have been collected unwittingly during the period it was deemed synonymous with *A. formosus*. The



Fig. 3 Dorsolateral (A) and ventral (B) views of a tadpole of *A. himalayanus* collected from Bodidrang stream. The ventral sucker (arrowed), is a principal character of *Amolops* tadpoles. Scale = 10 mm.



Fig. 4 Map showing the recorded distribution of *A. himalayanus*. The identification of individuals from the localities marked with a black circle is not supported by substantive data. The Indian state of Himachal Pradesh is represented by the shaded area. Localities: 1 – Kangra District; 2 – Kullu District; 3 – Shimla District; 4 – Sirmaur District; 5 – Chyangma; 6 – Darjeeling; 7 – Chungthang; 8 – Bodidrang stream; 9 – Jere Chhu; 10 – Khaling stream. BANG. = Bangladesh.

record of *A. himalayanus* from Khaling extends the taxon's range 330 km to the east. The distance stated, however, warrants a *caveat* insofar as it is not ascertainable whether the original collection locality of "Darjeeling" refers to the town or the district of that name.

The accession of *A. himalayanus* to Bhutan's known fauna increases the number of confirmed amphibian species in the country to 40, a figure that includes one salamander (*Tylototriton verrucosus*) and one caecilian (*Ichthyophis sikkimensis*) (Wangyal, 2014).

Bordoloi et al. (2004), who consider *A. himalayanus* and *A. formosus* to be conspecific (see Appendix I for the principal morphological differences between the two taxa), highlight the lack of information available on egg deposition and larval ecology. Accordingly, it is of note that breeding pairs and an egg clutch together with juveniles and tadpoles were observed in Jere Chhu on 5 April. By 19 April, no mating couples were seen and egg clutches were no longer evident. It would appear likely, therefore, that breeding occurs in late March or early April.

Measured against The World Health Organisation's maximum recommended nitrate level (in drinking water) of 50 ppm (www.who.int (a)), the 5 ppm concentration of nitrate in Jere Chhu is unremarkable as is the pH value of 8. which is within the same Organisation's suggested optimum range of 6.5-9.5 (www.who.int (b)). The phosphate level of 4 ppm in Jere Chhu is, however, of some concern as the reading is 40 times higher than the annual mean phosphate concentration in rivers of 0.1 mg per litre (0.1 ppm) recommended by the U.K. Government (www.gov.uk (2)). Phosphate pollution is caused principally by human activity, often through the treatment of crops with fertiliser (which may run off into adjacent watercourses) or by the discharge of effluent or detergents (White & Hammond, 2006). It is not known whether phosphate-based fertilisers are applied to the agricultural land bordering Jere Chhu but it is unlikely owing to the remoteness of the location. the cost of the chemicals, and the low income of the rural farming communities. A more probable cause of the high phosphate reading is the direct discharge into the stream of effluent, detergents, and other pollutants from Jigme Sherubling Higher Secondary School. The high levels of both tangible and chemical pollution in Jere Chhu seemed not to have a detrimental effect on the frogs' occupation of the locality.

The occurrence of *A. himalayanus* in freshwater habitats at three sites within an area of approximately 6.3 km^2 in Trashigang District points to the species being at least locally common. Extrapolation of these locality data suggests that *A. himalayanus* would be encountered reasonably often in similar habitats at an elevation of approximately 2,000 m (the elevation of Darjeeling is 2,042 m) (www.darjeeling.gov.in) both in Bhutan and in the wider Himalayan region.

It is recommended that further field research be undertaken in Bhutan, particularly in areas of Critical/ Endangered Himalayan subtropical broadleaf forest, in order to augment existing knowledge of the composition, distribution, and conservation requirements of the country's amphibian fauna.

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Appendix I. The principal morphological differences between *A. himalayanus* (Boulenger, 1888) and *A. formosus* (Günther, 1875).

A. himalayanus	A. formosus	Authority	
Tibia as long as the trunk	Tibia considerably shorter than the trunk	Boulenger (1888)	
Tibio-tarsal articulation of the hind limb reaches beyond the tip of the snout	Tibio-tarsal articulation of the hind limb reaches the nostril or the tip of the snout	Boulenger (1888, 1920)	
Web of toes feebly notched	Web of toes deeply notched	Boulenger (1920)	
Nostril nearer to eye than to tip of snout	Nostril midway between eye and tip of snout	Yang (1991)	
Tympanum indistinct	Tympanum distinct	Yang (1991)	
Small granules on side of body and temples; belly and posterior half of lower surface of thighs granulate	Belly granulate	Boulenger (1920)	
	Granules on side of head absent	Yang (1991)	
Spinules limited to side of the head and base of the forelimb		Yang (1991)	
Olive or greyish above with indistinct darker spots on the body	Bright green above with sharply defined black or blackish spots on the head and body	Boulenger (1920)	
Hinder side of thighs purplish brown	Hinder side of thighs marbled with black	Boulenger (1920)	
Lower parts brownish or pale olive	Lower parts and web between toes brown or marbled brown and whitish	Boulenger (1920)	
Ethmoid rounded in front	Ethmoid obtusely pointed in front	Boulenger (1920)	
Snout to vent length (males, n. 2): 74-75 mm	Snout to vent length (males, n. 1): 53 mm	Boulenger (1920), Yang (1991)	
Snout to vent length (males): 76 mm maximum		Yang (1991)	
Snout to vent length (females, n. 2): 80-83 mm	Snout to vent length (females, n. 6): 53-75 mm	Boulenger (1920), Yang (1991)	
Snout to vent length (females): 88 mm maximum		Yang (1991)	
Head length (males, n. 2): 24-24mm	Head length (males, n. 1): 18 mm	Boulenger (1920)	
Head length (females, n. 2): 27-27 mm	Head length (females, n. 6): 19-23 mm	Boulenger (1920)	
Head width (males, n. 2): 25-27 mm	Head width (males, n. 1): 18 mm	Boulenger (1920)	
Head width (females, n. 2): 28-29 mm	Head width (females, n. 6): 20-24 mm	Boulenger (1920)	
Tibia length (males, n. 2): 47-49 mm	Tibia length (males, n. 1): 32 mm	Boulenger (1920)	
Tibia length (females, n. 2): 50-52 mm	Tibia length (females, n. 6): 32-41 mm	Boulenger (1920)	

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