

# First record of *Amolops beibengensis* Jiang et al., 2020 from India, along with a detailed morphological description

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The genus *Amolops*, Cope, 1865 often referred to as Cascade frogs or Torrent frogs, is one of the oldest frog lineages (Pyron and Wiens, 2011; Chan and Brown, 2017; Wu et al., 2020) and a speciose genus of the family Ranidae, with 80 recognised species (AmphibiaWeb, 2023; Frost, 2023). The genus is restricted to Asia where it has a wide geographical range extending from north and northeast India, Nepal, Bhutan, Myanmar, western, southern, and eastern China, Laos, Vietnam, Thailand, down into Peninsular Malaysia (Ao et al., 2003; Nidup et al., 2016; Khatiwada et al., 2020; Gan et al., 2020; AmphibiaWeb, 2023; Frost, 2023). Although it has been a recognised ranid genus for 158 years, almost two-thirds of its species have only recently been discovered (Frost, 2023). Meanwhile, current research suggests that there still are many potential undescribed species (Stuart et al., 2010; Wu et al., 2020; Mahony et al., 2023).

Within *Amolops*, eight species groups have been delimited largely on the basis of available phylogenetic evidence: *A. daiyunensis* group, *A. hainanensis* group, *A. laurentis* group, *A. mantzorum* group, *A. marmoratus* group, *A. monticola* group, *A. ricketti* group, and *A. viridimaculatus* group, as well as an unassigned taxon *A. spinapectoralis*, recognised by Wu et al. (2020). The *marmoratus*, *monticola* and *viridimaculatus* groups occur in the southern Himalayas where they are broadly sympatric (Wu et al., 2020). Each of these species' groups contains cryptic species making in-field identification challenging. Several species are still poorly defined morphologically, and the recent use of DNA sequence data as the major means of species delimitation (Che

et al., 2020; Wu et al., 2020) has made comparisons with older, morphology-based literature records more difficult. Nonetheless, there has been an upsurge of new species descriptions from the Himalayan region in recent years (Zhao et al., 2005; Qi et al., 2019; Che et al., 2020; Khatiwada et al., 2020; Patel et al., 2021; Mahony et al., 2022; Saikia et al., 2022a, 2022b, 2023).

In the present study, we report on the populations of the *A. viridimaculatus* group sampled during herpetological surveys in the northeast Indian state of Arunachal Pradesh. The aim of this paper is to formally clarify the generic taxonomy of collected samples of the *A. viridimaculatus* species group and compare it to all similar congeners within the currently recognised *Amolops viridimaculatus* species complex (Wu et al., 2020; Mahony et al., 2022).

We carried out field surveys in the Dibang Valley district of Arunachal Pradesh, India in August of 2022. Nocturnal visual encounter surveys were carried out to locate the amphibians aided with torch light between 18:00–23:00 hrs. Collected specimens were euthanised using MS222 (Tricaine Methanesulfonate), fixed in 4% formalin, washed in water and stored in 70% ethanol. Prior to fixation, the frogs were photographed, muscle tissue was collected and kept in molecular grade ethanol for DNA extraction. Collected specimens were deposited in Wildlife Institute of India, Dehradun.

Genomic DNA was extracted from the collected tissue samples of the *Amolops* populations using Qiagen DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA, USA) following the manufacturer's protocol. Partial fragment (~570 base pairs) of the 16S rRNA was amplified and sequenced using previously published primers in Simon et al. (1994). Polymerase chain reaction (PCR) conditions were as follows: initial denaturation at 95°C for 3 minutes, followed by 39 cycles of denaturation at 94°C for 45 seconds, annealing at 52°C for 45 seconds, and extension at 72°C for 2 minutes. Final extension was at 72°C for 10 minutes. Amplified PCR products were run on a 2% agarose gel and viewed under UV transilluminator. Purified PCR product was sequenced

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directly in an Applied Biosystems Genetic Analyzer 3500 XL in both directions using BigDye v3.1.

The taxon sampling procedure for the phylogenetic analyses involved the retrieval of homologous sequences of the 16S mitochondrial gene from GenBank (Benson et al., 2007), representing 66 *Amolops* species along with one outgroup, following Mahony et al. (2022). We also added our newly generated DNA sequences from Arunachal Pradesh, India resulting in a dataset of 71 taxa (Appendix 1). All sequences were aligned using ClustalW (Thompson et al., 1994) with default settings in MEGA v10 (Kumar et al., 2018). Maximum Likelihood (ML) phylogenetic analysis was performed on the aligned dataset using the IQ-TREE online portal (Minh et al., 2020). The JModel Test v2.1.6 was used to select the best-fit model for nucleotide sequence substitution. Support for internal branches was quantified using the bootstrap approximation (UFB 1000 pseudo replicates) (Minh et al., 2013). The uncorrected pairwise distance (P-distance) was computed using MEGA v10 (Kumar et al., 2018) with pairwise deletion of missing data and gaps.

Morphometric measurements of the collected specimens were taken using a vernier calliper (Mitutoyo) to the nearest value 0.1 mm. All measurements were taken on the right side of the specimens. Morphometric abbreviations used in the text and tables are as followed by (Mahony et al., 2022): snout to vent length, from snout tip to cloacal opening (SVL); maximum head width, measured at posterior angle of jaws (HW); head length, measured from retroarticular process of mandible to snout tip (HL); snout depth, measured at anterior border of orbit (SD); snout length, measured from snout tip to anterior bony orbital border (SL); snout to nostril, distance from centre of nostril to snout tip (SN); orbit to nostril, distance from anterior bony orbital border to centre of nostril (EN); minimum distance between nostrils (IN); eye length, horizontal distance between anterior and posterior bony orbital borders (EL); inter upper eyelid width, shortest distance between upper eyelids (IUE); maximum upper eyelid width (UEW); internal front of eyes, distance between anterior (/inner) canthi (IFE); internal back of eyes, shortest distance between posterior (/outer) canthi (IBE); maximum tympanum diameter (TD); tympanum to eye, distance from anterior border of tympanum to posterior bony orbital border (TE); forearm length, from elbow to proximal border of inner metacarpal tubercle (FAL); hand length, from proximal border of inner metacarpal tubercle to tip of third digit (HAL); first finger length,

from tip of first digit to its base where it joins second digit (FIL); second finger length, from tip of second digit to its base where it joins first digit (FIIL); third finger length, from tip of third digit to its base where it joins second digit (FIIL); fourth finger length, from tip of fourth digit to its base where it joins third digit (FIVL); minimum third finger width, taken at approximately half distance between distal subarticular tubercle and base of disc (FIIW); maximum disc widths of fingers I–IV (FIDW, FIIDW, FIIDW, FIVDW); fourth toe width, taken dorsally on digit proximal to disc (TIVW); maximum disc widths of toes I–V (TIDW, TIIDW, TIIDW, TIVDW, TVDW); thigh length, from centre of cloacal opening to knee taken when femur is flexed at right angle to body (TL); shank (containing tibia) length, from knee to tibio-tarsal articulation taken when leg is held in naturally folded position (SHL); maximum width of shank (SHW), tarsus and foot length, from tibiotarsal articulation to tip of fourth digit (TFL); foot length, from proximal edge of inner metatarsal tubercle to tip of fourth digit (FOL); maximum length of inner metatarsal tubercle (IMT). Digits are numbered from preaxial (inner-FI/TI) to postaxial (outer-FIV/TV) side. Webbing formula between toes follows Savage and Heyer (1997).

## Results and Discussion

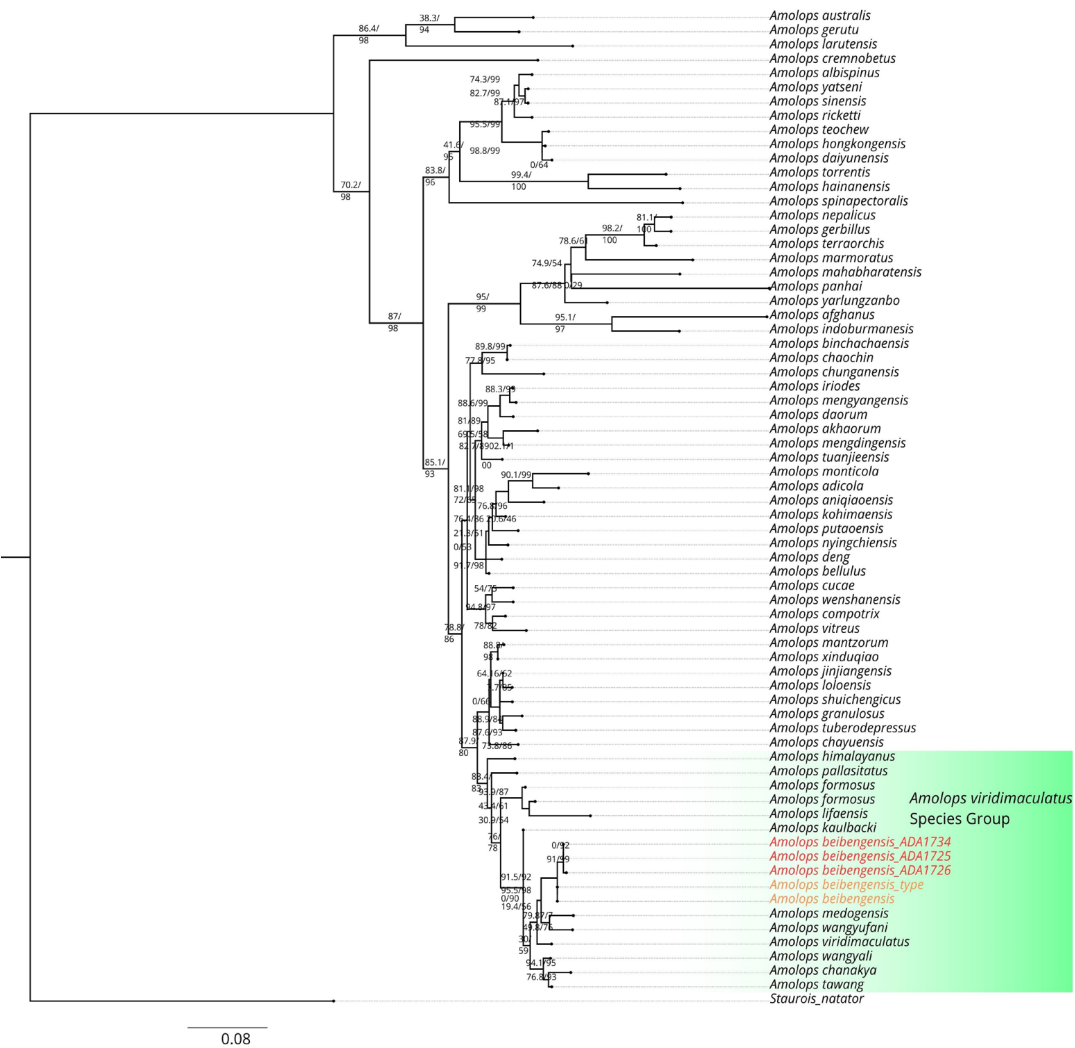
Based on our phylogenetic analysis, the newly collected *Amolops* samples from Arunachal Pradesh clustered with *Amolops beibengensis* Jiang et al., 2020 from Mêdog, China. Together these populations from Arunachal Pradesh and Mêdog County are placed as a sister taxon to *A. medogensis* Li and Rao, 2005 and *A. wagyufani* Jiang, 2020. The P-distance between the newly collected populations and Mêdog County was only 0.5%. The lowest interspecific P-distance for 16S between our samples and congeneric species of the *A. viridimaculatus* group varied between 2.6% to 6.2%. (i.e., 0.5% from *A. beibengensis* type specimen, 6.2% from *A. himalayanus* (Boulenger, 1888), 2.6% from *A. viridimaculatus* (Jiang, 1983) 4.1% from *A. medogensis*, 4.4% from *A. wangyali* Mahony et al., 2022, 3.4% from *A. wangyufani*, 4.1% from *A. tawang* Saikia et al., 2022, and 4.6% from *A. chanakya* Saikia et al., 2022). Tree topology of the present study shows a similar species group structure to Wu et al. (2020). The Indian lineage of the *Amolops viridimaculatus* group comprises of *A. formosus* (Günther, 1876), *A. himalayanus*, *A. chanakya* and *A. tawang* along with new addition of *A. beibengensis* (Fig. 1). Based on our

phylogenetic analysis and morphological examination of the collected specimens of *Amolops* from Arunachal Pradesh, we confirm the identity of these specimens as *A. beibengensis*. An expanded description of *A. beibengensis* based on the collected specimens in this study is provided herein.

**Material examined.** Arunachal Pradesh, Dibang Valley district: WII-ADA1720, collected from Mawa nullah (28.8316°N, 95.8879°E), Anini to Mippi road (~4 km northwest from Anini) on 15 August 2022; WII-ADA1725-1726, collected at Dri River bank

(28.8087°N, 95.9312°E), near Etabe village (~2.5 km northeast from Anini) on 16 August 2022; Lower Dibang Valley district: WII-ADA1734, collected at bank of Tangon river near Punli village (28.6327°N, 95.9430°E) (~8 km east from Etalin) on 21 August 2022.

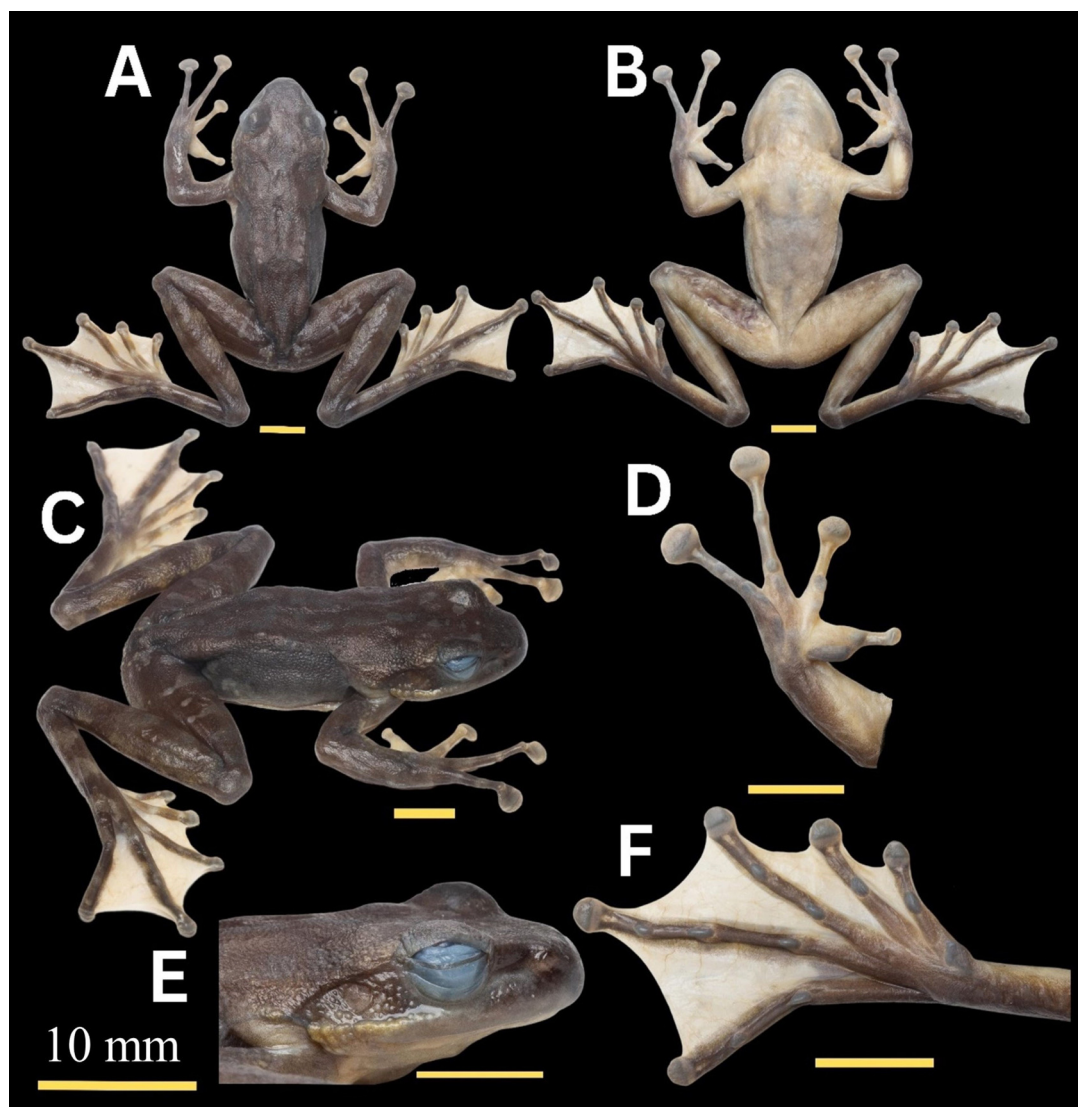
Morphological description of *Amolops beibengensis* based on specimens collected in this study: three adult males (WIIADA1720, WIIADA1734, WIIADA1725) and one adult female specimen (WIIADA1726). All measurements are reported in millimetre (mm). Robust bodies for males (SVL 53.5–67.5) and female (SVL



**Figure 1.** Maximum Likelihood phylogeny of selected species of the genus *Amolops* based on mitochondrial 16s gene partial sequences produced by 1000 bootstraps with the TIM2+G+R model for sequence substitution. Single branch tests (SH-aLRT) before the slash and Ultrafast bootstrap values (UFBoot) after the slash are represented by the numbers above the nodes; UFBoot values of 95 and SH-aLRT values of 80 are regarded as strong support, respectively.

91.8) (Figs. 2A–B); head dorsally sub elliptical (Fig. 2E), marginally longer than wide (HW 17.3–24.2, HL 19.3–24.9, HW:HL 0.8–0.9), flat above; snout rounded and slightly protruding in profile (Fig. 2E), its length (SL 8.4–10.9 in males; 13.7 in female), longer than the horizontal diameter of the eye (EL 5.7–7.0 in males; 8.2 in female); canthus rostralis rounded, loreal region strongly concave, obtuse (Fig. 2E; interorbital space flat, interorbital distance (IUE 5.6–7.8 in males; 11.5 in female) greater than width of the eye lids (UEW 4.4–5.7 in males; 6.9 in female), and narrower than the internarial

distance (IN 7.1–8.9 in males; 11.6 in female); nostrils laterally positioned, vertically ovular with slightly raised rim anteriorly, slightly closer to the eye (EN 3.7–4.6 in males; 5.7 in female) than to the snout (SN 4.3–6.2 in males; 7.8 in female); pupil horizontal; tympanum fully exposed (TD 2.3–2.4 in males; 4.8), horizontally oval in shape, tympanum–eye distance (TE 2.6–3.8 in males; 5.4 in female); pineal ocellus visible; vomerine ridge distinct, obtuse, positioned level to choanae which are oval and transverse.



**Figure 2.** An Adult male of *A. beibengensis* in preservation (Scale 10 mm): (A) dorsal view; (B) ventral view; (C) lateral view of head; (D) ventral view of hand; (E) Lateral view of head; (F) ventral view of foot. Photos by Naitik G. Patel.



Forelimbs moderately long and thick, forearms enlarged; relative length of fingers I<II<IV<III (FIL 7.8–10.0 in males; 12.0 in female, FIIL 8.9–11.4 in males; 16.0 in female, FIIL 14.4–18.8 in males; 23.5 in female, FIVL 10.4–12.5 in males; 17.1 in female); finger tips on II–IV dilated with wide oval disks (Fig. 2D), largest on digit IV (FIVDW 3.3–4.8 in males and 6.2 in female, FWIII 1.0–1.5 in males and 2.1 in female), finger I with distinct disk but only slightly dilated in relation to adjoining finger width, relative width of finger disks I<II<III<IV, circummarginal grooves present on digits II–IV only; terminal phalange shape unknown; fingers without distinct lateral fringes, webbing on fingers absent; subarticular tubercles prominently domed, circular; prepollex distinct, thenar tubercle elliptical, outer metacarpal tubercle not distinct; supernumerary tubercle on the base of II–IV fingers barely distinguishable (Fig. 2C).

Hindlimbs long, shank (SHL 33.1–41.5 in males; 52.6 in female) longer than thigh (TL 29.2–37.5 in males; 49.7 in female) and shorter than foot (FOL 29.8–38.6 in males; 52.4 in female); toes long and thin, relative lengths I<II<III<V<IV; tips of all toes expanded with transversely oval disks, smaller than those of the fingers (THIDW 2.6–3.6 in males; 4.8 in female), relative width of disks I<5<4<2<3, all with circummarginal grooves; toes completely webbed (Fig. 2F); post axial groove on toe V extends from tip of toe to the basal subarticular tubercle; subarticular tubercles all present, prominently domed and circular, inner metatarsal tubercle prominent, oval and relatively long (IMT 2.9–4.0 in males; 6.2 in female); tarsal glandular ridge, outer metatarsal and supernumerary tubercles all absent (Fig. 2F).

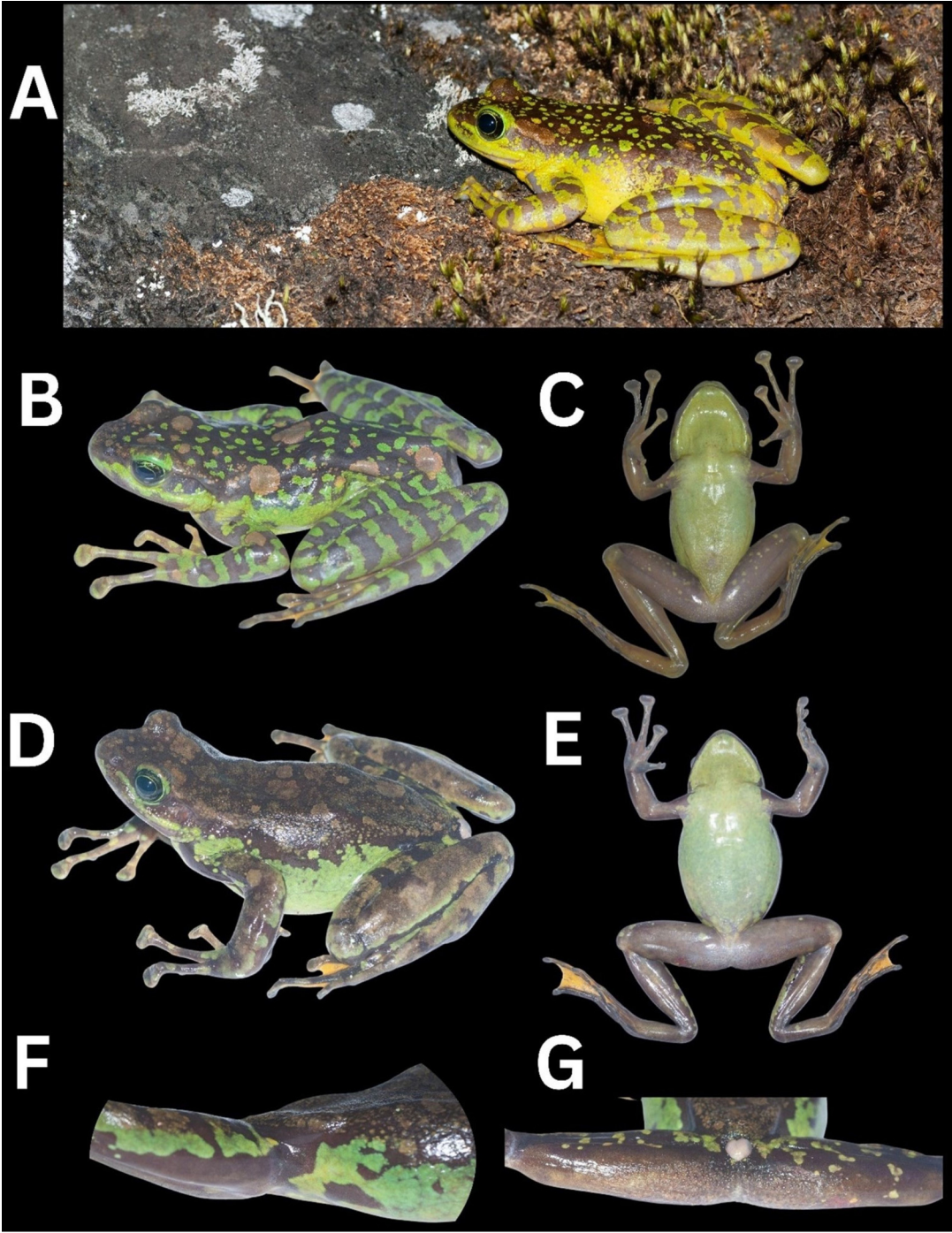
Skin on dorsal surface of head, flanks, throat, forelimbs and hindlimbs smooth; skin of dorsal surface of the body and ventral surface of the body smooth; area surrounding vent and adjoining posterior and ventral surface of the thighs granular, dorsal tubercles or pustular warts absent with exception of the temporal region posterior to the eye below the supratympanic fold, which is covered with enlarged glandular granules and warts; dorsolateral fold absent; supratympanic fold present but weakly developed; co-ossified skin absent; rectal and humeral glands absent (Fig. 2C); dorsal and ventral asperities and/or horny spinules absent.

**Colouration in life.** Dorsum of head and body dark greyish brown anteriorly, with speckles and blotches of light golden brown and yellow-green, remaining dorsum of the dark greyish brown; large brown irregularly shaped blotches on dorsum of body; lateral surfaces of

head and snout dark brown with a broken irregular stripe yellow green mottling around upper lip; pupil with near continuous pale metallic green border, remaining iris mottled metallic green and chocolate brown, more green than brown on dorsal third and ventral most portions of iris; supratympanic fold with golden brown speckles. The dorsolateral region of the flank is dark brown with continuation of the dorsal pattern with irregular shaped green and golden brown blotches. Yellow green blotches are densely present in dorsal and dorso-lateral region compared with golden brown blotches. Golden brown blotches are large in size compared to yellow-green blotches; gular region, chest and abdomen pale olive green in colour anteriorly; dorsum of hindlimbs dark brown with contrasting yellow green transverse to oblique crossbands. In the tibia region the green bands are bordered with light golden specks; dorsum of the forelimbs dark brown with contrasting yellow green transverse to oblique crossbands. One or two golden brown blotches are present on the dorsum of the forelimb; dorsum of fingers (including expanded discs) dark brown with irregularly arranged yellow green speckles; ventral surface of thighs brownish grey with irregularly arranged yellow spots and speckling, the ventral surface of tibia, tarsus and the forelimb is pinkish brown; nuptial pads dark grey ventrally (Fig. 3).

**Colouration in preservation.** Dorsum of head and body primarily dark chocolate brown with irregular shaped grey and cream coloured blotches; lateral surfaces of head dark brown with granules around the angle of the jaw are yellowish cream in colour; flanks dark grey with yellowish granules; dorsum of forelimb dark grey brown banded with light brown; dorsum of hindlimbs dark grey brown banded with light brown transverse or obliquely transverse stripes and speckles; granules in the vent region are yellowish in colour; dorsum of hands and feet grey brown with pale greyish cream speckles; inner/ posterior surface of thighs dark brown with irregular small yellowish-cream spots and speckles; ventral surfaces of head, body and limbs primarily light grey without any spots or speckles; ventral surfaces of hands and feet with tubercles and discs carbon grey; webbing between toes greyish white (Fig. 2).

Currently 18 species of *Amolops* are recorded from India, representing three species groups (Saikia et al., 2023). *Amolops adicola* Patel et al., 2021, *A. monticola* (Anderson, 1871), *A. aniqueanesis* Dong et al., 2005, *A. kohimaensis* Biju et al., 2010 and *A. chakrataensis* Ray, 1992 represent the *Amolops monticola* species



**Figure 3.** *Amolops beibengensis* in life: (A) an adult female in natural habitat; (B) dorsolateral view of an adult male; (C) Ventral view of an adult male; (D) dorsolateral view of an adult female; (E) Ventral view of an adult female; (F) Groin region of the adult female; (G) Posterior view of high. Photos by Bitupan Boruah.

complex (Andersson 1871; Chanda 1987; Ray 1992; Ray 1999; Biju et al., 2010; Patel et al., 2021; Saikia 2023; AmphibiaWeb 2023; Frost 2023). The, *A. cf. marmoratus* (Blyth, 1855), *A. gerbillus* (Annandale, 1912), *A. indoburmenus* Dever et al., 2012, *A. jaunsari* Ray, 1992, *A. assamensis* Sengupta et al., 2008, *A. siju* Saikia et al., 2023, and *A. terraochris* Saikia et al., 2022 belong to the *A. marmoratus* species group (Annadale, 1912; Ray, 1991; Ray, 1999; Sengupta et al., 2008; Sakia et al., 2023). *A. beibengensis*, *A. nidorbellus* Biju et al., 2010, *A. wangyali*, *A. formosus*, *A. himalayanus*, and the recently described *A. chanakya* and *A. tawang* that represent the *Amolops viridimaculatus* species group (Günther, 1876; Boulenger, 1888; Ray, 1999; Biju et al., 2010; Mahony et al., 2022; Saikia et al., 2023).

Within the *Amolops viridimaculatus* group, *A. beibengensis* differs from *A. formosus* by having a smooth dorsolateral vs. presence of broken irregular dorsolateral fold; from *A. himalayanus* by having dorsum large brown irregularly shaped blotches with yellow green blotches are densely present on greyish brown or yellow green dorsum vs. dorsal pattern with irregular large sized light brown blotches on yellow green (Nidup et al., 2016); from *A. wangyali*, *A. chanakya* and *A. tawang* by having a smooth dorsolateral skin vs. granular dorsolateral skin (Mahony et al., 2022; Saikia et al., 2023); from *A. pallasitatus* Qi et al., 2019 by a larger adult female size, female SVL 91.82 mm (vs. female SVL 70.6–72.3,  $n = 2$ ; Qi et al., 2019), smaller TD/ED 58% (vs. TD/EL 40–42%,  $n = 2$ ; Qi et al., 2019); from *A. nidorbellus* by having large brown irregularly shaped blotches with yellow green blotches are densely present on the dorsum vs. chocolate brown with small iridescent green rosette shaped spots ( $n = 6$ ; Biju et al., 2010); from *A. viridimaculatus* by having smooth flank vs. weakly granular flank (Orlov and Ho, 2007; Rao and Wilkinson, 2007); from *A. viridimaculatus* by having large brown irregularly shaped blotches with yellow green blotches are densely present on the dorsum vs. reddish-brown/dark purple/black/ black brown with very small to moderately large yellow, yellowish green or iridescent green smooth edged rounded spots (Orlov and Ho, 2007; Rao and Wilkinson, 2007); from *A. viridimaculatus* by having supratympanic fold present vs supratympanic fold weakly developed (Orlov and Ho, 2007; Rao and Wilkinson, 2007); from *A. longimanus* (Andersson, 1939) by having large brown irregularly shaped blotches with yellow green blotches are densely present on the dorsum vs. primarily dark brown with green reticulations (Andersson, 1939); from

*A. medogensis* by having a larger SVL, 72.4–82.5 mm vs 90.2–93.2 mm (female) and smaller TFL 0.83–0.86 vs. 0.77–0.79 (TFL/SVL ratio); from *A. senchalensis* Chanda, 1987 “1986” by a larger SVL (46.2 mm vs. 53.5–67.5 mm), the inner metatarsal tubercle IMT is bigger in size 0.049 vs. 0.055–0.059 (IMT/SVL ratio), and the snout to nostril distance of *A. beibengensis* is larger than *A. senchalensis* 0.060 vs. 0.080–0.092 (SN/SVL), and lastly the disc width of the forth finger of *A. beibengensis* is larger in size compare to *A. senchalensis* 0.047 vs 0.072–0.073 (FIVDW/SVL) (Mahony et al., 2022).

The previous taxonomic confusion within *Amolops* systematics arose largely due to the absence of genetic data and superficial morphological similarities, resulting in poorly understood cryptic diversity. In the last decade, more than 50% of the *Amolops* species have been described, which has helped in revealing the underestimated cryptic diversity within *Amolops* (Frost, 2023). The current availability of DNA data for 66 of the recognised species (80%), aside from numerous yet unnamed populations reported across the range, is evidence of extensive taxonomic and phylogenetic investigations on the genus *Amolops* (Cai et al., 2007; Stuart et al., 2010; Lu et al., 2014; Jiang et al., 2016; Qi et al., 2019; Gan et al., 2020; Khatiwada et al., 2020; Wu et al., 2020; Zeng et al., 2020; Mahony et al., 2023). However, it is equally important to provide detailed morphological descriptions to understand the morphological similarities within each species group. In the case of *A. beibengensis*, the original description by Che et al. (2020) is based on a single male holotype and two female paratypes and does not provide details regarding the morphology of the species, which is crucial information for cryptic species groups such as *Amolops* (Table 1). The present study provides more information regarding the morphology of this species and its distribution range (Table 1; Fig. 4). The aerial distance between the new distribution record and the type locality of *A. beibengensis* is 82–100 km (Fig. 4). The interspecific P-distance for 16S between the newly described *A. tawang* and *A. wangyali* is 1.3%, which is equal to the interspecies P-distance of 1.4% between the type of *A. formosus* and *A. formosus* from Nepal, suggesting that these species require further taxonomic investigation. *Amolops nidorbelus* and *A. senchalensis* lack molecular evidence to support their phylogenetic position within the *Amolops viridimaculatus* group. It is challenging to resolve the systematics within the *Amolops* species groups as the majority shares

**Table 1.** Specimen morphometrics for *Amolops beibengensis* from present study along with morphometric data from the holotype of *Amolops beibengensis* (Che et al., 2020).

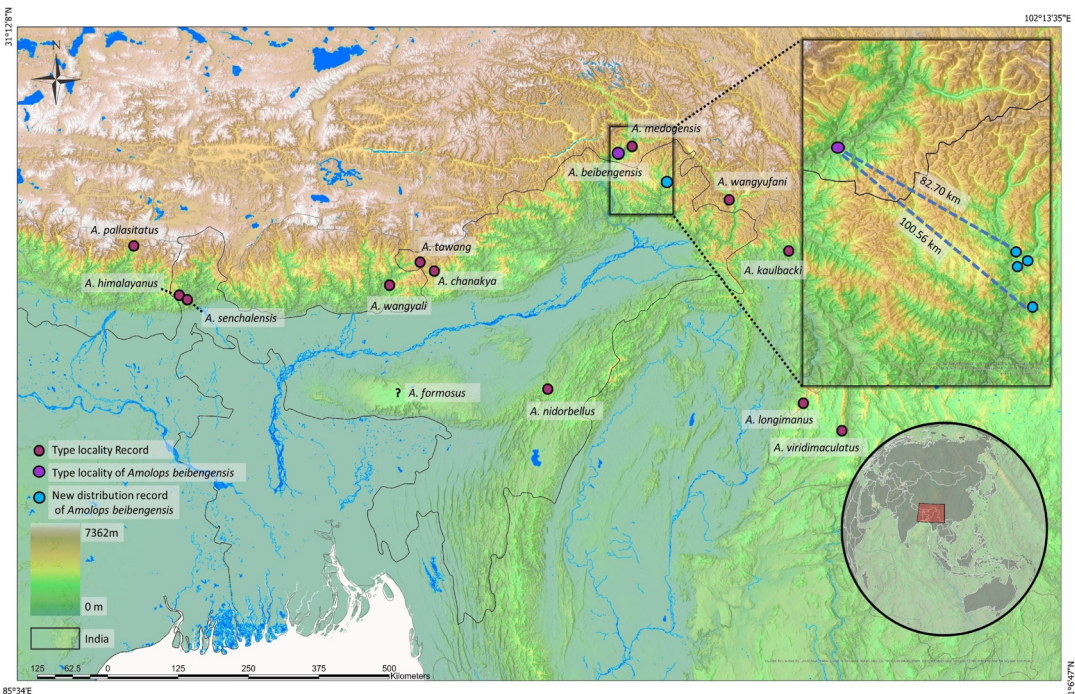
Morphometric variables	WIIADA1720	WIIADA1734	WIIADA1725	KIZ011015 Holotype (Che et al., 2020)	Range	WIIADA1726	KIZ016396 Paratype (Che et al., 2020)	KIZ016397 Paratype (Che et al., 2020)	Range
Sex	M	M	M	Male	M	F	F	F	F
SVL	53.5	61.1	67.5	75.8	53.5–75.8	91.8	90.2	93.2	90.2–93.2
HL	19.3	22.4	24.9	26.5	19.3–26.5	31.6	32	33.2	31.6–33.2
TE	2.6	3.3	3.81	—	2.6–3.8	5.4	—	—	—
SL	8.4	9.1	10.9	9.9	8.4–10.9	13.7	13.2	13.7	13.2–13.7
EN	3.7	4.2	4.6	—	3.7–4.6	5.7	—	—	—
IMT	2.9	3.5	4	—	2.9–4.0	6.2	—	—	—
HW	17.3	21.1	24.2	26.5	17.3–26.5	30.6	32.8	34.1	30.6–34.1
SD	5	5.4	6.3	—	5.0–6.3	7.5	—	—	—
IFE	10.3	12	12.7	—	10.3–12.7	17.4	—	—	—
IBE	14.8	17.1	18.4	—	14.8–18.4	23.2	—	—	—
EL	5.7	6.1	7	10.6	5.7–10.6	8.2	10	11.3	8.2–11.3
TD	2.3	2.4	2.4	2.9	2.3–2.9	4.8	2	3.1	2–4.8
SN	4.3	4.9	6.2	—	4.3–6.2	7.8	—	—	—
IN	7.1	8.1	8.9	8.9	7.1–8.9	11.6	10.5	10.7	10.5–11.6
IUE	5.6	5.9	7.8	6.3	5.6–7.8	11.5	7.4	9	7.4–11.5
UEW	4.4	5	5.7	6.3	4.4–6.3	6.9	7.4	8.6	6.94–8.6
HAL	19.3	21.74	25.8	26.8	19.3–26.8	32.8	32.8	33	32.8–33
TL	29.2	33.36	37.5	35.7	29.2–37.5	49.7	47.2	48.9	47.2–49.7
SHL	33.1	37	41.5	42.9	33.1–42.9	52.6	50.3	51	50.3–52.6
SHW	5.6	7.8	7.3	—	5.6–7.8	12.3	—	—	—
TFL	46	53.7	57.7	58.6	46.0–58.6	78.1	70	74.1	70–78.1
FOL	29.8	34.7	38.6	40	29.8–40	52.4	46.8	51.4	46.8–52.4
FIL	7.8	8.8	10	—	7.8–10.0	12	—	—	—
FIIL	8.9	9.7	11.4	—	8.9–11.4	16.1	—	—	—
FIHIL	14.4	15.2	18.8	—	14.4–18.8	23.5	—	—	—
FIVL	10.4	10.4	12.5	—	10.4–12.5	17.1	—	—	—
FIDW	1.7	1.7	2.2	—	1.7–2.2	3.6	—	—	—
FIIDW	3.2	3.2	4.3	—	3.2–4.3	5.5	—	—	—
FIIIDW	3.8	4	4.6	—	3.8–4.6	6	—	—	—
FIVDW	3.9	4.4	4.8	—	3.9–4.8	6.2	—	—	—
FIIIW	1.2	1	1.5	—	1.0–1.5	2.1	—	—	—
TIDW	2.4	2.2	2.9	—	2.2–2.9	4.5	—	—	—
TIIDW	2.6	2.9	3.5	—	2.6–3.5	4.8	—	—	—
TIIIDW	2.6	3	3.6	—	2.6–3.6	4.8	—	—	—
TIVDW	2.6	2.7	3	—	2.6–3.0	4.5	—	—	—
TVDW	2.2	2.5	2.7	—	2.2–2.7	4	—	—	—

geographical distributions across political boundaries of multiple countries, which makes the collection of the specimens difficult and requires collaboration. In the present time it is important to generate molecular data from the type specimen or topotypic material in order to resolve the cryptic species diversity. The recovery of new *Amolops* species and reports of new country records in recent years from northeast India indicates the need to carry out extensive surveys to understand *Amolops* species richness. Each species group has high diversity and requires intensive sampling across altitudinal and latitudinal gradients across Himalayas. This is the only geographic distribution identified using genetics and morphologically other than its type locality from Mèdog, China (Che et al., 2020). Furthermore,

recent discoveries and new distribution records from Arunachal Pradesh along with our study indicate the need for further herpetological exploration within this region.

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**Figure 4.** The type localities of all known members of the *Amolops viridimaculatus* species group along with a new distribution record of *A. beibengensis* from Arunachal Pradesh, India.

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# References

AmphibiaWeb (2023): Amphibiaweb: information on amphibian biology and conservation. Berkeley: University of California. Accessed 2023 Apr 30.

Anderson, J. (1871): A list of the reptilian accession to the Indian Museum, Calcutta from 1865 to 1870, with a description of some new species. *Journal of the Asiatic Society of Bengal* **40**: 12–39.

Andersson, L.G. (1939): Batrachians from Burma collected by Dr. R. Malaise, and from Bolivia and Ecuador collected by Dr. C. Hammarlund. *Arkiv för Zoologi, Stockholm* **30** (23 “1938”): 1–24.

Annandale, N. (1912): Zoological results of the Abor Expedition, 1911–1912. I. Amphibia. *Records of the Indian Museum* **8**: 7–36.

AO, J.M., Bordoloi S., Ohler A. (2003): Amphibian fauna of Nagaland with nineteen new records from the state including five new records for India. *Zoos’ Print Journal* **18**: 1117–1125.

Biju, S.D., Mahony, S., Kamei, R.G. (2010): Description of two new species of torrent frog, *Amolops* Cope (Anura: Ranidae) from a degrading forest in the northeast Indian state of Nagaland. *Zootaxa* **2408**: 31–46.

Benson, D.A., Karsch-Mizrachi, I., Lipman, D.J., Ostell, J.,

Wheeler, D.L. (2007): GenBank. *Nucleic Acids Research* **35** (Supplement1): D21–D25.

Boulenger, G.A. (1888): Descriptions of two new Indian species of *Rana*. *Annals and Magazine of Natural History Series* **6**(2): 506–508.

Cai, H.X., Che, J., Pang, J.F., Zhao, E.M., Zhang, Y.P. (2007): Paraphyly of Chinese *Amolops* (Anura, Ranidae) and phylogenetic position of the rare Chinese frog, *Amolops tormotus*. *Zootaxa* **1531**: 49–55.

Chan, K.O., Brown, R.M. (2017): Did true frogs “dispersify”? *Biology Letters* **13**: 20170299.

Chanda, S.K. (1987): On a collection of anuran amphibians from Darjeeling and Sikkim Himalayas, with description of a new species of *Rana* (Ranidae). *Journal of Bengal Natural History Society* **5**(2 “1986”): 140–155.

Chanda, S.K. (2002): Hand book - Indian amphibians. Kolkata: Zoological Survey of India, 335 p.

Chanda, S.K., Deuti, K. (1998): Endemic amphibians of India. *Records of the Zoological Survey of India* **96**(1–4 “1997”): 63–79.

Chanda, S.K., Das, I., Dubois, A. (2001): Catalogue of amphibian types in the collection of Zoological Survey of India. *Hamadryad* **25**(2 “2000”): 100–128.

Che, J., Jiang, K., Yan, F., Zhang, Y. (2020): *Amphibians and Reptiles in Tibet—Diversity and Evolution*. 803 p. Beijing: Chinese Academy of Sciences. Science Press.

Frost, D.R. (2021): *Amphibian Species of the World: an online*

- reference. Version 6. New York: American Museum of Natural History. Downloaded on 16 September 2021.
- Gan, Y.-L., Yu, G.-H., Wu, Z.-J. (2020): A new species of the genus *Amolops* (Anura: Ranidae) from Yunnan, China. *Zoological Research* **41**(2): 188–193.
- Gan, Y.-L., Qin, T., Lwin, Y.H., Li, G.-G., Quan, R.-C., Liu, S., et al. (2020): A new species of *Amolops* (Anura: Ranidae) from northern Myanmar. *Zoological Research* **4**(6): 1–7.
- Günther, A.C.L.G. (1876): Third report on collections of Indian reptiles obtained by the British Museum. *Proceedings of the Zoological Society of London* **1875**: 567–577.
- Jiang K., Wang K., Yan F., Xie, J., Zou, D.H., Liu, W.L., et al. (2016): A new species of the genus *Amolops* (Amphibia: Ranidae) from southeastern Tibet, China. *Zoological Research* **37**: 31–40.
- Khatiwada, J.R., Shu, G., Wang, B., Zhao, T., Xie, F. Jiang, J. (2020): Description of a new species of *Amolops* Cope, 1865 (Amphibia: Ranidae) from Nepal and nomenclatural validation of *Amolops nepalicus* Yang, 1991. *Asian Herpetological Research* **11**(2): 71–94.
- Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K. (2018): MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution* **35**: 1547–1549.
- Lu, B., Bi, K., Fu, J. (2014): A phylogeographic evaluation of the *Amolops mantzorum* species group: cryptic species and plateau uplift. *Molecular Phylogenetics and Evolution* **73**: 40–52.
- Mahony, S., Nidup, T., Streicher, J.W., Teeling, E.C., Kamei, R.G. (2022): A review of torrent frogs (*Amolops*: Ranidae) from Bhutan, the description of a new species, and reassessment of the taxonomic validity of some *A. viridimaculatus* group species aided by archival DNA sequences of century-old type specimens. *Herpetological Journal* **32**: 142–175.
- Minh, B., Schmidt, H., Chernomor, O., Schrempf, D., Woodhams, M., von Haeseler, A., et al. (2020): IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era. *Molecular Biology and Evolution* **37**: 1530–1534.
- Nidup, T., Gyeltshen, D., Penjor, Dorji, S. Pearce, M.J. (2016): The first record of *Amolops himalayanus* (Anura: Ranidae) from Bhutan. *Herpetological Bulletin* **136**(2016): 13–18.
- Patel, N.G., Das, A. (2020): Shot the spots: A reliable field method for individual identification of *Amolops formosus* (Anura, Ranidae). *Herpetozoa* **33**: 7–15.
- Patel, N.G., Garg, S., Das, A., Stuart B.L., Biju, S.D. (2021): Phylogenetic position of the poorly known montane cascadefrog *Amolops monticola* (Ranidae) and description of a new closely related species from Northeast India. *Journal of Natural History* **55**: 1403–1440.
- Qi, S., Zhou, Z., Lyu, Z., Lu, Y., Wan, H., Hou, M., et al. (2019): Description of a new species of *Amolops* (Anura: Ranidae) from Tibet, China. *Asian Herpetological Research* **10**(4): 219–229.
- Pyron, R.A., Wiens, J.J. (2011): A large-scale phylogeny of Amphibia including over 2800 species, and a revised classification of extant frogs, salamanders, and caecilians. *Molecular Biology and Evolution* **61**: 543–583.
- Rambaut, A. (2009): FigTree version 1.3.1. Downloaded in January 2012.
- Rao, D.Q., Wilkinson, J.A. (2007): A new species of *Amolops* (Anura: Ranidae) from southwest China. *Copeia* **2007**(4): 913–919.
- Ray, P. (1992): Two new hill-stream frogs of the genus *Amolops* Cope (Amphibia: Anura: Ranidae) from Uttar Pradesh (India). *Indian Journal of Forestry* **15**: 346–350.
- Ray, P. (1999): Systematic studies on the amphibian fauna of the district Dehradun, Uttar Pradesh, India. *Memoirs of the Zoological Survey of India* **18**(3): 1–102.
- Saikia, B., Laskar, M.A., Dinesh, K.P., Shabnam, A., Sinha, B. (2022): Description of two new species of *Amolops* (Anura: Ranidae) from Arunachal Pradesh, Northeast India under the morphological ‘*Viridimaculatus* species group’. *Records of the Zoological Survey of India* **122**: 247–266.
- Saikia, B., Sinha, B., Laskar, M.A., Shabnam, A., Dinesh, K.P. (2022): A new species of *Amolops* (Anura: Ranidae) representing the morphological ‘*Marmoratus* species group’ from Sessa Orchid Sanctuary, Arunachal Pradesh, Northeast India. *Records of the Zoological Survey of India* **122**: 303–322.
- Saikia, B., Sinha, B., Shabnam, A., Dinesh, K.P. (2023): Description of a new species of *Amolops* Cope (Anura: Ranidae) from a cave ecosystem in Meghalaya, Northeast India. *Journal of Animal Diversity* **5**: 36–54.
- Savage, J.M., Heyer, W.R. (1997): Digital webbing formulae for anurans: a refinement. *Herpetological Review* **28**(3): 131.
- Sengupta, S., Hussain, B., Choudhury, P.K., Gogoi, J., Ahmed, F.M., Choudhury, N.K. (2008): A new species of *Amolops* (Anura: Ranidae) from Assam, north-eastern India. *Hamadryad* **32**: 5–12.
- Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H., Flook, P. (1994): Evolution, weighting and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the Entomological Society of America* **87**: 651–701.
- Stuart, B.L., Bain, R.H., Phimmachak, S., Spence, K. (2010): Phylogenetic systematics of the *Amolops monticola* group (Amphibia: Ranidae), with description of a new species from northwestern Laos. *Herpetologica* **66**: 52–66.
- Thompson, J., Higgins, D., Gibson, T. (1994): ClustalW: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* **22**: 4673–4680.
- Wu, Y.H., Yan, F., Stuart, B.L., Prendini, E., Suwannapoom, C., Dahn, H.A., et al. (2020): A combined approach of mitochondrial DNA and anchored nuclear phylogenomics sheds light on unrecognized diversity, phylogeny, and historical biogeography of the torrent frogs, genus *Amolops* (Anura: Ranidae). *Molecular Phylogenetics and Evolution* **148**: 106789.
- Zeng, Z., Liang, D., Li, J., Lyu, Z., Wang, Y., Zhang, P. (2020): Phylogenetic relationships of the Chinese torrent frogs (Ranidae: *Amolops*) revealed by phylogenomic analyses of AFLP-Capture data. *Molecular Phylogenetics and Evolution* **146**: 106753.
- Zhao, W.G., Rao, D.Q., Lü, S.Q., Dong, B.J. (2005): Herpetological surveys of Xizang Autonomous Region 2. Mêdog. *Sichuan Journal of Zoology* **24**: 250–253.

**Appendix 1.** List of valid *Amolops* species with details for 16S GenBank/Sequence used in phylogenetic analysis.

Species	16S Genebank Number	References			
<i>A. adicola</i>	MZ229772.1	Patel et al., (2021)	<i>A. mengdingensis</i>	MK501809.1	Wu et al., (2020)
<i>A. afghanus</i>	MK604837.1	Lyu et al., (2019b)	<i>A. mengyangensis</i>	KR827703.2	Wu et al., (2020)
<i>A. akhaorum</i>	FJ417158.2	Stuart et al., (2010)	<i>A. monticola</i>	MZ229773.1	Patel et al., (2021)
<i>A. albispinus</i>	MK263247.1	Lyu et al., (2019a)	<i>A. nepalicus</i>	MT124521.1	Wu et al., (2020)
<i>A. aniqiaoensis</i>	MN953658.1	Wu et al., (2020)	<i>A. nyingchiensis</i>	MK573814.1	Lyu et al., (2019b)
<i>A. australis</i>	MF061745.1	Chan et al., (2017)	<i>A. pallasitatus</i>	MK573816.1	QJ et al., (2019)
<i>A. beibengensis</i>	KIZ016397	Wu et al., (2020)	<i>A. panhai</i>	MG909606.1	Arifin et al., (2018)
<i>A. beibengensis</i>	WIIADA1734	Present Study	<i>A. putaoensis</i>	MT901382.1	Gan et al., (2020a)
<i>A. beibengensis</i>	WIIADA1726	Present Study	<i>A. ricketti</i>	KX507303.1	Sung et al., (2016)
<i>A. beibengensis</i>	WIIADA1725	Present Study	<i>A. shuichengicus</i>	MK604845.1	Lyu et al., (2019b)
<i>A. beibengensis</i>	MN953662.1	Wu et al., (2020)	<i>A. sinensis</i>	MK263299.1	Lyu et al., (2019a)
<i>A. bellulus</i>	DQ204473.1	Ngo et al., (2006)	<i>A. spinapectoralis</i>	DQ204488.1	Ngo et al., (2006)
<i>A. chanakya</i>	ON025582.1	Saikia et al., (2022a)	<i>A. tawang</i>	ON025581.	Saikia et al., (2022a)
<i>A. chaochin</i>	MZ702027.1	Jiang et al., (2021)	<i>A. teochew</i>	MZ447970.1	Zeng et al., (2006)
<i>A. chaochin</i>	MZ702029.1	Jiang et al., (2021)	<i>A. terraorchis</i>	MW794282.1	Saikia et al., (2022b)
<i>A. chayuenis</i>	MK573820.1	lyu et al., (2019b)	<i>A. torrentis</i>	DQ204489.1	Gan et al., (2020a)
<i>A. chunganensis</i>	MK263263.1	lyu et al., (2019a)	<i>A. tuanjiensis</i>	MN832773.1	Gan et al., (2020a)
<i>A. compotrix</i>	FJ417142.2	Stuart et al., (2010)	<i>A. tuberodepressus</i>	MK573797.1	Lyu et al., (2019b)
<i>A. cremnobetus</i>	DQ204477.1	Ngo et al., (2006)	<i>A. viridimaculatus</i>	MK573793.1	Lyu et al., (2019b)
<i>A. cucae</i>	FJ417144.2	Stuart et al., (2010)	<i>A. vitreus</i>	FJ417164.2	Stuart et al., (2010)
<i>A. daiyunensis</i>	DQ204479.1	Ngo et al., (2006)	<i>A. wangyali</i>	ON462439.1	Mahony et al., (2022)
<i>A. daorum</i>	FJ417147.2	Stuart et al., (2010)	<i>A. wangyufani</i>	MN953740.1	Wu et al., (2020)
<i>A. deng</i>	MN953695.1	Wu et al., (2020)	<i>A. wenshanensis</i>	FJ417129.2	Stuart et al., (2010)
<i>A. formosus</i>	MN953685	Wu et al., (2020)	<i>A. xindugiao</i>	MN953764.1	Xia et al., (2014)
<i>A. formosus</i>	MT124519.1	Khatiwada et al., (2020)	<i>A. yarlungzanbo</i>	MN953744.1	Wu et al., (2020)
<i>A. gerbillus</i>	ON462437.1	Mahony et al., (2022)	<i>A. yatseni</i>	MK263290.1	Lyu et al., (2019a)
<i>A. gerutu</i>	MF061721.1	Chan et al., (2017)	<i>Staurois natator</i>	MN953754.1	Wu et al., (2020)
<i>A. granulosus</i>	MK573811.1	Lyu et al., (2019b)			
<i>A. hainanensis</i>	DQ204481.1	Mahony et al., (2022)			
<i>A. himalayanus</i>	MN953712.1	Wu et al., (2020)			
<i>A. hongkongensis</i>	AF206453.1	Chen et al., (2005)			
<i>A. indoburmanensis</i>	MG909571.1	Arifin et al., (2018)			
<i>A. iriodes</i>	FJ417152.2	Stuart et al., (2010)			
<i>A. jinjiangensis</i>	MK573801.1	Lyu et al., (2019b)			
<i>A. kaulbacki</i>	MN953737.1	Wu et al., (2020)			
<i>A. kaulbacki</i>	MN953736.1	Wu et al., (2020)			
<i>A. kohimaensis</i>	MZ229774.1	Patel et al., (2021)			
<i>A. larutensis</i>	AB211484.1	Matsui et al., (2006)			
<i>A. lifaensis</i>	MK573809.1	Lyu et al., (2019b)			
<i>A. loloensis</i>	MK604854.1	Lyu et al., (2019b)			
<i>A. mahabharatensis</i>	MT124507.1	Khatiwada et al., (2020)			
<i>A. mantzorum</i>	MK573808.1	Lyu et al., (2019b)			
<i>A. marmoratus</i>	JF794456.1	Dever et al., (2012)			
<i>A. medogensis</i>	MK573813.1	Lyu et al., (2019b)			

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