

Description of a new species of *Amolops* Cope (Anura: Ranidae) from a cave ecosystem in Meghalaya, Northeast India

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Abstract

Seventeen nominal species of the ranid frog genus *Amolops* Cope, some of which are based on unverified historical records, are reported from India. Herein, we describe a new species of *Amolops* of the *marmoratus* group from a cave ecosystem. This is an uncommon habitat for this group of frogs, which is commonly found around cascades. The Siju Cave, from which four specimens of the new species were collected is a natural limestone cave located in the South Garo Hills District of Meghalaya, Northeast India. The new species is separated from other congeners based on morphological and genetic differences and is also geographically isolated from its sister species.

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Introduction

The cascade dwelling frogs of the genus *Amolops* Cope, are predominantly found in Southeast and East Asian countries, and are currently represented by 77 species (Frost, 2023). In India, this genus is restricted to the Himalayas and the North-Eastern Hills, and is represented by 17 species (Saikia et al., 2022a, b; Frost, 2023), of which 14 have their type locality in the country (Saikia et al., 2022a-c). Of the species of *Amolops* described from India, the majority are restricted to either small geographic ranges or to their respective type localities, including *Amolops adicola* Patel, Garg, Das, Stuart, and Biju; *A. assamensis* Sengupta, Hussain, Choudhury, Gogoi, Ahmed, and Choudhury; *A. chakrataensis* Ray; *A. chanakya* Saikia, Laskar, Dinesh, Shabnam, and Sinha; *A. jaunsari* Ray; *A. kohimaensis* Biju, Mahony, and Kamei; *A. monticola* (Anderson); *A. nidorbellus* Biju, Mahony, and Kamei; *A. senchalensis* (Chanda); *A. tawang* Saikia, Laskar, Dinesh, Shabnam, and Sinha; and *A. terraorchis* Saikia, Sinha, Laskar, Shabnam, and Dinesh (Patel et al., 2021; Saikia et al., 2022a, b; Frost, 2023), whereas the distribution ranges of *A. formosus* (Günther), *A. gerbillus* (Annandale) and *A. himalayanus* (Boulenger) remain relatively uncertain. This is largely due to the

relative lack of exploration in the region and the reliance on morphological data only for identification of specimens.

Among the other species of *Amolops* described from neighboring countries but reported from India, namely, *A. aniqiaoensis* Dong, Rao, and Lü; *A. indoburmanensis* Dever, Fuiten, Konu, and Wilkinson; and *A. viridimaculatus* (Jiang) sensu stricto (hereafter s.s.), doubts have been raised regarding the distribution of the latter (Mahony et al., 2022; Saikia et al., 2022c; Frost, 2023). Mahony et al. (2022) provisionally allocated the population of *Amolops* reported by Athreya (2006) from Eaglenest Wildlife Sanctuary of Arunachal Pradesh—the easternmost state of India—to *A. wangyali* Mahony, Nidup, Streicher, Teeling, and Kamei, a species belonging to the *Amolops viridimaculatus* species group recently described from Bhutan, but this needs confirmation.

Indian *Amolops* can be categorized into three morphological species groups (see Jiang et al., 2021; Saikia et al., 2022a, b) mostly based on their morphological characters: *Amolops marmoratus* species group (*A. assamensis*, *A. gerbillus*, *A. indoburmanensis*, *A. jaunsari*, *A. senchalensis* and *A. terraorchis*) (henceforth *marmoratus* group); *Amolops monticola*

species group (*A. aniqiaoensis*, *A. adicola*, *A. chakrataensis*, *A. kohimaensis* and *A. monticola* s.s.) (henceforth *monticola* group); and *Amolops viridimaculatus* species group (*A. chanakya*, *A. formosus*, *A. himalayanus*, *A. nidorbellus*, *A. viridimaculatus* s.s. and *A. tawang*) (henceforth *viridimaculatus* group) (see Table 1). However, based on the molecular phylogenetic analysis of Saikia et al. (2022b) and the analysis presented in this paper, *A. formosus* appears to belong to *marmoratus* group. Jiang et al. (2021) also mentioned that the placement of *A. formosus* within the *viridimaculatus* group is based on morphological data only, while their species groupings were largely based on both molecular and morphological data. As such, we are placing this species in the *marmoratus* group. Additionally, Mahony et al. (2022), considered *A. senchalensis* likely to belong to the *viridimaculatus* group based on their limited examination of the shriveled holotype deposited in the collections of the Zoological Survey of India (ZSI), Kolkata. However, until fresh topotypic specimens of this species are collected and examined, we retain *A. senchalensis* in the *marmoratus* group.

In January 2020, a field survey was conducted to Siju Cave in the South Garo Hills District of Meghalaya, Northeast India. During the course of that survey, four *Amolops* specimens were collected from the twilight and the dark zones of the cave (Fig. 1). These were provisionally identified as *Amolops* cf. *assamensis*. However, further morphological examinations led to the conclusion that this population of *Amolops* is markedly different from the typical *A. assamensis*, as well as from any known species of the *marmoratus* group (Table 1). As such, based on the morphological and molecular data, as well as geographic isolation (in the cave environment), we here describe the Siju Cave frog as a new species of *Amolops* in the *marmoratus* group as *Amolops siju* sp. nov.

Material and Methods

Collection site—Description of Siju Cave

Siju Cave (25°21' N; 90°41' E; altitude 60 m), located in the South Garo Hills District of Meghalaya (Fig. 2) is a 4 km long limestone cave (oriented approximately on a North-west–South-east axis). In the Garo language this cave is called *Siju Do-bak Khol* (= bat cave), as the cave harbors a large population of fruit bats, probably *Eonycteris spelaea* (Dobson) and leaf-nosed bats, *Hipposideros lankadiva* (Kelaart) (Harries et al., 2020). The cave can be accessed from the huge entrance situated in a vertical cliff on the western bank of the Simsang River. Nearly all along its length, the cave floor is covered in water which flows towards the cave entrance. The cave entrance is a rather straight, high-ceiling passage (Fig. 3) of ca. 150 m in length; the bottom is filled with coarse sand through which meanders the *Do-bak Khol* stream that joins the Simsang River outside. The twilight zone of the cave persists (Fig. 4) from ca. 60 m from the entrance up to 100 m; beyond this lies the dark zone, where no light penetrates and the ambient temperature and humidity remain constant year-round. At ca. 150 m, there is a huge dome-shaped cavity where thousands of bats roost; the cave floor is filled with bat guano that sustains a large number of cave invertebrates, such as oligochaetes, arachnids, isopods, crickets, beetles and millipedes. It was from this area that one of the authors (Bhaskar Saikia) collected a pair of *Amolops* (a male and a gravid female), which were found sitting adjacent (not in amplexus) in the darkness. Another two female *Amolops* (one gravid and one juvenile) were collected from the twilight zone of the cave (Fig. 5).

Table 1: Morphological species groups for *Amolops* from India (after Khatiwada et al., 2020; Jiang et al., 2021; Patel et al., 2021; Saikia et al., 2022b).

Morphological character	<i>monticola</i> group	<i>marmoratus</i> group	<i>viridimaculatus</i> group?
Male SVL	< 55 mm	27–95 mm	>70 mm
Dorso-lateral fold	Present	Absent, instead incomplete row of glands are present dorso-laterally	Absent
Skin	Smooth	Tuberculate/Granular	Smooth or Granular
Lateral sides of Head	Dark, with light-colored stripe up to the shoulder	No distinct band or stripe	No distinct band or stripe
Vomerine teeth	Present	Present	Distinct
Circum-marginal groove on Finger I disc	Present or Absent	Present	Absent or Indistinct
Tarsal fold and tarsal glands	No data	Absent	Absent
Vocal sac in males	Present	Present	Absent
Nuptial pad on Finger I in males	Present	Present	Present
Species	<i>A. aniqiaoensis</i> , <i>A. adicola</i> , <i>A. chakrataensis</i> , <i>A. kohimaensis</i> , <i>A. monticola</i> s.s.	<i>A. assamensis</i> , <i>A. formosus</i> , <i>A. gerbillus</i> , <i>A. indoburmanensis</i> , <i>A. jaunsari</i> , <i>A. senchalensis</i> , <i>Amolops siju</i> sp. nov., <i>A. terraorchis</i>	<i>A. chanakya</i> , <i>A. himalayanus</i> , <i>A. nidorbellus</i> , <i>A. tawang</i> , <i>A. viridimaculatus</i> s.s.



Figure 1: Siju Cave entrance photographed from the inside.

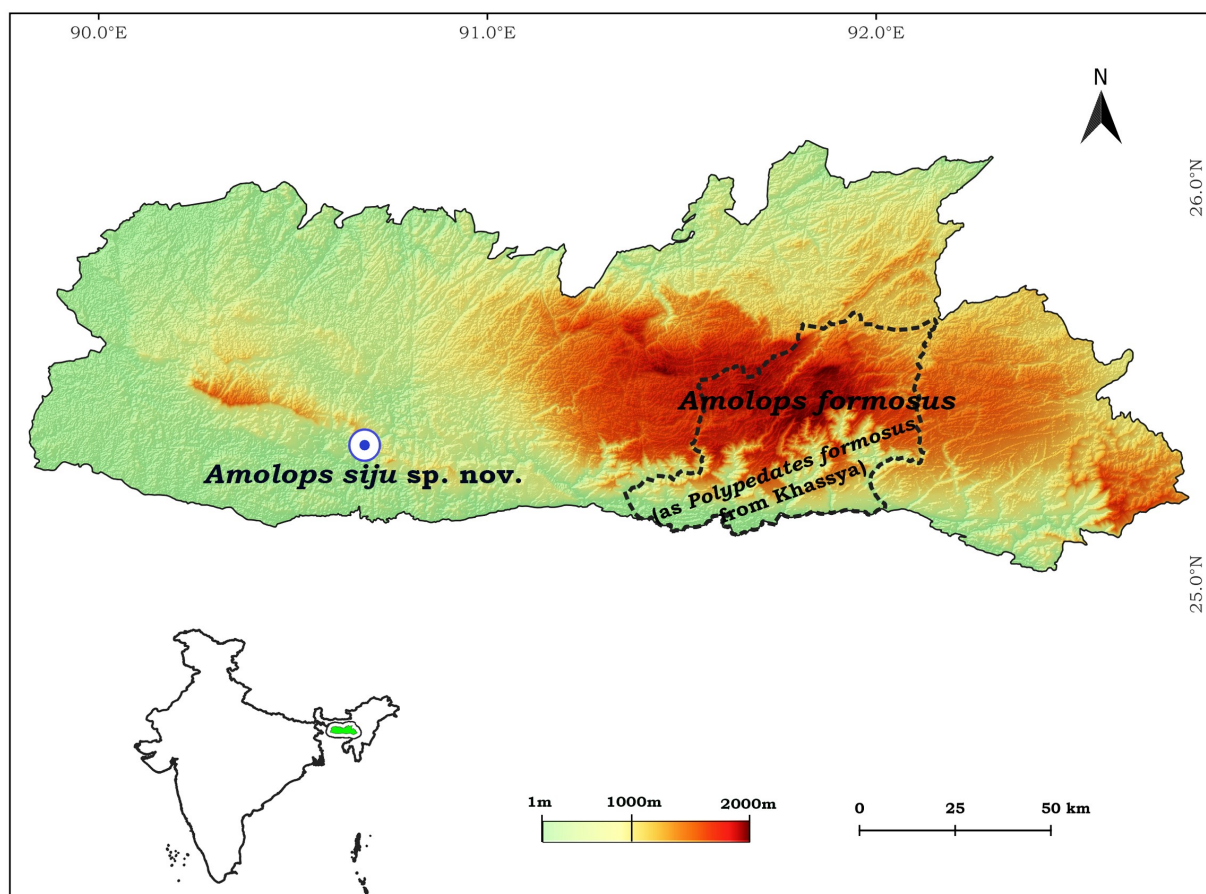


Figure 2: Map showing the type localities of *Amolops siju* sp. nov. and *A. formosus* (Günther).



Figure 3: The cave entrance and interiors of Siju and the *Do-bak Khol* stream.



Figure 4: The natural light condition of the twilight zone of Siju Cave.



Figure 5: One of the co-authors collecting a gravid *Amolops siju* sp. nov. from the twilight zone of the cave. The illumination is from searchlight used during the survey.

There are records of the Siju Cave written by Europeans since the 1870s (Harries et al., 2020). However, the first comprehensive biospeleological exploration of this cave was conducted by Stanley Kemp and B. Chopra of ZSI, Kolkata (then Calcutta) in 1922, which led to the publication of 102 species records including the description of many new taxa from there (Kemp and Chopra, 1924). Interestingly, in that report, the presence of two species of frogs was mentioned (identified by the former Director of ZSI and zoologist/limnologist, Nelson Annandale). These were *Megophrys* sp. (equivalent to *Xenophrys* sp.), based on tadpoles collected between 0–350 ft. (\approx 107 m) and *Rana afghana* Günther (currently, *Amolops afghanus* (Günther)) collected about 900 ft. (\approx 275 m) from the cave entrance. While previous reports of *A. afghanus* from India have been considered erroneous (Dever et al., 2012; Frost, 2023), recently, Saikia et al. (2022d) recorded the presence of a megophryid frog species, *Xenophrys megacephala* (Mahony, Sengupta, Kamei, and Biju), from Siju Cave based on the collection of an adult frog from ca. 400 m inside the cave. Interestingly, Harries et al. (2020) also spotted a single individual of *Amolops* at ca. 400 m from the cave entrance. The continued reporting of frog populations in a resource scarce, dark cave habitat over the course of a century is indeed intriguing. The only probable explanation for the source of food required to support the frog

populations would be troglobitic invertebrates, which in turn, are sustained by bat guano, as the food chain/web of Siju Cave lacks any autotrophic elements. Aside from bat guano, the heterotrophic trophic levels of Siju Cave could probably be supported by the supplementary supply of organic matter brought in by the *Do-bak Khol* stream. However, this is a mere postulation. A future study on the detritus food chain/web of this cave ecosystem would be of interest.

Collected specimens

Four specimens of *Amolops* were collected and were euthanized in 10% alcohol, fixed in 4% formalin overnight and finally transferred into 70% alcohol for preservation. Prior to fixation, liver tissues were extracted for molecular studies and preserved in absolute alcohol. Morphometric measurements were taken using a Mitutoyo™ digital calliper (Table 2) following Saikia et al. (2022c), while GPS coordinates were recorded using a GARMIN Oregon 550 (WGS84). The following morphological measurements were taken: AG—axilla to groin distance; EL—eye length, i.e., the horizontal distance between the bony orbital borders of the eye; FL1—first finger length (tip of finger to proximal palmar tubercle); FL2—second finger length (tip of finger to proximal palmar tubercle); FL4—fourth finger length (tip of finger to proximal palmar tubercle);

Table 2: Morphometric data (in mm) of *Amolops siju* sp. nov. described in this paper.

Regd. No.	V/A/NERC/1620	V/A/NERC/1856	V/A/NERC/1857	V/A/NERC/1858
Sex	M	F	F	F
SVL	63.4	94.4	84.5	43.9
HW	24.5	35.7	31.5	17.2
HL	22.8	32.3	28.9	16.9
IN	8.0	11.0	9.8	6.0
NE	4.9	6.8	6.6	4.0
NS	4.9	6.8	6.4	4.0
MN	18.0	25.0	23.1	13.7
MFE	13.3	18.1	16.7	10.8
MBE	7.1	10.1	9.2	5.3
SL	9.9	14.6	13.8	7.4
EL	8.5	9.7	9.2	6.1
IUE	4.6	7.7	7.2	3.7
UEW	6.5	8.2	8.1	5.1
IFE	13.8	18.5	17.6	9.6
IBE	20.1	25.9	24.8	13.6
TYD (Horizontal)	1.6	3.2	2.7	1.6
TYD (Vertical)	1.8	3.5	3.2	1.8
TE	3.6	5.9	5.1	2.6
FLL	14.4	20.4	19.8	10.3
HAL	20.5	28.6	25.7	13.8
FL1	10.8	16.5	Tip missing	7.9
FL2	13.9	20.3	18.6	9.8
TFL	20.5	29.1	26.2	13.6
FL4	17.8	25.7	22.8	12.2
AGL	26.4	40.3	32.7	18.1
WBS	23.8	34.9	30.1	13.7
WFG	10.0	21.1	19.4	9.5
ShL/ FL	40.4	52.7	51.0	26.4
TiL	40.6	54.7	52.9	30.0
Tal	51.6	71.3	66.9	37.4
FOL	34.0	48.4	44.9	24.8
FTL	18.6	27.5	23.8	12.8
ITL	7.1	10.6	9.5	4.0
IMT	4.8	6.1	5.7	2.5

FLL—forelimb length, measured from the elbow to the base of the outer palmar tubercle; FOL—foot length, measured from the base of the inner metatarsal tubercle to the tip of the fourth toe; FTL—fourth toe length, measured from base of proximal sub articular tubercle to toe tip; HAL—hand length, measured from the base of the outer palmar tubercle to the tip of the third finger; HL—head length, from the rear of the mandible to the tip of the snout; HW—head width, at the angle of the jaws; IBE—distance between posterior corner of eyes; IFE—distance between anterior corner of eyes; IMT—length of inner metatarsal tubercle; IN—inter-narial distance; ITL—inner toe length; IUE—inter upper eyelid width, i.e., the shortest distance between the upper eyelids; MBE—distance from the rear of the mandible to the posterior most orbital border; MFE—distance from the rear of the mandible to the anterior most orbital border; MN—distance from the rear of the mandible to the center of the nostril; NE—nostril to eye distance; NS—nostril to snout tip distance; ShL/FL—thigh length; SL—snout length, measured from the tip of the snout to the anterior most orbital border; SVL—snout to vent length; Tal—tarsus length; TE—tympanum to posterior corner of eye distance; TFL—third finger length (tip of finger to proximal palmar tubercle); TiL—tibia length; TYD—tympanum diameter; UEW—maximum upper

eyelid width; WBS—body width behind shoulders; and WFG—body width in front of groin.

Specimens were cataloged in the National Zoological Collections of the North Eastern Regional Centre, ZSI, Shillong with the registration numbers: V/A/NERC/ZSI/1620, 1856, 1857 and 1858. Paratypes of *A. assamensis* (V/A/ERC/606–612) available in ZSI, Shillong were examined for comparison with our new species. The following literature were also consulted for morphological comparisons: Dever et al. (2012); Saikia et al. (2022b, c); Sengupta et al. (2008).

Molecular protocols were followed as per Saikia et al. (2021). PCR amplification of 697 bp of the mitochondrial 16S rRNA gene was performed using the 16Sar-L and 16Sbr-H primers of Palumbi et al. (2002) from genomic DNA of the two amphibian samples mentioned in Appendix. Sanger sequencing with an ABI Prism 377 (Applied Biosciences) sequencer was outsourced to Barcode Biosciences Pvt. Ltd., Bangalore. Low quality base calls were filtered and end-trimmed from trace files of 16S rRNA gene sequence fragments using SeqTrace (Stucky, 2012). The resultant sequence was checked for corrections in MEGA XI software (Tamura et al., 2021). Phylogenetic analysis using maximum likelihood (ML) was based on a total of 221 sequences, inclusive of our

newly generated sequences of *Amolops* (Appendix) and those obtained from GenBank along with the outgroups in the IQ-TREE multicore version 1.6.12 (Trifinopoulos et al., 2016) web server. The TIM2+F+I+G4 nucleotide substitution model was auto-selected as per BIC (Bayesian Information Criterion) and the analysis was set for 1000 ultrafast bootstraps with default parameters and SH-aLRT branch test. The sequence names and outgroups follow Wu et al. (2020) and Saikia et al. (2022a–c), and the resultant tree was visualized in Fig. Tree v. 1.4.

Results

Phylogenetic analysis

In the phylogenetic tree of *Amolops* spp. with 221 sequences of mt 16S rRNA, *Amolops siju* sp. nov. is sister to *Amolops indoburmanensis* from the Rakhine region of Myanmar with a genetic distance of 4.5 % (Fig. 6). Additionally, the clade comprising the new species, *Amolops* sp. and *A. indoburmanensis* is sister to *A. afghanus* from the Yunnan Province of China and Myanmar with strong bootstrap support (Fig. 6).

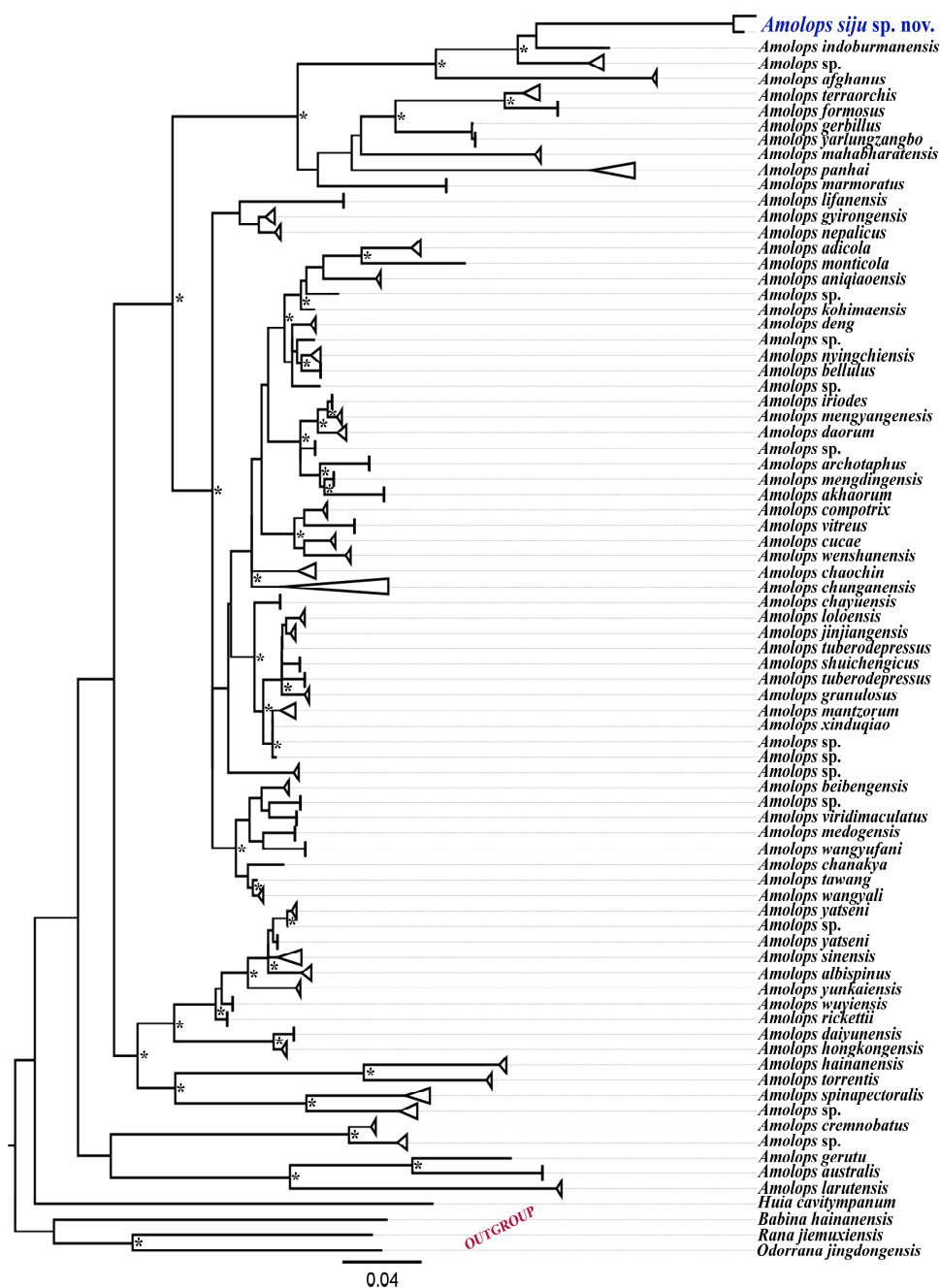


Figure 6: Maximum likelihood tree for the members of *Amolops* based on 560 bp of mt 16S rRNA (*ufbs \geq 95%).

New species description

Amolops siju sp. nov.

(Siju Cascade Frog)

(Figs. 7–9)

<http://zoobank.org/urn:lsid:zoobank.org:act:C71F607E-75FA-421B-9FD5-403EA097CAE5>

Holotype

V/A/NERC/ZSI/1620, adult male (Figs. 7 and 9), collected from the dark zone of Siju Cave (ca. 150 m from the entrance), South Garo Hills, Meghalaya, India on 3 January 2020 by Bhaskar Saikia and team.

Paratypes

V/A/NERC/ZSI/1856, adult gravid female (Fig. 8) collected along with the holotype; V/A/NERC/ZSI/1857, adult gravid female collected ca. 80 m from the entrance; and V/A/NERC/ZSI/1858, juvenile female collected ca. 65 m from the entrance, both from the twilight zone of Siju Cave, South Garo Hills, Meghalaya, India on 3 January 2020 by Bhaskar Saikia and team.

Diagnosis

Amolops siju sp. nov. is a member of the *marmoratus* species group isolated in geographical space from its phylogenetic sister species and having a distinct set of morphological characters. The new species can be diagnosed based on the following set of morphological characters: adult male size large (SVL 63.4 mm), adult female size very large (SVL 84.5–94.4 mm); head wider than long (HW > HL); snout rounded, longer than eye length (SL > EL); inter-orbital distance less than both upper eyelid width and inter-narial distance (IUE < UEW < IN); tympanum distinct and very small, less than 20% of eye length; fingers with circum-marginal grooves; tibia slightly longer than femur and foot (TiL > ShL > FOL), roughly 2/3rd of SVL; tibio-tarsal articulation reaches slightly beyond snout; supra-tympanic fold visible; dorsum smooth, lateral side with scattered warts.

Description of the Holotype

Adult male cascade frog (SVL 63.4 mm), body robust, head wider than long (HW > HL; HW 24.5 mm, HL 22.8 mm), flat above; snout (SL 9.9 mm) rounded, protruding in lateral view, snout longer than the eye diameter (EL 8.5 mm) (SL > EL); canthus rostralis distinct, loreal region depressed; supra-tympanic fold weak; inter-orbital distance (IUE 4.6 mm) less than both upper eyelid width (UEW 6.5 mm) and inter-narial distance (IN 8.0 mm) (IUE < UEW < IN); nostrils laterally placed, equidistant (NS = NE = 4.9 mm); distance between the back of eyes 1.5 times than the front of eyes (IFE 13.8 mm; IBE 20.1 mm); pupil horizontal; tympanum distinct (TYD (H) 1.6 mm) and concolorous, minute, elliptical and little depressed, about one-fifth of eye diameter (EL 8.5 mm); tympanum separated from the eye by a distance (TE 3.6 mm) more than twice its diameter; pineal ocellus visible; a pair of vomerine teeth situated between the choanae, choanae angular to the body axis; tongue large and bilobed. Vocal sac absent.

Arms robust, forearm length (FLL 14.4 mm) shorter than the hand length (HAL 20.5 mm); fingers free, tips ending in disk, fingers with circum-marginal grooves, relative length of fingers I < II < IV < III (FL1 10.8 mm, FL2 13.9, FL4 17.8 mm, TFL 20.5 mm); fingers without any webbing; sub-articular tubercles prominent and elliptical; prepollex oval.

Hindlimbs long, strongly overlap when folded at right angles to the body; tibia (TiL 40.6 mm) marginally longer than femur (ShL 40.4 mm); but distinctly longer than foot (34.0 mm) (TiL > ShL > FOL); tibio-tarsal articulation reaches slightly beyond the snout; toes long and thin, tips of all toes dilated into disks with circum-marginal grooves, toes completely webbed, no phalanges free on toes; sub-articular tubercles elliptical; inner metatarsal tubercle elongated (4.8 mm), outer circular. Dorsal skin smooth; lateral sides with scattered warts; ventral skin smooth.

Coloration of the Holotype

In life, dorsal and lateral ground color olive green (Fig. 7), with large irregular brown blotches. Limbs banded with alternating narrow bands of greenish-yellow and wider bands of dark brown. Venter dirty-white with dark patches around the upper region. In preservative, same as in life condition, except that all the colors are faded (Fig. 9).

Variation

The paratypes (all females; n = 3) show slight morphological variations with respect to the holotype (male): tibio-tarsal articulation reaches anterior eye corner; in V/A/NERC/1857 nostrils slightly nearer to the snout; in V/A/NERC/1858 tympanum is separated from the eye by a distance of less than two times of its diameter.

Condition of the type series

The type series is in good condition. The grooves on the dorsum and right thigh of the holotype (Fig. 9A, B) and paratypes are due to field tag impressions. The first fingertip in the paratype V/A/NERC/1857 is missing. A ventral incision was made to extract liver tissue used in the molecular study, and also to determine the sex of the specimens.

Natural history

All individuals of *Amolops siju* sp. nov. were collected at around 11:00 hours. The holotype and a gravid female were collected from the dark zone of the cave, near the bat chamber, beside the *Do-bak Khol* stream. One gravid female was collected from a cave wall crevice on the left side of *Do-bak Khol* stream, while one juvenile female was collected from the right side. The latter two specimens were found in the twilight zone of the cave. The associated amphibian fauna observed included individuals of *Ingerana* sp. swimming in the *Do-bak Khol* stream at the transition zone between the light and the twilight zones, and a few individuals of *Minervarya* sp. spotted at the cave entrance.



Figure 7: Field photo (in situ) of the holotype (V/A/NERC/ZSI/1620) of *Amolops siju* sp. nov.



Figure 8: Field photo (in situ) of a female paratype (V/A/NERC/ZSI/1856) of *Amolops siju* sp. nov.



Figure 9: Holotype image of *Amolops siju* sp. nov. in preserved condition (A: dorsal view; B: ventral view).

Distribution

Currently, this species is known only from its type locality of Siju Cave, South Garo Hills, Meghalaya, India. Since, we did not find any signs of troglobitic adaptation in this species, we assume that they migrate into the caves for the warmth and steady temperature and humidity that a limestone cave such as Siju can provide. Therefore, this species should be equally adapted for habitats outside the cave, and hence, could have a wider distribution.

Comparisons

The new species is compared with the members of the *marmoratus* species group (Table 1). There are no sympatric congeners and the only other *Amolops* species described from the state of Meghalaya is *A. formosus* (Type locality: Khasi Hills).

From other members of the Indian *marmoratus* species group, *Amolops siju* sp. nov. differs from *A. assamensis* (in parentheses) in having larger male SVL = 63.4 mm (vs. smaller, 52.8–61.7 mm), nostril equidistant (vs. closer to snout), supra-tympanic fold weak (vs. well-developed); tympanum very small, about 1/5th of eye diameter (vs. more than half of eye diameter), tympanum separated from the eye by a distance more than twice its diameter (vs. separated from the eye by a distance of about its diameter), tympanum elliptical, concolorous (vs. roughly rounded, reddish-brown), vocal sac absent in male (vs. present), tongue bifid (vs. notched), choanae in line with vomerine teeth (vs. choanae beside the vomerine teeth), male with smooth dorsal skin (vs. male with scattered warts dorsally); from *A. formosus* (in parentheses) in having small tympanum (vs. tympanum of the size of finger III disk), circum-

marginal groove in finger I (vs. absent), TiL > ShL (vs. TiL < ShL), inner metatarsal tubercle elongated (vs. indistinct), dorsal color dark olive green with large irregular brown blotches (vs. dark green with large brown distinct spots); from *A. gerbillus* (in parentheses) in having head wider than long in males (vs. as long as broad in males), nostrils equidistant (vs. nearer to eye), no gular pouch (vs. present); from *A. indoburmanensis* (in parentheses) in having smaller adult male size (vs. large), dorsum dark brown with no spots (vs. brown with scattered darker brown spots), male without gular pouch (vs. dual); from *A. jaunsari* (in parentheses) in having head wider than long (vs. equal), inter-narial distance smaller than inter-orbital (vs. equal), finger I < II (vs. finger I = II), thighs banded (vs. blotched with reticulum); from *A. senchalensis* (in parentheses) in having nostril equidistant (vs. nearer to eye), toes entirely webbed (vs. nearly webbed); from *A. terraorchis* (in parentheses) in having larger male SVL of > 63 mm (vs. smaller male SVL of < 43 mm), larger female SVL of > 84 mm (vs. smaller female SVL of < 83 mm), IUE < UEW (vs. IUE = UEW), nostril equidistant (vs. nearer to snout), tympanum elliptical, about 20% of EL (vs. tympanum circular, about 40% of EL), TE is more than twice of TD (vs. TE less than TD), gular pouch absent (vs. present), inner metatarsal tubercle elongated (vs. oval), outer metatarsal tubercle circular (vs. absent).

From members of the *marmoratus* species group found outside India, *Amolops siju* sp. nov. differs from *A. afghanus* in having larger (> 63 mm) male SVL (vs. smaller, < 63 mm in *A. afghanus*); supra-tympanic weak (vs. short and thick in *A. afghanus*), IUE < UEW (vs. IUE > UEW in *A. afghanus*), dorsal

skin smooth (vs. granular in *A. afghanus*); from *Amolops mahabharatensis* Khatiwada, Shu, Wang, Zhao, Xie, and Jiang in not having dorso-lateral glandular folds (vs. present in *A. mahabharatensis*), IUE < UEW < IN (vs. IUE > UEW and IN in *A. mahabharatensis*), tongue bifid (vs. lanceolate in *A. mahabharatensis*), TTA reaching beyond snout (vs. up to snout in *A. mahabharatensis*); from *A. marmoratus* (Blyth) s.s. in having distinct tympanum (vs. indistinct in *A. marmoratus*), dorsum smooth (vs. strongly granular in *A. marmoratus*); from *A. nepalicus* Yang in having head wider than long (vs. longer than wide in *A. nepalicus*), IUE < IN (vs. IUE > IN in *A. nepalicus*), Finger I < II (vs. Finger I = II in *A. nepalicus*); from *A. panhai* Matsui and Nabhitabhata, in having larger (85–95 mm) matured sized female (vs. medium sized, 47–57 mm in *A. panhai*); head wider than long (vs. longer than wide *A. panhai*).

Etymology

The species epithet is a toponym derived from the type locality. The name ‘Siju’ is treated as noun in apposition.

Suggested common name

We suggest Siju Cave frog as the common English name for this species.

Discussion

When Dever et al. (2012) delimited the range of *A. marmoratus* s.s. and *A. afghanus* (while resurrecting it from the synonymy of the former) to Myanmar along with the description of *A. indoburmanensis*, this left the previous reports of *A. marmoratus* s.s. and *A. afghanus* from adjacent Northeast India and Nepal in a state of flux. Of these three *Amolops* species, only *A. indoburmanensis*—which is restricted to a narrow tract of hills of Mizoram that is in continuum with the Burmese Hills—has been confirmed to occur in Northeast India (Lalronunga et al., 2020; Saikia et al., 2022c). This left *A. assamensis*, *A. formosus*, *A. gerbillus*, *A. jaunsari*, *A. senchalensis* and *A. terraorchis* as the other Indian members of the *marmoratus* group with the latter four species restricted to the Himalayas.

When *Amolops* specimens were collected from Siju Cave in 2020, they were tentatively identified as *A. assamensis* due to the superficial morphological similarities. However, when a detailed examination of the paratypes of *A. assamensis* available at ZSI, Shillong and a perusal of literature describing the species (Sengupta et al., 2008) were later conducted, it led to the documentation of the presence of many morphological characters differentiating the Siju population from a typical *A. assamensis*. Due to the presence of the documented morphological differences, along with the presence of a high genetic distance and the geographical isolation of Siju population from other congeners of the *marmoratus* group, we propose this cave-dwelling population as a new species.

Amolops assamensis was described from Mayeng Hill Reserved Forest (RF) and Kolaghat RF from the eastern part of the Kamrup District of Assam, located towards the north-east of Siju Cave and which is separated from it by an aerial distance of ca. 370 km across the Meghalaya Plateau—a geographic relief feature of about 1,950 m a.s.l.—separating the respective type localities of these two species. Additionally, we would like to point out that the ranges of the geographic coordinates from where the type series of *A. assamensis* was collected from the above two protected areas need to be verified (Kolaghat RF, 26°04′–09′ N, 90°24′–92°22′ E; 61.65 sq. km. and Mayeng Hill RF, 25°43′–25°55′ N, 90°32′–91°21′ E; 21 sq. km. vide Sengupta et al. 2008) because these represent large geographical areas beyond the jurisdictions of the actual protected areas. Additionally, the examination of the paratypes of *A. assamensis* revealed that the tongue in this species is distinctly notched, and not bifid as stated by Sengupta et al. (2008).

Currently, there are no genetic data for *A. assamensis* available in the public domain. It is critical that genetic sequences of this species are generated based on topotypic materials, for which it is important that proper type locality information (including altitudinal data) for this species be made available. A comparison of such novel data with the genetic data of our new species will help to show the degree of separation between the two cryptic species. A report on the occurrence of “*A. assamensis*” from Sohra (the current name of Cherrapunjee) in Meghalaya from an altitude of 1,280 m a.s.l. exists (Purkayastha and Basak, 2017), without any supporting data on the collected specimen. In light of our new species, the specific identity of this specimen from Sohra needs to be verified.

In the present study, 221 *Amolops* sequences of mt 16S rRNA were utilized to generate a single gene phylogenetic tree, wherein good bootstrap support was noted for our new lineages and its sister clades at the terminal nodes. In earlier studies, Wu et al. (2020) and Jiang et al. (2021) have presented multigene phylogenetic trees for the genus with sampling from Nepal, China and the Malayan region wherein genetic data from the Indian region were limited. Within the genus *Amolops*, type localities for 14 species (among the 17 species known from India) occur within the boundaries of India. Of these, genetic data are available for nine species—*A. adicola*, *A. chanakya*, *A. formosus*, *A. gerbillus*, *A. himalayanus*, *A. kohimaensis*, *A. monticola*, *A. tawang* and *A. terraorchis*. With this current study, genetic data is now available for *Amolops siju* sp. nov.

With this proposed new species, we are attributing all the previous reports of *Amolops* species from Siju Cave (Kemp and Chopra, 1924; Harries et al., 2020) to *Amolops siju* sp. nov.

Conclusion

With the description of *Amolops siju* sp. nov., 18 species of *Amolops* are recognized in India with 15 of these species having their respective type localities in the country. Genetic data are needed for five species (*A. assamensis*, *A. chakrataensis*, *A. jaunsari*, *A. nidorbellus* and *A. senchalensis*) described from India to understand the phylogenetic relationship among the congeners (77 species) of *Amolops* where distribution ranges (natural biogeographic ranges) are heterogenous and do not fit into our understanding of political boundaries.

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Author contributions

All the authors equally contributed to the design of the study. Field studies and photography were conducted by B.S. Morphometric measurements and morphological studies were conducted by B.S. and B.J.S. Taxonomic studies were carried out by B.S., K.P.D. and B.J.S. Molecular studies and phylogenetic analysis were performed by K.P.D. and A.S. Maps were generated by A.S. Manuscript writing, editing and proofreading was contributed to equally by B.S., B.J.S., A.S. and K.P.D.

Conflict of interest

All the authors declare that there are no conflicting issues related to this research article.

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Appendix 1: GenBank accession numbers for mt 16S rRNA sequences used in the maximum likelihood phylogenetic analysis of cascade frogs of *Amolops* (Saikia et al., 2022a-c).

Sr. No.	NCBI Accession no.	Species name	Collection locality	References
1	OM174172.1	<i>Amolops siju</i> sp. nov.	India: Meghalaya, Siju	Present Study
2	OM174173.1	<i>Amolops siju</i> sp. nov.	India: Meghalaya, Siju	Present Study
3	JF794460.1	<i>Amolops indoburmanensis</i> Dever, Fuiten, Konu, and Wilkinson	Myanmar: Rakhine	Dever et al. (2012); Khatiwada et al. (2020)
4	MN519705.1	<i>A. indoburmanensis</i> Dever, Fuiten, Konu, and Wilkinson	India: Mizoram, Murlen National Park	Saikia et al. (2022c)
5	MN953693.1	<i>A. indoburmanensis</i> Dever, Fuiten, Konu, and Wilkinson	Myanmar: Haka Township, Chin	Wu et al. (2020)
6	MN953692.1	<i>A. indoburmanensis</i> Dever, Fuiten, Konu, and Wilkinson	Myanmar: Baw village, Chin	Wu et al. (2020)
7	MN953694.1	<i>A. indoburmanensis</i> Dever, Fuiten, Konu, and Wilkinson	Myanmar: Upper Bee Hoe village, Mindat, Chin	Wu et al. (2020)
8	MN953654.1	<i>Amolops afghanus</i> (Günther)	China: Husa, Yunnan	Wu et al. (2020)
9	MN953774.1	<i>A. afghanus</i> (Günther)	Myanmar: Myitkyina	Wu et al. (2020)
10	MN953773.1	<i>A. afghanus</i> (Günther)	Myanmar: Indawgyi Lake Wildlife Sanctuary, Kachin	Wu et al. (2020)
11	JF794431.1	<i>A. afghanus</i> (Günther)	Myanmar: Kachin	Dever et al. (2012); Khatiwada et al. (2020)
12	MW794278.1	<i>Amolops terraorchis</i> Saikia, Sinha, Laskar, Shabnam, and Dinesh	India: Arunachal Pradesh, Sessa Wildlife Sanctuary	Saikia et al. (2022b)
13	MW794279.1	<i>A. terraorchis</i> Saikia, Sinha, Laskar, Shabnam, and Dinesh	India: Arunachal Pradesh, Sessa Wildlife Sanctuary	Saikia et al. (2022b)
14	MW794280.1	<i>A. terraorchis</i> Saikia, Sinha, Laskar, Shabnam, and Dinesh	India: Arunachal Pradesh, Sessa Wildlife Sanctuary	Saikia et al. (2022b)
15	MW794281.1	<i>A. terraorchis</i> Saikia, Sinha, Laskar, Shabnam, and Dinesh	India: Arunachal Pradesh, Sessa Wildlife Sanctuary	Saikia et al. (2022b)
16	MW794282.1	<i>A. terraorchis</i> Saikia, Sinha, Laskar, Shabnam, and Dinesh	India: Arunachal Pradesh, Sessa Wildlife Sanctuary	Saikia et al. (2022b)
17	MN953750.1	<i>Amolops</i> sp.	Nepal: Mabu, Ilam	Wu et al. (2020); Patel et al. (2021)
18	MT124518.1	<i>Amolops formosus</i> (Günther)	Nepal: Kimathanka, Sankhuwasabha	Khatiwada et al. (2020)
19	MT124519.1	<i>A. formosus</i> (Günther)	Nepal: Lamatar, Taplejung	Khatiwada et al. (2020)
20	MT124517.1	<i>A. formosus</i> (Günther)	Nepal: Kimathanka, Sankhuwasabha	Khatiwada et al. (2020)
21	OK138592.1	<i>A. gerbillus</i> (Annandale)	India: Arunachal Pradesh, Kamle	Saikia et al. (2022b)
22	MN953744.1	<i>Amolops yarlungzangbo</i> Jiang, Wang, Li, Qi, Li, and Che	China: Medog, Tibet	Wu et al. (2020)
23	MN953745.1	<i>A. yarlungzangbo</i> Jiang, Wang, Li, Qi, Li, and Che	China: Medog, Tibet	Wu et al. (2020)
24	MN953746.1	<i>A. yarlungzangbo</i> Jiang, Wang, Li, Qi, Li, and Che	China: Medog, Tibet	Wu et al. (2020)
25	MN953747.1	<i>A. yarlungzangbo</i> Jiang, Wang, Li, Qi, Li, and Che	China: Medog, Tibet	Wu et al. (2020)
26	MT124507.1	<i>Amolops mahabharatensis</i> Khatiwada, Shu, Wang, Zhao, Xie, and Jiang	Nepal: Hattibang, Chitwan district	Khatiwada et al. (2020)
27	MT124513.1	<i>A. mahabharatensis</i> Khatiwada, Shu, Wang, Zhao, Xie, and Jiang	Nepal: Barahakshetra, Sunsari district	Khatiwada et al. (2020)
28	MT124514.1	<i>A. mahabharatensis</i> Khatiwada, Shu, Wang, Zhao, Xie, and Jiang	Nepal: Barahakshetra, Sunsari district	Khatiwada et al. (2020)
29	MT124509.1	<i>A. mahabharatensis</i> Khatiwada, Shu, Wang, Zhao, Xie, and Jiang	Nepal: Hattibang, Chitwan district	Khatiwada et al. (2020)
30	MT124510.1	<i>A. mahabharatensis</i> Khatiwada, Shu, Wang, Zhao, Xie, and Jiang	Nepal: Hattibang, Chitwan district	Khatiwada et al. (2020)
31	MT124512.1	<i>A. mahabharatensis</i> Khatiwada, Shu, Wang, Zhao, Xie, and Jiang	Nepal: Pokhara, Kaski district	Khatiwada et al. (2020)
32	MT124511.1	<i>A. mahabharatensis</i> Khatiwada, Shu, Wang, Zhao, Xie, and Jiang	Nepal: Pokhara, Kaski district	Khatiwada et al. (2020)
33	MT124508.1	<i>A. mahabharatensis</i> Khatiwada, Shu, Wang, Zhao, Xie, and Jiang	Nepal: Hattibang, Chitwan district	Khatiwada et al. (2020)
34	MT124515.1	<i>A. mahabharatensis</i> Khatiwada, Shu, Wang, Zhao, Xie, and Jiang	Nepal: Latinath, Darchula district	Khatiwada et al. (2020)
35	MT124516.1	<i>A. mahabharatensis</i> Khatiwada, Shu, Wang, Zhao, Xie, and Jiang	Nepal: Latinath, Darchula district	Khatiwada et al. (2020)
36	MN953720.1	<i>Amolops panhai</i> Matsui and Nabhitabhata	Thailand: Huay Yang National Park, Prachuap Khiri Khan	Wu et al. (2020)

Appendix 1: (Continued).

Sr. No.	NCBI Accession no.	Species name	Collection locality	References
37	AB211488.1	<i>A. panhai</i> Matsui and Nabhitabhata	Thailand: Phetchaburi	Matsui et al. (2006); Khatiwada et al. (2020)
38	JF794451.1	<i>A. panhai</i> Matsui and Nabhitabhata	Myanmar: Tanintharyi	Dever et al. (2012); Khatiwada et al. (2020)
39	MN953722.1	<i>A. panhai</i> Matsui and Nabhitabhata	Myanmar: Pakchan Reserve Forest, Kawthoung, Tanintharyi	Wu et al. (2020)
40	MN953721.1	<i>A. panhai</i> Matsui and Nabhitabhata	Thailand: Ngao Falls National Park, Ranong	Wu et al. (2020)
41	MN953708.1	<i>Amolops marmoratus</i> (Blyth)	Thailand: Huai Hea, Chiang Mai	Wu et al. (2020)
42	MN953709.1	<i>A. marmoratus</i> (Blyth)	Thailand: Wachiratam Falls, Amphoe Chom Thong, Chiangmai	Wu et al. (2020)
43	JF794453.1	<i>A. marmoratus</i> (Blyth)	Myanmar: Mon	Dever et al. (2012); Khatiwada et al. (2020)
44	JF794454.1	<i>A. marmoratus</i> (Blyth)	Myanmar: Mon	Dever et al. (2012); Khatiwada et al. (2020)
45	MN953702.1	<i>Amolops lifanensis</i> (Liu)	China: Maoxian, Sichuan	Wu et al. (2020)
46	MN953703.1	<i>A. lifanensis</i> (Liu)	China: Maoxian, Sichuan	Wu et al. (2020)
47	AB211482.1	<i>A. lifanensis</i> (Liu)	China: Sichuan	Matsui et al. (2006); Khatiwada et al. (2020)
48	AB211481.1	<i>A. lifanensis</i> (Liu)	China: Sichuan	Matsui et al. (2006); Khatiwada et al. (2020)
49	MN953682.1	<i>Amolops gyirongensis</i> Jiang, Wang, Wang, Pan, and Che	China: Gyirong, Tibet	Wu et al. (2020)
50	MN953686.1	<i>A. gyirongensis</i> Jiang, Wang, Wang, Pan, and Che	China: Gyirong, Tibet	Wu et al. (2020)
51	MN953684.1	<i>A. gyirongensis</i> Jiang, Wang, Wang, Pan, and Che	China: Gyirong, Tibet	Wu et al. (2020)
52	MN953683.1	<i>A. gyirongensis</i> Jiang, Wang, Wang, Pan, and Che	China: Gyirong, Tibet	Wu et al. (2020)
53	MN953685.1	<i>A. gyirongensis</i> Jiang, Wang, Wang, Pan, and Che	China: Gyirong, Tibet	Wu et al. (2020)
54	MT124521.1	<i>Amolops nepalicus</i> Yang	Nepal: Dobhan, Sankhuwasabha	Khatiwada et al. (2020)
55	MT124523.1	<i>A. nepalicus</i> Yang	Nepal: Dobhan, Sankhuwasabha	Khatiwada et al. (2020)
56	MT124522.1	<i>A. nepalicus</i> Yang	Nepal: Dobhan, Sankhuwasabha	Khatiwada et al. (2020)
57	MT124524.1	<i>A. nepalicus</i> Yang	Nepal: Dobhan, Sankhuwasabha	Khatiwada et al. (2020)
58	OK138593.1	<i>Amolops adicola</i> Patel, Garg, Das, Stuart, and Biju	India: Arunachal Pradesh: Upper Subansiri	Saikia et al. (2022c)
59	MZ229772.1	<i>A. adicola</i> Patel, Garg, Das, Stuart, and Biju	India: Arunachal Pradesh	Patel et al. (2021)
60	MZ229773.1	<i>A. monticola</i> (Anderson)	India: South Sikkim	Patel et al. (2021)
61	MT636754.1	<i>Amolops aniqiaoensis</i> Dong, Rao, and Lü	India: Arunachal Pradesh: Upper Subansiri	Saikia et al. (2022c)
62	MN953658.1	<i>A. aniqiaoensis</i> Dong, Rao, and Lü	China: Tibet	Wu et al. (2020)
63	MT636755.1	<i>A. aniqiaoensis</i> Dong, Rao, and Lü	India: Arunachal Pradesh: Upper Subansiri	Saikia et al. (2022c)
64	MN953655.1	<i>A. aniqiaoensis</i> Dong, Rao, and Lü	China: Medog, Tibet	Wu et al. (2020)
65	MN953656.1	<i>A. aniqiaoensis</i> Dong, Rao, and Lü	China: Medog, Tibet	Wu et al. (2020)
66	MN953657.1	<i>A. aniqiaoensis</i> Dong, Rao, and Lü	China: Medog, Tibet	Wu et al. (2020)
67	MZ229774.1	<i>Amolops kohimaensis</i> Biju, Mahony, and Kamei	India: Nagaland	Patel et al. (2021)
68	MN953695.1	<i>Amolops deng</i> Jiang, Wang, and Che	China: Zayü, Tibet	Wu et al. (2020)
69	MN953696.1	<i>A. deng</i> Jiang, Wang, and Che	China: Zayü, Tibet	Wu et al. (2020)
70	MN953697.1	<i>A. deng</i> Jiang, Wang, and Che	China: Zayü, Tibet	Wu et al. (2020)
71	MN953698.1	<i>A. deng</i> Jiang, Wang, and Che	China: Zayü, Tibet	Wu et al. (2020)
72	MN953699.1	<i>A. deng</i> Jiang, Wang, and Che	China: Medog, Tibet	Wu et al. (2020)
73	MN953716.1	<i>Amolops nyingchiensis</i> Jiang, Wang, Xie, Jiang, and Che	China: Mainling, Tibet	Wu et al. (2020)
74	MN953718.1	<i>A. nyingchiensis</i> Jiang, Wang, Xie, Jiang, and Che	China: Medog	Wu et al. (2020)
75	MN953717.1	<i>A. nyingchiensis</i> Jiang, Wang, Xie, Jiang, and Che	China: Mainling, Tibet	Wu et al. (2020)
76	MN953715.1	<i>A. nyingchiensis</i> Jiang, Wang, Xie, Jiang, and Che	China: Mainling, Tibet	Wu et al. (2020)
77	MN953719.1	<i>A. nyingchiensis</i> Jiang, Wang, Xie, Jiang, and Che	China: Mainling, Tibet	Wu et al. (2020)
78	MN953664.1	<i>Amolops bellulus</i> Liu, Yang, Ferraris, and Matsui	China: Teng Chong Co., Yunnan	Wu et al. (2020)
79	FJ417127.2	<i>A. bellulus</i> Liu, Yang, Ferraris, and Matsui	China: Teng Chong Co., Yunnan	Stuart et al. (2010); Wu et al. (2020)

Appendix 1: (Continued).

Sr. No.	NCBI Accession no.	Species name	Collection locality	References
80	MN953665.1	<i>A. bellulus</i> Liu, Yang, Ferraris, and Matsui	China: Teng Chong Co., Yunnan	Wu et al. (2020)
81	FJ417126.2	<i>A. bellulus</i> Liu, Yang, Ferraris, and Matsui	China: Teng Chong Co., Yunnan	Stuart et al. (2010); Wu et al. (2020)
82	FJ417152.2	<i>Amolops iriodes</i> (Bain and Nguyen)	Vietnam: Vi Xuyen, Ha Giang	Stuart et al. (2010); Wu et al. (2020)
83	FJ417153.2	<i>A. iriodes</i> (Bain and Nguyen)	Vietnam: Vi Xuyen, Ha Giang	Stuart et al. (2010); Wu et al. (2020)
84	KR827703.1	<i>A. mengyangensis</i> Yu, Wu, and Yang	Vietnam: Sa Pa, Lao Cai	Grosjean et al., (2015); Patel et al. (2021)
85	KR827704.1	<i>Amolops mengyangensis</i> Yu, Wu, and Yang	Vietnam: Sa Pa, Lao Cai	Grosjean et al., (2015); Patel et al. (2021)
86	FJ417151.2	<i>A. mengyangensis</i> Yu, Wu, and Yang	Vietnam: Sa Pa, Lao Cai	Stuart et al. (2010); Patel et al. (2021)
87	FJ417147.2	<i>Amolops daorum</i> (Bain, Lathrop, Murphy, Orlov, and Ho)	Laos: Vieng Tong, Huaphahn	Stuart et al. (2010); Wu et al. (2020)
88	FJ417148.2	<i>A. daorum</i> (Bain, Lathrop, Murphy, Orlov, and Ho)	Laos: Vieng Tong, Huaphahn	Stuart et al. (2010); Wu et al. (2020)
89	MN953678.1	<i>A. daorum</i> (Bain, Lathrop, Murphy, Orlov, and Ho)	China: Jingdong, Yunnan	Wu et al. (2020)
90	MN953679.1	<i>A. daorum</i> (Bain, Lathrop, Murphy, Orlov, and Ho)	China: Jingdong, Yunnan	Wu et al. (2020)
91	MN953659.1	<i>Amolops archotaphus</i> (Inger and Chan-ard)	Thailand: Amphoe Chom Thong, Chiang Mai	Wu et al. (2020)
92	MN953660.1	<i>A. archotaphus</i> (Inger and Chan-ard)	Thailand: Doi Inthanon, Chiang Mai	Wu et al. (2020)
93	MN953661.1	<i>A. archotaphus</i> (Inger and Chan-ard)	Thailand: Doi Inthanon, Chiang Mai	Wu et al. (2020)
94	MK501809.1	<i>Amolops mengdingensis</i> Yu, Wu, and Yang	China: Mengding, Yunnan	Yu et al. (2019)
95	MK501810.1	<i>A. mengdingensis</i> Yu, Wu, and Yang	China: Mengding, Yunnan	Yu et al. (2019)
96	MK501808.1	<i>A. mengdingensis</i> Yu, Wu, and Yang	China: Mengding, Yunnan	Yu et al. (2019)
97	FJ417158.1	<i>Amolops akhaorum</i> Stuart, Bain, Phimmachak, and Spence	Laos: Vieng Phou Kha, Luang Namtha	Stuart et al. (2010); Wu et al. (2020)
98	FJ417159.1	<i>A. akhaorum</i> Stuart, Bain, Phimmachak, and Spence	Laos: Vieng Phou Kha, Luang Namtha	Stuart et al. (2010); Wu et al. (2020)
99	FJ417141.1	<i>Amolops compotrix</i> (Bain, Stuart, and Orlov)	Laos: Nakai	Stuart et al. (2010); Wu et al. (2020)
100	FJ417142.1	<i>A. compotrix</i> (Bain, Stuart, and Orlov)	Vietnam: Dak Gle, Kon Tum	Stuart et al. (2010); Wu et al. (2020)
101	FJ417164.1	<i>Amolops vitreus</i> (Bain, Stuart, and Orlov)	Laos: Phongsaly, Phongsaly	Stuart et al. (2010); Wu et al. (2020)
102	MN953739.1	<i>A. vitreus</i> (Bain, Stuart, and Orlov)	Laos: Phongsaly, Phou Dendin National Biodiversity Conservation Area	Wu et al. (2020)
103	FJ417145.1	<i>Amolops cucae</i> (Bain, Stuart, and Orlov)	Vietnam: Van Ban, Lao Cai	Stuart et al. (2010); Wu et al. (2020)
104	FJ417144.1	<i>A. cucae</i> (Bain, Stuart, and Orlov)	Vietnam: Van Ban, Lao Cai	Stuart et al. (2010); Wu et al. (2020)
105	MN953725.1	<i>Amolops wenshanensis</i> Yuan, Jin, Li, Stuart, and Wu	China: Xichou, Yunnan	Yuan et al. (2018); Wu et al. (2020)
106	MN953724.1	<i>A. wenshanensis</i> Yuan, Jin, Li, Stuart, and Wu	China: Xichou, Yunnan	Yuan et al. (2018); Wu et al. (2020)
107	FJ417129.1	<i>A. wenshanensis</i> Yuan, Jin, Li, Stuart, and Wu	China: Jinxiu, Guangxi	Stuart et al. (2010); Wu et al. (2020)
108	KU840605.1	<i>Amolops chaochin</i> Jiang, Ren, Lyu, and Li	China: Sichuan: Mt. Qingcheng	Wu et al. (2020); Jiang et al., (2021)
109	AB211477.1	<i>A. chaochin</i> Jiang, Ren, Lyu, and Li	China: Sichuan: Mt. Emei	Wu et al. (2020); Jiang et al. (2021)
110	MN953668.1	<i>A. chaochin</i> Jiang, Ren, Lyu, and Li	China: Sichuan: Anxian	Wu et al. (2020); Jiang et al. (2021)
111	MN953766.1	<i>A. chaochin</i> Jiang, Ren, Lyu, and Li	China: Gansu	Wu et al. (2020)
112	MN953669.1	<i>A. chaochin</i> Jiang, Ren, Lyu, and Li	China: Sichuan: Anxian	Wu et al. (2020); Jiang et al., (2021)
113	KX645666.1	<i>A. chaochin</i> Jiang, Ren, Lyu, and Li	China	Unpublished
114	MG991886.1	<i>Amolops chunganensis</i> (Pope)	China: Mt. Jinggang, Jiangxi	Lyu et al. (2018)
115	KX507310.1	<i>A. chunganensis</i> (Pope)	China: Mt. Wugong, Anfu, Jiangxi	Sung et al. (2016)

Appendix 1: (Continued).

Sr. No.	NCBI Accession no.	Species name	Collection locality	References
116	MN953762.1	<i>A. chunganensis</i> (Pope)	China: Chengkou, Chongqing	Wu et al. (2020)
117	MN953670.1	<i>A. chunganensis</i> (Pope)	China: Chengkou, Chongqing	Wu et al. (2020)
118	MN953671.1	<i>A. chunganensis</i> (Pope)	China: Fangxian, Hubei	Wu et al. (2020)
119	MN953666.1	<i>Amolops chayuensis</i> Sun, Luo, Sun, and Zhang	China: Baxoi, Tibet	Wu et al. (2020)
120	MN953667.1	<i>A. chayuensis</i> Sun, Luo, Sun, and Zhang	China: Baxoi, Tibet	Wu et al. (2020)
121	MK604877.1	<i>A. chayuensis</i> Sun, Luo, Sun, and Zhang	China: Xizang	Lyu et al. (2019) ; Khatiwada et al. (2020)
122	MN953704.1	<i>Amolops loloensis</i> (Liu)	China: Xichang, Sichuan	Wu et al. (2020)
123	MN953705.1	<i>A. loloensis</i> (Liu)	China: Yuexi, Sichuan	Wu et al. (2020)
124	MN953757.1	<i>A. loloensis</i> (Liu)	China: Xichang, Sichuan	Wu et al. (2020)
125	MN953756.1	<i>A. loloensis</i> (Liu)	China: Xichang, Sichuan	Wu et al. (2020)
126	AB211478.1	<i>A. loloensis</i> (Liu)	China: Sichuan	Matsui et al. (2006); Khatiwada et al. (2020)
127	EF453743.1	<i>A. loloensis</i> (Liu)	China: Xichang, Sichuan	Cai et al. (2007); Wu et al. (2020)
128	MN953700.1	<i>Amolops jinjiangensis</i> Su, Yang, and Li	China: Deqing, Yunnan	Wu et al. (2020)
129	MN953701.1	<i>A. jinjiangensis</i> Su, Yang, and Li	China: Chuxiong, Yunnan	Wu et al. (2020)
130	EF453741.1	<i>A. jinjiangensis</i> Su, Yang, and Li	China: Deqing, Yunnan	Cai et al. (2007); Wu et al. (2020)
131	MG991904.1	<i>Amolops tuberodepressus</i> Liu and Yang	China: Yunnan	Khatiwada et al. (2020)
132	MK604845.1	<i>Amolops shuichengicus</i> Lyu and Wang	China: Shuicheng County, Guizhou	Lyu et al. (2019)
133	MK604846.1	<i>A. shuichengicus</i> Lyu and Wang	China: Shuicheng County, Guizhou	Lyu et al. (2019)
134	MN953729.1	<i>A. tuberodepressus</i> Liu and Yang	China: Jingdong, Yunnan	Wu et al. (2020)
135	MN953730.1	<i>A. tuberodepressus</i> Liu and Yang	China: Jingdong, Yunnan	Wu et al. (2020)
136	MN953680.1	<i>Amolops granulosis</i> (Liu and Hu)	China: Dayi, Sichuan	Wu et al. (2020)
137	MN953681.1	<i>A. granulosis</i> (Liu and Hu)	China: Anxian, Sichuan	Wu et al. (2020)
138	MN953706.1	<i>Amolops mantzorum</i> (David)	China: Wolong, Sichuan	Wu et al. (2020)
139	MN953707.1	<i>A. mantzorum</i> (David)	China: Dayi, Sichuan	Wu et al. (2020)
140	AB211479.1	<i>A. mantzorum</i> (David)	China: Sichuan	Matsui et al. (2006); Khatiwada et al. (2020)
141	EF453742.1	<i>A. mantzorum</i> (David)	China	Unpublished
142	MN953764.1	<i>Amolops xinduiqiao</i> Fei, Ye, Wang, and Jiang	China: Kangding, Sichuan	Wu et al. (2020)
143	MN953765.1	<i>A. xinduiqiao</i> Fei, Ye, Wang, and Jiang	China: Kangding, Sichuan	Wu et al. (2020)
144	MN953712.1	<i>Amolops</i> "sp. 5"	Nepal: Rakshe Village, Mechi	Wu et al. (2020)
145	MN953713.1	<i>Amolops</i> "sp. 5"	Nepal: Mabu, Ilam	Wu et al. (2020)
146	MN953714.1	<i>Amolops</i> "sp. 5"	Nepal: Maimajhuwa, Ilam	Wu et al. (2020)
147	MN953733.1	<i>A. indoburmanensis</i> Dever, Fuiten, Konu, and Wilkinson	China: Pianma, Yunnan	Wu et al. (2020)
148	MN953734.1	<i>A. indoburmanensis</i> Dever, Fuiten, Konu, and Wilkinson	China: Pingbian, Yunnan	Wu et al. (2020)
149	MN953735.1	<i>A. indoburmanensis</i> Dever, Fuiten, Konu, and Wilkinson	China: Pingbian, Yunnan	Wu et al. (2020)
150	MN953731.1	<i>A. indoburmanensis</i> Dever, Fuiten, Konu, and Wilkinson	China: Tengchong, Yunnan	Wu et al. (2020)
151	MN953732.1	<i>A. indoburmanensis</i> Dever, Fuiten, Konu, and Wilkinson	China: Tengchong, Yunnan	Wu et al. (2020)
152	MN953738.1	<i>A. indoburmanensis</i> Dever, Fuiten, Konu, and Wilkinson	China: Gongshan, Yunnan	Wu et al. (2020)
153	AB211480.1	<i>A. indoburmanensis</i> Dever, Fuiten, Konu, and Wilkinson	China: Yunnan	Matsui et al. (2006); Khatiwada et al. (2020)
154	MN953736.1	<i>A. indoburmanensis</i> Dever, Fuiten, Konu, and Wilkinson	China: Pianma, Yunnan	Wu et al. (2020)
155	MN953737.1	<i>A. indoburmanensis</i> Dever, Fuiten, Konu, and Wilkinson	China: Pianma, Yunnan	Wu et al. (2020)
156	MN953662.1	<i>Amolops beibengensis</i> Jiang, Li, Zou, Yan, and Che	China: Medog, Tibet	Wu et al. (2020)
157	MN953663.1	<i>A. beibengensis</i> Jiang, Li, Zou, Yan, and Che	China: Medog, Tibet	Wu et al. (2020)
158	MN953710.1	<i>Amolops medogensis</i> Li and Rao	China: Medog, Tibet	Wu et al. (2020)
159	MN953711.1	<i>A. medogensis</i> Li and Rao	China: Medog, Tibet	Wu et al. (2020)
160	MK573813.1	<i>A. medogensis</i> Li and Rao	China, Xizang	Lyu et al. (2019)
161	MN953740.1	<i>Amolops wangyufani</i> Jiang	China: Zayü, Tibet	Wu et al. (2020)
162	MN953741.1	<i>A. wangyufani</i> Jiang	China: Zayü, Tibet	Wu et al. (2020)
163	ON025582.1	<i>Amolops chanakya</i> Saikia, Laskar, Dinesh, Shabnam, and Sinha	India: Arunachal Pradesh, West Kameng	Saikia et al. (2022a)
164	ON025581.1	<i>Amolops tawang</i> Saikia, Laskar, Dinesh, Shabnam, and Sinha	India: Arunachal Pradesh, Tawang	Saikia et al. (2022a)

Appendix 1: (Continued).

Sr. No.	NCBI Accession no.	Species name	Collection locality	References
165	ON462447.1	<i>Amolops wangyali</i> Mahony, Nidup, Streicher, Teeling, and Kamei	Bhutan	Mahony et al. (2022)
166	ON462446.1	<i>A. wangyali</i> Mahony, Nidup, Streicher, Teeling, and Kamei	Bhutan	Mahony et al. (2022)
167	MN953723.1	<i>Amolops yatseni</i> Lyu, Wang, and Wang	Vietnam: Sa Pa, Lao Cai	Wu et al. (2020)
168	MN953758.1	<i>A. yatseni</i> Lyu, Wang, and Wang	China: Jingxi, Guangxi	Wu et al. (2020)
169	DQ204486.1	<i>Amolops ricketti</i> (Boulenger)	China: Shanghang, Fujian	Orlov et al. (2006); Khatiwada et al. (2020)
170	MK263290.1	<i>Amolops yatseni</i> Lyu, Wang, and Wang	China: Zhongshan City, Guangdong	Lyu et al. (2019)
171	MK263250.1	<i>A. yatseni</i> Lyu, Wang, and Wang	China: Shangchuan Island, Guangdong	Lyu et al. (2019)
172	MN953748.1	<i>Amolops sinensis</i> Lyu, Wang, and Wang	China: Mao'er Shan, Guangxi	Wu et al. (2020)
173	MN953749.1	<i>A. sinensis</i> Lyu, Wang, and Wang	China: Mao'er Shan, Guangxi	Wu et al. (2020)
174	MK263262.1	<i>A. sinensis</i> Lyu, Wang, and Wang	China: Guangdong	Lyu et al. (2019); Khatiwada et al. (2020)
175	MN953775.1	<i>A. sinensis</i> Lyu, Wang, and Wang	China: Guidong, Hunan	Wu et al. (2020)
176	MN953763.1	<i>A. sinensis</i> Lyu, Wang, and Wang	China: Gongcheng, Guangxi	Wu et al. (2020)
177	MK263249.1	<i>Amolops albispinus</i> Sung, Wang, and Wang	China: Mt. Wutong, Guangdong	Sung et al. (2016); Lyu et al. (2019)
178	MK263248.1	<i>A. albispinus</i> Sung, Wang, and Wang	China: Mt. Wutong, Guangdong	Sung et al. (2016); Lyu et al. (2019)
179	KX507312.1	<i>Amolops yunkaiensis</i> Lyu, Wang, Liu, Zeng, and Wang	China: Yunkaienshan	Sung et al. (2016); Khatiwada et al. (2020)
180	MK263271.1	<i>A. yunkaiensis</i> Lyu, Wang, Liu, Zeng, and Wang	China: Yunkaishan Nature Reserve, Guangdong	Lyu et al. (2018), 2019
181	MK263277.1	<i>A. yunkaiensis</i> Lyu, Wang, Liu, Zeng, and Wang	China: Ehuangzhang Nature Reserve, Guangdong	Lyu et al. (2018), 2019
182	MN953742.1	<i>Amolops wuyiensis</i> (Liu and Hu)	China: Wuyishan, Fujian	Wu et al. (2020)
183	MN953761.1	<i>A. wuyiensis</i> (Liu and Hu)	China: Yiwu, Zhejiang	Wu et al. (2020)
184	MN953760.1	<i>A. wuyiensis</i> (Liu and Hu)	China: Wencheng, Zhejiang	Wu et al. (2020)
185	KX507304.1	<i>A. wuyiensis</i> (Liu and Hu)	China: Fujian	Sung et al. (2016); Khatiwada et al. (2020)
186	MN953743.1	<i>Amolops ricketti</i> (Boulenger)	China: Wuyishan, Fujian	Wu et al. (2020)
187	MN953759.1	<i>A. ricketti</i> (Boulenger)	China: Shicheng, Jiangxi	Wu et al. (2020)
188	MN953675.1	<i>Amolops daiyunensis</i> (Liu and Hu)	China: Daiyunshan, Fujian	Wu et al. (2020)
189	MN953676.1	<i>A. daiyunensis</i> (Liu and Hu)	China: Daiyunshan, Fujian	Wu et al. (2020)
190	MN953677.1	<i>A. daiyunensis</i> (Liu and Hu)	China: Daiyunshan, Fujian	Wu et al. (2020)
191	KX507306.1	<i>A. daiyunensis</i> (Liu and Hu)	China: Mt. Daiyu, Fujian	Sung et al. (2016); Khatiwada et al. (2020)
192	MN953689.1	<i>Amolops hongkongensis</i> (Pope and Romer)	China: Hong Kong	Wu et al. (2020)
193	MN953691.1	<i>A. hongkongensis</i> (Pope and Romer)	China: Hong Kong	Wu et al. (2020)
194	KX507317.1	<i>A. hongkongensis</i> (Pope and Romer)	China: Hong Kong	Sung et al. (2016); Khatiwada et al. (2020)
195	MN953690.1	<i>A. hongkongensis</i> (Pope and Romer)	China: Hong Kong	Wu et al. (2020)
196	MN953687.1	<i>Amolops hainanensis</i> (Boulenger)	China: Wuzhishan, Hainan	Wu et al. (2020)
197	MN953688.1	<i>A. hainanensis</i> (Boulenger)	China: Wuzhishan, Hainan	Wu et al. (2020)
198	KX507320.1	<i>A. hainanensis</i> (Boulenger)	China: Hainan	Sung et al. (2016); Khatiwada et al. (2020)
199	EF453744.1	<i>Amolops torrentis</i> (Smith)	China: Hainan	Wu et al. (2020)
200	MN953728.1	<i>A. torrentis</i> (Smith)	China: Diao Luo Shan Forest Park, Lingshui, Hainan	Wu et al. (2020)
201	MN953726.1	<i>Amolops spinaepectoralis</i> Inger, Orlov, and Darevsky	Vietnam: Ngoc Linh vicinity, Kon Tum	Wu et al. (2020)
202	MN953770.1	<i>A. spinaepectoralis</i> Inger, Orlov, and Darevsky	Vietnam: Tram Lap, Gia Lai	Wu et al. (2020)
203	MN953771.1	<i>A. spinaepectoralis</i> Inger, Orlov, and Darevsky	Vietnam: Central Highland, Ngok Linh, Kon Tum	Wu et al. (2020)
204	AF206456.1	<i>A. spinaepectoralis</i> Inger, Orlov, and Darevsky	Vietnam: Gia Lai	Chen et al. (2005); Khatiwada et al. (2020)
205	MN953727.1	<i>A. spinaepectoralis</i> Inger, Orlov, and Darevsky	Vietnam: Kon Ka Kinh, Gia Lai	Wu et al. (2020)
206	MN953769.1	<i>A. spinaepectoralis</i> Inger, Orlov, and Darevsky	Vietnam: Phong Dien Nature Reserve, Phong Dien, Thua Thien Hue	Wu et al. (2020)
207	MN953772.1	<i>A. spinaepectoralis</i> Inger, Orlov, and Darevsky	Vietnam: Phong Dien Nature Reserve, Phong Dien, Thua Thien Hue	Wu et al. (2020)
208	MN953768.1	<i>A. spinaepectoralis</i> Inger, Orlov, and Darevsky	Vietnam: Bana resort, Da Nang	Wu et al. (2020)
209	MN953672.1	<i>Amolops cremnobatus</i> Inger and Kottelat	Vietnam: Puhu National Reserve, Thanh Hoa	Wu et al. (2020)

Appendix 1: (Continued).

Sr. No.	NCBI Accession no.	Species name	Collection locality	References
210	MN953673.1	<i>A. cremnobatus</i> Inger and Kottelat	Vietnam: Puhu National Reserve, Thanh Hoa	Wu et al. (2020)
211	DQ204477.1	<i>A. cremnobatus</i> Inger and Kottelat	Vietnam: Khe Moi	Orlov et al. (2006); Khatiwada et al. (2020)
212	FJ417143.1	<i>A. cremnobatus</i> Inger and Kottelat	Laos: Kasi, Vientiane	Stuart et al. (2010); Wu et al. (2020)
213	MN953674.1	<i>A. cremnobatus</i> Inger and Kottelat	Thailand: Doi Phuka, Chom Poo Phuka nature trail, Nan	Wu et al. (2020)
214	MF061721.1	<i>Amolops gerutu</i> Chan, Abraham, Grismer, and Grismer	Malaysia: Gunung Tebu, Terengganu	Unpublished
215	MF061745.1	<i>Amolops australis</i> Chan, Abraham, Grismer, and Grismer	Malaysia: Endau-Rompin, Johor (Peta)	Unpublished
216	MF061749.1	<i>Amolops larutensis</i> (Boulenger)	Malaysia: Bukit Larut, Perak	Unpublished
217	MF061741.1	<i>A. larutensis</i> (Boulenger)	Malaysia: Gunung Bubu, Perak	Unpublished
218	MN953752.1	<i>Huia cavitympanum</i> (Boulenger)	Malaysia: Marak Parak, S. Tahobang, Kota Marudu, Sabah	Wu et al. (2020)
219	MN953751.1	<i>Babina hainanensis</i> (Fei, Ye, and Jiang)	China: Lingshui, Diao Luo Shan Forest Park, Hainan	Wu et al. (2020)
220	MN953753.1	<i>Rana jiemuxiensis</i> Yan, Jiang, Chen, Fang, Jin, Li, Wang, Murphy, Che, and Zhang	China: Jiemuxi, Hunan	Wu et al. (2020)
221	MN953755.1	<i>Odorrana jingdongensis</i> Fei, Ye, and Li	China: Jingdong, Yunnan	Wu et al. (2020)