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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

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Seven species of the Asian torrent frogs (genus *Amolops*) have previously been reported from the eastern Himalayan country of Bhutan. Species identifications from the region have been largely based on photographed animals with few voucher specimens available and no molecular sampling. Understanding the taxonomic status of Bhutan's torrent frogs has also been hampered by the poorly understood distributional limits of species from surrounding regions. Herein we utilised molecular phylogenetic and morphological data for vouchered specimens from Bhutan and provide a complete literature review of all *Amolops* populations reported from the country. Phylogenetic relationships were estimated by combining available sequence data (from GenBank) with newly generated sequences from recently collected Bhutanese *Amolops* populations. We also obtained archival DNA sequences from the type specimens of *Amolops formosus*, *A. himalayanus*, and *A. kaulbacki*, collected between 82 and 151 years ago. Our comparative analyses revealed a large, new (to science) species of the *Amolops viridimaculatus* group from eastern Bhutan. Morphological examinations of related taxa revealed that *A. senchalensis* from India is not a synonym of *A. marmoratus*. Molecular phylogenetic results supplemented by morphological data unambiguously demonstrate i) that *A. himalayanus* is present in eastern Nepal, ii) the presence of a previously undocumented population of *A. nepalicus* in eastern Nepal, iii) a 200 km range extention for *A. kaulbacki* into Yunnan, China, iv) that *A. gyirongensis* should be considered a junior subjective synonym of *A. formosus*, and v) that *A. splendissimus* from Vietnam should be considered a junior subjective synonym of *A. viridimaculatus*. Based on our results, we expand the *Amolops viridimaculatus* group to include nine species, including *A. formosus, A. himalayanus, A. kaulbacki*, and the new species described herein. We provisionally include a further three species in the *viridimaculatus* group based on morphology, *A. longimanus, A. nidorbellus,* and *A. senchalensis*. Combining our data with the literature review allowed us to identify several unidentified *Amolops* species from recent phylogenetic studies and remove nine frog species (including *Hyla, Sylvirana*, and seven *Amolops* species) from Bhutan's amphibian checklist. We recognise four species of *Amolops* in Bhutan, three of which cannot be confidently identified to the species level based on currently available data.

Keywords: Anura, taxonomy, Himalayas, conservation, vouchered-specimens

INTRODUCTION

The large radiation of Asian torrent or waterfall frogs, genus *Amolops* Cope, 1865 currently contains 72 valid species (Che et al., 2020; Wu et al., 2020; Jiang et al., 2021; Patel et al., 2021; Zeng et al., 2021; Zhang et al., 2021; Table 1) distributed throughout the hilly regions of mainland south-east Asia, much of southern and eastern China and along the southern Himalayas as far west as northern India (Dubois, 1974; Ray, 1999; Anders, 2002; Orlov et al., 2002; Wogan et al., 2008; Biju et al., 2010; Fei et al., 2012; IUCN Bangladesh, 2015; Chan et al., 2018; Streicher et al., 2020). Recent molecular phylogenetic analysis has identified eight distinct evolutionary radiations now treated as species groups, of which the *marmoratus*, *monticola* and *viridimaculatus* groups are represented in the southern Himalayas where they are broadly sympatric (Wu et al., 2020). Each of these species groups contain numerous species that are morphologically conserved and can be difficult or even impossible to reliably identify in the field. Several species remain poorly defined morphologically and the recent use of DNA sequence data as the primary means of species delimitation (Che et al., 2020; Wu et al., 2020) has complicated comparisons with older, morphology-based literature records. Nonetheless, there has been a flood of new species descriptions in recent years from the region (e.g. Zhao et al., 2005; Qi et al., 2019; Che et al., 2020; Khatiwada et al., 2020; Patel et al., 2021).

The Kingdom of Bhutan is a small country (38,394 km²) nestled between the Brahmaputra River Valley and

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the Tibetan Plateau in the eastern Himalayas, bordered by Tibet and the four Indian states of Arunachal Pradesh, Assam, Sikkim and West Bengal. The mountainous terrain with broad elevational range from 160 m up to 7,500 m a.s.l. (Ramashray & Dorji, 2011) creates varied environments with rich biodiversity potential (NBC, 2019). The amphibian fauna of Bhutan remained poorly studied, but a recent drive by local wildlife enthusiasts, academics, and research staff of the forest department has seen a rapid rise in the number of species reported from the country (DoFPS, 2020; Wangyal et al., 2020; 2021). Unfortunately, the amphibians of Bhutan are poorly represented in museum collections both nationally and internationally, and reports of species are largely based on anecdotal evidence and/or photographs of animals (Wangyal et al., 2020). The presence of morphologically cryptic species complexes and poorly described species named in the 19th and early 20th centuries in the region have contributed to a situation where many species identifications reported in the literature require taxonomic revision. Such revisions require the collection of vouchered specimens from the originally reported populations, direct morphological comparison to taxonomic descriptions in modern literature (when available) or type specimens (spread internationally across museum collections), or through molecular comparison with reliably identified published DNA sequences.

In this paper, we provide a comprehensive review of literature records for the genus *Amolops* from Bhutan to determine the basis of each reported population, and whether species identifications require revision. We sequenced mitochondrial DNA (mtDNA) from eleven specimens of *Amolops* collected in Bhutan (referable to the *marmoratus*, *monticola*, and *viridimaculatus* groups) to compare with available sequence data from other studies. Due to extensive confusion in the literature with respect to the identifications and taxonomic status of several poorly known *viridimaculatus* group species from the southern Himalayas, we also utilised a high-throughput DNA sequencing methodology to obtain mitochondrial data from the 82–151 year old type specimens of three species, *A. formosus* (Günther, 1876), *A. himalayanus* (Boulenger, 1888) and *A. kaulbacki* (Smith, 1940) from the collection of the Natural History Museum, London, UK. Our literature review demonstrates extensive confusion relating to the identification of *Amolops* species reported from Bhutan and allows us to recommend corrections to the national species checklist. Based on morphological and molecular analyses, we identified a new species of the *viridimaculatus* group from eastern Bhutan and we formally describe it herein. Morphological studies of related taxa demonstrate that one synonym, *Rana senchalensis* Chanda, 1987, required taxonomic re-evaluation and is here provisionally treated as valid. Molecular data obtained from the historical type specimens provide insights into the systematic status and distributions of several species, highlight some species identification errors on GenBank, and also necessitate the synonymisation of two species, one from Tibet Autonomous Region, China and one from Vietnam and adjacent Yunnan, China.

MATERIALS & METHODS

Taxon sampling

Fieldwork was carried out in Bhutan by Tshering Nidup, Nub Tshering Lepcha, Penjor, Dawa Gyeltshen, Namgay Dorji and Namgay Rinchen during the spring to monsoon season (March to August) between 2015 and 2019 (Research Permit No. 205474365BFF950D3594C; MTA Reference No. NBC/BRD-7/2019-2020). Specimens were collected during opportunistic visual surveys during both daytime and night-time. Search time was predominantly spent in the vicinity of streams flowing through natural (forested) habitat. Global Positioning System (GPS) coordinates and elevation (metres above sea level) were determined using a Garmin eTrex 10 hand-held GPS device. Representative specimens from each population were photographed live either in-situ (in the field, as observed when encountered) or ex-situ under staged conditions within 24 hours of collection, to provide data on colouration in life. Animals were humanely euthanised using the anaesthetics, aqueous solution of ethyl 3-aminobenzoic methane sulfonate salt (MS 222), or lidocaine. Specimens were fixed in absolute (99.99 %) ethanol (EtOH) for 12 hours and then transferred to 70 % EtOH for long-term storage in the collections of the Sherubtse College Zoology Museum (SCZM), Royal University of Bhutan, Trashigang, Bhutan. A portion of thigh muscle was excised from representative specimens of each population for molecular analyses. Tissue samples were stored at -20 °C in PCR grade absolute EtOH. For this study, we collected eleven specimens of *Amolops* from three localities in eastern Bhutan that we tentatively identified in the field as *A*. aff. *himalaynaus* (n=9), *A*. cf. *gerbillus* (n=1), and *A.* cf. *putaoensis* (n=1).

Museum collection acronyms mentioned in this study are as follows: AMNH (American Museum of Natural History, New York, USA); AMS (Australian Museum, Sydney, Australia); BMNH (British Museum [Natural History]: now the NHMUK - Natural History Museum, London, UK); BNHS (Bombay Natural History Society, Mumbai, Maharashtra, India); CAS (Department of Herpetology, California Academy of Sciences, San Francisco, California, USA); CDZMTU (Central Department of Zoology, Tribuvan University, Kirtipur, Kathmandu, Nepal); CIB (Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu, Sichuan, China); CUMZ (Chulalongkorn University Museum of Zoology, Bangkok, Thailand); FMNH (Field Museum of Natural History, Chicago, Illinois, USA); GXNU (Guangxi Normal University, Guilin, Guangxi, China); KIZ (Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan, China); HLNP (Hoang Lien National Park headquarters, Lao Cai, Vietnam); KUHE (Graduate School of Human and Environmental Studies, Kyoto University, Kyoto, Japan); KU KUH (University of Kansas Natural History Museum, Lawrence, Kansas, USA); LSUHC (La Sierra University Herpetological Collection, Riverside, California, USA); MCBT (Madras Crocodile Bank Trust, Mahabalipuram, Tamil Nadu, India); MNHN (Museum National d'Histoire Naturelle, Paris, France); NRM (Swedish Museum of Natural History, Stockholm, Sweden); RMB (Lee Kong

Chian Natural History Museum, Singapore); ROM (Centre for Biodiversity and Conservation Biology, Royal Ontario Museum, Toronto, Ontario, Canada); SDB (Systematics Lab, University of Delhi, Delhi, India); SWFU (Southwest Forestry University, Kunming, Yunnan, China); SYNU (Shenyang Normal University, Shenyang, Liaoning, China); SYS (The Museum of Biology, Sun Yat-sen University, Guangzhou, Guangdong, China); VNMN (Vietnam National Museum of Nature, Hanoi, Vietnam); WIIADA (Wildlife Institute of India, Dehradun, Uttarakhand, India); ZISP (Zoological Institute of St. Petersburg, St. Petersburg, Russia); ZSIK (Zoological Survey of India, Kolkata, West Bengal, India). Authors abbreviations are as follows: ECT (Emma C. Teeling); JWS (Jeffrey W. Streicher); RGK (Rachunliu G. Kamei); SM (Stephen Mahony); TN (Tshering Nidup).

Molecular analyses

Whole genomic DNA was extracted from the recently collected tissue samples from Bhutan using a DNeasy Blood and Tissue Kit® (Qiagen) following manufacturer's instructions. A mtDNA sequence that comprises a portion of the 16S ribosomal subunit (*16S*) gene was selected for phylogenetic analyses since it is available for all bar seven currently valid species of *Amolops* at the time of analyses (8 April 2022; see Table 1). We amplified either a continuous portion of the 12S ribosomal subunit, complete tDNA-Valine (*t-Val*) and a portion of the 16S ribosomal subunit (*12S-tVal-16S*) for seven samples (SCZM 2015.03.15.4; SCZM 2015.03.15.5; SCZM 2019.07.18.1; SCZM 2019.07.18.2; SCZM 2019.08.02.1; SCZM 2019.07.20.1; SCZM 2019.07.27.1) using the primers 12SAL(f), 16S2000H(r), LX12SN1(f) and LX16S1R(r) (Zhang et al., 2008), or just the *16S* sequences for four samples (SCZM 2014.07.19.1; SCZM 2015.06.29.1; SCZM 2015.03.28.1; SCZM 2015.03.28.2) using primers 16SAR(f) and 16SBR(r) (Palumbi, 1996). For the *12S-tVal-16S* sequences, we used a PCR reaction recipe of 25 µL aliquots comprised of 1.5 µL extracted DNA, 10 µL PCR grade water, 12.5 µL MyTaq™ Mix (Bioline) and 0.5 µL each of forward and reverse primers (10 µM). For the *16S* only sequences, we used a PCR reaction recipe of 20 µL aliquots comprised of: 2.0 µL extracted DNA; 5 µL Promega 5X Green GoTaq® Flexi Buffer; 4 µL 25 mM Mg CL_2; 0.2 µL dNTPs (10 mM each); 1 µL each of forward and reverse primers (10 mM concentrations); 0.1 µL GoTaq® G2 Flexi DNA Polymerase (Promega); 6.7 µL PCR grade H_2O. The following PCR reaction protocols were used: (1) for 12SAL, 16S2000H, LX12SN1 and LX16S1R primers, TD 55: initial denaturation at 95 °C for 3 minutes, 10 cycles of denaturation at 95 °C for 30 seconds, annealing at 65 °C (with a reduction of 1 °C each cycle) for 40 seconds, extension at 72 °C for 1 minute, followed by 35 cycles of denaturation at 95 °C for 3 minutes, annealing at 55 °C for 40 seconds, extension at 72 °C for 1 minute; (2) for 16SAR and 16SBR primers, initial denaturation at 95 °C for three minutes, then 35 cycles of denaturation at 95 °C for 30 seconds, annealing at 50 °C for 30 seconds, and extension at 72 °C for 1 minute. The final extension for both protocols was at 72 °C for 10 minutes. PCR products were purified, and Sanger sequenced for forward and reverse strands using PCR primers by the Core Research Labs at the Natural History Museum (London; NHMUK). Chromatograms of raw sequences were checked for quality, trimmed and assembled into contigs using Geneious® V.8.1.9 (Kearse et al., 2012) and sequences were checked on BLAST (Altschul et al., 1990) using the NCBI BLAST (website http://blast.ncbi.nlm.nih.gov) for verifying approximate identities against the GenBank nucleotide database (Benson et al., 2017).

For archival DNA sampling, small portions of muscle tissue (typically the triceps femoris or adductor magnus + gracilis major) from type specimens of three species, *A. formosus* (holotype, BMNH 1947.2.4.18), *A. himalayanus* (syntype, BMNH 1947.2.3.83) and *A. kaulbacki* (holotype, BMNH 1940.6.1.1; Table 1) were excised from the ventral thighs and stored in absolute EtOH at -20 °C for approximately one week before beginning DNA extractions. Samples were digested at 55 °C in a solution of 20 µL of proteinase K and 180 µL of lysis buffer (100 mM NaCL, 100 mM Tris, 25 mM EDTA, 0.5 % SDS), and checked at 10-minute intervals until fully digested. DNA extraction was performed using Sera-Mag™ SpeedBeads™ (Thermo Fisher Scientific) at a concentration of 2.1X (200 µL of digested tissue to 420 µL of beads) and eluted into 40 µL of 10 mM Tris Buffer. We quantified the amount of double-stranded DNA (dsDNA) using 1 µL of each extraction with a Qubit® dsDNA HS (High Sensitivity) Assay Kit on a Qubit® 2.0 Fluorometer (Thermo Fisher Scientific) which indicated the following concentrations: *A. formosus* (0.80 ng/µL), *A. himalayanus* (0.56 ng/µL), and *A. kaulbacki* (0.19 ng/µL). Given these relatively low concentrations, we used the entirety of the DNA extract as template for the library construction which was approximately 31.2 ng (*A. formosus*), 21.8 ng (*A. himalayanus*), and 7.41 ng (*A. kaulbacki*). We used a modified version of the shotgun preparation described in Streicher et al. (2016) to produce Illumina NextSeq® libraries. We used NEBNext® DNA Library Prep Master Mix Sets (New England Biolabs Inc.) for end-repair (NEB #E6050), dA-tailing (NEB #E6053), ligation (NEB #E6056) and PCR (NEB #E7805) to construct libraries. We did not fragment DNA prior to library construction because archival DNA from ethanol-preserved specimens is already highly degraded (McGuire et al., 2018).

End-repair reactions each contained 5 µL 10X Reaction Buffer, 2.5 µL enzyme mix, 2.5 µL PCR water and 40 µL degraded museum specimen DNA. Reactions were held for 30 minutes at 20 °C and then were cleaned using the same bead protocol as the DNA extraction (2.1X) eluting the cleaned, end-repaired fragments into 21 µL 10 mM Tris Buffer. For dA-tailing, each reaction contained 2.5 µL of 10X Reaction Buffer, 1.5 µL Klenow fragment, and 21 µL of cleaned, end-repaired sample. The dA-tailing reactions were held for 30 minutes at 37 °C and then were cleaned using the standard bead protocol (2.1X) and eluted into 12.5 µL of 10 mM Tris Buffer. For ligation, each reaction contained 5 µL of 5X Reaction Buffer, 2.5 µL Quick T4 DNA Ligase, 5 µL 1mM adapter (unique to each sample), and 12.5 µL cleaned, dA-tailed reaction. Adapters sequences for each sample are reported in Streicher et al. (2016)

Table 1. Complete list of valid *Amolops* species (as per 12 April 2022) giving relevant details for GenBank/Sequence Read Archive sequences (*12S-tVal-16S*, or *16S* only) used in maximum likelihood analysis, and *nd2* and *co1* used in *p*-distance analyses. NA, not applicable; NSA, no sequences available; Dist., District; Au. Co., Autonomous County; N.R., Nature Reserve; N.P., National Park; Mt., Mount; Prov., Province.

Species	Locality	Specimen #	*12S-tVal-16S / 16S*	*nd2*	*co1*	Reference & Notes
A. adicola Patel, Garg, Das, Stuart & Biju, *2021*	India: Arunachal Pradesh: Upper Siang Dist.	BNHS 6121 (holotype)	MZ229772.1	-	-	Patel et al. (2021)
A. afghanus (Günther, 1858a)	China: Yunnan: Yingjiang Co.	SYS a003852	MK604837.1	-	-	Lyu et al. (2019b); as *A.* "*marmoratus"* on GenBank
A. akhaorum Stuart, Bain, Phimmachak & Spence, 2010	Laos: LuangNamtha: Vieng Phou Kha.	FMNH 271355 (paratype)	FJ417158.2	-	-	Stuart et al. (2010); as *A.* "sp. BLS-2009" on GenBank
A. albispinus Sung, Hu, Wang, Liu & Wang, 2016	China: Guangdong: Shenzhen City: Mt. Wutong.	SYS a003452 (paratype)	MK263247.1	-	-	Lyu et al. (2019a)
A. aniqiaoensis Dong, Rao & Lü, 2005 (in Zhao et al., 2005)	China: Tibet.	KIZ 011136	MN953658.1	-	-	Wu et al. (2020)
A. archotaphus (Inger & Chan-ard, 1997)	Thailand: Chiang Mai: Doi Inthanon.	CUMZ A 2000.62	FJ417124.1	-	-	Stuart et al. (2010)
A. assamensis Sengupta, Hussain, Choudhury, Gogoi, Ahmed & Choudhury, 2008	NA	NSA	-	-	-	NA
A. australis Chan, Abraham, Grismer & Grismer, 2018	Malaysia: Peta, Endau-Rompin N.P.	LSUHC 7673 (paratype)	MF061745.1	-	-	"Chan K.O. direct submission" on GenBank, published in Chan et al. (2017); as *A.* "*larutensis*" on GenBank
A. beibengensis Jiang, Li, Zhou, Yan & Che, 2020 (in Che et al., 2020)	China: Tibet: Medog.	KIZ 016397 (paratype)	MN953662.1	MN958721.1	MN961359.1	Wu et al. (2020: as *A.* "sp. 2")
A. beibengensis	China: Tibet: Medog.	KIZ 011061 (topotype)	-	MN958722.1	MN961360.1	Wu et al. (2020: as *A.* "sp. 2")
A. bellulus Liu, Yang, Ferraris & Matsui, 2000	China: Yunnan: Lushui Co.	KIZ 9810021 (paratype)	DQ204473.1	-	-	Ngo et al. (2006)
A. chakrataensis Ray, 1992	NA	NSA	-	-	-	NA
A. chaochin Jiang, Ren, Lyu & Li, 2021	China: Sichuan: Chongzhou City	CIB 116971 (holotype)	MZ702027.1	-	-	Jiang et al. (2021); as *A.* "sp. c JLR-2021" on GenBank
A. chayuensis Sun, Luo, Sun & Zhang, 2013	China: Tibet: Baxoi Co.	SYS a007509	MK573820.1	-	-	Lyu et al. (2019b)
A. chunganensis (Pope, 1929)	China: Jiangxi: Mt. Jinggang.	SYS a004212	MK263263.1	-	-	Lyu et al. (2019a)
A. compotrix (Bain, Stuart & Orlov, 2006)	Vietnam: Kon Tum: Dak Glei Dist.	ZISP A7367 (paratype)	FJ417142.2	-	-	Stuart et al. (2010)
A. cremnobatus Inger & Kottelat, 1998	Vietnam: Nghe An: Khe Moi.	ROM 14528	DQ204477.1	-	-	Ngo et al. (2006)
A. cucae (Bain, Stuart & Orlov, 2006)	Vietnam: Lao Cai: Van Ban Dist.	AMNH 168727 (paratype)	FJ417144.2	-	-	Stuart et al. (2010)
A. daiyunensis (Liu & Hu, 1975)	China: Fujian: Dehua Co.	KIZ F93069 (topotype)	DQ204479.1	-	-	Ngo et al. (2006)
A. daorum (Bain, Lathrop, Murphy, Orlov & Ho, 2003)	Laos: Huaphahn: Vieng Tong Dist.	FMNH 255353	FJ417147.2	-	-	Stuart et al. (2010)
A. deng Jiang, Wang & Che, 2020 (in Che et al., 2020)	China: Tibet: Zayü.	KIZ 014116 (paratype)	MN953695.1	-	-	Wu et al. (2020: as *A.* "sp. 1")
A. formosus (Günther, 1876)	India: Meghalaya: "Khassya".	BMNH 1947.2.4.18 (holotype, *A. formosus*)	**SAMN28238801**	**SAMN28238801**	**SAMN28238801**	This study; Appendix I for assembled sequences.
A. formosus	Nepal: Prov. 1: Sankhuwasabha: Dobhan.	CDZMTU 0145	MT124521.1	-	-	Khatiwada et al. (2020); as *A.* "*nepalicus*" on GenBank
A. formosus [syn. *A. gyirongensis* Jiang, Wang, Wang, Pan & Che, 2020 (in Che et al., 2020)]	China: Tibet: Gyirong.	KIZ 012533 (paratype, *A. gyirongensis*)	MN953682.1	MN958739.1	MN961382.1	Wu et al. (2020: as *A.* "sp. 4")
A. formosus [syn. *A. gyirongensis*]	China: Tibet: Gyirong.	KIZ 012537 (paratype, *A. gyirongensis*)	MN953683.1	MN958740.1	MN961383.1	Wu et al. (2020: as *A.* "sp. 4")
A. formosus [syn. *A. gyirongensis*]	China: Tibet: Gyirong.	KIZ 012534 (paratype, *A. gyirongensis*)	MN953684.1	MN958741.1	MN961384.1	Wu et al. (2020: as *A.* "sp. 4")
A. formosus [syn. *A. gyirongensis*]	China: Tibet: Gyirong.	KIZ 012535 (paratype *A. gyirongensis*)	MN953685.1	MN958742.1	MN961385.1	Wu et al. (2020: as *A.* "sp. 4")
A. formosus [syn. *A. gyirongensis*]	China: Tibet: Gyirong.	KIZ 012536 (holotype *A. gyirongensis*)	MN953686.1	MN958743.1	MN961386.1	Wu et al. (2020: as *A.* "sp. 4")
A. cf. *gerbillus* (Annandale, 1912)	Bhutan: Trashigang: Bodidrang Chhu/Stream	SCZM 2015.06.06.1 (tissue no. D18)	**ON462437.1**	-	-	This study

Species	Locality	Specimen #	*12S-tVal-16S / 16S*	*nd2*	*co1*	Reference & Notes
A. gerutu Chan, Abraham, Grismer & Grismer, 2018	Malaysia: Terengganu: Gunung Tebu.	RMB 21077 (topotype)	MF061721.1	-	-	"Chan K.O. direct submission" on GenBank, published in Chan et al. (2017); as *A.* "*larutensis*" on GenBank
A. granulosus (Liu & Hu, 1961)	China: Sichuan: Mt. Guangwu.	SYS a005399	MK573811.1	-	-	Lyu et al. (2019b)
A. hainanensis (Boulenger, 1900)	China: Hainan: Lingshui.	KIZ 970512	DQ204481.1	-	-	Ngo et al. (2006)
A. himalayanus (Boulenger, 1888a)	India: West Bengal: Darjeeling.	BMNH 1947.2.3.83 (syntype)	**SAMN28238802**	-	-	This study; Appendix I for assembled sequence.
A. himalayanus	Nepal: Prov. 1: Ilam Dist.: Rakse Village.	SH 2789	MN953712.1	MN958770.1	MN961414.1	Wu et al. (2020: as *A.* "sp. 5") ["SH" abbreviation not explained]
A. himalayanus	Nepal: Prov. 1: Ilam Dist.: Mabu.	KIZ 040227	-	MN958771.1	MN961415.1	Wu et al. (2020: as *A.* "sp. 5")
A. himalayanus	Nepal: Prov. 1: Ilam Dist.: Maimajhuwa.	KIZ 040228	-	MN958772.1	MN961416.1	Wu et al. (2020: as *A.* "sp. 5")
A. hongkongensis (Pope & Romer, 1951)	China: Hong Kong.	ROM 16300 (topotype)	AF206453.1	-	-	Chen et al. (2005)
A. indoburmanensis Dever, Fuiten, Konu& Wilkinson, 2012	Myanmar: Chin: Mindat.	CAS 234720 (topotype)	MG909571.1	-	-	Arifin et al. (2018)
A. iriodes (Bain & Nguyen, 2004)	Vietnam: Ha Giang: Vi Xuyen Dist.	AMNH 163926 (paratype)	FJ417152.2	-	-	Stuart et al. (2010)
A. jaunsari Ray, 1992	NA	NSA	-	-	-	NA
A. jinjiangensis Su, Yang & Li, 1986	China: Yunnan: Mt. Gaoligong.	SYS a004571	MK573801.1			Lyu et al. (2019b)
A. kaulbacki (Smith, 1940)	Myanmar: Kachin: Pangnamdim.	BMNH 1940.6.1.1 (holotype)	**SAMN28238803**	-	-	This study; Appendix I for assembled sequence.
A. kaulbacki	China: Yunnan: Pianma.	SCUM 050402CHX	MN953736.1	MN958793.1	MN961437.1	Wu et al. (2020: as *A. "viridimaculatus"*)
A. kaulbacki	China: Yunnan: Pianma.	SCUM 050403CHX	MN953737.1	MN958794.1	MN961438.1	Wu et al. (2020: as *A.* "*viridimaculatus*")
A. kohimaensis Biju, Mahony & Kamei, 2010	India: Nagaland: Kohima Dist.	WIIADA 751 (topotype)	MZ229774.1	-	-	Patel et al. (2021)
A. larutensis (Boulenger, 1899a)	Malaysia: Perak.	KUHE 15488	AB211484.1	-	-	Matsui et al. (2006)
A. lifanensis (Liu, 1945)	China: Sichuan: Lixian Co.	SYS a005374	MK573809.1	-	-	Lyu et al. (2019b)
A. loloensis (Liu, 1950)	China: Sichuan: Zhaojue City.	SYS a005346	MK604854.1	-	-	Lyu et al. (2019b)
A. longimanus (Andersson, 1939)	NA	NSA	-	-	-	NA
A. mahabharatensis Khatiwada, Shu, Wang, Zhao, Xie & Jiang, 2020	Nepal: Bagmati: Chitwan Dist: Hattibang.	CDZMTU 0110 (holotype)	MT124507.1	-	-	Khatiwada et al. (2020)
A. mantzorum (David, 1872)	China: Sichuan: Fengtongzhai.	SYS a005365	MK573808.1	-	-	Lyu et al. (2019b)
A. marmoratus (Blyth, 1855)	Myanmar: Mon.	CAS 240593 (topotype)	JF794456.1	-	-	Dever et al. (2012)
A. medogensis Li & Rao, 2005 (in Zhao et al., 2005)	China: Tibet: Medog Co.	SYS a006657 (topotype)	MK573813.1	-	MK568328.1	Lyu et al. (2019b)
A. medogensis	China: Tibet: Medog Co.	SYNU 04II6219 (paratype)	-	MN958769.1	MN961413.1	Wu et al. (2020)
A. medogensis	China: Tibet: Medog Co.	SYNU 04II6216 (paratype)	-	MN958768.1	MN961412.1	Wu et al. (2020)
A. medogensis	not mentioned.	SYS a007531	-	-	MK568332.1	"Lyu et al. 2019[b]" according to GenBank, but not mentioned in this paper.
A. medogensis	not mentioned.	SYS a007530	-	-	MK568331.1	"Lyu et al. 2019[b]" according to GenBank, but not mentioned in this paper.
A. medogensis	China: Tibet: Medog Co.	KIZ 06638 (topotype)	-	-	KU243077.1	Jiang et al. (2016)
A. medogensis	China: Tibet: Medog Co.	KIZ 06635 (topotype)	-	-	KU243076.1	Jiang et al. (2016)
A. mengdingensis Yu, Wu & Yang, 2019	China: Yunnan: Mengding.	KIZ 20160266 (holotype)	MK501809.1	-	-	Yu et al. (2019)
A. mengyangensis Wu & Tian, 1995	Vietnam: Lao Cai: Sa Pa.	MNHN[?] 1999.5811	KR827703.1	-	-	Grosjean et al. (2015: museum collection not explicitly stated in reference)
A. minutus Orlov & Ho, 2007	NA	NSA	-	-	-	NA

Species	Locality	Specimen #	*12S-tVal-16S / 16S*	*nd2*	*co1*	Reference & Notes
A. monticola (Anderson, 1871)	India: Sikkim: Tarku Forest Block	WIIADA 544	MZ229773.1	-	-	Patel et al. (2021)
A. nepalicus Yang, 1991	Nepal: Prov. 1: Ilam: Mabu.	KIZ 040269	MN953750.1	-	-	Wu et al. (2020: as *A.* "sp. 7")
A. nepalicus	Nepal: Prov. 1: Taplejung: Lamatar.	CDZMTU 0135	MT124519.1	-	-	Khatiwada et al. (2020); as *A.* "*formosus*" on GenBank
A. nidorbellus Biju, Mahony & Kamei, 2010	NA	NSA	-	-	-	NA
A. nyingchiensis Jiang, Wang, Xie, Jiang & Che, 2016 (in Jiang et al., 2016)	China: Tibet: Medog Co.	SYS a006679 (topotype)	MK573814.1	-	-	Lyu et al. (2019b)
A. ottorum Pham, Sung, Pham, Le, Ziegler & Nguyen, 2019	NA	NSA	-	-	-	NA
A. pallasitatus Qi, Zhou, Lyu, Lu & Li, 2019 (in Qi et al., 2019)	China: Tibet: Dinggyê Co.	SYNU 1507034 (paratype)	MK573816.1	-	-	Qi et al. (2019); as *A.* "sp. n. ZTL-2019" on GenBank
A. panhai Matsui & Nabhitabhata, 2006	Myanmar: Tanintharyi: Dewei Dist.	CAS 229816	MG909606.1	-	-	Arifin et al. (2018)
A. putaoensis Gan, Qin, Lwin, Li, Quan, Liu & Yu, 2020	Myanmar: Kachin: Putao Co.	GXNU QT 20170200 (holotype)	MT901382.1	-	-	Gan et al. (2020b)
A. cf. *putaoensis*	Bhutan: Trashigang: Khaling Chhu/Stream (also called Mongnagkhola).	SCZM 2015.06.29.1 (tissue no. D17)	**ON462438.1**	-	-	This study
A. ricketti (Boulenger, 1899b)	China: Jiangxi: Wuyishan N.R.	SYS a001605	KX507303.1	-	-	Sung et al. (2016)
A. senchalensis (Chanda, 1987)	NA	NSA	-	-	-	NA
A. shuichengicus Lyu & Wang, 2019 (in Lyu et al., 2019b)	China: Guizhou: Shuicheng Co.	SYS a004956 (paratype)	MK604845.1	-	-	Lyu et al. (2019b)
A. sinensis Lyu, Wang & Wang, 2019	China: Guangdong: Shimentai N.R.	SYS a007107 (holotype)	MK263299.1	-	-	Lyu et al. (2019a)
A. spinapectoralis Inger, Orlov &Darevsky, 1999	Vietnam: Kon Tum: Ngoc Linh.	ROM 27424	DQ204488.1	-	-	Ngo et al. (2006)
A. teochew Zeng, Wang, Lyu & Wang, 2021	China: Guangdong: Mt Fenghuang	SYS a008705 (holotype)	MZ447970.1	-	-	Zeng et al. (2021)
A. torrentis (Smith, 1923)	China: Hainan: Lingshui.	KIZ 970543	DQ204489.1	-	-	Ngo et al. (2006)
A. tuanjieensis Gan, Yu & Wu, 2020	China: Yunnan: Gengma Dai and Wa Au. Co.	GXNU YU 110003 (paratype)	MN832772.1	-	-	Gan et al. (2020a)
A. tuanjieensis	China: Yunnan: Gengma Dai and Wa Au. Co.	GXNU YU 110005 (holotype)	MN832773.1	-	-	Gan et al. (2020a)
A. tuberodepressus Liu & Yang, 2000	China: Yunnan: Mt. Ailao.	SYS a003900	MK573797.1	-	-	Lyu et al. (2019b)
A. viridimaculatus (Jiang, 1983)	China: Yunnan: Mt. Gaoligong.	SYS a003753	MK573793.1	-	MK568310.1	Lyu et al. (2019b)
A. viridimaculatus	China: Yunnan: Mt. Gaoligong.	SYS a003754	MK573794.1	-	MK568311.1	Lyu et al. (2019b)
A. viridimaculatus	China: Yunnan: Mt. Gaoligong.	SYS a003812 (topotype)	MK604835.1	-	MK605596.1	Lyu et al. (2019b)
A. viridimaculatus	China: Yunnan: Mt. Gaoligong.	SYS a003813 (topotype)	MK604836.1	-	MK605597.1	Lyu et al. (2019b)
A. viridimaculatus	China: Yunnan: Tengchong.	KIZ 93501	DQ204490.1	-	-	Ngo et al. (2006)
A. viridimaculatus	China: Yunnan: Tengchong.	KIZ 048487	MN953731.1	MN958788.1	MN961434	Wu et al. (2020)
A. viridimaculatus	China: Yunnan: Tengchong.	KIZ 048488	MN953732.1	MN958789.1	MN961434	Wu et al. (2020)
A. viridimaculatus	China: Yunnan: Pingbian.	KIZ 047019	MN953734.1	MN958791.1	-	Wu et al. (2020)
A. viridimaculatus	China: Yunnan: Pingbian.	KIZ 047020	MN953735.1	MN958792.1	-	Wu et al. (2020)
A. viridimaculatus	China: Yunnan: Pianma.	SCUM 050423CHX	MN953733.1	MN958790.1	MN961436.1	Wu et al. (2020)
A. viridimaculatus	China: Yunnan: Gongshan.	CAS 242607	MN953738.1	-	-	Wu et al. (2020); *A.* "*medogensis*" on CAS Herpetology Collection Database
A. viridimaculatus	China: Yunnan: no locality given.	"C-green 05"	AB211480.1	-	-	Matsui et al. (2006)
A. viridimaculatus [syn. *A. splendissimus* Orlov & Ho, 2007]	Vietnam: Lao Cai: Bat Xat Dist.	VNMN 010923 [EBU 85309]	MZ484725.1	-	-	Zhang et al. (2021)
A. viridimaculatus [syn. *A. splendissimus*]	Vietnam: Lao Cai: Bat Xat Dist.	AMS R188526 [EBU 85336]	MZ484726.1	-	-	Zhang et al. (2021)
A. viridimaculatus [syn. *A. splendissimus*]	Vietnam: Lao Cai: Bat Xat Dist.	HLNP2017100900016 [EBU 95337]	MZ484727.1	-	-	Zhang et al. (2021)

Species	Locality	Specimen #	*12S-tVal-16S / 16S*	*nd2*	*co1*	Reference & Notes
A. viridimaculatus [syn. *A. caelumnoctis* Rao & Wilkinson, 2007]	China: Yunnan: Luchun Co.	SWFU 003995 [Yuan 16267]	MZ484728.1	-	-	Zhang et al. (2021)
A. viridimaculatus [syn. *A. caelumnoctis*]	China: Yunnan: Luchun Co.	SWFU 004525 [Yuan 16268]	MZ484729.1	-	-	Zhang et al. (2021)
A. viridimaculatus [syn. *A. caelumnoctis*]	China: Yunnan: Wenshan Co.	SWFU 004524 [Yuan 16447]	MZ484730.1	-	-	Zhang et al. (2021)
A. vitreus (Bain, Stuart & Orlov, 2006)	Laos: Phongsaly: Phongsaly Dist.	FMNH 258187 (paratype)	FJ417164.2	-	-	Stuart et al. (2010)
A. wangyali sp. nov.	Bhutan: Trashigang: Jere Chhu/Stream.	Specimen not collected (tissue no. D4)	**ON462439.1**	-	-	This study
A. wangyali sp. nov.	Bhutan: Trashigang: Jere Chhu/Stream.	Specimen not collected (tissue no. D7)	**ON462440.1**	-	-	This study
A. wangyali sp. nov.	Bhutan: Trashigang: Jere Chhu/Stream.	SCZM 2015.03.28.1 (tissue no. D10; field no. TND011)	**ON462446.1**	-	-	This study
A. wangyali sp. nov.	Bhutan: Trashigang: Jere Chhu/Stream.	SCZM 2015.03.28.2 (tissue no. D11; field no. TND012)	**ON462447.1**	-	-	This study
A. wangyali sp. nov.	Bhutan: Trashigang: Bodidrang Chhu/Stream.	SCZM 2019.07.18.1 [field no. MW 11585] (holotype)	**ON462441.1**	-	-	This study
A. wangyali sp. nov.	Bhutan: Trashigang: Bodidrang Chhu/Stream.	SCZM 2019.07.18.2 [field no. MW 11587] (paratype)	**ON462442.1**	-	-	This study
A. wangyali sp. nov.	Bhutan: Trashigang: Bodidrang Chhu/Stream.	SCZM 2019.08.02.1 [field no. SC0034] (paratype)	**ON462445.1**	-	-	This study
A. wangyali sp. nov.	Bhutan: Trashigang: Rongthong.	SCZM 2019.07.20.1 [field no. SC0001]	**ON462443.1**	-	-	This study
A. wangyali sp. nov.	Bhutan: Trashigang: Kanglung.	SCZM 2019.07.27.1 [field no. SC0017]	**ON462444.1**	-	-	This study
A. wangyufani Jiang, 2020 (in Che et al., 2020)	China: Tibet: Zayü.	KIZ 014067 (paratype)	MN953740.1	MN958796.1	MN961440.1	Wu et al. (2020: as *A.* "sp. 3")
A. wangyufani	China: Tibet: Zayü.	KIZ 014068 (holotype)	-	MN958797.1	MN961441.1	Wu et al. (2020: as *A.* "sp. 3")
A. wenshanensis Yuan, Jin, Li, Stuart & Wu, 2018	China: Guangxi: Jingxi City.	KU KUH 292045 (paratype)	FJ417129.2	-	-	Stuart et al. (2010)
A. wuyiensis (Liu & Hu, 1975)	China: Anhui: Qingyang.	[CIB] QLY53	KF771291.1	-	-	Xia et al. (2014)
A. xinduqiao Fei, Ye, Wang & Jiang, 2017	China: Sichuan: Kangding.	"KIZ041127" (fide Wu et al. 2020) / "KIZ 014127" (fide Fei et al. 2017) (paratype)	MN953764.1	-	-	Wu et al. (2020)
A. yarlungzangbo Jiang, Wang, Li, Qi, Li & Che, 2020 (in Che et al., 2020)	China: Tibet: Medog.	KIZ 014086 (paratype)	MN953744.1	-	-	Wu et al. (2020: as *A.* "sp. 6")
A. yatseni Lyu, Wang & Wang, 2019 (in Lyu et al., 2019a)	China: Guangdong: Zhongshan City.	SYS a006807 (holotype)	MK263290.1	-	-	Lyu et al. (2019a)
A. yunkaiensis Lyu, Wang, Liu, Zeng & Wang, 2018 (in Lyu et al., 2018)	China: Guangdong: Ehuangzhang N.R.	SYS a003979 (paratype)	MK263253.1	-	-	Lyu et al. (2019b)

and we used indexes 20, 22, and 23 on *A. formosus*, *A. himalayanus*, and *A. kaulbacki*, respectively. Reactions were held for 15 minutes at 20 °C, and then pooled together (also with some additional samples not reported here, see below) for size selection. We performed size selection using a BluePippin™ (Sage Science) and 2 % Agarose Gel Cassette (No. BDF2010) with internal size standards. Pooled ligations were cleaned using the standard bead protocol (2.1X) and eluted into 30 µL of 10 mM Tris, which was mixed with 10 µL of BluePippin™ size standard before being loaded into the sample well of the BluePippin™. We selected for a size range of 270–370 base pairs (bp) in order to target DNA insert sizes that were < 300 bp in length. We then performed an enrichment PCR using the size-selected sample by combining 6 µL water, 1 µL each of Illumina TruSeq® primers (i5 and i7), 10 µL Q5® HotStart HiFi PCR Master Mix, and 2 µL of sample. This recipe was repeated 15 times to use all 30 µL of size-selected adapter-ligated samples extracted from the BluePippin™. We ran the 15 PCRs along with a negative control for 18 cycles. Following the PCR-enrichment, we combined all reactions and cleaned them using the standard bead protocol (2.1X), eluting into 15 µL of 10 mM TRIS. We confirmed the successful enrichment of the pooled library using an Agilent® TapeStation System.

The three *Amolops* samples were processed with 45 additional anuran and squamate archival DNA samples (results will be discussed in detail elsewhere; Mahony

et al., in litt.). Sequencing was performed on an Illumina NextSeq® 500 using a mid-output paired end 150 kit (300 cycles) at the Core Research Labs at the NHMUK. Sequencing output was demultiplexed and converted into FASTQ format using the bcl2fastq v2.15.0.4 software from Illumina® (https://github.com/brwnj/bcl2fastq). Resulting paired-end FASTQ files were then processed through Illumiprocessor using Trimmomatic (Faircloth, 2013; Del Fabbro et al., 2013), to remove low quality bases and adapter contamination.

Sequence assembly for the three archival *Amolops* samples was performed by mapping read 1, read 2, and singleton reads to a reference mtDNA sequence. Reference mapping was conducted in Geneious® V.8.1.9 using the 'Map to reference' feature with the 'Medium sensitivity / Fast' setting and fine tuning of up to five iterations. Morphologically, *A. formosus*, *A. himalayanus* and *A. kaulbacki* most closely resemble *viridimaculatus* species group taxa, so *A. viridimaculatus* (Jiang, 1983), GenBank number DQ204490.1 was selected as the reference sequence. This sequence also represented the longest continuous sequence of the *12S-tVal-16S* for a *viridimaculatus* species group taxon available on GenBank which maximised the area for read mapping. We then inferred the *12S-tVal-16S* sequence of each type specimen by exporting the consensus sequence of all mapped reads.

Sequencing errors, environmental contamination and crosstalk (index hopping) can cause issues for consensus sequence inference when using reference mapping. These issues can be problematic in regions with low read coverage, when mapped reads are relatively short and when a reference sequence is not available for a closely related species. To mitigate erroneous mapping of reads, the following quality control measures were taken to avoid assembling chimeric sequences: i) we used a reference sequence from a morphologically-similar species (see above), ii) reads suspected to be erroneous because they contained many polymorphisms not observed in overlapping and adjacent mapped reads (particularly an issue for the *A. formosus* sample) were deleted from the assembly, and iii) reads from low coverage regions were compared (using BLAST) to the whole GenBank nucleotide database to confirm that they were most similar to other available *Amolops* sequences (instead of human and/or bacterial contaminants, or the other anuran and squamate species included in the NextSeq® 500 run).

Homologous sequences that comprise either *12S-tVal-16S*, or just the partial *16S* sequence were downloaded from GenBank, targeting at least one representative of all available species and particularly sequences for type specimens when available. As exceptions, we included all available sequences for *A. viridimaculatus* and all unique sequences for other *viridimaculatus* group taxa in order to identify additional populations of *A. formosus*, *A. himalayanus* and *A. kaulbacki* which may have been sequenced in previous studies but misidentified (Table 1). Downloaded sequences were generated in the following studies: Chen et al. (2005); Matsui et al. (2006); Ngo et al. (2006); Stuart et al. (2010); Dever et al. (2012); Xia et al. (2014); Grosjean et al. (2015); Sung et al. (2016); Arifin et al. (2018); Lyu et al. (2019a; 2019b); Qi et al. (2019); Yu et al. (2019); Gan et al. (2020a; 2020b); Khatiwada et al. (2020); Wu et al. (2020); Jiang et al. (2021); Patel et al. (2021); Zeng et al. (2021); Zhang et al. (2021). The dataset comprising newly generated and downloaded sequences was aligned using MUSCLE (Edgar, 2004) in MEGA7 (Kumar et al., 2016; Tamura & Nei, 1993) with default settings. The alignment was visualised in MEGA7 and ambiguously aligned regions were further adjusted by eye where necessary to ascertain homology. Phylogenetic relationships were estimated using RAxML-HPC2 (Stamatakis, 2014) on XSEDE (CIPRES platform: Miller et al., 2010), using default settings with the GTR CAT model on an unpartitioned alignment and 1000 rapid bootstrap (bs.) replicates. The resulting maximum likelihood phylogenetic tree was viewed using FigTree (Rambaut, 2009). The south-eastern China clade comprising *A. spinapectoralis* Inger, Orlov & Darevsky, 1999, and the *hainanensis*, *daiyunensis* and *ricketti* species groups, has been demonstrated to be the sister taxon to the clade containing all remaining *Amolops* species in phylogenetic studies that utilised high-throughput sequencing techniques (i.e. 330 loci in Wu et al. [2020]; 242 nuclear loci + mitogenomes in Zeng et al. [2020]). Therefore, we rooted the tree ("user selected" option in FigTree) with the south-eastern China clade.

Sequence comparisons involving additional mitochondrial genes were required to compare *A. gyirongensis* Jiang, Wang, Wang, Pan & Che, 2020 (in Che et al., 2020) to *A. formosus* due to limited *16S* data from the holotype of *A. formosus*. We used a NADH dehydrogenase 2 (*nd2*) and a cytochrome c oxidase subunit 1 (*co1*) sequence (MN958739.1 & MN961382.1, respectively; Wu et al., 2020) from a paratype (KIZ012533) of *A. gyirongensis* as the reference sequences for mapping archival DNA reads from the holotype of *A. formosus*. Read mapping was performed on Geneious as described above. The resulting consensus (assembled) sequences of *A. formosus* were aligned (as described above) against all available *nd2* and *co1* sequences on GenBank for other *viridimaculatus* species group taxa (Table 1): comparative sequences were generated in the following studies: Jiang et al. (2016); Lyu et al. (2019b); Wu et al. (2020). Uncorrected *p*-distances were generated for the resulting *nd2* and *co1* alignments in MEGA7 using default settings (Kumar et al., 2016; Tamura & Nei, 1993), to estimate distance between *A. gyirongensis* and the holotype of *A. formosus* relative to intraspecific and interspecific distances within and between other valid *viridimaculatus* species group taxa. All newly generated Sanger sequences and Illumina® reads are available on GenBank and the Sequence Read Archive (Leinonen et al., 2010) with the accession numbers ON462437–ON462447, and SAMN28238801–SAMN28238803, respectively (Table 1). Assembled sequence contigs used in analyses for the arcDNA samples are given in Appendix I.

Taxonomy and morphology

Specimens of geographically relevant species were directly examined from the following museum collections: BMNH, BNHS, CAS, SDB, ZSIK; images of type specimens were also obtained from NRM (Appendix II). Due to a prevalence of

misidentifications in the literature for *Amolops* (SM, pers. obs.), for species that could not be directly examined, relevant taxonomic literature is cited for each morphological character used in the 'Morphological comparisons' section. Only characters verified on all examined specimens are utilised to represent the new and known species in the 'Morphological comparisons' sections. Sex and maturity of specimens were confirmed by direct examination of the gonads. Sex is not provided for the character/s being discussed where characters are compared based on a combination of male, female and juvenile specimens. To assess dermal microstructures such as asperities coverage, the entire skin surface (of all examined specimens) was viewed under binocular microscope. All measurements on specimens examined in this study were made by SM using digital calipers, in millimetres rounded to the nearest 0.1 mm. Measurements were taken on the right side of the specimen, except when a character was damaged, in which case the measurement was taken on the left side (as noted in text). In the 'Morphological comparisons' section, percentages given for min.–max. ranges are rounded up or down to the nearest whole number. Morphometric abbreviations used in the text and tables are as follows: 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 (FIIIL); 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 (FIIIW); maximum disc widths of fingers I–IV (FIDW, FIIDW, FIIIDW, FIVDW); fourth toe width, taken dorsally on digit proximal to disc (TIVW); maximum disc widths of toes I–V (TIDW, TIIDW, TIIIDW, 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 tibio-tarsal 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 & Heyer (1997). To supplement morphometric data for the type series of the newly described species, SVL and SHL measurements for four males and four females from Nidup et al. (2016) are included in the 'Morphological comparisons' section as these standard measurements are not expected to be excessively subject to methodological differences between the studies (Hayek et al., 2001). We sequenced four of the measured animals in Nidup et al. (2016: i.e. TND005, TND008 [both not collected], TND011 [SCZM 2015.03.28.1], TND012 [SCZM 2015.03.28.2]) to confirm that they represent the new species described herein.

Map

The software Quantum GIS (QGIS v.2.14.3-Essen) was used to make a topographic map using the 250 m spatial resolution Shuttle Radar Topography Mission (SRTM) layer available from DIVA-GIS (http://www.diva-gis.org), and other basic layers from the Natural Earth Quick Start Kit (http://www.naturalearthdata.com). GPS coordinates and elevation given herein for localities reported in literature without this information were estimated using Google Maps (https://maps.google.com/, accessed April 2021). All coordinates plotted on the map are given in Table 2.

RESULTS & DISCUSSION

Molecular phylogenetics

The shotgun high-throughput sequencing of the three archival DNA samples was successful. These results demonstrate that standard DNA library preparation is a viable method for obtaining archival DNA sequence data from museum specimens. Further discussion of our findings for other taxa and suggestions for improving the protocol will be discussed elsewhere. Total post trimming reads for each sample were 1,914,338 for *A. formosus*, 7,525,416 for *A. himalayanus* and 32,886,900 for *A. kaulbacki*. The final *12S-tVal-16S* alignment used in the phylogenetic analyses had a total length of 1401 bp, in which the sequences of *A. himalayanus* and *A. kaulbacki* were complete, however, the *A. formosus* sequence had a total of 497 bp of missing data. The consensus sequences were generated using variable coverage depths from 2–777 reads per nucleotide for the three samples (read depth 2–59, n=146 total reads for *A. formosus*; read depth 8–120, n=614 total reads for *A. himalayanus*; read depth 29–777, n=3125 total reads for *A. kaulbacki*). The consensus sequence for the holotype of *A. formosus* (collected ca. 1870 based on the timing of Jerdon's visit to 'Khasia' fide, Mahony et al., 2018) and a syntype of *A. himalayanus* (collected sometime between 1870 and 1888; SM, unpublished) were identical to *16S* sequences identified as *A. formosus* in Kathiwada et al. (2020) and as *Amolops* "sp. 5" in Wu et al. (2020), respectively, generated

Table 2. Localities plotted in the distribution map (Fig. 7) for *Amolops wangyali* sp. nov. and for populations suspected to be conspecific with this species but requiring further taxonomic attention. NA. not applicable.

Species	Country	State	District	Locality	GPS	Elevation [m a.s.l.]	GPS / elevation notes	Reference
A. wangyali sp. nov.	Bhutan	N/A	Trashigang	"Jere Chhu (27°12'21.90"N, 91°36'12.20"E), Khaling, … 2,073 m"	27.206083, 91.603389	2073	GPS & elevation as reported in the reference.	Nidup et al. (2016)
A. wangyali sp. nov.	Bhutan	N/A	Trashigang	"Khaling [Chhu/Stream] (27°11´26.18´´N,91° 36´09.40´´E)"	27.190606, 91.602611	2070	GPS as reported in the reference, elevation & coordinate conversion based on plotted GPS point in Google Maps.	Nidup et al. (2016)
A. wangyali sp. nov.	Bhutan	N/A	Trashigang	"Bodidrang [Chhu/ Stream] (27°17´20.33´´N, 91°30´56.28´´E)"	27.288981, 91.515633	1640	GPS as reported in the reference, elevation & coordinate conversion based on plotted GPS point in Google Maps.	Nidup et al. (2016); Limbu et al. (2020)
A. wangyali sp. nov.	Bhutan	N/A	Trashigang	"Kanglung: Road from Sherubtse College to Trashigang: Bodidrang [Chhu/Stream]: Namla"	27.27223, 91.53129	1750	GPS & elevation as reported in the reference.	Streicher et al. (2020)
A. wangyali sp. nov.	Bhutan	N/A	Trashigang	"Kanglung: Road from Sherubtse College to Trashigang: Bodidrang [Chhu/Stream]: Namla"	27.27023, 91.53043	1738	GPS & elevation as reported in the reference.	Streicher et al. (2020)
A. wangyali sp. nov.	Bhutan	N/A	Trashigang	"Rongthong"	27.2808, 91.53937	1520	GPS & elevation as reported in the reference.	Streicher et al. (2020)
A. wangyali sp. nov.	Bhutan	N/A	Trashigang	Thragom, a small stream above Kanglung BHU Hospital, Kanglung Gewog (village block)	27.28031, 91.51456	1950	GPS & elevation as reported herein.	This study.
A. wangyali sp. nov.	Bhutan	N/A	Trashiyangtse	"Serkang Chu, … Choetenkora town [27°36'50.00"N,91° 29'32.00"E], … Trashiyangtse District … 1745 m"	27.605298, 91.493766	1745	GPS as reported in the reference, plotted and converted in Google Maps.	Wangyal (2013)
A. cf. *wangyali*	India	Arunachal Pradesh	West Kameng	Bompu (1950–2200 m a.s.l.), Eaglenest Wildlife Sanctuary	27.066411, 92.405947	1950	GPS taken as Bompu camp ca. 1950 m a.s.l., coordinates estimated from Google Maps.	Athreya (2006)
A. cf. *wangyali*	India	Arunachal Pradesh	West Kameng	"New Khellong (1250 m)", Eaglenest Wildlife Sanctuary	27.022367, 92.414367	1250	GPS taken as "New Khellong" 27° 01.342 N 92° 24.862 E, 1270 m from Sondhi & Kunte (2016) though the plotted point is at an elevation of ca. 1040 m a.s.l. in Google Maps.	Athreya (2006)
A. cf. *wangyali*	India	Arunachal Pradesh	West Kameng	"Sessni (1250 m)", Eaglenest Wildlife Sanctuary	27.047500, 92.418611	1250	GPS taken as "Sessni (27° 02'51" N, 92° 25'07" E; 1250 m)" from Agarwal et al. (2010), plotted and coordinate converted in Google Maps.	Athreya (2006)

from recently collected tissue samples of populations from eastern Nepal. The consensus sequence for the holotype of *A. kaulbacki* (collected between 82 and 84 years ago; Smith, 1940) was identical to two *16S* sequences from Wu et al. (2020) identified as *A. "viridimaculatus"* from Yunnan, China (Fig. 1).

Our overall phylogeny of *Amolops* resolved an identical systematic arrangement of major species groups identified elsewhere (Wu et al., 2020; Zeng et al., 2020; Fig. 1). Support for monophyly was moderate to high (bs. 76–100) for most species groups except the *mantzorum* group (bs. 63) as observed elsewhere (Wu et al., 2020, Fig. 1). Within the various species groups the topology of our tree differed slightly from the mitochondrial tree in Wu et al. (2020, fig. 1), and support values for relationships within the species groups were generally low (Fig. 1). Our phylogeny placed the recently named *A. pallasitatus* Qi Zhou, Lyu, Lu & Li, 2019 (in Qi et al., 2019) as a member of the *viridimaculatus* group, which was previously placed within the broader concept of the '*mantzorum* group' where it was sister to a clade containing *A. viridimaculatus* and *A. medogensis* Li & Rao, 2005 (in Zhao et al., 2005) (Qi et al., 2019). The expanded phylogeny of Wu et al. (2020) included *A. viridimaculatus* and *A. medogensis* and four unidentified taxa labelled as *Amolops* sp. 2–5, the six lineages forming a distinct clade which they defined as the '*viridimaculatus* group'. Wu et al's. (2020) analyses did not include sequences for *A. pallasitatus* or *A. formosus* (from Qi et al., 2019 & Khatiwada et al., 2020, respectively) which might not have been available to them due to the short timeframe in which the three studies were published. Our study demonstrates that specimen numbers associated with sequences for three of the unidentified species (*Amolops* sp. 2–4) in Wu et al. (2020) match three of the recently described species in Che et al. (2020). Our archival DNA sequencing also confirms the identity of the lineage *Amolops* "sp. 5" of Wu et al. (2020; labelled *A.* "cf. *monticola*" in Che et al., 2020: fig. 59) represented by sequences from three localities in eastern Nepal ("Rakse Village", "Mabu", and "Maimajhuwa" in Ilam District, Province No. 1) as *A. himalayanus* (Fig. 1), representing the first genetically verified populations of this species from Nepal.

Amolops kaulbacki is another enigmatic species

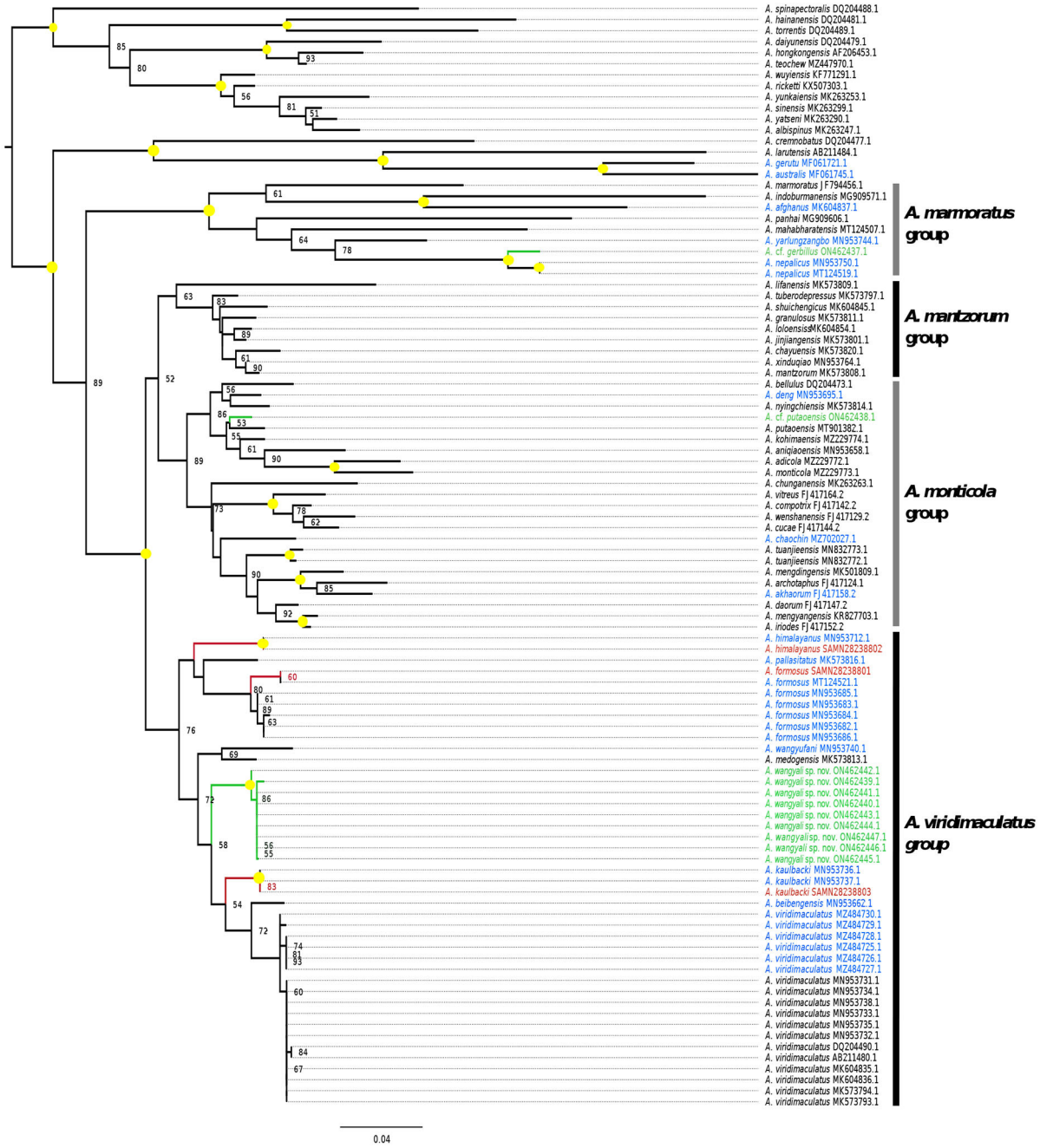

Figure 1. Maximum likelihood phylogeny based on the *12S-tVal-16S* genes for the genus *Amolops* showing the systematic position of the eleven Bhutan samples (green) and the archival DNA sequences for the three type specimens (red). Species identifications in blue differ from those given in GenBank which were misidentified, not identified, or required updating based on the results of this study (Table 1). GenBank/Sequence Read Archive numbers follow species names. Bootstrap support values ≥95 are represented by yellow spots, values >50 are given next to relative nodes, values <50 are not given.

described based on two specimens from Pangnamdim (27° 42’ N, 97° 54’ E), Kachin State in northern-most Myanmar (Smith, 1940). Aside from a questionable report of this species from north-east India (see Lalronunga et al., 2020), no verifiable subsequent accounts of this species are known since its original description (Smith, 1940). Our archival DNA sequencing of the holotype allowed us to correct the identification of two sequences previously identified as *A. “viridimaculatus”* from China (Wu et al., 2020). These two sequences are reported to have come from “Pianma”, Yunnan, which could either refer to Pianma Town, Zhenyuan County, Puer Prefecture-level City, central Yunnan Province (according to Google Maps), or more likely Pianma Township (ca. 26° 01.9' N, 98° 38.01' E), situated close to the China-Myanmar border, in Lushui County, Nujiang Prefecture, north-western Yunnan Province (Liu et al., 2000). The latter locality lies ca. 200 km southeast of the type locality of *A. kaulbacki*, and represents a new country record for China and a second verified locality for the species. Wu et al. (2020) also included a sequence from *A. viridimaculatus* sensu stricto (hereafter s.s.) from “Pianma” Yunnan, indicating that the two species might even be sympatric at this locality (assuming the authors were not referring to a different

place called "Pianma"), and Liu et al. (2000) mentioned that *A. "viridimaculatus"* was collected at Pianma (Lushui) along with the type specimens of *A. bellulus* (Liu et al., 2000). We recommend that specimens in museum collections previously identified as *A. "viridimaculatus"* from western Yunnan be re-examined to determine which species they represent. We also recommend that authors of molecular phylogenetic studies either provide specific locality information (e.g. GPS coordinates) for all newly sequenced specimens, or refer to a permanent source for this information (e.g. a publication/accessible online museum collection database) to enable georeferencing of sequenced specimens.

The recently collected eastern Bhutan populations of *Amolops* we sequenced represent three distantly related species. One of the species (formally described below as new to science) is nested within the *viridimaculatus* group though its relationship with other members of the group is not clear due to poor support for internal nodes within this clade. Our analysis demonstrates that the *viridimaculatus* group now contains the following nine species confirmed by DNA sequence data: *A. baibengensis* Jiang, Li, Zhou, Yan & Che, 2020 (in Che et al., 2020), *A. formosus* (including *A. gyirongensis*, see taxonomic discussion below), *A. himalayanus*, *A. kaulbacki*, *A. medogensis*, *A. pallasitatus, A. viridimaculatus* (including *A. splendissimus* Orlov & Ho, 2007, see taxonomic discussion below), *A. wangyufani* Jiang, 2020 (in Che et al., 2020) and the new species described below (Fig. 1). The second Bhutan species (SCZM 2015.06.06.1 [tissue no. D18]), collected from Bodidrang Chhu/Stream, Trashigang District, is resolved as sister to the closely related Nepal population identified as *A. nepalicus* Yang, 1991 (Khatiwada et al., 2020; Fig. 1). The Bhutan population lies geographically closer to the type locality of *A. gerbillus* (Annandale, 1912) (360 km to the east) than to the nearest reported locality of *A. nepalicus* (the type locality, ca. 417 km to the west). Until further comparisons can be made, we recommend the use of "*A.* cf. *gerbillus*" for populations representing the *marmoratus* group from Bhutan. Our phylogenetic analysis (Fig. 1) demonstrates that the sequence from "Mabu", Ilam District, Province No. 1, Nepal referred to as *Amolops* "sp. 7" in Wu et al. (2020) is conspecific with the species identified as *A. nepalicus* in Khatiwada et al. (2020). This adds a third population to the known distribution of this species (Khatiwada et al., 2021) which lies ca. 76 km south-east of the nearest previously reported locality (type locality), and only ca. 5 km from the border with West Bengal State, India, where this species is likely to be present in the mountainous Darjeeling and Kalimpong districts. The third Bhutan species (SCZM 2015.06.29.1 [tissue no. D17]), collected from Khaling Chhu (27.190606, 91.602611, ~2,070 m a.s.l), Trashigang District, included in our phylogenetic analyses is a member of the *monticola* group. Low bootstrap support obtained for relationships within this group prevent us from determining its closest relative, but the analysis suggests that it might be closest to *A. putaoensis* (Gan et al., 2020) from northern Myanmar (Gan et al., 2020b; Fig. 1).

At the time of writing (06 July 2021), GenBank records for *Amolops* were difficult to navigate due to species identification errors and a large number of sequences for unidentified species. For example, the *16S* sequences identified as *A. "nepalicus"* on GenBank from Khatiwada et al. (2020) represented *A. formosus*, and their *A. "formosus"* sequences represented *A. nepalicus* (Table 1). The sequences on GenBank generated in Wu et al. (2020) identified as *Amolops* "sp. 1–7" must now be updated to reflect the new species descriptions in Che et al. (2020) and the results presented herein as follows: *Amolops* "sp. 1" = *A. deng* Jiang, Wang & Che, 2020 (in Che et al., 2020); *Amolops* "sp. 2" = *A. baibengensis*; *Amolops* "sp. 3" = *A. wangyufani*; *Amolops* "sp. 4" = *A. gyirongensis* (herein considered a synonym of *A. formosus*, see discussion below); *Amolops* "sp. 5" = *A. himalayanus*; *Amolops* "sp. 6" = *A. yarlungzangbo* Jiang, Wang, Li, Qi, Li & Che, 2020 (in Che et al., 2020); *Amolops* "sp. 7" = *A. nepalicus* (Table 1). Outdated and erroneous species identifications are common with GenBank sequences (Mahony et al., 2017: suppl. info.; Mahony & Kamei, 2022; Streicher et al., 2020; Fig. 1; Table 1). Until such time as GenBank incorporates required corrections identified in published literature, the responsibility of maintaining the accuracy of GenBank data is retained by the submitting authors (see Mahony & Kamei, 2022 for detailed discussion).

The taxonomic status of *Amolops gyirongensis*

The assembled *16S* sequence from the *A. formosus* holotype and sequences from recently collected samples identified as this species from Nepal (Khatiwada et al., 2020) comprised of 139 bp of homologous sequence and were found to be identical in nucleotide composition. The same 139 bp sequence of *16S* obtained from the holotype of *A. formosus* was found to be 99–100 % identical to those of the recently described *A. gyirongensis* from Tibet, though the missing data in our alignment produced the erroneous impression on the phylogenetic tree that these taxa are as deeply diverged from each other as other sister taxa on the tree (Fig. 1). A BLAST comparison of a *16S* sequence of *A. formosus* from Khatiwada et al. (2020: MT124521.1, 434 bp length) against homologous sequences for all five available *A. gyirongensis* samples (Table 1) demonstrated that they are only marginally divergent (98.2–98.6 % similar).

To further test whether *A. formosus* and *A. gyirongensis* are genetically distinct we used *nd2* and *co1* sequences (Wu et al., 2020) for a paratype (KIZ012533) of *A. gyirongensis* as reference sequences for mapping archival DNA reads from the holotype of *A. formosus*. For the *nd2* gene, 93 *A. formosus* reads mapped to the reference, and the consensus sequence had a total length of 572 bases (excluding two short stretches of missing data; read depth 2–27 reads). Uncorrected *p*-distance analyses on a *nd2* alignment comprising all available *viridimaculatus* group sequences (572 homologous nucleotide loci), demonstrated that the *A. formosus* holotype differed from *A. gyirongensis* sequences by only 2.1–2.6 % (Table S1). The lowest interspecific *p*-distance for *nd2* between *A. formosus* and other *viridimaculatus* group taxa was 12.1 % (i.e. 13.1 % from *A. baibengensis*, 12.2–12.8 % from *A.*

himalayanus, 12.1 % from *A. medogensis*, 12.4–14.0 % from *A. viridimaculatus*, 12.1–12.2% from *A. wangyufani*). The minimum interspecific *p*-distance observed using this *nd2* alignment was 6.6 % both between *A. viridimaculatus* and *A. baibengensis* and between *A. medogensis* and *A. wangyufani*.

For the *co1* gene, 87 *A. formosus* reads mapped to the reference, and the consensus sequence had a total length of 539 bases (excluding two short stretches of missing data; read depth 2–54 reads). Uncorrected *p*-distance analyses on a *co1* alignment comprising all available *viridimaculatus* group sequences (448 homologous nucleotide loci) demonstrated that the *A. formosus* holotype differed from *A. gyirongensis* sequences by only 2.2 % (Table S2). The lowest interspecific *p*-distance for *co1* between *A. formosus* and other *viridimaculatus* group taxa was 8.0 % (i.e. 9.6 % from *A. baibengensis*, 8.7 % from *A. himalayanus*, 8.7–8.9 % from *A. medogensis*, 8.3–8.5 % from *A. viridimaculatus*, 8.0 % from *A. wangyufani*). The minimum interspecific *p*-distance observed using this alignment dataset is 5.6 % each between *A. viridimaculatus* and *A. baibengensis, A. medogensis* and *A. wangyufani* and *A. viridimaculatus* and *A. kaulbacki*. Our results demonstrate that the genetic distance based on mitochondrial DNA sequences between the holotype of *A. formosus* and the type series of *A. gyirongensis* is significantly lower than that seen between closely related species in the *viridimaculatus* species group and is likely representative of intraspecific variation within a single species. The archival DNA sequencing of the *A. formosus* holotype did not produce homologous reads to compare nuclear sequence data between the two populations. We recommend considering *A. gyirongensis* as a junior subjective synonym of *A. formosus* (also see 'Taxonomic accounts' section below).

The taxonomic status of *Amolops splendissimus* and *Amolops caelumnoctis*

Our phylogeny identified a second taxonomic issue in the *viridimaculatus* group based on sequences obtained from GenBank, where six *Amolops splendissimus* Orlov & Ho, 2007 sequences were nested within a clade otherwise comprising *A. "viridimaculatus"*. The *A. splendissimus* sequences were recently published in a study that aimed to resolve the taxonomic status of *A. splendissimus* named from northern Vietnam and the morphologically indistinct species *A. caelumnoctis* Rao & Wilkinson, 2007 named from neighbouring Yunnan, China (Zhang et al., 2021). Zhang et al. (2021) concluded, based on morphological and molecular *16S* data, that *A. caelumnoctis* represents a junior subjective synonym of *A. splendissimus*. Their phylogeny included only two sequences of other *viridimaculatus* group taxa, *A. medogensis* and *A. "viridimaculatus"* (MN953737: identified in our study as *A. kaulbacki*; Fig. 1). Our phylogeny, that includes all sequences identified as *A. "viridimaculatus"* and *A. splendissimus* available on GenBank demonstrates minimal divergences between the most north-western population nearby the China-Myanmar border from Gongshan County in Yunnan Province to the most south-eastern population from Lao Cai District in northern Vietnam (straight line distance >800 km). A BLAST search for one of the *A. splendissimus 16S* sequences (MZ484729.1) against the 17 other sequences of these two 'species' show they are >99.4 % identical. These sequenced populations include topotype specimens of *A. viridimaculatus* (MK604835.1 and MK604836.1 from Lyu et al., 2019b; see Yang et al., 2019 for account and more precise locality information for these specimens) and *A. splendissimus* (from Zhang et al., 2021). Orlov & Ho (2007) and Rao & Wilkinson (2007) relied primarily on details of markings and colouration to diagnose *A. splendissimus* (and *A. caelumnoctis*) from *A. viridimaculatus*: small yellow spots on a dark purple/black/black-brown background (vs. larger green or yellowish green spots on a red-brown background), fore and hindlimbs spotted (vs. with transverse bands). Zhang et al. (2021: figs. 4 & 5) show individuals of *A. splendissimus* with distinctly larger green spots and one individual having banded forelimbs, demonstrating considerable plasticity in colouration range and markings, with some individuals looking more like *A. viridimaculatus* from the type locality (e.g. Yang et al., 2019: fig. 3f) than the type series of *A. splendissimus* and *A. caelumnoctis* (Orlov & Ho, 2007; Rao & Wilkinson, 2007). Rao & Wilkinson (2007) further diagnosed *A. caelumnoctis* from *A. viridimaculatus* by absence (vs. presence) of a pineal body. The presence or absence of a pineal body was not mentioned by Orlov & Ho (2007), but considered to be absent on all specimens examined by Zhang et al. (2021). Based on comparative specimens examined in this study (Appendix II), the pineal body can be externally visible or appear absent (i.e. probably present, just not visible through the skin) within species, e.g. *A. kaulbacki* visible on holotype, not visible on paratype, visible or not visible within the type series of *A. nidorbellus* Biju, Mahony & Kamei, 2010, and in the referred specimens of *A. viridimaculatus*, not externally visible (CAS 242251), indistinct (CAS 242250, CAS 242252) or distinctly visible (CAS 242214, CAS 242215, CAS 242249). Presence/absence of an externally visible pineal body should therefore not be considered a reliable diagnostic character in *viridimaculatus* group taxa. Considering the lack of species-level genetic differentiation in the *16S* sequences, largely overlapping colouration and markings, and a lack of other clearly diagnostic characters to suggest that the sequences represent more than one species, we formally recognise *A. splendissimus* Orlov & Ho, 2007 (including its junior subjective synonym *A. caelumnoctis* Rao & Wilkinson, 2007) to represent a junior subjective synonym of *A. viridimaculatus* (Jiang, 1983).

Taxonomic accounts

The southern Himalayan *marmoratus* and *monticola* groups require a more comprehensive taxonomic review which is beyond the scope of this current paper (see Patel et al., 2021 for progress on the *monticola* group), thus we refrain from further discussions on the identifications of the two Bhutan species sampled herein from these groups. The holotype of *A. gerbillus* is a juvenile, and no additional specimens representing the *marmoratus* group were available for us to study from the vicinity of the type

locality, preventing us from morphologically comparing the Bhutan population with *A. gerbillus* s.s. The Bhutan *viridimaculatus* group species, however, has been studied extensively by one of us (TN: Nidup et al., 2016; Limbu et al., 2020) and the morphological comparison of specimens with regional congeners enabled the identification of sufficient characters to demonstrate that this species represents a previously undescribed taxon (formally described below). The morphological examination of type specimens from the southern Himalayas (Appendix II) included *Rana senchalensis* which has been considered conspecific with *Amolops marmoratus* (Blyth, 1855) (Dubois, 2000). We provide an account for this species below to summarise the current state of knowledge on the taxon and make a recommendation for its treatment as valid (see 'Remarks' section of *Amolops senchalensis*).

Consistent with the small divergence in mitochondrial genes we described above, we found no obvious morphological differences between the holotype of *A. formosus* (examined herein, Appendix II) and the original description of *A. gyirongensis* (Jiang et al., 2020 [in Che et al., 2020]). However, we recommend further morphological study including more specimens of *A. formosus* and/or molecular analyses that includes a combination of mitochondrial and nuclear sequence data to reassess the validity of *A. gyirongensis* in the future. We have compared the newly described species with both *A. formosus* s.s. and *A. gyirongensis* (Jiang et al., 2020 [in Che et al., 2020]) separately in case our proposal for synonymisation is not universally accepted by the community.

***Amolops senchalensis* (Chanda, 1987)**

Rana senchalensis Chanda, 1987 ("1986"). J. Bombay Nat. Hist. Soc., 5(2): 140, 146–147, fig. 1: cited by Dutta (1992:2); Sarkar et al. (1992:67, 81); Duellman (1993:275); Dutta (1997:162–163); Chanda & Deuti (1998:72); Ray (1999:3); Chanda (2002:133–134).

- *Rana sinchalensis*[sic]: Chanda & Ghosh (1988:626); Swan (1993:143).
- *Amolops senchalensis*: Das & Dutta (1998:64).

Holotype

Adult male (ZSI A8753, formerly ZSI KZ 982; Fig. 2), from "Senchal Lake, Darjeeling District, West Bengal" that currently refers to Senchal Lake (ca. 26.993902, 88.264908, 2,260–2,280 m a.s.l.; estimated from Google Maps), Senchal Wildlife Sanctuary, Darjeeling Sadar Sub-division, Darjeeling District, West Bengal State, India, collected by S.K. Dey on 8 October 1983 (Chanda, 1987; Dutta, 1997; specimen jar label).

Etymology

The specific epithet '*senchalensis*' is a toponym, meaning from/of Senchal, with reference to the type locality, Senchal Lake. Considering all *Amolops* species are torrent frogs, thus not associated with lentic waterbodies, presumably the holotype was collected from a stream associated with/nearby the lake, rather than the lake itself.

Suggested common name

The following three alternative common names have been suggested for this species during the short period that it was considered valid (between 1987 and 2000): "Senchal Lake Frog" (Frank & Ramus, 1995), "Senchal Stream Frog" (Das & Dutta, 1998), "Senchal Frog" (Chanda, 2002). Though any of these names could be considered suitable, we favour using common names that indicate the systematic position of species to facilitate the end users of common names to distinguish between different taxonomic groups. Species of the genus *Amolops* are commonly referred to as "torrent frogs", a suitable group name that reflects the lotic habitat in which they are found and morphologically adapted to (e.g. tadpoles possess a large ventral gastromyzophorus disc and post metamorphic frogs possess expanded discs on digits), so we suggest a modification to the common name that reflects this - Senchal torrent frog.

Condition of type specimen

The holotype specimen was in poor preservation condition when examined in 2008 (by SM), appearing to have been completely dehydrated at some point, and then placed back into EtOH (Fig. 2). It might be possible that this specimen can be mostly restored via water or trisodium phosphate rehydration (e.g. Moore, 1999) so that some taxonomic characters can be assessed that are not discernible in its current condition. Chanda (1987) did not mention that the specimen was desiccated in the description, and the illustration provided indicated that the specimen examined by Chanda was probably in reasonably good condition (assuming artistic interpretation was not excessive). We provide measurements taken in this study (by SM) for the holotype in Table 3, but we caution the reader to not overly rely on many of these measurements (particularly soft characters like HL, disc widths, etc.) which would differ significantly if the specimen was hydrated.

Remarks

Chanda (1987) first named the species in the then catchall genus *Rana* Linnaeus, 1758, which at the time included *Amolops* as a synonym. Chanda (1987) primarily used the keys provided by Boulenger (1920) to infer the taxonomic affinities of *Rana senchalensis*, leading to several misleading comparisons, i.e. *Rana senchalensis* was compared with the dicroglossid *Nanorana annandalii* (Boulenger, 1920) rather than other sympatric species of *Amolops*. Duellman (1993:275) erroneously credited "Pillai and Chanda, 1990. J. Bengal Nat. Hist. Soc, N.S., 9:146." as the authorship of the name. We have confirmed that the J. Bengal Nat. Hist. Soc., volume 9 (not available online) comprises issues 1 and 2 which are separately paginated and neither of the two issues reach 146 pages. In addition, the indexes of both issues have been searched and no paper by these authors were published in either issue (H. Pethers, pers. comm. 01/02/2022). Dubois (2000) is typically credited for the synonymisation of *Rana senchalensis* under *Amolops marmoratus* (Blyth, 1855) (e.g. Chanda et al., 2001; Deuti & Ayyaswamy, 2008; Frost, 2021, online). However, Dubois (2000) merely placed the name as a

Figure 2. *Amolops senchalensis* adult male holotype (ZSIK A8753) showing preservation condition in 2008 at the time of examination: A. dorsal view; B. ventral view; C. dorsolateral view; D. profile view of head; E. dorsal view of right forearm; F. posterior view of left thigh. Images not to scale.

synonym of *Amolops marmoratus* without any discussion or justification, and with the wrong authorship (i.e. "*Rana senchalensis* Chanda, 1990"- reference not provided) and therefore this action should not have been regarded as a formal synonymisation of *Rana senchalensis* Chanda, 1987. Dutta (1997) gave the holotype number as "ZSI A8753" which is the number on the specimen label that was still attached to the specimen when examined by SM in December 2008 (Fig. 2). Morphologically this specimen corresponds relatively well with the original description, and details present on the specimen jar label regarding the specimen's collector and collection date agree with details provided for the holotype by Chanda (1987). Chanda et al. (2001) inexplicibly and erroneously gave the holotype number as "ZSI A8474", which has since been followed by Frost (2021).

Despite the poor condition of the holotype, the following characters are obvious enough to determine that

Amolops senchalensis is not conspecific with either of the *marmoratus* group species likely to be present in Darjeeling (i.e. *A. nepalicus* and *A. mahabharatensis* Khatiwada, Shu, Wang, Zhao, Xie & Jiang, 2020 from neighbouring Nepal): 1) external vocal sacs absent (vs. present and distinct in males of *A. nepalicus* and *A. mahabharatensis*; Wang et al., 2020 and Kathiwada et al., 2020, respectively); 2) third finger distinctly elongated, FIIIL/SVL = 27.7% (vs. <24 % in *A. nepalicus* and *A. mahabharatensis*; Wang et al., 2020 and Kathiwada et al., 2020, respectively); 3) outer metatarsal tubercle absent (vs. present in *A. nepalicus* and *A. mahabharatensis* Wang et al., 2020 and Kathiwada et al., 2020, respectively); 4) forearms are very enlarged relative to upper arms (Fig. 2E) (vs. much less enlarged relative to upper arms in *A. nepalicus* and *A. mahabharatensis*; Wang et al., 2020: figs. 3 & 4 and Kathiwada et al., 2020: figs. 4 & 5, respectively). Though some of these character states observed on the holotype of *A. senchalensis* (i.e. 1, 3 & 4) are also observed on a few geographically distant members of the *marmoratus* group (Dever et al., 2012; examined specimens), all are more typically seen in mature male *viridimaculatus* group taxa (Che et al., 2020; examined specimens, Appendix II), so we consider it more likely that *A. senchalensis* is a member of the *viridimaculatus* group.

Within the *viridimaculatus* group, the systematic position of *A. senchalensis* cannot be confirmed due to the poor preservation condition of the holotype (as described above). Based on adult size alone, the holotype (SVL 46.2 mm) approaches the adult male size for *A. formosus* (SVL 53–65 mm, n=2; Dubois, 1974), a species known from the vicinity of the type locality in Darjeeling (Boulenger, 1920) that also has the smallest adult male size in the group. However, the posterior thighs of *A. senchalensis* appear to have transverse broad dark brown stripes separated by narrow lighter intervening areas (Fig. 2F), whereas *A. formosus* has narrow dark brown stripes separated by broad green intervening areas (e.g. on the examined holotype; see also Dubois, 1974: fig. 4 and Kathiwada et al. 2020: fig. 10), so based on some apparent differences in markings we refrain from considering *A. senchalensis* as a junior subjective synonym of *A. formosus*. We therefore recommend that *Amolops senchalensis* (Chanda, 1987) is considered valid pending the collection of fresh material from the type locality that can permit a further evaluation of its taxonomic status.

***Amolops wangyali* sp. nov.**

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- *Amolops mantzorum* (nec. *Polypedates mantzorum* David, 1872): Wangyal (2013:4774, 4777, 4779, 4780, image 14): cited by Wangyal (2014:29, as "*Amolops mantzarum*" [sic.]); Nidup et al. (2016:13); Wangyal & Gurung (2017:52).
- *Amolops himalayanus* (nec. *Rana himalayana* Boulenger, 1888): Nidup et al. (2016:13–18, fig. 2 & possibly fig. 3: cited by Wangyal & Gurung (2017:52); Khatiwada et al. (2020:87); Nokhbatolfoghahai et al. (2020:326); Limbu et al. (2020:57, 59, 62).
- *Amolops* aff. *himalayanus*: Streicher et al. (2020:494, 495, fig. 1 [S10 & S11]).
- *Amolops himalayanus* (nec. *Rana himalayana* Boulenger, 1888): Streicher et al. (2020:494; error, should read "*Amolops* aff. *himalayanus*").
- *Amolops himalayanus* (nec. *Rana himalayana* Boulenger, 1888): Limbu et al. (2020:56–64, figs. 2B, 2G, 2H & 6 [left upper & lower]).

Holotype

Adult male (SCZM 2019.07.18.1 [field no. MW 11585]; Figs. 3, 4A & 4B), from Bodidrang Chhu/Stream (27.27023, 91.53043, 1,740–1,750 m a.s.l.), Namla Village, Kanglung Gewog (village block), Trashigang District, eastern Bhutan, collected by Tshering Nidup on 18 July 2019.

Paratypes

Adult female (SCZM 2019.07.18.2 [field no. MW 11587]; Figs. 4C, 4D & 5), collection details same as for holotype; adult female (SCZM 2019.08.02.1 [field no. SC 0034]; Figs. 4E, 4F & 6F), from Bodidrang Chhu/Stream (27.27223, 91.53129, 1,700 m a.s.l.), Namla Village, Kanglung Gewog (village block), Trashigang District, eastern Bhutan, collected by Tshering Nidup and Namgay Rinchen on 2 August 2019.

Etymology

The specific epithet is a patronym, named in recognition of Mr. Jigme Tshelthrim Wangyal, a Forest Officer with the Department of Forest and Park Services, Ministry of Agriculture and Forests, Government of Bhutan. Jigme is an accomplished Bhutanese herpetologist and has published many papers on the subject (Wangyal, 2011, 2013, 2014; Wangyal & Gurung, 2012, 2017; Wangyal & Das, 2014; Wangyal et al., 2020). Jigme's extensive network of Forest Officers, researchers and wildlife enthusiasts have supplemented his extensive personal observations in several of his publications, and as a consequence, many of the species currently on Bhutan's amphibian and reptile checklist were first documented in the country through his efforts. He continues to support and inspire interest in amphibian and reptile research through seminars and field training workshops and is a vocal proponent for improving standards of herpetological research in Bhutan.

Suggested common name

Wangyal's torrent frog.

Condition of type series

All specimens are fully intact, except for a portion of the ventral thigh muscle removed for molecular analysis. Specimens are well hydrated and in a good state of preservation (Fig. 4).

Description of holotype

Adult male, body habitus slender (Figs. 3A, 3C, 3E, 4A & 4B); head dorsally subovoid, wider than long, flat above; snout rounded and strongly protruding in profile (Fig. 3B), its length, longer than horizontal diameter of eye; canthus rostralis distinct, rounded, loreal region concave, obtuse; interorbital space flat, interorbital distance less than width

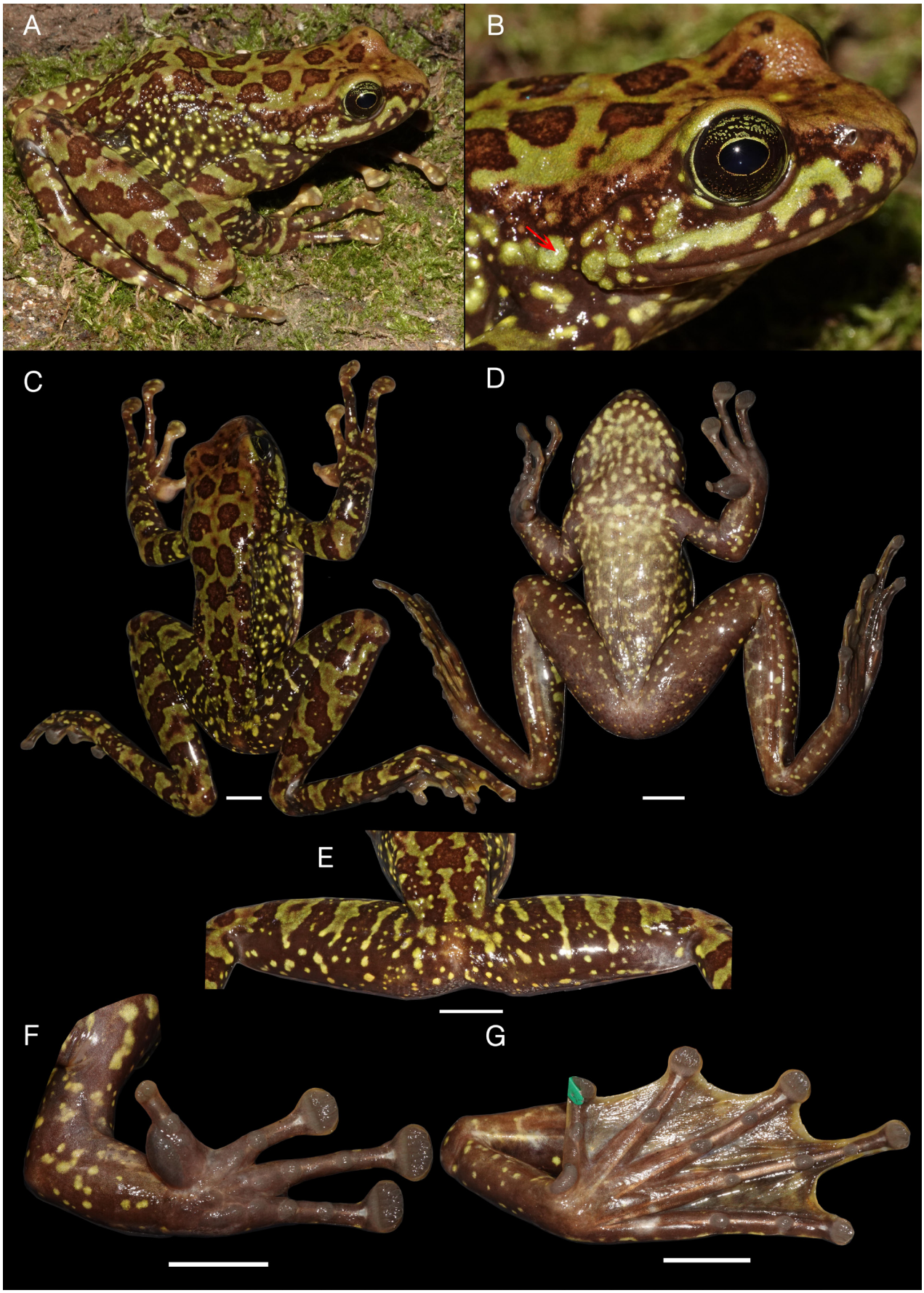

Figure 3. *Amolops wangyali* sp. nov. adult male holotype (SCZM 2019.07.18.1) in life (A & B: images taken ex-situ) and immediately after euthanisation, prior to fixation (C–G): A. dorsolateral view; B. lateral view of head, red arrow shows the shoulder gland; C. dorsolateral view; D. ventral view; E.posterior view of thighs; F. palmar view of left hand; G. plantar view of left foot. Scale bars represent 10 mm.

of upper eyelids, narrower than internarial distance; nostrils laterally positioned, obtusely oval with raised rim (Fig. 3B), closer to eye than snout; pupil horizontal (Fig. 3B); tympanum fully exposed, oval, obliquely orientated, flat, slightly recessed relative to temporal region, rim barely distinct (Fig. 3B), tympanum-eye distance greater than maximum tympanum diameter; pineal ocellus distinct (Figs. 3A–C, 4A); vomerine ridges well developed, more raised posteriorly, narrow, slightly obtuse, equidistant from each other and choanae, anterior ends positioned between choanae, transversely orientated; vomerine teeth small; choanae oval; tongue large, deeply emarginated distally with tips bluntly pointed, covered with tiny papillae, lingual processes absent; external vocal pouch indistinct, represented by patch of longitudinally wrinkled skin near posterior axis of mandible on each side (Figs. 3D & 4B), internal vocal slits small, one positioned on floor of mouth near posterior articulation of jaw on each side.

Forelimbs moderately long, thick, forearms significantly enlarged relative to upper arms (Figs. 3C & 4A), forearm length shorter than hand length; actual and relative finger length formula same, FI<FII<FIV<FIII; fingers relatively long and thin, all finger tips dilated with oval discs, but disc considerably reduced on FI (Fig. 3F), circummarginal grooves present on FII–FIV only (absent on FI); terminal phalange shape not determined; lateral dermal fringes absent on all fingers (Fig. 3F); webbing between fingers absent; subarticular tubercles prominently domed, circumference circular (FI–FIV: 1, 1, 2, 2); supernumerary tubercles prominent, circumference circular to longitudinally oval, one present on base of all digits (Fig. 3F), a secondary smaller supernumerary tubercle present between primary supernumerary tubercle and subarticular tubercle on FIII of left hand and FIV of right hand; prepollex absent; thenar tubercle long, preaxial half obscured by ventral portion of nuptial pad (Fig. 3F); outer and inner metacarpal tubercles distinct, separate, circumference oval, flat, outer long and thin, inner wider and ~30 % shorter (Fig. 3F); smooth nuptial pad restricted to FI, moderately large (notably larger on right hand than on left - asymmetry considered atypical; Fig. 4A & 4B), covering preaxial dorsal surface of proximal phalange extending ventrally on digit (Fig. 3F).

Hindlimbs long, shanks thin (Fig. 3C & 3D), longer than thighs and feet; toes long and thin (Fig. 3G), relative lengths TI<TII<TIII=TV<TIV; tips of all toes expanded with relatively small transversely oval discs, all with circummarginal grooves; webbing between toes extensive, webbing formula: I0–0II0–0III0–1IV1–0V (Fig. 3G); postaxial fringe on TV absent, preaxial groove on TI extends from base of disc to subarticular tubercle (Fig. 3G); subarticular tubercles all present (TI–TV: 1, 1, 2, 3, 2), prominent, circumference longitudinally oval; inner metatarsal tubercle flat, oval, relatively short (Fig. 3G); outer metatarsal tubercle absent; tarsal glandular ridge and supernumerary tubercles absent.

Skin on dorsal surface of head, body and limbs smooth (Figs. 3A–C, 3E & 4A); posterior lateral surface of head below supratympanic ridge and entire flanks covered with small dense tubercles, few small scattered tubercles on posterior dorsum and surrounding cloacal opening (Fig. 3E); supratympanic ridge well developed; a distinct patch of rictal glands at rear of jaw on either side (Fig. 3B); a distinctly enlarged gland positioned anterodorsally above forelimb insertion on either side (hereafter “shoulder gland”; Fig. 3B, indicated by an arrow); dorsolateral ridges absent; a weak parotoid-like swelling posterior to upper supratympanic ridge level with arm insertion, upper edge of parotoid-like swelling straight, lower edge curved and in contact with upper border of shoulder gland (Fig. 3A & 3B); ventral surfaces smooth except a patch of weakly granular skin on proximal posteroventral thighs (Figs. 3D & 4B); small white dermal spinules present, dense on tympanic region and rear of jaw, located on tips of tubercles (Fig. 3B), a moderately dense patch dorsolaterally above forelimb insertions, few scattered on granules of upper flanks, absent on remaining surfaces; other obvious macroglands absent.

Colouration in life (Fig. 3): Dorsum of head light brown anteriorly, blending to light green posteriorly, remaining dorsum of the body light green; large brown irregularly shaped blotches on dorsum of head and body; lateral surfaces of head and snout light green with dark brown mottling and blotches; a broken irregular stripe from tip of snout to eye passing along lateral edge of canthus rostralis on either side; 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 and parotoid region light brown; flanks evenly mottled green and brown, with contrasting light green tubercles; gular region, chest and abdomen brown with dense pale green spots anteriorly, becoming more mottled posteriorly on abdomen; dorsum of forelimbs and hindlimbs green with contrasting dark brown transverse to oblique cross-bands, all cross-bands with distinct light green specks; dorsum of fingers (including expanded discs) dark brown with irregularly arranged green specks; posterior surface of thighs and entire ventral surfaces of forelimbs and hindlimbs dark brown with irregularly arranged pale green spots and speckling (spots absent from palmar and plantar surfaces); nuptial pads yellowish-grey dorsally, dark grey ventrally.

Colouration in preservation (Fig. 4A & 4B): Dorsum of head and body primarily pale grey with large dark brown blotches, blotches with slightly darker borders, those on posterior half of body with cream coloured speckles; lateral surfaces of head marbled dark brown and light grey; flanks densely mottled grey and dark brown with creamish yellow granules; dorsum of fore and hindlimbs pale grey banded with dark brown transverse or obliquely transverse stripes; dorsum of hands and feet primarily dark brown with pale greyish cream speckles that increase in density distally onto expanded discs; inner/posterior surface of thighs dark brown with irregular small yellowish-cream spots and speckles; ventral surfaces of head, body and limbs primarily dark brown with dense yellowish-cream spots and speckles; ventral surfaces of hands and feet plain brown with tubercles and discs light

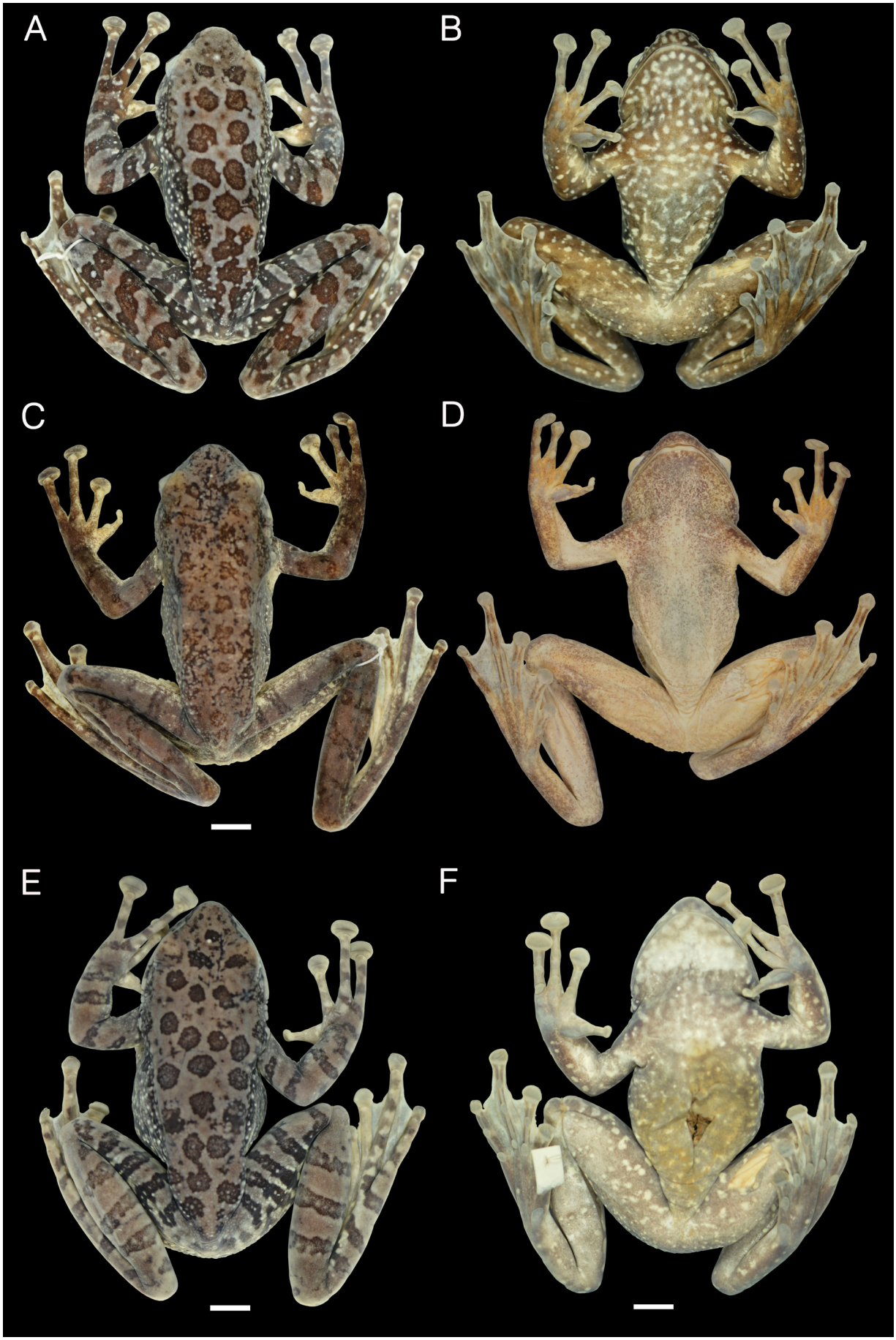

Figure 4. *Amolops wangyali* sp. nov. dorsal and ventral views of the type series in preservation showing variation in colouration and markings: A & B. adult male holotype (SCZM 2019.07.18.1); C & D. adult female paratype (SCZM 2019.07.18.2); E & F. adult female paratype (SCZM 2019.08.02.1). Scale bars represent 10 mm. A, B & D not to scale.

Table 3. Specimen morphometrics for *Amolops wangyali* sp. nov. and the holotype of *A. senchalensis*. Refer to the 'Methods & materials' section for explanation of measurement abbreviations and collection acronyms. HT holotype; PT paratype; M adult male; F adult female; – measurement not taken; * for measurements taken on left side.

Species	*A. wangyali* sp. nov.			*A. senchalensis*	Species	*A. wangyali* sp. nov.			*A. senchalensis*
Specimen #	SCZM 2019.07.18.1	SCZM 2019.07.18.2	SCZM 2019.08.02.1	ZSIK A8753	Specimen #	SCZM 2019.07.18.1	SCZM 2019.07.18.2	SCZM 2019.08.02.1	ZSIK A8753
Status	HT	PT	PT	HT	Status	HT	PT	PT	HT
Sex	M	F	F	M	Sex	M	F	F	M
SVL	71.4	87.5	89.6	46.2	SHW	10.3	10.8	13.1	–
HW	25.7	30.5	31.6	13.9	TFL	61.5	69.8	70	brittle
HL	24.8	30.3	31.6	15.9	FOL	42.1	47.3	47.6	26.6
SD	7.5	8.7	9.5	4.8	IMT	4.2	4.6	5.2	2.3
IFE	11.8	13.8	15	7.7	FIL	8.8	10.3*	13.2	6.3
IBE	20.1	23.9	25.1	12.5	FIIL	13.2	15.7*	17.9	8.4
EL	8.6	10.8	9.8	5.5	FIIIL	19.3	23.3*	24.9	12.8
TD	2.8	3.2	3.3	1.8	FIVL	15.4	16.4*	18.2	9.3
TE	3.6	4.1	3.6	2.1	FIDW	2.3	2.9*	3.3	1.1
SL	9.9	11.3	13.1	5.6	FIIDW	4.8	5.6*	6	1.9
EN	4.3	4.5	5	3.1	FIIIDW	5.1	6.6*	7.1	2.2
SN	5.8	7.2	8.1	2.8	FIVDW	5.5	6.9*	7.2	2.2
IN	7.9	8.4	9.6	5.2	FIIIW	2.2	2.3*	2.4	–
IUE	5.8	6.5	7.0	4.6	TIDW	3.4	3.9	4	–
UEW	6.3	8.4	9.3	3.9	TIIDW	4.1	4.8	4.3	–
FAL	17.6	21.8*	20.3	12.2	TIIIDW	3.4	4.7	5.2	1.5
HAL	25.7	30.6*	33.5	16.7	TIVDW	3.3	4.5	4.9	1.6
TL	41.5	48.9	48.2	26.1	TVDW	3.1	3.7	4.1	1.2
SHL	46.5	51.4	51	28.8	TIVW	2.6	2.7	3	–

grey; webbing between toes mottled grey and brown.

Variation

Mensural data for the type series is provided in Table 3. Paratypes morphologically agree with the holotype description with the following exceptions: webbing between toes slightly less extensive on the two paratypes, I0–1II0–1III0–1.5IV1.5–0V in SCZM 2019.07.18.2 (Fig. 5G) and I0–1II0–1III0–1IV1–0V on SCZM 2019.08.02.1; on SCZM 2019.07.18.2, vomerine ridges are slightly closer to the choanae than to each other, and on SCZM 2019.08.02.1, vomerine ridges are notably more developed (than the holotype and the other paratype), are linear (not obliquely orientated), positioned posterior to the choanae, are much closer to each other than to the choanae, and are evenly raised throughout their length (i.e. posterior ends more raised than anterior ends in the holotype and the other paratype); on SCZM 2019.07.18.2, tubercles are absent from the posterior dorsum and surrounding the cloacal region (Fig. 5E), and on SCZM 2019.08.02.1, tubercles are more dense on the posterior dorsum (than on the holotype), and surrounding the cloacal region, and the loreal region is very weakly granular (this specimen was not fixed in 90 % EtOH so some skin characters are more distinct than on the holotype and the other paratype); on SCZM 2019.07.18.2, a distinct dorsolateral row of tubercles is present on the body (Fig. 5A–C); on SCZM 2019.08.02.1, the tympanum is not distinctly sunken relative to the surrounding tympanic region; on SCZM 2019.07.18.2, dermal asperities are absent from all surfaces, and on SCZM 2019.08.02.1, the tips of some of the upper flank tubercles appear to have small, flat calcifications; on SCZM 2019.07.18.2, the shoulder gland is positioned directly above the forelimb insertion (Fig. 5A–C); colouration and markings vary extensively between specimens, see Figs. 3–6 and the following figures published elsewhere: Wangyal (2013: image 14); Nidup et al. (2016: figs. 2 & possibly 3); Limbu et al. (2020:56–64, figs. 2B, 2G, 2H & 6 [left upper & lower]); Streicher et al. (2020: fig. 1 [S10 & S11]).

Secondary sexual characters

Male (the holotype) with a large nuptial pad on F1; forearms significantly enlarged relative to upper arms; external paired subgular vocal sacs indistinct (e.g. not opaque relative to surrounding skin colour) but present as longitudinal folds; internal vocal slits small, one positioned on each side on floor of mouth close to posterior axes of jaws; testes yellow in colour. Female paratypes have forearms only slightly enlarged relative to upper arms; fallopian tubes convoluted; ova pigmentless; nuptial pads, external vocal sacs and internal vocal slits absent. Examination of additional specimens is required to determine whether presence of dermal asperities is unique to males.

Morphological comparisons

Amolops wangyali sp. nov. is herein compared to the

following species confirmed to be members of the *viridimaculatus* group based on molecular phylogenetic analyses: *A. baibengensis*, *A. formosus* (*A. gyirongensis* is compared separately), *A. himalayanus, A. kaulbacki, A. medogensis, A. pallasitatus, A. viridimaculatus* (including *A. caelumnoctis* and *A. splendissimus*), and *A. wangyufani*. Based on the examination of specimens (Appendix II), photographs of specimens or the original descriptions, we consider the following species to likely be members of the *viridimaculatus* group but await confirmation based on molecular data: *A. longimanus* (Andersson, 1939), *A. nidorbellus* and *A. senchalensis*. The new species is not compared with the following *Amolops* species from Bhutan and surrounding regions of the southern Himalayas (north and north-east India, Nepal, Myanmar, and Tibet Autonomous Region and Yunnan Province in China) that have not yet been assigned to a species group based on molecular data as they are not here considered to represent *viridimaculatus* group members: *A. assamensis* Sengupta, Hussain, Choudhury, Gogoi, Ahmed & Choudhury, 2008, *A. gerbillus* and *A. jaunsari* Ray, 1992 are clearly *marmoratus* group members (examined specimens, Appendix II; Ray, 1999); and based on the original morphological description, *A. chakrataensis* Ray, 1992 is either a *marmoratus* or *monticola* group member (Ray, 1999). SVL and SHL measurements for additional *Amolops wangyali* sp. nov. are from Nidup et al. (2016), see 'Materials & Methods' section.

Amolops wangyali sp. nov. differs from *A. baibengensis* by smaller adult female size, female SVL 80.5–89.6 mm, n=6 (vs. female SVL 90.2–93.2, n=2; Jiang et al., 2020 [in Che et al., 2020]; note: English language diagnosis erroneously stated "SVL 75.8 mm in females, SVL 90.2–93.2 in females" in Jiang et al., 2020 [in Che et al., 2020] - should read "SVL 75.8 mm in male, SVL 90.2–93.2 in females" based on data given in their table 27), tympanum distinct, larger TD/EL 30–34 %, n=3 (vs. "tympanum indistinct", TD/EL 20–27 %, n=3; Jiang et al., 2020 [in Che et al., 2020]), adult colouration in life - dorsum of body primarily green with brown blotches (vs. adult colouration in life - dorsal and lateral surfaces of body brown with small green spots; Jiang et al., 2020 [in Che et al., 2020:184–185 figs. [x5]]); from *A. formosus* by larger adult size, male SVL 71.4–76.7 mm, n=5, female SVL 80.5–89.6 mm, n=6 (vs. male SVL 53–65 mm, n=2, female SVL 53–75 mm, n=6; Boulenger, 1920; Dubois, 1974), higher SN/EN 135–162 %, n=3 (vs. SN/EN 125 % on holotype, n=1; examined specimen, Appendix II), lower IUE/IN 73–77 % and IUE/UEW 75–92 %, n=3 (vs. IUE/IN 89 % and IUE/UEW 112 % on holotype, n=1; examined specimen, Appendix II), lower FOL/SHL 91–93 %, n=3 (vs. FOL/SHL 100 % on holotype, n=1; examined specimen, Appendix II), tympanum oval, n=3 (vs. circular on holotype, n=1; examined specimen, Appendix II); from *A. gyirongensis* by larger adult male size, SVL 71.4–76.7 mm, n=5 (vs. male SVL 61.3–63.1, n=3; Jiang et al., 2020 [in Che et al., 2020]), relatively longer shanks, male SHL/SVL mean 63.6 %, min.–max. range 61–66 %, n=5, female SHL/SVL mean 58.7 %, min.–max. range 57–61 %, n=6 (vs. male SHL/SVL mean 58.3 %, n=3, female SHL/SVL mean 54.9 %, n=7; Jiang et al., 2020 [in Che et al., 2020] - measurements for individual specimens were not provided so min.–max. SHL/SVL ranges cannot be determined), tympanum distinct (vs. "indistinct"; Jiang et al., 2020 [in Che et al., 2020]); from *A. himalayanus* by lower TE/TD 109–129 %, n=3 (vs. TE/TD 136–150 %, n=3; examined specimens, Appendix II; tympanum border indistinct on fourth examined specimen, BMNH 1947.2.3.83, so measurement was not taken), lateral dermal fold on preaxial edge of TI extends from disc to subarticular tubercle, n=3 (vs. extends from disc to inner metatarsal tubercle, n=4; examined specimens, Appendix II); from *A. kaulbacki* by higher SN/EN 135–162 % and FIIIL/SVL 27–28 %, n=3 (vs. SN/EN 120–127 % and FIIIL/SVL 24–25 %, n=2; examined specimens, Appendix II), lower FOL/SHL 91–93 %, n=3 (vs. FOL/SHL 88–90 %, n=2; examined specimens, Appendix II), adult dorsal colouration of body in life primarily green with dark brown blotches (vs. primarily dark brown with green reticulations, n=2; examined specimens, Appendix II); from *A. longimanus* by skin on flanks distinctly tubercular, n=3 (vs. smooth on holotype, n=1; determined from photograph, Appendix II), nostril closer to eye than to snout tip, SN/EN 135–162 %, n=3 (vs. "nostrils a little nearer the tip of the snout than the eye" on holotype, n=1; Andersson, 1939), interorbital distance narrower than upper eyelid width, IUE/UEW 75–92 %, n=3 (vs. "Interorbital space….broader than the upper eyelid" on holotype, n=1; Andersson, 1939); from *A. medogensis* by skin on flanks with small dense tubercles (vs. smooth, n=6; Fei et al., 2012:424 figs. [6x]), smaller adult size, male SVL 71.4–76.7 mm, n=5, female SVL 80.5–89.6 mm, n=6 (vs. male SVL 95 mm, n=1, female SVL 93 mm, n=1; Fei et al., 2012), dorsum of body primarily green with dark brown blotches (vs. primarily dark brown with dense green speckling and mottling, n=6; Fei et al., 2012:424 figs. [6x]); from *A. nidorbellus* by typically smaller adult size, male SVL 71.4–76.7 mm, n=5, female SVL 80.5–89.6 mm, n=6 (vs. male SVL 76.4–82.3 mm, n=3, female SVL 85.4–98.0 mm, n=3; examined specimens, Appendix II; Biju et al., 2010), lower TE/TD 109–129 % and IUE/UEW 75–92 %, n=3 (vs. TE/TD 163–230 % and IUE/UEW 105–144 %, n=6; examined specimens, Appendix II; Biju et al., 2010), typically longer SHL/SVL, male 61–66 %, n=5, female 57–61 %, n=6 (vs. SHL/SVL, male 57–60 %, n=3, female 52–57 mm, n=3; examined specimens, Appendix II; Biju et al., 2010), dorsal colouration primarily green with dark brown blotches (vs. chocolate brown with small iridescent green rosette shaped spots, n=6; examined specimens, Appendix II; Biju et al., 2010), skin on flanks tubercular (vs. smooth, n=6; examined specimens, Appendix II; Biju et al., 2010); from *A. pallasitatus* by larger adult female size, SVL 80.5–89.6 mm, n=6 (vs. female SVL 70.6–72.3 mm, n=2; Qi et al., 2019), skin on flanks tubercular (vs. smooth, n=2; Qi et al., 2019), smaller TD/EL 30–34 %, n=3 (vs. TD/EL 40–42 %, n=2; Qi et al., 2019), longer female SHL/SVL 57–61 %, n=6 (vs. female SHL/SVL 53–55 %, n=2; Qi et al., 2019); from *A. senchalensis* by larger adult male size, SVL 71.4–76.7 mm, n=5 (vs. adult male SVL 46.2 mm on holotype, n=1; examined specimen, Appendix II), lower IUE/IN 73–77 % and FAL/HAL 61–71 %, n=3 (vs. IUE/IN 89 % and FAL/HAL 73 % on holotype, n=1; examined specimen, Appendix II);

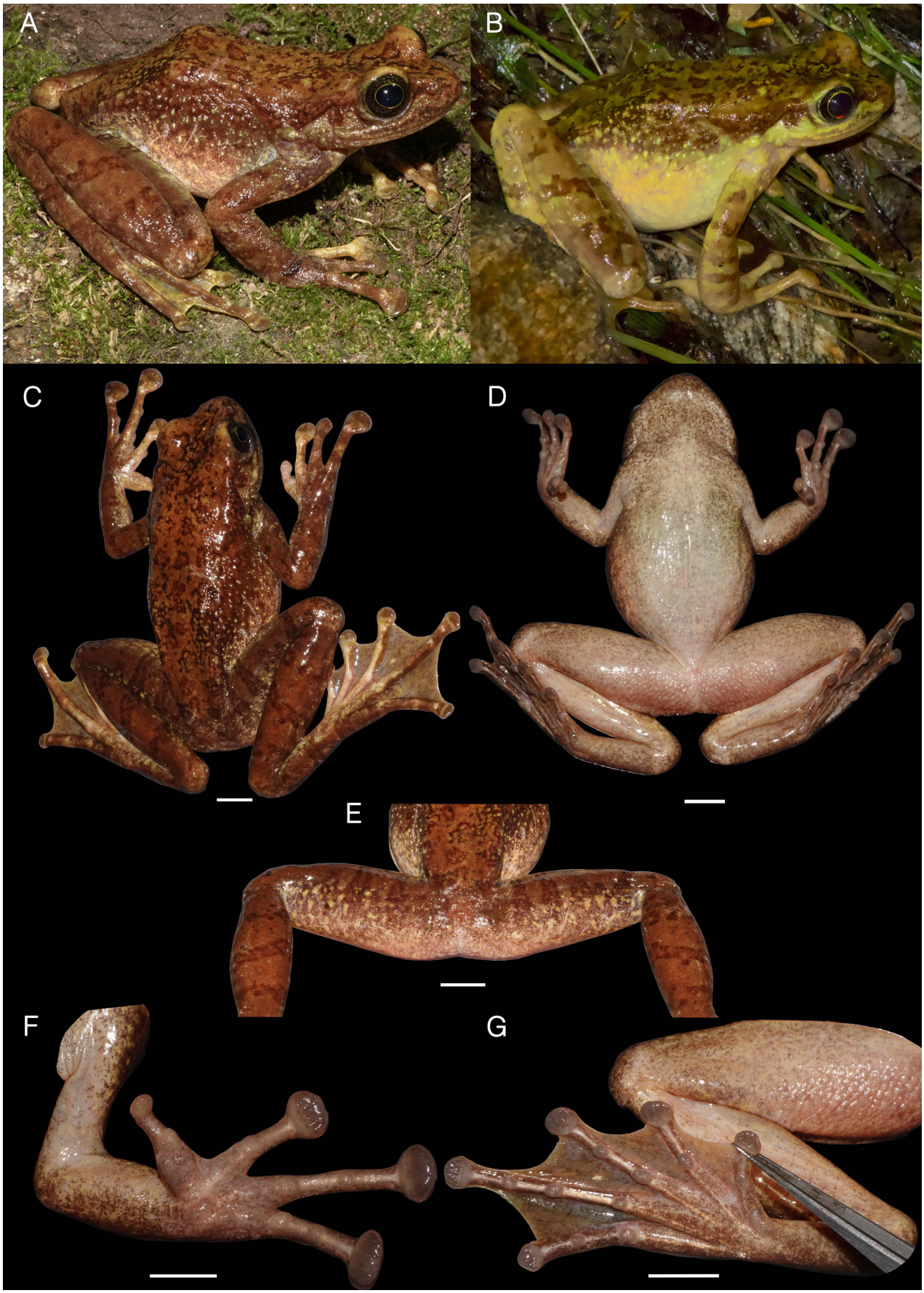

Figure 5. *Amolops wangyali* sp. nov. adult female paratype (SCZM 2019.07.18.2) in life (A & B) and immediately after euthanisation, prior to fixation (C–G): A. dorsolateral view, image taken ex-situ; B. dorsolateral view, image taken in-situ; C. dorsolateral view; D. ventral view; E. posterior view of thighs; F. palmar view of left hand; G. plantar view of right foot. Scale bars represent 10 mm.

from *A. viridimaculatus* (including *A. caelumnoctis* and *A. splendissimus*) by lower IUE/UEW 75–92 %, n=3 (vs. IUE/UEW 110–150 %, n=6; examined specimens, Appendix II; IUE/UEW 93–125 %, n=10, of which only two specimens had IUE/UEW<108 %; Orlov & Ho, 2007; IUE or UEW not measured; Zhang et al., 2021; Rao & Wilkinson, 2007), higher male SHL/SVL 61–66 %, n=5 (vs. male SHL/SVL 55–60 %, n=4; examined specimens, Appendix II; SHL/SVL 52–62 %, n=18, of which only one specimen had SHL/SVL>59 %; Orlov & Ho, 2007; Rao & Wilkinson, 2007; SHL not measured; Zhang et al., 2021), lower FOL/SHL 91–93 %, n=3 (vs. FOL/SHL 96–100 %, n=5 [FOL not taken for one specimen CAS 242252 due to missing digits]; examined specimens, Appendix II; FOL/SHL 89–105 %, n=8 of which only one specimen had FOL/SHL<94 %; Rao & Wilkinson, 2007; FOL not measured; Orlov & Ho, 2007; Zhang et al., 2021), TVL=TIIIL, n=3 (vs. TVL>TIIIL, n=5 [not taken for one specimen CAS 242252 due to missing digits]; examined specimens, Appendix II; TVL>TIIIL, n=1; Orlov & Ho, 2007; not mentioned; Rao & Wilkinson, 2007; Zhang et al., 2021), supratympanic fold well-developed, n=3 (vs. weakly developed, n=6; examined specimens, Appendix II; Orlov & Ho, 2007; Rao & Wilkinson, 2007), tympanum oval shaped, n=3 (vs. circular, n=6; examined specimens, Appendix II; "round"; Orlov & Ho, 2007; shape not mentioned; Rao & Wilkinson, 2007; Zhang et al., 2021), skin on flanks distinctly tubercular (vs. weakly granular, n=6; examined specimens, Appendix II; or "smooth"; Orlov & Ho, 2007; Rao & Wilkinson, 2007; Zhang et al., 2021), adult dorsal colouration in life primarily green with dark brown blotches (vs. in life, reddish-brown/dark purple/black/black-brown with very small to moderately large yellow, yellowish-green or iridescent green smooth edged rounded spots; Orlov & Ho, 2007; Rao & Wilkinson, 2007; Yang et al., 2019; Zhang et al., 2021); from *A. wangyufani* by larger adult male size, SVL 71.4–76.7 mm, n=5 (vs. male SVL 68.3–69.0, n=2; Jiang, 2020 [in Che et al., 2020]), relatively longer shanks, male SHL/SVL mean 63.6 %, min.–max. range 61–66 %, n=5, female SHL/SVL mean 58.7 %, min.–max. range 57–61 %, n=6 (vs. male SHL/SVL mean 60.6 %, n=2, female SHL/SVL mean 56.5 %, n=1; Jiang, 2020 [in Che et al., 2020] - individual specimen measurements were not provided so min.–max. SHL/SVL range cannot be determined for males), adult dorsal colouration of body in life primarily green with dark brown blotches (vs. primarily brown with green reticulations; Jiang, 2020 [in Che et al., 2020]:226, 228–229 figs. [x7]); shanks with 3–4 distinct dark brown transverse stripes (vs. 6–7 distinct dark brown transverse stripes; Jiang, 2020 [in Che et al., 2020]:226, 228–230 figs. [x8]).

Distribution

This species is currently known with certainty based on the results of sequence data from four localities in Trashigang District, Bhutan (Table 1): 1) the type locality at Bodidrang Chhu/Stream (1,640–1,750 m a.s.l.), Kanglung Gewog (village block) (Figs. 6E & 7; Nidup et al., 2016; Limbu et al., 2020; Streicher et al., 2020; results herein); 2) Jere Chhu/Stream (27.206083, 91.603389; 2,073 m a.s.l.), Khaling Town, Khaling-Kharungla Forest Management Unit (Nidup et al., 2016: fig. 2B & 2C); 3) a small perennial stream (27.2808, 91.53937; 1,520 m a.s.l.) that bisects the Trashigang-Samdrumjongkhar Highway (the stream eventually joins the Bodidrang Chhu/Stream further north at ca. 1,300 m a.s.l.), Rongthong Village (Streicher et al., 2020; Fig. 6C); 4) Thragom, a small stream above Kanglung BHU Hospital (27.28031, 91.51456; 1950 m a.s.l.), Kanglung Gewog (village block). We also consider the following localities to be included in the distribution based on published photographs that we regard as identifiable to this species (see 'Review of *Amolops* reports from Bhutan' section below for more details; Table 2; Fig. 7): 1) Serkang Chhu/Stream, a tributary of the Kulong Chhu/River, Choetenkora Town (ca. 27.605298, 91.493766; 1,745 m a.s.l.), Trashiyangtse District (Wangyal, 2013: image 14); 2) Khaling Chhu/Stream (27.190606, 91.602611; ca. 2,070 m a.s.l.), 1.7 km south of the Khaling Town, Khaling-Kharungla Forest Management Unit, Trashigang District (Nidup et al., 2016: fig. 2F). All six of these streams are within the Drangme Chhu/River Basin indicating that the species is likely to be widespread along tributaries on both sides of the Drangme Chhu/River in Mongar, Trashigang and Trashiyangtse districts of eastern Bhutan, at least within the elevation range 1,520–2,073 m a.s.l. The species possibly ranges further east into Tawang District, Arunachal Pradesh State, India where the Drangme Chhu/River is known as the Tawang Chhu/River.

Populations of *Amolops* photographed in Athreya (2006:145–146) referred to as "*Staurois* cf *viridimaculatus*" (adults) from "Bompu (2200 m) to New Khellong (1250 m)" and "*Amolops* sp." (juvenile) from "Sessni (1250 m)" in Eaglenest Wildlife Sanctuary, West Kameng District, Arunachal Pradesh State, India, superficially resemble *Amolops wangyali* sp. nov. These Eaglenest Wildlife Sanctuary populations are ca. 90 km east of the type locality of *Amolops wangyali* sp. nov. Measurements provided for two adult individuals from the Eaglenest Wildlife Sanctuary population differ from the type series of *Amolops wangyali* sp. nov., most notably by the head being considerably wider than long, however, HL measurements in Athreya (2006) may have been taken differently to our study. We suggest that the taxonomic status of populations from West Kameng District be reassessed to verify their identities.

Natural history and conservation

Nidup et al. (2016) and Limbu et al. (2020) discussed details on the general habitat, ecology and reproductive biology of this species. Limbu et al. (2020) reported the breeding season as March to June based on field observations, however, one of the paratypes (SCZM 2019.07.18.2) contained large pigmentless ova extending the reproductive season at least to late July when it was collected. Nidup et al. (2016) provided an image of an uncollected tadpole (fig. 3) from Bodidrang Chhu/Stream which they identified as *A. "himalayanus"*, however, at least two, or possibly three species of *Amolops* are sympatric in this stream (Limbu et al., 2020), so the identity of this photographed tadpole would require verification. Nidup et al. (2016) identified domestic stream

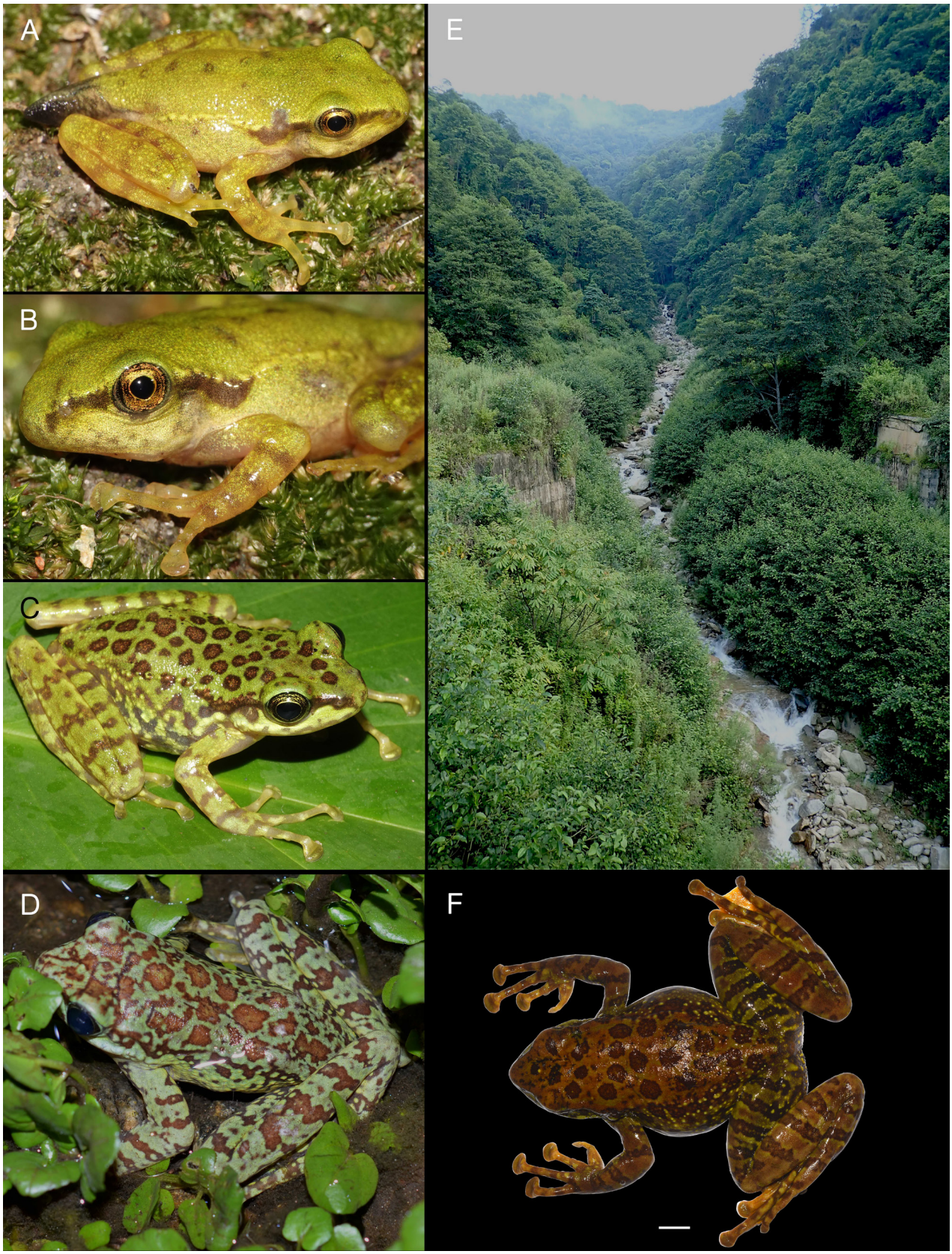

Figure 6. *Amolops wangyali* sp. nov. juveniles in life (A–D) showing ontogenetic variation in colouration and markings: A & B. dorsolateral and profile views of a nearly metamorphosed juvenile (SCZM 2019.07.18.3), from the type locality, images taken ex-situ; C. dorsolateral view of larger juvenile (SCZM 2019.07.20.1), from Rongthong (27.2808, 91.53937, ca. 1,520 m a.s.l.), Trashigang District, Bhutan, image taken ex-situ; D. dorsal view of uncollected halfgrown juvenile, from Jere Chhu/Stream, Khaling Town, Bhutan, image taken in-situ; E. habitat at the type locality, Bodidrang Chhu/Stream, taken from the Singye Thegchog Bridge two days after the collection of the holotype (20 July 2019); F. adult female paratype (SCZM 2019.08.02.1) from Bodidrang Chhu/Stream, image taken immediately after euthanisation, prior to fixation. Scale bar represents 10 mm.

pollution as a potential threat to the population at the Jere Chhu/Stream locality. Streicher et al. (2020) included the holotype and one of the paratypes in a preliminary survey for the amphibian pathogen *Batrachochytrium dendrobatidis* (Longcore et al., 1999), in Bhutan, which found no positive infections. The forested areas in Bhutan are afforded considerably more protection than in surrounding countries and frogs are not commonly collected for food/medicinal consumption in Bhutan, so deforestation and overharvesting are unlikely to threaten this species. However, a growing body of research is finding catastrophic biodiversity decline in even pristine and protected habitats worldwide (e.g. Hallmann et al., 2017; Zipkin et al., 2020) demonstrating the immediate need for long term monitoring of amphibian populations and extensive biodiversity surveys to understand the taxonomic diversity of the region. Patel & Das (2020) obtained high success rates for identifying and counting individuals of *Amolops formosus* during a multiday minimally invasive photographic survey using pattern recognition software. This could be a valuable tool of determining population sizes of adult individuals of *Amolops wangyali* sp. nov. (and other taxa with contrasting markings). However, juveniles are almost plain green becoming blotched as they mature (Fig. 6A–D; Nidup et al., 2016: fig. 2F; Limbu et al., 2020: fig. 2G & 2H) demonstrating that ontogenetic changes in colouration and markings are observable in this species. More work would be necessary to determine the extent of individual ontogenetic change, and at which point (if at all) adult colour pattern stabilises to assess the usefulness of Patel & Das's (2020) technique for long term studies (over months or years).

Review of *Amolops* reports from Bhutan

In this section, we review the *Amolops* species reported from Bhutan in the literature. Based on thus-far available published data, we clarify some misidentifications (provisionally) of species referred to this taxon in an effort to reduce taxonomic confusion of Bhutanese anurans. A relatively recent surge in progress among Bhutan's herpetological researchers to document the amphibians of this historically poorly studied country has led to a dramatic increase in the numbers of publications and number of species reported from the country. The following publications have provided original information relating to members of the genus *Amolops* from Bhutan: Das & Palden (2000); Wangyal & Gurung (2012, 2017); Wangyal (2013; 2014); Wangyal & Das (2014); Nidup et al. (2016); Tshewang & Letro (2018); Koirala et al. (2019); Limbu et al. (2020); Streicher et al. (2020); Wangyal et al. (2020).

Das & Palden (2000) provided the first country record of an *Amolops* species in Bhutan, *A. marmoratus*, based on two specimens collected from "Sershong, Sarpang District" and deposited in the Royal Manas National Park Museum, Gelephu, Sarpang District, Bhutan. The name *Amolops marmoratus* has long been a catch-all name that comprised a complex of superficially similar species reported from throughout much of the southern and eastern Himalayas from Nepal, through north-east India, Myanmar and neighbouring parts of Thailand and southern China. Dever et al. (2012) subsequently restricted the geographic distribution of *A. marmoratus* s.s. to eastern Myanmar and western Thailand. Pending further molecular verification and taxonomic scrutiny of the populations in Bhutan from the *marmoratus* species group, we recommend that species in this group are provisionally referred to as *A.* cf. *gerbillus*.

Wangyal & Gurung (2012) reported *A.* cf. *monticola* for the first time in Bhutan from "Rurichu [or Ruri Chhu/ Stream] (27020.30' N & 89054.49' E), Wangdue Phodrang District at an altitude of 982m", however, the photograph provided of an example of the species (Wangyal & Gurung, 2012: image 7) represents a *marmoratus* species group member most similar to a species we have elsewhere identified as *A.* cf. *gerbillus* (Streicher et al., 2020). *Amolops monticola* (Anderson, 1871) is a poorly known species with few known specimens that are correctly identified in museum collections (SM pers. obs.; Patel et al. 2021). The name *A. monticola* has commonly been used to refer to *Amolops* that have dorsolateral ridges (e.g. Stuart et al., 2010) but many individuals of *A.* cf. *gerbillus* can possess weak dorsolateral ridges, and thus the confusion may arise.

Wangyal (2013: image 14) identified a photograph of a live frog as *A. mantzorum* that we consider to morphologically resemble a female *A. wangyali* sp. nov. The photographed individual was reported from a "perennial stream called Serkang Chu, that runs through the suburban Choetenkora Town [also called Chorten Kora, ca. 27.605298, 91.493766], the headquarters of Trashiyangtse District at an altitude of 1745m" (Wangyal, 2013). Serkang Chhu is a tributary of the Kulong Chhu, and the reported locality lies ca. 40 km north of the type locality of the new species, *A. wangyali* sp. nov. Wangyal (2013: image 17) identified a photograph of a pair of frogs as "*Sylvirana* cf. *guentheri*" from "Zhonggarchu, Lingmethang, Mongar [District]". Wangyal (2014) cautioned that this report of *Sylvirana* cf. *guentheri* required confirmation. The distinctly expanded discs on the digit tips and overall morphology of the photographed individuals (Wangyal, 2013: image 17) agree well with members of the *Amolops monticola* species group (possibly conspecific with the species included in our molecular analyses as "*A.* cf. *putaoensis*"; Fig. 1). *Hylarana (Sylvirana) guentheri* (Boulenger, 1882) has not been reported elsewhere in the southern Himalayas and should be removed from the faunal list of Bhutan. Wangyal & Das (2014) listed *A. formosus, A. gerbillus, A. himalayanus, A. marmoratus* and *A. monticola* in a checklist with no clarification regarding the basis for the inclusion of these species and thus must be regarded as anecdotal. They provided a figure (Wangyal & Das, 2014: fig. 3b) of a live frog identified as *Nanorana liebigii* (Günther, 1860) which we here identify as a female *A.* cf. *gerbillus*. Wangyal (2014) listed *Amolops "farmosus"* [sic.], *A. himalayanus* and *A. gerbillus* as expected to occur in Bhutan based on the proximity of their known distributions bordering Bhutan, indicating that their inclusion of these species on the aforementioned checklist (Wangyal & Das, 2014) was

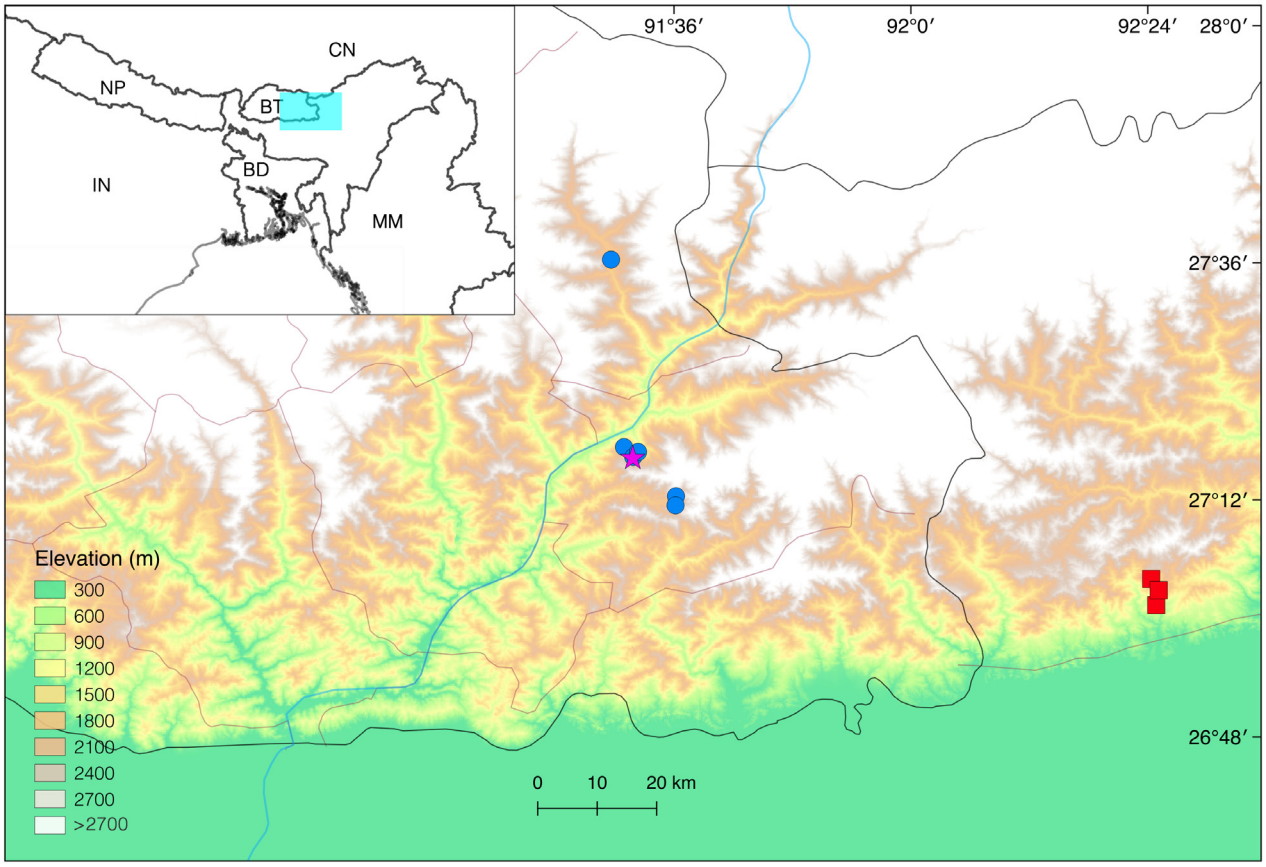

Figure 7. Topographic map of eastern Bhutan and bordering areas of north-east India showing the distribution of *Amolops wangyali* sp. nov. (star for type locality, circles for additional localities) and populations suspected to be conspecific with this species (squares). Inset: map of Bhutan and neighbouring north-east India with cyan box representing the area depicted in the main map. Country borders in black; district/state borders in brown; major river in blue; BD Bangladesh; BT Bhutan; CH China; IN India; MM Myanmar; NP Nepal.

premature.

Nidup et al. (2016) reported *A. himalayanus* for the first time in Bhutan giving the first in-depth study on an *Amolops* species in the country. This paper gave some basic specimen measurements, details of biology and conservation concerns and a summary of several previous reports of *A. himalayanus* from the literature. The authors discussed differences in SVL between their new material and specimens of *A. himalayanus* at BMNH, but other subtle morphological differences between the BMNH type specimens and the Bhutan populations were overlooked by the authors (see 'Morphological comparisons' section above). This is understandable as species diversity within the group was not known to be so high at the time of their study, and subtle morphological differences may have been considered as intraspecies variation when not informed by molecular data. The populations discussed in Nidup et al. (2016) represent *A. wangyali* sp. nov. Therefore, subsequent listing of *A. himalayanus* in Bhutan based on Nidup et al.'s (2016) study are corrected herein (see 'chresonymy of *A. wangyali* sp. nov.'). Limbu et al. (2020) further expanded on the reproductive ecology of *A. wangyali* sp. nov. (as *A. "himalayanus"*), from the type locality, Bodidrang Chhu/Stream. Limbu et al. (2020: fig. 6) also provided four photographs of other unidentified species of *Amolops* from the same locality, their identifications are provisionally revised here as follows: fig. 6, top left, undetermined *viridimaculatus* species group member, superficially similar to *A. formosus* but possibly representing a juvenile *A. wangyali* sp. nov.; fig. 6, bottom left, represents *A. wangyali* sp. nov.; fig. 6, top and bottom right, both represent *A.* cf. *gerbillus*. The morphological examination of specimens preferably accompanied by molecular sampling will be necessary to confirm the identities of these populations.

Tshewang & Letro (2018) reported *A. formosus* from "Langthel" and *A. marmoratus* from "Langthel, Nabji, Taksha, Tingtibi" in the Jigme Singye Wangchuck National Park in central Bhutan. The photograph they provided (Tshewang & Letro, 2018: image 33) of a live frog identified as *A. formosus* does not show diagnosing morphological characters to enable us to determine a reliable species-level identification beyond that it is a member of the *viridimaculatus* species group. The frogs shown in two photographs identified as *A. marmoratus* represent *Duttaphrynus* cf. *himalayanus* and *A.* cf. *gerbillus* (Tshewang & Letro, 2018: images 34a & 34b, respectively). The cited literature used for making species identifications in Tshewang & Letro's (2018) study do not provide adequate details of morphological characters to correctly identify species of *Amolops* so we recommend that the population identified as *A. formosus* should be

referred to in future as "*Amolops* sp. 1. (*viridimaculatus* group)" pending a detailed taxonomic study of voucher specimens from the locality.

Koirala et al. (2019) reported four species of *Amolops* (*A. marmoratus, A. mantzorum, A. monticola* and an unidentified *Amolops* species) from Jigme Dorji National Park (but gave no specific localities) in western Bhutan. A photograph of *A. marmoratus* (image 4) represents an *A.* cf. *gerbillus* female, *A. mantzorum* (image 5) represents an *A.* cf. *formosus*, and *Amolops* sp. (image 6) represents another *A.* cf. *gerbillus*. No image of the species they identified as *A. monticola* was given so their report of *A. monticola* must be considered as anecdotal. They also provided an image labelled as "*Hyla* sp." (Koirala et al., 2019: image 8) which represents a juvenile *viridimaculatus* group member, possibly the same species as that shown in their image 4. Wangyal (2011) listed "*Hyla* cf. *annectans*" in a checklist as a new country record from the Royal Manas National Park, Bhutan, but provided no accompanying information. Wangyal (2014) subsequently highlighted the need for verification of his earlier report of a "*Hyla*" (Laurenti, 1768) species in Bhutan, but despite the uncertainty, he included "*Hylo*[sic.] cf. *onnectans*[sic.]" in the checklist of Bhutan species. Wangyal & Gurung (2017) listed "*Hyla annectans*" (Jerdon, 1870) in their updated amphibian checklist for Bhutan, without "cf." therefore implying that the species' identification had been confirmed, but no supporting evidence was provided. We are unaware of a verifiable report for the genus *Hyla* in Bhutan, so this genus should not be included on future national faunal checklists.Wangyal & Gurung's (2017) amphibian checklist of Bhutan listed six species of *Amolops*: *A. formosus, A. gerbillus, A. himalayanus, A. mantzorum, A. marmoratus*, and *A. monticola*.

In a study to determine whether the amphibian pathogen *Batrachochytrium dendrobatidis* was present in Bhutan, Streicher et al. (2020: fig. 1 S10 & S11) provided photographs of frogs identified as *A.* aff. *himalayanus* which represent the holotype and one of the paratypes (SCZM 2019.07.18.2) of *Amolops wangyali* sp. nov., and a specimen of *A.* cf. *gerbillus* (fig. 1, S12). Most recently, *Amolops wenshanensis* Yuan, Jin, Li, Stuart and Wu, 2018 was reported by Wangyal et al. (2020) from Goenshari Rimchu (27.694890 °N, 89.769082 °E) in Punakha District based on a photo voucher (ZRC (IMG) 1.208). The individual photographed is the same as that given in Koirala et al. (2019: image 8) as "*Hyla* sp.", taken from a slightly different angle, but on the same substrate. Wangyal et al. (2020) did not refer to Koirala et al.'s (2019) prior identification. The field work in Koirala et al. (2019) was reported to have been carried out from November 2017 to February 2019, however, the photo voucher in Wangyal et al. (2020) was reported to have been taken on 20 July 2019, indicating a discrepancy in the date that the photo vouchered animal was observed between these two papers. As mentioned above the individual photographed in both studies represents a juvenile *viridimaculatus* species group member; *A. wenshanensis* is a member of the morphologically disparate *monticola* species group (Yuan et al., 2018). The basis for the identification in Wangyal et al. (2020) is not clear and is considered here to be erroneous.

CONCLUSIONS

In summary, we identified four species of *Amolops* from Bhutan: (1) *Amolops* sp. 1. (*viridimaculatus* group: from Tshewang & Letro, 2018), (2) *A.* cf. *gerbillus* (*marmoratus* group), (3) *A.* cf. *putaoensis* (*monticola* group), and (4) *A. wangyali* sp. nov. (*viridimaculatus* group). Outside of the new species described herein, we were unable to determine species identities for these taxa given the available data. Until such time as vouchered specimens are clearly identified from the country by means of a detailed morphological comparison of vouchered specimens with relevant taxonomic literature, and/or with the aid of DNA sequence data, the following nine species must be formally removed from the amphibian checklist of Bhutan: (1) *Amolops formosus*, (2) *A. gerbillus*, (3) *A. himalayanus* (including *A.* aff. *himalayanus*), (4) *A. mantzorum*, (5) *A. marmoratus*, (6) *A. monticola*, (7) *A. wenshanensis*, (8) *Sylvirana* cf. *guentheri*, (9) *Hyla annectans* (including *Hyla* cf. *annectans*). Unintentional misidentifications in the literature can result in significantly overestimated/ erroneous geographic distributions for species, a situation which undermines conservation efforts. Inaccuracies in such assessments could even result in the redirection of conservation resources (funds and efforts) away from vulnerable range restricted species that require urgent attention. For these reasons, we encourage authors not to assign species names to taxa in publications if there is any uncertainty regarding the identification of the species. Many populations of amphibians reported from Bhutan (and elsewhere in Asia) are provided non-specific locality details (e.g. lack GPS coordinates, elevation details), are not represented in museum/university collections by vouchered specimens, and are often published without photographic evidence. Locally abundant species can often be dismissed as "common", or of little scientific interest, and subsequently ignored by researchers; however, studies on Himalayan amphibians have demonstrated that "common" or widespread species occasionally represent complexes of morphologically similar species (e.g. Dubois, 1975; Kamei et al., 2009; Dever et al., 2012; Khatiwada et al., 2017; Mahony et al., 2013, 2018, 2020), so careful attention to document every species should be made when possible. Our review of *Amolops* reports in literature demonstrate that some taxonomic information can be obtained from good quality images of uncollected animals, but inevitably an accurate species inventory for Bhutan's amphibian fauna will not be possible without permanently maintained reference collections of vouchered specimens. Range restricted species may be only one drought, forest fire or hydroelectric dam away from extinction, thus the urgency to catalogue the Himalayan biodiversity has never been more urgent.

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Authors' contribution

SM examined museum specimens, did molecular phylogenetic analyses and drafted the manuscript. SM & JWS designed arcDNA experiments. SM, JWS, RGK did molecular lab work. SM, JWS, ECT obtained funding. TN collected specimens and field data. SM, TN, JWS organised permits. SM, JWS, TN photographed specimens. RGK, SM prepared figures and map. JWS drafted arcDNA lab protocol. SM, RGK, JWS, TN, ECT read, commented and edited working drafts of the manuscript.

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APPENDIX I

***12S-tVal-16S* assembled arcDNA sequences used in Maximum Likelihood analyses**

>Amolops_himalayanus_BMNH1947.2.3.83_12S-tVal-16S
ACAAAACATTTTAACATTTTAGTACAGGTGATCGAAAAATT
CCTAGGCGCTTCAGATAAAGTACCGCAAGGGAATAATGAA
ATAGAAATGAAATAATCTTAAAGCCCTAAATAGCAGAGCTT
CCCCCTCGTACCTTTTGCATCATGGTCTAGCCAGTCTCCCCA
GGCAAAATGAAAATTTTAGTCTGTTTCCCCGAAACTAAGCG
AGCTACTTCAAAACAGCCCAAAGGGGCTAACCCGTCTCTGTT
GCAAAAGAGTGGGAAGATTTTTAAGTAGAGGTGACAAGCCT
ACCGAGCTTAGAGATAGCTGGTTGTTCAGGAAAAGAGTCTCA
GCTCTACCTTAAATCTCTTTATTAAACCAAACAAAACACTAA
ATTTAAGAGCTATTCAAATAAGGCACAGCTTATTTGAAGCGG
GGTACAACCCCTAATACTGGGTCAATATAGATGCTATTAATA
AAGTGGGCCTAAAAGCAGCCACCTTCTAAAAAGCGTTAAAG
CTTAATACACCATATCTACAATTCCACAATCTTTAAACAACC
CTTCATTACTACTGAACTATTTTATAACCTTATAAAAGCCATT
ATGCTAGAACTAGTAACAAGAAATTGTCCTTCTCCAAAATGT
ATGTTTAAACCAAAATGGACCCACCACTGGTAGTTAACGCA
AATGTAAAAATTATAGCACCACACGGCCAGAAAACCCTATA
ATCCCCTAACGTTAACCTTACACTAGAACATTACAGGAAAGA
TTAAAAGAGGGAGAAGGAACTCGGCAAATCACAGTCCCGCC
TGTTTACCAAAAACATCGCCTCTTGACTAAACATAAGAGGTC
CAGCCTGCCCAGTGACATAGTTTAACGGCCGCGGTACCCTAA
CCGTGCAAAGGTAGCATAATCACTTGTTCTTTAAATGAGGAC
TCGTATCAACGGCATCACGAGGACTGTACTGTCTCCTCCCTCC
AATCAGTGAAACTGATCCCCCCGTGAAGAAGCGGGGATTAAC
CTATAAGACGAGAAGACCCCATGGAGCTTTAAACCCAACACA
CACCTCTACGCACTCAAATCAACGTAGCCCAAGAGACTTGCGT
GTTGGTTTTAGGTTGGGGGGACCGCGGAGTAAAATTAAACCTC
CACGACAGACGGACTAACACCCTTATCCACGAACTACAATTCT
AAGAGCCAATAAATTGTTGTCTAATGACCCAATTACTTGATCAA
CGAACCAAGTTACCCTGGGGATAACAGCGCAATCTACTTCAAG
AGCCCCTATCGACAAGTGGGTTTACGACCTCGATGTTGGATCAG
GGTATCCTGGTGGTGCAGCCGCTACTAACGGTTCGTTTGTTCAA
CGATTAAAACCCT

>Amolops_formosus_BMNH1947.2.4.18_12S-tVal-16S
ACAAAACATTTTAACATTTTAGTACAGGTGATCGAAAAATT
TCTAAGCGCTTCAGATAAAGTACCACAAGGGAACAATGAA
ATAGAAATGAAATAATATTAAAGCCCTAAATAGCAGAGTTT
CCCCCTCGTACCTTTTGCATCATGGTCTAGCCAGTCTCCACA
GGCAAAACGAGAATTTTAGTCTGCTTCCCCTAAACTAAGCG
AGCTACTNNNNNNNNNNNNNNNNNNGTTAACCCGTCTCTGT
TGCAAAAGAGTGGGAAGATTTTTAAGTAGAGGTGACAAGCC
TACTGAGCTTAGAGATAGCTGGTTGTTCAGGAAAAGAGTCTC
AGCTCTACCTTAAATTTTTCTATTACACCAAACAAAACACTA
AATTTAAGAGCTATTCAAATAAGGCACAGCTTATTTGAAGCG
GGGTACAAACCACAATACTGGGTTAATCCAGATATTATAATT
TAAGTGGGCCTAAAAGCAGCCATCTTCTAAAAAGCGTTAAAG
CTTAATACACCATGTCTACAAATTCCAAAATTTTTAAACAACC
CTTCATTACTACTGAACTATTTTATAACTTTATAAAAGTCATTA
TGCTAGAACTAGTAACAAGAAATTATCCTTCTCCAAAATGTA
TGTTTGAACCAAAATGGACCCACCACTGGTAGTTAACGCAAA
TGTAAAAATCATAGCACACACGCTAGAAAACCCTATGATTTC
TAAGCGTCAACCNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
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NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
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CACGAACTACAATTCTAAGAGCCAATAAATTGTTGTCTA
ATGACCCAATTACTTGATCAACGAACCAAGTTACCCTGG
GGATAACAGCGCAATCTACTTCAAGAGCCCCTATCGACA
AGTAGGTTTACGACCNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNGGTTCGTTTGTTCA
ACGATTAAAACCCT

>Amolops_kaulbacki_BMNH1940.6.1.1_12S-tVal-16S
ACAAAACATTTTAACACTTTAGTACAGGTGATCGAAAAATT
TCTAAGCGCTTCAGATAAAGTACCGTAAGGGAATAATGAAA
TAGAAATGAAATAATCTTAAAGCCCTAAATAGCAGAGCTTC
CCCCTCGTACCTTTTGCATCATGGTCTAGCCAGTCCCCCCCA
GGCAAAACGAAAATTTTAGTCTGATTCCCCGAAACTAAGCG
AGCTACTTCAAAACAGCCCAAAAGGGCCAACCCGTCTCTGT
TGCAAAAGAGTGGGAAGATTTTTAAGTAGAGGTGACAAGCC
TACCGAGCTTAGAGATAGCTGGTTGTTCAGGAAAAGAGTCTC
AGCTCTACCTTAAATTTTTTTTATTGAAACCAAACAAAACACT
AAATTTAAGAGCTATTCAAATAAGGCACAGCTTATTTGAAGC
GGGGTACAACCCCAAATACTGGGTTAGTCTAGATGTAGTAAA
TAAAGTGGGCCTAAAAGCAGCCATCTTCTGAAAAGCGTTAAA
GCTTAATATACCTTATCTACAATTCCACAATTTTTAAACAACCC
TTCATTACTACTGAACTATTTTATAACCTTATAAAAGTTATTAT
GCTAGAACTAGTAACAAGAAATTGCCCTTCTCCCAAATGTATG
TTTAAACCAAGATGGACCTACCACTGGTAGTTAACGCAAGTGT
AAAAATCATAGCACCACACGGCTAGAAAACCCTATGGTTTCTC
AGCGTTAACCTTACACTAGAACATTACAGGAAAGATTAAAAGA
GAAAGAAGGAACTCGGCAAATCACAGTCCCGCCTGTTTACCAA
AAACATCGCCTCTTGACCAAGTATAAGAGGTCCAGCCTGCCCAG
TGACATAGTTTAACGGCCGCGGTACCCTAACCGTGCAAAGGTAG

CATAATCACTTGTTCTCTAAATAGGGACTCGTATCAATGGCATCA
CGAGGGCTGTACTGTCTCCTTTCTCCAATCAGTGAAACTGATCTC
CCCGTGAAGAAGCGGGGATTGACCTATAAGACGAGAAGACCCC
ATGGAGCTTTAAACCCAATATACACCTCCTACGCACTCAAATCA
ATATAGCCCAAGAGACTTGTGTATCGGTTTTAGGTTGGGGGGACC
GCGGAGTAAAATTAAACCTCCACGACAGACGGATTAACACCCTT
ATCCACGAACTACAATTCTAAGAGCCAATAAATTGTTGTCTAAT
GACCCAATTACTTGATCAACGGACCAAGTTACCCTGGGGATAAC
AGCGCAATCTACTTCGAGAGCCCCTATCGACAAGTAGGTTTACG
ACCTCGATGTTGGATCAGGGTATCCTAGTGGTGCAGCCGCTACT
AAAGGTTCGTTTGTTCAACGATTAAAACCCT

***nd2* assembled arcDNA sequence used for *p*-distance analyses**

>Amolops_formosus_BMNH1947.2.4.18_nd2
ATTAATACCCTCGCCATCATCCCAATTATAACTAAAACCNN
NNNNNNNNNNGCCATTGAAGCTGCCACAAAATACTTCCTCA
CTCAAGCCACAGCCTCCGCTCTAGTTCTATTTTCAAGTCTCAT
TAATGCCTGACAAGTAGGACAATGAAATATCTCCCACCTAAC
TGACTTACCAATAAATGTACTTGCTATTGCCCTCATAATAAAA
CTAGGCCTAGCCCCCTTACATTTTTNNNNNNNNNNNNNNNNNN
NNNNNATTTCATTGTCTACAGGGCTTATTTTATCAACCTGACAA
AAAATTGCCCCAATAGCCCTTCTTCTCCAAATTTCCCACCTAAT
TGACCTAAATTTAACCATTATTATTGGTCTCACCTCAATTCTAGT
TGGGGGATGAGGCGGCATTGCCCAAACCCAATTACGTAAGATTA
TTGCCTTCTCTTCAATCGGCCACCTTGGGTGAATAGTCATTGTATT
AAAATTTAGCCCCCAGCTCACACTTTTTAACTTTCTACTTTATATC
TTAATAACAACCTCAATATTTATCTCCCTCATAGCAATTTCTGCCA
CAAAAATATCGGAAATCTCCACATCCTGACCAAAAACCC

***co1* assembled arcDNA sequence used for *p*-distance analyses**

>Amolops_formosus_BMNH1947.2.4.18_co1
ATTGGCACAGCCTTAAGCCTACTCATTCGAGCAGAACTAAG
CCAACCMGGAGCCCTTCTTGGTGATGACCAAATTTACAATG
TAATTGTAACCGCCCACGCATTTGTAATAATTTTTTTTATAGT
AATGCCCATCTTAATCGGAGGCTTCGGAAACTGATTAATCCC
ATTAATAATTGGAGCCCCAGACATAGCTTTCCCCCGGATAAA
TAATATGANNNNNNNNNNNNNNNNNNCATCTTTTTTCCTCCT
CCTAGCATCCTCTGCAGTCGAGGCTGGGGCCGGAACAGGCTGAAC
TGTTTATNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNCTCATCAATCTTGGGGGCTATTAACTTTATTACAA
CAATTATTAATATAAAACCTGCATCAATTACACAATACCAAA
CACCCCTCTTTGTTTGGTCCGTNTTGATTACTGCCGTCCTATT
ACTACTTTCCCTCCCAGTCTTAGCCGCCGGCATCACAATACTT
TTAACTGACCG

APPENDIX II

Comparative specimens examined

Amolops afghanus: BMNH 1947.2.27.93 (previously BMNH 1933.3.7.55: lectotype of *Polypedates afghanus*—Dever et al., 2012), female, "Afghanistan" (in error).

Amolops assamensis: ZSIK 10272 (holotype), adult male, "Mayeng Hill", Assam, India; ZSIK 10273 (paratype), adult female, "Kolahat R.F.", Assam, India; ZSIK 10274 (nontype), adult female, "Garbhanga R.F.", Assam, India.

Amolops formosus: BMNH 1947.2.4.18 (previously BMNH [18]72.4.17.281: holotype), adult female, "Khassya" [=Khasi Hills], Meghalaya, India.

Amolops gerbillus: ZSIK 16925 (holotype of *Rana gerbillus*), juvenile not sexed, "Yembung, alt. 1100 ft", Arunachal Pradesh, India; ZSIK 16950 (holotype of *Ixalus argus* Annandale, 1912), juvenile not sexed, "Upper Renging, alt. 2150 ft., Abor", Arunachal Pradesh, India.

Amolops himalayanus: BMNH 1947.2.27.94 and BMNH 1947.2.3.83 (previously BMNH [18]72.4.17.347 and BMNH [18]80.11.10.120, respectively: syntypes), two adult females and BMNH 1947.2.27.95 (previously BMNH [18]87.11.2.20: syntype), adult male, "Darjeeling", West Bengal, India; ZSIK (unregistered when examined in 2009), adult female, "Gangtok, Sikkim", India.

Amolops kaulbacki: BMNH 1947.2.3.96 (previously BMNH 1940.6.1.1: holotype) and BMNH 1947.2.3.65 (previously BMNH 1940.6.1.2: paratype), two adult males, "Pangnamdim, Triangle, Upper Burma", Kachin State, Myanmar.

Amolops kohimaensis: ZSIK A 10972 (holotype), ZSIK A 10966, ZSIK A 10971, BNHS 5282 (paratypes), SDB 2007.129 (referred specimen), five adult males, "Lorü, 25°38'23"N 94°03'31"E, 1,575 m asl., Jotsoma village, Kohima district, Nagaland, northeast India".

Amolops longimanus: NRM1861 (holotype), "Kambaiti", Kachin State, Myanmar.

Amolops monticola: ZSIK 10036 (holotype), adult female, "Darjeeling", West Bengal, India.

Amolops nidorbellus: ZSIK A 10965 (holotype), BNHS 5276 (paratype), SDB 2009.335 (referred specimen), three adult males, and ZSIK A 10968–10970 (paratypes), three adult females, "Lorü, 25°38'23"N 94°03'31"E, 1,575 m asl., Jotsoma village, Kohima district, Nagaland, northeast India".

Amolops senchalensis: ZSIK A8753 (holotype), adult male, "Senchal Lake, Darjeeling", West Bengal, India.

Amolops viridimaculatus: CAS 242214, adult male, and CAS 242215, adult female, "XiaoDiFang Village (24°52'51.4" N, 98°45'7.9" E), Zhengding, LongYang Co. [error, should be Tengchong Co.], Baoshan Pref., Yunnan, China"; CAS 242250–242252, three adult males, and CAS 242249, adult female, "XiaoDiFang River (24°51'45.5" N, 98°45'21.7" E, 2133 m) at XiaoDiFang Village, TengChong Co., Baoshan Pref., Yunnan, China".

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