A review of torrent frogs \textit{(Amolops: Ranidae)} from Bhutan, the description of a new species, and reassessment of the taxonomic validity of some \textit{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 \textit{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 voucher specimens from Bhutan and provide a complete literature review of all \textit{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 \textit{Amolops} populations. We also obtained archival DNA sequences from the type specimens of \textit{Amolops formosus}, \textit{A. himalayanus}, and \textit{A. kaupbacki}, collected between 82 and 151 years ago. Our comparative analyses revealed a large, new (to science) species of the \textit{Amolops viridimaculatus} group from eastern Bhutan. Morphological examinations of related taxa revealed that \textit{A. senchalensis} from India is not a synonym of \textit{A. marmoratus}. Molecular phylogenetic results supplemented by morphological data unambiguously demonstrate i) that \textit{A. himalayanus} is present in eastern Nepal, ii) the presence of a previously undocumented population of \textit{A. nepalis} in eastern Nepal, iii) a 200 km range extention for \textit{A. kaupbacki} into Yunnan, China, iv) that \textit{A. gyirongensis} should be considered a junior subjective synonym of \textit{A. formosus}, and v) that \textit{A. splendissimus} from Vietnam should be considered a junior subjective synonym of \textit{A. viridimaculatus}. Based on our results, we expand the \textit{Amolops viridimaculatus} group to include nine species, including \textit{A. formosus}, \textit{A. himalayanus}, \textit{A. kaupbacki}, and the new species described herein. We provisionally include a further three species in the \textit{viridimaculatus} group based on morphology, \textit{A. longimanus}, \textit{A. nidorbellus}, and \textit{A. senchalensis}. Combining our data with the literature review allowed us to identify several unidentified \textit{Amolops} species from recent phylogenetic studies and remove nine frog species (including \textit{Hyla}, \textit{Sylvirana}, and seven \textit{Amolops} species) from Bhutan’s amphibian checklist. We recognise four species of \textit{Amolops} in Bhutan, three of which cannot be confidently identified to the species level based on currently available data.

\textit{Keywords:} Anura, taxonomy, Himalayas, conservation, vouchered-specimens

\section*{INTRODUCTION}

The large radiation of Asian torrent or waterfall frogs, genus \textit{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 \textit{marmoratus}, \textit{monticola} and \textit{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 km$^2$) nestled between the Brahmaputra River Valley and
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 (referred 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 nighttime. 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); GNGU (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); KUJE (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); VNLM (Vietnam National Museum of Nature, Hanoi, Vietnam); WIIADA (Wildlife Institute of India, Dehradun, Uttarakhand, India); ZISP (Zoological Institute of St. Petersburg, St. Petersburg, Russia); ZSK (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-tVox-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., 2014). For the 16S only sequences, we used 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 1 mM 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.

<table>
<thead>
<tr>
<th>Species</th>
<th>Locality</th>
<th>Specimen #</th>
<th>12S-tVal-16S / 16S</th>
<th><em>nd2</em></th>
<th><em>co1</em></th>
<th>Reference &amp; Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. afghanus (Günther, 1858a)</td>
<td>China: Yunnan: Yingjiang Co.</td>
<td>SYS a003852</td>
<td>MK604837.1</td>
<td>-</td>
<td>-</td>
<td>Lu et al. (2019a); as A. &quot;marmoratus&quot; on GenBank</td>
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<tr>
<td>A. alaskorum Stuart, Bain, Phimmachak &amp; Spence, 2010</td>
<td>Laos: LuangNamtha: Vient Phou Kha.</td>
<td>FMNH 217155 (paratype)</td>
<td>FJ417158.2</td>
<td>-</td>
<td>-</td>
<td>Stuart et al. (2010); as A. &quot;sp. BLS-2009&quot; on GenBank</td>
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<tr>
<td>A. assamensis Sengupta, Hussain, Choudhury, Gogoi, Ahmed &amp; Choudhury, 2008</td>
<td>NA</td>
<td>NSA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>NA</td>
</tr>
<tr>
<td>A. australis Chan, Abraham, Grismer &amp; Grismer, 2018</td>
<td>Malaysia: Peta, Endau-Rompin N.P.</td>
<td>LSJHC 7673 (paratype)</td>
<td>MF061745.1</td>
<td>-</td>
<td>-</td>
<td>&quot;Chan K.O. direct submission&quot; on GenBank, published in Chan et al. (2017); as A. &quot;ibnengensis&quot; on GenBank</td>
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<tr>
<td>A. beibengensis Jiang, Li, Zhou, Yan &amp; Che, 2020 (in Che et al., 2020)</td>
<td>China: Tibet: Medog.</td>
<td>KIZ 016397 (paratype)</td>
<td>MN953662.1</td>
<td>MN958721.1</td>
<td>MN961359.1</td>
<td>Wu et al. (2020; as A. &quot;sp. 2&quot;)</td>
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<td>KIZ 011061 (topotype)</td>
<td>MN958722.1</td>
<td>MN961360.1</td>
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<td>Wu et al. (2020; as A. &quot;sp. 2&quot;)</td>
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<td>A. chalaxoramei Ray, 1992</td>
<td>NA</td>
<td>NSA</td>
<td>-</td>
<td>-</td>
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<td>NA</td>
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<td>A. chochin Jiang, Ren, Lyu &amp; Li, 2021</td>
<td>China: Sichuan: Chongzhou City</td>
<td>CIB 116971 (holotype)</td>
<td>MZ702027.1</td>
<td>-</td>
<td>-</td>
<td>( \text{as } A. \text{ chochin} )</td>
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<tr>
<td>A. choyunensis Sun, Luo, Sun &amp; Zhang, 2013</td>
<td>China: Tibet: Basu Co.</td>
<td>SYS a007509</td>
<td>MK573820.1</td>
<td>-</td>
<td>-</td>
<td>Lyu et al. (2019b)</td>
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<td>A. chungangensis (Pope, 1929)</td>
<td>China: Jiangxi: Mt. Jinggang.</td>
<td>SYS a004212</td>
<td>MK263263.1</td>
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<td>-</td>
<td>Lyu et al. (2019a)</td>
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<td>A. chumchum (Bain, Stuart &amp; Orlov, 2006)</td>
<td>Vietnam: Kon Tum: Dak Glei Dist.</td>
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<td>FJ417142.2</td>
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<td>Stuart et al. (2010)</td>
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<td>Laos: Luangphabong: Vient Tong Dist.</td>
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<td>Stuart et al. (2010)</td>
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<td>A. dengjiang, Wang &amp; Che, 2020 (in Che et al., 2020)</td>
<td>China: Tibet: Zayu.</td>
<td>KIZ 014116 (paratype)</td>
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<td>-</td>
<td>-</td>
<td>Wu et al. (2020; as A. &quot;sp. 3&quot;)</td>
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<td>A. formosus (Günther, 1876!)</td>
<td>India: Meghalaya: &quot;Khasiya&quot;.</td>
<td>BMNH 1947.2.4.18 (holotype, A. formosus)</td>
<td>SAMN28238801</td>
<td>SAMN28238801</td>
<td>SAMN28238801</td>
<td>This study; Appendix I for assembled sequences.</td>
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<td>A. formosus</td>
<td>Nepal: Prok: 1: Sankhuwasabah: Dobhan.</td>
<td>C02MTU 0145</td>
<td>MT124521.1</td>
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<td>Khatiwada et al. (2020); as A. &quot;nepalicus&quot; on GenBank</td>
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<tr>
<td>A. formosus (syn. A. gyorgyensis, Liang, Wang, Wang, Pan &amp; Che, 2020 (in Che et al., 2020))</td>
<td>China: Tibet: Gyirong.</td>
<td>KIZ 012533 (paratype, A. gyorgyensis)</td>
<td>MN953682.1</td>
<td>MN958739.1</td>
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<td>KIZ 012537 (paratype, A. gyorgyensis)</td>
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<td>MN958740.1</td>
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<td>Wu et al. (2020; as A. &quot;sp. 4&quot;)</td>
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<td>KIZ 012534 (paratype, A. gyorgyensis)</td>
<td>MN953684.1</td>
<td>MN958741.1</td>
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<td>KIZ 012535 (paratype, A. gyorgyensis)</td>
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<td>KIZ 012536 (holotype, A. gyorgyensis)</td>
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<td>MN958743.1</td>
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<td>Wu et al. (2020; as A. &quot;sp. 4&quot;)</td>
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<td>A. cf. genibillus (Annandale, 1912)</td>
<td>Bhutan: Trashigang: Bodidrang Chhu/Stream</td>
<td>SCZM 2015.06.06.1 (issue no. D138)</td>
<td>ON462437.1</td>
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<td>Species</td>
<td>Locality</td>
<td>Specimen #</td>
<td>12S-Vol-16S / 16S</td>
<td>nd2</td>
<td>co1</td>
<td>Reference &amp; Notes</td>
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<td>(Boulenger, 1900)</td>
<td>China: Hainan: Lingshui.</td>
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<td>DG204481.1</td>
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<td>A. himalayanus</td>
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<td>India: West Bengal: Darjeeling.</td>
<td>BMNH 1947.2.3.83 (syntype)</td>
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<td>A. himalayanus</td>
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<td>MN953712.1</td>
<td>MN958770.1</td>
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<td>Nepal: Prov. 1: Iham Dist.: Mabu.</td>
<td>KIZ 040227</td>
<td>MN958771.1</td>
<td>MN961415.1</td>
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<td>Nepal: Prov. 1: Iham Dist.: Marmajhuwa.</td>
<td>KIZ 040228</td>
<td>MN958772.1</td>
<td>MN961416.1</td>
<td>Wu et al. (2020: as A. &quot;sp. 5&quot;)</td>
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<td>(Poole &amp; Romer, 1951)</td>
<td>China: Hong Kong.</td>
<td>ROM 16300 (topotype)</td>
<td>AF206453.1</td>
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<td>A. jaunsari</td>
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<td>(Smith, 1940)</td>
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<td>BMNH 1940.6.1.1 (holotype)</td>
<td>SAMN28238803</td>
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<td>MN961438.1</td>
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<td>A. lichenensis</td>
<td>(liu, 1945)</td>
<td>China: Sichuan: Liexian Co.</td>
<td>SYS a005374</td>
<td>MK573809.1</td>
<td>-</td>
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<td>A. longimanus</td>
<td>(Andersson, 1939)</td>
<td>NA</td>
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<td>A. mantzorum</td>
<td>(David, 1872)</td>
<td>China: Sichuan: Fengtongghai.</td>
<td>SYS a005365</td>
<td>MK573808.1</td>
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<td>A. marmoratus</td>
<td>(Blaby, 1855)</td>
<td>Myanmar: Mon.</td>
<td>CAS 240593 (topotype)</td>
<td>JF794456.1</td>
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<td>(in Zhao et al., 2005)</td>
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<td>Locality</td>
<td>Specimen #</td>
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<td>coI</td>
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<td>A. montecola [Anderson, 1871]</td>
<td>India: Sikkim: Tarku Forest Block</td>
<td>WIADA 544</td>
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<td>Patel et al. (2021)</td>
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<td>A. nepalicus Yang, 1991</td>
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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 (also with some additional samples not reported here, see below) for size selection after being loaded into the sample well of the BluePippin™. We selected for a 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 (5′ and 3′), 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 and coauthors, 20...
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 bc12fasta v2.15.0.4 software from Illumina (https://github.com/bc12/fastq). Resulting paired-end FASTQ files were then processed through Illuminoprocessor 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 archDNA 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 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 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 (FIl); third finger length, from tip of third digit to its base where it joins second digit (FIIIiL); 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 (FIIIiW); maximum disc widths of fingers I–V (FIDW, FIIDW, FIIIiDW, FIVWD); fourth toe width, taken dorsally on digit proximal to disc (TIVW); maximum disc widths of toes I–V (TIDW, TIIDW, TIIIiDW, TIVWD, TIVWD); 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 tibiotalar articulation to tip of fourth digit (TFI); 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-Fi/Ti) 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 Katiwada 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.

<table>
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<tr>
<th>Species</th>
<th>Country</th>
<th>State</th>
<th>District</th>
<th>Locality</th>
<th>GPS</th>
<th>Elevation (m a.s.l.)</th>
<th>GPS / elevation notes</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>A. wangyali sp. nov.</td>
<td>Bhutan</td>
<td>N/A</td>
<td>Trashigang</td>
<td>“Jere Chhu [27°12'21.90&quot;N, 91°36’12.07’E], Khaling, … 2.073 m”</td>
<td>27.206083, 91.603389</td>
<td>2073</td>
<td>GPS &amp; elevation as reported in the reference.</td>
<td>Nidup et al. (2016)</td>
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<td>Bhutan</td>
<td>N/A</td>
<td>Trashigang</td>
<td>“Khaling (Chhu/Stream) [27°11'12.16&quot;N,91°36’09.40’E]”</td>
<td>27.190606, 91.602611</td>
<td>2070</td>
<td>GPS as reported in the reference; elevation &amp; coordinate conversion based on plotted GPS point in Google Maps.</td>
<td>Nidup et al. (2016)</td>
</tr>
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<td>Bhutan</td>
<td>N/A</td>
<td>Trashigang</td>
<td>“Bodiodrang (Chhu/Stream) [27°17’20.33”N, 91°30’56.28’E]”</td>
<td>27.288981, 91.513563</td>
<td>1640</td>
<td>GPS as reported in the reference; elevation &amp; coordinate conversion based on plotted GPS point in Google Maps.</td>
<td>Nidup et al. (2016); Limbu et al. (2020)</td>
</tr>
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<td>A. wangyali sp. nov.</td>
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<td>Trashigang</td>
<td>“Kanglung: Road from Sherubtse College to Trashigang: Bodiodrang (Chhu/Stream): Namla”</td>
<td>27.272233, 91.53129</td>
<td>1750</td>
<td>GPS &amp; elevation as reported in the reference.</td>
<td>Streicher et al. (2020)</td>
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<td>Bhutan</td>
<td>N/A</td>
<td>Trashigang</td>
<td>“Kanglung: Road from Sherubtse College to Trashigang: Bodiodrang (Chhu/Stream): Namla”</td>
<td>27.27023, 91.53043</td>
<td>1738</td>
<td>GPS &amp; elevation as reported in the reference.</td>
<td>Streicher et al. (2020)</td>
</tr>
<tr>
<td>A. wangyali sp. nov.</td>
<td>Bhutan</td>
<td>N/A</td>
<td>Trashigang</td>
<td>“Rongthong”</td>
<td>27.2808, 91.53937</td>
<td>1520</td>
<td>GPS &amp; elevation as reported in the reference.</td>
<td>Streicher et al. (2020)</td>
</tr>
<tr>
<td>A. wangyali sp. nov.</td>
<td>Bhutan</td>
<td>N/A</td>
<td>Trashigang</td>
<td>Thragom, a small stream above Kanglung BHU Hospital, Kanglung Gewog (village block)</td>
<td>27.28031, 91.51456</td>
<td>1950</td>
<td>GPS &amp; elevation as reported herein.</td>
<td>This study.</td>
</tr>
<tr>
<td>A. wangyali sp. nov.</td>
<td>Bhutan</td>
<td>N/A</td>
<td>Trashiyangtse</td>
<td>“Serkiang Chu, … Choetenkora town [27°36’50.00”N, 91°29’32.00’E], Trashiyangtse District … 1745 m”</td>
<td>27.605298, 91.493766</td>
<td>1745</td>
<td>GPS as reported in the reference, plotted and coordinate converted in Google Maps.</td>
<td>Wangyal (2013)</td>
</tr>
<tr>
<td>A. cf. wangyali</td>
<td>India</td>
<td>Arunachal Pradesh</td>
<td>West Kameng</td>
<td>“New Khelling (1250 m)”, Eaglenest Wildlife Sanctuary</td>
<td>27.022367, 92.414367</td>
<td>1250</td>
<td>GPS taken as “New Khelling” 27°01.342 N 92°24.862 E, 1270 m from Songdi &amp; Kunte (2016) though the plotted point is at an elevation of ca. 1040 m a.s.l. in Google Maps.</td>
<td>Athreya (2006)</td>
</tr>
<tr>
<td>A. cf. wangyali</td>
<td>India</td>
<td>Arunachal Pradesh</td>
<td>West Kameng</td>
<td>“Sessni (1250 m)”, Eaglenest Wildlife Sanctuary</td>
<td>27.047500, 91.418611</td>
<td>1250</td>
<td>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.</td>
<td>Athreya (2006)</td>
</tr>
</tbody>
</table>

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
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, Nuijiang 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 clade. Our analysis demonstrates that the sequence from the type 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 2015.06.06.1 [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).

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. wanyufanisi.

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. wanyufanisi.

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. gyirongesis 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. wanyufanisi).

The minimum interspecific p-distance observed using this alignment dataset is 5.6 % each between A. viridimaculatus and A. baibengensis, A. medogensis and A. wanyufanisi 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. gyirongesis 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. gyirongesis 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 (M2484729.1) 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 congener 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[sic]: Chanda & Ghosh (1988:626); Swan (1993:143).

**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 Subdivision, 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 Bouleneger (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
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, FIII/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 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 wangyal sp. nov.
{urn:lsid:zoobank.org:pub:729D12FC-6EAD-49C5-B17A-B901FD636FCA}
- Amolops aff. himalayanus: Streicher et al. (2020:494, 495, fig. 1 [S10 & S11]).
- 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 Boidirang 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 Tshe Ringbup 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 Bodirang Chhu/Stream (27.27223, 91.53129, 1,700 m a.s.l.), Namla Village, Kanglung Gewog (village block), Trashigang District, eastern Bhutan, collected by Tshe Ringbup 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 positioned between choanae, transversely orientated; more equidistant from each other and choanae, anterior ends more raised posteriorly, narrow, slightly obtuse, distinct (Figs. 3A–C, 4A); vomerine ridges well developed, 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 tubercules; 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 speckles; dorsum of fingers (including expanded discs) dark brown with irregularly arranged green speckles; 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 limbs 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 wongyali* 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.
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–I1I0–I1I1–I1V1.5–0V in SCZM 2019.07.18.2 (Fig. 5G) and I0–I1I0–I1I1–I1V–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 more developed (than 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); and on SCZM 2019.07.18.2, 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 *Amolops senchalensis* species group, with a special emphasis on *A. senchalensis*, as well as additional Bhutan torrent frogs described in recent years (Table 3 & Figs. 5 & 6).

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.

<table>
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following species confirmed to be members of the *viridimaculatus* group based on molecular phylogenetic analyses: *A. baibengensis*, *A. formosus* (*A. gyironensis* 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. jainti* Ray, 1999, 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 mm, n=2; Jiang et al., 2020 [in Che et al., 2020]; note: English language diagnosis erroneously stated “SVL 75.8 mm in females, 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. gyironensis* 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 T1 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 FILL/SVL 27–28 %, n=3 (vs. SN/EN 120–127 % and FILL/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; not mentioned; Zhang et al., 2021), supratympanic fold well-developed, 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; 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-Samdrujongkhar 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.), Trashi Yangtse 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, Trashi Yangtse 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 Trashi Yangtse 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 “*Staurops 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
Review of Bhutan torrent frogs, a new species, and taxonomic validity of other species

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 Bodirang 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); Tshawang & 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 982 m”, 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 1745 m” (Wangyal, 2013). Serkang Chu 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. puttaensis*”; 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 liebigi* (Günter, 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
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 “Hylo 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 “Hylo 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 “Hylo” (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 “Hylo 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 Hylo 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 “Hylo 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 vouchedered 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 vouchedered 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.

ACKNOWLEDGEMENTS

The Department of Forest and Park Services, Bhutan
kindly provided specimen collection permits to TN ( Permit no. 2054743658FF950D3594C) and the National Biodiversity Centre, Ministry of Agriculture & Forests, Royal Government of Bhutan issued the Material Transfer Agreement (Ref. no. NBC/BRD-7/2019–2020) that made this research possible. For logistical support in the field and assistance organising permits TN thanks the Sherubtse College management, particularly Tshering Wangdi (President) and Dedup Tshering (Dean of Research & Industrial Linkages), and Malcolm J. Pearch (previously in Harrison Institute, UK), and for their assistance in the field, Nub Tshering Lepcha, Penjor, Dawa Gyeltshen, Namgay Rinchen, Ash B. Limbu and Namgay Dorji; SM thanks Kaushik Deuti and K. Venkataraman, and staff of the amphibian sections (ZSIK) for access to specimen collections and support during museum visits, Jens Vindum (CAS) for providing images for a type specimen, Hellen Pethers (NHM library) for confirming the missing reference in J. Bengal Nat. Hist. Soc. We thank Tshering Wangdi (President) and Karma Wangchuck (Head of Department of Environment & Life Sciences) of Sherubtse College for hosting SM, JWS and RGK. SM, JWS and RGK thank Ugyen Norbu and Parop Wangdi for their companionship and/or logistical assistance during our Bhutan visit. This research was supported by the following funding: Irish Research Council-Marie Skłodowska-Curie CAROLINE Fellowship (CLNE/2017/482) to SM; Natural History Museum, London support from the Department of Life Sciences to JWS and SM; Percy Sladen Memorial Fund from the Linnean Society to SM; first (2015–2016) and second (2018–2019) Rufford Small Foundation Grants to TN. We thank the two anonymous reviewers for their suggestions and comments on our submitted manuscript. 

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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Zootaxa, 3722(2), 143–169.


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[172]
12S-tVal-16S assembled arcDNA sequences used in Maximum Likelihood analyses

>Amolops_himalayanus_BMNH1947.2.3.83_12S-tVal-16S
ACGACGTTTTACACTGACTAGTTGCTAGGATTCACAAAATT
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CTAACACTCTCAGTATCAAGCTGAGTGCAGAACAAAAT
ATGACGTTTTATACACTGACTAGTTGCTAGGATTCACAAAATT
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**: 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 *Iaxlus 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]87.11.2.20: 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 monticolae**: ZSIK 10036 (holotype), adult female, “Darjeeling”, West Bengal, India.

**Amolops nidorbellus**: ZSIK A 10965 (holotype), BNHS 5276 (paratypes), SDB 2007.129 (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 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”.

**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 *Iaxlus 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 monticolae**: ZSIK 10036 (holotype), adult female, “Darjeeling”, West Bengal, India.

**Amolops nidorbellus**: ZSIK A 10965 (holotype), BNHS 5276 (paratypes), SDB 2007.129 (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”.

Accepted: 17 May 2022