

# A new species of *Amolops* (Anura: Ranidae) from northern Myanmar

## DEAR EDITOR,

A new species of the genus *Amolops*, *Amolops putaoensis* sp. nov., is described from northern Myanmar. The new species can be distinguished from its congeners by the following characters: (1) dorsolateral fold distinct; (2) upper-lip stripe white; (3) male body size 37.6–40.2 mm; (4) ground color of dorsal surface brown, flank green, small warts on dorsum; (5) two internal subgular vocal sacs present; (6) HL slightly shorter than HW; (7) two palmar tubercles present, supernumerary tubercles and outer metatarsal tubercle absent; (8) tympanum smaller than half of eye diameter; (9) vomerine teeth present; (10) tibiotarsal articulation reaching beyond snout tip; (11) supratympanic fold indistinct; (12) pineal body present; (13) finger webbing absent, presence of circummarginal groove on tip of first finger; (14) nuptial pads present. The population from Myanmar represented a distinct maternal lineage within the *Amolops monticola* group and was recovered as a sister taxon to *Amolops aniqiaoensis* with strong support (100) based on concatenated data. Average uncorrected pairwise distances (*P*-distances) between the specimens from Myanmar and other species in the genus ranged from 2.69% (vs. *A. aniqiaoensis*) to 12.24% (vs. *A. indoburmanensis*) for 16S rRNA, 6.14% (vs. *A. aniqiaoensis*) to 15.79% (vs. *A. panhai*) for *COI*, and 9.66% (vs. *A. aniqiaoensis*) to 19.52% (vs. *A. afghanus*) for *ND2*.

The genus *Amolops* Cope, 1865 is distributed widely from Nepal and northern India to western and southern China and southward to the Malay Peninsula, with 61 species currently recorded (Frost, 2020; Gan et al., 2020). The *Amolops monticola* species group, characterized by smooth skin, dark side of head with light-colored upper lip stripe extending to axilla, and distinct dorsolateral folds (Jiang et al., 2016; Stuart et al., 2010; Yu et al., 2019; Yuan et al., 2018), currently contains 19 species (Gan et al., 2020), including *Amolops akhaorum* Stuart, Bain, Phimmachak, & Spence, *Amolops*

*aniqiaoensis* Dong, Rao, & Lü, *Amolops archotaphus* (Inger & Chanard), *Amolops bellulus* Liu, Yang, Ferraris, & Matsui, *Amolops chakrataensis* Ray, *Amolops chunganensis* (Pope), *Amolops compotrix* (Bain, Stuart, & Orlov), *Amolops cucae* (Bain, Stuart, & Orlov), *Amolops chayuensis* Sun, Luo, Sun and Zhang, *Amolops daorum* (Bain, Lathrop, Murphy, Orlov, & Ho), *Amolops gerbillus* (Annandale), *Amolops iriodes* (Bain & Nguyen), *Amolops mengdingensis* Yu, Wu, & Yang, *Amolops mengyangensis* Wu & Tian, *Amolops monticola* (Anderson), *Amolops nyingchiensis* Jiang, Wang, Xie, Jiang, & Che, *Amolops tuanjieensis* Gan, Yu, & Wu, *Amolops vitreus* (Bain, Stuart, & Orlov), and *Amolops wenshanensis* Yuan, Jin, Li, Stuart, & Wu. The group is distributed throughout southern and eastern Himalaya, southern China, and mainland Southeast Asia (Yuan et al., 2018), but has not been recorded in Myanmar, where currently seven *Amolops* species are known: i.e., *Amolops afghanus* (Günther), *Amolops kaulbacki* (Smith), *Amolops longimanus* (Andersson), *Amolops marmoratus* (Blyth), *Amolops indoburmanensis* Dever, Fuiten, Konu, and Wilkiinson, *Amolops panhai* Matsui and Nabhitabhata, and *Amolops viridimaculatus* (Jiang) (Dever et al., 2012).

During recent field surveys in northern Myanmar between 2016 and 2017, we collected three *Amolops* specimens resembling species from the *A. monticola* group, i.e., skin smooth, head side dark with light-colored upper lip stripe extending to axilla, and distinct dorsolateral folds. However, they differed from known members of *Amolops* in both morphological and molecular characters. Thus, we considered these specimens to represent a new species of the genus

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*Amolops*, which we describe herein.

The *Amolops* specimens collected during field surveys at Putao Township, Kachin State, Myanmar (Figure 1A) were photographed, euthanized, fixed in 75% ethanol, and stored in 75% ethanol. Liver and muscle tissues were preserved in 99% ethanol. Specimens were deposited at Guangxi Normal University (GXNU). *Amolops ricketti* (Boulenger) and *Amolops cremnobatus* Inger & Kottelat were selected as outgroup species according to Gan et al. (2020) and their sequences were downloaded from GenBank.

Total genomic DNA was extracted from the liver and muscle tissues. Tissue samples were digested using proteinase K, and subsequently purified following standard phenol/chloroform isolation and ethanol precipitation. Fragments encoding partial 16S ribosomal RNA (16S), partial cytochrome oxidase subunit I (COI), and complete NADH dehydrogenase subunit 2 (ND2) genes were amplified and sequenced following Yu et al. (2019). All new sequences were deposited in GenBank under accession Nos. MT901382–MT901384 and MT901210–MT901214 (Supplementary Table S1). Sequences were aligned using MUSCLE with default parameters in MEGA 7 (Kumar et al., 2016) and phylogeny was inferred using Bayesian inference. Uncorrected pairwise distances (*P*-distances) between species were calculated in MEGA 7. The best substitution model of the concatenated data of 16S, COI, and ND2 was selected using Akaike Information Criterion (AIC) in MODELTEST v3.7 (Posada & Crandall, 1998). Bayesian inference was performed in MRBAYES v3.2 (Ronquist et al., 2012) under the selected substitution model: GTR + I + G. Two runs were performed simultaneously with four Markov chains starting from a random tree. The chains were run for 5 000 000 generations and sampled every 100 generations. Convergence and burn-in were checked in Tracer v1.6 (Rambaut et al., 2014). The first 25% of sampled trees were discarded as burn-in and the remaining trees were used to create a consensus tree and to estimate Bayesian posterior probabilities (BPPs).

Morphometric data were taken using digital calipers to the nearest 0.1 mm. Morphological terminology followed the China Wildlife Protection Association (1999). Measurements included: snout-vent length (SVL, tip of snout to vent); head length (HL, tip of snout to rear of jaws); head width (HW, width of head at widest point); snout length (SL, tip of snout to anterior border of eye); internarial distance (IND, distance between nares); interorbital distance (IOD, minimum distance between upper eyelids); upper eyelid width (UEW, maximum width of upper eyelid); eye diameter (ED, diameter of exposed portion of eyeball); tympanum diameter (TD, greater of tympanum vertical and horizontal diameters); forearm and hand length (FHL, elbow to tip of third finger); thigh length (THL, vent to knee); tibia length (TL, knee to heel); tarsus and foot length (TFL, tibiotarsal joint to tip of fourth toe); and foot length (FL, proximal end of inner metatarsal tubercle to tip of fourth toe). Comparative morphological data of congeners were taken from their original descriptions or re-descriptions

(Bain et al., 2003; Bain & Truong, 2004; Bain et al., 2006; Biju et al., 2010; Boulenger, 1888; Chinese Zoology Editorial Committee & Chinese Academy of Sciences, 2009; Dever et al., 2012; Fei et al., 2017; Gan et al., 2020; Inger & Chanard, 1997; Inger & Kottelat, 1998; Inger et al., 1999; Jiang, 1983; Jiang et al., 2016; Liu, 1945, 1950; Liu & Hu, 1961, 1975; Liu et al., 2000; Lu et al., 2014; Lyu et al., 2018, 2019a, 2019b; Matsui & Nabhitabhata, 2006; Onn et al., 2018; Orlov & Ho, 2007; Pope, 1929; Pope & Romer, 1951; Pham et al., 2019; Qi et al., 2019; Rao & Wilkinson, 2007; Ray, 1992, 1999; Sengupta et al., 2008; Smith, 1923; Stuart et al., 2010; Su et al., 1986; Sung et al., 2016; Wu & Tian, 1995; Yang, 1991; Yu et al., 2019; Yuan et al., 2018; Zhao et al., 2005).

The obtained alignments of the 16S rRNA, COI, and ND2 genes were 855 bp, 629 bp, 1017 bp, respectively. Average *P*-distances between the specimens from Myanmar and other species in *Amolops* ranged from 2.69% (vs. *A. aniqiaoensis*) to 12.24% (vs. *A. indoburmanensis*) for 16S rRNA (Supplementary Table S2), 6.14% (vs. *A. aniqiaoensis*) to 15.79% (vs. *A. panhai*) for COI (Supplementary Table S3), and 9.66% (vs. *A. aniqiaoensis*) to 19.52% (vs. *A. afghanus*) for ND2 (Supplementary Table S4). The population from Myanmar represented a distinct maternal lineage within the *A. monticola* group and was recovered as a sister taxon to *A. aniqiaoensis* with strong support (100) based on the concatenated data (Figure 1B). Moreover, morphologically these specimens were distinguished from all other species of *Amolops* by a series of characters. Thus, we describe these specimens as a new *Amolops* species.

#### Taxonomic account

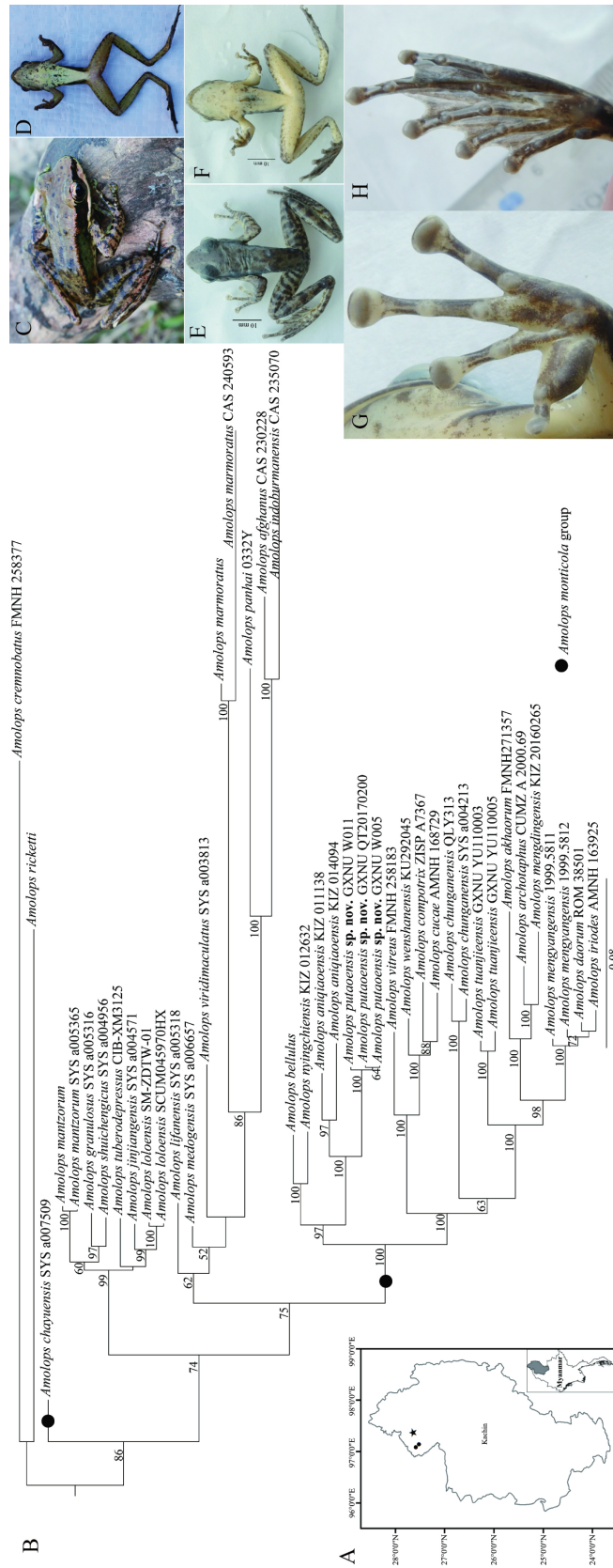
*Amolops putaoensis* sp. nov. (Figure 1C–H; Table 1)

**Holotype:** GXNU QT20170200, adult male, collected on 9 December 2017 by Tao Qin from a small stream of upper Mali Kha River, Putao County, Kachin State, Myanmar (N27°38'48.7", E97°22'28.7", 544 m a.s.l.).

**Paratypes:** GXNU W005, adult male, collected from Putao County, Kachin State, Myanmar (N27°34'55.2", E97°5'9.59", 1098 m a.s.l.) on 13 December 2016. GXNU W011, adult male, collected from Putao County, Kachin State, Myanmar (N27°31'12.0", E97°8'31.2", 940 m a.s.l.) on 20 December 2016.

**Etymology:** The specific epithet is named for Putao Township, where the type locality is located. We suggest the English common name as “Putao cascade frog” and the Chinese common name as “葡萄湍蛙”.

**Diagnosis:** Morphologically, *Amolops putaoensis* sp. nov. can be distinguished from its congeners by a combination of the following characters: (1) dorsal skin smooth; (2) dorsolateral fold distinct; (3) head side dark with light-colored upper lip stripe extending to axilla; (4) finger webbing absent, presence of circummarginal groove on tip of first finger; (5) body size of males 37.6–40.2 mm; (6) ground color of dorsal surface brown, flank green, small warts on dorsum; (7) two internal subgular vocal sacs present; (8) two inner palmar tubercles present, supernumerary tubercles and outer



**Figure 1** Distribution, phylogenetic position, and holotype of *Amolops putaoensis* sp. nov. A: Map showing collection sites of *Amolops putaoensis* sp. nov. from Putao Township, Kachin State, Myanmar. Star indicates type locality and two circles indicate sampling sites of paratypes. B: Bayesian phylogram of *Amolops* species inferred from combined 16S rRNA, COI, and ND2 data. C, D: Dorsolateral and ventral views of holotype of *Amolops putaoensis* sp. nov. (GXNU QT20170200) in life. E, F: Dorsal and ventral views of holotype in preservative. Ventral view of hand (G) and foot (H) of holotype in preservative. (Photos C and D were taken by Tao Qin, photos E–H were taken by Guo-Hua Yu).

**Table 1 Morphological measurements (mm) of holotype and paratypes of *Amolops putaoensis* sp. nov.**

	GXNU W011	GXNU QT20170200(Holotype)	GXNU W005
Sex	M	M	M
SVL	38.1	40.2	37.6
HL	11.9	13.6	12.9
HW	12.3	13.8	13.4
SL	4.9	5.5	5.1
IND	3.8	4.4	4.2
IOD	3.4	3.9	3.8
UEW	3.2	3.9	3.3
ED	5.3	6.0	5.2
TD	2.3	2.7	2.4
FHL	21.3	21.5	22.7
THL	21.6	21.4	22.2
TL	25.6	23.7	25.2
TFL	33.4	34.3	33.4
FL	20.9	22.6	21.6

See text for abbreviations. M: Male.

metatarsal tubercle absent; (9) tooth-like apophysis on anterior part of lower jaw absent; (10) “/ \”-shaped mark on chest absent; (11) HL slightly shorter than HW; (12) tympanum smaller than half of eye diameter; (13) vomerine teeth present; (14) tibiotarsal articulation reaching beyond snout tip; (15) supratympanic fold indistinct; (16) pineal body present; and (17) nuptial pads present.

**Description of holotype** (all measurements in mm; see Table 1): Adult male (SVL 40.2 mm); head slightly wider (HW 13.8 mm) than long (HL 13.6 mm); snout obtusely pointed, projecting beyond margin of lower jaw in ventral view, rounded in profile; canthus rostralis distinct, nearly straight; loreal region sloping, concave; nostrils oval, slightly protuberant, closer to snout tip than to eye; internarial distance (IND 4.4 mm) greater than interorbital distance (IOD 3.9 mm); upper eyelid width (UEW 3.9 mm) equal to interorbital distance; pineal spot present; pupil oval, horizontal; tympanum distinct, rounded, less than half eye diameter; supratympanic fold indistinct; vomerine teeth in two oblique rows; maxillary teeth present; choanae oval; tongue attached anteriorly, cordiform, notched posteriorly; vocal sac opening on floor of mouth at each corner; pair of internal subgular vocal sacs present (Figure 1C–F).

Forelimbs robust, relative length of fingers I<II<IV<III; all finger tips expanded into discs with circummarginal and transverse grooves; velvety nuptial pad present on first finger; webbing between fingers absent; subarticular tubercles prominent and rounded, formula 1, 1, 2, 2; supernumerary tubercle absent; two metacarpal tubercles present, oval (Figure G).

Hindlimbs long, heels overlapping when legs positioned at right angle to body, tibiotarsal articulation reaching beyond snout tip; tibia length (TL 23.7 mm) longer than thigh length (THL 21.4 mm) and foot length (FL 22.6 mm); relative length

of toes I<II<III<V<IV; dermal fringe on preaxial side of toe I and postaxial side of toe V; all toe tips expanded into discs with circummarginal and transverse grooves, slightly narrower than discs of outer fingers; webbing between toes well developed, webbing formula I1-1.5II1-2III1-2IV2-1V (Myers & Duellman, 1982); subarticular tubercles distinct, formula 1, 1, 2, 3, 2; inner metatarsal tubercle prominent, oval; outer metatarsal tubercle absent; supernumerary tubercle absent (Figure 1H).

Dorsolateral folds distinct, extending from rear of eye to near vent; skin on dorsal and ventral surfaces of head, body, limbs, and flanks smooth, with exception of small white warts on posterior part of dorsum; two rectal glands present, continuous with upper lip; humeral gland absent.

**Color of holotype in life:** Upper one-fourth of iris bronze with black reticulations, lower three-fourths dark; top of head and dorsum brown with dark spots; head sides black, white upper lip stripe extending to axilla, mottled with red brown; flanks green, scattered with sparse black spots; groin orangish; limbs dorsally brown with dark bars on hindlimbs and lower arms; throat grayish, scattered with irregular dark spots; chest and venter dirty white, scattered with irregular dark spots; ventral surface of limbs grayish with small dark spots; anterior and posterior sides of thigh yellow, scattered with large dark blotches.

**Color of holotype in preservative:** Dorsal surface grayish brown; throat, chest, abdomen, and ventral surface of limbs faded to white, scattered with brown spots.

**Morphological variation:** Morphological measurements of holotype and paratypes are presented in Table 1. Because all types are male, sexual dimorphism could not be determined.

**Distribution and ecology:** The new species is known only from Kachin State, Northern Myanmar. The holotype was found sitting on a branch (ca. 0.5 m above the ground) of a withered tree near the bank of a fast-flowing stream. No tadpoles or vocal recordings were collected for the new species.

**Comparisons (Supplementary Table S5; Supplementary Note S1):** Both morphological characters and phylogenetic analyses indicated that the new species belongs to the *A. monticola* group with strong support. Morphologically, the new species can be distinguished from the 19 congeners in the *A. monticola* group by the following characteristics: (1) body size of males 37.6–40.2 mm (vs. larger male body size in *A. aniqiaoensis* (SVL 52.0 mm) and *A. bellulus* (SVL 45.9–50.1 mm)); (2) vomerine teeth present (vs. absent in *A. daorum*); (3) dorsolateral fold distinct (vs. weak or absent in *A. archotaphus*); (4) tibiotarsal articulation reaching beyond snout tip (vs. reaching tympanum or just posterior to corner of eye in *A. chakrataensis*; reaching end of snout in *A. chunganensis*; reaching end of snout or between eye and snout in *A. mengyangensis*); (5) two internal subgular vocal sacs present (vs. two external vocal sacs present in *A. akhaorum*, *A. cucae*, *A. compotrix*, *A. iriodes*, *A. mengdingensis*, *A. monticola*, *A. tuanjiensis*, *A. vitreus*, and *A. wenshanensis*, and vocal sac absent in *A. nyingchiensis*); (6) webbing between fingers

absent (vs. rudimentary webbing between third and fourth fingers in *A. gerbillus*); (7) dorsal surface brown (vs. green in *A. chayuensis*).

There are seven species of *Amolops* known from Myanmar, including *A. afghanus*, *A. kaulbacki*, *A. longimanus*, *A. marmoratus*, *A. indoburmanensis*, *A. panhai*, and *A. viridimaculatus* (Dever et al., 2012). The new species can be distinguished from *A. afghanus*, *A. kaulbacki*, *A. marmoratus*, *A. indoburmanensis*, *A. panhai*, and *A. viridimaculatus* by dorsolateral fold distinct (vs. absent), two internal subgular vocal sacs present (vs. two external vocal sacs present in *A. afghanus*, *A. marmoratus*, *A. indoburmanensis*, *A. panhai*; and absent in *A. kaulbacki* and *A. viridimaculatus*); and from *A. longimanus* by parotoid-like swelling above tympanum absent (vs. present) and tympanum visible (vs. invisible). The new species further differs from these species by light-colored upper lip stripe present (vs. absent).

*Amolops putaoensis* sp. nov. is distinguishable from the remaining 36 congeners based on the following characters: (1) dorsolateral fold distinct (vs. absent in *A. albispinus* Sung, Hu, Wang, Liu, and Wang, *A. australis* Onn, Abraham, Grismer, and Grismer, *A. caelumnoctis* Rao and Wilkinson, *A. daiyunensis* (Liu & Hu), *A. formosus* (Günther), *A. gerutu* Onn, Abraham, Grismer, and Grismer, *A. hainanensis* (Boulenger), *A. himalayanus* (Boulenger), *A. hongkongensis* (Pope & Romer), *A. larutensis* (Boulenger), *A. lifanensis* (Liu), *A. loloensis* (Liu), *A. mantzorum* (David), *A. minutus* Orlov & Ho, *A. ottorum* Pham, Sung, Pham, Le, Ziegler, and Nguyen, *A. pallasitatus* Qi, Zhou, Lyu, Lu, and Li, *A. ricketti* (Boulenger), *A. sinensis* Lyu, Wang, and Wang, *A. spinapectoralis* Inger, Orlov, and Darevsky, *A. torrentis* (Smith), *A. tuberodepressus* Liu and Yang, *A. wuyiensis* (Liu & Hu), *A. xinduiqiao* Fei, Ye, Wang, and Jiang, *A. yatseni* Lyu, Wang, and Wang, and *A. yunkaiensis* Lyu, Wang, Liu, Zeng, and Wang); (2) smaller or larger male body size (SVL 37.6–40.2 mm vs. 52.8–61.5 mm in *A. assamensis* Sengupta, Hussain, Choudhury, Gogoi, Ahmed, and Choudhury, 32–34 mm in *A. cremnobatus* Inger & Kottelat, 43–52 mm in *A. jinjiangensis* Su, Yang, and Li, 42.8–48.6 in *A. kohimaensis* Biju, Mahony, and Kamei, 95 mm in *A. medogensis* Li & Rao, 76.4–82.3 mm in *A. nidorbellus* Biju, Mahony, and Kamei, 62.6–75.6 mm in *A. splendissimus* Orlov & Ho); (3) outer metatarsal tubercle absent (vs. present in *A. jaunsari* Ray); (4) two internal vocal sacs present (vs. absent in *A. shuichengicus* Lyu and Wang); (5) circummarginal groove on tip of first finger present (vs. absent in *A. formosus*); and (6) dorsal surface of limbs smooth (vs. rough with small white spines in *A. granulatus* (Liu & Hu)).

**Remarks:** Myanmar is an important component of the Indo-Burma biodiversity hot-spot (Conservation International, available at: [http://www.biodiversityhotspots.org/xp/Hotspots/indo\\_burma/](http://www.biodiversityhotspots.org/xp/Hotspots/indo_burma/)) and its northern region lies at a biogeographic crossroads where the faunas of China, Indochina, India, and Himalaya converge (Wogan et al., 2008). However, for nearly half a century, there has been a great shortage of faunal surveys in Myanmar and its herpetofauna remain poorly known. This has hindered our full understanding of the

patterns of distribution and evolutionary histories of Asia's amphibian and reptile species.

Although various studies have shown that northern Myanmar is similar to southern Tibet and western Yunnan in amphibian fauna (e.g., Hui et al., 2019; Yu et al., 2018, 2019), the region seems to be a vacuum in terms of *A. monticola* species group distribution, which is widely reported in neighboring regions (e.g., Annandale, 1912; Gan et al., 2020; Jiang et al., 2016; Liu et al., 2000; Yu et al., 2019; Zhao et al., 2005). The discovery of the new species from northern Myanmar in this study fills a gap in the distribution of the *A. monticola* group between eastern Himalaya and southwestern China. Due to the rich diversity of this species group in neighboring countries, it is expected that more species will be discovered in Myanmar.

Like most previous studies, the *A. monticola* group was not recovered as a monophyly in this study, as *A. chayuensis* did not cluster with the clade consisting of other members of the group (Figure 1B). This may be the result of insufficient genetic information in the present data because Wu et al. (2020) recently supported the monophyly of the *A. monticola* group using data generated from anchored hybrid enrichment.

#### NOMENCLATURE ACTS REGISTRATION

The electronic version of this article in portable document format represents a published work according to the International Commission on Zoological Nomenclature (ICZN), and hence the new names contained in the electronic version are effectively published under that Code from the electronic edition alone (see Articles 8.5–8.6 of the Code). This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information can be viewed through any standard web browser by appending the LSID to the prefix <http://zoobank.org/>.

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#### SCIENTIFIC FIELD SURVEY PERMISSION INFORMATION

Permission for field surveys was granted by the Southeast Asia Biodiversity Research Institute of the Chinese Academy of Sciences (SEABRI, CAS) and Natural Resources and Environmental Conservation of Myanmar.

#### SUPPLEMENTARY DATA

Supplementary data to this article can be found online.

#### COMPETING INTERESTS

The authors declare that they have no competing interests.

## AUTHORS' CONTRIBUTIONS

G.H.Y., S.L., and R.C.Q. conceived and designed the study. Y.L.G., T.Q., Y.H.L., and G.G.L. conducted field surveys. Y.L.G., T.Q., and G.G.L. performed the molecular experiments. Y.L.G. measured the specimens, analyzed the data, and prepared the manuscript. All authors read and approved the final version of the manuscript.

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