

# A new species of the genus *Amolops* (Anura: Ranidae) from Yunnan, China

## DEAR EDITOR,

A new species of the genus *Amolops*, *Amolops tuanjieensis* sp. nov., is described from Yunnan, China. The new species can be distinguished by the following characters: dorsolateral folds present; dorsal and ventral surfaces smooth; top of head and dorsum brown-red with irregular gray and dark spots; flank green; side of head black, from tip of snout, diffusing posteriorly to axilla, continuing as black streak below edge of dorsolateral fold; SVL 39.5–40.4 mm in males, 56.8–60.7 mm in females; tympanum distinct; supratympanic fold indistinct; vomerine teeth in two oblique rows between choanae, closer to each other than choanae; vocal sacs present; nuptial pads present; outer metatarsal tubercle absent, supernumerary tubercles absent; all fingertips expanded into discs; limbs dorsally brown with dark brown bars and irregular dark brown blotches.

The genus *Amolops* Cope, 1865 is distributed throughout Southeast Asia, southern China, and southern and eastern Himalaya. The genus currently contains 59 species (Frost, 2019), 18 of which belong to the *Amolops monticola* species group (Lyu et al., 2019a), characterized by smooth skin, side of head dark with light-colored upper lip stripe extending to axilla, and dorsolateral folds present (Jiang et al., 2016; Stuart et al., 2010; Yuan et al., 2018), including *Amolops aniqiaoensis* Dong, Rao, and Lü, 2005, *Amolops akhaorum* Stuart, Bain, Phimmachak, and Spence, 2010, *Amolops archotaphus* (Inger and Chanard, 1997), *Amolops bellulus* Liu, Yang, Ferraris, and Matsui, 2000, *Amolops chakrataensis* Ray, 1992, *Amolops chunganensis* (Pope, 1929), *Amolops compotrix* (Bain, Stuart, and Orlov, 2006), *Amolops cucae* (Bain, Stuart, and Orlov, 2006), *Amolops chayuensis* Sun, Luo, Sun and Zhang, 2013, *Amolops daorum* (Bain, Lathrop, Murphy, Orlov, and Ho, 2003), *Amolops gerbillus* (Annandale, 1912), *Amolops iriodes* (Bain and Nguyen, 2004), *Amolops mengyangensis* Wu and Tian, 1995, *Amolops monticola*

(Anderson, 1871), *Amolops mengdingensis* and Yu, Wu, Yang, 2019, *Amolops nyingchiensis* Jiang, Wang, Xie, Jiang, and Che, 2016, *Amolops vitreus* (Bain, Stuart, and Orlov, 2006) and *Amolops wenshanensis* Yuan, Jin, Li, Stuart, and Wu, 2018. There are ten species of *A. monticola* group in China (*A. aniqiaoensis*, *A. bellulus*, *A. chunganensis*, *A. chayuensis*, *A. gerbillus*, *A. mengyangensis*, *A. monticola*, *A. nyingchiensis*, *A. wenshanensis* and *A. mengdingensis*) and four occur in Yunnan including *A. bellulus*, *A. mengyangensis*, *A. wenshanensis*, and *A. mengdingensis* (Frost, 2019; Yu et al., 2019).

During recent fieldwork at Tuanjie Township, Gengma Dai and Wa Autonomous County, Yunnan Province, China (Figure 1A), five *Amolops* specimens were collected. These specimens resemble members of the *A. monticola* group in that they have smooth skin, light-colored upper lip stripe extending to axilla, and dorsolateral folds present. Based on morphological comparison and molecular phylogenetic analyses, we considered these specimens to represent a new species of the genus *Amolops*, which is described herein.

Specimens were fixed in 80% ethanol and then stored in 80% ethanol. Muscle tissues were preserved in 99% ethanol. Specimens were deposited at Guangxi Normal University (GXNU).

Total genomic DNA was extracted from the muscle tissues of the five individuals. Fragments encoding partial 16S rRNA (16S), partial cytochrome oxidase subunit I (COI), and complete NADH dehydrogenase subunit 2 (ND2) genes were amplified and sequenced following the protocols of Yu et al. (2019). All new sequences were deposited in GenBank under accession Nos. MN832750–MN832759 and MN832772–MN832776 (Supplementary Table S1). The phylogenetic position of these individuals in *Amolops* was reconstructed based on the three fragments using Bayesian inference (BI) (see Supplementary Methods). Sequence divergence (uncorrected *P* distance) was calculated in MEGA 7 (Kumar et

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al., 2016).

Morphometric data were taken using digital calipers to the nearest 0.1 mm. Measurements followed Fei et al. (1999) (Supplementary Methods). Comparative morphological data of *Amolops* were taken from previous publications (Anderson, 1871; Annandale, 1912; Bain et al., 2003, 2006; Bain & Truong, 2004; Dever et al. 2012; Dong et al., 2005; Fei et al., 2009; Inger & Chanard, 1997; Jiang et al., 2016; Liu et al., 2000; Lu et al. 2014; Lyu et al., 2018, 2019a, 2019b; Orlov & Ho, 2007; Pope, 1929; Rao & Wilkinson, 2007; Ray, 1999; Stuart et al., 2010; Sung et al., 2016; Wu & Tian, 1995; Yu et al., 2019; Yuan et al., 2018).

The specimens from Tuanjie Township represented a distinct lineage and sister taxon to the clade consisting of *A. akhaorum*, *A. archotaphus*, *A. mengdingensis*, *A. mengyangensis*, *A. daorum*, and *A. iriodes*, with strong support (Figure 1B). In addition, the new specimens possess a combination of morphological characters different from all known congeners. Therefore, we describe them as a new species of the genus *Amolops* below.

**Taxonomic account**

*Amolops tuanjieensis* sp. nov. (Figures 1C–J; Table 1)

**Holotype:** GXNU YU110005, adult male, collected on 18 April 2019 by Guo-Hua Yu from Tuanjie Township (N23°32'54.00", E99°20'12.00"; Figure 1A), Gengma Dai and Wa Autonomous County, Yunnan Province, China.

**Paratypes:** GXNU YU110003, GXNU YU110007, and GXNU YU110034, three adult females; GXNU YU110006, adult male, collected at the same time as the holotype from the type locality by Guo-Hua Yu.

**Etymology:** The specific epithet is named for the type locality, Tuanjie Township, Gengma Dai and Wa Autonomous County, Yunnan Province, China. We suggest the English common name as “Tuanjie cascade frog” and the Chinese common name as “团结湍蛙”.

**Diagnosis:** *Amolops tuanjieensis* sp. nov. differs from other members in the genus *Amolops* by the following characters: (1) SVL 39.5–40.4 mm in males and 56.8–60.7 mm in females; (2) dorsolateral folds present; (3) side of head dark with light-colored upper lip stripe extending to axilla; (4) skin on dorsal and ventral surfaces smooth; (5) tympanum distinct, less than half of eye diameter; (6) supratympanic fold indistinct; (7) vomerine teeth in two oblique rows between choanae, closer to each other than to choanae; (8) top of head and dorsum brown-red with irregular black and gray spots; (9) loreal regions dark black; (10) lateral green; (11) pineal body present; (12) nuptial pad velvety; (13) two external subgular vocal sacs in males; (14) all fingertips expanded; (15) two palmar tubercles present; (16) inner metatarsal tubercle oval, outer metatarsal tubercle absent; (17) supernumerary tubercles absent.

**Description of holotype** (all measurements in mm; see Table 1): GXNU YU110005, adult male (SVL 39.5 mm); head longer

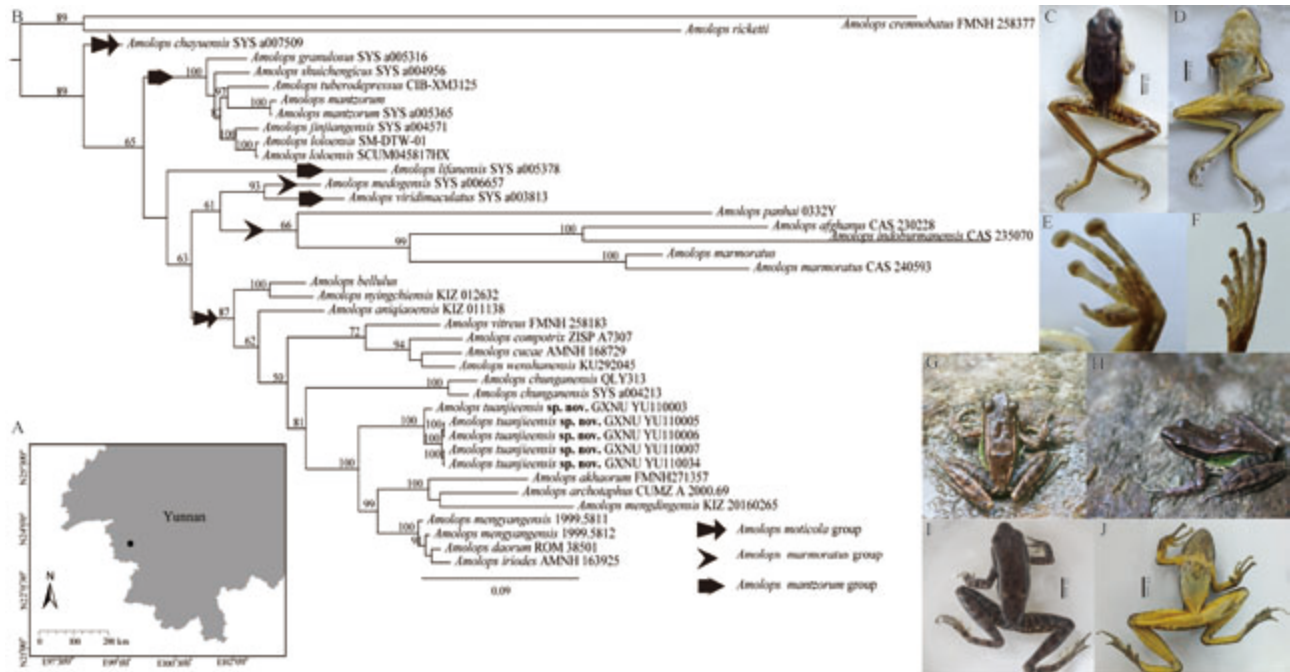


Figure 1 Collection site of *Amolops tuanjieensis* sp. nov. from Yunnan, China (A) and Bayesian phylogram of *Amolops* species inferred from a combination of 16S rRNA, CO1, and ND2 (B). Dorsal (C) and ventral (D) views of holotype of *Amolops tuanjieensis* sp. nov. (GXNU YU110005) in preservative. Ventral view of hand (E) and foot (F) of holotype in preservative. Dorsal (G) and lateral (H) views of paratype of *Amolops tuanjieensis* (GXNU YU110034) in life and dorsal (I) and ventral (J) views of paratype (GXNU YU110034) in preservative. Numbers above branches are Bayesian posterior probabilities (only values above 50% are shown).

**Table 1** Measurements (mm) of holotype and paratypes of *Amolops tuanjiensis* sp. nov.

	GXNU YU110003	GXNU YU110005(Holotype)	GXNU YU110006	GXNU YU110007	GXNU YU110034
Sex	♀	♂	♂	♀	♀
SVL (Snout-Vent Length)	60.7	39.5	40.4	57.3	56.8
HL (Head Length)	20.2	13.7	14.7	18.6	18.6
HW (Head Width)	20.1	11.7	12.9	18.0	18.1
SL (Snout Length)	9.1	5.5	6.5	8.2	7.5
IND (Internarial Distance)	6.4	4.4	4.3	6.2	6.4
IOD (Interorbital Distance)	6.2	4.1	3.9	6.1	6.3
UEW (Upper Eyelid Width)	5.6	4.0	4.0	4.3	4.5
ED (Eye Diameter)	8.4	5.7	5.9	7.9	7.7
TD (Tympanum Diameter)	3.1	2.7	2.6	2.6	2.1
FHL (Forearm and Hand Length)	34.4	22.5	19.2	30.1	31.9
THL (Thigh Length)	31.1	20.6	20.3	29.4	33.7
TL (Tibia Length)	36.7	24.1	24.8	35.7	37.0
TFL (Length of Foot and Tarsus)	50.3	34.0	31.8	45.1	48.6
FL (Foot Length)	28.3	18.3	19.1	26.0	29.7
F3DSC (Horizontal Diameter of Digital Disc of Finger III)	3.1	1.9	1.6	2.3	3.1

(HL 13.7 mm) than wide (HW 11.7 mm); snout obtusely pointed, projecting beyond margin of lower jaw; canthus rostralis distinct; loreal region sloping, concave; nostrils oval, lateral, closer to eye than snout tip; internarial distance (IND 4.4 mm) larger than interorbital distance (IOD 4.1 mm); upper eyelid width (UEW 4.0 mm) narrower than interorbital space; tympanum distinct (TD 2.7 mm), less than half eye diameter (ED 5.7 mm); supratympanic fold indistinct; vomerine teeth in two oblique rows between choanae, closer to each other than to choanae; tongue attached anteriorly, cordiform deeply notched posteriorly (Figure 1C–D).

Forelimbs moderately long with slender fingers; relative length of fingers I<II<IV<III; all fingertips expanded into discs with circummarginal grooves; webbing between fingers absent; subarticular tubercles prominent and rounded, formula 1, 1, 2, 2; supernumerary tubercle present; two metacarpal tubercles, oval (Figure 1E).

Hindlimbs long, tibiotarsal articulation reaching beyond tip of snout; tibia length (TL 24.1 mm) longer than thigh length (THL 20.6 mm) and foot length (FL 18.3 mm); relative length of toes I<II<III<V<IV; all toe tips expanded into discs with circummarginal and transverse grooves; webbing between toes well developed, webbing formula I1–2II2–2III1–2IV2–1V; subarticular tubercles distinct, formula 1, 1, 2, 3, 2; inner metatarsal tubercle prominent, oval; outer metatarsal tubercle absent; supernumerary tubercles absent (Figure 1F).

Wide and flattened dorsolateral fold present; skin on dorsal and ventral surfaces smooth; dorsal limbs smooth; flanks granular; small warts above vent.

**Color of holotype in life:** Top of head and dorsum brown-red with irregular gray and dark spots; side of head black, from tip of snout, diffusing posteriorly to axilla, continuing as black streak below edge of dorsolateral fold; golden upper lip stripe extending to axilla; narrow golden stripe along above edge of

dorsolateral fold; limbs dorsally brown with dark brown bars and irregular dark brown blotches; upper part of flanks green with dark blotches, lower part of flanks white with large dark blotches.

**Color of holotype in preservative:** Top of head and dorsum red-black; dorsal surface of limbs yellow with black bands; dorsolateral fold gray-white; lateral faded to black; throat, chest, venter, and ventral surface of limbs light yellow, scattered with light blotches on chest (Figure 1C–F).

**Male secondary sexual characteristics:** Adult males possess nuptial pads covering dorsal surface of base of first finger; two external subgular vocal sacs with slit-like opening at posterior of jaw.

**Morphological variation:** Measurements of holotype and paratypes are given in Table 1. The new species is sexually dimorphic, with females being obviously larger than males and having no vocal sacs or nuptial pads. Paratype GXNU YU110034 has more streaks on throat and chest than others (Figure 1G–J).

**Distribution and ecology:** The new species is known only from the type locality (Supplementary Figure S1). The holotype and paratypes were found on leaves and small branches, less than 1 m above the ground along a stream. No tadpoles or vocal recordings were collected for the new species.

**Comparisons:** Within the *A. monticola* group, the new species (SVL 39.5–40.4 mm in males, 56.8–60.7 mm in females) is distinguishable from *A. akhaorum* (SVL 34.9–37.2 mm in males), *A. chakrataensis* (SVL 55.0 mm in females), *A. chunganensis* (SVL 34.0–39.0 mm in males, SVL 44.0–54.0 mm in females), *A. daorum* (SVL 34.8–38.1 mm in males, SVL 53.3–57.6 mm in females), and *A. wenshanensis* (SVL 35.7–39.9 mm in males, SVL 43.7–45.6 mm in females) by having larger body size and from *A. aniqiaoensis* (SVL

52.0 mm in males), *A. bellulus* (SVL 45.9–50.1 mm in males, SVL 63.6 mm in females), *A. cucae* (SVL 40.7–44.6 mm in males, SVL 65.9–68.0 mm in females), *A. chayuensis* (SVL > 42.0 mm in males), and *A. nyingchiensis* (SVL 48.5–58.3 mm in males) by having smaller body size. The new species further differs from *A. akhaorum*, *A. aniqiaoensis*, *A. archotaphus*, *A. compotrix*, *A. cucae*, *A. chayuensis*, *A. daorum*, *A. iriodes*, *A. mengyangensis*, *A. mengdingensis*, *A. vitreus*, and *A. wenshanensis* by dorsum red-brown (vs. green); from *A. archotaphus* and *A. chunganensis* by distinct dorsolateral folds present (vs. weakly developed); and from *A. bellulus* and *A. nyingchiensis* by vocal sacs present (vs. absent). *Amolops tuanjieensis* **sp. nov.** is further distinguished from *A. chakrataensis* by supratympanic fold absent (vs. distinct) and from *A. archotaphus*, *A. compotrix*, *A. cucae*, and *A. vitreus* by outer metatarsal tubercle absent (vs. present). The new species differs from *A. gerbillus* by distinct tympanum present (vs. small or indistinct) and finger webbing absent (vs. rudimentary webbing between fingers III and IV) and from *A. monticola* by dorsum brown-red (vs. dorsal surface brown or yellow), limb dorsally brown with dark brown bars (vs. upper surface of legs grayish, obscurely banded), and line from eye to glandular fold absent (vs. pale bluish line from eye along glandular fold present).

*Amolops tuanjieensis* **sp. nov.** differs from members of the *Amolops marmoratus* group (*A. afghanus* (Günther, 1858), *A. marmoratus* (Blyth, 1855), *A. medogensis* Li and Rao, 2005, *A. indoburmanensis* Dever, Fuiten, Konu and Wilkinson, 2012, and *A. panhai* Matsui and Nabhitabhata, 2006) by distinctive dorsolateral folds present (vs. absent).

Compared to the *Amolops mantzorum* group, *Amolops tuanjieensis* **sp. nov.** can be easily distinguished from *A. lifanensis* (Liu, 1945), *A. loloensis* (Liu, 1950), *A. mantzorum* (David, 1872), *A. tuberodepressus* Liu and Yang, 2000, *A. xinduqiao* (Fei, Ye, Wang, and Jiang, 2017), and *A. viridimaculatus* (Jiang, 1983) by dorsolateral folds present (vs. absent in all) and from *A. jinjiangensis* Su, Yang, and Li, 1986, *A. shuichengicus* Lyu and Wang, 2019, and *A. granulosus* (Liu and Hu, 1961) by having two external vocal sacs (vs. vocal sac absent in *A. jinjiangensis* and *A. shuichengicus* and vocal sac internal in *A. granulosus*).

In addition, *Amolops tuanjieensis* **sp. nov.** differs from *Amolops caelumnoctis* Rao & Wilkinson, 2007 and *Amolops splendissimus* Orlov & Ho, 2007, both of which occur in Yunnan but are not assigned to any species group, by having smaller body size (SVL 36.9–40.2 mm in males, SVL 64.3 mm in females vs. SVL 71.3–73.7 mm in males, SVL 78.0–90.6 mm in females in *A. caelumnoctis* and SVL 62.6–75.6 mm in males, SVL 69.3–96.8 mm in females in *A. splendissimus*), dorsolateral folds present (vs. absent), white upper lip stripe present (vs. absent), two external subgular vocal sacs present (vs. vocal sac absent), and light yellow spots on dorsum absent (vs. numerous small light yellow spots on dorsum present in *A. caelumnoctis* and *A. splendissimus*).

In China, there are ten other *Amolops* species that belong to three species groups, but are not distributed in Yunnan,

including the *A. ricketti* group (*A. albispinus* Sung, Wang and Wang, 2016, *A. ricketti*, *A. sinensis* Lyu, Wang and Wang, 2019, *A. wuyiensis* (Liu and Hu, 1975), *A. yatseni* Lyu, Wang and Wang, 2019, and *A. yunkaiensis* Lyu, Wang, Liu, Zeng and Wang, 2018), *A. daiyunensis* group (*A. daiyunensis* (Liu and Hu, 1975) and *A. hongkongensis* (Pope and Romer, 1951)), and *A. hainanensis* group (*A. hainanensis* (Boulenger, 1900) and *A. torrentis* (Smith, 1923)) according to Lyu et al. (2019a). *Amolops tuanjieensis* **sp. nov.** can be distinguished from these species by distinctive dorsolateral folds present (vs. absent). Moreover, the new species differs from *A. albispinus*, *A. ricketti*, *A. sinensis*, *A. wuyiensis*, *A. yatseni*, *A. daiyunensis*, *A. hongkongensis*, *A. hainanensis*, and *A. torrentis* by two external subgular vocal sacs present (vs. absent in *A. albispinus*, *A. ricketti*, *A. sinensis*, *A. yatseni*, and *A. hainanensis*, and two internal vocal sacs present in *A. wuyiensis*, *A. daiyunensis*, *A. hongkongensis*, and *A. torrentis*).

**Comments:** In China, species of *Amolops* have been assigned to different species groups based on morphological characters (Fei et al., 2009). However, consistent with Lyu et al. (2019a), our phylogenetic analysis revealed that the division of some species groups needs further investigation. Firstly, *A. chayuensis*, which was placed in the *A. monticola* group by Sun et al. (2013) based on the presence of dorsolateral folds, did not group together with the clade consisting of the new species and other members of the same group, indicating that the *A. monticola* group is not monophyletic and that assignment of species groups based on dorsolateral folds only is problematic. Comprehensive morphological and molecular comparisons using *A. monticola* data are necessary to clarify the division of the *A. monticola* group.

In addition to the problems at the species group level in *Amolops*, species diversity within this genus also needs further investigation. *Amolops marmoratus*, which has been confused with *A. afghanus* and *A. indoburmanensis* (Dever et al., 2012; Lyu et al., 2019a), is mainly distributed in southern Tibet, as well as Myanmar, Bangladesh, Nepal, and eastern Himalaya in India (Frost, 2019), with distribution in Thailand according to Chan-ard (2003). This species is certainly known from Myanmar, but the statuses of other populations remain problematic (Frost, 2019). In this study, we found that the genetic distance between *A. marmoratus* from Thailand and *A. marmoratus* from Myanmar reached 4.48% for the 16S sequences, indicating that *A. marmoratus* from Thailand possibly represents a cryptic species.

## NOMENCLATURAL 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

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## SCIENTIFIC FIELD SURVEY PERMISSION INFORMATION

Permission for field surveys in Gengma County, Yunnan Province was granted by the Forestry Bureau of Gengma County.

## SUPPLEMENTARY DATA

Supplementary data to this article can be found online.

## COMPETING INTERESTS

The authors declare that they have no competing interests.

## AUTHOR CONTRIBUTIONS

G.H.Y. and Z.J.W conceived and designed the study. Y.L.G performed the experiments, analyzed the data, and prepared the manuscript. G.H.Y. collected materials. All authors read and approved the final version of the manuscript.

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