



# Taxonomic relationship between *Amolops minutus* Orlov & Ho, 2007 and *A. ottorum* Pham, Sung, Pham, Le, Zieger & Nguyen, 2019, with first record of *A. minutus* from China (Anura, Ranidae)

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## Abstract

## Background

Based on the examination of specimens of *Amolops minutus* Orlov & Ho, 2007 and *A. ottorum* Pham, Sung, Pham, Le, Zieger & Nguyen, 2019, we found that there is no significant morphological difference between them. Phylogenetic analysis also showed

that *A. minutus* and *A. ottorum* belong to the same taxon. In addition, we discovered the distribution of *A. minutus* in China.

## New information

In this study, we provide the first molecular data of *Amolops minutus* and regard *A. ottorum* as a junior synonym of *A. minutus*. In addition, we report the first record of *A. minutus* from China, based on nine specimens collected from Guanyinshan Provincial Nature Reserve in southern Yunnan Province and present an updated diagnosis of this species, based on literature data and newly-collected specimens.

## Keywords

16S, Guanyinshan Provincial Nature Reserve, morphology, synonym, taxonomy, torrent frogs

## Introduction

The torrent frogs of *Amolops* Cope, 1865 inhabits torrents or waterfalls and they usually have an abdominal sucker in larvae and enlarged digital discs in adults (Fei et al. 2009, Fei et al. 2012). This genus is distributed broadly from southern and eastern Himalayas to Peninsular Malaysia (Dever et al. 2012, Pham et al. 2019, Wu et al. 2020, Mahony et al. 2022, Tang et al. 2023, Li et al. 2024, Frost 2024). It currently contains 86 recognised species (Frost 2024) and they are placed into ten species groups, namely the *A. chayuensis* group, *A. daiyunensis* group, *A. hainanensis* group, *A. larutensis* group, *A. mantzorum* group, *A. marmoratus* group, *A. monticola* group, *A. ricketti* group, *A. spinapectoralis* group and *A. viridimaculatus* group (Jiang et al. 2021, Wu et al. 2024).

The *Amolops mantzorum* group currently comprises twelve species, namely *A. ailao* Tang, Sun, Liu, Luo, Yu & Du, 2023, *A. dafangensis* Li, Liu, Ke, Cheng & Wang, 2024, *A. granulosus* (Liu & Hu, 1961), *A. jinjiangensis* Su, Yang & Li, 1986, *A. lifanensis* (Liu, 1945), *A. loloensis* (Liu, 1950), *A. mantzorum* (David, 1872), *A. minutus* Orlov & Ho, 2007, *A. ottorum* Pham, Sung, Pham, Le, Zieger & Nguyen, 2019, *A. sangzhiensis* Qian, Xiang, Jiang, Yang & Gui, 2023, *A. shuichengnicus* Lyu & Wang, 2019 and *A. tuberodepressus* Liu & Yang, 2000 (Tang et al. 2023, Li et al. 2024).

*Amolops minutus* was described by Orlov and Ho (2007), based on morphological characteristics from Tam Duong District, Lai Chau Province, Vietnam, and molecular data of the species is unavailable. *Amolops ottorum* was discovered by Pham et al. (2019), based on two female specimens from Muong La District, Son La Province, Vietnam. The type localities of both species are located in north-western Vietnam and they are approximately 90 km distant from each other.

During our fieldwork in northern Vietnam, we collected fifteen specimens of *Amolops minutus* from its type locality in Lai Chau Province. As these specimens morphologically resemble *A. ottorum*, we suspected that these two species may be conspecific. To confirm their taxonomic status, we sequenced the homologous gene of the topotypic specimens of *A. minutus* and the type specimens of *A. ottorum* and phylogenetic analysis supported that they are conspecific with a genetic divergence of 0.7% (16S gene). Morphological examination of the type specimens of *A. minutus* showed that the original description of the species was not very accurate and there was no significant morphological difference between *A. minutus* and *A. ottorum*. According to the order of naming time, we herein consider *A. ottorum* as a junior synonym of *A. minutus*. In addition, we recently collected nine specimens of *Amolops* from Guanyinshan Provincial Nature Reserve in Yunnan Province, China. The sequences of these specimens clustered with those of the topotypic specimens of *A. minutus*. We herein report the record of *A. minutus* from China for the first time.

## Materials and methods

Field surveys were conducted in Lai Chau Province, northern Vietnam, in 2020 and in Guanyinshan Provincial Nature Reserve, Yunnan Province, China, in 2023 and 2024. Voucher specimens collected from Vietnam were deposited at the Institute of Ecology and Biological Resources (IEBR), Hanoi, Vietnam and specimens collected from China were deposited at Kunming Natural History Museum of Zoology, Kunming Institute of Zoology, Chinese Academy of Sciences (KIZ), Kunming, China.

Measurements were taken with a digital caliper to the nearest 0.1 mm. The following morphological characteristics were used: snout-vent length (SVL), from the tip of the snout to the cloacal; head length (HL), from the rear of the lower jaw to the tip of the snout; head width (HW), at the greatest cranial width; snout-eye distance (ESL), from the tip of the snout to the anterior corner of the eye; eye diameter (ED), horizontal diameter of the eye; tympanum diameter (TD), horizontal diameter of the tympanum; fore-limb length (FLL), from the tip of the disc of the third finger to the axilla; hind-limb length (HLL), from the tip of the disc of the fourth toe to the groin; tibia length (TL), from the knee to the tarsus; foot and tarsus length (FOT), from the tip of the disc of the fourth toe to the posterior edge of the tibia.

Total genomic DNA was extracted from tissues. A fragment of the 16S ribosomal RNA (16S) gene was amplified and sequenced. The primer pairs L2188: 5'-AAAGTGGGCCTAAAAGCAGCCA-3' and 16H1: 5'-CTCCGGTCTGAACTCAGATCACG TAGG-3' (Hedges 1994, Matsui et al. 2006) and 16SAR: 5'-CGCCTGTTTAYCAA AACAT-3' and 16SBR: 5'-CCGGTYTGAACCTCAGATCAYGT-3' (Simon et al. 1994) were used in polymerase chain and cycling reactions. All newly-generated sequences have been deposited in GenBank and homologous sequences of other *Amolops* species were obtained from GenBank (Table 1).

Table 1.

Sequences (16S) used for molecular analyses in this study.

| Species                      | Voucher                                    | Locality                     | Accession number         |
|------------------------------|--|------------------------------|--------------------------|
| <i>Amolops ailao</i>         | GXNU YU000001                              | Xinping, Yunnan, China       | <a href="#">MN650751</a> |
| <i>Amolops ailao</i>         | GXNU YU000002                              | Xinping, Yunnan, China       | <a href="#">MN650752</a> |
| <i>Amolops ailao</i>         | GXNU YU000003                              | Xinping, Yunnan, China       | <a href="#">MN650753</a> |
| <i>Amolops ailao</i>         | GXNU YU000004                              | Xinping, Yunnan, China       | <a href="#">MN650754</a> |
| <i>Amolops ailao</i>         | GXNU YU20160273                            | Xinping, Yunnan, China       | <a href="#">MN650755</a> |
| <i>Amolops ailao</i>         | GXNU YU20160274                            | Xinping, Yunnan, China       | <a href="#">MN650756</a> |
| <i>Amolops ailao</i>         | KIZ 2022041                                | Xinping, Yunnan, China       | <a href="#">OP879227</a> |
| <i>Amolops dafangensis</i>   | MT DF20230601002                           | Dafang, Guizhou, China       | <a href="#">OR936315</a> |
| <i>Amolops dafangensis</i>   | MT DF20230601001                           | Dafang, Guizhou, China       | <a href="#">OR936314</a> |
| <i>Amolops dafangensis</i>   | MT DF20230601003                           | Dafang, Guizhou, China       | <a href="#">OR936316</a> |
| <i>Amolops dafangensis</i>   | MT DF20230601004                           | Dafang, Guizhou, China       | <a href="#">OR936317</a> |
| <i>Amolops dafangensis</i>   | MT DF20230601005                           | Dafang, Guizhou, China       | <a href="#">OR936318</a> |
| <i>Amolops granulopus</i>    | SYS a005315                                | Hongya, Sichuan, China       | <a href="#">MK604850</a> |
| <i>Amolops granulopus</i>    | SYS a005316                                | Hongya, Sichuan, China       | <a href="#">MK604851</a> |
| <i>Amolops granulopus</i>    | 20130258                                   | Hongya, Sichuan, China       | <a href="#">MH922934</a> |
| <i>Amolops jinjiangensis</i> | SCUM 050435CHX                             | Deqing, Yunnan, China        | <a href="#">EF453741</a> |
| <i>Amolops jinjiangensis</i> | CIB-XM6120                                 | Deqing, Yunnan, China        | <a href="#">MZ292455</a> |
| <i>Amolops jinjiangensis</i> | KIZ 047095                                 | Chuxiong, Yunnan, China      | <a href="#">MN953701</a> |
| <i>Amolops lifanensis</i>    | SYS a005378                                | Lixian, Sichuan, China       | <a href="#">MK604870</a> |
| <i>Amolops loloensis</i>     | SYS a005351                                | Zhaojue, Sichuan, China      | <a href="#">MK573806</a> |
| <i>Amolops loloensis</i>     | SYS a005346                                | Zhaojue, Sichuan, China      | <a href="#">MK604854</a> |
| <i>Amolops loloensis</i>     | SYS a005347                                | Zhaojue, Sichuan, China      | <a href="#">MK604855</a> |
| <i>Amolops mantzorum</i>     | SYS a005366                                | Baoxing, Sichuan, China      | <a href="#">MK604862</a> |
| <i>Amolops mantzorum</i>     | SYS a005362                                | Baoxing, Sichuan, China      | <a href="#">MG991893</a> |
| <i>Amolops mantzorum</i>     | SYS a005336                                | Hongya, Sichuan, China       | <a href="#">MK573804</a> |
| <i>Amolops minutus</i>       | IEBR A.5142                                | Tam Duong, Lai Chau, Vietnam | <a href="#">PQ346023</a> |
| <i>Amolops minutus</i>       | IEBR A.6300                                | Tam Duong, Lai Chau, Vietnam | <a href="#">PQ346024</a> |
| <i>Amolops minutus</i>       | IEBR 4342 (Holotype of <i>A. ottorum</i> ) | Muong La, Son La, Vietnam    | <a href="#">PQ346025</a> |
| <i>Amolops minutus</i>       | TBU 06 (Paratype of <i>A. ottorum</i> )    | Muong La, Son La, Vietnam    | <a href="#">PQ346026</a> |
| <i>Amolops minutus</i>       | KIZ 2023064                                | Yuanyang, Yunnan, China      | <a href="#">PQ346027</a> |
| <i>Amolops minutus</i>       | KIZ 2023065                                | Yuanyang, Yunnan, China      | <a href="#">PQ346028</a> |

| Species                        | Voucher     | Locality                     | Accession number         |
|--------------------------------|-------------|------------------------------|--------------------------|
| <i>Amolops minutus</i>         | KIZ 2023066 | Yuanyang, Yunnan, China      | <a href="#">PQ346029</a> |
| <i>Amolops minutus</i>         | KIZ 2023067 | Yuanyang, Yunnan, China      | <a href="#">PQ346030</a> |
| <i>Amolops minutus</i>         | KIZ 2023068 | Yuanyang, Yunnan, China      | <a href="#">PQ346031</a> |
| <i>Amolops minutus</i>         | KIZ 2023069 | Yuanyang, Yunnan, China      | <a href="#">PQ346032</a> |
| <i>Amolops minutus</i>         | KIZ 2023070 | Yuanyang, Yunnan, China      | <a href="#">PQ346033</a> |
| <i>Amolops minutus</i>         | KIZ 2023102 | Yuanyang, Yunnan, China      | <a href="#">PQ346034</a> |
| <i>Amolops sangzhiensis</i>    | CSUFT 901   | Sangzhi, Hunan, China        | <a href="#">OQ079538</a> |
| <i>Amolops sangzhiensis</i>    | CSUFT 905   | Sangzhi, Hunan, China        | <a href="#">OQ079539</a> |
| <i>Amolops sangzhiensis</i>    | CSUFT 907   | Sangzhi, Hunan, China        | <a href="#">OQ079540</a> |
| <i>Amolops shuichengicus</i>   | SYS a004956 | Shuicheng, Guizhou, China    | <a href="#">MK604845</a> |
| <i>Amolops shuichengicus</i>   | SYS a004957 | Shuicheng, Guizhou, China    | <a href="#">MK604846</a> |
| <i>Amolops tuberodepressus</i> | CIB-XM3125  | Jingdong, Yunnan, China      | <a href="#">KR559270</a> |
| <i>Amolops tuberodepressus</i> | YU20160272  | Xinping, Yunnan, China       | <a href="#">MN650757</a> |
| <i>Amolops tuberodepressus</i> | SYS a003931 | Jingdong, Yunnan, China      | <a href="#">MG991904</a> |
| <i>Amolops viridimaculatus</i> | SYS a003813 | Mt. Gaoligong, Yunnan, China | <a href="#">MK604836</a> |

Sequences were aligned using MAFFT 7.471 (Kato and Standley 2013). The best fit substitution models were selected for Bayesian inference (BI) and maximum likelihood (ML) analyses, respectively, using the corrected Akaike Information Criterion (AICc) in ModelFinder (Kalyaanamoorthy et al. 2017). Bayesian inference was performed in MrBayes 3.2.7 (Ronquist et al. 2012) using the GTR+F+I+G4 model. Markov chains were run for 5,000,000 generations and sampled every 100 generations. The first 25% of the sampled trees was discarded as burn-in and the remaining trees were used to estimate Bayesian posterior probabilities (BPPs). Maximum likelihood analysis was performed in IQ-TREE 1.6.12 (Nguyen et al. 2015) with the TIM2+F+I+G4 model. Branch support was assessed using 5,000 ultrafast bootstrap replicates (UFB). The values of BPPs and UFB  $\geq$  95% are considered strong support for a clade (Ronquist et al. 2012, Nguyen et al. 2015). The uncorrected pairwise distances between species were calculated in MEGA 11 (Tamura et al. 2021).

## Taxon treatment

### *Amolops minutus* Orlov & Ho, 2007

#### Materials

- a. scientificName: *Amolops minutus*; country: Vietnam; stateProvince: Lai Chau; locality: Ho Thau Village, Ho Thau Commune, Tam Duong District; verbatimElevation: 2440 m;

- verbatimCoordinates: 22°24'42"N 103°36'35"E; eventRemarks: collected by Anh Van Pham, Chung Van Hoang, Tien Phan Quang, and Nenh Ba Sung in May 2020; individualCount: 1; sex: male; lifeStage: adult; catalogNumber: IEBR A.5142; basisOfRecord: preserved specimen; occurrenceID: D85CF383-2685-58BF-B761-02FA97632765
- b. scientificName: *Amolops minutus*; country: Vietnam; stateProvince: Lai Chau; locality: Ho Thau Village, Ho Thau Commune, Tam Duong District; verbatimElevation: 2440 m; verbatimCoordinates: 22°24'42"N 103°36'35"E; eventRemarks: collected by Anh Van Pham, Chung Van Hoang, Tien Phan Quang, and Nenh Ba Sung in May 2020; individualCount: 1; sex: male; lifeStage: adult; catalogNumber: IEBR A.5143; basisOfRecord: preserved specimen; occurrenceID: 220E4937-E84B-5FA0-B0DE-88D8039C4940
- c. scientificName: *Amolops minutus*; country: Vietnam; stateProvince: Lai Chau; locality: Ho Thau Village, Ho Thau Commune, Tam Duong District; verbatimElevation: 2440 m; verbatimCoordinates: 22°24'42"N 103°36'35"E; eventRemarks: collected by Anh Van Pham, Chung Van Hoang, Tien Phan Quang, and Nenh Ba Sung in May 2020; individualCount: 1; sex: male; lifeStage: adult; catalogNumber: IEBR A.5144; basisOfRecord: preserved specimen; occurrenceID: B1C10D49-59B9-5180-9D18-54C1BC04E812
- d. scientificName: *Amolops minutus*; country: Vietnam; stateProvince: Lai Chau; locality: Ho Thau Village, Ho Thau Commune, Tam Duong District; verbatimElevation: 2440 m; verbatimCoordinates: 22°24'42"N 103°36'35"E; eventRemarks: collected by Anh Van Pham, Chung Van Hoang, Tien Phan Quang, and Nenh Ba Sung in May 2020; individualCount: 1; sex: male; lifeStage: adult; catalogNumber: IEBR A.5145; basisOfRecord: preserved specimen; occurrenceID: 24C4943D-F3CA-5BF0-B133-87C5CB7A1603
- e. scientificName: *Amolops minutus*; country: Vietnam; stateProvince: Lai Chau; locality: Ho Thau Village, Ho Thau Commune, Tam Duong District; verbatimElevation: 2440 m; verbatimCoordinates: 22°24'42"N 103°36'35"E; eventRemarks: collected by Anh Van Pham, Chung Van Hoang, Tien Phan Quang, and Nenh Ba Sung in May 2020; individualCount: 1; sex: male; lifeStage: adult; catalogNumber: IEBR A.5146; basisOfRecord: preserved specimen; occurrenceID: 5CEB76ED-5893-519D-9452-E3D31153B175
- f. scientificName: *Amolops minutus*; country: Vietnam; stateProvince: Lai Chau; locality: Ho Thau Village, Ho Thau Commune, Tam Duong District; verbatimElevation: 2440 m; verbatimCoordinates: 22°24'42"N 103°36'35"E; eventRemarks: collected by Anh Van Pham, Chung Van Hoang, Tien Phan Quang, and Nenh Ba Sung in May 2020; individualCount: 1; sex: male; lifeStage: adult; catalogNumber: IEBR A.5147; basisOfRecord: preserved specimen; occurrenceID: C23C4993-DBF6-512A-BA1F-007CFABBA6BA
- g. scientificName: *Amolops minutus*; country: Vietnam; stateProvince: Lai Chau; locality: Ho Thau Village, Ho Thau Commune, Tam Duong District; verbatimElevation: 2440 m; verbatimCoordinates: 22°24'42"N 103°36'35"E; eventRemarks: collected by Anh Van Pham, Chung Van Hoang, Tien Phan Quang, and Nenh Ba Sung in May 2020; individualCount: 1; sex: male; lifeStage: adult; catalogNumber: IEBR A.5148; basisOfRecord: preserved specimen; occurrenceID: 9319A5D0-DB68-5859-BD38-3E0623489A2C
- h. scientificName: *Amolops minutus*; country: Vietnam; stateProvince: Lai Chau; locality: Ho Thau Village, Ho Thau Commune, Tam Duong District; verbatimElevation: 2440 m; verbatimCoordinates: 22°24'42"N 103°36'35"E; eventRemarks: collected by Anh Van

- Pham, Chung Van Hoang, Tien Phan Quang, and Nenh Ba Sung in May 2020; individualCount: 1; sex: male; lifeStage: adult; catalogNumber: IEBR A.5149; basisOfRecord: preserved specimen; occurrenceID: FD7307D9-575F-559F-B23E-5A7DBC70CC7B
- i. scientificName: *Amolops minutus*; country: Vietnam; stateProvince: Lai Chau; locality: Ho Thau Village, Ho Thau Commune, Tam Duong District; verbatimElevation: 2440 m; verbatimCoordinates: 22°24'42"N 103°36'35"E; eventRemarks: collected by Anh Van Pham, Chung Van Hoang, Tien Phan Quang, and Nenh Ba Sung in May 2020; individualCount: 1; sex: female; lifeStage: adult; catalogNumber: IEBR A.5150; basisOfRecord: preserved specimen; occurrenceID: 0F19C455-DBF5-5A49-9C5C-B047533698E6
- j. scientificName: *Amolops minutus*; country: Vietnam; stateProvince: Lai Chau; locality: Ho Thau Village, Ho Thau Commune, Tam Duong District; verbatimElevation: 2440 m; verbatimCoordinates: 22°24'42"N 103°36'35"E; eventRemarks: collected by Anh Van Pham, Chung Van Hoang, Tien Phan Quang, and Nenh Ba Sung in May 2020; individualCount: 1; sex: female; lifeStage: adult; catalogNumber: IEBR A.6299; basisOfRecord: preserved specimen; occurrenceID: 14D431C9-4554-500C-BD95-364B33E742E2
- k. scientificName: *Amolops minutus*; country: Vietnam; stateProvince: Lai Chau; locality: Ho Thau Village, Ho Thau Commune, Tam Duong District; verbatimElevation: 2440 m; verbatimCoordinates: 22°24'42"N 103°36'35"E; eventRemarks: collected by Anh Van Pham, Chung Van Hoang, Tien Phan Quang, and Nenh Ba Sung in May 2020; individualCount: 1; sex: female; lifeStage: adult; catalogNumber: IEBR A.6300; basisOfRecord: preserved specimen; occurrenceID: AF82375D-18FF-54D1-B211-8AC61B87CF47
- l. scientificName: *Amolops minutus*; country: Vietnam; stateProvince: Lai Chau; locality: Ho Thau Village, Ho Thau Commune, Tam Duong District; verbatimElevation: 2440 m; verbatimCoordinates: 22°24'42"N 103°36'35"E; eventRemarks: collected by Anh Van Pham, Chung Van Hoang, Tien Phan Quang, and Nenh Ba Sung in May 2020; individualCount: 1; sex: female; lifeStage: adult; catalogNumber: IEBR A.6301; basisOfRecord: preserved specimen; occurrenceID: F4EB3970-70D7-5F67-8EFA-6A5734985252
- m. scientificName: *Amolops minutus*; country: Vietnam; stateProvince: Lai Chau; locality: Ho Thau Village, Ho Thau Commune, Tam Duong District; verbatimElevation: 2440 m; verbatimCoordinates: 22°24'42"N 103°36'35"E; eventRemarks: collected by Anh Van Pham, Chung Van Hoang, Tien Phan Quang, and Nenh Ba Sung in May 2020; individualCount: 1; sex: female; lifeStage: adult; catalogNumber: IEBR A.6302; basisOfRecord: preserved specimen; occurrenceID: B11DEB88-8877-5909-AC5D-806099C697B0
- n. scientificName: *Amolops minutus*; country: Vietnam; stateProvince: Lai Chau; locality: Ho Thau Village, Ho Thau Commune, Tam Duong District; verbatimElevation: 2440 m; verbatimCoordinates: 22°24'42"N 103°36'35"E; eventRemarks: collected by Anh Van Pham, Chung Van Hoang, Tien Phan Quang, and Nenh Ba Sung in May 2020; individualCount: 1; sex: female; lifeStage: adult; catalogNumber: IEBR A.6303; basisOfRecord: preserved specimen; occurrenceID: 7B75B4E5-270B-5227-9928-67B20CB682FB
- o. scientificName: *Amolops minutus*; country: Vietnam; stateProvince: Lai Chau; locality: Ho Thau Village, Ho Thau Commune, Tam Duong District; verbatimElevation: 2440 m; verbatimCoordinates: 22°24'42"N 103°36'35"E; eventRemarks: collected by Anh Van Pham, Chung Van Hoang, Tien Phan Quang, and Nenh Ba Sung in May 2020;

- individualCount: 1; sex: female; lifeStage: adult; catalogNumber: IEBR A.6304; basisOfRecord: preserved specimen; occurrenceID: B7189F6D-EFF6-5110-A7B6-DE4DBB8C2E2E
- p. scientificName: *Amolops minutus*; country: China; stateProvince: Yunnan; locality: Ganiang Township, Yuanyang County, Honghe Prefecture; verbatimElevation: 2410 m; verbatimCoordinates: 23°0'57"N 102°56'57"E; eventRemarks: collected by Shuo Liu on 16 May 2023; individualCount: 1; sex: male; lifeStage: adult; catalogNumber: KIZ 2023064; basisOfRecord: preserved specimen; occurrenceID: 518014E8-3FCE-56F9-B84A-BC2D935425CA
- q. scientificName: *Amolops minutus*; country: China; stateProvince: Yunnan; locality: Ganiang Township, Yuanyang County, Honghe Prefecture; verbatimElevation: 2410 m; verbatimCoordinates: 23°0'57"N 102°56'57"E; eventRemarks: collected by Shuo Liu on 16 May 2023; individualCount: 1; sex: male; lifeStage: adult; catalogNumber: KIZ 2023065; basisOfRecord: preserved specimen; occurrenceID: 0FC83528-7760-5F20-84F7-3B4465EEECBD
- r. scientificName: *Amolops minutus*; country: China; stateProvince: Yunnan; locality: Ganiang Township, Yuanyang County, Honghe Prefecture; verbatimElevation: 2410 m; verbatimCoordinates: 23°0'57"N 102°56'57"E; eventRemarks: collected by Shuo Liu on 16 May 2023; individualCount: 1; sex: male; lifeStage: adult; catalogNumber: KIZ 2023066; basisOfRecord: preserved specimen; occurrenceID: 89044FE6-F2FA-5991-9BC8-A22EBEFFDD45
- s. scientificName: *Amolops minutus*; country: China; stateProvince: Yunnan; locality: Ganiang Township, Yuanyang County, Honghe Prefecture; verbatimElevation: 2410 m; verbatimCoordinates: 23°0'57"N 102°56'57"E; eventRemarks: collected by Shuo Liu on 16 May 2023; individualCount: 1; sex: male; lifeStage: adult; catalogNumber: KIZ 2023067; basisOfRecord: preserved specimen; occurrenceID: DFC7F447-5B74-5FFD-9254-C729C75CC841
- t. scientificName: *Amolops minutus*; country: China; stateProvince: Yunnan; locality: Ganiang Township, Yuanyang County, Honghe Prefecture; verbatimElevation: 2410 m; verbatimCoordinates: 23°0'57"N 102°56'57"E; eventRemarks: collected by Shuo Liu on 16 May 2023; individualCount: 1; sex: male; lifeStage: adult; catalogNumber: KIZ 2023068; basisOfRecord: preserved specimen; occurrenceID: DC4AB2B4-70F8-516E-A561-24B34E657C26
- u. scientificName: *Amolops minutus*; country: China; stateProvince: Yunnan; locality: Ganiang Township, Yuanyang County, Honghe Prefecture; verbatimElevation: 2410 m; verbatimCoordinates: 23°0'57"N 102°56'57"E; eventRemarks: collected by Shuo Liu on 16 May 2023; individualCount: 1; sex: female; lifeStage: adult; catalogNumber: KIZ 2023069; basisOfRecord: preserved specimen; occurrenceID: 2467D2C6-A15C-5EE0-8C7E-B63F9F489211
- v. scientificName: *Amolops minutus*; country: China; stateProvince: Yunnan; locality: Ganiang Township, Yuanyang County, Honghe Prefecture; verbatimElevation: 2410 m; verbatimCoordinates: 23°0'57"N 102°56'57"E; eventRemarks: collected by Shuo Liu on 16 May 2023; individualCount: 1; sex: female; lifeStage: adult; catalogNumber: KIZ 2023070; basisOfRecord: preserved specimen; occurrenceID: FDAC765F-29ED-5487-9B51-46F6331679CF
- w. scientificName: *Amolops minutus*; country: China; stateProvince: Yunnan; locality: Xiaoxinjie Township, Yuanyang County, Honghe Prefecture; verbatimElevation: 2490 m; verbatimCoordinates: 22°59'20"N 102°59'26"E; eventRemarks: collected by Shuo Liu on 20 May 2023; individualCount: 1; sex: female; lifeStage: adult; catalogNumber: KIZ



2023102; basisOfRecord: preserved specimen; occurrenceID: A891C6DE-2573-5D59-A021-CE1C3EA24FD3

- x. scientificName: *Amolops minutus*; country: China; stateProvince: Yunnan; locality: Ganiang Township, Yuanyang County, Honghe Prefecture; verbatimElevation: 2200 m; verbatimCoordinates: 23°2'54"N 102°54'45"E; eventRemarks: collected by Shuo Liu on 13 April 2024; individualCount: 1; sex: male; lifeStage: adult; catalogNumber: KIZ 2024112; basisOfRecord: preserved specimen; occurrenceID: F47A3080-9562-550A-B16F-1828B9EA9F16

### Extended diagnosis

This species is assigned to the *Amolops mantzorum* species group on the basis of the absence of a dorsolateral fold and the absence of circum-marginal groove on the disc of the first finger. It is distinguishable from other members of this species group by a combination of the following morphological characters: size small (SVL 29.7–38.3 mm in adult males and 38.5–51.1 mm in adult females); head moderate large, longer than wide (HL/SVL 0.33–0.37 in males and 0.31–0.35 in females, HW/SVL 0.28–0.33 in males and 0.26–0.32 in females); tympanum distinct, small (TD/HL 0.10–0.23 in males and 0.09–0.27 in females); pineal spot present; pupil oval, horizontal; vomerine teeth absent or weakly developed; vocal sac absent in males; fore-limbs robust (FLL/SVL 0.70–0.87 in males and 0.67–0.85 in females), relative finger lengths I < II < IV < III; hind-limbs long (HLL/SVL 1.79–2.09 in males and 1.70–2.00 in females), relative toe length I < II < III < V < IV; dorsal skin smooth, with a few flattened tubercles on flanks and posterior surface of dorsum; supratympanic fold absent or weakly developed; true dorsolateral folds absent, but dorsolateral glandular folds distinct; circummarginal groove on tip of first finger absent; inner metatarsal tubercle small; outer metatarsal tubercle absent; nuptial pad present on finger I of adult males. Colour of dorsal surface from nearly uniform green to mostly brown, flanks mostly green, dark bars on dorsal limbs distinct or indistinct.

### Distribution

*Amolops minutus* is currently known from Lai Chau and Son La provinces in northern Vietnam and Guanyinshan Provincial Nature Reserve in Yuanyang County, Honghe Prefecture, Yunnan Province, China (Fig. 1).

## Analysis

Morphological characteristics of the specimens collected from the type locality of *Amolops minutus* in Lai Chau Province (Vietnam) were similar to those in the original description of Orlov and Ho (2007), except for the vomerine teeth which were absent or weakly developed and the gular pouches being absent in males (vs. vomerine teeth strongly developed and paired gular pouches present in males in the original description) and small variations in the relative size of the tympanum and the relative length of the fore-limbs (Table 2). However, we found that the type specimens of *A.*

*minutus* have no vomerine teeth or only weakly-developed vomerine teeth and no gular pouches in males (Fig. 2), which were not as described in Orlov and Ho (2007).

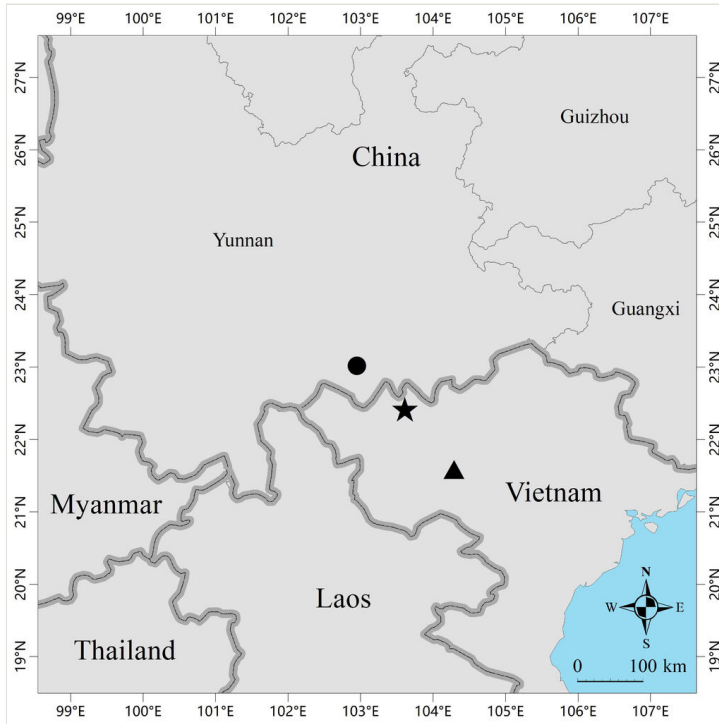


Figure 1. doi

Map showing the type locality (black star) of *Amolops minutus* in Lai Chau Province, Vietnam, the type locality (black triangle) of *Amolops ottorum* in Son La Province, Vietnam and the collection site (black dot) of the specimens from Guanyinshan Provincial Nature Reserve in Yuanyang County, Honghe Prefecture, Yunnan Province, China.

Table 2.

Comparisons amongst the type specimens of *Amolops minutus*, the topotypic specimens of *A. minutus*, the type specimens of *A. ottorum* and the specimens of *A. minutus* collected from China. Measurements in mm. Abbreviations defined in the text. Data for the type specimens of *A. minutus* and *A. ottorum* were from the original descriptions (Orlov and Ho 2007; Pham et al. 2019).

|     | Types of <i>Amolops minutus</i> |                 | Topotypes of <i>Amolops minutus</i> |                 | Types of <i>Amolops ottorum</i> | <i>Amolops minutus</i> from China |                 |
|-----|---------------------------------|-----------------|-------------------------------------|-----------------|---------------------------------|-----------------------------------|-----------------|
|     | Males (n = 8)                   | Females (n = 5) | Males (n = 8)                       | Females (n = 7) | Females (n = 2)                 | Males (n = 6)                     | Females (n = 3) |
| SVL | 29.7–36.4                       | 38.5–50.2       | 30.8–34.0                           | 42.5–47.5       | 47.5–48.2                       | 34.6–38.3                         | 46.7–51.1       |
| HL  | 11.0–12.9                       | 12.5–15.7       | 10.8–12.0                           | 14.3–16.0       | 15.1–16.0                       | 12.2–13.6                         | 16.0–17.1       |
| HW  | 9.7–11.4                        | 10.1–14.4       | 10.0–11.0                           | 13.5–14.0       | 14.6–14.9                       | 10.9–11.8                         | 15.1–16.6       |

|         | Types of <i>Amolops minutus</i> |                 | Topotypes of <i>Amolops minutus</i> |                 | Types of <i>Amolops ottorum</i> | <i>Amolops minutus</i> from China |                 |
|---------|---------------------------------|-----------------|-------------------------------------|-----------------|---------------------------------|-----------------------------------|-----------------|
|         | Males (n = 8)                   | Females (n = 5) | Males (n = 8)                       | Females (n = 7) | Females (n = 2)                 | Males (n = 6)                     | Females (n = 3) |
| ED      | 4.3–5.0                         | 5.4–6.4         | 4.7–5.0                             | 5.5–6.0         | 5.7–5.8                         | 4.4–4.7                           | 5.5–5.6         |
| TD      | 2.1–2.7                         | 3.1–3.5         | 1.8–2.0                             | 2.2–2.4         | 2.1                             | 1.3–1.4                           | 1.5–1.8         |
| ESL     | 4.8–5.4                         | 6.5–7.6         | 4.9–5.4                             | 6.4–7.0         | 6.8–7.0                         | 5.6–6.0                           | 7.2–7.7         |
| FLL     | 25.7–28.2                       | 32.7–40.3       | 22.5–25.5                           | 30.5–32.5       | 32.3–32.5                       | 26.6–28.6                         | 34.8–38.8       |
| HLL     | 59.5–67.2                       | 74.3–85.5       | 58.2–65.6                           | 81.5–87.7       | 82.3–83.5                       | 72.4–75.9                         | 92.3–99.3       |
| TL      | 17.7–20.3                       | 22.3–25.6       | 17.4–20.4                           | 24.8–27.0       | 27.2–27.8                       | 21.6–22.7                         | 27.0–29.1       |
| FOT     | 25.7–29.4                       | 31.2–36.0       | 25.0–28.6                           | 34.8–37.2       | 37.4–38.0                       | 30.5–32.2                         | 39.2–42.6       |
| HL/SVL  | 0.33–0.37                       | 0.31–0.33       | 0.34–0.36                           | 0.32–0.35       | 0.32–0.33                       | 0.35–0.36                         | 0.33–0.34       |
| HW/SVL  | 0.28–0.33                       | 0.26–0.31       | 0.30–0.33                           | 0.29–0.32       | 0.31                            | 0.30–0.32                         | 0.32            |
| ESL/SVL | 0.14–0.17                       | 0.15–0.17       | 0.15–0.16                           | 0.15            | 0.14–0.15                       | 0.15–0.16                         | 0.14–0.16       |
| ED/HL   | 0.36–0.44                       | 0.36–0.44       | 0.41–0.44                           | 0.36–0.39       | 0.36–0.38                       | 0.34–0.36                         | 0.33–0.34       |
| TD/HL   | 0.19–0.23                       | 0.20–0.27       | 0.17                                | 0.14–0.16       | 0.13–0.14                       | 0.10–0.11                         | 0.09–0.11       |
| FLL/SVL | 0.77–0.87                       | 0.71–0.85       | 0.70–0.75                           | 0.68–0.72       | 0.67–0.68                       | 0.72–0.77                         | 0.73–0.76       |
| HLL/SVL | 1.79–2.00                       | 1.70–1.93       | 1.87–1.94                           | 1.81–1.92       | 1.71–1.76                       | 1.98–2.09                         | 1.85–2.00       |
| TL/SVL  | 0.54–0.60                       | 0.51–0.58       | 0.56–0.60                           | 0.54–0.59       | 0.57–0.58                       | 0.59–0.63                         | 0.54–0.59       |
| FOT/SVL | 0.77–0.86                       | 0.72–0.81       | 0.78–0.84                           | 0.76–0.82       | 0.78–0.80                       | 0.83–0.88                         | 0.79–0.86       |

Moreover, morphological comparison showed that the type specimens of *A. ottorum* from Son La Province (Vietnam) and the newly-collected specimens of *Amolops* from Yunnan Province (China) are very similar to *A. minutus* (Table 2).

The newly-generated sequences are approximately 850 bp or 570 bp. BI and ML analyses yielded similar results. The sequences of the type specimens of *Amolops ottorum* from Son La Province (Vietnam), the specimens collected from the type locality of *A. minutus* in Lai Chau Province (Vietnam) and the *Amolops* specimens collected from Yunnan Province (China), clustered in the same clade with strong support by both BI and ML (BPPs = 1, UFB = 96) (Fig. 3). The uncorrected pairwise distance between the sequences of the type specimens of *A. ottorum* and the specimens collected from the type locality of *A. minutus* was 0.7% and the uncorrected pairwise distance between the sequences of the *Amolops* specimens collected from Yunnan and the specimens collected from the type locality of *A. minutus* was 0.6% (Table 3).

Based on morphological data and phylogenetic analysis, we assign the specimens collected from the type locality of *A. minutus* in Lai Chau Province (Vietnam) and from

Yunnan Province (China) to *A. minutus* and regard *A. ottorum* as a junior synonym of *A. minutus*.

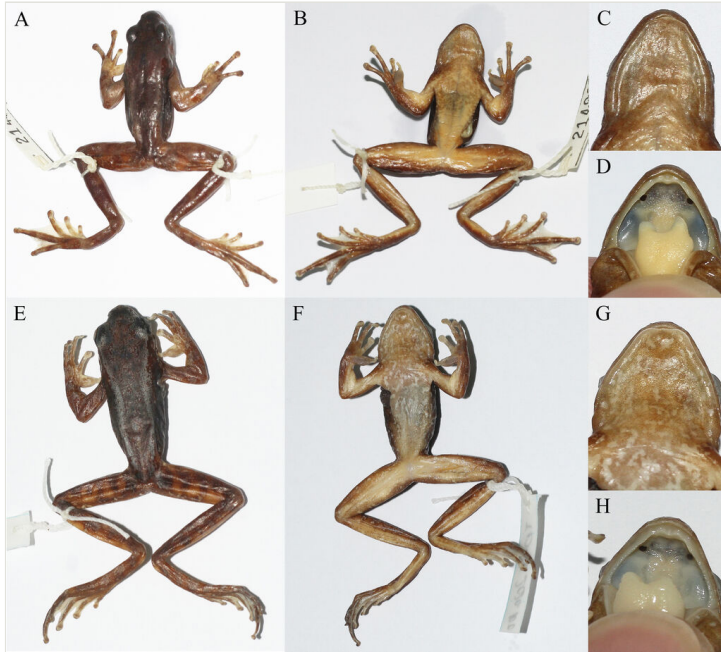


Figure 2. [doi](#)

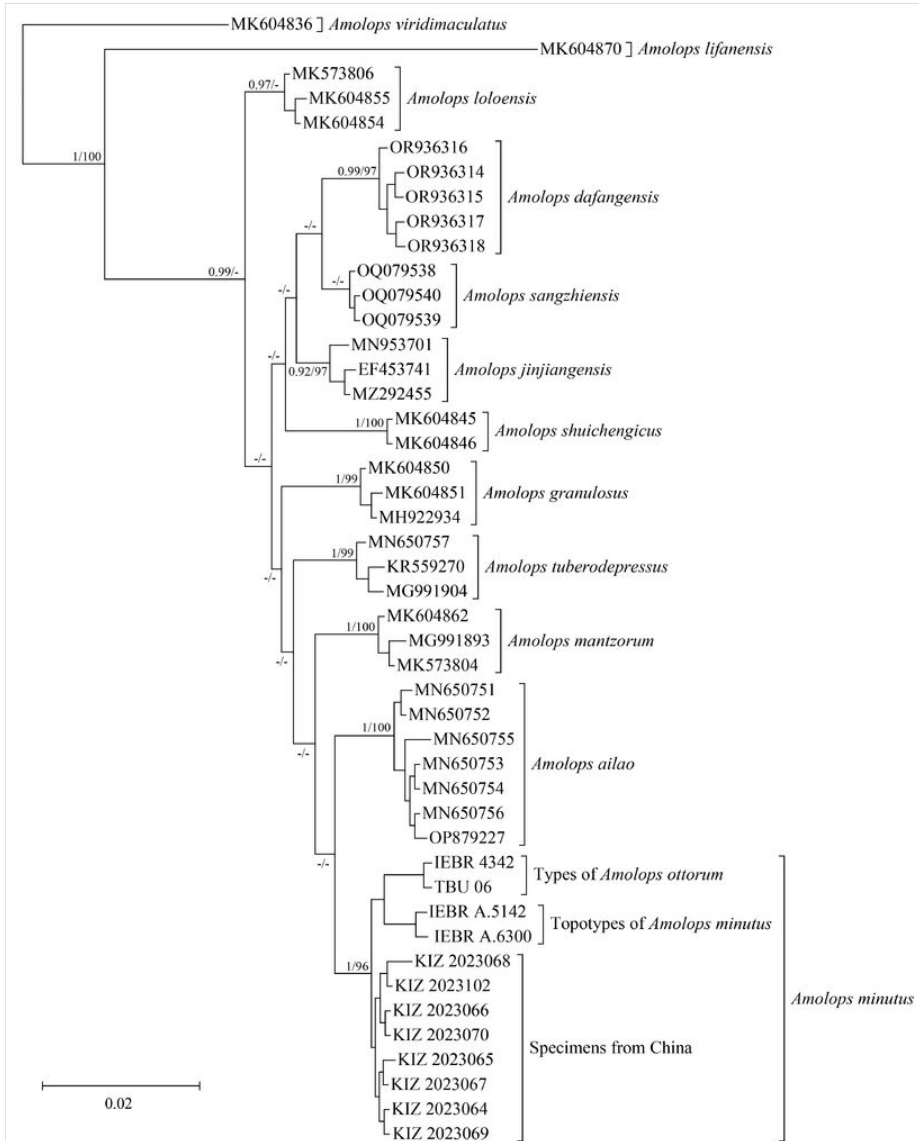
Dorsal view (A), ventral view (B), close-up view the gular region (C) and close-up view of the upper oral wall (D) of the paratype (ZISP 7615) of *Amolops minutus*; and dorsal view (E), ventral view (F), close-up view the gular region (G) and close-up view of the upper oral wall (H) of the topotypic specimen (IEBR A.5142) of *A. minutus*.

Table 3.

Uncorrected pairwise genetic distances (%) estimated from 16S rRNA sequences.

|                                       | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10 | 11 | 12 |
|---------------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|----|----|----|
| 1 Types of <i>Amolops ottorum</i>     |     |     |     |     |     |     |     |     |     |    |    |    |
| 2 Topotypes of <i>Amolops minutus</i> | 0.7 |     |     |     |     |     |     |     |     |    |    |    |
| 3 <i>Amolops minutus</i> from China   | 0.6 | 0.6 |     |     |     |     |     |     |     |    |    |    |
| 4 <i>Amolops ailao</i>                | 1.2 | 1.8 | 1.6 |     |     |     |     |     |     |    |    |    |
| 5 <i>Amolops dafangensis</i>          | 2.1 | 1.9 | 1.8 | 1.7 |     |     |     |     |     |    |    |    |
| 6 <i>Amolops granulosis</i>           | 2.2 | 2.8 | 2.5 | 2.7 | 1.8 |     |     |     |     |    |    |    |
| 7 <i>Amolops jinjiangensis</i>        | 1.6 | 1.6 | 1.4 | 2.1 | 1.1 | 2.1 |     |     |     |    |    |    |
| 8 <i>Amolops lifanensis</i>           | 5.8 | 8.0 | 8.4 | 8.3 | 4.8 | 7.6 | 7.3 |     |     |    |    |    |
| 9 <i>Amolops loloensis</i>            | 2.0 | 2.1 | 1.8 | 2.3 | 1.5 | 1.9 | 1.1 | 7.6 |     |    |    |    |
| 10 <i>Amolops mantzorum</i>           | 2.0 | 2.2 | 1.7 | 1.9 | 2.2 | 2.5 | 1.9 | 8.5 | 2.0 |    |    |    |

|                                   | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  | 11  | 12  |
|-----------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 11 <i>Amolops sangzhiensis</i>    | 1.4 | 1.9 | 1.6 | 1.8 | 0.8 | 2.0 | 0.8 | 7.8 | 1.4 | 1.7 |     |     |
| 12 <i>Amolops shuichengicus</i>   | 1.6 | 2.8 | 2.7 | 2.9 | 1.7 | 2.7 | 1.9 | 7.9 | 2.4 | 3.0 | 1.8 |     |
| 13 <i>Amolops tuberodepressus</i> | 1.8 | 2.7 | 2.2 | 2.5 | 2.1 | 2.1 | 1.7 | 8.2 | 1.8 | 1.8 | 1.8 | 2.8 |

Figure 3. [doi](#)

Bayesian phylogram of the *Amolops mantzorum* group inferred from the 16S rRNA sequences. Numbers after and behind “/” are BPPs and UFB values (only above 0.90/90 are shown), respectively.

## Discussion

As some discrepancies between the morphological characters of the specimens we collected from the type locality of *Amolops minutus* and the original descriptions of this species, in order to verify whether the specimens we collected belong to *A. minutus*, we rely on the type specimen of this species. Re-examination of the type specimens verified that the specimens collected from the type locality *A. minutus* belong to *A. minutus*. In addition, morphological comparison between the type specimens of *A. ottorum* and the type and topotypic specimens of *A. minutus* showed that these two species could not be clearly distinguished. Combined with phylogenetic analysis, we confirmed that *A. ottorum* and *A. minutus* are conspecific and *A. ottorum* should be regarded as a junior synonym of *A. minutus*. Moreover, we found that *A. minutus* is also distributed in China and the colouration of this species in life was quite variable (Fig. 4), which extends the distribution range and diagnosis of this species.



Figure 4. [doi](#)

The specimens of *Amolops minutus* in life. A, the male topotypic specimen (IEBR A.5142); B, the female topotypic specimen (IEBR A.6300); C, the male specimen (KIZ 2024112) from China; D, the male specimen (KIZ 2023067) from China; E, the male specimen (KIZ 2023066) from China; F, the female specimen (KIZ 2023070) from China.

Guanyinshan Provincial Nature Reserve is located in southern Yunnan Province of China, with relatively high altitudes (approximately 1640–2746 m) and well-preserved natural habitat. There have been few previous field surveys in this nature reserve, resulting in a serious underestimation of its species diversity. Some new species of plants and animals have been discovered in this nature reserve recently, such as *Primula weimingii* Bin Yang & Y.H. Tan and *Pareas guanyinshanensis* Liu, Mo, Li, Li, Luo, Rao & Li, 2024 (Yang et al. 2023, Liu et al. 2024). The discovery of *Amolops minutus* in this nature reserve further enriches its species diversity. More field surveys will likely reveal more new species or new records of amphibians from this nature reserve.

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