



## A new species of *Amolops* (Anura: Ranidae) from southern China

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### Abstract

A new species, *Amolops albispinus* **sp. nov.** is described based on a series of specimens collected from Mt. Wutong, Shenzhen, Guangdong Province, China. The new species can be distinguished from other known congeners by molecular divergence in the mitochondrial COI and 16S rRNA gene and morphological characters including presence of white conical spines on the lips, loreal and temporal regions, excluding the tympanum; small body, SVL 36.7–42.4 mm in adult males and 43.1–51.9 mm in adult females; very rough dorsal skin of body with numerous raised large warts; olive-brown dorsum with dark brown blotches; strongly developed vomerine teeth; absence of vocal sacs; absence of tarsal glands; absence of dorsolateral folds; presence of circummarginals groove on the disk of first finger; and absence of outer metatarsal tubercles. At present, the genus *Amolops* contains 51 species, of which 23 occur in China.

**Key words:** *Amolops albispinus* **sp. nov.**, Anura, China, mitochondrial DNA, morphology, Ranidae

### Introduction

The genus *Amolops* (Cope 1865) currently contains 50 recognized species and is widely distributed in the southern and eastern Himalayas, through the Hengduan Range, eastward to south-eastern China, and southward to Malaysia (Fei *et al.* 2010; Jiang *et al.* 2016). Only seven species occur in eastern and southern China, six of these species were divided into three groups by Fei *et al.* (2009): the *Amolops ricketti* group, consisting of *A. ricketti* (Boulenger, 1899) and *A. wuyiensis* (Liu & Hu, 1975); the *Amolops daiyunensis* group, consisting of *A. daiyunensis* (Liu & Hu, 1975) and *Amolops hongkongensis* (Pope & Romer, 1951); and the *Amolops hainanensis* group, consisting of *A. hainanensis* (Boulenger, 1900) and *Amolops torrentis* (Smith, 1923). The seventh species, *Amolops chunganensis* (Pope, 1929), was assigned to the *Amolops monticola* group by Fei *et al.* (2009).

During field surveys in mountainous areas of Shenzhen City, Guangdong Province, China, we collected a series of specimens of a small-sized species of *Amolops* that at first appeared to be *Amolops ricketti* based on the following morphological features: presence of circummarginal grooves on the disk of first finger, presence of vomerine teeth, and adult males with nuptial pads and conical nuptial spines on the first finger (Fei *et al.* 2009). However, based on further examination we observed other characters not known from *A. ricketti*. After molecular analyses, we confirmed that these specimens were distinct from *A. ricketti* and herein describe them as a new species.

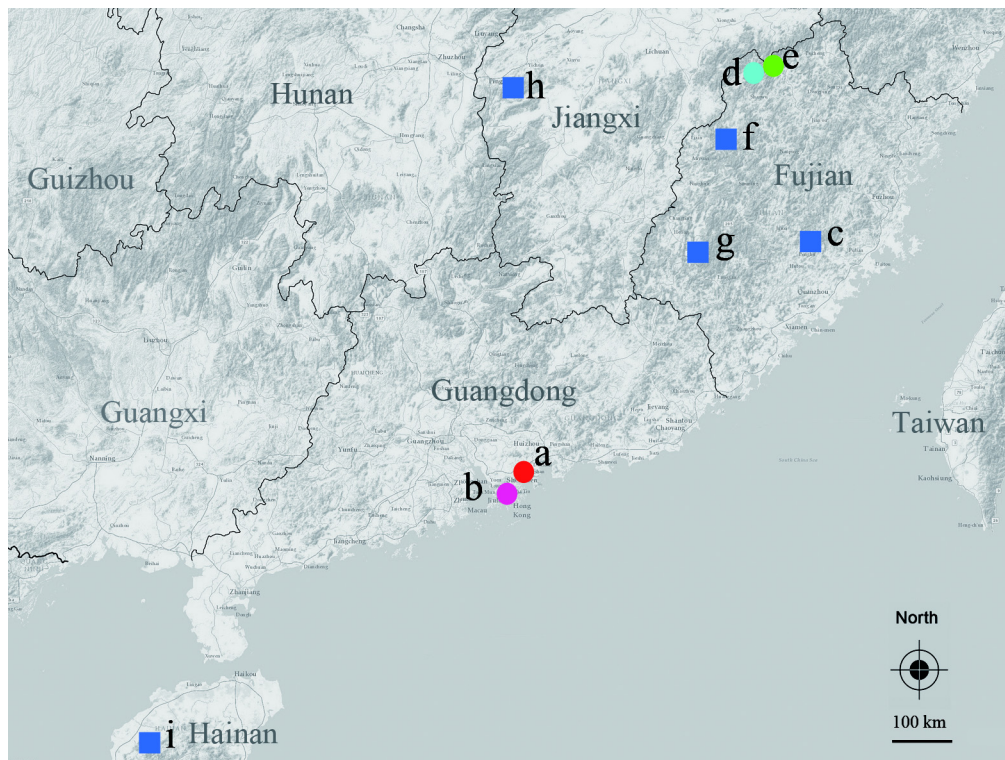
### Material and methods

**Taxon sampling.** For molecular analyses, we collected a total of 20 specimens of seven species, included all specimens of *Amolops* occurring in eastern and southern China, i.e., three specimens of *A. hongkongensis* from Hong Kong; three specimens of *A. daiyunensis* from its type locality of Mt. Daiyun, Fujian Province; three

specimens of *A. ricketti* from Wuyishan Nature Reserve of Jiangxi Province, [10 km from its type locality of Guadun (= Kuatun) Village], from Mt. Emeifeng in Taining County, and from Gutian Town in Shanghang County, Fujian Province; two specimens of *A. wuyiensis* from its type locality Sangang (= Sanchiang) Village in Mt. Wuyi, Fujian Province; two specimen of *A. torrentis* from Bawangling National Nature Reserve, Hainan Province; three specimens of *A. hainanensis* from Bawangling National Nature Reserve, Hainan Province; and one specimen of *A. chunganensis* from Mt. Wugong, Jiangxi Province (Table 1 and Fig. 1). All specimens were fixed in 10% buffered formalin after preserving muscle tissue in 95% ethanol, and subsequently transferred to 70% ethanol.

**TABLE 1.** Localities, voucher information, and Genbank accession numbers for all specimens used in this study.

ID.	Species name	Locality	Specimen voucher No.	16S gene	COI gene
(1)	<i>A. albispinus</i> <b>sp. nov.</b>	China; Mt. Wutong, Shenzhen City, Guangdong	SYS a003452	KX507312	KX507332
(2)	<i>A. albispinus</i> <b>sp. nov.</b>	China; Mt. Wutong, Shenzhen City, Guangdong	SYS a003453	KX507313	KX507333
(3)	<i>A. albispinus</i> <b>sp. nov.</b>	China; Mt. Wutong, Shenzhen City, Guangdong	SYS a003454	KX507314	KX507334
(4)	<i>A. hongkongensis</i>	China; Hong Kong	SYS a004577	KX507317	KX507337
(5)	<i>A. hongkongensis</i>	China; Hong Kong	SYS a004578	KX507318	KX507338
(6)	<i>A. hongkongensis</i>	China; Hong Kong	SYS a004579	KX507319	KX507339
(7)	<i>A. daiyunensis</i>	China; Mt. Daiyun, Dehua County, Fujian	SYS a001737 (topotype)	KX507306	KX507326
(8)	<i>A. daiyunensis</i>	China; Mt. Daiyun, Dehua County, Fujian	SYS a001738 (topotype)	KX507307	KX507327
(9)	<i>A. daiyunensis</i>	China; Mt. Daiyun, Dehua County, Fujian	SYS a001739 (topotype)	KX507308	KX507328
(10)	<i>A. ricketti</i>	China; Wuyishan Nature Reserve, Jiangxi	SYS a001605	KX507303	KX507323
(11)	<i>A. ricketti</i>	China; Mt. Emeifeng, Taining County, Fujian	SYS a002492	KX507309	KX507329
(12)	<i>A. ricketti</i>	China; Gutian, Shanghang County, Fujian	SYS a003342	KX507311	KX507331
(13)	<i>A. wuyiensis</i>	China; Sangang (= Sanchiang) Village, Mt. Wuyi, Fujian	SYS a001716 (topotype)	KX507304	KX507324
(14)	<i>A. wuyiensis</i>	China; Sangang Village, Mt. Wuyi, Fujian	SYS a001717 (topotype)	KX507305	KX507325
(15)	<i>A. torrentis</i>	China; Bawangling National Nature Reserve, Hainan	SYS a004573	KX507315	KX507335
(16)	<i>A. torrentis</i>	China; Bawangling National Nature Reserve, Hainan	SYS a004574	KX507316	KX507336
(17)	<i>A. hainanensis</i>	China; Bawangling Nature Reserve, Hainan	SYS a004580	KX507320	KX507340
(18)	<i>A. hainanensis</i>	China; Bawangling National Nature Reserve, Hainan	SYS a004581	KX507321	KX507341
(19)	<i>A. hainanensis</i>	China; Bawangling National Nature Reserve, Hainan	SYS a004582	KX507322	KX507342
(20)	<i>A. chunganensis</i>	China; Mt. Wugong, Anfu County, Jiangxi	SYS a003136	KX507310	KX507330
(21)	<i>O. margaretae</i>	Not provided	HNNU:1207003	NC024603	NC024603
(22)	<i>O. schmackeri</i>	China; Huangshan, Anhui	Not provided	KP732086	KP732086



**FIGURE 1.** Collection localities of specimens of *Amolops*: **a**: Mt. Wutong, Shenzhen, China, here collected type specimens of *Amolops albispinus* sp. nov. SYS a003452, 3453 and 3454; **b**: Hong Kong, here collected topotypes of *A. hongkongensis* SYS a004577, 4578 and 4579; **c**: Mt. Daiyun, Fujian, China, here collected topotypes of *A. daiyunensis* SYS a001737, 1738 and 1739; **d**: Guadun Village (Kuaton Village), Mt. Wuyi, Fujian, here collected topotypes of *A. ricketti* SYS a001605; **e**: Sangang Village, Mt. Wuyi, Fujian, here collected topotypes of *A. wuyiensis* SYS a001716 and 1717; **f**: Mt. Emeifeng, Taining County, Fujian, specimen SYS a002492 referred to *A. ricketti*; **g**: Gutian, Shanghang County, Fujian, here collected specimen SYS a003342 referred to *A. ricketti*; **h**: Mt. Wugong, Anfu County, Jiangxi, here collected specimen SYS a003136 referred to *A. chunganensis*; **i**: Bawangling Nature Reserve, Hainan, here collected specimens referred to *A. hainanensis* and *A. torrentis*, respectively. Circle indicates the locations where topotypic specimens were collected.

**Extraction, PCR amplification, and sequencing.** We extracted DNA from the muscle tissue removed from the specimens using a standard phenol-chloroform extraction protocol (Sambrook *et al.* 1989). We sequenced the mitochondrial COI and 16S rRNA gene from all samples. We amplified fragments of the genes using the primer pairs L3975 (5'-CGCCTGTTTACCAAAAACAT-3') and H4551 (5'-CCGGTCTGAACTCAGATCACGT-3'; Sumida & Ogata 1998) for the 16S gene, and dgLCO (5'-GGTCAACAAATCATAAAGAYATYGG-3') and dgHCO (5'-AAACTTCAGGGTGACCAARAAYCA-3'; Meyer *et al.* 2005), and Chmf4 (5'-TYTCWACWAAAYCAYA AAGAYATCGG-3') and Chmr4 (5'-ACYTCRGGRTGRCCRAARAATCA-3'; Che *et al.* 2012) for the COI gene. We performed PCR amplifications in a 30 reaction volume with the following cycling conditions: an initial denaturing step at 95°C for 4 min; 33 cycles of denaturing at 94°C for 30 s, annealing at 52°C for 30 s and extending at 72°C for 1 min, and a final extending step of 72°C for 7 min. PCR products were purified with spin columns. We sequenced the purified products with both forward and reverse primers using the BigDye Terminator Cycle Sequencing Kit according to the guidelines of the manufacturer. We sequenced the products on an ABI Prism 3730 automated DNA sequencer in Shanghai. All sequences were deposited in GenBank (Table 1).

**Phylogenetic analyses.** We included sequences of *A. ricketti* and its genetically closest congeners; *A. daiyunensis*, *A. hainanensis*, *A. hongkongensis* and *A. wuyiensis* (Ngo *et al.* 2006). We also included sequences available from GenBank of *A. chunganensis*, *Odorrana margaretae* and *Odorrana schmackeri* (Table 1). Alignments were first conducted using Clustal X 2.0 (Thompson *et al.* 1997), with default parameters and the alignment being checked and manually revised, if necessary. The GTR model (Posada & Crandall 2001), assuming a gamma-shaped distribution across sites (Felsenstein 2004), was selected as the best-fitting nucleotide substitution model using MEGA 5.2 (Tamura *et al.* 2011). We analyzed the sequence data using maximum likelihood (ML) implemented in

MEGA 5.2, and Bayesian inference (BI) using MrBayes 3.12 (Ronquist & Huelsenbeck 2003). We constructed the phylogenetic tree using ML and BI methods. For the ML analysis, the bootstrap consensus tree inferred from 500 replicates was used to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% of bootstrap replicates were collapsed. For BI analysis, two independent runs with four Markov Chain Monte Carlo simulations were performed for one million iterations and sampled every 100th iteration. The first 25% of samples were discarded as burn-in. We assessed the convergence of the Markov Chain Monte Carlo simulations using Tracer v.1.4 (<http://tree.bio.ed.ac.uk/software/tracer/>). Apart from phylogenetic tree-based methods, we also calculated pairwise sequence divergence based on uncorrected *p*-distance using MEGA 5.2 to determine the genetic distance between groups (Tamura *et al.* 2011).

**Measurements.** We took the following measurements from the specimens of the new species to the nearest 0.1 mm with digital calipers: Snout-vent length (SVL); head length from tip of snout to rear of jaws (HDL); head width at commissure of jaws (HDW); snout length from tip of snout to anterior corner of eye (SNT); horizontal diameter of exposed portion of eyeball (EYE); interorbital distance (IOD); horizontal diameter of tympanum (TMP); distance from anterior edge of tympanum to posterior corner of eye (TEY); tibia length with hindlimb flexed (TIB); manus length from tip of third digit to proximal edge of inner palmar tubercle (ML), pes length from tip of fourth toe to proximal edge of inner metatarsal tubercle (PL), width of finger II digital disc (F2D), width of finger III digital disc (F3D) and width of toe IV digital disc (T4D). We determined sex by observation of secondary sexual characters, i.e. the presence of nuptial pads in males.

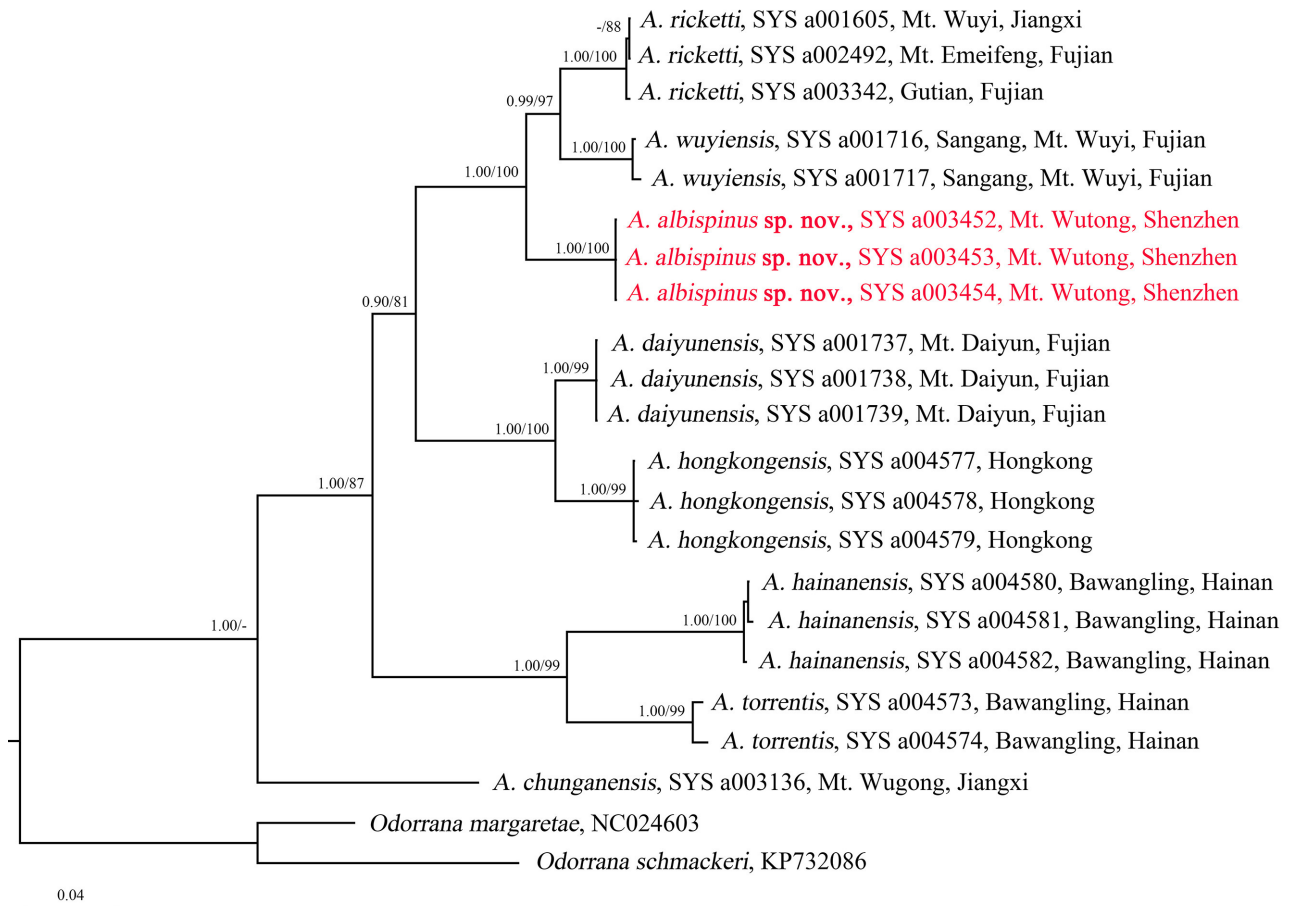
We obtained comparative morphological data from examination of museum specimens and photographs of multiple species of *Amolops*, and from the literature for *A. afghanus* (Günther 1858), *A. akhaorum* (Stuart *et al.* 2010), *A. aniqiaoensis* (Fei *et al.* 2009; Zhao *et al.* 2004), *A. archotaphus* (Inger & Chanard 1997), *A. assamensis* (Sengupta *et al.* 2008), *A. bellulus* (Fei *et al.* 2009; Liu *et al.* 2000), *A. caelumnoctis* (Rao & Wilkinson 2007), *A. chakrataensis* (Ray 1992), *A. chayuenensis* (Sun *et al.* 2013), *A. chunganensis* (Fei *et al.* 2009; Pope 1929), *A. compotrix* (Bain *et al.* 2006), *A. cremnobatus* (Inger & Kottelat 1998), *A. cucuae* (Bain *et al.* 2006), *A. daiyunensis* (Fei *et al.* 2009; Liu & Hu 1975), *A. daorum* (Bain *et al.* 2003), *A. formosus* (Günther 1875), *A. gerbillus* (Annandale 1912; Fei *et al.* 2009), *A. granulatus* (Fei *et al.* 2009; Liu & Hu 1961), *A. hainanensis* (Boulenger 1899b; Fei *et al.* 2009), *A. himalayanus* (Boulenger 1888), *A. hongkongensis* (Fei *et al.* 2009; Pope & Romer 1951), *A. indoburmanensis* (Dever *et al.* 2012), *A. iriodes* (Bain & Truong 2004), *A. jaunsari* (Ray 1992), *A. jinjiangensis* (Yang *et al.* 1983), *A. kangtingensis* (Liu 1950), *A. kaulbacki* (Smith 1940), *A. kohimaensis* (Biju *et al.* 2010), *A. larutensis* (Boulenger 1899c), *A. liangshanensis* (Wu & Zhao 1984), *A. lifanensis* (Fei *et al.* 2009; Liu 1945), *A. loloensis* (Fei *et al.* 2009; Liu 1950), *A. longimanus* (Andersson 1939), *A. mantzorum* (David 1872; Fei *et al.* 2009), *A. marmoratus* (Blyth 1853; Fei *et al.* 2009), *A. medogensis* (Fei *et al.* 2009; Zhao *et al.* 2004), *A. mengyangensis* (Wu & Tian 1995), *A. minutus* (Orlov & Ho 2011), *A. monticola* (Anderson 1871; Fei *et al.* 2009), *A. nidorbellus* (Biju *et al.* 2010), *A. nyingchiensis* (Jiang *et al.* 2016), *A. panhai* (Matsui & Nabhitabhata 2006), *A. ricketti* (Boulenger 1899a; Fei *et al.* 2009), *A. spinapectoralis* (Inger *et al.* 1999), *A. splendissimus* (Orlov & Ho 2011), *A. torrentis* (Fei *et al.* 2009; Smith 1923), *A. tuberodepressus* (Liu & Yang 2000), *A. viridimaculatus* (Fei *et al.* 2009; Jiang 1983), *A. vitreus* (Bain *et al.* 2006) and *A. wuyiensis* (Fei *et al.* 2009; Liu & Hu 1975). The examined specimens of *A. ricketti*, *A. wuyiensis*, *A. hongkongensis*, *A. daiyunensis*, *A. chunganensis*, *A. hainanensis*, *A. marmoratus*, *A. torrentis*, *A. tuberodepressus* and *A. viridimaculatus* are listed in the Appendix.

## Results

**Molecular phylogenetic analyses.** The Maximum likelihood (ML) and Bayesian inference (BI) phylogenetic trees were constructed based on concatenated DNA sequences of the mitochondrial COI and 16S gene with a total length of 1137bp. The two analyses resulted in essentially identical topologies with strong node supporting values (Fig. 2). The phylogenetic trees suggested that the population of *Amolops* from Mt. Wutong, Shenzhen is a sister taxon to a clade composed of *A. ricketti* and *A. wuyiensis*, and should be assigned to the *A. ricketti* species group, which is a sister clade to the *A. daiyunensis* species group (Fei *et al.* 2009) in the phylogenetic analyses. Furthermore, uncorrected *p*-distances among the three specimens from Mt. Wutong were <0.001% (Table 2) but the lowest *p*-distances between them and a known species of *Amolops* (*A. ricketti*) were 7.1–7.3%. These values are higher than those observed between described species, for example, between *A. hongkongensis* and *A. daiyunensis* (*p*-distances of 4.8–5.0%), and between *A. ricketti* and *A. wuyiensis* (*p*-distances of 5.5–5.8%). These results indicate that there



are substantial genetic divergences between the specimens from Mt. Wutong and other *Amolops* species, and that this population is a separately evolving lineage, likely representing an undescribed species. This population also presents a combination of morphological characteristics that are not observed in other known congener species including the presence of white conical spines on the upper and lower lips, loreal and temporal regions, excluding the tympanum, and the presence of numerous raised large warts on the dorsal skin of the body. Based on molecular and morphological evidence, we hereby describe the specimens collected from Mt. Wutong, Shenzhen, Guangdong Province, China as a new species.



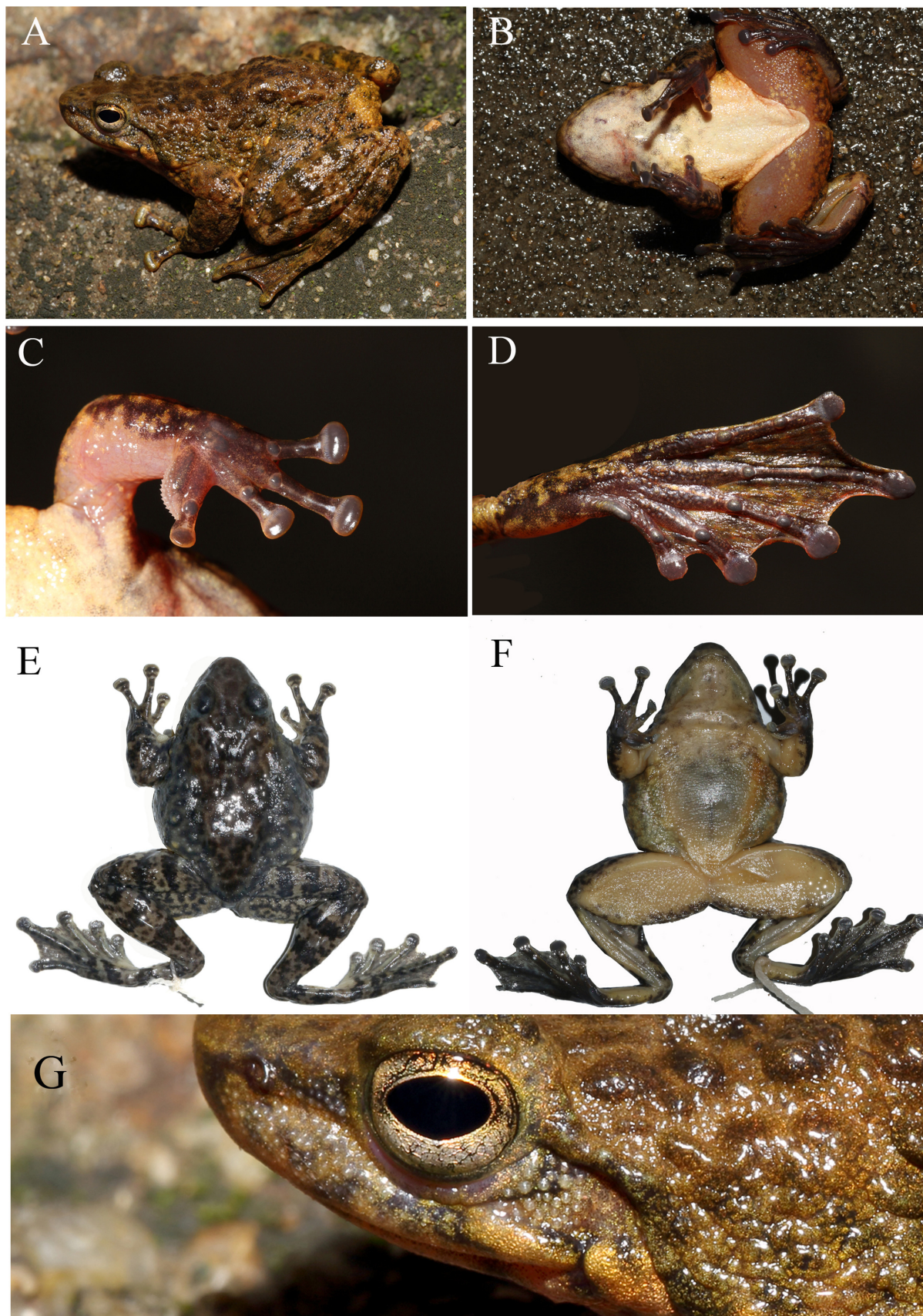
**FIGURE 2.** Bayesian inference tree derived from partial DNA sequences of the mitochondrial 16S rRNA and COI genes. Numbers above branches are bootstrap support values for ML/BI inferences respectively.

***Amolops albispinus* Sung, Wang and Wang sp. nov.**

(Fig. 3 and 4)

**Holotype:** SYS a003454, adult male, collected by Jian Wang, Zu-Yao Liu and Zhi-Tong Lyu on 26 January, 2015 from Mt. Wutong (22°34'54.8"N, 114°12'2.7"E; 260 m a.s.l.), Shenzhen City, Guangdong Province, China.

**Paratype:** 13 adult specimens, collected Ying-Yong Wang, Jian-Huan Yang, Run-Lin Li, Zu-Yao Liu, Jian Wang, and Zhi-Tong Lyu from Mt. Wutong at elevations between 85–500 m. Seven males: SYS a003364 by Ying-Yong Wang on 13 January 2012, SYS a001509 by Jian-Huan Yang and Run-Lin Li on 5 March 2012, SYS a003271 and 3272 by Zu-Tao Liu, Jian Wang and Zhi-Tong Lyu on 16 September 2014, SYS a003452 by Jian Wang and Run-Lin Li on 26 January 2015, SYS a003473 by Zu-Yao Liu, Jian Wang and Zhi-Tong Lyu on 13 March 2015, and SYS a004511 by Zhi-Tong Lyu and Jian Wang on 22 October 2015; six females: SYS a001508, 1513, 1514, and 1526 by Ying-Yong Wang, Jian-Huan Yang, Run-Lin Li on 8 March 2012, SYS a003270 by Zu-Yao Liu, Jian Wang and Zhi-Tong Lyu on 16 September 2014, SYS a003453 by Jian Wang and Run-Lin Li on 26 January 2015.



**FIGURE 3.** Dorsolateral view of adult male holotype SYS a003454 of *Amolops albispinus* sp. nov. in life; **B:** ventral view of the holotype in life; **C:** hand of the holotype in life; **D:** foot of the holotype in life; **E:** Dorsal view of the holotype in preserve; **F:** ventral view of the holotype in preserve; **G:** close-up of the head of the holotype in life.

Other specimens examined include five juveniles: SYS a001510 collected by Jian-Huan Yang and Run-Lin Li on 5 March 2012, SYS a001532 collected by Jian-Huan Yang and Run-Lin Li on 10 March 2012, and SYS a003474, 3475 and 3481 collected by Jian Wang and Zhi-Tong Lyu on 13 March 2015 from Mt. Wutong.

**Diagnosis.** The presence of an abdominal sucker in the tadpole is the diagnostic character for the genus *Amolops* (Rao & Wilkinson 2007). However, because the tadpole of the new species remains to be discovered, we assigned the new species to this genus based on the morphological and genetic similarity of the adult specimens to those of *A. ricketti* and *A. wuyiensis*.

*Amolops albispinus* **sp. nov.** is distinguished from its congeners by a combination of the following morphological characteristics: (1) presence of white conical spines on the upper and lower lips, loreal and temporal regions, excluding the tympanum; (2) relatively small body size, SVL 36.1–42.4 mm in adult males and 43.1–50.9 mm in adult females; (3) dorsal skin of body very rough with numerous raised large warts; (4) dorsal body olive-brown with dark brown blotches; (5) presence of strongly developed vomerine teeth; (6) absence of vocal sacs; (7) absence of tarsal glands; (8) absence of the dorsolateral folds; (9) presence of a circummarginal groove on the disk of the first finger; (10) absence of outer metatarsal tubercles.

**Description of Holotype:** Head width approximately equal to head length (HDW/HDL 1.0); snout short (SNT/HDL 0.4) and rounded in profile, projecting beyond lower jaw; nostril closer to tip of snout than eye; loreal region concave; top of head flat; eye large and convex (EYE/HDL 0.3); eye diameter shorter than snout length (EYE/SNT 0.9); canthus rostralis distinct; pineal body barely visible; tympanum small, edge faintly distinct; supratympanic fold broad, from back of eye to shoulder; choanae large; vomerine teeth on well-developed ridges, converging posteriorly; tongue cordiform, deeply notched posteriorly; vocal sacs absent.

Forelimbs moderately robust; hands moderately long (ML/SVL 0.3); relative finger lengths I<II<IV<III; finger tips on I–IV dilated to wide oval disks with circummarginal grooves, relative width of finger disks I<II<III=IV; nuptial pad on first finger prominent with strongly developed white conical spines; subarticular tubercles prominent, rounded; inner and outer metacarpal tubercle slightly elongated; no finger webbing or lateral fringes.

Hindlimbs long and robust (TIB/SVL 0.5; PL/SVL 0.5); relative toe lengths I<II<V<III<IV; tips of all toes expanded to well-developed oval discs with circummarginal grooves; subarticular tubercles oval and distinct; inner metatarsal tubercles laterally compressed and pronounced; outer metatarsal tubercles absent; toes fully webbed, webbing formula I1–III1–2<sup>3</sup>/<sub>4</sub>III1–3IV3–1 following Savage (1997); lateral fringe present; tibio-tarsal articulation reaching snout, when hindlimb stretched alongside of body.

Skin on dorsal surface of head, trunk, and limbs very rough with numerous tubercles and large raised warts; prominent conical spines on the upper and lower lips, loreal and temporal regions, excluding the tympanum; numerous small tubercles and ridges on the throat and ventral surfaces of trunk and limbs; dorsolateral fold absent; posterior part of upper lip swollen; rictal gland prominent and ellipsoidal, posterior to corner of mouth.

**Measurement of holotype (in mm).** SVL 36.8; HDL 13.2, HDW 13.4; SNT 5.2; IOD 3.3; EYE 4.6; TMP 1.5; TEY 1.3; TIB 17.3; ML 11.4; PL 19.2; F2D 1.8; F3D 2.2; T4D 1.4.

**Color in life.** Dorsal surface olive-brown with raised dark brown blotches on the dorsal surface of head and trunk and small dark brown spots on the dorsal surface of fingers, upper arms, tarsi, tibias, thighs and toes; faint dark transverse bars on dorsal surface of fingers, lower arms, tarsi, tibias, thighs and toes; posterior edge of upper lip and rictal gland copper; white conical spines on upper and lower lips, loreal and temporal regions, excluding tympanum; nuptial pad and spines white; dorsal surfaces of discs on fingers copper; copper flecks on the lateral sides of body; ventral surface of the throat, chest, and belly opaque creamy white, some grey flecks on the throat and chest; ventral surface of the hands and feet dark grey; ventral surfaces of the upper arms, lower arms, tarsi, tibias, and thighs pink with copper flecks; rear of thighs copper with dark mottling.

**Color in preservative.** On dorsum, color fades to dark olive with dark brown blotches, transverse bars, and spots; ventral surface yellow with grey mottling on throat and chest; edge of the ventral surface of the belly with a hint of orange (Fig. 3).

**Variation.** Measurements of type series are given in Table 3. All specimens were very similar in morphology and color pattern. However, the main diagnostic character of this species (i.e., white conical spines on upper and lower lips, temporal and loreal regions), are obvious on adult males but subtle on adult females, which indicates that it is a secondary sexual character.



**TABLE 3.** Measurements [in mm; mean±SD (range)] of the type series of *Amolops albispinus* sp. nov. See Material and methods for abbreviations.

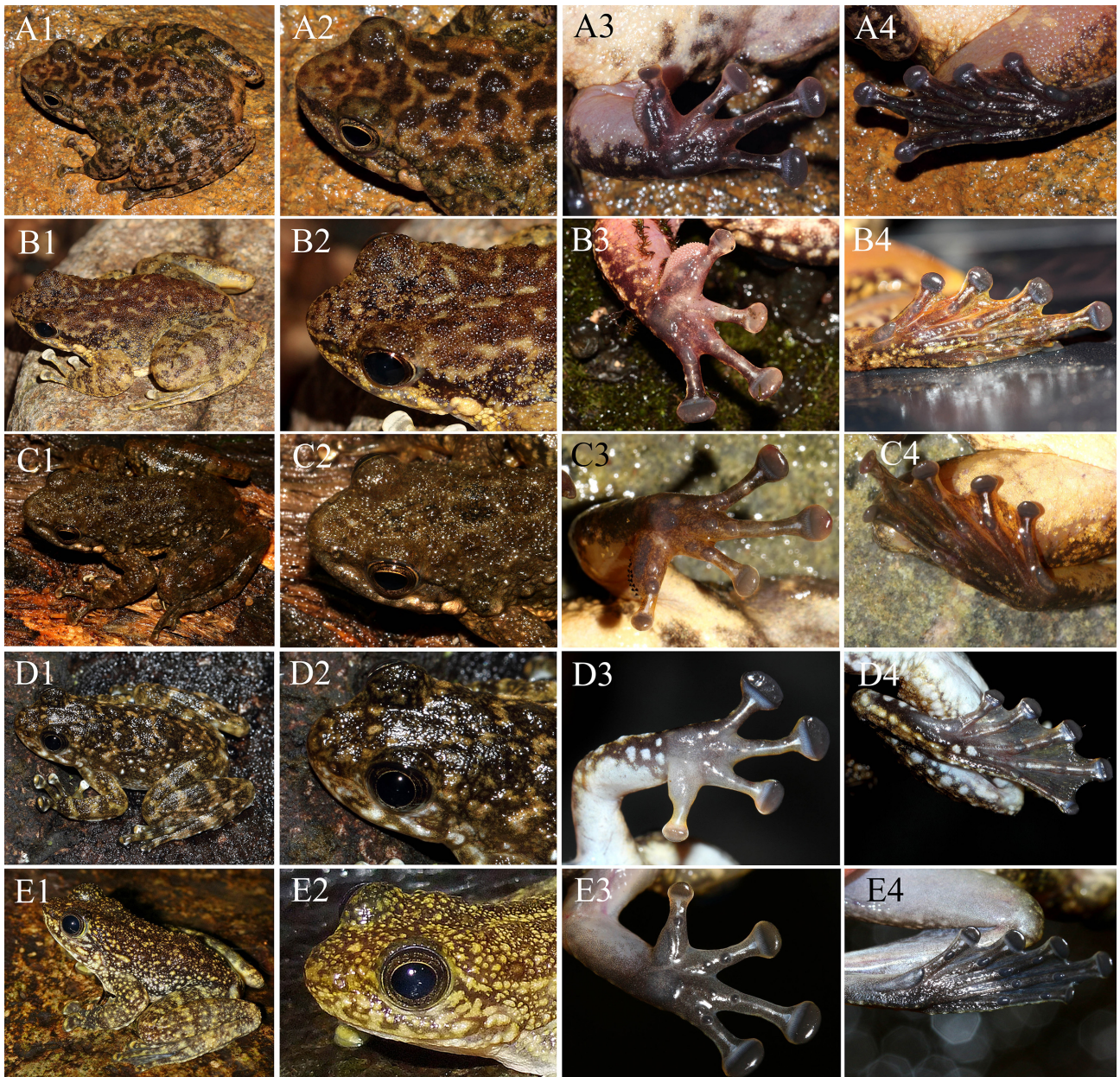
	<i>A. albispinus</i> sp. nov.		
	Males (n=8)	Females (n=6)	Juveniles (n=5)
SVL	40.0±2.2 (36.7–42.4)	48.3±2.8 (43.1–51.9)	25.7±5.2 (21.5–33.5)
HDL	14.3±0.7 (13.2–15.1)	17.4±0.8 (16.2–18.4)	8.2±1.3 (6.9–9.9)
HDW	15.2±1.1 (13.4–16.9)	17.8±1.2 (16.0–19.5)	9.5±2.0 (7.3–12.0)
SNT	6.0±0.7 (5.0–7.0)	6.5±0.9 (5.8–8.1)	4.0±0.6 (3.4–4.6)
IOD	3.5±0.5 (2.8–4.0)	4.0±0.5 (3.2–4.5)	2.4±0.5 (1.6–3.0)
EYE	5.0±0.6 (3.9–5.8)	5.6±0.4 (5.1–6.3)	3.7±0.9 (2.9–5.1)
TMP	2.1±0.3 (1.5–2.5)	2.8±0.5 (2.2–3.3)	1.2±0.4 (0.7–1.8)
TEY	1.4±0.2 (1.3–1.7)	1.8±0.5 (0.8–2.2)	0.7±0.2 (0.5–0.9)
TIB	19.4±1.9 (17.2–22.4)	22.9±2.1 (21.0–26.4)	12.9±2.7 (10.0–17.1)
ML	11.2±0.9 (9.7–12.3)	13.1±0.6 (12.1–13.8)	7.9±1.5 (6.5–9.8)
PL	20.1±1.4 (18.2–22.4)	22.7±1.5 (20.2–24.1)	12.5±2.3 (10.7–16.1)
F2D	2.1±0.2 (1.8–2.4)	2.4±0.4 (1.8–2.9)	1.3±0.4 (1.0–1.8)
F3D	2.5±0.2 (2.2–2.7)	2.8±0.3 (2.2–3.2)	1.5±0.4 (1.1–2.2)
T4D	1.4±0.2 (1.2–1.7)	1.6±0.2 (1.4–1.9)	0.8±0.2 (0.6–1.1)
HDW/HDL	1.1±0.1 (1.0–1.2)	1.0±0.0 (1.0–1.1)	1.2±0.1 (1.0–1.3)
HDL/SVL	0.4±0.0 (0.3–0.4)	0.4±0.0 (0.3–0.4)	0.3±0.0 (0.3–0.4)

**Comparisons.** Morphologically, *Amolops albispinus* sp. nov. differs from *A. ricketti*, *A. wuyiensis*, *A. daiyunensis*, and *A. hongkongensis* (in parenthesis), to which it is most closely related (Fig. 2), by the presence of white strongly developed conical spines on upper and lower lips and loreal and temporal regions, excluding tympanum (vs. absence; Fig. 3), and by the presence of numerous raised large warts on the dorsal skin of the body (vs. dorsal skin without such warts). Further, it differs from *A. ricketti* by having a relative small body size at SVL 36.7–42.4 mm in adult males (vs. 42.0–60.5), and 43.1–51.9 mm in adult females (vs. 53.5–67.0), and a dorsal color of olive-brown with dark brown blotches (vs. dorsal color olive-brown or brown with light-colored worm-like marks); from *A. wuyiensis* by the presence of vomerine teeth (vs. absence), absence of vocal sacs (vs. presence), and white nuptial spines (vs. black); from *A. daiyunensis* by the presence of vomerine teeth (vs. absence), absence of vocal sacs (vs. presence), prominent conical nuptial spines (vs. fine, particle-like), and absence of tarsal gland (vs. presence); and from *A. hongkongensis* by the presence of vomerine teeth (vs. absence), absence of vocal sacs (vs. presence), and prominent conical nuptial spines (vs. fine, particle-like).

In addition to the presence of white, strongly developed, conical spines on the upper and lower lips and loreal and temporal region, *Amolops albispinus* sp. nov. differs from the remaining 46 species of *Amolops* as follows: from *A. akhaorum*, *A. aniqiaoensis*, *A. archotaphus*, *A. bellulus*, *A. chakrataensis*, *A. chayuenensis*, *A. chunganensis*, *A. compotrix*, *A. cremnobatus*, *A. cucae*, *A. gerbillus*, *A. iriodes*, *A. jaunsari*, *A. kohimaensis*, *A. longimanus*, *A. mengyangensis*, *A. minutus*, *A. monticola*, *A. nyingchiensis*, and *A. vitrea* by the lack of dorsolateral folds (vs. presence); from *A. formosus*, *A. granulosus*, *A. jinjiangensis*, *A. kangtingensis*, *A. liangshanensis*, *A. lifanensis*, *A. loloensis*, *A. mantzorum*, *A. nidorbellus*, *A. tuberodepressus*, and *A. viridimaculatus* by the presence of a circummarginal groove on disk of first finger (vs. absence); from *A. afghanus*, *A. assamensis*, *A. himalayanus*, *A. indoburmanensis*, *A. marmoratus*, and *A. spinapectoralis* by the absence of vocal sacs (vs. presence); from *A. daorum*, *A. hainanensis*, *A. panhai*, and *A. torrentis* by the presence of strongly developed vomerine teeth (vs. vomerine teeth weak or absent); from *A. caelumnoctis*, *A. medogensis*, and *A. splendissimus* by a dorsal color of olive-brown with dark brown blotches (vs. dorsal color of green or bright yellow); and from *A. larutensis* and *A. kaulbacki* by the absence of outer metatarsal tubercles (vs. presence).

**Etymology.** The specific name, *albispinus*, refers to the “white spines” on the upper and lower lips, and loreal and temporal regions, which are the diagnostic features of this new species. As an English common name we suggest “White-spined Cascade Frog”.

**Distribution and ecology.** Currently, *A. albispinus* **sp. nov.** is known from the type locality of Mt. Wutong, and from Mt. Paiya, which is 30 km from Mt. Wutong, in Shenzhen City, Guangdong Province, China. This species is common in Mt. Wutong throughout the year, whereas, it was observed to be rare in Mt. Paiya (only one specimen (SYS a002436) found). It inhabits low to mid-elevation (60–500 m) rocky, fast-flowing streams surrounding by moist subtropical secondary evergreen broadleaved forests.



**FIGURE 4.** Comparisons of morphological characteristics between *Amolops albispinus* **sp. nov.** and species in the *Amolops ricketti* and *Amolops daiyunensis* species groups. **A:** *Amolops albispinus* **sp. nov.**; **B:** *Amolops ricketti*; **C:** *Amolops wuyiensis*; **D:** *Amolops daiyunensis*; **E:** *Amolops hongkongensis*. **1:** dorsolateral view; **2:** close-up of the head; **3:** ventral view of the hand; **4:** ventral view of the leg.

## Discussion

The original description of *Amolops ricketti* was based on an examination of only a few specimens only and only a limited number of diagnostic characters were presented (Boulenger 1899a). This has resulted in considerable challenges in accurate species identification by morphology and may have led to the oversight of morphologically

cryptic species. Advances in molecular analysis have dramatically improved our ability to address these issues (Funk *et al.* 2012). Among all *Amolops* species in China, *Amolops ricketti* has the widest distribution, including the Provinces of Sichuan, Guizhou, Anhui, Hubei, Zhejiang, Guangdong, Jiangxi, Guangxi, Yunnan, and Fujian, and is even found north to central Vietnam (Frost 2015). A relatively high variation in morphological characters was observed among *A. ricketti* specimens collected from Vietnam (Ngo *et al.* 2006), which suggests that cryptic species may occur within what is presently considered *A. ricketti*. This situation is similar to other Asian stream-dwelling genera, for example, *Odorrana*, *Leptolalax* and *Megophrys*, from which a number of new species have been recently described (Li *et al.* 2014; Rowley *et al.* 2015; Wang *et al.* 2015). The discovery of *A. albispinus* **sp. nov.** may represent only the beginning of uncovering additional cryptic diversity within *A. ricketti*.

*Amolops albispinus* **sp. nov.** is currently only found in several rocky, fast-running streams in the Mt. Wutong and Mt. Paiya, Shenzhen City, China. These areas are being threatened by alien invasive species (e.g. *Gambusia affinis* and *Trachemys scripta*) and the construction of dams (Dudgeon 1995). Therefore, surveys are urgently needed in south China to investigate the population status and distribution of this species and we recommend the species to be listed as Data Deficient in the IUCN Red List of Threatened Species.

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#### APPENDIX 1. List of specimens examined in this study.

- Amolops albispinus* sp. nov. (1): China: Guangdong Province, Mt. Paiya: SYS a002436.
- Amolops chunganensis* (9): China: Jiangxi Province: Yanshan County: Wuyishan Nature Reserve: SYS a001331; Jinggangshan City: Mt. Jinggang: SYS a001214, 1262, 1263 and 1264; Anfu County: Mt. Wugong: SYS a003139; Guangdong Province: Ruyuan County: Naling Nature Reserve: SYS a000826; Guangxi Zhuang Autonomous Region: Longsheng County: Huaping Nature Reserve: SYS a000897 and 0898.
- Amolops daiyunensis* (9): China: Fujian Province: Dehua County: Mt. Daiyun: SYS a001737–1745.
- Amolops hainanensis* (4): China: Hainan Province: Mt. Diaoluo: SYS a000397; Mt. Bawangling: SYS a004580, 4581 and 4582.
- Amolops hongkongensis* (3): China: Hong Kong: SYS a004577, 4578 and 4579.
- Amolops marmoratus* (9): China: Yunnan Province: Ruili City: Tongbiguan Nature Reserve: SYS a003852, 3853, 3854, 3855, 3869, 3870, 3871, 3872 and 3873.
- Amolops ricketti* (19): SYS a004141 and 4142 from Guadun (=Kwadun) Village, Mt. Wuyi, Wuyishan City, Fujian Province, China; SYS a002492 from Mt. Emeifeng, Taining County, Fujian Province, China; SYS a003342 from Gutian Town, Shanghang County, Fujian Province, China; SYS a001605, 1342 and 1343 from Wuyishan Nature Reserve, Yanshan

County, Jiangxi Province, China; SYS a000214, 0240, 0314, 0354 and 0355 from Yangjifeng Nature Reserve, Guixi City, Jiangxi Province, China; SYS a000125 and 0126, 3346 and 3348 from Heishiding Nature Reserve, Fengkai County, Guangdong Province, China; SYS a003614, 3997 and 3998 from Mt. Nankun, Longmen County, Guangdong Province, China.

*Amolops torrentis* (6): China: Hainan Province: Mt. Diaoluo: SYS a000405; Mt. Jiangfengling: SYS a001243; Mt. Bawangling: SYS a004573, 4574, 4575 and 4576.

*Amolops tuberodepressus* (21): China: Yunnan Province: Yuxi City: Simenxia: SYS a003884, 3885 and 3886, 3896, 3897, 3898, 3999, 3900, 3901, 3902; Pu'er City: Huangcaoling: SYS a003931 and 3932; Zhenyuan County: Ailaoshan Nature Reserve: SYS a002996, 2997, 2998, 2999, 3000, 3001, 3002, 3003 and 3004.

*Amolops viridimaculatus* (3): China: Yunnan Province: Tengchong County: Gaoligong Nature Reserve: SYS a003754, 3812 and 3813.

*Amolops wuyiensis* (13): China: Fujian Province: Wuyishan City: Sangang (=Sanchiang) Village: SYS a001716 and 1717, 4139 and 4140; Shaowu City: Jiangshi Nature Reserve: SYS a004122. Jiangxi Province: Yanshan County: Wuyishan Nature Reserve: SYS a001606; Guixi City: Yangjifeng Nature Reserve: SYS a000324, 0358, 0359 and 0360; Guangfeng County: Tongboshan Nature Reserve: 1668 and 1688; Zhejiang Province: Jingning County: Makeng Village: SYS a002723.