



A new species of *Amolops monticola* group (Amphibia: Ranidae) from Hoang Lien Range, northwest Vietnam

LUAN THANH NGUYEN^{1,2}, BENJAMIN TAPLEY^{3,*}, TOI VAN LA⁴ & JODI J. L. ROWLEY^{5,6}

¹EDGE of Existence Programme, Zoological Society of London, Regent's Park, London, UK

²Indo Myanmar Conservation, R.1301, CT1 Bac Ha C14 Building, To Huu Str., Nam Tu Liem Dist., Hanoi, Vietnam

✉ nguyenluanbio@gmail.com; <https://orcid.org/0000-0002-4663-125X>

³Zoological Society of London, Regent's Park, London, NW1 4RY, UK

⁴Hoang Lien National Park, 89 Nguyen Chi Thanh Str., Sapa Dist., Lao Cai Prov., Vietnam

⁵Australian Museum Research Institute, Australian Museum, 1 William St, Sydney, NSW, 2010, Australia

✉ jodi.rowley@australian.museum; <https://orcid.org/0000-0002-2011-9143>

⁶Centre for Ecosystem Science, School of Biological, Earth and Environmental Sciences, University of New South Wales, Sydney NSW 2052, Australia

*Corresponding author: ✉ ben.tapley@zsl.org; <https://orcid.org/0000-0002-9787-3793>

Abstract

Of the 19 species of *Amolops* reported from Vietnam, 10 are known to occur in the Hoang Lien Range in northwest Vietnam. During field surveys in the Hoang Lien Range, we collected individuals from a population of *Amolops* that we could not assign to any known species; morphological and molecular data assign these individuals to the *Amolops monticola* group and we describe a new species to science, *Amolops spicalinea* **sp. nov.**, in reference to a line of horny spinules running along the body below the dorsolateral fold in males. The new species is morphologically and molecularly most similar to *Amolops bellulus* from Yunnan Province, China but is distinguished from *A. bellulus* and other congeneric species by a combination of the following characters: (1) SVL of adult males 46.6–52.2 mm, $N=3$; adult females 60.1–63.0 mm, $N=3$; (2) vomerine teeth in two oblique rows between choanae; (3) tympanic annulus visible; (4) all finger and toe tips expanded to discs with circummarginal grooves present; (5) skin smooth, except for lateral surfaces of head, below the dorsolateral fold, and the area surrounding cloaca where the texture varies from tiny spinules to large tubercles; (6) dorsolateral fold present with horny spinules along the lower half in males; (7) creamy-white lip stripe extending from tip of snout, terminating in a broken, rugose line above axilla; (8) dorsum reddish brown or greenish brown with numerous small black spots; flanks yellowish brown to dark brown; ventral surface of body orange-yellow with small brown spots present on chest and throat; (9) interdigital finger webbing absent; toes fully webbed to discs except on Toes IV where webbing reaches discs as a fringe, webbing formula: I0–0II0–0III0–1⁺IV1⁺–0V; (10) outer metatarsal tubercles absent; (11) vocal sac absent in males, and (12) nuptial pad velvety without spines. Our phylogenetic analysis using 16S ribosomal RNA mitochondrial gene and cytochrome c oxidase subunit 1 (CO1) genes show that *Amolops spicalinea* **sp. nov.** is sister to *Amolops bellulus* 2 from Yunnan, China. The new species is currently known from only three localities in the Hoang Lien Range between 2288–2493 m asl and is threatened by habitat loss and degradation. This species likely qualifies for being assessed as Endangered in accordance with the IUCN Red List of Threatened Species categories and criteria (B1ab).

Key words: Amphibians, Anura, cascade frog, Fansipan, Nam Kang Ho Tao, Pu Ta Leng

Tóm tắt tiếng Việt

Trong số 19 loài ếch bám đá (giống *Amolops*) ghi nhận ở Việt Nam, 10 loài phân bố tại Dãy Hoàng Liên Sơn, Tây Bắc Việt Nam. Trong quá trình khảo sát thực địa tại dãy Hoàng Liên Sơn, chúng tôi đã thu thập một số mẫu vật của một quần thể ếch bám đá *Amolops* nhưng không thể xác định được loài đã biết nào; kết quả phân tích dữ liệu hình thái và di truyền cho thấy các cá thể này cùng một loài và thuộc nhóm *Amolops monticola* và là một loài mới cho khoa học, có tên là *Amolops spicalinea* **sp. nov.**, đặc trưng bởi đặc điểm gai nhỏ dọc cơ thể và phần sau của gờ da trên lưng ở các cá thể đực. Loài ếch mới này có đặc điểm hình thái và di truyền gần gũi với loài *Amolops bellulus*

phân bố ở tỉnh Vân Nam, Trung Quốc nhưng có đặc điểm khác biệt với loài *Amolops bellulus* và các loài khác trong giống *Amolops* bởi: (1) chiều dài mõm-huyết (SVL) con đực 46.6–52.2 mm, $N=3$; con cái 60.1–63.0 mm, $N=3$; (2) răng lá mía 2 hàng phát triển, giữa hai lỗ mũi trong; (3) màng nhĩ rõ; (4); mút ngón tay phình rộng với rãnh mút ngón; (5) da lưng hầu hết nhẵn ngoại trừ bên đầu, sau gờ hông và quanh lỗ huyết có các mụn gai hoặc mụn nhỏ (6) gờ da bên hông phát triển với các đốm trắng ở phần sau; (7) trên môi có vệt trắng kéo dài tới trên bả vai thành hàng mụn; (8) lưng màu đỏ nâu hoặc xanh với một số đốm đen nhỏ; hông màu vàng nâu hoặc nâu đen; bụng màu cam vàng với các đốm đen ở ngực và họng; (9) màng bơi chân sau phát triển tới mút ngón ngoại trừ ngón IV chạm tới mút ngón bởi rèm da, công thức màng bơi I0–0II0–0III0–1⁺IV1⁺–0V; (10) không có củ bàn chân ngoài; (11) con đực không có túi kêu và (12) chai sinh dục ở con đực rất phát triển và phủ bởi hạn mịn. Kết quả phân tích 2 đoạn gen ti thể (16S và CO1) cho thấy loài *Amolops spicalinea* **sp. nov.** có quan hệ gần gũi với loài *Amolops bellulus* 2 từ tỉnh Vân Nam, Trung Quốc. Loài ếch mới hiện ghi nhận ở ba địa điểm dọc dãy Hoàng Liên Sơn, tỉnh Lào Cai ở độ cao 2288 tới 2493 m so với mực nước biển và chắc chắn đủ điều kiện để phân hạng bảo tồn ở mức Nguy cấp theo các tiêu chí “B1ab” trong Danh lục đỏ thế giới IUCN.

Introduction

Cascade frogs of the genus *Amolops* Cope 1865 are the most speciose genera of the Family Ranidae, with 85 species currently described (Frost 2024). Members of the genus are widely distributed from Nepal, northern India, western and southern China to Malaysia, including Indochina (Frost 2024). Recent phylogenetic studies on this genus support the existence of up to eight species groups (Mahony *et al.* 2022). The 24 species of the *Amolops monticola* group have several distinct morphological characters that delimit them from other *Amolops* species groups including: the presence of true dorsolateral folds (not formed by an incomplete series of glands); smooth skin on the dorsum; lateral sides of the head dark in colouration, and a light-colored upper lip stripe extending to shoulder (Stuart *et al.* 2010; Jiang *et al.* 2021; Patel *et al.* 2021). Species in this group are distributed from India to southern China and Indochina (Frost 2024; Patel *et al.* 2021). Up to 16 species from the *Amolops monticola* group have been reported from Vietnam (Frost 2024).

During field work in the Hoang Lien Range, northwest Vietnam, we encountered a population of *Amolops*. These were assigned to the *Amolops monticola* group based on examination of morphological characters and phylogenetic analysis, but this population could not be assigned to any described species within this group, nor to any other *Amolops* species. We describe this population as a new species to science on the basis of both morphological and genetic differences and biogeography.

Materials and methods

Specimens were collected at night in forest stream habitats in Lao Cai Province, Vietnam: on Mount Fansipan, Sa Pa District in September 2018; Mount Pu Ta Leng, Bat Xat District in October 2018; and on Mount Nam Kang Ho Tao, Sa Pa District in September 2020 (Fig. 1). Geographic coordinates were obtained using a Garmin GPSMAP 60CSx GPS receiver (Garmin Ltd., Kansas, USA) and recorded in datum World Geodetic System 1984. Specimens were photographed in life before being humanely euthanised using a 20% solution of benzocaine applied to the ventral surface of the frog. Tissue samples (liver) for molecular analyses were extracted from freshly euthanised specimens and stored in absolute ethanol prior to the fixation of specimens with 10% formalin and subsequent storage in 70% ethanol (Simmons 2002). Type specimens were deposited at the Institute of Tropical Biology Collection of Zoology (ITBCZ) in Ho Chi Minh City, Vietnam. A referred specimen was deposited at the Hoang Lien National Park headquarters (HLNP) as a reference for National Park scientists.

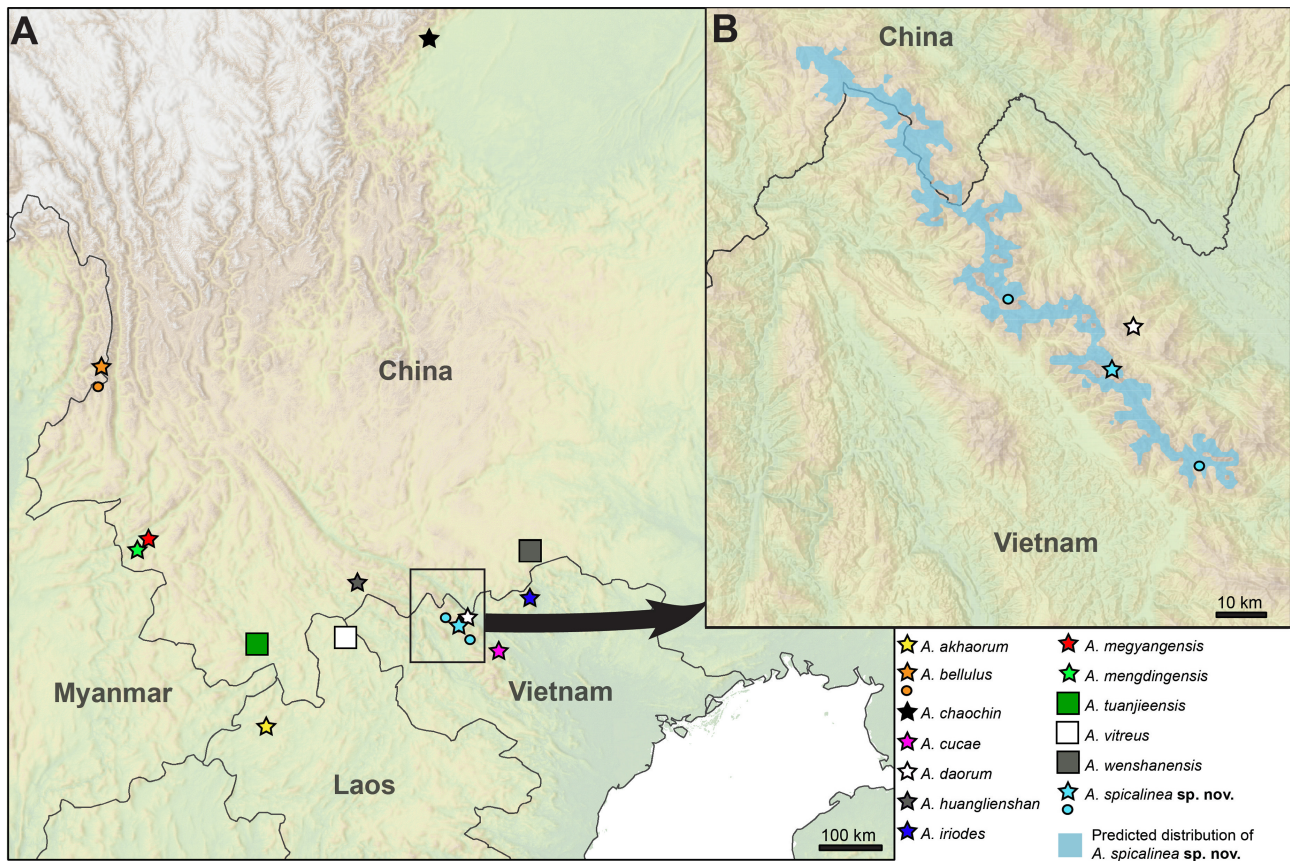


FIGURE 1. (A–B) Type localities (stars and squares) and other relevant localities (circles) of species in the *Amolops monticola* group and (B) zoomed in area showing the predicted distribution of *Amolops spicalinea* sp. nov. (B). Pale grey areas indicative of higher elevation, dark green indicative of lowest elevation.

Laboratory methods

Molecular data: Total genomic DNA was extracted with a DNeasy® Blood and Tissue Kit (QIAGEN GmbH, Hilden, Germany), following the manufacturer's protocols for purification of genomic DNA from animal tissues. We amplified a ~550 bp section of 16S ribosomal RNA mitochondrial gene (here after 16S gene) using the primers (5'-3') 16Sar CGCCTGTTTATCAAAAACAT and 16Sbr CCGGTCTGAACTCAGATCACGT (Palumbi *et al.* 1991) and a ~570 bp section The cytochrome c oxidase subunit 1 gene (here after CO1 gene) using the primers (5'-3') Chmf4 TYTCWACWAAYCAYAAAGAYATCGG and Chmr4 ACYTCRGGRTGRCCRAARAATCA (Che *et al.* 2012). PCR amplification was carried out in 25 µl reactions with 1000 ng of genomic DNA, 2xES Taq MasterMix (CW BIO, China), 2 pmol of corresponding primers and Bionline MyTaq™ Red DNA Polymerase (0.75 unit). Negative controls were included in each PCR batch. Thermocycling was performed on an Eppendorf Mastercycler EpS (Eppendorf, Hamburg, Germany) under the following conditions: initial denaturation 94 °C (5 mins), two cycles of 94 °C (1 min) denaturation, 55 °C (1 min) annealing, followed by 35 cycles of 94 °C (20 s) denaturation, 50 °C (40 s) annealing and 72 °C (50 s) extension, followed by a final extension step at 72 °C (10 mins) for 16S, and initial denaturation 94 °C (5 min), 35 cycles of 94 °C (60 s) denaturation, 55 °C (60 s) annealing and 72 °C (60 s) extension, followed by a final extension step at 72 °C (10 min) for CO1. All PCR products were purified using ExoSap-IT™ (USB Corporation, Ohio USA), and sequenced in both directions at Macrogen (Seoul, South Korea). Sequence chromatograms were edited and checked for quality using BioEdit 7.0.5.2 (Hall 1999). The new sequences were then checked using BLAST (Altschul *et al.* 1990) on NCBI BLASTN (The National Center for Biotechnology Information, <http://blast.ncbi.nlm.nih.gov>) to verify their approximate identity (Benson *et al.* 2017). Sequences were deposited in GenBank (see Table 1).

TABLE 1. Specimens, localities, and GenBank accession numbers of *Amolops* used in this study.

S.No	Taxa	Collection Locality	Voucher No.	Accession Number		Reference
				16S	CO1	
	<i>A. monticola</i> Group					
1	<i>Amolops spicalinea</i> sp. nov.	Vietnam: Sapa, Lao Cai	ITBCZ 3648	PQ856365	-	This study
2	<i>Amolops spicalinea</i> sp. nov.	Vietnam: Bat Xat, Lao Cai	ITBCZ 3649	PQ856352	PQ856504	This study
3	<i>Amolops spicalinea</i> sp. nov.	Vietnam: Bat Xat, Lao Cai	HLNP2019 1003 00029	PQ856366	-	This study
4	<i>Amolops spicalinea</i> sp. nov.	Vietnam: Bat Xat, Lao Cai	ITBCZ 3650	PQ856392	PQ856505	This study
5	<i>Amolops spicalinea</i> sp. nov.	Vietnam: Sapa, Lao Cai	ITBCZ 3652	PQ856363	PQ856431	This study
6	<i>A. adicola</i>	India: Arunachal Pradesh	BNHS 6121	MZ229772	MZ221140	Patel <i>et al.</i> 2021
7	<i>A. adicola</i>	India: Arunachal Pradesh	WIIADA 433	-	MZ221141	Patel <i>et al.</i> 2021
8	<i>A. akhaorum</i>	Laos: Vieng Phou Kha, Luang Namtha	FMNH 271355	FJ417158	MN961350	Stuart <i>et al.</i> 2010; Wu <i>et al.</i> 2020
9	<i>A. akhaorum</i>	Laos: Vieng Phou Kha, Luang Namtha	FMNH 271406	FJ417159	MN961351	Stuart <i>et al.</i> 2010; Wu <i>et al.</i> 2020
10	<i>A. aniqiaoensis</i> 1	China: Medog, Tibet	SYNU 04II6015	MN953655	MN961352	Wu <i>et al.</i> 2020
11	<i>A. aniqiaoensis</i> 1	China: Medog, Tibet	SYNU 04II6016	MN953656	MN961353	Wu <i>et al.</i> 2020
12	<i>A. aniqiaoensis</i> 1	China: Tibet	KIZ 011136	MN953658	MN961355	Wu <i>et al.</i> 2020
13	<i>A. aniqiaoensis</i> 2	China: Medog, Tibet	KIZ 014094	MN953657	MN961354	Wu <i>et al.</i> 2020
14	<i>A. archotaphus</i>	Thailand: Amphoe Chom Thong, Chiang Mai	FMNH 271708	MN953659	MN961356	Wu <i>et al.</i> 2020
15	<i>A. archotaphus</i>	Thailand: Doi Inthanon, Chiang Mai	KIZ030888	MN953660	MN961357	Wu <i>et al.</i> 2020
16	<i>A. archotaphus</i>	Thailand: Doi Inthanon, Chiang Mai	KIZ 030948	MN953661	MN961358	Wu <i>et al.</i> 2020
17	<i>A. bellulus</i>	China: Lushui, Yunnan	KIZ 9810021	DQ204473	-	Ngo <i>et al.</i> 2006
18	<i>A. bellulus</i> 1	China: Teng Chong Co., Yunnan	KIZYPX9037	MN953664	KU243078	Jiang <i>et al.</i> 2016; as <i>A. bellulus</i> 1 Wu <i>et</i> <i>al.</i> 2020
19	<i>A. bellulus</i> 1	China: Teng Chong Co., Yunnan	KIZYPX9038	MN953665	KU243079	Jiang <i>et al.</i> 2016; as <i>A. bellulus</i> 1 Wu <i>et</i> <i>al.</i> 2020
20	<i>A. bellulus</i> 1	China: Teng Chong Co., Yunnan	CAS 233991	FJ417127	MN961361	Stuart <i>et al.</i> 2010; Wu <i>et al.</i> 2020
21	<i>A. bellulus</i> 2	China: Teng Chong Co., Yunnan	CAS 233986	FJ417126	MN961362	Stuart <i>et al.</i> 2010; as <i>A. bellulus</i> 2 Wu <i>et</i> <i>al.</i> 2020
22	<i>A. chaochin</i>	China: Sichuan: Dayi	CIB 116971	MZ702027	MZ706968	Jiang <i>et al.</i> 2021
23	<i>A. chaochin</i>	China: Sichuan: Dayi	CIB 116977	MZ702026	MZ706969	Jiang <i>et al.</i> 2021
24	<i>A. chaochin</i>	China: Sichuan: Dayi	CIB 116979	MZ702029	MZ706971	Jiang <i>et al.</i> 2021
25	<i>A. chunganensis</i>	China: Jiangxi: Mt. Wuyi	SYS a008411	MZ702021	MZ706964	Jiang <i>et al.</i> 2021
26	<i>A. chunganensis</i>	China: Jiangxi: Mt. Wuyi	SYS a008412	MZ702022	MZ706965	Jiang <i>et al.</i> 2021
27	<i>A. chunganensis</i>	China: Jiangxi: Mt. Wuyi	SYS a008412	MZ702023	MZ706966	Jiang <i>et al.</i> 2021

.....continued on the next page

TABLE 1. (Continued)

S.No	Taxa	Collection Locality	Voucher No.	Accession Number		Reference
				16S	CO1	
	<i>A. monticola</i> Group					
28	<i>A. chunganensis</i>	China: Jiangxi: Mt. Wuyi	SYS a008414	MZ702024	MZ706967	Jiang <i>et al.</i> 2021
29	<i>A. cf. chunganensis</i> 2	China: Hubei: Fangxian	KIZ 03756	MN953671	MN961365	Jiang <i>et al.</i> 2021
30	<i>A. compotrix</i>	Laos: Nakai	FMNH 256500	FJ417141	MN961366	Stuart <i>et al.</i> 2010; Wu <i>et al.</i> 2020
31	<i>A. compotrix</i>	Vietnam: Dak Glei, Kon Tum	ZISP A7367	FJ417142	MN961367	Stuart <i>et al.</i> 2010; Wu <i>et al.</i> 2020
32	<i>A. cucae</i>	Vietnam: Van Ban, Lao Cai	AMNH 168729	FJ417145	MN961372	Stuart <i>et al.</i> 2010; Wu <i>et al.</i> 2020
33	<i>A. cucae</i>	Vietnam: Van Ban, Lao Cai	AMNH 168727	FJ417144	MN961373	Stuart <i>et al.</i> 2010; Wu <i>et al.</i> 2020
34	<i>A. daorum</i> 1	China: Jingdong, Yunnan	KIZ047048	MN953678	MN961377	Wu <i>et al.</i> 2020
35	<i>A. daorum</i> 1	China: Jingdong, Yunnan	KIZ047049	MN953679	MN961378	Wu <i>et al.</i> 2020
36	<i>A. daorum</i> 2	Laos: Vieng Tong, Huaphahn	FMNH 255353	FJ417147	MN961379	Stuart <i>et al.</i> 2010; Wu <i>et al.</i> 2020
37	<i>A. daorum</i> 2	Laos: Vieng Tong, Huaphahn	FMNH 255354	FJ417148	MN961380	Stuart <i>et al.</i> 2010; Wu <i>et al.</i> 2020
38	<i>A. deng</i>	China: Zayü, Tibet	KIZ 014116	MN953695	MN961395	Wu <i>et al.</i> 2020
39	<i>A. deng</i>	China: Zayü, Tibet	KIZ014069	MN953696	MN961396	Wu <i>et al.</i> 2020
40	<i>A. deng</i>	China: Zayü, Tibet	KIZ014070	MN953697	MN961397	Wu <i>et al.</i> 2020
41	<i>A. iriodes</i>	Vietnam: Vi Xuyen, Ha Giang	AMNH 163926	FJ417152	MN961400	Stuart <i>et al.</i> 2010; Wu <i>et al.</i> 2020
42	<i>A. iriodes</i>	Vietnam: Vi Xuyen, Ha Giang	AMNH 163928	FJ417153	MN961401	Stuart <i>et al.</i> 2010; Wu <i>et al.</i> 2020
43	<i>A. kohimaensis</i>	India: Nagaland	WIIADA 751	MZ229774	MZ221145	Patel <i>et al.</i> 2021
44	<i>A. mengdingensis</i>	China: Mengding, Yunnan	KIZ 20160266	MK501809	MK501812	Yu <i>et al.</i> 2019
45	<i>A. mengdingensis</i>	China: Mengding, Yunnan	KIZ20160317	MK501810	MK501813	Yu <i>et al.</i> 2019
46	<i>A. mengyangensis</i>	Vietnam: Sa Pa, Lao Cai	MNHN 1999.5811	KR827703	KR087618	Grosjean <i>et al.</i> 2015
47	<i>A. mengyangensis</i>	Vietnam: Sa Pa, Lao Cai	MNHN 1999.5812	KR827704	KR087619	Grosjean <i>et al.</i> 2015
48	<i>A. mengyangensis</i>	Vietnam: Sa Pa, Lao Cai	ROM 38503	FJ417151	-	Stuart <i>et al.</i> 2010
49	<i>A. monticola</i>	India: South Sikkim	WIIADA 544	MZ229773	MZ221142	Patel <i>et al.</i> 2021
50	<i>A. monticola</i>	India: South Sikkim	WIIADA 546	-	MZ221143	Patel <i>et al.</i> 2021
51	<i>A. monticola</i>	India: South Sikkim	BNHS 6120	-	MZ221144	Patel <i>et al.</i> 2021
52	<i>A. nyingchiensis</i>	China: Mainling, Tibet	KIZ012636	MN953716	MN961418	Wu <i>et al.</i> 2020
53	<i>A. nyingchiensis</i>	China: Mainling, Tibet	KIZ012633	MN953719	MN961420	Wu <i>et al.</i> 2020
54	<i>A. nyingchiensis</i>	China: Mainling, Tibet	KIZ012629	MN953715	MN961417	Wu <i>et al.</i> 2020
55	<i>A. putaoensis</i>	Myanmar: Putao, Kachin	GXNU QT20170200	MT901383	MT901211	Gan <i>et al.</i> 2020a
56	<i>A. putaoensis</i>	Myanmar: Putao, Kachin	GXNU W011	MT901384	MT901212	Gan <i>et al.</i> 2020a
57	<i>A. tuanjieensis</i>	China: Tuanjie: Yunnan	GXNU YU110003	MN832772	MN832750	Gan <i>et al.</i> 2020b

.....continued on the next page

TABLE 1. (Continued)

S.No	Taxa	Collection Locality	Voucher No.	Accession Number		Reference
				16S	CO1	
<i>A. monticola</i> Group						
58	<i>A. tuanjieensis</i>	China: Tuanjie: Yunnan	GXNU YU110005	MN832773	MN832751	Gan <i>et al.</i> 2020b
59	<i>A. vitreus</i>	Laos: Phongsaly, Phongsaly	FMNH 258187	FJ417164	MN961439	Stuart <i>et al.</i> 2010; Wu <i>et al.</i> 2020
60	<i>A. wenshanensis</i>	China: Xichou, Yunnan	KIZ021425	MN953724	MN961426	Yuan <i>et al.</i> 2018; Wu <i>et al.</i> 2020
61	<i>A. wenshanensis</i>	China: Xichou, Yunnan	KIZ 021426	MN953725	MN961427	Yuan <i>et al.</i> 2018; Wu <i>et al.</i> 2020
62	<i>A. wenshanensis</i>	China: Jinxiu, Guangxi	KU 292045	FJ417129	MN961425	Stuart <i>et al.</i> 2010; Wu <i>et al.</i> 2020
Outgroup						
63	<i>A. rickettii</i>	China: Wuyishan, Fujian	HDSK0043	MN953743	MN961443	Wu <i>et al.</i> 2020
64	<i>A. rickettii</i>	China: Shicheng, Jiangxi	KIZ07150	MN953759	MN961460	Wu <i>et al.</i> 2020

Phylogenetic analysis

For phylogenetic analyses, 61 sequences of 16S mtDNA (including five new sequences) and 60 sequences of CO1 (including three new sequences) belonging to 27 taxa of the *Amolops monticola* species group were included. Sequences for *A. chakrataensis*, *A. gerbillus* and *A. truongi* were not available to be included in the phylogenetic analysis. Two sequences of *Amolops rickettii* were used to root the tree following Jiang *et al.* (2021). New and previously published sequences were aligned using MAFFT (Katoh *et al.* 2019) on the CIPRES Science Gateway (http://www.phylo.org/sub_sections/portal; Miller *et al.* 2010). The 16S and CO1 datasets were concatenated for our final matrix using MEGA 11 for the tree/phylogenetic analysis. Uncorrected *p*-distances (with partial deletion of gaps and missing data) were calculated based on separate 16S and CO1 sequence datasets using MEGA11 (Tamura *et al.* 2021).

The matrilineal genealogy was inferred using two methods: Bayesian inference (BI) and Maximum Likelihood (ML).

BI analyses were conducted in MRBAYES v.3.2 for both datasets (Ronquist *et al.* 2012). The optimal evolutionary models for the BI analysis were tested using MODELTEST v.3.06 (Posada and Crandall 1998), and the best-fit model for BI was the GTR+G+I model of DNA evolution (suggested by the Akaike Information Criterion).

Metropolis-coupled Markov chain Monte Carlo (MCMCMC) analyses were run for 30 million generations and sampled every 1000 generations. Five independent MCMCMC runs were performed and the first 25% of trees were discarded as “burn-in”. Confidence in topology was assessed by posterior probability (BPP, Huelsenbeck *et al.* 2001).

ModelFinder (Kalyaanamoorthy *et al.* 2017) was used to estimate the optimal evolutionary model for each partition for ML analysis, and the best fit model was the TPM2+F+I+G4 for the dataset. The IQ-TREE v.2.1.2 (Minh *et al.* 2020) was used to perform the ML analysis with 10,000 ultrafast bootstrap replications (Hoang *et al.* 2018).

We considered Bayesian posterior probability (BPP) and ultrafast bootstrap (UFB) support values of ≥ 0.95 and ≥ 95 , respectively, as strongly supported (Minh *et al.* 2013; Hoang *et al.* 2018; Huelsenbeck *et al.* 2001; Wilcox *et al.* 2002).

Morphological analysis

The following measurements were taken from fixed specimens to the nearest 0.1 mm using a digital calliper. Morphological characters and measurements follow Stuart *et al.* (2010). SVL = snout-vent length; HDL = head length from tip of snout to rear of the jaws; HDW = maximum head width (between posterior angle of the jaws and

snout tip); IND = internarial distance measured from the center of each nare; SNT = snout length from tip of snout to the anterior corner of the eye; EYE = diameter of the exposed portion of the eyeball; IOD = interorbital distance at narrowest point; TMP = horizontal diameter of tympanum; TEY = tympanum — eye distance from anterior edge of tympanum to posterior corner of the eye; TIB = tibia length, measured from the knee to the tibiotarsal articulation; TL = thigh length, from vent to outer edge of knee; HND = hand length, from base of palm to tip of Finger III; F3DSC = width of Finger III digital disc; T4DSC = width of Toe IV digital disc; FTL = foot length, from proximal edge of inner metatarsal tubercle to tip of fourth toe. In addition, SN = snout tip to center of nare and NE = center of nare to the anterior edge of the eye were measured. All measurements were taken from the right side of the specimen and by the first author for consistency. Interdigital toe webbing formula follows Savage and Heyer (1997).

We obtained comparative morphological data from original descriptions and the literature for all other species within the *Amolops monticola* group: *A. adicola* (Patel *et al.* 2021); *A. akhaorum* (Patel *et al.* 2021; Stuart *et al.* 2010); *A. aniqiaoensis* (Jiang *et al.* 2021; Patel *et al.* 2021; Zhao *et al.* 2005); *A. archotaphus* (Inger & Chanard 1997; Patel *et al.* 2021); *A. bellulus* (Liu *et al.* 2000); *A. chakrataensis* (Ray 1992); *A. chaochin* (Jiang *et al.* 2021); *A. chunganensis* (Jiang *et al.* 2021; Patel *et al.* 2021); *A. compotrix* (Bain *et al.* 2006; Patel *et al.* 2021); *A. cucae* (Bain *et al.* 2006); *A. daorum* (Bain *et al.* 2003); *A. deng* (Che *et al.* 2020; Patel *et al.* 2021); *A. gerbillus* (Annandale 1912; Patel *et al.* 2021); *A. iriodes* (Bain *et al.* 2004); *A. kohimaensis* (Biju *et al.* 2010); *A. mengdingensis* (Yu *et al.* 2019); *A. mengyangensis* (Patel *et al.* 2021); *A. monticola* (Patel *et al.* 2021); *A. nyingchiensis* (Jiang *et al.* 2016); *A. putaoensis* (Gan *et al.* 2020a); *A. tuanjieensis* (Gan *et al.* 2020b); *A. truongi* (Pham *et al.* 2023); *A. vitreus* (Bain *et al.* 2006); and *A. wenshanensis* (Pham *et al.* 2020; Yuan *et al.* 2018). Terminology for the species comparisons follows Patel *et al.* (2021).

Species distribution mapping

An estimated species distribution map was created in ArcMap 10.3.1 (Esri, California, USA). The species' distribution was generated using the International Union for Conservation of Nature (IUCN) elevation raster (IUCN 2017). The range for the newly described species was estimated by clipping the elevation to above 2000 m and below 2600 m. Areas of habitat were deemed suitable and included in maps if they are within species' estimated elevation range, and are not separated from known localities by any continuous stretch of unsuitable habitat with a distance equal to or greater than 1 km. Extent of occurrence (EOO), defined as the area of a minimum convex polygon that passes all known and inferred sites occupied by the species, was measured using the IUCN EOO Calculator tool v. 1.5.

Results

Sequence variation, genetic distances, and genealogy

The aligned CO1 and 16S mtDNA dataset contained 1109 characters and 64 taxa including gaps and missing data. In the BI runs, the standard deviation of split frequencies was 0.001705. Monophyly of the *Amolops monticola* group was well supported in both analyses (UFB/BPP 100/1 respectively; Fig. 2). Our phylogeny was congruent with other published studies (e.g. Jiang *et al.* 2021; Patel *et al.* 2021).

Sequences from five specimens from the Hoang Lien Range formed a distinct and strongly supported clade (UFB/BPP 99/1; Fig. 2.) (hereafter *Amolops* sp.) and this clade was sister to *Amolops bellulus* 2 (Wu *et al.* 2020) from Yunnan, China (UFB/BPP 100/1). Specimens assigned to *Amolops bellulus* on the basis of morphology are known to be paraphyletic (Wu *et al.* 2020), and it is possible that *Amolops bellulus* 2 is a species undescribed by science. In the 16S mtDNA dataset, the mean uncorrected *p*-distance from *Amolops* sp. and the *A. bellulus* paratype was 3.4% (no CO1 mtDNA available for comparison), the mean uncorrected *p*-distance between *Amolops* sp. and other taxa in the *Amolops monticola* group (Table 2) ranged from 1.4% (*A. bellulus* 2) to 7.2% (*A. monticola*) in the 16S mtDNA dataset and from 5.7% (*A. bellulus* 2) to 17.3% (*A. archotaphus*) in the CO1 dataset.

TABLE 2. Mean uncorrected *p*-distances between *Amolops* species in this study. Mean values of genetic distance are given in the lower half of the table for the 16S rRNA gene and upper half for COI. The in-group mean uncorrected *p*-distances are shown on the diagonal and shaded in bold for 16S and COI, respectively. “-“ Not applicable.

Species name	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1 <i>Amolops</i>	0.36	12.9	13.3	11.3	10.7	17.3	-	11.4	5.7	13.4	15.3	13.8	13.7	14.1	12.0	9.9	12.1	12.2	11.7	15.2	12.2	11.5	10.1	9.6	11.4	13.5	14.2	21.2
<i>spicalinea</i> sp. nov.	1.54																											
2 <i>A. adicola</i>	6.3	-	17.0	9.2	8.1	18.0	-	12.7	13.3	16.1	16.8	16.2	16.7	14.2	13.3	12.6	12.7	13.1	8.3	17.7	14.3	6.6	11.1	8.3	12.9	14.8	13.2	22.9
	0.19																											
3 <i>A. akhaorum</i>	6.3	7.7	0.00	16.8	15.2	14.9	-	14.0	14.6	17.8	18.7	17.9	17.6	17.9	11.4	11.3	16.0	12.6	15.7	14.1	13.0	14.9	14.3	14.6	12.2	16.9	17.0	26.2
	0.00																											
4 <i>A. aniqtaoensis</i>	4.9	5.2	6.3	0.13	5.7	16.1	-	11.8	12.1	12.7	15.9	15.4	15.2	15.4	11.1	10.1	11.4	11.2	6.6	16.0	11.0	6.9	11.3	6.4	12.1	15.2	13.9	26.1
	0.00																											
5 <i>A. aniqtaoensis</i> 2	3.5	4.5	5.8	3.8	-	15.4	-	9.8	11.8	13.9	16.7	15.1	14.7	13.5	11.4	10.7	9.7	11.1	5.5	14.9	11.8	6.1	10.0	7.1	11.4	14.7	13.2	25.0
6 <i>A. archotaphus</i>	5.0	8.5	4.5	6.3	5.8	0.00	-	13.8	19.5	17.5	22.1	18.0	19.6	19.0	11.8	10.6	14.7	13.0	16.3	15.5	12.0	15.1	14.8	15.9	11.7	20.1	19.9	27.7
	0.00																											
7 <i>A. bellulus</i> (paratype)	3.4	5.6	5.8	4.4	3.1	5.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8 <i>A. bellulus</i> 1	3.5	5.5	6.0	5.2	3.1	5.9	2.3	0.00	11.5	13.4	16.1	13.9	13.5	12.8	10.9	10.2	5.8	11.2	10.3	13.9	11.9	9.6	5.5	9.4	11.6	14.0	14.0	23.6
	0.12																											
9 <i>A. bellulus</i> 2	1.4	6.4	5.7	5.0	2.9	4.7	2.3	-	13.8	16.3	15.9	14.5	14.4	12.1	10.8	10.8	12.9	13.0	13.1	16.5	12.9	11.8	10.4	11.0	12.3	13.2	14.3	25.0
10 <i>A. chaochin</i>	4.3	7.1	6.5	5.4	4.3	5.0	4.0	4.6	3.8	0.13	11.0	14.2	17.0	15.7	12.5	12.0	14.4	13.3	13.3	15.0	12.7	15.0	12.5	11.7	13.6	14.0	15.3	23.5
	0.23																											
11 <i>A. chunganensis</i>	7.1	10.4	7.2	7.8	7.3	7.1	6.5	6.8	6.2	5.6	0.19	14.1	18.3	16.5	14.3	14.8	16.4	15.0	18.0	17.5	15.8	17.1	15.1	15.2	16.1	17.3	18.2	24.0
	0.00																											
12 <i>A. cf.</i> <i>chunganensis</i>	4.8	8.0	5.9	5.5	5.3	5.9	4.6	5.0	4.2	4.2	4.9	-	15.2	14.5	13.0	12.2	14.5	12.9	15.3	16.6	13.6	15.6	11.5	14.3	14.8	17.1	14.9	21.5
13 <i>A. compotrix</i>	5.4	6.8	5.3	6.5	5.1	5.1	4.3	4.7	4.3	4.8	6.6	5.1	0.38	6.0	15.2	13.7	13.6	14.7	13.7	18.9	15.7	14.6	12.7	13.9	15.6	10.4	7.8	24.5
	1.96																											
14 <i>A. cucae</i>	5.5	6.2	5.8	6.3	4.8	6.0	4.8	4.8	4.4	4.9	6.4	5.6	2.6	0.1	15.4	13.4	14.9	13.7	13.1	17.8	14.9	15.2	12.1	12.3	15.1	9.2	7.7	26.5
	0.18																											

.....continued on the next page

TABLE 2. (Continued)

Species name	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
15 <i>A. daorum</i> 1	4.1	6.2	3.9	4.4	3.3	3.3	2.9	3.7	2.9	4.0	6.5	4.8	4.5	4.2	0.00 / 0.00	4.4	12.7	3.0	13.4	12.4	2.9	10.0	10.4	11.3	6.7	15.9	13.4	23.7
16 <i>A. daorum</i> 2	4.8	7.4	4.7	5.8	4.5	3.9	4.1	4.7	3.5	4.4	5.8	5.1	4.6	4.8	2.3	0.38 \	11.7	4.5	11.9	10.8	5.0	7.9	9.2	10.6	6.0	14.6	13.5	23.1
17 <i>A. deng</i>	3.2	5.6	5.9	4.3	2.8	5.3	1.7	2.0	2.0	4.3	6.5	4.7	4.5	4.7	3.4	4.1	0.13 \	13.7	10.4	13.8	13.8	11.0	5.7	10.4	14.3	16.1	14.9	23.6
18 <i>A. iriodes</i>	4.5	7.0	4.3	5.6	4.3	3.7	4.1	4.5	3.3	4.0	6.5	4.4	4.1	4.2	1.9	1.5	4.2	0.00 / 0.18	12.1	12.9	2.2	9.7	10.8	11.2	7.2	14.9	13.2	23.0
19 <i>A. kohimaensis</i>	3.3	4.7	6.0	3.6	2.1	6.0	2.3	2.7	2.7	3.7	6.9	4.6	4.3	4.2	3.7	4.7	2.3	4.1	—	16.5	12.9	7.6	8.7	6.2	12.9	13.2	13.0	25.0
20 <i>A. mengdingensis</i>	4.4	6.6	2.7	4.8	4.3	2.5	3.7	4.1	3.7	4.4	6.4	4.4	3.7	4.6	1.9	2.9	3.9	2.7	4.5	0.00 / 0.00	13.9	15.9	15.1	15.4	14.2	19.2	17.9	24.2
21 <i>A. mengyangensis</i>	5.4	7.8	5.2	6.4	5.4	4.4	4.6	5.6	4.1	5.0	7.5	5.4	5.1	5.2	2.6	2.0	5.3	0.8	5.1	3.6	0.16 / 0.35	10.9	11.0	11.2	7.4	15.2	14.3	23.0
22 <i>A. monticola</i>	7.2	5.8	8.6	7.4	6.1	8.4	7.3	7.9	7.4	7.7	10.8	7.9	8.6	8.5	7.0	8.4	7.4	7.4	6.1	7.5	8.3	—	9.3	8.3	10.2	15.4	13.1	23.7
23 <i>A. nyingchiensis</i>	2.6	5.9	6.2	4.7	3.1	5.6	1.9	1.2	1.5	4.2	7.2	5.1	4.8	4.9	3.3	4.4	1.7	4.1	2.3	4.2	5.1	7.4	0.00 / 0.12	8.5	11.4	13.9	12.6	23.6
24 <i>A. putaoensis</i>	2.8	5.4	6.8	4.4	2.9	5.2	2.5	2.7	2.2	4.4	7.3	4.9	4.6	4.5	3.9	4.6	2.4	4.4	2.2	4.8	5.2	7.0	2.1	0.00 / 0.18	11.5	13.0	13.5	24.1
25 <i>A. tuanjieensis</i>	3.8	7.1	4.1	4.7	3.9	3.8	3.0	3.4	2.6	4.1	5.9	4.3	4.6	4.7	2.0	2.6	2.6	2.8	3.7	2.4	3.7	7.9	3.0	3.5	0.57 / 0.71	13.9	15.7	21.6
26 <i>A. vitreus</i>	4.8	6.1	5.1	6.4	5.1	5.7	5.3	5.3	4.5	5.2	6.3	4.8	2.9	3.4	4.7	5.3	5.2	4.7	4.5	4.3	5.9	8.6	5.4	5.4	4.8	—	11.0	24.5
27 <i>A. wenshanensis</i>	4.7	7.3	6.0	6.4	5.3	4.6	4.2	4.6	4.2	4.6	6.9	5.4	2.4	2.9	4.2	4.8	4.4	4.6	5.1	3.6	5.7	8.6	4.7	4.1	4.6	4.6	0.13 / 0.59	25.6
28 <i>A. rickettii</i>	10.6	13.6	9.7	11.7	9.5	10.6	9.8	9.7	9.3	8.8	9.9	7.7	8.8	9.1	9.1	8.9	9.4	8.2	9.5	8.8	10.8	14.6	10.1	10.4	9.2	9.7	9.0	0.00 / 0.53

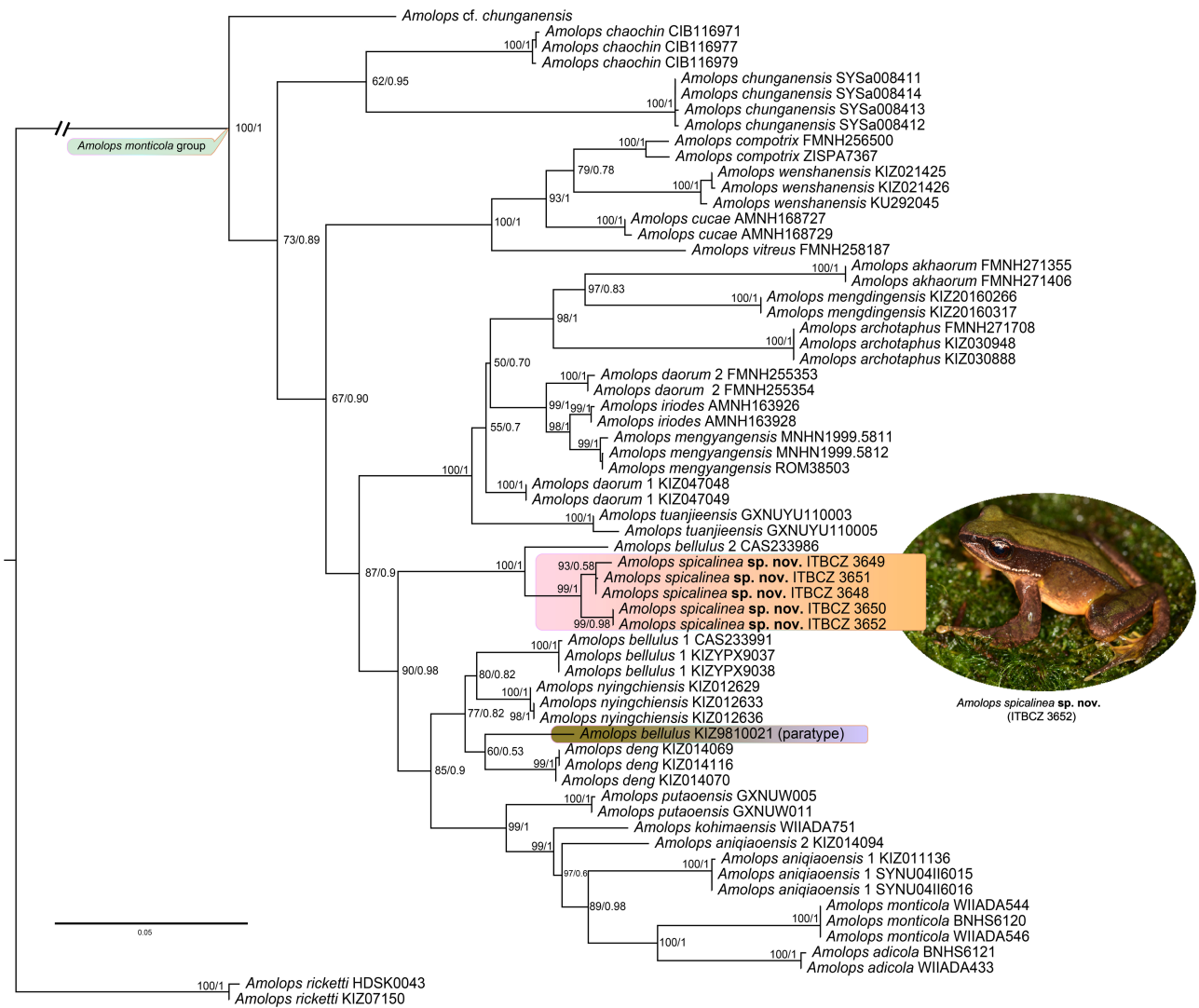


FIGURE 2. Fifty percent majority-rule consensus phylogram resulting from Bayesian analysis of the mitochondrial 16S rRNA and COI genes of *Amolops monticola* group and two outgroups (*Amolops ricketti*). Numbers at nodes are Ultrafast bootstrap (UFB) support values and Bayesian posterior probabilities (BPP) respectively, and numbers at terminal tips are Voucher numbers with details shown in Table 1.

Taxonomic conclusion

Our data demonstrate concordant differences in mtDNA sequences and morphology. The range of *Amolops* sp. is isolated by breaks of low elevation habitat below 2000 m asl at least 10 km from all other species in the *Amolops monticola* group with the exception of *A. daorum* which has a type locality approximately 9 km away from the nearest collection site of *Amolops* sp. *Amolops* sp. is morphologically most similar to *Amolops bellulus* but the latter does not have circummarginal grooves on Finger I or white horny spinules running alongside the lower edge of the dorsolateral fold in males, around the cloaca, tympanic region, or lips in life, all of which are present in *Amolops* sp.. *Amolops* sp is sister to *Amolops bellulus* in the mtDNA 16S dataset; the type locality for *A. bellulus* is approximately 670 km to the northwest of sites where *Amolops* sp. were collected and separated by several areas of unsuitable low elevation habitat (Fig. 1). These three lines of evidence suggest that *Amolops* sp. from the Hoang Lien Range, Lao Cai Province, Vietnam clearly represents a new species to science, which we describe below:

Amolops spicalinea sp. nov.

Holotype. Adult male (ITBCZ 3648; field tag FAN50) found on a tree branch beside a 2.0 m wide, fast flowing, clear, rocky stream in a patch of primary forest dominated by the endemic subspecies of Fansipan fir (*Abies delavayi fansipanensis* (Q.P.Xiang) Rushforth) on Mount Fansipan, Sa Pa District, Lao Cai Province, Vietnam (N22.29595, E103.80435; 2493 m asl). Collected at 20:30h on 15th September 2018 by Luan Thanh Nguyen, Chung Thanh Nguyen, and Hoang Van Luong.

Paratypes. One adult male (ITBCZ 3649; field tag FAN103) and two adult females (ITBCZ 3650 and ITBCZ 3651; field tags FAN105 and FAN106, respectively) found 0.5–2.0 m from the ground on tree branches alongside the bank of a 3 m wide, fast flowing, clear water, rocky stream in a patch of primary forest on Mount Pu Ta Leng, Bat Xat District, Lao Cai Province, Vietnam (N22.42634, E103.61299; 2426 m asl). All three specimens were collected on 3rd October 2019 by Luan Thanh Nguyen and Luong Van Nong; one adult male (ITBCZ 3652; field tag LNT2976) found on branch of tree, 1.5 m above a fast flowing, clear water, rocky stream in a patch of primary forest on Mount Nam Kang Ho Tao, Sa Pa District, Lao Cai Province, Vietnam (N22.12860, E103.96222; 2288 m asl), collected on 13th September 2020 by Luan Thanh Nguyen, Hoang Truong Giang, and Tan A Quay.

Referred specimen. One adult female HLNP2019 1003 00029 (field tag FAN104) found 0.5–2.0 m from the ground on tree branches alongside the bank of a 3 m wide, fast flowing, clear water, rocky stream in a patch of primary forest on Mount Pu Ta Leng, Bat Xat District, Lao Cai Province, Vietnam (N22.42634, E103.61299; 2426 m asl) on 3rd October 2019 by Luan Thanh Nguyen and Luong Van Nong. This specimen is not included in the type series due to it being deposited in a local collection. The taxonomic identity of the specimen is not in question.

Etymology: Specific epithet “*spicalinea*” from *spica*, the Latin word for a point or ear of grain and *lineata*, the Latin noun meaning line or thread in reference to the line of spinules running along the body, below the dorsolateral fold in males. We suggest common name as Hoang Lien cascade frog (English) andẾch bảm đá hoàng liên sơn (Vietnamese).

Diagnosis. *Amolops spicalinea* sp. nov. is placed within the *Amolops monticola* group based on the following morphological characters: Finger I shorter than Finger II; true dorsolateral folds present (not formed by incomplete series of glands); skin smooth; lateral sides of head dark, with light-colored upper lip stripe extending to shoulder (Jiang *et al.* 2021; Patel *et al.* 2021; Stuart *et al.* 2010; Wu *et al.* 2020). The new species can be diagnosed from congeneric species by the combination of the following characters: (1) SVL of adult males 46.6–52.2 mm, $N=3$; adult females 60.1–63.0 mm, $N=3$; (2) vomerine teeth in two oblique rows between choanae; (3) tympanic annulus visible; (4) all finger and toe tips expanded to discs with circummarginal grooves present; (5) skin smooth, except for lateral surfaces of head, below the dorsolateral fold, and the area surrounding cloaca where the texture varies from tiny spinules to large tubercles; (6) dorsolateral fold present with horny spinules along the lower half in males; (7) creamy-white lip stripe extends from the tip of the snout terminating in a broken, rugose line above axilla; (8) dorsum reddish brown or greenish brown with numerous small black spots; flank yellowish brown to dark brown; ventral surface orange-yellow with brown spots present on chest and throat; (9) interdigital finger webbing absent; toes fully webbed to discs except on Toes IV where the webbing reaches the discs as a fringe, webbing formula: I0–0II0–0III0–1⁺IV1⁺–0V; (10) outer metatarsal tubercle absent; (11) males vocal sacs absent, and (12) nuptial pad velvety without spines.

Description of holotype. Figs. 3 & 4. A mature male, SVL 48.1 mm; habitus robust and stocky; head slightly shorter than wide (HDL 95% HDW); snout short (SNT 47% of HDL), pointed in dorsal view, broadly rounded in profile, protruding beyond margin of lower jaw; eye prominent (EYE 80% of SNT), eyelid broader than interorbital distance; pupil broadly horizontal oval; top of head flat; distinct canthus rostralis; loreal region weakly concave; nares vertical, located slightly closer to snout than eye; tympanum round, distinctly visible, small, and vertical, (TMP 30% of EYE), separated from eye by distance nearly equal to tympanum diameter; pineal ocellus present on middle line connecting anterior corners of orbits; choanae rounded; vomerine dentigerous processes weakly developed, located between the choanae, closer to each other than to choanae; tongue cordiform, distinctly notched posteriorly, free for approximately one-third its length; vocal sac absent.

Forearms very robust; fingers moderately short, HND 14.5 mm, relative lengths of Fingers I < II < IV < III; no interdigital webbing or lateral fringes on fingers; discs on tips of fingers rounded, greatly expanded, except for Finger I which is small and rounded, relative finger disc width I<II<III<IV; width of Fingers III 2.8 mm, 156% of TMP; ventral circummarginal grooves present on all finger discs (Fig 3); subarticular tubercles strongly developed, oval in shape; formula: 1:1:2:2 (given for Fingers I:II:III:IV, respectively); supernumerary tubercles small, rounded

and close to first subarticular tubercle of each finger; two palmar tubercles present, outer elongated, inner rounded, small; well-developed thenar tubercle, covered by a velvety nuptial pad along base of Finger I, and extending along inner edges to intercalary cartilages; nuptial spines absent.

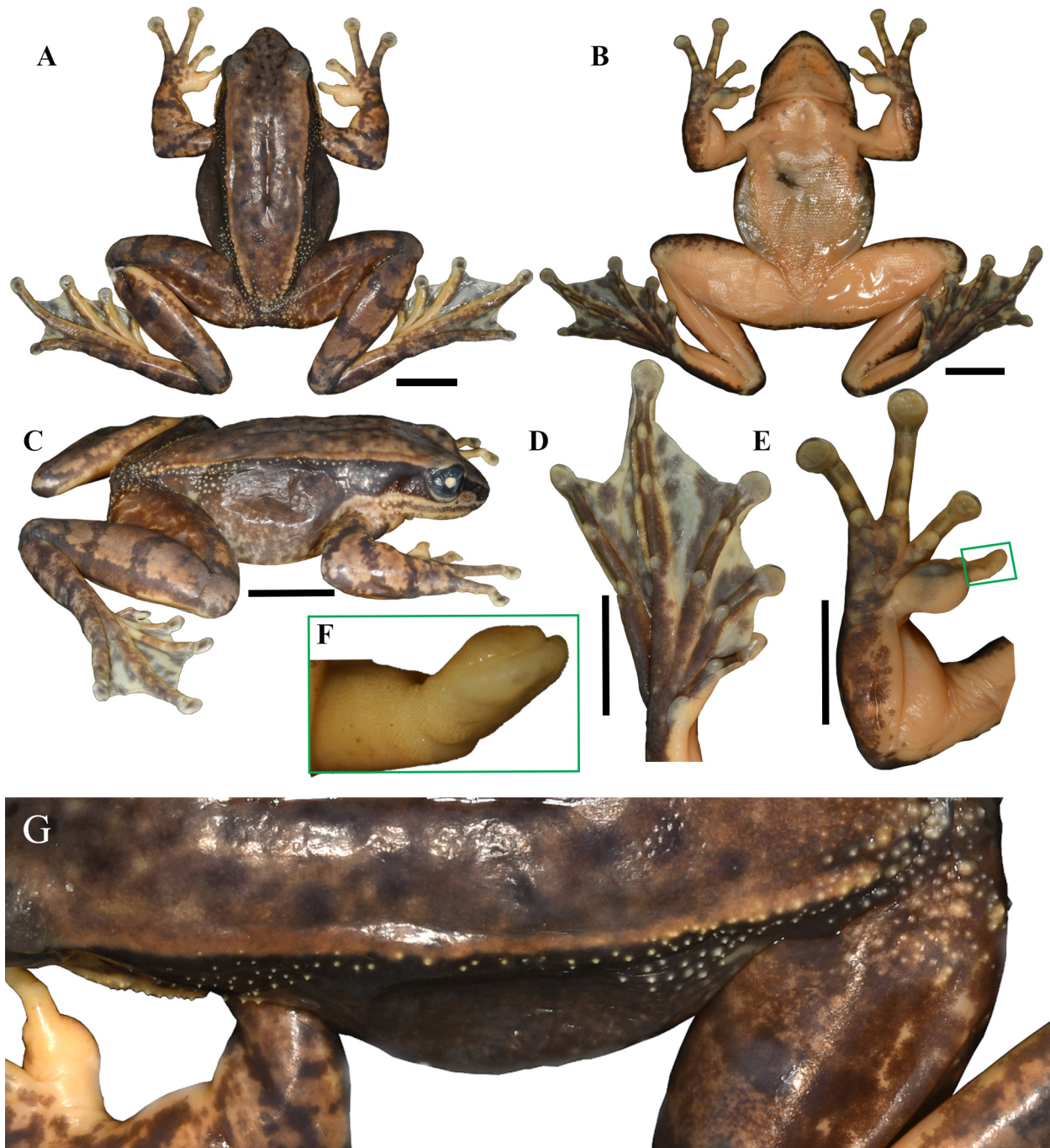


FIGURE 3. *Amolops spicalinea* sp. nov. in preservative, adult male holotype ITBCZ 3648. (A) Dorsal view, (B) ventral view, (C) lateral view, (D) plantar aspect of right foot, (E) palmar aspect of right hand, (F) close up of Finger I of right hand, and (G) close up of dorsal surface highlighting the horny white spinules. 10 mm scale bar except for F & G which are not to scale.

Hindlimbs robust; tibia length greater than half of snout-vent length (TIB 63% of SVL), and greater in length than thigh length (FTL 55% of SVL); tibiotarsal articulation of adpressed limb reaching far beyond snout; heels overlapping when thighs and tibiae are placed at right angles to body; relative toe lengths $I < II < III < V < IV$; inner tarsal fold absent; toes fully webbed to base of toe disc except on Toe IV where webbing reaches distal to the 3rd subarticular tubercle before joining terminal disc as a fringe, webbing formula: $I0-0II0-0III0-1^+IV1^+-0V$; toes long, slender, with large, rounded discs, disc width of Toe IV slightly greater than tympanum (2.4 mm in width,

133% TMP), toe discs smaller than finger discs, with ventral circummarginal groove; subarticular tubercles oval, formula: 1:1:2:3:2 (for toes I:II:III:IV:V, respectively), supernumerary tubercles absent; outer metatarsal tubercles absent, inner metatarsal tubercles well developed, oval in shape, 2.6 mm in length.

Skin on head, dorsum, flanks, and venter smooth; supratympanic folds indistinct; dorsolateral folds present, thick and flattened with no distinct apex; spinules present on lateral surfaces of head, above insertion of arm and around tympanum and on surface of tympanum; upper and lower surfaces lips with tiny spinules; lower edge of dorsolateral folds bordered by a line of horny spinules, especially in anterior and posterior parts; skin around cloaca and base of thigh with numerous tubercles; limbs smooth on dorsal and ventral surfaces; humeral glands absent. A line of small sharp tubercles present running from posterior margin of mandible to axillary region (Fig. 3).

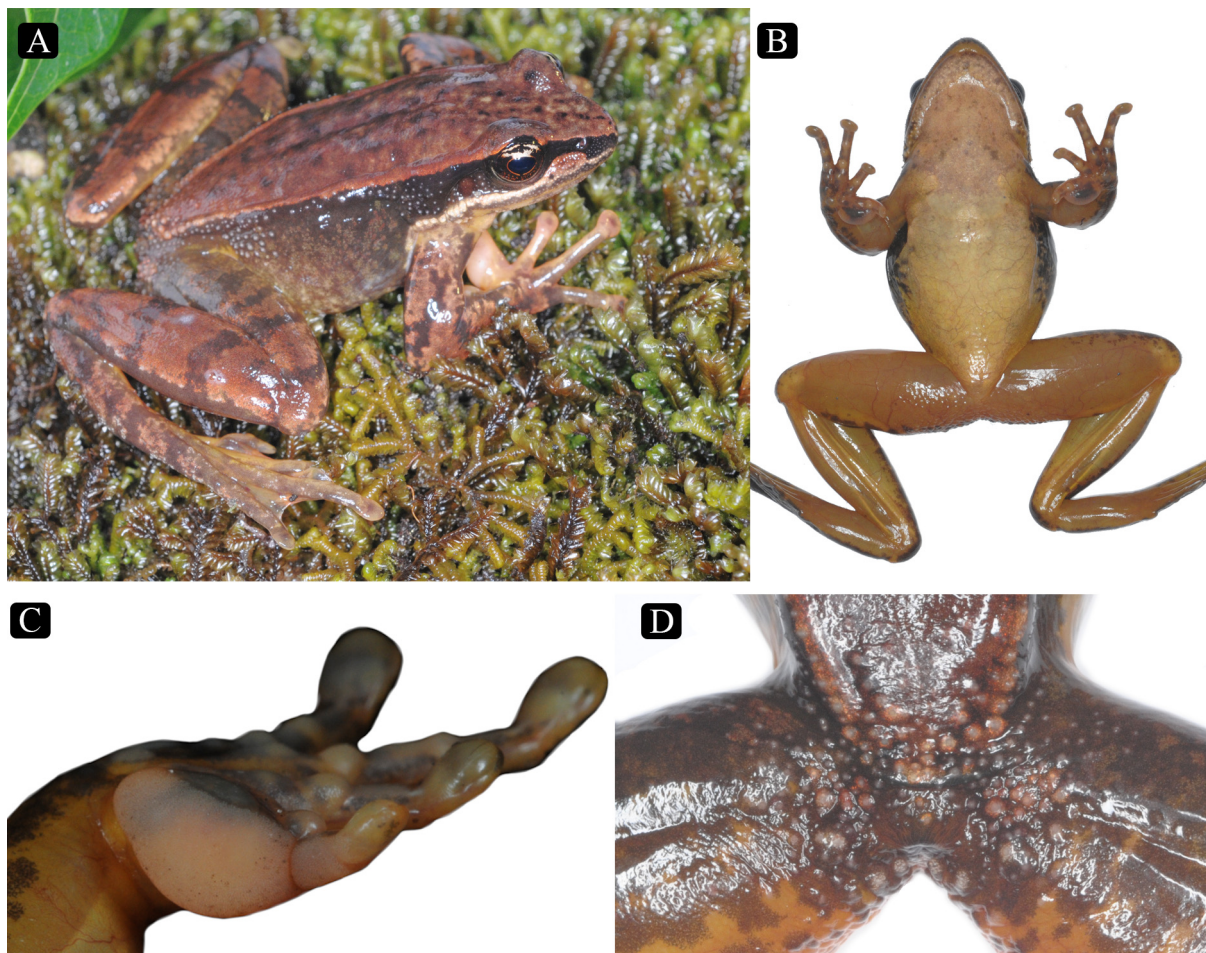


FIGURE 4. *Amolops spicalinea* sp. nov. adult male holotype ITBCZ 3648. (A) Dorsolateral view in life, (B) ventral view under sedation, (C) view of nuptial pad on first right finger under sedation, and (D), view of cloaca and base of thighs to illustrate glandular character under sedation. Not to scale.

Color of holotype in life: Dorsal surfaces of head and body reddish brown with numerous small black spots; lateral surfaces of head very dark brown except for reddish brown loreal region; a creamy-white lip stripe extends from area below and posterior to nares to form a broken line above axilla; dorsolateral folds reddish brown on upper surface and very dark brown on lower surface; flanks yellowish brown, becoming increasingly darker brown near the forelimb; groin yellow; throat orange-yellow with some small brown spots; chest, belly, and ventral surfaces of limbs orange-yellow; dorsal surfaces of limbs reddish brown with dark brown transverse bars, posterior surfaces of thigh dark brown with yellowish reticulations; ventral surfaces of fingers and toes orange-yellow with brown blotches; interdigital toe webbing light yellow with brown marbling; nuptial pad creamy; iris bicoloured, upper half golden yellow with black reticulations, lower half red with black reticulations; tiny spinules and glandular tubercles on lips, dorsolateral folds, and around cloaca white (Fig. 4).

Colour of holotype in preservative. surface of head and body, dark brown; flanks dark brown; ventral surfaces

light orange; yellowish brown lip stripe; dorsal surfaces of limbs brown with brown transverse bars more visible than in life; whitish brown interdigital webbing with dark brown blotches; eye black with creamy-white iris (Fig. 3).

Variation. Morphometric measurements of the type series are shown in Table 3 and representative photographs of paratypes and referred specimen in life are shown in Fig. 5. Species varies in body size and colouration. Paratypes have fewer glandular tubercles on lower part of dorsolateral fold than holotype; all paratypes are brighter in colour relative to holotype, especially on flanks. All specimens with small white spinules on the tympanum except female ITBCZ 3651. Male ITBCZ 3649 has yellowish green dorsum without black spots; females HLNP2019 1003 00029 and ITBCZ 3650 only have small black spots on dorsal surfaces of head; ventral surfaces of all paratype specimens with black blotches on throat and chest, black throat blotches absent in holotype.

TABLE 3. Morphometric measurements (in mm) of *Amolops spicalinea* sp. nov. Abbreviations are defined in the Material and methods. HT = holotype; PR = paratype; RS = referred specimen.

Museum tag	ITBCZ 3648	ITBCZ 3649	ITBCZ 3652		ITBCZ 3650	HLNP2019 1003 00029	ITBCZ 3651	
	HT	PR	PR		PR	RS	PR	
Field tag	FAN50	FAN103	NT2976		FAN105	FAN104	FAN106	
Sex	Male	Male	Male	Mean (Min–Max)	Female	Female	Female	Mean (Min–Max)
SVL	48.1	46.6	52.2	49.0 (46.6–52.2)	62.1	60.1	63.0	61.73 (60.10–63.00)
HDL	16.1	15.5	18.5	16.7 (15.5–18.5)	22.3	23.9	22.5	22.90 (22.30–23.90)
HDW	17.0	15.6	17.7	16.8 (15.6–17.7)	21.5	20.9	21.7	21.37 (20.90–21.70)
IND	5.7	5.7	6.8	6.1 (5.7–6.8)	8.0	6.8	7.8	7.53 (6.80–8.00)
SNT	7.5	7.0	7.5	7.3 (7.0–7.5)	8.6	9.8	9.5	9.30 (8.60–9.80)
EYE	6.0	8.6	6.0	6.9 (6.0–8.6)	7.5	6.4	6.7	6.87 (6.40–7.50)
NS	3.7	4.9	4.7	4.4 (3.7–4.9)	5.2	5.1	4.8	5.03 (4.80–5.20)
EN	3.8	2.1	2.8	2.9 (2.1–3.8)	3.4	4.7	4.7	4.27 (3.4–4.7)
IOD	4.2	5.0	6.4	5.2 (4.2–6.4)	6.0	5.6	5.0	5.53 (5.00–6.00)
TMP	1.8	2.0	2.0	1.9 (1.8–2.0)	2.0	2.5	2.2	2.23 (2.00–2.50)
TEY	1.9	1.5	2.1	1.8 (1.5–2.1)	1.7	2.7	2.8	2.40 (1.70–2.80)
TL	28.5	27.3	27.1	27.6 (27.1–28.5)	33.0	34.0	34.5	33.83 (33.00–34.50)
TIB	30.2	29.5	30.3	30.0 (29.5–30.3)	36.4	36.1	38.6	37.03 (36.10–38.60)
HND	14.5	14.4	14.7	14.5 (14.4–14.7)	19.3	19.2	19.6	19.37 (19.20–19.60)
FTL	26.4	27.4	27.2	27.0 (26.4–27.4)	33.7	33.0	35.0	33.90 (33.00–35.00)
F3DSC	2.8	2.4	2.5	2.6 (2.4–2.8)	3.0	2.7	3.0	2.90 (2.70–3.00)
T4DSC	2.4	2.0	2.5	2.3 (2.0–2.5)	2.5	2.4	2.7	2.53 (60.10–63.00)
TMP/ SVL %	3.7	4.2	3.8		3.2	4.2	3.5	3.76 (3.2–4.2)

Secondary sexual characters. Three males with a creamy coloured, velvety nuptial pad along base of Finger I (Figs. 3 and 4). Three females gravid with yellow, rounded eggs about 2.7–3.0 mm in diameter (10 eggs measured, eggs uncounted). The presence of white spinules on the dorsolateral fold, around cloaca, and on lips in life are present only in males, which indicates that these are secondary sexual character (Sung *et al.* 2016). Body size of females greater than body size of males (male SVL 60.1–63.0 mm, $N=3$, versus female SVL 46.6–52.2 mm, $N=3$).

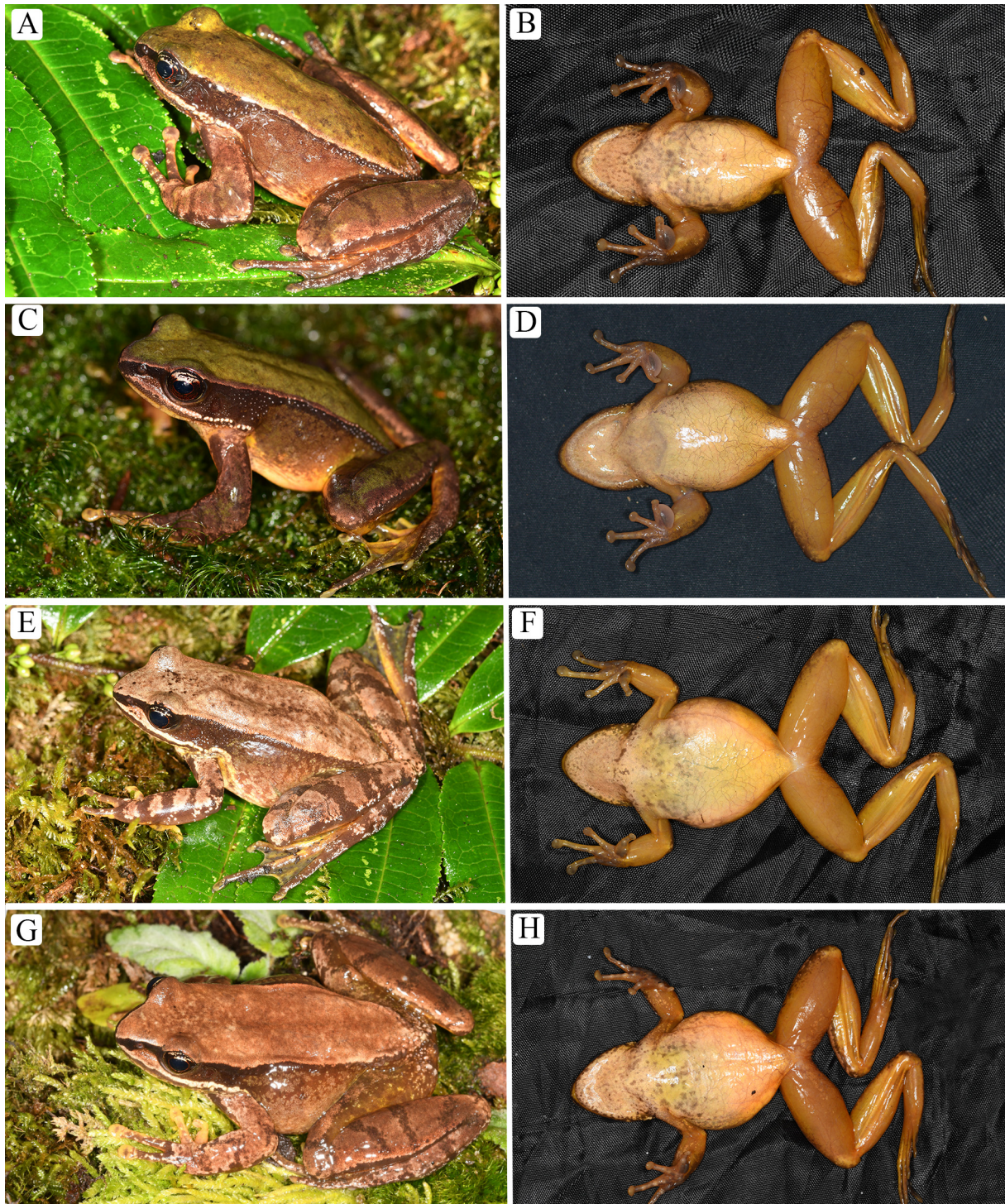


FIGURE 5. Dorsolateral and ventral view of paratypes of *Amolops spicalinea* **sp. nov.** in life. (A and B) adult male ITBCZ 3649, (C and D) adult male ITBCZ 3652, (E and F) adult female ITBCZ 3650, and (G and H) adult female ITBCZ 3651.

Natural history. *Amolops spicalinea* **sp. nov.** were encountered in relatively undisturbed primary forest with a relatively closed canopy (Fig. 6). On Mount Fansipan, the type locality is dominated by the endemic subspecies of Fansipan fir and *Rhododendron* forest. All individuals have been found at night, perched 0.5–2.0 m above streams on tree branches, in riparian habitat along clear, fast-moving water, along 2–3 m wide streams. All male specimens collected in late September and October had nuptial pads and females were gravid. *Amolops spicalinea* **sp. nov.** were not heard calling. No individuals of this species were encountered during surveys at the same locations on Mount Fansipan and Mount Pu Ta Leng in March 2018; June 2018; April 2019, and June 2019. *Amolops spicalinea*

sp. nov. is sympatric with *A. minutus* Orlov and Ho on Mount Fansipan. On Mount Pu Ta Leng, this species is sympatric with *A. minutus* and *A. viridimaculatus* (Jiang). On Mount Nam Kang Ho Tao this species is sympatric with *A. minutus* and *Amolops daorum*. The tadpole and breeding behaviour of this species were not observed.



FIGURE 6. Habitat of *Amolops spicalinea* **sp. nov.** in the Hoang Lien Range. (A) Collection site on Mount Nam Kang Ho Tao, Lao Cai Province, (B) Collection site on Mount Pu Ta Leng, Lao Cai Province, (C) Male *Amolops spicalinea* **sp. nov.** *in situ* ITBCZ 3649 on tree branch, 2.0 m above stream, Mount Pu Ta Leng, Lao Cai Province.

Distribution and conservation status: This new species is currently known from between 2288–2493 m asl at three localities in Lao Cai Province, up to 50 km apart in the Hoang Lien Range (Mount Nam Kang Ho Tao, Mount Fansipan and Mount Pu Ta Leng). It is likely that the new species also occurs in neighbouring Lai Chau Province as the collection sites are close to the Lao Cai-Lai Chau provincial borders. Based upon current data, the species' EOO is predicted to 1917 km² (Fig. 1). The habitat of this species in all three locations is relatively intact and forest clearance for agriculture was not observed. However, at elevations below 2000 m asl, forest is being degraded, particularly in riparian habitat as a result of cardamom plantations. On both Mount Fansipan and Mount Pu Ta Leng, the forest in which this species occurs is being degraded by fuelwood collection for the tourism industry. Tourist camps are located just 500 m from the streams where this species was recorded on Mount Fansipan and Mount Pu Ta Leng. On Mount Fansipan there are ongoing infrastructural developments for tourism in close proximity to where this species occurs which has resulted in localised forest clearance and the discharge of effluent from accommodation for hikers. The fungal pathogen, *Batrachochytrium dendrobatidis*, was not detected from 17 samples collected from

seven different anuran amphibian species at the type locality on Mount Fansipan in September 2017; including one sample from *Amolops spicalinea* **sp. nov.** (reported as *Amolops* sp. 2; Tapley *et al.* 2020a). Given the relatively small range and ongoing habitat loss and modification, this species qualifies for being assessed as Endangered in accordance with the IUCN Red List of Threatened Species categories and criteria (B1ab; see IUCN 2012).

Comparisons

We follow Jiang *et al.* (2021); Patel *et al.* (2021); and Stuart *et al.* (2010) by comparing the new species with 25 members of the *Amolops monticola* group. *Amolops spicalinea* **sp. nov.** can be distinguished from all species in the *Amolops monticola* group on the basis of morphology and for species where comparable sequences are available, molecular data. The following comparisons are based on three adult males and three adult females of *Amolops spicalinea* **sp. nov.** Comparisons between the new species and other species in the *Amolops monticola* group are summarised Table 4.

Amolops spicalinea **sp. nov.** is genetically most similar to *A. bellulus*, but it differs from *A. bellulus* by its stocky habitus (vs. habitus moderately depressed and slender in *A. bellulus*), presence of circummarginal grooves on Finger I (vs. absent in *A. bellulus*), presence of white spinules on the dorsolateral fold, around cloaca, tympanic region, and on lips in males, in life (vs. absent in *A. bellulus*), and flanks yellowish brown to dark brown (vs. flanks bluish green to olive green in *A. bellulus*).

Amolops spicalinea **sp. nov.** differs from *A. adicola* by tympanic annulus visible (vs. indistinct in *A. adicola*), absence of external subgular vocal sacs in males (vs. present in *A. adicola*), and dorsolateral folds with white horny spinules alongside the lower edge in males (vs. without spinules in *A. adicola*).

Amolops spicalinea **sp. nov.** differs from *A. akhaorum* by its larger adult male body size, 46.6–52.2 mm, $N=3$, (vs. male 34.9–37.2 mm, $N=9$, in *A. akhaorum*), stocky habitus (vs. slender in *A. akhaorum*), dorsum olive green to reddish brown without large black spots (vs. dorsum green with large, dark black spots in *A. akhaorum*), skin on dorsal body surface smooth with spinules (vs. smooth with glandular tubercles in *A. akhaorum*), dorsolateral folds with white horny spinules alongside the lower edge in males (vs. glandular dorsolateral folds without spinules *A. akhaorum*), absence of subgular vocal sacs in males (vs. present in *A. akhaorum*), and interdigital webbing less developed, reaching to below on Toe IV (vs. reaching disc on Toe IV in *A. akhaorum*).

Amolops spicalinea **sp. nov.** differs from *A. aniqiaoensis* by its smaller adult female body size, 60.1–63.0 mm, $N=3$, (vs. 69.5–71.7 mm, $N=4$, in *A. aniqiaoensis*), absence of subgular vocal sacs in males (vs. present in *A. aniqiaoensis*), and absence of “/” shaped pattern on throat (vs. present in *A. aniqiaoensis*).

Amolops spicalinea **sp. nov.** differs from *A. archotaphus* by its larger adult male body size, 46.6–52.2 mm, $N=3$, (vs. 38.2–42.1 mm, $N=8$, in *A. archotaphus*), stocky habitus (vs. slender in *A. archotaphus*), dorsolateral folds with white horny spinules alongside the lower edge in males (vs. dorsolateral folds without spinules in *A. archotaphus*), presence of circummarginal groove on Finger I (vs. absent in *A. archotaphus*), absence of subgular vocal sacs in males (vs. present in *A. archotaphus*), and absence of outer metatarsal tubercles (vs. present in *A. archotaphus*).

Amolops spicalinea **sp. nov.** differs from *A. chakrataensis* by its larger adult female body size, 60.1–63.0 mm, $N=3$, (vs. 55 mm, $N=1$, in *A. chakrataensis*), snout rounded in dorsal view (vs. snout pointed in *A. chakrataensis*), and small tubercles forming a glandular line in axilla region (vs. a single, large rounded axillary gland in *A. chakrataensis*).

Amolops spicalinea **sp. nov.** differs from *A. chaochin* by its larger adult body size, male 46.6–52.2 mm, $N=3$, female 60.1–63.0 mm, $N=3$, (vs. males 35.3–39.2, $N=7$, female 50.5–54.4, $N=7$, in *A. chaochin*), body stocky (vs. slender in *A. chaochin*), absence of subgular vocal sacs in males (vs. present in male *A. chaochin*), absence of outer metatarsal tubercles (vs. present in *A. chaochin*), and presence of tiny spinules on lips and lores in males (vs. absent in *A. chaochin*).

Amolops spicalinea **sp. nov.** differs from *A. chunganensis* by its larger body size, males 46.6–52.2 mm, $N=3$, females 60.1–63.0 mm, $N=3$, (vs. male 37.2–41.5 mm, $N=5$, females 52.6 mm, $N=1$, in *A. chunganensis*), absence of subgular vocal sacs in males (vs. present in male *A. chunganensis*), and dorsolateral folds with white spinules alongside the lower edge and in a cluster on the temporal region, tympanic region, and lips in adult males (vs. absent in *A. chunganensis*).

TABLE 4. Comparison of major diagnostic characters among the 22 described species in the *Amolops monticola* group with *Amolops spicalinea* sp. nov., modified from Patel *et al.* (2021). Abbreviations/definitions for characters are as follows: SVL: snout-vent length (in mm); N: number of specimens (NR=Not Reported); Finger I groove: circummarginal groove on Finger I present (+) or absent (-); Fold: supratympanic fold distinct (+) or indistinct (-); Vocal sac: two gular pouches on either side of the throat in mature males present (+) or absent (-) and Nuptial pad: presence of nuptial pad on Finger I (+) or absence (-); OMT: outer metatarsal tubercle present (+) or absent (-); Flank gland: white gland present (+) or absent (-); Web: extent of webbing on feet (toe IV webbing up to the disc = D, toe IV webbing below the disc = B); Dorsal skin (Dor. skin): dorsal skin texture, S = smooth, ST = smooth with glandular tubercles, Sh = shagreened, G = granular, Sp. = spinular; Data sources Biju *et al.* (2010), Jiang *et al.* (2021), Gan *et al.* (2020b) Patel *et al.* (2021), Pham *et al.* (2023), Yu *et al.* (2019) and this study. Grey shading indicates non-overlapping characters when compared to *Amolops spicalinea* sp. nov.

Taxon	Male S. Sex										Spinules in males			
	SVL ♂	N	SVL ♀	N	Finger I groove	Fold	Vocal Sac	Nuptial pad	OMT	Flank gland	Web	Dor. skin	On lips, lores and tympanum, and dorsolateral folds	In a line below
1 <i>Amolops spicalinea</i> sp. nov.	46.6-52.2	3	60.1-63.0	3	+	-	-	+	-	-	B	S,Sp.	+	+
2 <i>A. adicola</i>	44.0-47.0	3	62.0-72.0	3	+	-	+	+	-	-	B	S	-	-
3 <i>A. akhaorum</i>	34.9-37.2	9	58.8-62.5	NR	+	-	+	-	-	-	D	ST	-	-
4 <i>A. aniqiaoensis</i>	51.6	1	69.5-71.7	4	+	-	+	+	-	-	B	S, Sp	NR	+
5 <i>A. archotaphus</i>	38.2-42.1	8	58.8-62.5	2	-	-	+	+	+	-	B	S, Sp	NR	-
6 <i>A. bellulus</i>	45.9-50.1	3	63.6	1	-	-	-	+	-	-	B	S	-	-
7 <i>A. chakrataensis</i>	NR	NR	55	1	+	-	-	+	-	-	B	S	-	-
8 <i>A. chaochin</i>	35.3-39.2	7	50.5-54.4	7	+	-	+	+	+	-	B	S	-	+
9 <i>A. chunganensis</i>	37.2-41.5	5	52.6	1	+	-	+	+	-	-	B	S	-	-
10 <i>A. compotrix</i>	31.4-42.6	10	55.6-56.9	3	+	-	+	+	+	-	B	S	-	-
11 <i>A. cucae</i>	40.7-44.6	4	65.8-68.0	4	+	-	+	+	+	-	B	G,Sp.	-	-
12 <i>A. daorum</i>	32.0-38.1	8	53.3-57.6	8	+	-, +	+	+	-	+	B	G,Sp.	+	+
13 <i>A. deng</i>	50.3-57.6	9	68.5-72.0	3	+	+	-	+	-	-	B	S	-	-
14 <i>A. gerbillus</i>	43.0-48.0	NR	80	1	+	+	+	+	-	-	D	G,Sp.	-	NR
15 <i>A. iriodes</i>	38.8-39.4	2	61.9	1	NR	-	+	-	-	+	B	S	-	-
16 <i>A. kohimaensis</i>	43.0-49.0	5	59	1	+	+	+	+	-	-	B	S,Sp.	+	+
17 <i>A. mengdingensis</i>	36.9-40.2	6	64.3	1	+	-	+	+	-	+	B	S,Sp.	-	-
18 <i>A. mengyangensis</i>	39.0-40.0	2	60.0	1	NR	-	+	+	NR	-	B	S	-	-
19 <i>A. monticola</i>	37.0-41.0	4	59.0-63.0	2	-, weak	-	+	+	-	-	B	S	-	-
20 <i>A. nyingchiensis</i>	52.3-58.3	3	57.6-70.7	10	+	-	-	+	-	-	B	S	-	-
21 <i>A. putaoensis</i>	37.6-40.2	3	NR	NR	+	+	+	+	-	-	B	S	-	-
22 <i>A. truongi</i>	37.5-41.3	7	61.5-62.5	3	-	-	+	+	-	-	B	S	-	-
23 <i>A. tuanjiensis</i>	39.5-40.4	3	56.8-60.7	2	+	-	+	+	-	+	B	S	-	-
24 <i>A. vitreus</i>	37.5-43.6	7	NR	NR	+	-	+	+	+	-	B	S	-	-
25 <i>A. wenshanensis</i>	35.7-39.9	7	43.7-45.6	2	+	-	+	+	-	-	B	S	-	-

Amolops spicalinea **sp. nov.** differs from *A. compotrix* by its larger adult body size, male 46.6–52.2 mm, $N=3$, female 60.1–63.0 mm, $N=3$, (vs. male 31.4–42.6 mm, $N=10$, female 55.6–56.9 mm, $N=3$, in *A. compotrix*), body stocky (vs. body slender in *A. compotrix*), absence of subgular vocal sacs in males (vs. present in *A. compotrix*), absence of outer metatarsal tubercles (vs. present in *A. compotrix*), and dorsolateral folds with white spinules alongside the lower edge and in a cluster on the temporal region, tympanic region, and lips in adult males (vs. absent in *A. compotrix*).

Amolops spicalinea **sp. nov.** differs from *A. cucae* by its larger adult male body size (46.6–52.2 mm, $N=3$, vs. 40.7–44.6 mm, $N=4$, in *A. cucae*), smaller adult female body size (60.1–63.0 mm, $N=3$, vs. 65.8–68.0 mm, $N=4$, in *A. cucae*), stocky habitus (vs. slender in *A. cucae*), dorsum olive green or reddish brown (vs. light green in *A. cucae*), absence of outer metatarsal tubercle (vs. present in *A. cucae*), and dorsolateral folds with white spinules alongside the lower edge and in a cluster on the temporal region, tympanic region, and lips in adult males (vs. absent in *A. cucae*).

Amolops spicalinea **sp. nov.** differs from *A. daorum* by its larger adult body size, males 46.6–52.2 mm, $N=3$, females 60.1–63.0 mm, $N=3$, (vs. males 32.0–38.1 mm, $N=8$, females 53.3–57.6 mm, $N=8$, in *A. daorum*), stocky habitus (vs. slender in *A. daorum*), dorsum olive green or reddish brown (vs. green in *A. daorum*), absence of white blotches on flank (vs. present in *A. daorum*), presence of vomerine teeth (vs. absent in *A. daorum*), and absence of subgular vocal sacs in males (vs. present in *A. daorum*).

Amolops spicalinea **sp. nov.** differs from *A. deng* by its smaller adult female body size 60.1–63.0 mm, $N=3$, (vs. 68.5–72.0 mm, $N=3$, in *A. deng*), absence of patch of grayish white microgranules on chest in males (vs. present in *A. deng*), and dorsolateral folds with white spinules alongside the lower edge and in a cluster on the temporal region, tympanic region, and lips in adult males (vs. absent in *A. deng*).

Amolops spicalinea **sp. nov.** differs from *A. gerbillus* by its smaller adult female body size (60.1–63.0 mm, $N=3$, vs. 80.0 mm, $N=1$, in *A. gerbillus*), stocky habitus (vs. slender in *A. gerbillus*), absence of large black spots on dorsal and ventral surfaces (vs. present in *A. gerbillus*), skin on dorsal body surface smooth with spinules (vs. granular in *A. gerbillus*), absence of subgular vocal sacs in males (vs. present in *A. gerbillus*), and interdigital webbing less developed, reaching to below on Toe IV (vs. reaching disc on Toe IV in *A. gerbillus*).

Amolops spicalinea **sp. nov.** differs from *A. iriodes* by its larger adult male body size 46.6–52.2 mm, $N=3$, (vs. 38.8–39.4 mm, $N=2$, in *A. iriodes*), stocky habitus (vs. slender in *A. iriodes*), dorsum olive green or reddish brown (vs. iridescent green in *A. iriodes*), absence of white glandular spots on flank (vs. present in *A. iriodes*), and dorsolateral folds with white spinules alongside the lower edge and in a cluster on the temporal region, tympanic region, and lip in adult males (vs. absent in *A. iriodes*), and absence of subgular vocal sacs in males (vs. present in *A. iriodes*).

Amolops spicalinea **sp. nov.** differs from *A. kohimaensis* by absence of subgular vocal sacs in males (vs. present in male *A. kohimaensis*), and no microgranules on chest (vs. microgranules covering the entire chest in *A. kohimaensis*).

Amolops spicalinea **sp. nov.** differs from *A. mengdingensis* by its larger adult male body size (46.6–52.2 mm, $N=3$, vs. 36.9–40.2 mm, $N=6$, in *A. mengdingensis*), stocky habitus (vs. slender in *A. mengdingensis*), dorsum olive green to reddish brown (vs. light green in *A. mengdingensis*), belly orange-yellow (vs. light green in *A. mengdingensis*), absence of white spots on flank near groin (vs. present in *A. mengdingensis*), absence of subgular sacs in males (vs. present in *A. mengdingensis*), and presence of white spinules and in a cluster on the temporal region, tympanic region, and lips in adult males (vs. absent in *A. mengdingensis*).

Amolops spicalinea **sp. nov.** differs from *A. mengyangensis* by its larger adult male body size (46.6–52.2 mm, $N=3$, vs. 39.0–40.0 mm, $N=2$, in *A. mengyangensis*), absence of subgular vocal sacs in males (vs. present in *A. mengyangensis*), and more developed interdigital toe webbing, webbing reaching to disc on Toe IV (vs. webbing reaching distal subarticular tubercle on Toe IV in *A. mengyangensis*).

Amolops spicalinea **sp. nov.** differs from *A. monticola* by its larger adult male body size (46.6–52.2 mm, $N=3$, vs. 37.0–41.0 mm, $N=4$, in *A. monticola*), stocky habitus (vs. slender in *A. monticola*), dorsolateral folds with horny white spinules alongside the lower edge in males (vs. weak dorsolateral folds without spinules in *A. monticola*), and absence of subgular sacs in males (vs. present in *A. monticola*).

Amolops spicalinea **sp. nov.** differs from *A. nyingchiensis* body stocky (vs. compressed in *A. nyingchiensis*), a smaller tympanum that is on average 3.8% of SVL and width < than width of F3DSC (vs. 5.3% and approximately = F3DSC in *A. nyingchiensis*), and dorsolateral folds with horny white spinules alongside the lower edge in males (vs. dorsolateral folds without spinules in *A. nyingchiensis*).

Amolops spicalinea **sp. nov.** differs from *A. putaoensis* by its larger adult male body size (46.6–52.2 mm, $N=3$, vs. 37.6–40.2 mm, $N=3$, in *A. putaoensis*), absence of subgular sacs in males (vs. present in *A. putaoensis*), and flanks flank yellowish brown to dark brown (vs. green in *A. putaoensis*).

Amolops spicalinea **sp. nov.** differs from *Amolops truongi* by its larger adult male body size (46.6–52.2 mm, $N=3$, vs. 37.5–41.3 mm, $N=3$, in *A. truongi*), absence of subgular sacs in males (vs. present in male *A. truongi*), dorsum olive green to reddish brown (vs. light grey in *A. truongi*), and presence of circummarginal groove on Finger I (vs. absent in *A. truongi*).

Amolops spicalinea **sp. nov.** differs from *A. tuanjieensis* by its larger adult male body size (46.6–52.2 mm, $N=3$, vs. 39.5–40.4 mm, $N=3$, in *A. tuanjieensis*), dorsolateral folds with white horny spinules alongside the lower edge in males (vs. dorsolateral folds without spinules in *A. tuanjieensis*), absence of subgular sacs in males (vs. present in *A. tuanjieensis*), and flanks yellowish brown to dark brown (vs. green with black spots in *A. tuanjieensis*).

Amolops spicalinea **sp. nov.** differs from *A. vitreus* by its larger adult male body size (46.6–52.2 mm, $N=3$, vs. 37.5–43.6 mm, $N=7$, in *A. vitreus*), dorsum olive green or reddish brown with small black spots (vs. green with large black spots in *A. vitreus*), dorsolateral folds with horny white spinules alongside the lower edge in males (vs. dorsolateral folds without spinules in *A. vitreus*), absence of subgular sacs in males (vs. present in *A. vitreus*), and absence of outer metatarsal tubercle (vs. present in *A. vitreus*).

Amolops spicalinea **sp. nov.** differs from *A. wenshanensis* by its larger adult male body size (46.6–52.2 mm, $N=3$, vs. 35.7–39.9 mm, $N=7$, in *A. wenshanensis*), and larger adult female (60.1–63.0 mm, $N=3$, vs. 43.7–45.6 mm, $N=2$, in *A. wenshanensis*), stocky habitus (vs. slender in *A. wenshanensis*), olive green or reddish brown on dorsum (vs. pure green in *A. wenshanensis*), dorsolateral folds with horny white spinules alongside the lower edge in males (vs. dorsolateral folds without spinules in *A. wenshanensis*), and absence of subgular sacs in males (vs. present in *A. wenshanensis*).

Discussion

The presence of white spinules on the tympanum region, running alongside the dorsolateral folds and around the cloaca of *Amolops spicalinea* **sp. nov.** (Figs. 3A, 3C, 3G, 4A & 4D) is a character shared with very few other *Amolops*; within the *Amolops monticola* group, only five other species are reported to have this character (*A. aniqiaoensis*, *A. daorum*, *A. chaochin*, *A. kohimaensis*, and *A. mengdingensis*). This is reported as an important feature that could be used to distinguish between several species with similar morphology in this species group (e.g., between *A. daorum* and *A. iriodes* and between *A. mengyangensis*, *A. chaochin*, and *A. chunganensis*) (Stuart *et al.* 2010). *Amolops spicalinea* **sp. nov.** were only collected in September and October, the presence of these spinules was consistent in individuals in the series but as we did not observe individuals of this species at other times of year, we are unable to determine if the prominence of these spinules varies with season or reproductive status.

Amolops spicalinea **sp. nov.** is likely distributed north of known localities, into Yunnan Province, China. Suitable habitats in these areas should be surveyed, and establishing the true elevation range of this species will be a priority to inform extinction risk assessments and subsequent conservation planning. Additional research should be undertaken to ascertain whether this species vocalises, and if it does, to document and describe the call. Further research to determine the breeding habitat and to describe the larvae of this species would aid future taxonomic studies.

Nine amphibians have been described from the Hoang Lien Range as new species to science within the last decade (Matsui *et al.* 2017; Tapley *et al.* 2017, 2018, 2020b; Kropachev *et al.* 2019; Nguyen *et al.* 2021, 2024a,b). This discovery increases the number of *Amolops* reported to occur in Vietnam to 20 and recorded from the Hoang Lien Range to 11 species (Ohler *et al.* 2000; Bain *et al.* 2003, 2006; Orlov and Ho 2007; Nguyen *et al.* 2009). It is highly likely that there is further undiagnosed species diversity within morphologically cryptic groups, including the genus *Amolops*, and further genetic sampling and genomic data will likely be needed to elucidate the true species richness in the genus. This further emphasises the need to continue taxonomic research in this biodiverse mountain range.

Acknowledgements

We are grateful to the staff at Hoang Lien National Park for their assistance and collaboration. In particular we would like to thank Director Nguyen Huu Hanh for continued support and partnership. We thank Lao Cai FPD for support in arranging the office's work at Bat Xat. We thank Timothy Cutajar for sequencing specimens at the Australian Museum. We thank the Asian Turtle Program for institution support for Luan Nguyen. Duong Thi Lan, Tran Van Tu, Nguyen Manh Hung, Luong Van Hoang, Nong Vang Luong, and Hoang Truong Giang assisted with field work. We extend our thanks to the People's Committee of Lao Cai Province for supporting this programme of research. Ethical approval was granted by the Zoological Society of London's ethics committee (project ZFP1). This work was supported by Ocean Park Conservation Foundation Hong Kong (B. Tapley, L. Nguyen, and J. Rowley) and a ZSL EDGE Fellowship (L. Nguyen). We thank Evan S.H. Quah, Cuong The Pham and Minh Le for their comments on an earlier version of this work.

References

- Altschul, S.F., Gish, W., Miller, W., Myers, E.W. & Lipman, D.J. (1990) Basic local alignment search tool. *Journal of Molecular Biology*, 215, 403–410.
[https://doi.org/10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2)
- Annandale, N. (1912) Zoological results of the Abor Expedition, 1911–1912. I. Amphibia. *Records of the Indian Museum*, 8, 7–36.
- Bain, R.H., Lathrop, A., Murphy, R.W., Orlov, N.L. & Cuc, H.T. (2003) Cryptic Species of a Cascade Frog from Southeast Asia: Taxonomic Revisions and Descriptions of Six New Species. *American Museum Novitates*, 3417, 1–60.
[https://doi.org/10.1206/0003-0082\(2003\)417<0001:csoacf>2.0.co;2](https://doi.org/10.1206/0003-0082(2003)417<0001:csoacf>2.0.co;2)
- Bain, R.H., Stuart, B.L. & Orlov, N.L. (2006) Three new Indochinese species of cascade frogs (Amphibia: Ranidae) allied to *Rana archotaphus*. *Copeia*, 2006, 43–59.
[https://doi.org/10.1643/0045-8511\(2006\)006\[0043:TNISOC\]2.0.CO;2](https://doi.org/10.1643/0045-8511(2006)006[0043:TNISOC]2.0.CO;2)
- Bain, R.H., Truong, N.Q. & Nguyen, Q.T. (2004) Herpetofaunal diversity of Ha Giang Province in northeastern Vietnam, with descriptions of two new species. *American Museum Novitates*, 3453, 1–42.
[https://doi.org/10.1206/0003-0082\(2004\)453<0001:hdohgp>2.0.co;2](https://doi.org/10.1206/0003-0082(2004)453<0001:hdohgp>2.0.co;2)
- Benson, D.A., Cavanaugh, M., Clark, K., Karsch-Mizrachi, I., Lipman, D.J., Ostell, J. & Sayers, E.W. (2017) GenBank. *Nucleic Acids Research*, 45, D37–D42.
<https://doi.org/10.1093/nar/gkw1070>
- Biju, S.D., Mahony, S. & Kamei, R.G. (2010) Description of two new species of torrent frog, *Amolops* Cope (Anura: Ranidae) from a degrading forest in the Northeast Indian State of Nagaland. *Zootaxa*, 2408 (1), 31–46.
<https://doi.org/10.11646/zootaxa.2408.1.2>
- Che, J., Chen, H., Yang, J.X., Jin, J.Q., Jiang, K., Yuan, Z.-Y., Murphy, R.W. & Zhang, Y.-P. (2012) Universal COI primers for DNA barcoding amphibians. *Molecular Ecology Resources*, 12 (2), 247–258.
<https://doi.org/10.1111/j.1755-0998.2011.03090.x>
- Che, J., Jiang, K., Yan, F. & Zhang, Y. (2020) *Amphibians and Reptiles in Tibet—Diversity and Evolution*. Chinese Academy of Sciences. Science Press, Beijing, China, 803 pp. [in Chinese with English abstracts and species descriptions]
- Cope, E.D. (1865) Sketch of the primary groups of Batrachia s. Salientia. *Natural History Review*, New Series, 5, 97–120.
- Frost, D.R. (2024) *Amphibian Species of the World: an Online Reference. Version 6.2. Electronic Database*. American Museum of Natural History, New York, New York. Available from: <http://research.amnh.org/herpetology/amphibia/index.html> (accessed 5 December 2024)
<https://doi.org/10.5531/db.vz.0001>
- Gan, Y.L., Qin, T., Lwin, Y.H., Li, G.G., Quan, R.C., Liu, S. & Yu, G.H. (2020a) A new species of *Amolops* (Anura: Ranidae) from northern Myanmar. *Zoological Research*, 41 (6), 733–739.
<https://doi.org/10.24272/j.issn.2095-8137.2020.125>
- Gan, Y.L., Yu, G.H. & Wu, Z.J. (2020b) A new species of the genus *Amolops* (Anura: Ranidae) from Yunnan, China. *Zoological Research*, 41, 188–193.
<https://doi.org/10.24272/j.issn.2095-8137.2020.018>
- Grosjean, S., Ohler, A., Chuaynkern, Y., Cruaud, C. & Hassanin, A. (2015) Improving biodiversity assessment of anuran amphibians using DNA barcoding of tadpoles. Case studies from Southeast Asia. *Comptes rendus biologiques*, 338, 351–361.
<https://doi.org/10.1016/j.crv.2015.03.015>
- Hall, T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *In: Nucleic acids symposium series*. Information Retrieval Ltd., [London], pp. 95–98. [c1979–c2000]
- Hoang, D.T., Chernomor, O., von Haeseler, A., Minh, B.Q. & Vinh, L.S. (2018) UFBoot2: Improving the ultrafast bootstrap

- approximation. *Molecular Biology and Evolution*, 35 (2), 518–522.
<https://doi.org/10.1093/molbev/msx281>
- Huelsenbeck, J.P., Ronquist, F., Nielsen, R. & Bollback, J.P. (2001) Bayesian inference of phylogeny and its impact on evolutionary biology. *Science*, 294, 2310–2314.
<https://doi.org/10.1126/science.1065889>
- Inger, R.F. & Chanard, T. (1997) A new species of ranid frog from Thailand, with comments on *Rana livida* (Blyth). *Natural History Bulletin of the Siam Society*, 45, 65–70.
- IUCN (2012) *IUCN Red List Categories and Criteria: Version 3.1. 2nd Edition*. IUCN, Gland and Cambridge, iv + 32 pp.
- IUCN (2017) The IUCN Red List of Threatened Species 2017–3. Available from: <http://www.iucnredlist.org/technicaldocuments/red-list-training/iucnspatialresources> (accessed 20 February 2024)
- Jiang, K., Ren, J.L., Lyu, Z.T., Wang, D., Wang, Z., Lv, K., Wu, J.W. & Li, J.T. (2021) Taxonomic revision of *Amolops chunganensis* (Pope, 1929) (Amphibia: Anura) and description of a new species from southwestern China, with discussion on *Amolops monticola* group and assignment of species groups of the genus *Amolops*. *Zoological Research*, 42, 574–591.
<https://doi.org/10.24272/J.ISSN.2095-8137.2021.107>
- Jiang, K., Wang, K., Yan, F., Xie, J., Zou, D.H., Liu, W.L., Jiang, J.P., Li, C. & Che, J. (2016) A new species of the genus *Amolops* (Amphibia: Ranidae) from southeastern Tibet, China. *Dong wu xue yan jiu = Zoological research*, 37, 31–40.
<https://doi.org/10.13918/j.issn.2095-8137.2016.1.31>
- Kalyaanamoorthy, S., Minh, B.Q., Wong, T.K.F., von Haeseler, A. & Jermin L.S. (2017) ModelFinder: Fast model selection for accurate phylogenetic estimates. *Nature Methods*, 14, 587–589.
<https://doi.org/10.1038/nmeth.4285>
- Katoh, K., Rozewicki, J. & Yamada, K.D. (2019) MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Briefings in bioinformatics*, 20, 1160–1166.
<https://doi.org/10.1093/BIB/BBX108>
- Kropachev, I.I., Orlov, N.L., Ninh, H.T. & Nguyen, T.T. (2019) A new species of *Rhacophorus* genus (Amphibia: Anura: Rhacophoridae: Rhacophorinae) from Van Ban district, Lao Cai province, Northern Vietnam. *Russian Journal of Herpetology*, 26, 325–334.
<https://doi.org/10.30906/1026-2296-2019-26-6-325-334>
- Liu, W., Yang, D., Ferraris, C. & Matsui, M. (2000) *Amolops bellulus*: A new species of stream-breeding frog from Western Yunnan, China (Anura: Ranidae). *Copeia*, 2000, 536–541.
[https://doi.org/10.1643/0045-8511\(2000\)000\[0536:ABANSO\]2.0.CO;2](https://doi.org/10.1643/0045-8511(2000)000[0536:ABANSO]2.0.CO;2)
- Mahony, S., Nidup, T., Streicher, J.W., Teeling, E.C. & Kamei, R.G. (2022) A review of torrent frogs (*Amolops*: Ranidae) from Bhutan, the description of a new species, and reassessment of the taxonomic validity of some *A. viridimaculatus* group species aided by archival DNA sequences of century-old type specimens. *The Herpetological Journal*, 32, 142–175.
<https://doi.org/10.33256/32.3.142175>
- Matsui, M., Ohler, A., Eto, K. & Nguyen, T.T. (2017) Distinction of *Gracixalus carinensis* from Vietnam and Myanmar, with description of a new species. *Alytes*, 33, 25–37.
- Miller, M.A., Pfeiffer, W. & Schwartz, T. (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: *Proceedings of the Gateway Computing Environments Workshop*, 2010, pp. 1–8.
<https://doi.org/10.1109/GCE.2010.5676129>
- Minh, B.Q., Nguyen, M.A.T. & von Haeseler, A. (2013) Ultrafast approximation for phylogenetic bootstrap. *Molecular Biology and Evolution*, 30, 1188–1195.
<https://doi.org/10.1093/molbev/mst024>
- Minh, B.Q., Schmidt, H.A., Chernomor, O., Schrempf, D., Woodhams, M.D., von Haeseler, A. & Lanfear, R. (2020) IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era. *Molecular Biology and Evolution*, 37 (5), 1530–1534.
<https://doi.org/10.1093/molbev/msaa015>
- Ngo, A., Murphy, R., Liu, W., Lathrop, A. & Orlov, N. (2006) The phylogenetic relationships of the Chinese and Vietnamese waterfall frogs of the genus *Amolops*. *Amphibia-Reptilia*, 27 (1), 81–92.
<https://doi.org/10.1093/molbev/msaa015>
- Nguyen, L.T., Tapley, B., Nguyen, C.T., Luong, V.H. & Rowley, J.J.L. (2021) A new species of *Leptobranchella* (Anura, Megophryidae) from Mount Pu Ta Leng, northwest Vietnam. *Zootaxa*, 5016 (3), 301–332.
<https://doi.org/10.11646/zootaxa.5016.3.1>
- Nguyen, L.T., Tapley, B., Kane, D., yet-Dzung, T.T., Cui, J. & Rowley, J.J.L. (2024a) A new *Oreolalax* (Anura: Megophryidae) from the Hoang Lien Range, northwest Vietnam. *Zootaxa*, 5514 (6), 501–524
<https://doi.org/10.11646/zootaxa.5514.6.1>
- Nguyen, T.T., Nguyen, H.H., Ninh, H.T., Le, L.T., Bui, H.T., Orlov, N., Van Hoang, C. & Ziegler, T. (2024b) *Zhangixalus thaoae* sp. nov., a new green treefrog species from Vietnam (Anura, Rhacophoridae). *ZooKeys*, 1197, 93–113.
<https://doi.org/10.3897/zookeys.1197.104851>
- Nguyen, S. Van, Ho, C.T. & Nguyen, T.Q. (2009) Herpetofauna of Vietnam. Edition chimaira, frankfurt am Main, 768 pp.
- Ohler, A., Marquis, O., Grosjean, S. & Swan, S. (2000) Amphibian biodiversity of Hoang Lien Nature Reserve (Lao Cai Province, northern Vietnam) with description of two new species. *Herpetozoa*, 13 (1/2), 71–87.

- Orlov, N.L. & Ho, C.T. (2007) Two new species of Cascade Ranids of *Amolops* genus (Amphibia: Anura: Ranidae) from Lai Chau province (northwest Vietnam). *Russian Journal of Herpetology*, 14 (3), 211–228.
<https://doi.org/10.30906/1026-2296-2007-14-3-211-228>
- Palumbi, S.R., Martin, A., Romano, S., McMillan, W.O., Stice, L. & Grabowski, G. (1991) *The simple fool's guide to PCR*. Department of Zoology, University of Hawaii, Honolulu, 47 pp.
- Patel, N.G., Garg, S., Das, A., Stuart, B.L. & Biju, S.D. (2021) Phylogenetic position of the poorly known montane cascade frog *Amolops monticola* (Ranidae) and description of a new closely related species from Northeast India. *Journal of Natural History*, 55, 1403–1440.
<https://doi.org/10.1080/00222933.2021.1946185>
- Pham, V. A., Ngo, H.T., Nenh, S.B., Ziegler, T. & Le, M.D. (2023) A new species of *Amolops* (Anura: Ranidae) from Son La Province, northwestern Vietnam. *Raffles Bulletin of Zoology*, 71, 51–69.
- Pham, C.T., Le, M.D., Hoang, C. Van, Pham, A. Van, Ziegler, T. & Nguyen, T.Q. (2020) First records of *Bufo luchunnicus* (Yang & Rao, 2008) and *Amolops wenshanensis* Yuan, Jin, Li, Stuart & Wu, 2018 (Anura: Bufonidae, Ranidae) from Vietnam. *Russian Journal of Herpetology*, 27, 81–86.
<https://doi.org/10.30906/1026-2296-2020-27-2-81-86>
- Posada, D. & Crandall, K.A. (1998) Modeltest: testing the model of DNA substitution. *Bioinformatics*, 14 (9), 817–818.
<https://doi.org/10.1093/bioinformatics/14.9.817>
- Ray, P. (1992) Two new hill-stream frogs of the genus *Amolops* Cope (Amphibia: Anura: Ranidae) from Uttar Pradesh (India). *Indian Journal of Forestry*, 15, 346–350.
- Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A. & Huelsenbeck, J.P. (2012) MRBAYES 3.2: Efficient Bayesian phylogenetic inference and model selection across a large model space. *Systematic Biology*, 61(3), 539–542.
<https://doi.org/10.1093/sysbio/sys029>
- Savage, J.M. & Heyer, W.R. (1997) Digital webbing formulae for anurans: a refinement. *Herpetological Review*, 28 (3), 131.
- Simmons, J.E. (2002) Herpetological collecting and collections management. Revised edition. Society for the Study of Amphibians and Reptiles. *Herpetological Circular*, 31, 1–153.
- Stuart, B.L., Bain, R.H., Phimmachak, S. & Spence, K. (2010) Phylogenetic systematics of the *Amolops monticola* group (Amphibia: Ranidae), with description of a new species from Northwestern Laos. *Herpetologica*, 66, 52–66.
<https://doi.org/10.1655/08-073.1>
- Sung, Y.H., Hu, P., Wang, J., Liu, H.J. & Wang, Y.Y. (2016) A new species of *Amolops* (Anura: Ranidae) from southern China. *Zootaxa*, 4170 (3), 525–538.
<https://doi.org/10.11646/zootaxa.4170.3.6>
- Tapley, B., Cutajar, T., Mahony, S., Nguyen, C.T., Dau, V.Q., Nguyen, T.T., Luong, H.V. & Rowley, J.J. (2017) The Vietnamese population of *Megophrys kuatunensis* (Amphibia: Megophryidae) represents a new species of Asian horned frog from Vietnam and southern China. *Zootaxa*, 4344 (3), 465–492.
<https://doi.org/10.11646/zootaxa.4344.3.3>
- Tapley, B., Cutajar, T., Mahony, S., Nguyen, C.T., Dau, V.Q., Luong, A.M., Le, D.T., Nguyen, T.T., Nguyen, T.Q., Portway, C., Luong, H. Van & Rowley, J.J.L. (2018) Two new and potentially highly threatened *Megophrys* Horned frogs (Amphibia: Megophryidae) from Indochina's highest mountains. *Zootaxa*, 4508 (3), 301–333.
<https://doi.org/10.11646/zootaxa.4508.3.1>
- Tapley, B., Jervis, P., Nguyen, L.T., Portway, C., Nguyen, C.T., Luong, H.V., Kane, D., Brookes, L., Perkins, M.W., Ghosh, P., Wierzbicki, C., Shelton, J., Fisher, M.C. & Rowley, J.J.L. (2020a) Low prevalence of *Batrachochytrium dendrobatidis* detected in amphibians from Vietnam's highest mountains. *Herpetological review*, 51 (4), 726–732.
- Tapley, B., Cutajar, T., Nguyen, L.T., Portway, C., Mahony, S., Nguyen, C.T., Harding, L., Luong, H. Van & Rowley, J.J.L. (2020b) A new potentially Endangered species of *Megophrys* (Amphibia: Megophryidae) from Mount Ky Quan San, north-west Vietnam. *Journal of Natural History*, 54, 2543–2575.
<https://doi.org/10.1080/00222933.2020.1856952>
- Tamura, K., Stecher, G. & Kumar, S. (2021) MEGA11: molecular evolutionary genetics analysis version 11. *Molecular biology and evolution*, 38 (7), 3022–3027.
<https://doi.org/10.1093/molbev/msab120>
- Wilcox, T.P., Zwickl, D.J., Heath, T.A. & Hillis, D.M. (2002) Phylogenetic relationships of the Dwarf Boas and a comparison of Bayesian and bootstrap measures of phylogenetic support. *Molecular Phylogenetics and Evolution*, 25, 361–371.
[https://doi.org/10.1016/S1055-7903\(02\)00244-0](https://doi.org/10.1016/S1055-7903(02)00244-0)
- Wu, Y.H., Yan, F., Stuart, B.L., Prendini, E., Suwannapoom, C., Dahn, H.A., Zhang, B.L., Cai, H.X., Xu, Y.B., Jiang, K., Chen, H.M., Lemmon, A.R., Lemmon, E.M., Raxworthy, C.J., Orlov, N.L., Murphy, R.W. & Che, J. (2020) A combined approach of mitochondrial DNA and anchored nuclear phylogenomics sheds light on unrecognized diversity, phylogeny, and historical biogeography of the torrent frogs, genus *Amolops* (Anura: Ranidae). *Molecular Phylogenetics and Evolution*, 148, 106789.
<https://doi.org/10.1016/j.ympev.2020.106789>
- Yu, G., Wu, Z. & Yang, J. (2019) A new species of the *Amolops monticola* group (Anura: Ranidae) from southwestern Yunnan, China. *Zootaxa*, 4577 (3), 548–560.

<https://doi.org/10.11646/zootaxa.4577.3.8>

Yuan, Z., Jin, J., Li, J., Stuart, B.L. & Wu, J. (2018) A new species of cascade frog (Amphibia: Ranidae) in the *Amolops monticola* group from China. *Zootaxa*, 4415 (3), 498–512.

<https://doi.org/10.11646/zootaxa.4415.3.5>

Zhao, W., Rao, D., Lu, S. & Dong, B. (2005) Herpetological surveys of Xizang autonomous region 2. Medog. *Herpetological surveys of Xizang autonomous region 2. Medog*, 24, 250–253.