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## A new *Oreolalax* (Anura: Megophryidae) from the Hoang Lien Range, northwest Vietnam

LUAN THANH NGUYEN<sup>1</sup>, BENJAMIN TAPLEY<sup>2\*</sup>, DANIEL KANE<sup>2</sup>, TUYET-DZUNG THI TRAN<sup>1</sup>, JIAXIN CUI<sup>3</sup> & JODI J. L. ROWLEY<sup>4,5</sup>

<sup>1</sup>Asian Turtle Program of Indo-Myanmar Conservation, R.1301, CT1 Bac Ha C14 Building, To Huu Str., Nam Tu Liem Dist., Hanoi, Vietnam.

 [nguyenluanbio@gmail.com](mailto:nguyenluanbio@gmail.com);  <https://orcid.org/0000-0002-4663-125X>

 [tuyetdung26@gmail.com](mailto:tuyetdung26@gmail.com);  <https://orcid.org/0000-0002-0192-426X>

<sup>2</sup>Zoological Society of London, Regent's Park, London, NW1 4RY, UK.

<sup>3</sup>Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu, Sichuan 610213, China.

 [cuijiixin23@mailsucas.ac.cn](mailto:cuijiixin23@mailsucas.ac.cn);  <https://orcid.org/0009-0002-1305-2929>

<sup>4</sup>Australian Museum Research Institute, Australian Museum, 1 William St, Sydney, NSW, 2010, Australia.

 [jodi.rowley@australian.museum](mailto:jodi.rowley@australian.museum);  <https://orcid.org/0000-0002-2011-9143>

<sup>5</sup>Centre 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](mailto:ben.tapley@zsl.org);  <https://orcid.org/0000-0002-9787-3793>

### Abstract

The megophryid genus *Oreolalax* is comprised of 19 species, most of which are endemic to China. A single species, *Oreolalax sterlingae*, is the only member of the genus known from Vietnam. During fieldwork on Mount Po Ma Lung in the Hoang Lien Range of northwest Vietnam, we encountered individuals of both *Oreolalax sterlingae* and another, morphologically divergent species of *Oreolalax*. Analyses of morphological and molecular data reveal that these individuals represent a previously undescribed species of *Oreolalax* which we describe as new to science. The new species, *Oreolalax adelphos* **sp. nov.**, is sister to *Oreolalax xiangchengensis* and can be diagnosed from all other congeneric species by the combination of the following characters: (1) SVL of adult male 38.0 mm,  $N=1$ ; adult female 46.2 mm,  $N=1$ ; (2) narrow supratympanic fold; (3) no visible tympanum; (4) head longer than wide; (5) vocal sac absent; (6) absence of subarticular tubercles on hands; (7) basal interdigital webbing on hind feet; (8) ventral surface mottled with grey and creamy white; (9) dorsal surface of head and body covered in rounded, evenly spaced and similar sized tubercles; (10) presence of dark bars on limbs; (11) greyish white and creamy white spots on the flanks, and (12) a bicoloured iris. *Oreolalax adelphos* **sp. nov.** is only known from a single site of high elevation bamboo forest, where it is sympatric with *Oreolalax sterlingae*. Both *Oreolalax adelphos* **sp. nov.** and *O. sterlingae* are almost certain to occur in neighbouring Jinping County in Yunnan Province, China. The new species of *Oreolalax* likely qualifies for being assessed as Data Deficient in accordance with the IUCN Red List of Threatened Species categories and criteria.

**Key words:** Amphibian, China, Leptobrachiinae, Po Ma Lung, taxonomy

### Tóm tắt tiếng Việt

Các loài cóc trong giống *Oreolalax* hiện có 19 loài được ghi nhận, hầu hết trong số đó là đặc hữu ở Trung Quốc. Một loài duy nhất, Cóc núi Sterling, *Oreolalax sterlingae* là có ghi nhận tại Việt Nam. Trong quá trình điều tra thực địa về đa dạng lưỡng cư tại núi Pô Ma Lung thuộc dãy Hoàng Liên Sơn, chúng tôi đã thu thập các mẫu vật thuộc loài *Oreolalax sterlingae* và một số mẫu vật chưa rõ loài có đặc điểm hình thái khác biệt với các loài trong giống *Oreolalax* hiện biết. Kết quả phân tích hình thái ngoài và trình tự di truyền cho thấy những mẫu vật này là một loài mới và được đặt tên là Cóc răng anh em, *Oreolalax adelphos* **sp. nov.** Loài cóc mới có một số đặc điểm đặc trưng sau: (1) chiều dài mút mõm-lỗ huyệt (SVL) 38.0 mm ở 1 cá thể đực và 26.2 mm ở 1 cá thể cái; (2) gờ trên màng nhĩ mảnh; (3) màng nhĩ ẩn; (4) đầu dài hơn rộng; (5) túi kêu ngoài không rõ; (6) các củ bàn phụ dưới bàn tay không phát triển; (7) màng bơi phát triển ít ở giữa các ngón chân sau; (8) mặt bụng có màu xám với các đốm trắng xen kẽ; (9) đầu và thân có những mụn tròn và đều; (10)

đuôi và ống chân có các vệt ngang màu nâu đen; (11) hông có các đốm trắng xám hoặc trắng kem và (12) mắt có 2 màu khác biệt ở nửa trên và dưới. Loài mới này hiện chỉ xuất hiện ở đỉnh núi Pờ Ma Lung và có cùng sinh cảnh sống với loài *Oreolalax sterlingae*, và cả hai loài này đều có khả năng phân bố ở Kim Bình (Jinping), Vân Nam, Trung Quốc. Đây là loài thứ 2 trong giống *Oreolalax* được phát hiện tại Việt Nam cho tới nay.

## 中文摘要:

角蟾科 (Megophryidae) 齿蟾属 (*Oreolalax*) 目前共记录有19个物种, 大部分为中国特有, *Oreolalax sterlingae*为该属唯一分布在越南的物种。在对越南西北部黄连山脉 Po Ma Lung山的野外调查中, 我们发现了*O. sterlingae*及一些与之形态特征相异的齿蟾。形态和分子的数据分析结果表明, 后者系未被描述过的齿蟾属新种, 本文将其命名为 *Oreolalax adelphos* **sp. nov.**。该新种为乡城齿蟾 (*Oreolalax xiangchengensis*) 的姐妹种, 与同属其他物种可通过以下特征的组合进行区分: (1) 雄性成体体长 (SVL) 38.0 mm, N=1; 雌性成体体长46.2 mm, N=1; (2) 有狭窄的鼓上棱; (3) 无明显鼓膜; (4) 头部长度大于头部宽度; (5) 无声囊; (6) 无指关节下瘤; (7) 趾间基部有蹼; (8) 腹面有灰色和奶油白色斑点; (9) 头部和身体背面有圆形、大小相似且间距均匀的疣粒; (10) 四肢有深色条状斑纹; (11) 体侧有灰白色和奶油白色斑点; (12) 双色虹膜。*Oreolalax adelphos* **sp. nov.** 仅发现于一海拔较高的竹林中, 在该处与 *O. sterlingae* 同域分布。二者极可能在邻近的中国云南省金平县也有分布。根据IUCN濒危物种红色名录等级和标准, 本研究中的齿蟾属新种*Oreolalax adelphos* **sp. nov.**可能被评估为数据缺乏 (Data Deficient)。

## Introduction

Toothed toads of the genus *Oreolalax* Myers & Leviton, 1962 are an Asian radiation, with 19 species scientifically described to date (Hou *et al.* 2020; Frost 2024). Phylogenetic relationships within the genus remain poorly understood and are largely based on mitochondrial DNA (Liang *et al.* 2016; Dufresnes & Litvinchuk 2022). These studies using mitochondrial DNA suggested that some taxa are closely related and exhibit mitochondrial sequence divergences that would typically be considered to represent intraspecific variation, however, analyses of mitogenomes showed that these taxa are distinct and robustly monophyletic (Dufresnes & Litvinchuk 2022). Various species groups have been suggested within *Oreolalax* (Fei *et al.* 2005, 2012; Wei *et al.* 2007; Dufresnes & Litvinchuk 2022) but not all species have been clearly assigned to the groups.

All known *Oreolalax* species occur above 1000 m asl (Wei *et al.* 2007; Fei *et al.* 2012) and most of them are endemic to the mountains of southwest China, although some are known to also occur in the border regions of neighbouring Vietnam and Myanmar (Nguyen *et al.* 2013; Rahman *et al.* 2020). The genus *Oreolalax* was first reported from Vietnam with the description of *Oreolalax sterlingae* Nguyen, Phung, Le, Ziegler & Böhme 2013, from the peak of Mount Fansipan, in the Hoang Lien Range of northwest Vietnam (Nguyen *et al.* 2013). This species was subsequently discovered on Mount Pu Ta Leng, Bat Xat District, Lao Cai Province (Tapley *et al.* 2020). Despite surveys of other high elevation peaks in the Hoang Lien Range, no other *Oreolalax* species have been recorded from Vietnam (Tapley *et al.* 2020; Nguyen *et al.* 2023) and Mount Fansipan remains the southernmost limit of the genus *Oreolalax*.

In August 2023 we encountered a population of *Oreolalax* during field surveys on Mount Po Ma Lung in Lai Chau Province in northwest Vietnam, adjacent to the China-Vietnam border. This population did not resemble any of the currently described *Oreolalax* species. We describe this species as new based on molecular and morphological evidences.

## Materials and methods

Specimens were collected at night in bamboo forest on Mount Po Ma Lung in Lai Chau Province, in August 2023. Geographic coordinates were obtained using a Garmin GPSMAP 64CSx 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 ethanol prior to the fixation

of specimens with 10% formalin and subsequent storage in 70% ethanol. Type specimens were deposited at the Institute of Tropical Biology Collection of Zoology (ITBCZ), Ho Chi Minh City, Vietnam.

## Laboratory methods

**Molecular data:** Total genomic DNA was extracted from ethanol-preserved tissues using 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 section of 16S (mtDNA) using the primers 16SL2021 (5' CCTACCGAGCTTAGTAATAGCTGGTT-3') modified from Hedges (1994) 16SH1 (5'-CTCCGGTCTGAACTCAGATCACGTAGG-3') by Hedges and Maxson (1993). PCR amplification was carried out in 25-μL reactions volume, including 12.5-μL 2 x ES Master Mix (CWBIO, China), 0.75-μL of each primer (10 pmol/μL), 1-μL of cDNA template, and 10-μL H<sub>2</sub>O. 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), followed by 35 cycles of 94°C (1 min) denaturation, 55°C (1 min) annealing and 72°C (1 min) extension, followed by a final extension step at 72°C (10 mins). All PCR products were purified using ExoSap-ITTM (USB Corporation, Ohio USA), and sequenced in both 5' and 3' directions at Macrogen (Seoul, South Korea). Sequence chromatograms were edited and checked by eye for quality using BioEdit V. 7.0.5.3 (Hall 1999). The new sequences were then checked on BLAST (The National Center for Biotechnology Information) (Altschul *et al.* 1990) to verify their approximate identity and sequences were deposited in GenBank under accession numbers PQ191257, PQ198697, PQ198698 and PQ198699 (Table 1).

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

ID	Species	Voucher no.	16S Genbank accession no.	Locality (County/District/City, Province, Country)	Distance from type locality	Reference
1	<i>Oreolalax adelphos</i> <b>sp. nov.</b>	ITBCZ 3619	PQ198697	Phong Tho District, Lai Chau, Vietnam	Type locality	This study
2	<i>Oreolalax adelphos</i> <b>sp. nov.</b>	ITBCZ 3620	PQ198698	Phong Tho District, Lai Chau, Vietnam	Type locality	This study
3	<i>Oreolalax adelphos</i> <b>sp. nov.</b>	ITBCZ 3621	PQ198699	Phong Tho District, Lai Chau, Vietnam	Type locality	This study
4	<i>Oreolalax chuanbeiensis</i>	CIB-ZYC074	EF397266	Mao County, Sichuan, China	≈125 km	Fu <i>et al.</i> 2007
5	<i>Oreolalax chuanbeiensis</i>	RDQ106	EU180887	Wangbachu, Ping Wu, Sichuan, China	Type locality	Rao & Wilkinson 2008
6	<i>Oreolalax jingdongensis</i>	IOZCAS2691	EF397255	Jingdong, Yunnan, China	Type locality	Fu <i>et al.</i> 2007
7	<i>Oreolalax jingdongensis</i>	/	MF953479	Xujiaba, Jingdong County, Yunnan, China	Type locality	Jiang <i>et al.</i> 2018
8	<i>Oreolalax jingdongensis</i>	RDQ162	EU180888	Ailao Mount, Jingdong County, Yunnan, China	Type locality	Rao & Wilkinson 2008
9	<i>Oreolalax liangbeiensis</i>	IOZCAS3796	EF397253	Puxiong, Yuexi, Sichuan, China	Type locality	Fu <i>et al.</i> 2007
10	<i>Oreolalax lichuanensis</i>	IZCASH30036	EF544237	Lichuan, Hubei, China	Type locality	Zheng <i>et al.</i> 2008
11	<i>Oreolalax lichuanensis</i>	CIB-ZYC787	EF397260	Chongqing Municipality, China	≈275 km	Fu <i>et al.</i> 2007
12	<i>Oreolalax longmenmontis</i>	CIB20180526002	MN688671	Pengzhou, Sichuan, China	Type locality	Hou <i>et al.</i> 2020

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TABLE 1. (Continued)

ID	Species	Voucher no.	16S Genbank accession no.	Locality (County/District/City, Province, Country)	Distance from type locality	Reference
13	<i>Oreolalax longmenmontis</i>	CIB2018041501	MN688666	Pengzhou, Sichuan, China	Type locality	Hou <i>et al.</i> 2020
14	<i>Oreolalax longmenmontis</i>	CIB2018052201602	MN688668	Pengzhou, Sichuan, China	Type locality	Hou <i>et al.</i> 2020
15	<i>Oreolalax major</i>	CIB2019bx05	MN688654	Baoxing, Sichuan, China	≈97 km	Hou <i>et al.</i> 2020
16	<i>Oreolalax major</i>	/	KU127230	China	Unknown	Liang <i>et al.</i> 2016
17	<i>Oreolalax major</i>	ROM40452	EF397252	Sichuan, China	Unknown	Fu <i>et al.</i> 2007
18	<i>Oreolalax multipunctatus</i>	CIB2013wb091	NC037382	Emei, Sichuan, China	Type locality	Li <i>et al.</i> 2019
19	<i>Oreolalax multipunctatus</i>	ROM40463	EF397268	Sichuan, China	Unknown	Fu <i>et al.</i> 2007
20	<i>Oreolalax nanjiangensis</i>	CIBSCNJJN2006004	MN688658	Nanjiang, Sichuan, China	Type locality	Hou <i>et al.</i> 2020
21	<i>Oreolalax nanjiangensis</i>	CIB-XM804	EF397265	Nanjiang, Sichuan, China	Type locality	Fu <i>et al.</i> 2007
26	<i>Oreolalax omeimontis</i> 1	CIB-XM379	EF397262	Da Yi County, Sichuan, China	≈110 km	Fu <i>et al.</i> 2007
27	<i>Oreolalax omeimontis</i> 1	CIB-XM297	EF397263	Omei Mount, Omei County, Sichuan, China	Type locality	Fu <i>et al.</i> 2007
28	<i>Oreolalax omeimontis</i> 1	ROM40454	EF397261	Wa Wu Shan, Hongya County, Sichuan, China	≈40 km	Fu <i>et al.</i> 2007
29	<i>Oreolalax omeimontis</i> 1	CIB-XM439	EF397264	Omei County, Sichuan, China	Type locality	Fu <i>et al.</i> 2007
22	<i>Oreolalax omeimontis</i> 2	CIBWWS180610022	MN688662	Emei, Sichuan, China	Type locality	Hou <i>et al.</i> 2020
23	<i>Oreolalax omeimontis</i> 2	CIBEMS18061203	MN688659	Emei, Sichuan, China	Type locality	Hou <i>et al.</i> 2020
24	<i>Oreolalax omeimontis</i> 2	20120068	OP722573	China	Unknown	Luo <i>et al.</i> 2022
25	<i>Oreolalax omeimontis</i> 2	KIZ-O.O96002	EU180886	Emei Mount, Emei City, Sichuan, China	Type locality	Rao & Wilkinson 2008
30	<i>Oreolalax pingii</i>	CIB-XM980	EF397259	Xi Chang, Sichuan, China	≈58 km	Fu <i>et al.</i> 2007
31	“ <i>Oreolalax popei</i> ”	CIB-XM0107	EF397267	Pengxian County, Sichuan, China	≈130 km	Fu <i>et al.</i> 2007
32	<i>Oreolalax rhodostigmatus</i>	CIB-ZYCA 746	EF397248	Da Fang, Guizhou, China	≈150 km	Fu <i>et al.</i> 2007
33	<i>Oreolalax rhodostigmatus</i>	/	MF770485	Suiyang County, Guizhou, China	≈40 km	Zhao <i>et al.</i> 2018
34	<i>Oreolalax rhodostigmatus</i>	CIB-ZYV724	EF397249	Siuyang County, Guizhou Province, China	≈40 km	Fu <i>et al.</i> 2007
35	<i>Oreolalax rugosus</i>	CIB-XM340	EF397254	Shi Mian, Sichuan, China	≈145 km	Fu <i>et al.</i> 2007
36	<i>Oreolalax schmidtii</i>	ROM40457	EF397257	Hongya, Sichuan, China	≈35 km	Fu <i>et al.</i> 2007
37	<i>Oreolalax schmidtii</i>	CIB-XM417	EF397258	Da Yi County, Sichuan, China	≈110 km	Fu <i>et al.</i> 2007
38	<i>Oreolalax</i> sp.	CIB-XM092	EF397256	Peng Xian County, Sichuan, China	NA	Fu <i>et al.</i> 2007

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TABLE 1. (Continued)

ID	Species	Voucher no.	16S Genbank accession no.	Locality (County/District/ City, Province, Country)	Distance from type locality	Reference
39	<i>Oreolalax sterlingae</i>	IEBR A.2012.1	KC569981	Sa Pa District, Lao Cai, Vietnam	Type locality	Nguyen <i>et al.</i> 2013
40	<i>Oreolalax sterlingae</i>	ITBCZ 3623	PQ191257	Phong Tho District, Lai Chau, Vietnam	≈45 km	This study
41	<i>Oreolalax</i> cf. <i>xiangchengensis</i>	CIB-3LW008	EF397250	Li Jiang, Yunnan, China	≈220 km	Fu <i>et al.</i> 2007
42	<i>Oreolalax</i> cf. <i>xiangchengensis</i>	CIB-3LW032	EF397251	Zhongdian County, Yunnan Province, China	≈118 km	Fu <i>et al.</i> 2007
43	<i>Oreolalax xiangchengensis</i>	CIB2013642	MH727696	Xiangcheng County, Sichuan, China	Type locality	Li <i>et al.</i> 2018
44	<i>Scutiger ningshanensis</i>	/	NC031426	China	Unknown	Hou <i>et al.</i> 2020
45	<i>Leptobranchella oshanensis</i>	CIB20050095	NC020610	China	Unknown	Xiang <i>et al.</i> 2013

## Phylogenetic analysis

For phylogenetic analyses, 39 sequences of 16S mtDNA from 16 of the 19 described species and another undescribed taxon available on GenBank (Benson *et al.* 2017) were downloaded (Table 1). Molecular data are lacking for *O. granulosus* Fei, Ye & Chen 1990, *O. puxiongensis* (Liu & Fei 1979) and *O. weigoldi* (Vogt 1924). The sequence from “*O. popei*” (Liu 1947) from Pengzhou City in Sichuan Province, China is likely to have been erroneously assigned to *O. popei*, the sequenced animal is likely to be *O. longmenmontis* Hou, Shi, Hu, Deng, Jiang, Xie & Wang 2020 see discussion in Hou *et al.* (2020). A single sequence from *Scutiger ningshanensis* Fang 1985 and a single sequence from *Leptobranchella oshanensis* (Liu 1950) were included as outgroups following Hou *et al.* (2020). The four new and 41 previously published sequences were aligned using MAFFT (Kato *et al.* 2019) on the CIPRES Science Gateway ([http://www.phylo.org/sub\\_sections/portal](http://www.phylo.org/sub_sections/portal); Miller *et al.* 2010). Uncorrected *p*-distance was calculated using MEGA11 with pairwise deletion of gaps (Tamura *et al.* 2021). There have been cases where GenBank sequences have been incorrectly assigned to species (Hou *et al.* 2020). Therefore the distance from the collection site of sequenced specimens from the type locality of each species were estimated using the ruler function of Google Earth. This measurement may provide some inference on the reliability of the species identification associated with the sequence, especially for species (e.g., *O. popei*) where there may be no molecular data associated with the type series.

The matrilineal genealogy was inferred using two methods: Bayesian inference (BI) and Maximum Likelihood (ML). In the BI analysis the optimal evolutionary model was tested using MODELTEST v.3.06 (Ronquist and Huelsenbeck 2003), and the best-fit model for BI was the GTR+G+I model of DNA evolution (suggested by the Akaike Information Criterion). BI analysis was conducted in MRBAYES v.3.2 (Ronquist *et al.* 2012); Metropolis-coupled Markov chain Monte Carlo (MCMCMC) analyses were run for 10 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). In the ML tree, the optimal evolutionary models for the analysis were tested using ModelFinder implemented in IQ-TREE (Kalyaanamoorthy *et al.* 2017), and the best-fit model was TIM2+F+I+R2 chosen according to BIC. 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 to indicate strong support (Huelsenbeck *et al.* 2001; Wilcox *et al.* 2002 Minh *et al.* 2013; Hoang *et al.* 2018).

## Morphological analysis

The following measurements were taken to the nearest 0.1 mm using a digital calliper from fixed specimens following Nguyen *et al.* (2013), although not all measurements reported by Nguyen *et al.* (2013) were taken as measurements of several characters are not reported for any other *Oreolalax* species and there is no widely adopted series of measurements for the genus: snout–vent length (SVL); head length from tip of snout to rear of jaw (HDL); head width at commissure of jaw (HDW); head height at largest point (HH); snout length from tip of snout to anterior corner of eye (SNT); internarial distance (IN); front of eye–nostril distance (EN); nostril–snout distance (distance from the tip of the snout to the naris) (NS); horizontal diameter of exposed portion of eyeball (EYE); interorbital distance (IOD); femur length, from vent to outer edge of knee (FEL); tibia length with hindlimb flexed (TIB); tarsus length (TaL); manus length from tip of third digit to proximal edge of inner metacarpal tubercle (ML); pes length from tip of fourth toe to proximal edge of the inner metatarsal tubercle (PL); maximum length of inner metacarpal tubercle (IPL); maximum length outer metacarpal tubercle (OPL); F1DSC: width of Finger I digital disc; F3DSC: width of Finger III digital disc; T4DSC: width of Toe IV digital disc; maximum length of inner metatarsal tubercle (IML); maximum diameter of pectoral gland (PEC); and maximum diameter of femoral gland (FEM). Sex and maturity were determined by gonadal inspection of both sexes.

We obtained comparative morphological data from the examination of material (see Appendix I) at the Australian Museum (AMS) and Chengdu Institute of Biology (CIB) original descriptions and the literature (Fei *et al.* 1990, 2012; Yang & Rao 2008; Fei & Ye 2016; Tapley *et al.* 2023) for all other *Oreolalax* species: *Oreolalax chuanbeiensis* Tian 1983; *O. granulatus*; *O. jingdongensis* Ma, Yang & Li 1983; *O. liangbeiensis* Liu & Fei 1979; *O. lichuanensis* Hu & Fei 1979; *O. longmenmontis*; *O. major* (Liu & Hu 1960); *O. multipunctatus* Wu, Zhao, Inger & Shaffer 1993; *O. nanjiangensis* Fei & Ye 1999; *O. omeimontis* (Liu & Hu 1960); *O. pingii* (Liu 1943); *O. popei*; *O. puxiongensis*; *O. rhodostigmatus* Hu & Fei 1979; *O. rugosus* (Liu 1943); *O. schmidtii* (Liu 1947); *O. sterlingae*; *O. weigoldi*; and *O. xiangchengensis* Fei & Huang 1983.

## Species distribution mapping

The distribution map was created in ArcMap 10.3.1 (Esri, California, USA). The species' distribution was generated using the Shuttle Radar Topography Mission (SRTM) 3-arc second digital elevation layer the International Union for Conservation of Nature (IUCN) elevation raster (IUCN 2017). The estimated range for the newly described species was estimated by clipping the elevation to above 2500 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. We followed the IUCN Red List (Red List Technical Working Group 2018). 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

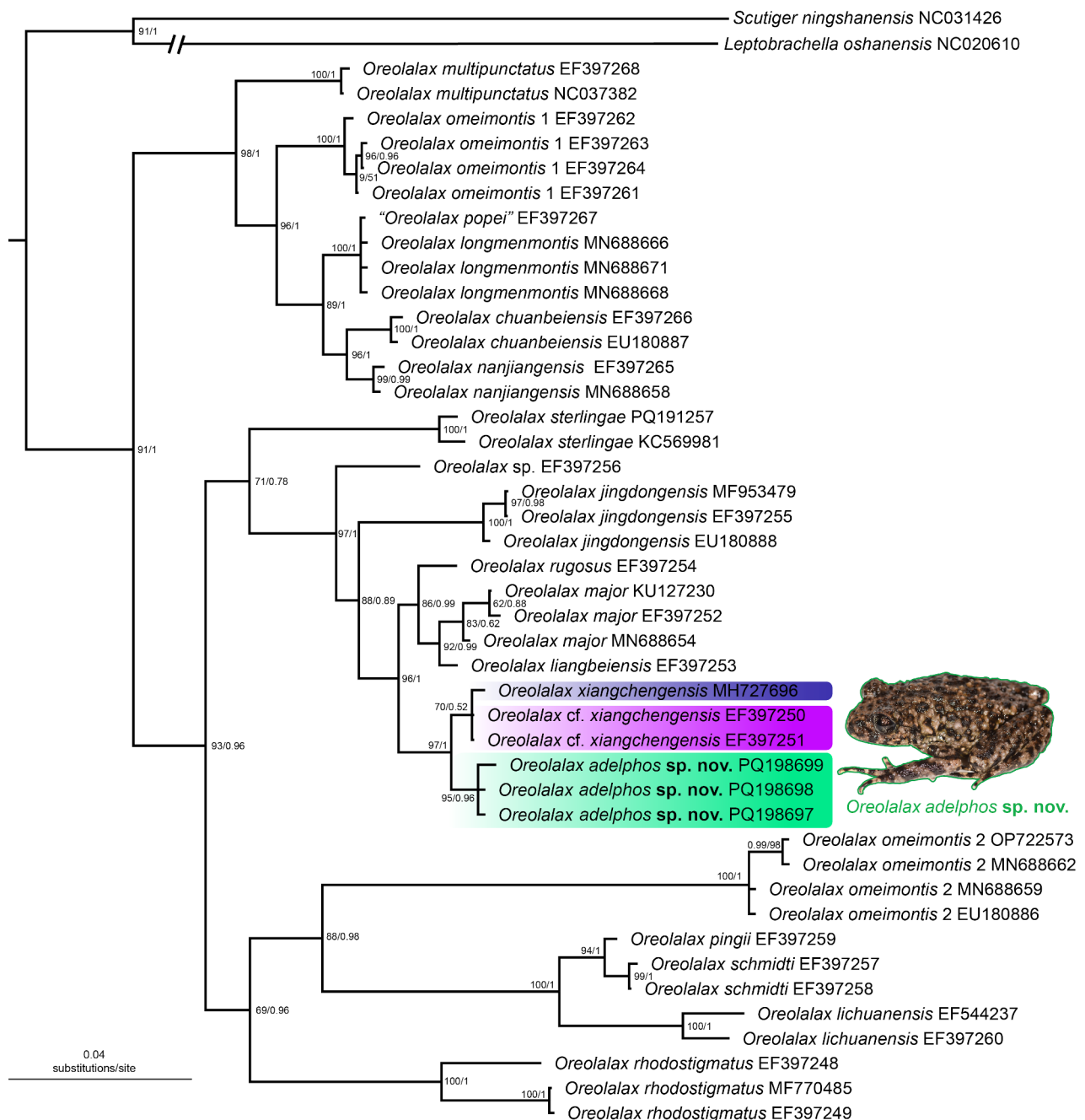
### Molecular data

The aligned 16S mtDNA dataset contained 1499 characters and 45 taxa including gaps and missing data. There were 923 conserved sites, 516 variable sites, and 343 parsimony-informative characters in the dataset (calculated in MEGA 11; Tamura *et al.* 2021). In the BI run, the standard deviation of split frequencies was 0.001705. Monophyly of the genus *Oreolalax* was supported in BI analyses (UFB/BPP 91/1 respectively; Fig. 1).

One specimen collected from Mount Po Ma Lung (ITBCZ 3623) was assigned to *O. sterlingae* using both morphological and molecular data. The uncorrected *p*-distance between the new 16S mtDNA sequence generated from this specimen and from the holotype specimen collected 48 km away on Mount Fansipan was 0.6% (GenBank accession number KC569981). The three remaining samples from specimens collected from Mount Po Ma Lung formed a strongly supported and distinct clade (UFB/BPP 95/0.96; Fig. 1.) (here after *Oreolalax* Po Ma Lung) that

is nested within the *Oreolalax* “*rugosus*” group (after Fei *et al.* 2005), which includes *O. rugosus*, *O. jingdongensis*, *O. xiangchengensis*, *O. major* and *O. liangbeiensis* (Dufresnes & Litvinchuk 2022). The mean uncorrected *p*-distance between *Oreolalax* Po Ma Lung and sympatric *Oreolalax sterlingae* was 5.8%. *Oreolalax* Po Ma Lung is a sister clade to *Oreolalax xiangchengensis* and *Oreolalax* cf. *xiangchengensis* from Sichuan and Yunnan provinces, China (UFB/BPP 97/1%).

Genetic diversity was low within sequences of *Oreolalax* Po Ma Lung (uncorrected *p*-distance 0.14%, *N*=3; Table 2). The mean uncorrected *p*-distance between *Oreolalax* Po Ma Lung and other taxa in the genus *Oreolalax* (Table 2) ranged from 1.1% (*O. xiangchengensis* from its type locality) to 9.9% (*O. omeimontis*). The mean uncorrected *p*-distance between *Oreolalax* Po Ma Lung and other species in this clade ranged from 1.1% (*O. major*) to 3.0% (*O. jingdongensis*).



**FIGURE 1.** Fifty percent majority-rule consensus phylogram resulting from Bayesian analysis of the mitochondrial 16S rRNA gene of *Oreolalax* and two outgroups in the genera *Leptobranchella* and *Scutiger*. Numbers at nodes are Ultrafast bootstrap (UFB) support values and Bayesian posterior probabilities (BPP) respectively, and numbers at terminal tips are GenBank accession numbers.



**TABLE 2.** Mean uncorrected p-distance between *Oreolalax* species of the 16S rRNA gene. Mean values of genetic distance are given in the lower half of the table. The in-group mean uncorrected p-distances are shown on the diagonal and shaded in bold.

ID	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	<i>Oreolalax adelphos</i> sp. nov.	<b>0.14</b>																			
2	<i>Oreolalax chuanbetensis</i>	6.7	<b>0.00</b>																		
3	<i>Oreolalax jingdongensis</i>	3.0	6.4	<b>0.06</b>																	
4	<i>Oreolalax liangbeiensis</i>	1.3	7.3	3.6	<b>NA</b>																
5	<i>Oreolalax lichuanensis</i>	7.9	10.6	10.0	11.4	<b>2.20</b>															
6	<i>Oreolalax longmenmontis</i>	6.6	1.6	6.2	6.5	10.4	<b>0.00</b>														
7	<i>Oreolalax major</i>	1.1	6.7	2.9	0.8	9.8	6.1	<b>0.07</b>													
8	<i>Oreolalax multipunctatus</i>	6.4	3.7	8.1	8.9	11.8	3.1	8.0	<b>0.14</b>												
9	<i>Oreolalax naniangensis</i>	6.4	0.9	6.7	7.2	10.7	1.4	6.7	3.8	<b>0.00</b>											
10	<i>Oreolalax omeimontis</i> 1	6.3	2.8	7.4	8.4	11.8	3.0	7.7	4.2	2.6	<b>0.27</b>										
11	<i>Oreolalax omeimontis</i> 2	9.9	10.1	9.6	10.1	12.6	8.8	9.5	10.3	9.9	10.9	<b>0.37</b>									
12	<i>Oreolalax pingii</i>	5.9	10.0	8.6	9.8	4.4	8.4	8.0	11.1	9.8	11.2	10.7	<b>NA</b>								
13	" <i>Oreolalax popei</i> "	7.6	2.1	8.0	8.6	11.9	0.0	8.1	4.2	1.8	3.2	10.4	11.5	<b>NA</b>							
14	<i>Oreolalax rhodostigmatus</i>	5.7	8.8	9.0	9.9	12.2	6.9	8.1	10.3	9.3	10.8	11.0	10.8	10.3	<b>2.80</b>						
15	<i>Oreolalax rugosus</i>	1.8	7.3	3.8	1.6	11.3	6.0	1.7	8.7	7.1	8.1	10.0	9.5	8.2	9.1	<b>NA</b>					
16	<i>Oreolalax schmidtii</i>	6.6	10.6	9.0	9.9	4.5	9.1	8.3	11.2	10.4	11.5	11.4	0.8	11.7	11.0	<b>0.14</b>					
17	<i>Oreolalax</i> sp.	3.0	7.1	4.0	3.8	11.3	5.5	3.4	7.9	6.9	8.0	9.9	9.9	8.0	9.4	3.7	10.1	<b>NA</b>			
18	<i>Oreolalax sterlingae</i>	5.8	7.5	6.0	5.8	8.3	7.5	5.5	6.8	7.2	7.3	10.4	6.5	8.1	7.2	5.1	6.9	4.9	<b>0.62</b>		
19	<i>Oreolalax xiangchengensis</i>	1.2	7.1	3.8	2.2	10.7	6.1	2.1	8.5	7.1	8.1	9.7	9.9	8.3	9.7	2.4	10.3	4.1	5.9	<b>NA</b>	
20	<i>Oreolalax</i> cf. <i>xiangchengensis</i>	0.5	7.4	3.7	2.3	10.8	6.7	1.9	8.6	7.4	8.2	10.4	9.8	8.4	9.7	2.3	10.2	4.2	5.9	0.2	<b>0.00</b>

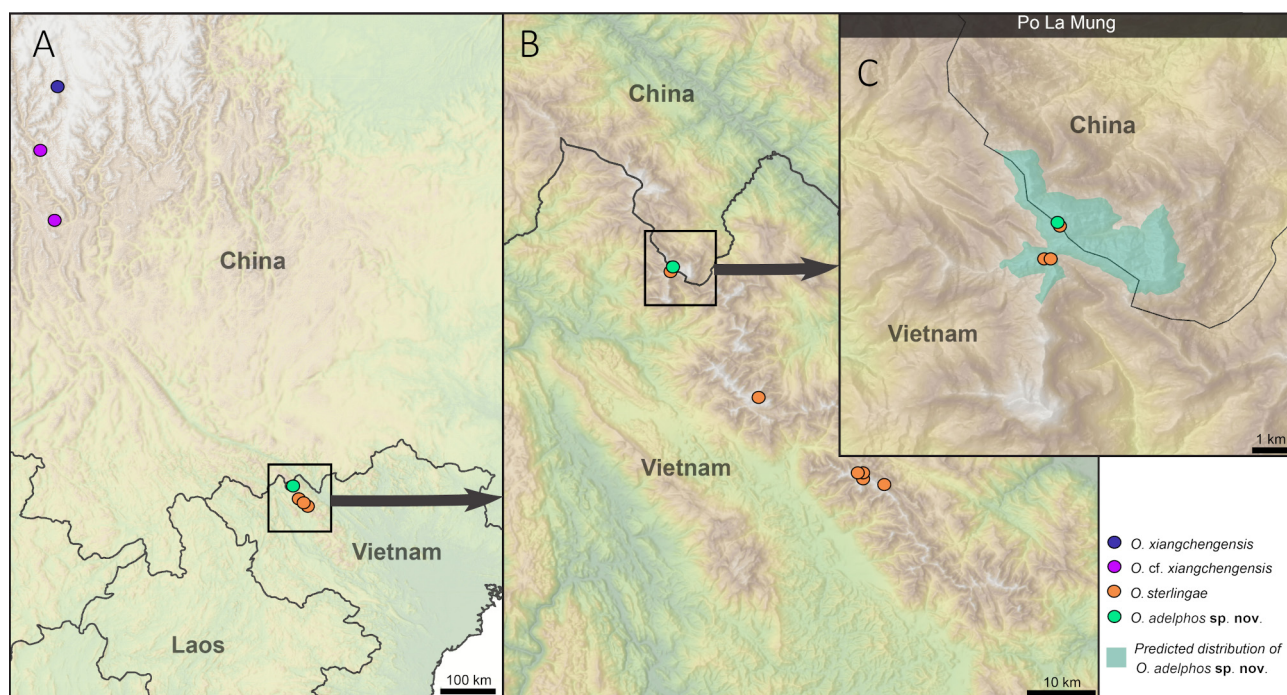
## Taxonomic implications

Uncorrected p-distances of 16S mtDNA between recognised species within the genus *Oreolalax* are typically low (Hou *et al.* 2020; Dufresnes & Litvinchuk 2022). The lowest value between *Oreolalax* Po Ma Lung and a congeneric species was 1.1% and this exceeds that of other named taxa in the genus (e.g., 0.6% between *O. major* and *O. xiangchengensis* and 0.4% between *O. schmidtii* and *O. pingii*; Hou *et al.* 2020) and these taxa have been shown to be robustly supported in the phylogenetic analyses of mitogenomes (Zhao *et al.* 2018; Huang *et al.* 2020; Dufresnes & Litvinchuk 2022). Our data demonstrate congruent differences in mtDNA sequences and morphological characters (see below in “Comparisons”) and show that *Oreolalax* Po Ma Lung from Lai Chau Province of Vietnam is an undescribed taxon. We describe this species as new to science below:

### *Oreolalax adelphos* sp. nov.

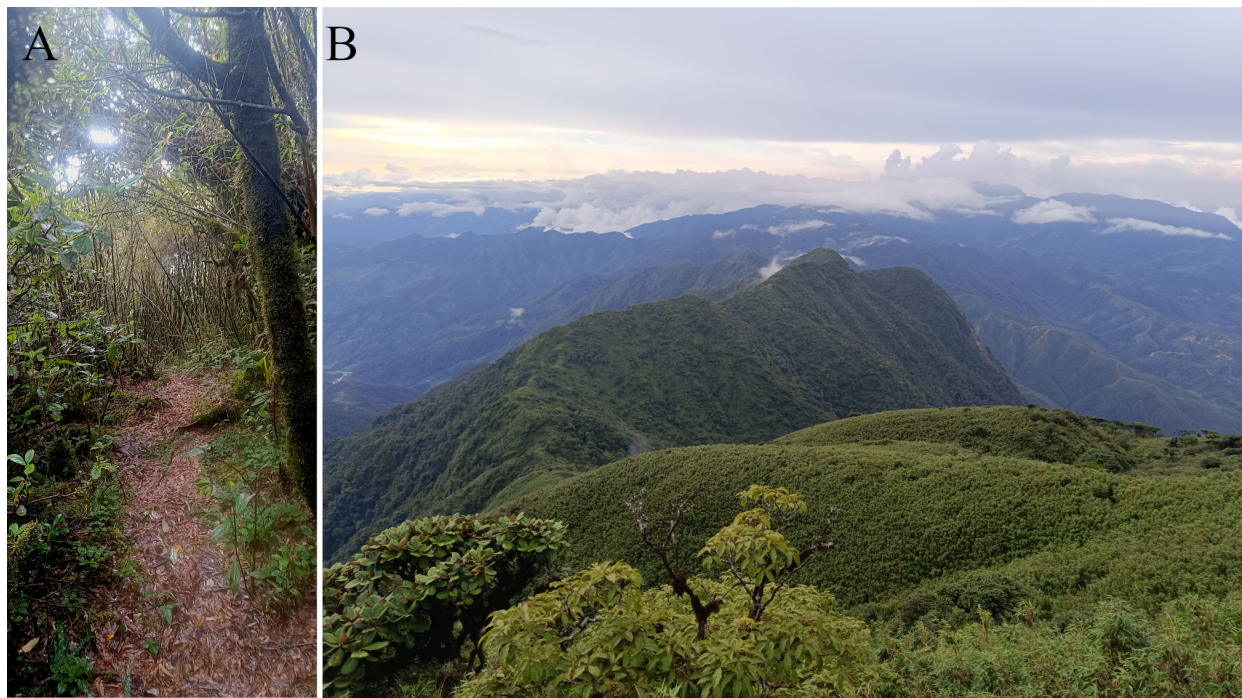
Figs. 4–7

**Holotype.** Adult male (ITBCZ 3619; field tag LNT1144) encountered on a path through bamboo forest on Mount Po Ma Lung, Ban Lang Commune, Phong Tho District, Lai Chau Province, Vietnam (N22 37.581 E103 29.104; 2959 m elevation; Figs 2 & 3.). Collected at 19:53 h on August 10, 2023 by Luan Thanh Nguyen, Chao Van Dat, Ly Manh Ha, and Hoang Van Hung.



**FIGURE 2.** Localities where *Oreolalax adelphos* sp. nov., *Oreolalax sterlingae* and *Oreolalax xiangchengensis* and *Oreolalax* cf. *xiangchengensis* are known to be present, confirmed with molecular data (A), Localities of *Oreolalax adelphos* sp. nov. and *Oreolalax sterlingae* in the Hoang Lien Range of Vietnam confirmed with molecular data (B), the predicted distribution of *Oreolalax adelphos* sp. nov. (C). Pale grey areas indicative of higher elevation, dark green indicative of lowest elevation.

**Paratypes.** Female (ITBCZ 3620; field tag LNT1145) encountered on a path through bamboo forest on Mount Po Ma Lung, Ban Lang Commune, Phong Tho District, Lai Chau Province, Vietnam (N22 37.538 E103 29.112; 2920 m elevation) and an adult female (ITBCZ 3621; field tag LNT1146) encountered on a path through bamboo forest on Mount Po Ma Lung, Phong, Ban Lang Commune, Phong Tho District, Lai Chau Province, Vietnam, (N22 37.476 E103 29.148; 2914 m elevation). Both collected at 20:30 h on August 10, 2023 by Luan Thanh Nguyen, Chao Van Dat, Ly Manh Ha, and Hoang Van Hung.



**FIGURE 3.** Habitat *Oreolalax adelphos* **sp. nov.** (A) Microhabitat of *Oreolalax adelphos* **sp. nov.** Mount Po Ma Lung Lai Chau Province, northwest Vietnam, and (B) Macrohabitat of *Oreolalax adelphos* **sp. nov.** Mount Po Ma Lung Lai Chau Province, northwest Vietnam.

**Etymology.** Specific epithet ‘*adelphos*’ the masculine adjective of the transliterated Greek word meaning brother, in reference to the fact that this is the second *Oreolalax* species known from Vietnam and that the two species are sympatric at one site.

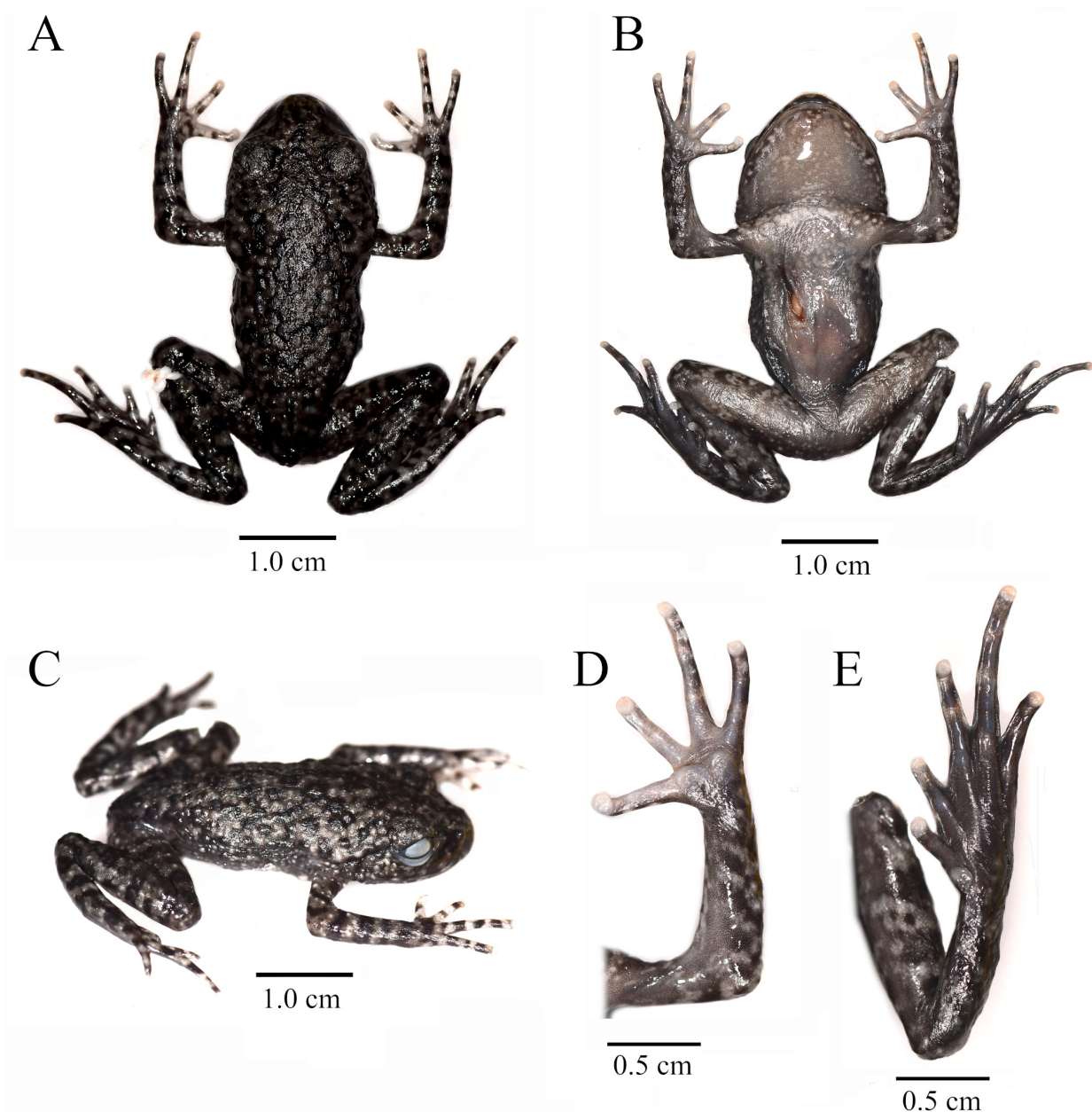
**Suggested vernacular name.** Mount Po Ma Lung toothed toad (English), Cóc răng pò ma lung (Vietnamese).

**Diagnosis.** *Oreolalax adelphos* **sp. nov.** is placed in the genus *Oreolalax* based on its molecular phylogenetic position and the following morphological characters: prominent maxillary teeth; a rough dorsal surface with scattered large warts, covered with oval black spots; vertical pupil; and an oval tongue, notched posteriorly (Myers & Leviton 1962; Delorme *et al.* 2006; Fei & Ye 2016). The new species can be diagnosed from congeneric species by the combination of the following characters: (1) SVL of adult male 38.0 mm,  $N=1$ ; adult female 46.2 mm,  $N=1$ ; (2) narrow supratympanic fold; (3) no visible tympanum; (4) head longer than wide; (5) vocal sac absent; (6) absence of subarticular tubercles on hands; (7) basal interdigital webbing on hind feet; (8) ventral surface mottled with grey and creamy white; (9) dorsal surface of head and body covered in rounded, evenly spaced and similar sized tubercles; (10) presence of dark bars on limbs; (11) greyish white and creamy white spots on the flanks, and (12) a bicoloured iris.

**Description of holotype.** Small sized (SVL 38.0 mm); head longer than wide, head rounded anteriorly; snout rounded in profile, protrudes slightly beyond lower jaw; loreal region concave; nares oval and positioned laterally, canthus rostralis distinct, interorbital area flat; tympanum not visible, narrow supratympanic fold extends from behind eye, terminates above axilla; ridges on head lacking; eyes large, eye diameter smaller than snout length; pupil vertical; vomerine teeth and ridges absent, maxillary teeth present; tongue free at back and posteriorly notched; vocal sac absent.

Forelimbs long and gracile; lower arm thicker than upper arm; fingers long and slender, tips of fingers rounded, finger length:  $II < I < IV < III$ , fingers lacking distinct tubercles, fringes, and interdigital webbing; inner metacarpal tubercle distinct large and rounded, palmar tubercle distinct and rounded, smaller than inner metacarpal tubercle; hind limbs, long and gracile; toes with basal interdigital webbing and narrow fringes, lacking distinct tubercles, relative toe length:  $I < II < V < III < IV$ ; toe tips rounded, without discs; inner metatarsal tubercle small and rounded; outer metatarsal tubercle absent.





**FIGURE 4.** *Oreolalax adelphos* sp. nov. adult male holotype ITBCZ 3619 in preservative. (A) Dorsal view, (B) ventral view, (C) dorsolateral view, (D) palmar surface of left hand, and (E) plantar surface of left foot.

Dorsal surfaces of head, body and limbs densely covered with rounded, evenly spaced, and similar sized tubercles; sparse scattering of very small tubercles on upper eyelid and lateral sides of head; large tubercles on flanks, less rounded than those on dorsal surfaces; crest of supratympanic fold covered in rounded tubercles; no tubercles with black tipped asperities; throat smooth, although some small rounded tubercles clustered directly below commissure of jaw; chest, belly, and ventral surfaces of limbs smooth; chest lacking small black spines.

**Colour of holotype in life:** Head and dorsal surface of body greyish brown with numerous, evenly spaced brown-black spots, black spots typically covering tubercles; numerous creamy grey spots, smaller in diameter than brown-black spots but also covering tubercles; flanks greyish brown with greyish white and creamy white spots which are larger on flanks than on dorsal surface; dorsal surface of upper arms greyish brown with two brown-black bars and few creamy grey spots, lower arm greyish brown with four brown-black bars; dorsal surfaces of fingers cream with brown-black bars; dorsal surface of hind limbs dark brown, anterior surface of thigh with dark bars, posterior surface of thighs with small greyish-white spots; dorsal surface of lower leg with brown-black bars;

dorsal surfaces of fingers cream with brown-black bars; throat, chest, belly mottled with grey and creamy white; iris bicolored with black reticulations, metallic silver on lower half, with a hint of copper in the upper half.

**Colour of holotype in preservative:** Head, dorsal surfaces of body and limbs dark brown, black; flanks with greyish white spots; dorsal surfaces of fingers grey with black bars; throat, chest, belly mottled with dark and light grey.

**Variation.** Ventral surface of ITBCZ 3621 is mottled with cream and grey; tibia length is greater than femur length in ITBCZ 3621 (versus femur length greater than tibia length in ITBCZ 3620 and ITBCZ 3619). See Table 3 for measurements of all individuals in the type series.

**TABLE 3.** Morphometric measurements (in mm) of *Oreolalax adelphos* **sp. nov.** Abbreviations are defined in the Materials and methods. HT = holotype; PR = paratype.

Specimens	ITBCZ 3619 (HT)	ITBCZ 3620 (PR)	ITBCZ 3621 (PR)
Sex	M	F	F
SVL	38.0	39.2	46.2
HL	15.0	14.8	19.0
HW	14.8	14.5	17.3
HH	6.7	6.2	7.9
SNT	6.8	6.3	8.1
IN	4.3	5.8	5.2
EN	4.1	3.8	4.0
NS	3.1	3.3	3.4
EYE	5.0	5.2	5.0
IOD	5.0	5.0	6.3
FEL	19.8	19.2	23.5
TIB	18.2	19.0	23.8
TaL	9.4	10.4	13.1
ML	11.2	12.9	13.1
PL	18.2	9.4	24.4
IPL	2.1	2.0	3.1
OPL	1.8	1.9	2.7
F1DSC	1.1	1.0	1.3
F3DSC	0.8	0.9	1.2
T4DSC	1.0	1.0	1.1
IML	1.3	1.7	2.4
PG	1.5	1.8	1.8
FG	1.3	1.8	2.0

**Sex determination and secondary sexual characters:** No evidence of secondary sexual characters. Sex determined by dissection and assessment of gonads or ovaries. Large testes present in ITBCZ 3619. Small eggs present in ITBCZ 3621. No eggs observed in ITBCZ 3620.

**Natural history:** All individuals were associated with a high elevation bamboo forest with scattered *Rhododendron* (Fig. 3A–B). There was rain in the morning before the survey and the air temperature was 15.5 °C at the time of collection. Ambient humidity was 100%. The three individuals were encountered at night on the forest floor about 400 m away from the nearest stream. During the surveys, males were not heard calling and the females we collected were not heavily gravid. Tadpoles were not observed. A single spider was found in the digestive tract of the adult female specimen (ITBCZ 3621). *Oreolalax adelphos* **sp. nov.** is sympatric with *O. sterlingae*.

**Distribution and conservation status:** *Oreolalax adelphos* **sp. nov.** is currently known from a single location at elevations between 2914 and 2920 m, near the summit of Mount Po Ma Lung in the Hoang Lien Range (Figs. 2 & 3). All individuals were collected within 100 m of the border with China. It is almost certain that the species also



occurs in Jinping County, Yunnan Province, China. The species' EOO is currently predicted to be 19.8 km<sup>2</sup> (Fig. 2C). The habitat of this species at the collection site is relatively intact. However, the forest in which this species occurs is being negatively impacted by fuelwood collection for the tourism industry. At elevations below 2300 m asl, the forest is being degraded to establish cardamom plantations. Not enough is currently known about the range of this species. If this species were to be restricted to elevations above 2500 m on Mount Po Ma Lung, and the habitat were to be increasingly degraded due to fuelwood collection this species could qualify for being assessed as Critically Endangered B1ab(iii) in accordance with the IUCN Red List of Threatened Species categories and criteria (see IUCN 2012). However, there is currently only limited data available on this species and so it likely qualifies for being assessed as Data Deficient in accordance with the IUCN Red List of Threatened Species categories and criteria (see IUCN 2012).



**FIGURE 5.** *Oreolalax adelphos* **sp. nov.** in situ. (A) adult male holotype ITBCZ 3619, (B) female ITBCZ 3620, and (C) adult female ITBCZ 3621.

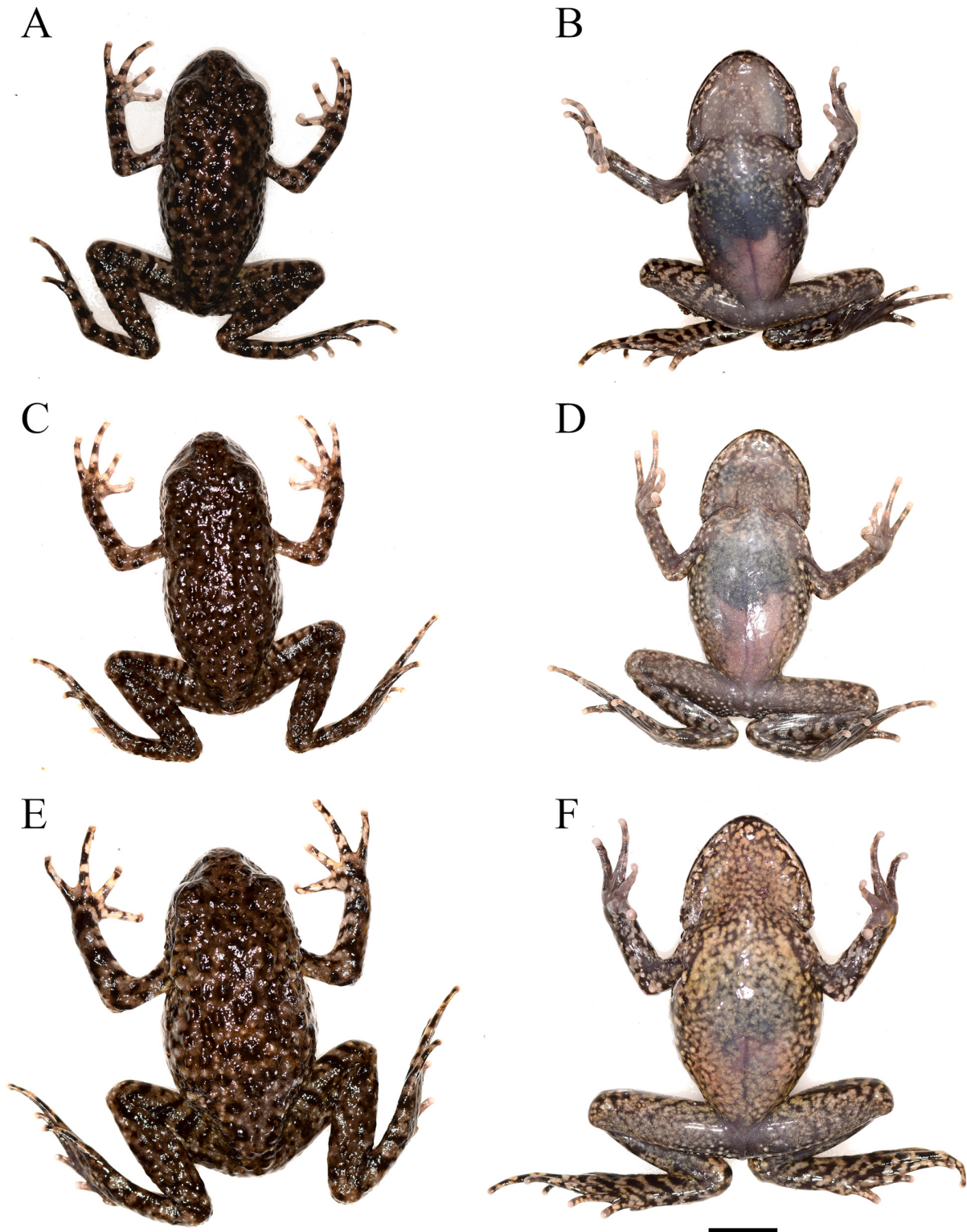
**Comparisons:** *Oreolalax adelphos* **sp. nov.** can be distinguished from all congeneric species on the basis of morphology and for species where comparable sequences exist, molecular data. The following comparisons are based on one adult male and two adult females of *Oreolalax adelphos* **sp. nov.** The SVL of the third specimen (ITBCZ 3620) is not included in the comparisons as sexual maturity of this female was not ascertained when the specimen was dissected, and it could be a subadult.

*Oreolalax adelphos* **sp. nov.** differs from *O. chuanbeiensis* by having a smaller adult male size, SVL 38.0 mm (versus 46.7–56.0 mm,  $N=13$ , in *O. chuanbeiensis*; Hou *et al.* 2020), a smaller adult female size SVL 46.2 mm



(versus 55.0–59.0 mm,  $N=3$ , mm in *O. chuanbeiensis*; Fei & Ye 2016), interdigital toe webbing basal (versus  $\frac{1}{3}$  webbed in *O. chuanbeiensis*; Fei & Ye 2016), and a mottled belly (versus immaculate in *O. chuanbeiensis*).

*Oreolalax adelphos* **sp. nov.** differs from *O. granulosus* by having a smaller adult male size, SVL 38.0 mm (versus 48.6–61.0 mm in *O. granulosus*; Fei & Ye 2016), a smaller adult female size SVL 46.2 mm (versus 47.4–60.0 mm in *O. granulosus*; Fei & Ye 2016), and a grey and creamy white mottled belly (versus yellowish white, without spots or with light grey, fine spots in *O. granulosus*; Fei *et al.* 2012).



**FIGURE 6.** *Oreolalax adelphos* **sp. nov.** in life under sedation. (A) dorsal view of adult male holotype ITBCZ 3619, (B) ventral view of adult male holotype ITBCZ 3619, (C) dorsal view of female paratype ITBCZ 3620, (D) ventral view of female paratype ITBCZ 3620, (E) dorsal view.

**TABLE 4.** Selected diagnostic characters for all species in the genus *Oreolalax*. Grey shading indicates non-overlapping characters when compared to *Oreolalax adelphos* sp. nov. References: 1. Fei & Huang 1983; 2. Ohler & Dubois 1992; 3. Yang & Rao 2008; 4. Fei *et al.* 2012; 5. Nguyen *et al.* 2013; 6. Fei & Ye, 2016; 7. Hou *et al.* 2020; 8. Tapley *et al.* 2023.

Species	SVL adult male (mm)	SVL adult female (mm)	Tympanum visible?	Vocal sac	Interdigital toe webbing	Belly pattern	Skin texture on belly	Tubercles on dorsum	Dark bars on hindlimbs	Dark triangular marking between eyes	Ref
<i>Oreolalax adelphos</i> sp. nov.	38.0 (N=1)	46.2 (N=1)	No	Absent	Basal	Mottled	Smooth	Rounded, evenly spaced, similar sized tubercles	Present	Absent	This study
<i>O. chuanbeiensis</i>	46.7–56.0 (N=13)	55.0–59.0 (N=3)	No	Absent	1/3 webbed	Immaculate	Smooth	Covered in spiny tubercles	Present	Absent	5, 6, 7
<i>O. granulosus</i>	48.6–61.0 (N=?)	47.4–60.0 (N=?)	No	Absent	1/2 – 2/3 webbed	Immaculate or with small spots	Smooth	Covered in small spiny tubercles	Present	Absent	5, 6
<i>O. jingdongensis</i>	49.3–60.3 (N=?)	48.7–56.5 (N=?)	No	Absent	1/3 webbed	Grey spots	Smooth	Covered in large tubercles with thick spines	Present	Present	3, 5, 6
<i>O. liangbeiensis</i>	47.5–56.3 (N=20)	56.0–65.7 (N=8)	No	Absent	1/3 webbed	Immaculate	Smooth or granular	Covered in large and small spiny tubercles	Present	Absent	5, 6
<i>O. lichuanensis</i>	52.9–64.8 (N=20)	57.3–62.2 (N=4)	No	Absent	Basal	Dark flecks	Smooth	Large and small spineless tubercles	Not reported	Absent	4, 6
<i>O. longmenmontis</i>	51.6–64.2 (N=3)	Not reported	No	Basal	Basal	Marbled	Smooth	Large, scattered tubercles	Present	Present	7
<i>O. major</i>	59.2–68.7 (N=20)	65.0–70.0 (N=2)	No	Absent	1/3 webbed	Dark spots	Smooth	Covered in large and small tubercles with black spines	Present	Absent	5, 6
<i>O. multipunctatus</i>	47.4–49.8 (N=4)	Not reported	No	Absent	Basal	With few or without spots	Not reported	Covered in tubercles	Indistinct	Present	5, 6
<i>O. nanjiangensis</i>	52.6–60.0 (N=10)	53.3–58.2 (N=8)	No	Absent	Basal	Immaculate	Smooth	Rough with small tubercles	Not reported	Absent	6, 7
<i>O. omeimontis</i>	49.5–58.4 (N=15)	51.2–56.1 (N=3)	No	Present	Basal	Cloudy spots	Smooth	Round or small elliptic spiny tubercles	Present	Present	4, 5, 6
<i>O. pingii</i>	43.4–51.0 (N=20)	46.8–54.4 (N=20)	No	Absent	Basal	Immaculate	Smooth	Very small tubercles	Present	Absent	5, 6
<i>O. popei</i>	60.0–69.0 (N=20)	51.7–67.0 (N=10)	No	Absent	Basal or absent	Small spots	Smooth	Large tubercles	Present	Absent	5, 6

..... Continued on the next page

TABLE 4. (continued)

Species	SVL adult male (mm)	SVL adult female (mm)	Tympanum visible?	Vocal sac	Interdigital toe webbing	Belly pattern	Skin texture on belly	Tubercles on dorsum	Dark bars on hindlimbs	Dark triangular marking between eyes	Ref
<i>O. puxiongensis</i>	41.3–45.3 (N=20)	43.0–50.0 (N=10)	No	Absent	Absent	Not clearly reported	Granular	Dense tubercles forming long spiny ridges	Not reported	Present	4, 5, 6
<i>O. rhodostigmatus</i>	57.5–73.5 (N=7)	62.4–70.6 (N=2)	Yes	Absent	Basal	Some marbling	Smooth	Small spiny tubercles	Not reported	Absent	6
<i>O. rugosus</i>	44.3–52.6 (N=10)	45.0–54.0 (N=10)	No	Absent	1/4 webbed	Mottled	Smooth	Covered in large spiny tubercles	Not reported	Absent	3, 4, 5, 6
<i>O. schmidtii</i>	40.0–47.0 (N=30)	48.0–54.0 (N=3)	No	Absent	Absent	Immaculate	Smooth	Large and small spiny tubercles	Indistinct	Present	5, 6
<i>O. sterlingae</i>	34.3–41.3 (N=7)	39.5–46.3 (N=11)	No	Absent	Basal	Marbled	Smooth	Head and body covered in irregular shaped warts	Present	Absent	5, 8, This study
<i>O. weigoldi</i>	58.2 (N=1)	Not reported	No	Absent	Not recorded	Marbling around edges	Granular	Rough with spiny tubercles	Present	Not recorded	2, 5
<i>O. xiangchengensis</i>	46.7–48.7 (N=2)	47.3–61.4 (N=10)	No	Absent	Fully webbed	Immaculate	Smooth	Covered in very small spiny tubercles	Absent	Absent	1, 6, This study

*Oreolalax adelphos* **sp. nov.** differs from *O. jingdongensis* by having a smaller adult male size, SVL 38.0 mm (versus 49.3–60.3 mm in *O. jingdongensis*; Fei & Ye 2016), a smaller adult female size SVL 46.2 mm (versus 48.7–56.5 mm in *O. jingdongensis*; Fei & Ye 2016), head longer than wide (versus head wider than long in *O. jingdongensis* Yang & Rao 2008), and the absence of a dark triangular marking between the eyes (versus present in *O. jingdongensis*; Nguyen *et al.* 2013; Fei & Ye 2016).

*Oreolalax adelphos* **sp. nov.** differs from *O. liangbeiensis* by having a smaller adult male size, SVL 38.0 mm (versus 47.5–56.3 mm, *N*=20, in *O. liangbeiensis*; Fei & Ye 2016), a smaller adult female size SVL 46.2 mm (versus 56.0–65.7 mm, *N*=8, in *O. liangbeiensis*; Fei & Ye 2016), and basal interdigital toe webbing (versus  $\frac{1}{3}$  webbed in *O. liangbeiensis*; Fei & Ye 2016), and a mottled belly (versus immaculate in *O. liangbeiensis*; Fei & Ye 2016).

*Oreolalax adelphos* **sp. nov.** differs from *O. lichuanensis* by having a smaller adult male size, SVL 38.0 mm (versus 52.9–64.8 mm, *N*=20, in *O. lichuanensis*; Fei & Ye 2016), a smaller adult female size SVL 46.2 mm (versus 57.3–62.2 mm, *N*=4, in *O. lichuanensis*; Fei & Ye 2016), and head longer than wide (versus head wider than long in *O. lichuanensis*; Fei *et al.* 2012).

*Oreolalax adelphos* **sp. nov.** differs from *O. longmenmontis* by having a smaller adult male size, SVL 38.0 mm (versus 51.6–64.2 mm, *N*=3, in *O. longmenmontis*; Hou *et al.* 2020), interorbital region without dark triangular pattern (versus present in *O. longmenmontis*; Hou *et al.* 2020), and dorsum covered in rounded, evenly spaced and similar sized tubercles (versus large, scattered tubercles in *O. longmenmontis*; Hou *et al.* 2020).

*Oreolalax adelphos* **sp. nov.** differs from *O. major* by having a smaller adult male size, SVL 38.0 mm (versus 59.2–68.7 mm, *N*=20, in *O. major*; Fei & Ye 2016), a smaller adult female size SVL 46.2 mm (versus 65.0–70.0 mm, *N*=2, in *O. major*; Fei & Ye 2016), and interdigital webbing basal (versus  $\frac{1}{3}$  webbed in *O. major*; Fei & Ye 2016).

*Oreolalax adelphos* **sp. nov.** differs from *O. multipunctatus* by having a smaller adult male size, SVL 38.0 mm (versus 47.4–49.8 mm, *N*=4, in *O. multipunctatus*; Fei & Ye 2016), and the absence of a dark triangular marking between the eyes (versus indistinct triangular marking present in *O. multipunctatus*; Fei & Ye 2016).

*Oreolalax adelphos* **sp. nov.** differs from *O. nanjiangensis* by having a smaller adult male size, SVL 38.0 mm (versus 52.6–60.0 mm, *N*=10, in *O. nanjiangensis*; Fei & Ye 2016), a smaller adult female size SVL 46.2 mm (versus 53.3–58.2 mm, *N*=8, in *O. nanjiangensis*; Fei & Ye 2016), and a mottled belly (versus immaculate in *O. nanjiangensis*; Fei & Ye 2016).

*Oreolalax adelphos* **sp. nov.** differs from *O. omeimontis* by having a smaller adult male size, SVL 38.0 mm (versus 49.5–58.4 mm, *N*=15, in *O. omeimontis*; Fei & Ye 2016), a smaller adult female size SVL 46.2 mm (versus 51.2–56.1 mm, *N*=3, in *O. omeimontis*; Fei & Ye 2016), head longer than wide (versus head wider than long in *O. omeimontis*; Fei *et al.* 2012), the absence of a vocal sac (versus presence in *O. omeimontis*; Fei *et al.* 2012), and interorbital region without dark triangular pattern (versus present in *O. omeimontis*; Nguyen *et al.* 2013).

*Oreolalax adelphos* **sp. nov.** differs from *O. pingii* by having a smaller adult male size, SVL 38.0 mm (versus 43.4–51.0 mm, *N*=20, in *O. pingii*; Fei & Ye 2016), and a mottled belly (versus immaculate in *O. pingii*; Fei & Ye 2016).

*Oreolalax adelphos* **sp. nov.** differs from *O. popei* by having a smaller adult male size, SVL 38.0 mm (versus 60.0–69.0 mm, *N*=20, in *O. popei*; Fei & Ye 2016), a smaller adult female size SVL 46.2 mm (versus 51.7–67.0 mm, *N*=10, in *O. popei*; Fei & Ye 2016), and a mottled belly (versus covered by small grey spots in *O. popei* Fei and Ye. 2016).

*Oreolalax adelphos* **sp. nov.** differs from *O. puxiongensis* by having a smaller adult male size, SVL 38.0 mm (versus 41.3–45.3 mm, *N*=20, in *O. puxiongensis*; Fei & Ye 2016), dorsum covered in rounded, evenly spaced and similar sized tubercles (versus dorsum with dense tubercles forming spiny ridges in *O. puxiongensis*; Fei & Ye 2016); the absence of a dark triangular marking between the eyes (versus present in *O. puxiongensis*, Fei & Ye 2016), by having smooth skin on the belly (versus granular skin on the belly in *O. puxiongensis*; Nguyen *et al.* 2013), and head longer than wide (versus head wider than long in *O. puxiongensis*; Fei *et al.* 2012).

*Oreolalax adelphos* **sp. nov.** differs from *O. rhodostigmatus* by having a smaller adult male size, SVL 38.0 mm (versus 57.5–73.5 mm, *N*=7, in *O. rhodostigmatus*; Fei & Ye 2016), a smaller adult female size SVL 46.2 mm (versus 62.4–70.6 mm, *N*=2, in *O. rhodostigmatus*; Fei & Ye 2016), no visible tympanum (versus distinct tympanum in *O. rhodostigmatus*; Fei & Ye 2016), dorsum covered in rounded, evenly spaced and similar sized tubercles (versus dorsum fully covered in small spiny tubercles in *O. rhodostigmatus* (Nguyen *et al.* 2013), and greyish white and creamy white spots on the flanks (versus orange-red spots in *O. rhodostigmatus*; Fei & Ye 2016).



*Oreolalax adelphos* **sp. nov.** differs from *O. rugosus* by having a smaller adult male size, SVL 38.0 mm (versus 44.3–52.6 mm,  $N=10$ , in *O. rugosus*; Fei & Ye 2016), head longer than wide (versus head wider than long in *O. rugosus* Yang & Rao 2008), and a belly colour of mottled grey and creamy white (versus entirely beige yellow, sometimes with some grey brown mottling in *O. rugosus*; Fei *et al.* 2012).

*Oreolalax adelphos* **sp. nov.** differs from *O. schmidtii* by having a smaller adult female size SVL 46.2 mm (versus 48.0–54.0 mm,  $N=3$ , in *O. schmidtii*; Nguyen *et al.* 2013), the absence of a dark triangular marking between the eyes (versus present in *O. schmidtii*; Nguyen *et al.* 2013), and a mottled belly (versus immaculate in *O. schmidtii*; Fei & Ye 2016).

*Oreolalax adelphos* **sp. nov.** differs from *O. sterlingae* by having a rounded snout in dorsal and ventral view (versus almost semicircular in *O. sterlingae*; material examined Fig. 7), supratympanic fold relatively narrow (versus thick and well developed in *O. sterlingae*; Nguyen *et al.* 2013; material examined; Fig. 7), dorsal surface of head and body covered in rounded, evenly spaced and similar sized tubercles (versus head and body covered in irregular shaped warts in *O. sterlingae*; Nguyen *et al.* 2013; Fig. 7), and a bicolored iris (versus uniform gold iris with black reticulations in *O. sterlingae*; Nguyen *et al.* 2013; Tapley *et al.* 2020).

*Oreolalax adelphos* **sp. nov.** differs from *O. weigoldi* by having a smaller adult male size, SVL 38.0 mm (versus 58.2 mm,  $N=1$ , in *O. weigoldi*; Ohler & Dubois 1992), a mottled belly (versus marbling around the edges in *O. weigoldi*; Ohler & Dubois 1992), and by having smooth skin on the belly (versus granular skin on the belly in *O. weigoldi*; Nguyen *et al.* 2013).

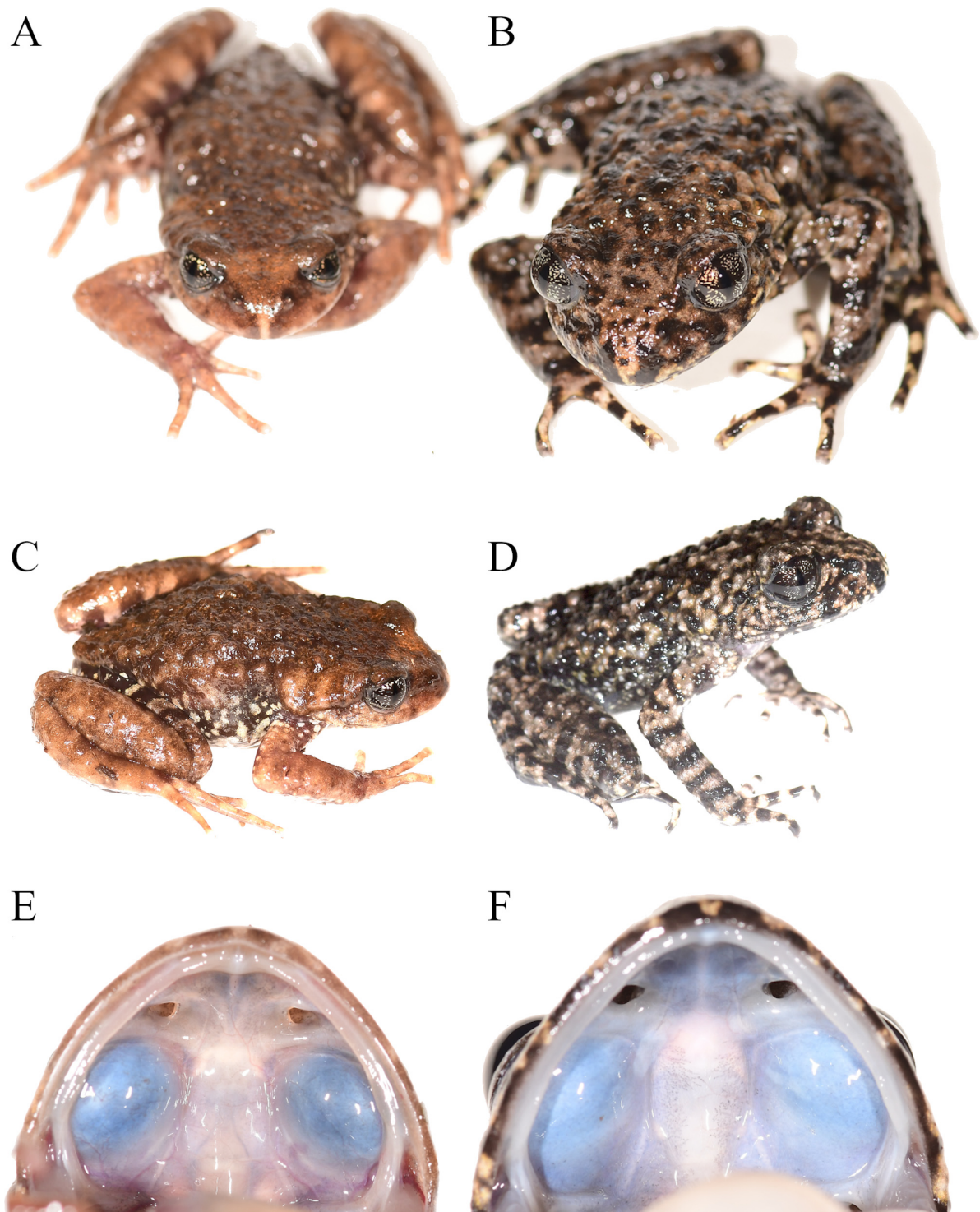
*Oreolalax adelphos* **sp. nov.** differs from *O. xiangchengensis* by having a smaller adult male size, SVL 38.0 mm (versus 46.7–48.7 mm,  $N=2$ , in *O. xiangchengensis*; examined material; Table 5; Fig. 8), dorsum covered in rounded, evenly spaced and similar sized tubercles (versus dorsum fully covered in very small spiny tubercles in *O. xiangchengensis*; examined material; Fig. 8), dorsal belly colour mottled with grey and creamy white (versus entirely beige yellow in *O. xiangchengensis*; Fei & Huang 1983; examined material; Fig. 8), by having rudimentary interdigital webbing on feet (vs. fully webbed in *O. xiangchengensis*; Fei & Huang 1983; examined material; Fig. 8B), the absence of subarticular tubercles on the hands (versus presence in *O. xiangchengensis*; examined material; Fig. 8B), and the presence of dark bars on limbs (versus absent in *O. xiangchengensis*; Fei & Huang 1983).

## Discussion

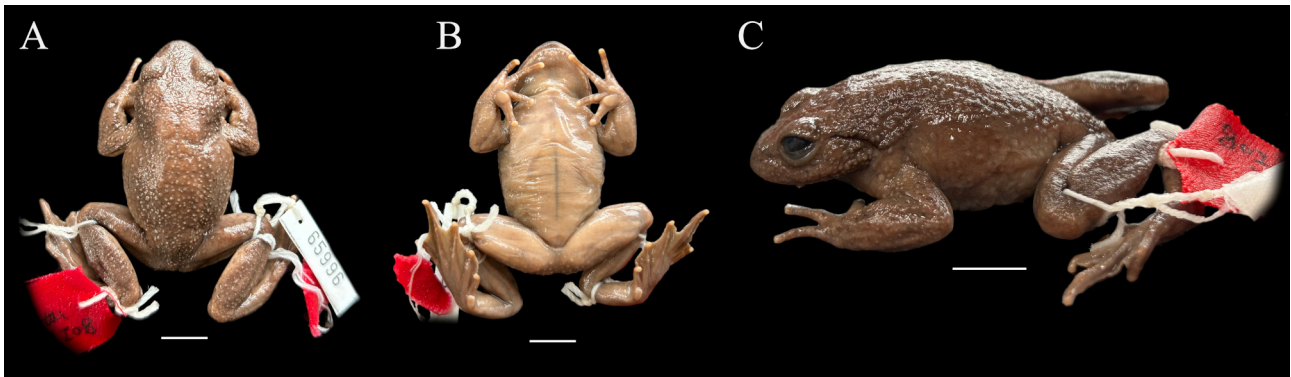
*Oreolalax adelphos* **sp. nov.** is the second species of *Oreolalax* recorded from Vietnam. It is sympatric with *O. sterlingae* on Mount Po Ma Lung. *Oreolalax adelphos* **sp. nov.** has only been recorded once and from elevations above 2900 m whereas *Oreolalax sterlingae* has been recorded from several sites in the Hoang Lien Range at elevations as low as 2345 m (Tapley *et al.* 2020). *Oreolalax adelphos* **sp. nov.** might be restricted to high elevation sites which limits dispersal further south in Vietnam due to the presence of valleys. Both *Oreolalax adelphos* **sp. nov.** and *O. sterlingae* almost certainly occur in China as both species we encountered less than 100 m from the China-Vietnam border. It is unknown what the quality of the habitat is on the Chinese side of the border or if there are any different potential threats to the species in this location.

Sympatry has been documented among other *Oreolalax* species (e.g. Zhao *et al.* 1994; Wang *et al.* 2020); on Mount Emei in China, four species of *Oreolalax* occur at the same elevation (Wang *et al.* 2020) and would be expected to compete with one another (Wang *et al.* 2022). Such competition might be reduced by breeding at different times of year and this likely occurs with *Oreolalax* occurring on Mount Emei with slightly staggered breeding periods between many of the species distributed there (Fei & Ye 2016) or by larval specialism (e.g. Sun *et al.* 2023). The reproductive biology of *Oreolalax adelphos* **sp. nov.** is unknown. Male *Oreolalax* develop patches of black spines on their chest and nuptial spines on their fingers during the breeding season; in some species, males also develop black spines on their upper lips. The documented breeding season for *Oreolalax* is variable and ranges from January to March in *O. granulosus* and *O. jingdongensis* (Liang & Ye 2016); April in *O. popei* (Liang & Ye 2016), April and May in *O. rugosus*, *O. xiangchengensis* (Liang & Ye 2016); May in *O. lichuanensis*, *O. multipunctatus* (Liang & Ye 2016) and *O. sterlingae* (authors unpublished data); May to June in *O. chuanbeiensis*, *O. major*, *O. pingii* and *O. schmidtii* (Liang & Ye 2016), and June in *O. liangbeiensis*, *O. omeimontis* and *O. puxiongensis* (Liang & Ye 2016). We collected the specimens of *Oreolalax adelphos* **sp. nov.** in August, and male secondary sexual characters may not have been developed at this time. The collected male did have well developed testes indicative of sexual

maturity. The absence of spines on the adult male specimen of *Oreolalax adelphos* **sp. nov.** indicate that the animals were collected outside the breeding season. However, further work would need to be undertaken to confirm if this secondary sexual character is present in males of this species. We suggest that field work should be conducted from March to May to document the presence or absence of male secondary sexual characters. The description of these characters could improve species identification in the field during the breeding season.



**FIGURE 7.** Comparison between *Oreolalax sterlingae* and *Oreolalax adelphos* **sp. nov.** (A) male *Oreolalax sterlingae* ITBCZ 3622 in life, (B) female *Oreolalax adelphos* **sp. nov.** ITBCZ 3621 in life, (C) dorsolateral view of male *Oreolalax sterlingae* ITBCZ 3622 in life, (D) dorsolateral view of male *Oreolalax adelphos* **sp. nov.** ITBCZ 3619, (E) oral cavity of male *Oreolalax sterlingae* (ITBCZ 3622) under sedation, and (F) oral cavity of female *Oreolalax adelphos* **sp. nov.** (ITBCZ 3621) under sedation.



**FIGURE 8.** *Oreolalax xiangchengensis* adult male holotype CIB 8011286 in preservative. (A) Dorsal view, (B) ventral view, and (C) dorsolateral view.

**TABLE 5.** Morphometric measurements (in mm) of *Oreolalax xiangchengensis*. Abbreviations are defined in the Materials and methods. HT = holotype; PR = paratype.

Specimens	CIB065996 (HT)	CIB065994 (PT)	CIB065995 (PT)	CIB089134	CIB089142
Sex	M	F	F	M	F
SVL	48.7	53.0	60.4	46.7	47.3
HL	17.3	17.7	21.0	16.8	16.6
HW	18.2	18.9	23.8	18.2	17.0
HH	8.0	7.8	9.2	7.1	8.7
SNT	8.6	8.1	8.6	7.7	8.0
IN	4.7	5.6	6.2	4.6	5.1
EN	3.8	3.7	4.0	3.4	3.8
EYE	5.2	4.4	6.0	4.7	4.6
IOD	4.5	5.2	5.8	5.6	5.4
FEL	23.3	24.2	26.9	21.9	21.8
TIB	22.4	22.8	23.9	21.1	21.4
TaL	32.7	36.2	36.3	32.3	32.1
ML	13.9	14.1	14.8	11.9	14.0
PL	23.5	24.6	25.5	22.3	23.0
IPL	3.2	3.2	3.1	3.0	2.4
OPL	3.4	2.9	2.6	2.6	2.7
F1DSC	No visible disc	No visible disc	No visible disc	No visible disc	No visible disc
F3DSC	No visible disc	No visible disc	No visible disc	No visible disc	No visible disc
T4DSC	No visible disc	No visible disc	No visible disc	No visible disc	No visible disc
IML	2.7	2.9	2.9	2.9	2.1
PG	12.1	Not visible	Not visible	10.8	Not visible
FG	Not visible	3.1	3.6	Not visible	3.2

In our Bayesian analysis, *Oreolalax adelphos* **sp. nov.** is sister to *Oreolalax xiangchengensis* which is known to occur in Sichuan and Yunnan provinces in China (Fei & Huang 1983; Fei *et al.* 2012; Yuan *et al.* 2022). The closest known record of *O. xiangchengensis* to mount Po Ma Lung is 580 km to the northwest in Lanping County in Yunnan Province (Fig. 2A). Fu *et al.* (2007) assigned *Oreolalax* sequences obtained from specimens collected from Zhongdian and Li Jiang counties in Yunnan to *O. xiangchengensis*, but no description of morphology was provided.



The collection sites in Zhongdian and Li Jiang counties are at least 70 and 180 km to the south of the type locality for *O. xiangchengensis* respectively, and 719 and 615 km to the northeast of the type locality of *Oreolalax adelphos* **sp. nov.** The uncorrected *p*-distances of 16S mtDNA between *Oreolalax adelphos* **sp. nov.** and the sequences collected from Zhongdian and Li Jiang counties in Yunnan were 0.5% which still greater than the uncorrected *p*-distances reported between named species e.g. Hou *et al.* (2020), but the uncorrected *p*-distance between these two samples to *O. xiangchengensis* collected from its type locality was 0.2% (i.e. a lower uncorrected *p*-distance between the Zhongdian and Li Jiang counties in Yunnan and *Oreolalax adelphos* **sp. nov.**). Results of phylogenetic analyses (both ML and BI) nest the samples collected from Zhongdian and Li Jiang counties Yunnan with *O. xiangchengensis* obtained from a specimen collected at the type locality but with a low support (UFB/BPP 70/0.52, respectively). Based on these results which utilise a small fragment of mtDNA and because that mitochondrial discordance is commonplace in frogs, we refer to the Yunnan population as *O. cf. xiangchengensis* and suggest further research is undertaken, especially on morphology of this population, to assess its taxonomic status. The only known localities for *Oreolalax adelphos* **sp. nov.** and localities for both *O. xiangchengensis* and *O. cf. xiangchengensis* are separated by distances of at least 580 km, furthermore there are large areas of low elevation habitat between these sampling points which are not suitable for *Oreolalax* (Fig. 2A).

The Hoang Lien Range is the southernmost range limit for *Oreolalax* and several other amphibian genera (Bain & Hurley 2011), e.g., *Atympanophrys* (Luong *et al.* 2019) and *Bombina* (Bain *et al.* 2011) and the Range is a zone of exceptionally high amphibian phylogenetic diversity (Fritz & Rahbek 2012) and a global hotspot for threatened amphibians (Luedke *et al.* 2023). Further work is needed to study and conserve this imperilled amphibian community.

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## Appendix I

*Oreolalax sterlingae* (N=17, AMS R. 177528, AMS R. 177530–1, AMS R. 177535–6, AMS R. 177545–46 (adult males), AMS R. 177532, AMS R. 177534, AMS R. 177537–42, AMS R. 177544, AMS R. 177533 collected from Mount Fansipan (22.3146°N 103.7657°E, elevation 2783 m, Hoang Lien National Park, Sa Pa District, Lao Cai Province, Vietnam) in June 2012.

*Oreolalax xiangchengensis* (N=5), CIB80I1286 (holotype), CIB80I1305 (paratype), CIB80I1342 (allotype) collected from 2,680–2,800 m, Yajin, Xiangcheng, in July 1980, and CIB089134 and CIB089142 collected from 2,700 m, Weidongxiaba, Xiangcheng, in June 1982.