

THE FIRST RECORDS OF *Limnonectes kohchangae* (SMITH, 1922) AND
Sylvirana mortenseni (BOULENGER, 1903)
FROM PHU QUOC ISLAND, SOUTHERN VIETNAM

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SUMMARY

We provide the first detailed records of *Limnonectes kohchangae* (Smith, 1922) and *Sylvirana mortenseni* (Boulenger, 1903) for Phu Quoc island, Phu Quoc National Park, Kien Giang province, Southern Vietnam. These records are supported by morphology and molecular data. *Sylvirana mortenseni* was associated with large streams between 23 – 102 m asl. This species has an adult snout–vent length of 46.0 – 80.9 mm in 3 adult males and 47.7 – 69.2 mm in 3 adult females. Males have large humeral glands and well developed nuptial pads; a lack of oblique, triangular or teardrop-shaped marking posterior to the tympanum; a dark stripe beneath the dorsolateral fold which lacks a strong demarcation between the dark upper surfaces of the flank and the lighter lower surface; and distinct dark marbling on the posterior surfaces of the thighs. *Limnonectes kohchangae* was associated with stream headwaters and small streams between 34–201 m asl. Individuals from Phu Quoc Island had an adult snout – vent length ranging from 34.3 – 41.8 mm in 9 males and 33.3 – 38.2 mm in 2 females. Dorsum with distinctly elongated tubercles and arranged in rows; a bicoloured tympanum with heavily pigmented upper half; dark spots present on flanks; and a broad band on shank with maximum shank band width > 50% horizontal diameter of eye. This report increases the number of amphibians known to occur on Phu Quoc Island from 14 to 16 species.

Keywords: Amphibians, Dicroglossidae, Ranidae, Vietnam.

1. INTRODUCTION

Vietnam harbours a large number of amphibian species, with 275 species recorded so far (Krzikowski *et al.*, 2022). A total of 14 anuran amphibian species are reported to occur on Phu Quoc Island in Kien Giang province, Southern Vietnam (Vassilieva *et al.*, 2016). During field work in Phu Quoc National Park, we encountered two populations of frogs that have not been reported as occurring on Phu Quoc Island previously, or had been reported without any supporting data. We provide the first detailed records of *Limnonectes kohchangae* (Smith, 1922) and *Sylvirana mortenseni* (Boulenger, 1903) from Phu Quoc Island. Our observations are based on 11 specimens of *L. kohchangae* and 6 specimens of *S. mortenseni*.

2. RESEARCH METHODOLOGY

Field work was undertaken at night in June 2020, along forest streams in evergreen

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lowland forest in Phu Quoc National Park, Phu Quoc Island, Kien Giang province, Vietnam. Geographic coordinates were obtained using a Garmin GPSMAP 60CSx Global Positioning System receiver (Garmin Ltd., Kansas, USA) and recorded in the World Geodetic System 1984 datum. All specimens were photographed in life before being euthanised using a 20% solution of benzocaine applied to the ventral surface of the frog; prior to fixation in 5% formalin with consequent storing in 70% ethanol. Specimens were deposited at the Institute of Tropical Biology Zoological Collection (ITBCZ), Ho Chi Minh City, Vietnam. Tissues for genetic analyses were taken from liver prior to preservation and stored in 95% ethanol. Sex was determined by direct observation of calling males in life or by gonadal dissection (for female specimens).

DNA extraction, amplification, and sequencing of a 970 – 973 bp fragment of mtDNA that encodes part of the 16S ribosomal

RNA (16S) gene followed Phimmachak *et al.* (2018) for *Limnonectes kohchangae* and Sheridan & Stuart (2018) for *Sylvirana mortenseni*. The raw nucleotide sequence generated was initially verified manually by eye using BioEdit 7.0.5.2 (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. The new sequences were deposited in GenBank under the accession numbers ON951664 (*Limnonectes kohchangae*) and ON952479 (*Sylvirana mortenseni*).

We recorded morphological data (to the nearest 0.1 mm) with digital callipers from fixed specimens. Selected measurements and morphometric abbreviations follow Phimmachak *et al.* (2018) and Sheridan & Stuart (2018): 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); diameter of exposed portion of eyeball (EYE); interorbital distance (IOD); horizontal diameter of tympanum (TMP); distance from anterior edge of tympanum to posterior corner of eye (TEY); 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 of outer metacarpal tubercle (OPL); maximum length of inner metatarsal tubercle (IML), and maximum length of outer metatarsal tubercle (OML).

3. RESULTS

Limnonectes kohchangae were encountered in small streams and the headwaters of streams (Fig. 1). Individuals were collected at Bai Thom village, Bai Thom commune on the 12th

of June 2020 (ITBCZ 3596 – 9; 10.3780°N, 104.0096°E, 102 m asl); Bai Thom village, Bai Thom commune on the 16th of June 2020 (ITBCZ 3600–1; 10.3989°N, 104.0221°E, 34 m asl); Ong Cu Pagoda Area, Bai Bon village, Ham Ninh commune on the 20th of June 2020 (ITBCZ 3602 – 4; 10.3227°N, 104.0612°E, 201 m asl); and Rach Tram Stream, Bai Thom commune on the 22nd of June 2020 (ITBCZ 3605–6; 10.4306°N, 103.9958°E, 41 m asl). The three individuals collected at Ong Cu Pagoda Area, Bai Bon village, Ham Ninh commune were male frogs that were located by the sound of their calls. All specimens were collected by Luan Thanh Nguyen, Manh Van Le and Ngon Quang Lam. Measurements, colouration and morphological characters (Table 1; Fig. 1B–D) of *L. kohchangae* collected from Phu Quoc National Park agree with those reported by Phimmachak *et al.* (2018): dorsum with distinctly elongated tubercles and arranged in rows; a bicoloured tympanum with heavily pigmented upper half; dark spots present on flanks; broad band on shank with maximum shank band width > 50% horizontal diameter of eye. Adult male SVL is reported to range from 37.6 – 46.4 mm (Phimmachak *et al.*, 2018), whereas the SVL ranged from 34.3 – 41.8 mm in specimens collected in Phu Quoc National Park. Adult female SVL is reported to range from 36.0 – 41.2 mm (Phimmachak *et al.*, 2018), whereas adult females in our series ranged from 33.3 – 38.2 mm. In the original species description, the head is described as being broader than it is long (Smith, 1922) and this character has been used as a diagnostic character in other taxonomic works (e.g., Pham *et al.*, 2018). Head length was greater than head width in several individuals collected in Phu Quoc National Park (ITBCZ 3599, ITBCZ 3601 – 6), therefore, this character can no longer be considered diagnostic. Species identity of was confirmed using molecular analysis of tissue samples from one adult male specimen (ITBCZ 3602).

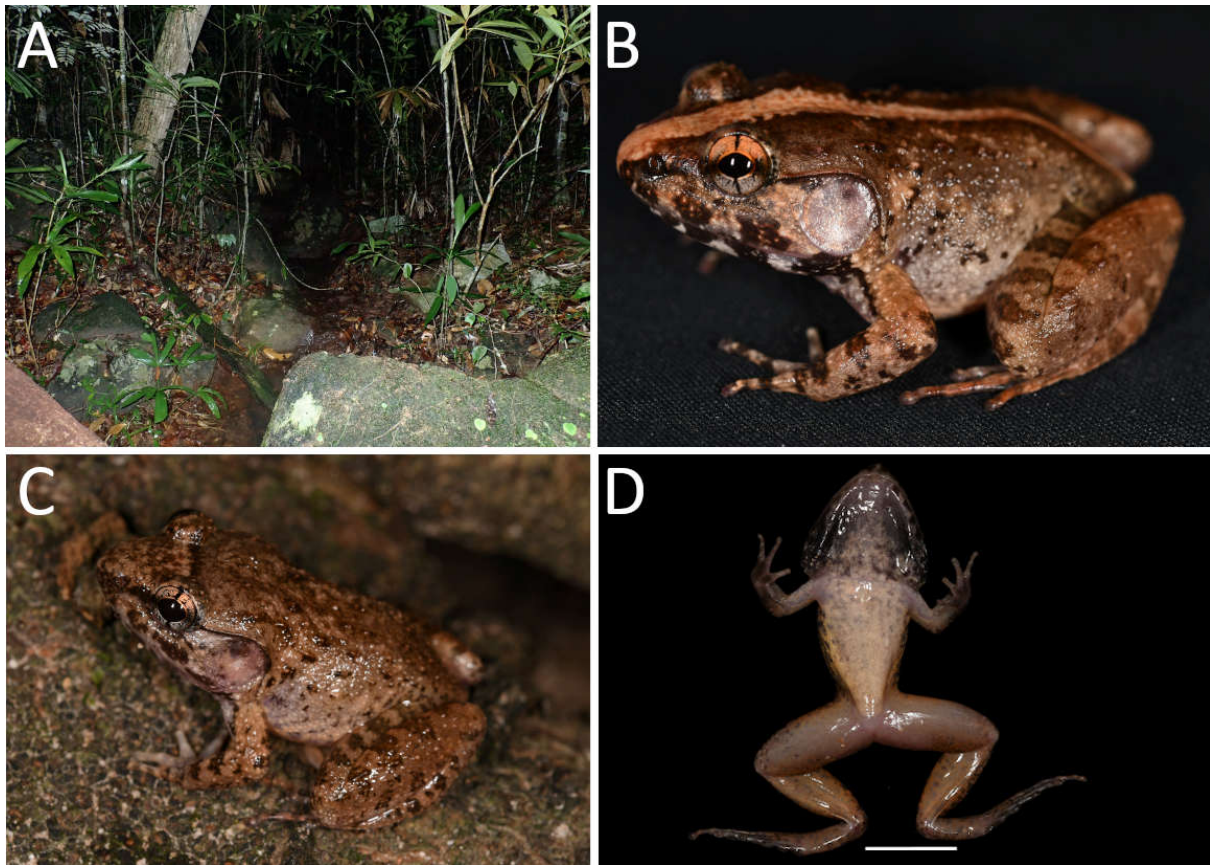


Figure 1. The small stream in Phu Quoc National Park, Bai Thom village, Bai Thom commune, where *Limnonectes kohchangae* were collected (A); dorsolateral view of adult ITBCZ3600 in life (B); dorsolateral view of adult male *Limnonectes kohchangae* ITBCZ 3601 in life (C); ventral view of adult male *Limnonectes kohchangae* ITBCZ 3601 under sedation (D) (Scale bar is 10 mm)

The 16S rDNA new sequence (accession number ON951664) was 98.86% identical to a sequence from *L. kohchangae* collected 30 km away in Kampot province, Cambodia (GenBank accession number KY768802; Phimmachak *et al.*, 2018).

Sylvirana mortenseni were commonly encountered along large streams (Fig 2A).

Individuals were collected at a stream that flows towards Duong Dong Lake on the 11th of June 2020 (ITBCZ 3590 – 2; 10.2505°N, 104.0205°E, 27 m asl); Bai Thom village, Bai Thom commune on the 12th of June 2020 (ITBCZ 3593; 10.3780°N, 104.0096°E, 102 m asl); Da Ban Stream, Cua Duong commune on the 18th of June 2020 (ITBCZ 3594; 10.2444°N, 104.0309°E, 23 m asl); and Da Chong village, Bai Thom commune on the 19th

of June 2020 (ITBCZ 3595; 10.3513°N, 104.0679°E, 45 m asl). All specimens were collected by Luan Thanh Nguyen, Manh Van Le and Ngon Quang Lam. Measurements, colouration and morphological characters (Table 2; Fig. 2B–C) of *S. mortenseni* largely agree with those reported by Sheridan and Stuart (2018): expanded digit tips with circummarginal grooves; finely granular skin on the dorsal surface; adult male SVL of 46.0–80.9 mm, adult female SVL of 47.7 – 69.2 mm (although ITBCZ 3594 had a SVL of 45.2 mm); males with large humeral glands and well developed nuptial pads; a lack of oblique, triangular or teardrop-shaped marking posterior to the tympanum; a dark stripe beneath the dorsolateral fold but lacking a strong demarcation between the dark upper

surfaces of the flank and the lighter lower surface; and distinct dark marbling on the posterior surfaces of the thighs. Species identity of was confirmed using molecular analysis of tissue samples from one adult male specimen (ITBCZ 3595); the 16S rDNA new

sequence (accession number ON952479) was 98.97% identical to a sequence from *S. mortenseni* collected approximately 170 km away in Koh Kong province, Cambodia (GenBank accession number KR264076; Oliver *et al.*, 2015).

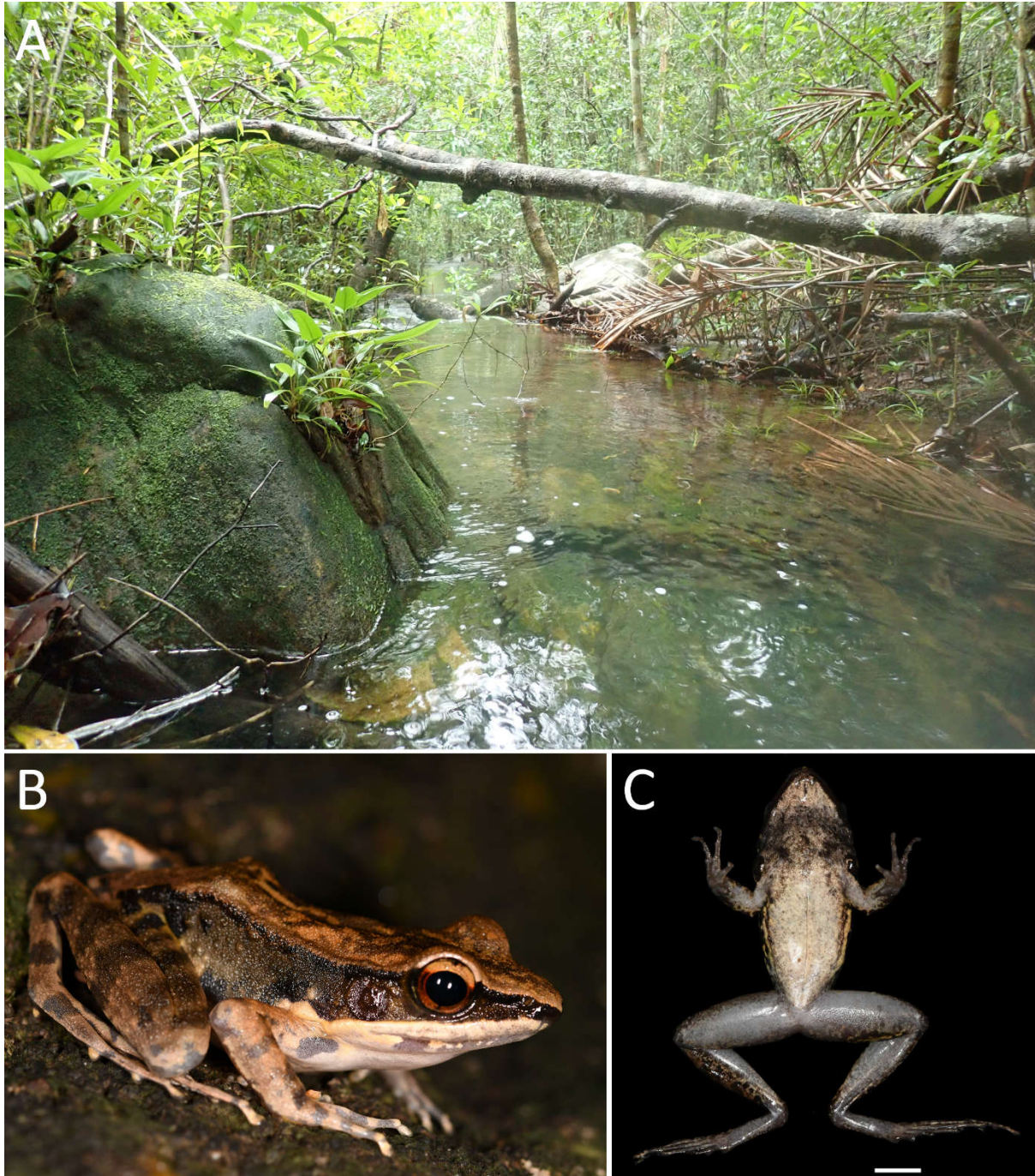


Figure 2. The large stream in Phu Quoc National Park, Bai Thom village, Bai Thom commune where *Sylvirana mortenseni* were collected - A; dorsolateral view of adult male *Sylvirana mortenseni* ITBCZ 3595 in life - B; ventral view of adult male *Sylvirana mortenseni* ITBCZ 3595 under sedation -C
(Scale bar is 10 mm)

Table 1. Detailed morphometric measurements of *Limnonectes kohchangae* collected in Phu Quoc National Park

Voucher #	ITBCZ 3596	ITBCZ 3597	ITBCZ 3598	ITBCZ 3599	ITBCZ 3600	ITBCZ 3601	ITBCZ 3602	ITBCZ 3603	ITBCZ 3604	ITBCZ 3605	ITBCZ 3606
Field tag #	LNT 2603	LNT 2604	LNT 2605	LNT 2611	LNT 2697	LNT 2698	LNT 2755	LNT 2756	LNT 2757	LNT 2773	LNT 2774
Sex	Male	Male	Male	Male	Male	Male	Male	Male	Male	Female	Female
SVL	35.3	41.1	36.7	41.8	37.7	37.1	41.0	34.3	41.5	38.2	33.1
HDL	16.3	19.6	17.6	19.0	18	17.7	18.8	15.7	18.5	16.7	14.1
HDW	17.5	19.6	17.8	18.8	17.7	17.0	17.4	15.6	18.2	14.4	12.7
HH	8.9	9.5	8.5	8.7	8.2	8.1	8.7	7.8	8.6	7.6	7.5
SNT	6.1	7.7	7.1	5.8	6.3	6.5	7.3	6.0	7.4	6.0	5.8
IN	4.3	5.6	4.5	4.8	5.2	4.3	4.5	3.2	5.1	4.3	4.0
EN	4.0	4.3	3.7	4.0	4.2	3.4	5.0	3.4	3.9	4.2	3.5
EYE	4.6	5.4	4.3	4.6	4.2	4.6	5.0	4.7	4.7	5.0	4.2
IOD	4.1	5.0	4.4	4.4	4.5	4.1	4.1	3.6	4.7	3.8	4.0
TMP	5.2	6.1	5.6	6.0	5.0	5.8	5.6	5.0	5.6	3.8	3.0
TEY	1.3	2.7	2.8	2.9	2.5	2.6	3.0	2.9	2.7	1.4	1.5
FEL	17.6	19.7	16.4	19.2	18.9	17.0	18.8	17.7	21.3	17.0	15.4
TIB	18.0	21.5	17.7	20.8	18.3	17.7	20.2	17.0	20.0	18.0	16.6
TaL	9.0	11.4	8.2	10.2	9.1	8.2	9.3	9.2	9.4	8.7	7.5
ML	9.4	11.0	9.1	10.8	9.1	9.1	10.5	8.6	9.1	9.5	8.5
PL	16.1	20.3	18.3	20.8	18.5	17.3	20.0	15.8	19.7	19.1	16.8
IPL	1.0	1.2	1.3	1.3	1.5	1.3	1.3	1.3	1.1	1.4	1.1
OPL	1.6	1.6	1.2	2.0	1.7	1.5	1.5	1.6	1.7	1.5	1.1
IML	1.7	2.1	2.4	3.3	2.0	1.7	2.4	1.8	2.5	2.0	2.3

Table 2. Detailed morphometric measurements of *Sylvirana mortenseni* collected in Phu Quoc National Park

Voucher #	ITBCZ 3590	ITBCZ 3593	ITBCZ 3595	ITBCZ 3592	ITBCZ 3591	ITBCZ 3594
Field tag #	LNT 2564	LNT 2602	LNT 2735	LNT 2569	LNT 2590	LNT 2734
Sex	Male	Male	Male	Female	Female	Female
SVL	48.3	56.9	52.7	61.1	52.5	45.2
HDL	19.1	23.2	22.2	24.4	20.4	19.2
HDW	17.0	20.7	19.9	22.0	19.8	16.2
HH	8.0	9.5	10.7	12.6	8.0	7.4
SNT	8.4	9.0	9.0	10.3	8.9	7.8
IN	5.1	6.3	5.2	6.0	5.5	4.7
EN	4.8	4.5	5.3	6.0	5.9	5.2
EYE	5.4	8.6	6.8	7.5	7.7	5.5
IOD	4.7	4.9	5.3	6.1	6.7	4.6
TMP	4.2	4.6	5.0	6.7	4.6	4.8
TEY	2.3	2.7	2.0	3.2	2.8	2.0
FEL	24.2	29.6	25	32.7	28.0	24.2
TIB	24.9	31.8	29.8	35.6	30.4	27.2
TaL	12.7	15.9	13.1	16.6	15.6	12.1
ML	12.3	15.1	15.9	16.7	15.1	12.6
PL	22.7	27.8	29.0	32.0	28.5	25.2
IPL	2.5	2.1	2.5	2.4	1.7	2.1
OPL	2.0	2.4	2.2	2.3	1.3	2.3
IML	2.4	2.7	2.6	3.0	2.8	2.0
OML	1.8	1.4	1.7	1.6	1.5	1.3

4. DISCUSSION

This report increases the number of amphibians known to occur on Phu Quoc island from 14 to 16 species. These data for *Sylvirana mortenseni* have already been shared with the IUCN amphibian Red List authority and the new records incorporated into recently published extinction risk assessments (IUCN SSC, 2021). The presence of *Limnonectes kohchangae* has been record for Phu Quoc Island, Vietnam (Poyarkov *et al.*, 2021) without any supporting data (voucher specimens, molecular data or morphological data). Both *S. mortenseni* and *L. kohchangae* are currently assessed as Least Concern (IUCN SSC, 2017; IUCN SSC, 2021). Little is known about the reproductive ecology of both *S. mortenseni* and *L. kohchangae* and their larvae remain undescribed. Further research should be undertaken to address these knowledge gaps.

5. CONCLUSION

We provide the first detailed records for *Limnonectes kohchangae* and *Sylvirana mortenseni* from Phu Quoc Island. These records are supported by morphological and molecular data. This report increases the

number of amphibians known to occur on Phu Quoc Island from 14 to 16 species.

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GHI NHẬN BỔ SUNG HAI LOÀI LƯỠNG CƯ *Limnonectes kohchangae* (SMITH, 1922) VÀ *Sylvirana mortenseni* (BOULENGER, 1903) TẠI ĐẢO PHÚ QUỐC, MIỀN NAM VIỆT NAM

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TÓM TẮT

Chúng tôi lần đầu tiên ghi nhận sự phân bố của hai loài lưỡng cư gồm ếch nhèo koh-chang (*Limnonectes kohchangae*) và ếch suối koh-chang (*Sylvirana mortenseni*) tại đảo Phú Quốc, thành phố Phú Quốc, tỉnh Kiên Giang dựa vào dữ liệu hình thái và di truyền. Ếch suối koh-chang (*Sylvirana mortenseni*) được tìm thấy dọc các con suối lớn tại Vườn Quốc gia Phú Quốc ở độ cao khoảng 23 – 102 m. Loài này có kích thước từ 46,0 – 80,9 mm ở 3 con đực trưởng thành và 47,7 – 69,2 mm ở 3 con cái trưởng thành. Con đực có tuyến bã vai lớn và chai sinh dục phát triển; không có gờ da tam giác sau màng nhĩ; một gờ da bên hông phát triển và màu sắc khá đồng nhất giữa trên và dưới hông. Loài ếch nhèo koh-chang (*Limnonectes kohchangae*) được ghi nhận tại các con suối nhỏ trong rừng thường xanh ở độ cao khoảng 34 – 201 m. Các cá thể loài này tại Phú Quốc có kích thước khoảng 34,3 – 41,8 mm ở 9 con đực trưởng thành và 33,3 – 38,2 mm ở 2 con cái trưởng thành. Lưng có các mụn da nổi rõ sắp xếp thành các hàng không liên tục; màng nhĩ lớn, rõ và có hai màu, đậm ở phần trên; đùi có các đốm đen rõ. Ghi nhận này nâng số loài lưỡng cư ở đảo Phú Quốc từ 14 lên 16 loài.

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