



## Research article

# A new species of the genus *Hoplobatrachus* Peters, 1863 (Anura, Dicroglossidae) from northwestern Thailand

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## Article Info

### Article history:

Received 20 July 2022

Revised 4 October 2022

Accepted 13 October 2022

Available online 19 December 2022

### Keywords:

*Hoplobatrachus*,  
*Hoplobatrachus litoralis*,  
*Hoplobatrachus rugulosus*,  
*Hoplobatrachus tigerinus*,  
Tiger frog

## Abstract

**Importance of the work:** Northwestern Thailand lacks any intensive survey of amphibian fauna; thus, this area is suspected to have undiscovered populations of amphibians. Therefore, conducting field surveys in such areas is essential to fill knowledge gaps about species richness in this animal group.

**Objectives:** To describe a new species of frog genus *Hoplobatrachus* as new to science.

**Materials & Methods:** A new species was described based on specimens collected from Salawin National Park, Mae Sariang District, Mae Hong Son province, northwestern Thailand. The new species was separated from its congeners inferred from molecular (16S and Cytb genes) and morphological information.

**Results:** The new species was distinguished from its congeners based on mitochondrial DNA sequence analyses and morphological comparisons. The species was characterized by the following characters: pointed snout; large size (snout-vent length [SVL] of adult males = 94.8–102.4 mm, SVL of females = 93.2–96.3 mm); presence of mid-dorsal stripe; absence of thin stripe on inner side of tibia; presence of sexual dichromatism; yellow external vocal sac; presence of nuptial pad on base of first finger in adult males; relative length of finger II < IV < I < III, toe I < II < V < III < IV.

**Main finding:** A new frog species was described based on specimens from Thailand.

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<https://doi.org/10.34044/j.anres.2022.56.6.08>

## Introduction

The dicroglossid frogs of the genus *Hoplobatrachus* Peter, 1863 (family Dicroglossidae Anderson, 1871) consist of five species (Frost, 2022): *H. crassus* (Jerdon, 1853), *H. litoralis* Hasan, Kuramoto, Islam, Alam, Khan and Sumida, 2012, *H. occipitalis* (Günther, 1858), *H. rugulosus* (Wiegmann, 1834) and *H. tigerinus* (Daudin, 1802). Members of the genus occur widely from Sub-Saharan Africa, south to Angola to northeastern Zambia and Tanzania, Peninsular India, Sri Lanka, Peninsular Malaysia, Myanmar, Thailand, Laos, Cambodia, Vietnam and southern China (introduced to Borneo and Madagascar) (Frost, 2022). Their global conservation status is not critical. Four of these species have been assessed for the IUCN Red List as species of Least Concern (LC): *H. crassus* (Padhye et al., 2008a), *H. occipitalis* (International Union for Conservation of Nature and Natural Resources, 2022), *H. rugulosus* (Diesmos et al., 2004) and *H. tigerinus* (Padhye et al., 2008b). The LC status results from their wide distribution, tolerance to a broad range of habitats, presumed large population and because their populations are unlikely to be declining rapidly enough to qualify for listing in a more threatened category. The remaining species, *H. litoralis*, has been considered as endemic in the southeastern corner (Ukhia Upazila, Teknaf district) of Bangladesh (Hasan et al., 2012).

*H. tigerinus* was included (as subspecies *Rana tigerina pantherina*) in the work of Taylor (1962) on amphibian fauna in Thailand; however, subsequent work on amphibians in Thailand did not comment on this species. Only *H. rugulosus* has been listed more recently in the amphibian fauna of Thailand (Chan-ard et al., 1999; Nabhitabhata, 2000; Khonsue and Thirakhupt, 2001; Chan-ard, 2003; Nabhitabhata et al., 2004; Nabhitabhata and Chan-ard, 2005; Chuaynkern and Chuaynkern, 2012; Chuaynkern and Duengkak, 2014; Duengkak et al., 2016; Niyomwan et al., 2019). Prior to these studies, Taylor and Elbel (1958) included *Rana tigerina pantherina* and Taylor (1962) subsequently added one more (*R. rugulosa*) to Thai amphibians. *H. rugulosus* is large, ranging in size (50–117 mm) and weighing 25–500 g. Female lays 2,649 eggs with an average length of  $1.81 \pm 0.23$  mm (1.15–2.43 mm) and width of  $1.79 \pm 0.24$  mm (1.15–2.43 mm) (Rongchapho et al., 2021). Its dorsal color ranges from yellow to olive green or grey, with irregular dark markings. *H. tigerinus* is distributed throughout India, Bangladesh and much of northern Pakistan, southern parts of Nepal,

Bhutan and upper and northern central Myanmar (Smith, 1940; Zug et al., 1998; Hasan et al., 2012) and is considered a complex of several cryptic species. This species was introduced to Madagascar (Frost, 2022). Ongoing taxonomic research in Sri Lanka has revealed that *H. tigerinus* does not occur in this country, and frogs previously assigned to *H. tigerinus* are misidentifications of *H. crassus* (Dutta, 1997; Batuwita et al., 2019). However, the current authors recently collected some specimens from the herpetological survey at Salawin National Park, Mae Hong Son province, northwestern Thailand. These specimens were primarily assigned to the genus *Hoplobatrachus* based on external morphology alone, according to Taylor (1962). The investigation of their identity at the time has led the current authors to present a new species of the genus *Hoplobatrachus* based on molecular and morphological analyses.

## Materials and Methods

### Study area, permission and specimens

Salawin National Park occupies the western part of Mae Hong Son province, northwestern Thailand. It is neighbored by the Salawin Wildlife Sanctuary (Mae Hong Son province) to the north, Chiang Mai province to the east, Tak province to the south and Myanmar to the west. The national park area is approximately 721 km<sup>2</sup>, extending from 17.989971° to 18.368046°N and 97.609530° to 97.893191° E. Most of the area is covered with high mountains interspersed with lowlands. The elevation is on the range 200–1,027 m above sea level (a.s.l.), while the lowland rice fields, where samples were collected, are in the range 86–106 m. a.s.l. The vegetation includes dry evergreen forest, mixed deciduous forest and dry dipterocarp forest (Department of National Parks, Wildlife and Plant Conservation, 2022).

The specimens were caught in the field at night by hand. Live photographs were taken *ex situ*, and then the frogs were euthanized using chloretone solution, preserved in 10% buffer formalin and later transferred into 70% ethanol. Before preservation in formalin, tissue samples were taken by cutting pieces from the liver and preserving them in 99% ethanol for molecular analyses. The specimens were deposited at the Khon Kaen University Vertebrate Collection (KKUC, Khon Kaen University, Khon Kaen province, northeastern Thailand) and the Thailand Natural History Museum (THNHM, the National Science Museum,

Pathum Thani province, central Thailand). Most specimens are cross-catalogued at both institutions, in which case voucher numbers are reported as KKUC/THNHM. Permission for conducting research in a protected area (Salawin National Park) and for specimen collection were obtained from the Department of National Parks, Wildlife and Plant Conservation (permission number: 0907.4/25571). The research was reviewed and approved by the Institutional Animal Care and Use Committee of Khon Kaen University, based on the Ethics of Animal Experimentation of National Research Council of Thailand (reference no. 660201.2.11/235[44]).

### DNA extraction, polymerase chain reaction amplification and sequencing

Genomic DNA of three unidentified individuals of *Hoplobatrachus* was extracted from liver tissue samples using the FavorPrep™ Tissue Genomic DNA Extraction Mini Kit (Favorgen Biotech Corp.) according to the manufacturer's instructions. The extracted DNA solutions were used to amplify partial portions of the 16S rRNA gene (16S) and cytochrome b gene (Cytb). Polymerase chain reaction (PCR) amplification of partial fragments of 16S was performed using the F51 (5'-CCCGCCTGTTTACCAAAACAT-3'), R51 (5'-GGTCTGAACTCAGATCACGTA-3') primer pair (Sumida et al., 2002) and the 16SA-L (5'-CGCCTGTTTATCAAAACAT-3') and 16SB-H (5'-CCGGTCTGAACTCAGATCACGT-3') primer pair (Palumbi et al., 1991). PCR amplification of a partial fragment of Cytb was performed using the HoploGluF-18 (5'-TAACTCGGACCTGTAGTCTGA-3') and HoploDloopR-46 (5'-GAGTGTACTTGAAGATATGCTTG-3') primer pair (Sultana et al., 2016). PCR was performed with an initial denaturation at 95 °C for 4 min followed by 35 cycles of denaturation at 94 °C for 40 s, annealing at 55 °C (for 16S)/53 °C (for Cytb) for 40 s and extension at 72 °C for 60 s, with a final extension at 72 °C for 10 min. The PCR products were purified using a FavorPrep GEL/PCR Purification Kit (Favorgen Biotech Corp.) according to the manufacturer's instructions. The purified PCR products were sequenced in the Molecular Informatics Laboratory of Macrogen Company, Korea, using the same primers as those used for PCR amplification. All sequences were deposited in GenBank as MW244087–MW244089 for 16S and MW310223–MW310225 for the Cytb gene, respectively (Table 1).

**Table 1** Localities, voucher information and GenBank accession numbers for all samples of *Hoplobatrachus* and *Euphylyctis* used in this study

ID	Identified species	Current study	Voucher	Locality	16S	Cytb	Reference
1	<i>H. salween</i> sp. nov.		KKUC 01173/THNHM 26829 holotype	Salawin National Park, Mae Hong Son, Thailand	MW244089	MW310223	This study
2	<i>H. salween</i> sp. nov.		THNHM 26827 paratype	Salawin National Park, Mae Hong Son, Thailand	MW244088	MW310224	This study
3	<i>H. salween</i> sp. nov.		THNHM 26828 paratype	Salawin National Park, Mae Hong Son, Thailand	MW244087	MW310225	This study
4	<i>H. crassus</i>		Cra_18	Dacope, Khulna, Bangladesh	-	LC121021	Sultana et al., 2016
5	<i>H. crassus</i>		Cra_19	Dacope, Khulna, Bangladesh	-	LC121022	Sultana et al., 2016
6	<i>H. crassus</i>		Cra_1A_20698	Assam, India	-	LC121023	Sultana et al., 2016
7	<i>H. crassus</i>		IABHU 3973	Ukhia, Cox's Bazar, Bangladesh	AB671184	AB671196	Hasan et al., 2012
8	<i>H. crassus</i>		cra-assa-A	Assam, India	AB290413	AB290597	Alam et al., 2008
9	<i>H. litoralis</i>		IABHU 3975	Ukhia, Cox's Bazar, Bangladesh	AB671173	AB671185	Hasan et al., 2012
10	<i>H. litoralis</i>		IABHU 3974 paratype	Ukhia, Cox's Bazar, Bangladesh	AB671174	AB671186	Hasan et al., 2012
11	<i>H. litoralis</i>		IABHU 3985	Teknaf, Cox's Bazar, Bangladesh	AB671175	AB671187	Hasan et al., 2012
12	<i>H. litoralis</i>		IABHU 3982	Ukhia, Cox's Bazar, Bangladesh	AB671176	AB671188	Hasan et al., 2012
13	<i>H. litoralis</i>		IABHU 3977	Ukhia, Cox's Bazar, Bangladesh	AB671177	AB671189	Hasan et al., 2012
14	<i>H. litoralis</i>		IABHU 3983	Teknaf, Cox's Bazar, Bangladesh	AB671178	AB671190	Hasan et al., 2012
15	<i>H. litoralis</i>		IABHU 3976	Ukhia, Cox's Bazar, Bangladesh	AB671179	AB671191	Hasan et al., 2012
16	<i>H. litoralis</i>		IABHU 3988	Teknaf, Cox's Bazar, Bangladesh	AB671180	AB671192	Hasan et al., 2012
17	<i>H. litoralis</i>		IABHU 3978	Ukhia, Cox's Bazar, Bangladesh	AB671181	AB671193	Hasan et al., 2012
18	<i>H. litoralis</i>		USNMFS 35607	Bago, Myanmar	MG935818	-	Mulcahy et al., 2018

Table 1 Continued

ID	Identified species		Voucher	Locality	16S	Cytb	Reference
	Other study	Current study					
19	<i>H. litoralis</i>	<i>H. salween</i> sp. nov.	USNM 587325	Yangon, Mingalardon Township, Hlawga Wildlife Park, Myanmar	MG935819	-	Mulcahy et al., 2018
20	<i>H. litoralis</i>	<i>H. salween</i> sp. nov.	USNM 587404	Yangon, Mingalardon Township, Hlawga Wildlife Park, Myanmar	MG935820	-	Mulcahy et al., 2018
21	<i>H. litoralis</i>	<i>H. litoralis</i>	Lit_Uk_03	Ukhia, Cox's Bazar, Bangladesh	-	LC121012	Sultana et al., 2016
22	<i>H. litoralis</i>	<i>H. litoralis</i>	Lit_Cx_01	Cox's Bazar, Bangladesh	-	LC121001	Sultana et al., 2016
23	<i>H. litoralis</i>	<i>H. litoralis</i>	Lit_Cx_02	Cox's Bazar, Bangladesh	-	LC121002	Sultana et al., 2016
24	<i>H. litoralis</i>	<i>H. litoralis</i>	Lit_Tk_05	Teknaf, Cox's Bazar, Bangladesh	-	LC121013	Sultana et al., 2016
25	<i>H. occipitalis</i>	<i>H. occipitalis</i>	ZMB 79256	Guinea	-	KF991310	Barej et al., 2014
26	<i>H. occipitalis</i>	<i>H. occipitalis</i>	-	Mauritania	-	AJ564733	Grosjean et al., 2004
27	<i>H. occipitalis</i>	<i>H. occipitalis</i>	occ-afri-3	Tanzania	-	AB274150	Alam et al., 2008
28	<i>H. occipitalis</i>	<i>H. occipitalis</i>	occ-afri-1	Tanzania	AB272599	AB274148	Alam et al., 2008
29	<i>H. occipitalis</i>	<i>H. occipitalis</i>	occ-afri-2	Tanzania	AB272600	AB274149	Alam et al., 2008
30	<i>H. occipitalis</i>	<i>H. occipitalis</i>	occ-afri-4	Tanzania	-	AB290607	Alam et al., 2008
31	<i>H. occipitalis</i>	<i>H. occipitalis</i>	ZFMK 65186	Uganda	-	AY014388	Kosuch et al., 2001
32	<i>H. chinensis</i>	<i>H. rugulosus</i>	Chi_ViHL_20629	Huu Lien, Vietnam	-	LC121019	Sultana et al., 2016
33	<i>H. chinensis</i>	<i>H. rugulosus</i>	Chi_ViHL_20630	Huu Lien, Vietnam	-	LC121020	Sultana et al., 2016
34	<i>H. chinensis</i>	<i>H. rugulosus</i>	Chi_LLNP_20696	Long Nai, Phongsaly, Laos	-	LC121018	Sultana et al., 2016
35	<i>H. chinensis</i>	<i>H. rugulosus</i>	Chi_LLNP_20697	Long Nai, Phongsaly, Laos	-	LC121017	Sultana et al., 2016
36	<i>H. chinensis</i>	<i>H. rugulosus</i>	Chi_Laos_20626	Vang Vieng, Laos	-	LC121016	Sultana et al., 2016
37	<i>H. chinensis</i>	<i>H. rugulosus</i>	Chi_LaLP_20647	Luang Prabang, Laos	-	LC121014	Sultana et al., 2016
38	<i>H. chinensis</i>	<i>H. rugulosus</i>	Chi_Laos_20625	Vang Vieng, Laos	-	LC121015	Sultana et al., 2016
39	<i>H. chinensis</i>	<i>H. rugulosus</i>	Chin-Chacho-3663	Chachoengsao, Thailand	AB636617	AB636599	Alam et al., 2012
40	<i>H. chinensis</i>	<i>H. rugulosus</i>	Chin-Chacho-3909	Chachoengsao, Thailand	AB636616	AB636598	Alam et al., 2012
41	<i>H. chinensis</i>	<i>H. rugulosus</i>	Chin-Chacho-3911	Chachoengsao, Thailand	AB636615	AB636597	Alam et al., 2012
42	<i>H. rugulosus</i>	<i>H. rugulosus</i>	THW1	Hainan, China	JX181763	JX181763	Yu et al., 2015
43	<i>H. chinensis</i>	<i>H. rugulosus</i>	Chin-Nongkh-3	Nong Khai, Thailand	-	AB636600	Alam et al., 2012
44	<i>H. chinensis</i>	<i>H. rugulosus</i>	Hchi-Th1	Nong Khai, Thailand	AB272596	AB274144	Alam et al., 2008
45	<i>H. chinensis</i>	<i>H. rugulosus</i>	Hchi-Ve	Huu Lien, Vietnam	AB290414	AB290603	Alam et al., 2008
46	<i>H. chinensis</i>	<i>H. rugulosus</i>	Hchi-La	Long Nai, Phongsaly province, Laos	AB290417	AB290601	Alam et al., 2008
47	<i>H. chinensis</i>	<i>H. rugulosus</i>	Hchi-Th4	Phangnga, Thailand	AB290416	AB290606	Alam et al., 2008
48	<i>H. rugulosus</i>	<i>H. rugulosus</i>	Phangnga-nga1	Phangnga, Thailand	AB514546	AB514487	Pansook et al., 2012
49	<i>H. chinensis</i>	<i>H. rugulosus</i>	Hchi-Th2, 3	Ko Chang, Thailand	-	AB274146	Alam et al., 2008
50	<i>H. chinensis</i>	<i>H. rugulosus</i>	JHHWW001	Zhejiang, Jinhua, China	HM104684	HM104684	Yu et al., 2012
51	<i>H. rugulosus</i>	<i>H. rugulosus</i>	Anhui10359.1	Jinhua, China	KC196066	KC196066	Unpublished
52	<i>H. tigerinus</i>	<i>H. tigerinus</i>	Tig_Fulp_HT20	Phulpur, Bangladesh	-	LC120980	Sultana et al., 2016
53	<i>H. tigerinus</i>	<i>H. tigerinus</i>	Tig_Rang_HT31	Rangpur, Bangladesh	-	LC120958	Sultana et al., 2016
54	<i>H. tigerinus</i>	<i>H. tigerinus</i>	Tig_Syd_HT20	Saidpur, Bangladesh	-	LC120955	Sultana et al., 2016
55	<i>H. tigerinus</i>	<i>H. tigerinus</i>	Tig_Syd_HT28	Saidpur, Bangladesh	-	LC120956	Sultana et al., 2016
56	<i>H. tigerinus</i>	<i>H. tigerinus</i>	Tig_Rang_HT29	Rangpur, Bangladesh	-	LC120957	Sultana et al., 2016
57	<i>H. tigerinus</i>	<i>H. tigerinus</i>	Tig_Din_HT17	Dinajpur, Bangladesh	-	LC120954	Sultana et al., 2016



**Table 1** Continued

ID	Identified species		Voucher	Locality	16S	Cytb	Reference
	Other study	Current study					
58	<i>H. tigrinus</i>	<i>H. tigrinus</i>	Tig_Net_HT30	Netrokona, Bangladesh	-	LC120973	Sultana et al., 2016
59	<i>H. tigrinus</i>	<i>H. tigrinus</i>	Tig_CNSM_HT22	Sonamasjid, Bangladesh	-	LC120960	Sultana et al., 2016
60	<i>H. tigrinus</i>	<i>H. tigrinus</i>	Tig_Pabna_HT21	Pabna, Bangladesh	-	LC120964	Sultana et al., 2016
61	<i>H. tigrinus</i>	<i>H. tigrinus</i>	Tig_Shamb_HT26	Shamvuganj, Bangladesh	-	LC121000	Sultana et al., 2016
62	<i>H. tigrinus</i>	<i>H. tigrinus</i>	Tig_NG_HT27	Narsingdi, Bangladesh	-	LC120983	Sultana et al., 2016
63	<i>H. tigrinus</i>	<i>H. tigrinus</i>	Tig_Lal_HT19	Lalmonirhat, Bangladesh	-	LC120953	Sultana et al., 2016
64	<i>H. tigrinus</i>	<i>H. tigrinus</i>	Tig_Jamal_HT18	Jamalpur, Bangladesh	-	LC120969	Sultana et al., 2016
65	<i>H. tigrinus</i>	<i>H. tigrinus</i>	Tig_BAU_HT15	BAU campus, Bangladesh	-	LC120978	Sultana et al., 2016
66	<i>H. tigrinus</i>	<i>H. tigrinus</i>	Tig_BAU_HT16	BAU campus, Bangladesh	-	LC120979	Sultana et al., 2016
67	<i>H. tigrinus</i>	<i>H. tigrinus</i>	Tig_SK_HT04	Sathkira, Bangladesh	-	LC120986	Sultana et al., 2016
68	<i>H. tigrinus</i>	<i>H. tigrinus</i>	Tig_Shamb_HT23	Shamvuganj, Bangladesh	-	LC120999	Sultana et al., 2016
69	<i>H. tigrinus</i>	<i>H. tigrinus</i>	Tig_Jamal_HT24	Jamalpur, Bangladesh	-	LC120970	Sultana et al., 2016
70	<i>H. tigrinus</i>	<i>H. tigrinus</i>	Tig_Fulp_HT23	Phulpur, Bangladesh	-	LC120981	Sultana et al., 2016
71	<i>H. tigrinus</i>	<i>H. tigrinus</i>	Tig_Net_HT23	Netrokona, Bangladesh	-	LC120972	Sultana et al., 2016
72	<i>H. tigrinus</i>	<i>H. tigrinus</i>	Tig_Jagan_HT23	Jagannathganj, Bangladesh	-	LC120967	Sultana et al., 2016
73	<i>H. tigrinus</i>	<i>H. tigrinus</i>	Tig_Jagan_HT25	Jagannathganj, Bangladesh	-	LC120968	Sultana et al., 2016
74	<i>H. tigrinus</i>	<i>H. tigrinus</i>	Tig_BAU_HT02	BAU campus, Bangladesh	-	LC120977	Sultana et al., 2016
75	<i>H. tigrinus</i>	<i>H. tigrinus</i>	Tig_IS_HT01	Iswardi, Bangladesh	-	LC120965	Sultana et al., 2016
76	<i>H. tigrinus</i>	<i>H. tigrinus</i>	Tig_Chur_HT06	Churkhai, Bangladesh	-	LC120998	Sultana et al., 2016
77	<i>H. tigrinus</i>	<i>H. tigrinus</i>	Tig_Chur_HT06	Chittagong, Bangladesh	-	LC120996	Sultana et al., 2016
78	<i>H. tigrinus</i>	<i>H. tigrinus</i>	Tig_Bn_HT06	Bandarban, Bangladesh	-	LC120995	Sultana et al., 2016
79	<i>H. tigrinus</i>	<i>H. tigrinus</i>	Tig_NG_HT06	Narsingdi, Bangladesh	-	LC120982	Sultana et al., 2016
80	<i>H. tigrinus</i>	<i>H. tigrinus</i>	Tig_C_HT06	Dirai, Bangladesh	-	LC120975	Sultana et al., 2016
81	<i>H. tigrinus</i>	<i>H. tigrinus</i>	Tig_Syl_HT06	Sylhet, Bangladesh	-	LC120974	Sultana et al., 2016
82	<i>H. tigrinus</i>	<i>H. tigrinus</i>	Tig_IS_HT06	Iswardi, Bangladesh	-	LC120966	Sultana et al., 2016
83	<i>H. tigrinus</i>	<i>H. tigrinus</i>	Tig_Pabna_HT06	Pabna, Bangladesh	-	LC120963	Sultana et al., 2016
84	<i>H. tigrinus</i>	<i>H. tigrinus</i>	Tig_Br_HT07	Barguna, Bangladesh	-	LC120988	Sultana et al., 2016
85	<i>H. tigrinus</i>	<i>H. tigrinus</i>	Tig_Vo_HT09	Bhola, Bangladesh	-	LC120992	Sultana et al., 2016
86	<i>H. tigrinus</i>	<i>H. tigrinus</i>	Tig_Pkun_HT09	Patuakhali University, Bangladesh	-	LC120990	Sultana et al., 2016
87	<i>H. tigrinus</i>	<i>H. tigrinus</i>	Tig_Khul_HT09	Khulna, Bangladesh	-	LC120985	Sultana et al., 2016
88	<i>H. tigrinus</i>	<i>H. tigrinus</i>	Tig_CN_HT10	Rajshahi, Bangladesh	-	LC120962	Sultana et al., 2016
89	<i>H. tigrinus</i>	<i>H. tigrinus</i>	Tig_CNSM_HT11	Sonamasjid, Bangladesh	-	LC120959	Sultana et al., 2016
90	<i>H. tigrinus</i>	<i>H. tigrinus</i>	Tig_No_HT05	Noakhali, Bangladesh	-	LC120989	Sultana et al., 2016
91	<i>H. tigrinus</i>	<i>H. tigrinus</i>	Tig_Khul_HT08	Khulna, Bangladesh	-	LC120984	Sultana et al., 2016
92	<i>H. tigrinus</i>	<i>H. tigrinus</i>	Tig_SK_HT12	Sathkira, Bangladesh	-	LC120987	Sultana et al., 2016
93	<i>H. tigrinus</i>	<i>H. tigrinus</i>	Tig_Snd_HT13	Sandwip, Bangladesh	-	LC120993	Sultana et al., 2016
94	<i>H. tigrinus</i>	<i>H. tigrinus</i>	Tig_Pkun_HT13	Patuakhali University, Bangladesh	-	LC120991	Sultana et al., 2016
95	<i>H. tigrinus</i>	<i>H. tigrinus</i>	Tig_Chur_HT14	Chittagong, Bangladesh	-	LC120997	Sultana et al., 2016
96	<i>H. tigrinus</i>	<i>H. tigrinus</i>	Tig_C_HT14	Dirai, Bangladesh	-	LC120976	Sultana et al., 2016
97	<i>H. tigrinus</i>	<i>H. tigrinus</i>	Tig_CN_HT03	Rajshahi, Bangladesh	-	LC120961	Sultana et al., 2016
98	<i>H. tigrinus</i>	<i>H. tigrinus</i>	Tig_Raoj_HT32	Raojan, Chittagong, Bangladesh	-	LC120994	Sultana et al., 2016
99	<i>H. tigrinus</i>	<i>H. tigrinus</i>	Tig_Kish_HT33	Kishorganj, Bangladesh	-	LC120971	Sultana et al., 2016

Table 1 Continued

ID	Identified species		Voucher	Locality	16S	Cytb	Reference
	Other study	Current study					
100	<i>H. tigerinus</i>	<i>H. tigerinus</i>	Tig_Ishr_20325	Shirva, India	-	LC121011	Sultana et al., 2016
101	<i>H. tigerinus</i>	<i>H. tigerinus</i>	Tig_IBM_20337	Bajpe, India	-	LC121004	Sultana et al., 2016
102	<i>H. tigerinus</i>	<i>H. tigerinus</i>	Tig_Ishr_20324	Shirva, India	-	LC121010	Sultana et al., 2016
103	<i>H. tigerinus</i>	<i>H. tigerinus</i>	Tig_IPM_20030	Padil, India	-	LC121006	Sultana et al., 2016
104	<i>H. tigerinus</i>	<i>H. tigerinus</i>	Tig_Ikm_20137	Karnoor, India	-	LC121008	Sultana et al., 2016
105	<i>H. tigerinus</i>	<i>H. tigerinus</i>	Tig_Ikm_20138	Karnoor, India	-	LC121009	Sultana et al., 2016
106	<i>H. tigerinus</i>	<i>H. tigerinus</i>	Tig_IBM_20338	Bajpe, India	-	LC121005	Sultana et al., 2016
107	<i>H. tigerinus</i>	<i>H. tigerinus</i>	Tig_IBM_20332	Bajpe, India	-	LC121003	Sultana et al., 2016
108	<i>H. tigerinus</i>	<i>H. tigerinus</i>	Tig_IPM_20031	Padil, India	-	LC121007	Sultana et al., 2016
109	<i>H. tigerinus</i>	<i>H. tigerinus</i>	Htig-ln1	Padil, India	AB272594	AB274137	Alam et al., 2008
110	<i>H. tigerinus</i>	<i>H. tigerinus</i>	Htig-ln2	Bajpe, India	AB290412	AB274139	Alam et al., 2008
111	<i>H. tigerinus</i>	<i>H. tigerinus</i>	IABHU 4001	Mymensingh, Bangladesh	AB671183	AB671195	Hasan et al., 2012
112	<i>H. tigerinus</i>	<i>H. tigerinus</i>	IABHU 4000	Mymensingh, Bangladesh	AB671182	AB671194	Hasan et al., 2012
113	<i>H. tigerinus</i>	<i>H. tigerinus</i>	-	Mymensingh, Bangladesh	AP011543	AP011543	Alam et al., 2010
114	<i>E. cyanophlyctis</i>	<i>E. cyanophlyctis</i>	Ecya-Ba1, 2	BAU campus, Mymensingh, Bangladesh	AB272601	AB274151	Alam et al., 2008
115	<i>E. hexadactylus</i>	<i>E. hexadactylus</i>	hex-khul-1	Khulna, Bangladesh	AB272605	AB274163	Alam et al., 2008

### Phylogenetic analyses

The combined 16S and Cytb data matrix consisted of 37 accessions; Cytb alone consisted of 110 accessions. Both data matrices represented six species of *Hoplobatrachus*. Most of the DNA sequences (other than the new species) were obtained from previous studies (Kosuch et al., 2001; Grosjean et al., 2004; Alam et al., 2008, 2010, 2012; Hasan et al., 2012; Pansook et al., 2012; Yu et al., 2012, 2015; Barej et al., 2014; Sultana et al., 2016; Mulcahy et al., 2018). Two *Euphlyctis* species (*E. cyanophlyctis* and *E. hexadactylus*) were used as the outgroup. DNA sequences were aligned using the SeaView version 4 software (Gouy et al., 2010). Phylogenetic analyses were performed using Bayesian inference (BI) and Maximum Parsimony (MP). BI was performed using the MrBayes 3.2.1 program (Ronquist et al., 2012). The Bayesian analysis parameters were estimated from the data set using the MrModelTest 2.2 program (Nylander, 2004). Nucleotide substitution models for BI analyses were chosen based on their Akaike information criterion. Two independent runs were conducted, each of which was performed for 10 million generations and sampled every 1,000 generations with the first 25% samples discarded as burn-in, resulting in a potential scale reduction factor of < 0.005. A 50% majority-rule consensus of the sampled trees was constructed to calculate the posterior probabilities of the tree nodes. A heuristic search for the MP was performed using the PAUP\* 4.0b10 program (Swofford, 2002) with 1,000 replicates, sequences random addition and tree-bisection-reconnection branching. Non-parametric bootstrap support was estimated using 1,000 replicates of full heuristic searches. Uncorrected pairwise sequence divergences (*p*-distances) were calculated using the MEGA X program (Kumar et al., 2018).

### Morphology

The frog description format followed other previous studies (such as Ohler, 1996; Ohler and Dubois, 1999; Chuaynkern et al., 2010). The specimen photographs were taken using a digital camera and improved using the Adobe® Photoshop CS6 software (Adobe Systems Inc.). The habitat and live photographs of frogs were taken in the field using a digital camera. Measurements were made with a pair of digital calipers to the nearest 0.1 mm. The abbreviations used for measurements were: SVL, snout-vent length; HW, head width; HL, head length (from the back of the mandible to the tip of the snout); MN, distance from the back of the mandible

to the nostril; MFE, distance from the back of the mandible to the front of the eye; MBE, distance from the back of the mandible to the back of the eye; IFE, distance between the fronts of the eyes; IBE, distance between the back of the eyes; IN, internarial space; EN, distance from the front of the eye to the nostril; EL, eye length; SN, distance from the nostril to the tip of the snout; SL, distance from the front of the eye to the tip of the snout; TYD, greatest tympanum diameter; TYE, distance from tympanum to the back of the eye; IUE, minimum distance between upper eyelids; UEW, maximum inter upper eyelid width; HAL, hand length (from the base of the outer palmar tubercle to the tip of the toe); FLL, forelimb length (from the elbow to the base of the outer tubercle); TFL, third finger length (from the base of the first subarticular tubercle); fd1–fd4, width of pads of fingers 1 to 4; fw1–fw4, width of fingers 1–4; (hindlimb) FL, femur length (from vent to knee); TL, tibia length; TW, tibia width; FOL, foot length (from the base of the inner metatarsal tubercle to the tip of the toe); FTL, fourth toe length (from the base of the first subarticular tubercle to the tip of toe 4); TFOl, length of tarsus and foot (from base of tarsus to tip of fourth toe); td1–td5, width of pads of the toes 1–5; tw1–tw5, width of toes 1 to 5; IMT, length of inner metatarsal tubercle; ITL, inner toe length; (webbing) MTTF, distance from the distal edge of the metatarsal tubercle to the maximum incurvation of the web between third and fourth toe; TFTF, distance from the maximum incurvation of the web between the third and fourth toes to the tip of the fourth toe; MTFF, distance from the distal edge of the metatarsal tubercle to the maximum incurvation of the web between the fourth and fifth toes; FFTF, distance from the maximum incurvation of the web between the fourth and fifth toes to the tip of the fourth toe; WTF, webbing between the third and fourth toes (from the base of the first subarticular tubercle); WFF, webbing between the fourth and fifth toes (from the base of the first subarticular tubercle); WI, webbing between the third and fourth toes when folded along the fourth toe (from the base of the first subarticular tubercle); WII, webbing between the fourth and fifth toes when folded along the fourth toes (from the base of the first subarticular tubercle). Abbreviations of the museums and institutions in which the specimens were deposited are as follows: BMNH, British Museum of Natural History (London, UK), KKUC, Khon Kaen University Vertebrate Collection (Khon Kaen, Thailand), THNHM, Thailand Natural History Museum (Pathum Thani, Thailand).

## Results

### Molecular analysis

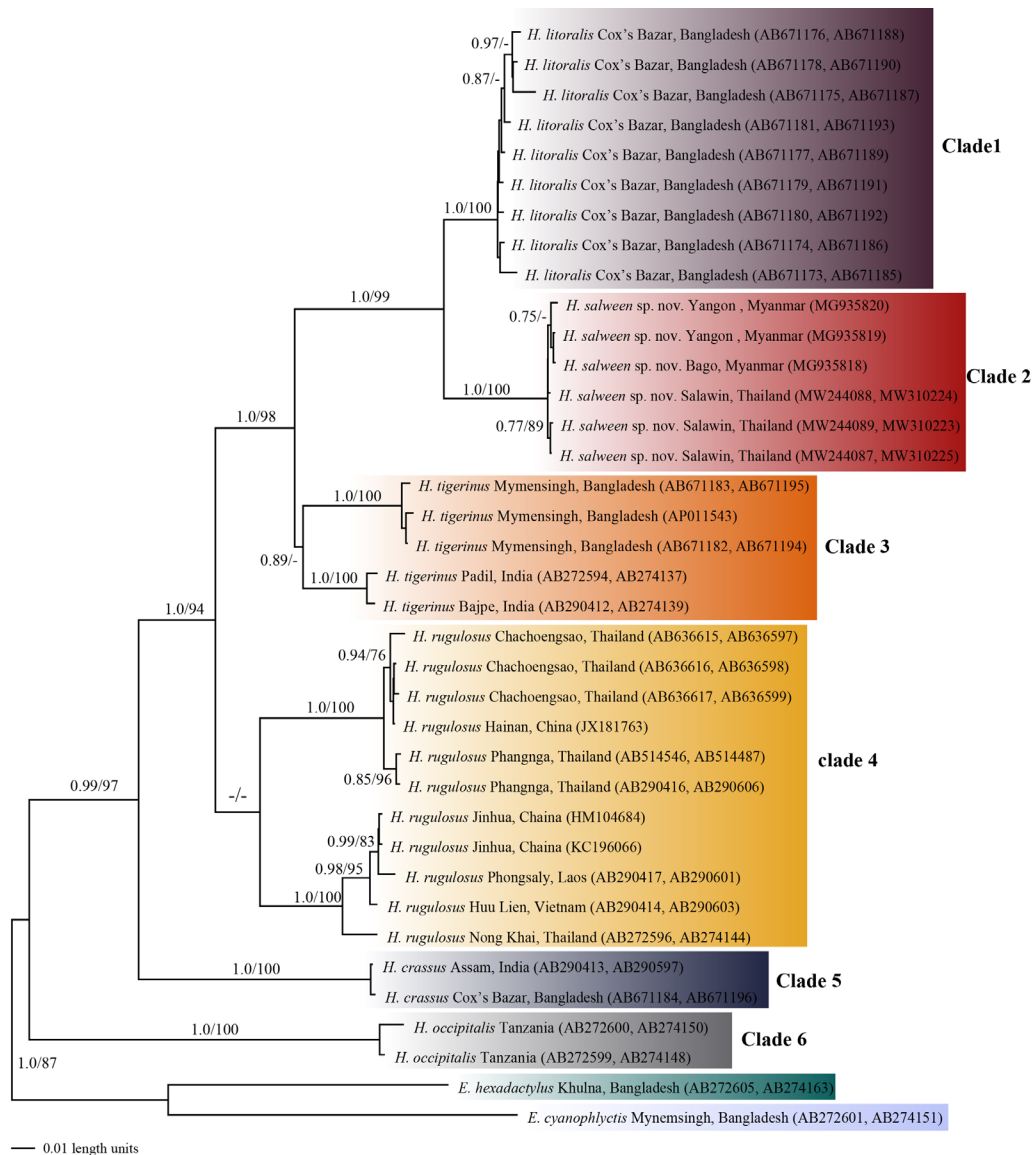
The aligned combined 16S and Cytb dataset contained 37 individuals and 1,188 characters, whereas the Cytb data set comprised only 110 individuals and 1,139 characters. BI analyses produced trees of almost identical topology (Figs. 1 and 2). *Hoplobatrachus* was a monophyletic group, as indicated by a high posterior probability of BI (BPP  $\geq$  95%) and high bootstrap supports of MP (MP-BS  $\geq$  95%). The African *H. occipitalis* was a sister group of the other five Asian species—*H. crassus*, *H. rugulosus*, *H. tigerinus*, *H. litoralis* and the unidentified species. A Bayesian tree of the combined 16S and Cytb dataset revealed six well-demarcated clades, identified as clades 1–6 (Fig. 1). Clade 1 contained *H. litoralis* from Bangladesh, while clade 2 contained the new species from Thailand and Myanmar (previously *H. litoralis*). Clade 3 contained two groups of *H. tigerinus* (BPP  $\geq$  75%, MP-BS  $\leq$  75%), with the first from Bangladesh and the second from India. Clade 4 contained 11 *H. rugulosus* individuals and was further separated into two groups (BPP and MP-BS  $\leq$  75%), with the first from southeastern Thailand (Chachoengsao province) and China (Hainan) and the second from upper northeastern Thailand (Nong Khai province), northern Laos (Phongsaly), northern Vietnam (Huu Lien) and China (Jinhua). Clades 5 and 6 contained two individuals of *H. occipitalis* from Tanzania and *H. crassus* from India and Bangladesh, respectively. The Bayesian tree of the dataset of the Cytb sequences was similar to the tree of concatenated 16S and Cytb tree (Fig. 2). The Asian *Hoplobatrachus* species formed a monophyletic group with respect to the African *Hoplobatrachus* (*H. occipitalis*). Separation of *H. litoralis* and new species was well supported by BPP 1.0 and MP-BS 99%. The relationship among the *Hoplobatrachus* species was concordant with other studies (Kosuch et al., 2001; Hasan et al., 2012).

The sequences of unidentified individuals from Thailand (Salawin National Park, Mae Hong Son) and *H. litoralis* from Myanmar (Bago and Yangon) differed from those of other *Hoplobatrachus* species. This species appeared to be closer to *H. litoralis* than other species because the genetic divergence was reduced (Table 2). However, the sequences of the unidentified specimens, and *H. litoralis*, from Myanmar formed a clade separate from *H. litoralis* of Bangladesh, according to both the combined gene set and Cytb-only trees. The sequences from Salawin National Park and *H. litoralis* from

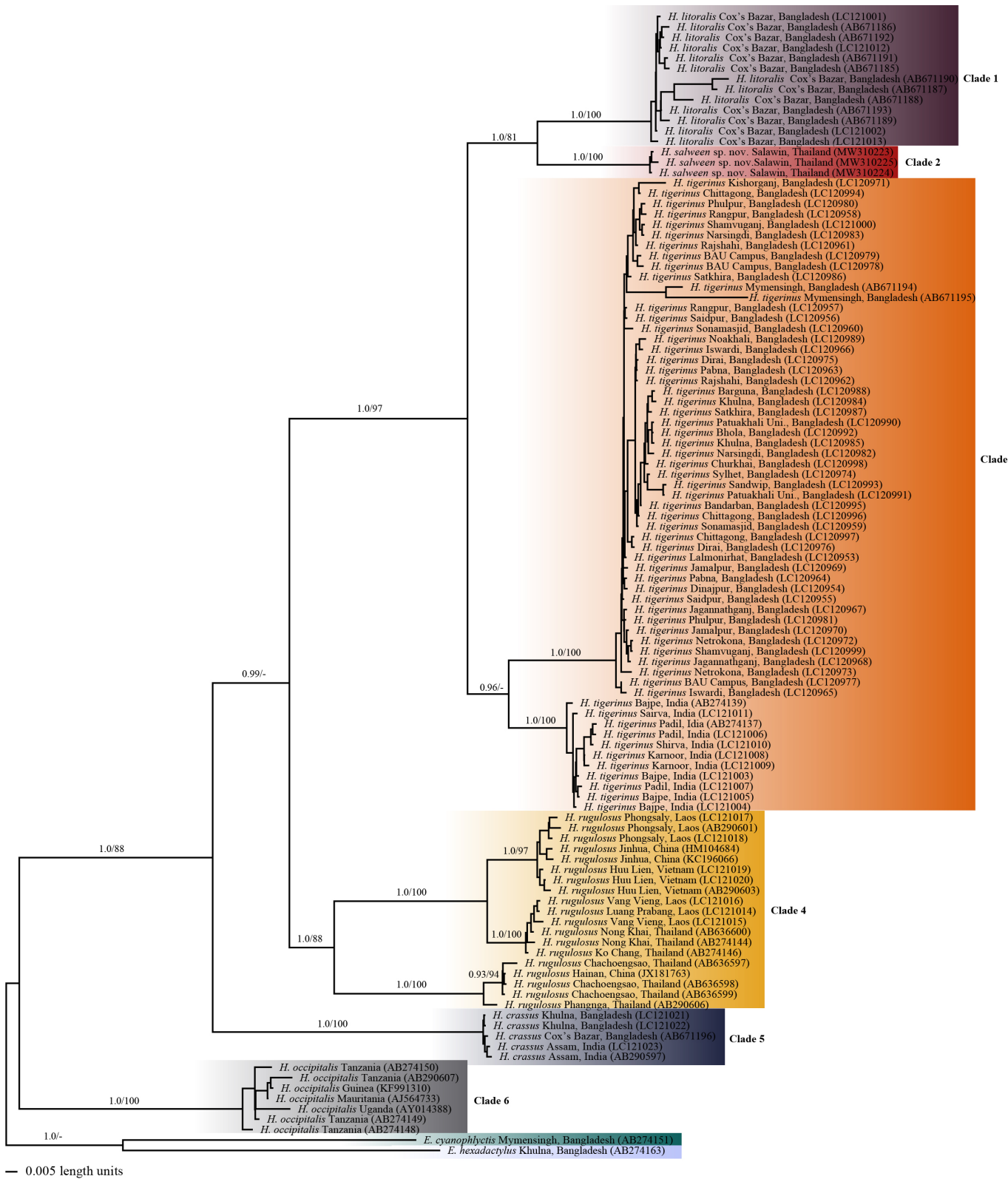
Myanmar formed a monophyletic group (clade 2, BPP = 1.0, MP-BS 99%), which was further divided into two subclades with a low BPP (Fig. 1). This suggested that these subgroups have the same taxon. The average sequence divergence levels for 16S between *H. salween* and *H. occipitalis*, *H. crassus*, *H. rugulosus*, *H. tigerinus* and *H. litoralis* were 13.8%, 9.3%, 5.7%, 5.2% and 3.1%, respectively, whereas the divergence levels of Cytb between *H. salween* and *H. occipitalis*, *H. crassus*, *H. rugulosus*, *H. tigerinus* and *H. litoralis* were 22.5%, 19.8%, 16.8%, 12.3% and 11.5% respectively

(Table 2). The average intraspecific 16S genetic divergence levels of the new species, *H. occipitalis*, *H. crassus*, *H. rugulosus*, *H. tigerinus* and *H. litoralis* were 1.4%, 0.2%, 1.2%, 2.0%, 1.2% and 1.8%, respectively, compared to 0.1%, 1.5%, 0.12%, 7.4%, 10.2% and 1.0%, respectively, in Cytb.

Based on the molecular and morphological differences, the specimens from Salawin National Park, Mae Hong Son province, northwestern Thailand represent an unnamed species, which is described here as a new species of the genus *Hoplobatrachus*.



**Fig. 1** Bayesian tree of 1,188 bp nucleotide sequence of mitochondrial (16S + Cytb) genes for samples of *Hoplobatrachus salween* sp. nov. and related species, with *Euphlyctis cyanophlyctis* and *E. hexadactylus* as out groups, where numbers above nodes represent Bayesian posterior probabilities  $\geq 75\%$  and bootstrap support for MP  $\geq 75\%$  (BPP/MP-BS). BPP and MP-BP values below 75% are indicated as ‘-’.



**Fig. 2** Bayesian tree of 1,139 bp nucleotide sequence of mitochondrial Cytb gene for samples of *Hoplobatrachus salween* sp. nov. and related species, with *Euphlyctis cyanophlyctis* and *E. hexadactylus* as out groups, where numbers above nodes represent Bayesian posterior probabilities  $\geq 75\%$  and bootstrap support for  $MP \geq 75\%$  (BPP/MP-BS). BPP and MP-BP values below 75% are indicated as ‘-’.



**Table 2** Average of uncorrected pairwise sequence divergences (*p*-distances) in 16S and Cytb of *Hoplobatrachus salween* sp. nov. and related species

16S	<i>H. occipitalis</i>	<i>H. crassus</i>	<i>H. rugulosus</i>	<i>H. tigerinus</i>	<i>H. litoralis</i>	<i>H. salween</i> sp. nov.
<i>H. occipitalis</i> (n = 2)	-	-	-	-	-	-
<i>H. crassus</i> (n = 2)	12.1	-	-	-	-	-
<i>H. rugulosus</i> (n = 11)	12.2	7.9	-	-	-	-
<i>H. tigerinus</i> (n = 5)	13.0	7.6	4.0	-	-	-
<i>H. litoralis</i> (n = 9)	13.9	8.8	5.7	4.5	-	-
<i>H. salween</i> sp. nov. (n = 6)	13.8	9.3	5.7	5.2	3.1	-
Cytb						
<i>H. occipitalis</i> (n = 7)	-	23.7	20.7	20.7	22.1	22.5
<i>H. crassus</i> (n = 5)	-	-	19.4	19.3	20.97	19.8
<i>H. rugulosus</i> (n = 20)	-	-	-	16.8	17.0	16.8
<i>H. tigerinus</i> (n = 62)	-	-	-	-	12.6	12.3
<i>H. litoralis</i> (n = 13)	-	-	-	-	-	11.5
<i>H. salween</i> sp. nov. (n = 3)	-	-	-	-	-	-

*Hoplobatrachus salween* sp. nov.

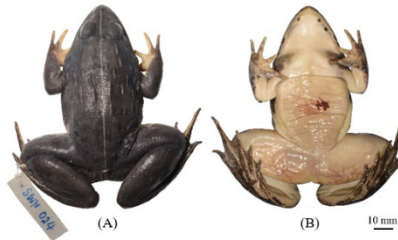
Holotype: KKUC 01173/THNHM 26829 (field no. SWN 024; Figs. 3 and 4), adult female, collected by Likhit Waiprom on 18 November 2016 from Baan Thataphung (18.078992°N, 97.696349°E; ca. 106 m a.s.l.), Salawin National Park, Mae Sariang district, Mae Hong Son province, northwestern Thailand.

Paratypes: KKUC 01175/ THNHM 26830 (field no. SM 06976, adult female), KKUC 01174/THNHM 26831 (field no. SM 06975, adult male) and KKUC01176/THNHM 16832 (field no. SM 06977, adult male), collected by Yodchaiy Chuaynkern, Sunchai Makchai, Likhit Waiprom on 14 June 2017, 18.030062°N, 97.722268°E; ca 86 m a.s.l.), Salawin National Park, Mae Sariang district, Mae Hong Son province, northwestern Thailand. THNHM 26827–28 (2 adult females), data as the holotype.

Comparative materials: *Hoplobatrachus rugulosus* (28 adult females, 25 adult males): Thailand: Bangkok: THNHM 09297–98; Chanthaburi: THNHM 03197–98, 07262–63; Chiang Mai: THNHM 06858, 09410;

Chiang Rai: BMNH 1974.2085, THNHM 11067–70; Chon Buri: THNHM 00124, 07561, 09210–11, 09291–95, 09299; Lampang: BMNH 1974.2083; Nakhon Nayok: THNHM 26746; Nakhon Ratchasima: THNHM 07032–34, 24281; Nakhon Si Thammarat: THNHM 28349; Narathiwat: THNHM 18726; Pattani: THNHM 26526; Phayao: THNHM 25836; Phitsanulok: THNHM 12654; Songkhla: THNHM 08322; Ubon Ratchathani: THNHM 28194–95; Uthai Thani: THNHM 19205, 19207–10; No locality: THNHM 02346, 04467, 05736, 06635, 07342–43, 09296, 12690, 3 uncatalogued specimens.

Etymology and other names: The specific name “salween” refers to the Salween River (known in Myanmar as the Thanlyin River and in China as the Nu River). The Salween, which is the 26<sup>th</sup> longest river (about 2,880 km) in the world, is a very important river for China, Myanmar and Thailand. The river flows from the Tibetan Plateau through China, Myanmar, and Thailand into the Andaman Sea. An English name “Salween Bullfrog” and a Thai name “Kob Salawin” are suggested by the current study.



**Fig. 3** Photographs of the adult female *Hoplobatrachus salween* sp. nov. holotype (KKUC 01173/THNHM 26829) in preservative: (A) dorsal view; (B) ventral view



**Fig. 4** *Hoplobatrachus salween* sp. nov. female holotype (KKUC 01173/ THNHM 26829): (A) lateral view of head; (B) right hand; (C) right foot

Diagnosis: *Hoplobatrachus salween* sp. nov. is characterized by the following combination of characters: body slightly stout; pointed snout; SVL of adult males  $98.6 \pm 5.42$  (94.8–102.4 mm,  $n = 2$ ), females  $94.1 \pm 1.49$  (93.2–96.3 mm,  $n = 4$ ); presence of mid-dorsal stripe; absence of thin stripe on inner side of tibia; presence of sexual dichromatism (completely yellow in whole body and limbs); presence of external vocal sac and vocal sac opening in adult males; presence of nuptial pad on base of the first finger in adult males; relative finger lengths  $II < IV < I < III$ , toe  $I < II < V < III < IV$ ; phylogenetic topological position close to *H. litoralis* (3.1% of 16S and 11.5% of Cytb) and *H. tigrinus* (5.1% of 16S and 12.3% of Cytb).

#### Description of the holotype (Figs. 3 and 4)

Frog of large size (SVL 93.2 mm), body slightly stout.

Head of moderate size, widely ovate in dorsal view; longer than wide (HW 33.0 mm; HL 35.3 mm; MN 29.6 mm; MFE 23.4 mm; MBE 14.5 mm), flat above. Snout pointed in dorsal and lateral views, protruding, its length (SL 15.3 mm) longer than horizontal diameter of eye (EL 8.8 mm). Canthus rostralis rounded, loreal region concave, vertical in cross-section. Interorbital space concave, narrower (IUE 3.9 mm) than upper eyelid (UEW 6.4 mm) and internarial distance (IN 5.4 mm); distance between fronts of eyes (IFE 13.5 mm) 1.5 times in distance between backs of eyes (IBE 20.0 mm). Nostril oval, directed posterolaterally, with flap of skin posteriorly, closer to tip of snout (SN 5.3 mm) than to eye (EN 8.3 mm). Pupil rounded (EL 8.8 mm). Tympanum (TYD 5.8 mm) distinct, oblique, 66% of eye diameter; tympanum to eye distance (TYE 2.8 mm) 50% of tympanum diameter. Pineal ocellus absent. Vomerine ridge present and large, bearing numerous large teeth ( $n = 9/10$  in left and right, respectively), teeth curve towards to body axis; with an angle of  $50^\circ$  to body axis, closer to choanae than each other, 6.2 times longer than distance between them. Tongue large, cordate, not emarginated, bearing no median lingual process. Tooth-like projections on lower jaw absent.

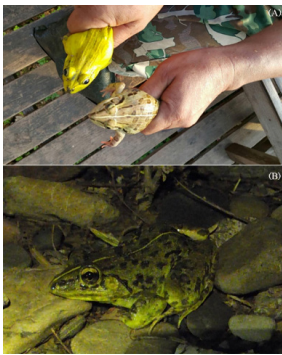
Arm long and strong; forearm (FLL 20.1 mm) longer than hand (HAL 18.5 mm); slightly enlarged. Finger I longer than II; fingers III (TFL 9.6 mm) and IV long and strong. Relative finger lengths:  $II < IV < I < III$ . Tips of fingers rounded, not enlarged without lateroventral grooves on fingers I–IV, fingers I and IV, slightly wide compared to finger width (fd1 1.6 mm, fw1 1.1 mm; fd2 1.0 mm, fw2 1.4 mm; fd3 1.2 mm, fw3 1.3 mm; fd4 1.1 mm, fw4 1.0 mm). Fingers with dermal fringe on inner side of fingers II and III; webbing on fingers absent. Subarticular tubercles

prominent rounded, single, all present. Prepollex distinct, oval; two distinct palmar tubercle oval; supernumerary tubercles absent.

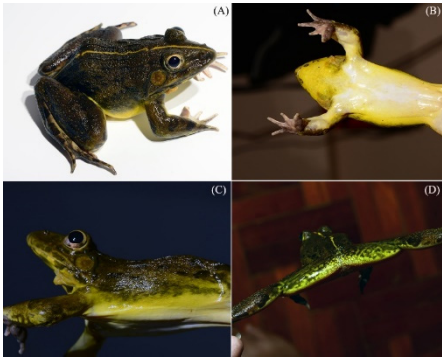
Hind limbs long; heels overlapping when limbs are folded at right angles to body; tibia 2.9 times longer (TL 50.1 mm) than wide (TW 17.5 mm), longer than thigh (FL 46.6 mm) and longer than distance from base of internal metatarsal tubercle to tip of toe IV (FOL 46.4 mm). Toes long and strong, toe IV (FTL 27.1 mm) 2.9 times distance from base of tarsus to tip of toe IV (TFOL 71.3 mm). Relative lengths of toes:  $I < II < V < III < IV$ . Tips of all toes rounded, not enlarged, without lateroventral grooves, slightly wide compared to toe width (td1 1.2 mm, tw1 1.1 mm; td2 1.2 mm, tw2 1.0 mm; td3 1.3 mm, tw3 0.9 mm; td4 1.1 mm, tw4 0.9 mm; td5 1.0 mm, tw5 0.8 mm). Webbing present, large:  $I \frac{1}{2}$ –0  $II \frac{1}{2}$ –0  $III \frac{1}{2}$ –1  $IV$ –0  $V$  (WTF 10.4 mm, WFF 9.6 mm; WI 10.2 mm, WII 6.8 mm; MTTF 25.9 mm, MTFF 26.9 mm, TTF 17.3 mm, FTF 20.2 mm). Dermal fringe along toe V present: from tip of toe to the position of the outer metatarsal tubercle. Subarticular tubercle prominent, elliptical or rounded, simple, all present. Inner metatarsal tubercle prominent, distinct, elongate, its length (IMT 4.6 mm) 2.5 times in length of toe I (ITL 11.2 mm). Tarsal folds present. Outer metatarsal tubercle absent; supernumerary tubercles absent; tarsal tubercle absent.

Snout, between eyes and side of head smooth; anterior and posterior parts of back smooth with longitudinal fold; upper and lower parts of flank smooth. Dorsolateral folds absent; supratympanic fold distinct, prominent from posterior eye to mandible; parotoid glands absent; cephalic ridges absent; co-ossified skin absent. Dorsal parts of forelimb, thigh, tibia, and tarsus smooth. Tibia with tubercles. Chest, belly, and ventral part of anterior part of thighs smooth. Macroglands absent.

In alcohol (Figs. 3 and 4), dorsal parts of head and back dark grey with cream strip from snout to upper vent; lateral part of head dark grey with tympanum dark brown; upper part of flank dark grey, lower part creamy white; loreal region, upper lip, dorsal part of thigh, leg, foot dark grey; posterior part of thigh greyish brown with dark blotches. Dorsal part of forelimb dark grey without crossbars; dorsal part of thigh, tibia, and tarsus brownish dark grey without crossbars. Throat, chest, anterior and posterior parts of belly, ventral part of forearm and thigh, tibia creamy white. Tarsus and webbing dark brown. In live (Figs. 5 and 6), dorsal parts of head and back light brown with light brown stripe from snout to upper vent; lateral part of head reddish brown; upper part of flank yellow-beige, lower part creamy white; loreal region, tympanic region, tympanum, upper lip, dorsal part of thigh, leg, foot reddish brown; posterior part of thigh creamy white. Throat, chest, anterior and posterior parts of belly, ventral part of forearm, thigh tibia, tarsus and webbing creamy white.



**Fig. 6** Photographs of live adult *Hoplobatrachus salween* sp. nov. specimens showing coloration and pattern: (A) dichromatism of adult male (left) and female (right); (B) dorsolateral view of adult male in its natural habitat, where all specimens were released into their natural habitat after photographing



**Fig. 5** Photographs of a live adult *Hoplobatrachus salween* sp. nov. male paratype (KKUC 01176/THNHM 26832) showing the coloration and pattern: (A) dorsolateral view; (B) ventral part of head and body; (C) lateral view; (D) posterior thigh

Adult males have a nuptial pad on first finger, pouch of external vocal sac under side of jaw corners and vocal sac opening inside the mouth. During mating at night, the male specimens showed sexual dichromatism in having yellow coloration in all parts of the body and limbs. After the frogs were collected and kept overnight, the color changed to normal coloration, similar to females. Finally, the coloration of male specimens changed completely to become similar to females. During mating, coloration of females showed little yellow on the flanks. Many tubercles on the dorsal tibia in males, fewer tubercles in females.

Comparison

A morphological comparison of *Hoplobatrachus* species (*H. crassus*, *H. litoralis*, *H. rugulosus*, and *H. tigerinus*) and specimens from Salawin National Park (Mae Hong Son province, northwestern Thailand) is given in Table 3.

Son province, northwestern Thailand					
Characteristic	<i>H. crassus</i>	<i>H. litoralis</i>	<i>H. rugulosus</i>	<i>H. tigerinus</i>	<i>H. salween</i> sp. nov.
SVL in adult males (mm)	70.2, <i>n</i> = 1 (92.9 in one unknown sex specimen)	90.0 ± 5.92, <i>n</i> = 14	90.2 ± 11.9 (74.4–122.3), <i>n</i> = 25	114.0 ± 6.52, <i>n</i> = 9	98.6 ± 5.42 (94.8–102.4), <i>n</i> = 2
SVL in adult females (mm)	No data	101.4 ± 12.01, <i>n</i> = 13	106.5 ± 16.4 (78.3–143.7), <i>n</i> = 28	113.7 ± 15.43, <i>n</i> = 6	94.1 ± 1.49 (93.2–96.3), <i>n</i> = 4
IMT in adult males (mm)	No data	4.9 ± 0.47, <i>n</i> = 14	7.1 ± 1.01, <i>n</i> = 25	6.9 ± 1.01, <i>n</i> = 9	5.2 ± 0.29, <i>n</i> = 2
IMT in adult females (mm)	No data	5.3 ± 0.99, <i>n</i> = 13	6.3 ± 1.72, <i>n</i> = 28	6.2 ± 1.92, <i>n</i> = 6	4.5 ± 0.24, <i>n</i> = 4
Snout shape	Obtusely pointed	Pointed	Rounded	Pointed	Pointed
Snout to anterior eye strip	No data	Black stripe	Absent	Indistinct	Indistinct
Supralabial stripe	Dark stripe	Black stripe	Absent	Indistinct	Indistinct
Mid-dorsal stripe	No data	Present	Absent	Present	Present
Dark supratympanic tripe	No data	Absent	Absent	Absent	Present
Thin inner side strip along tibia	No data	Absent	Absent	Present	Absent
Hand pattern	No data	Uniform	Mottled	No data	Mottled
Web pattern	No data	Uniform grey	Mottled	Mottled with an irregular pattern	Mottled with an irregular pattern

Table 3 Continued

Characteristic	<i>H. crassus</i>	<i>H. litoralis</i>	<i>H. rugulosus</i>	<i>H. tigerinus</i>	<i>H. salween</i> sp. nov.
Dorsal dermal ridges	5–6 broken longitudinal ridges	Thin and long	Wide and short	Thin and long	Thin and long
Tubercles on dorsal tibia of male	No data	Few	Few	No data	Densely
Sexual dichromatism	No data	Absent	Absent	Lemon yellow	Yellow
Vocal sac coloration in life	Blackish	No data	Dark brown	Blue	Yellow
Finger length	$IV < II < I < III$	$IV < II < I < III$	$II < I < IV < III$	No data	$II < IV < I < III$
Toe length	$I < II < III < V < IV$	$I < II < III < V < IV$	$I < II < V < III < IV$	No data	$I < II < V < III < IV$
Sources	Prasad et al. (2020)	Hasan et al. (2012)	This study	Hasan et al. (2012)	This study

SVL = snout-vent length; IMT = length of inner metatarsal tubercle

These results are similar to those of the molecular analyses, with the morphological characteristics of the specimens from Salawin National Park being similar to those of *H. litoralis* and *H. tigerinus* (see Hasan et al., 2012). However, the average body size of adult male specimens ( $98.7 \pm 5.42$  mm,  $n = 2$ ) was larger than that of *H. litoralis* ( $90.0 \pm 5.96$  mm,  $n = 14$ ). In contrast, adult females from the Salawin National Park ( $94.1 \pm 1.49$  mm,  $n = 4$ ) were smaller than *H. litoralis* ( $101.4 \pm 12.01$  mm,  $n = 13$ ). Measurements of the type series of adult *H. salween* sp. nov. are given in Table 4. The Salawin specimens could be differentiated from *H. litoralis* based on the following characteristics: snout to anterior eye stripe (indistinct stripe in *H. salween* sp. nov. vs. black stripe in *H. litoralis*), supralabial stripe (indistinct stripe in *H. salween* sp. nov. vs. black stripe in *H. litoralis*), dark supratympanic stripe (present in *H. salween* sp. nov. vs. absent in *H. litoralis*), hand pattern (mottled in *H. salween* sp. nov. vs. uniform in *H. litoralis*), web pattern (mottled with an irregular pattern in *H. salween* sp. nov. vs. uniform grey in *H. litoralis*), tubercles on the dorsal tibia of males (densely in *H. salween* sp. nov. vs. few in *H. litoralis*), sexual dichromatism (completely yellow except for the eye in *H. salween* sp. nov. vs. absent in *H. litoralis*), relative finger lengths ( $II < IV < I < III$  in *H. salween* sp. nov. vs.  $IV < II < I < III$  in *H. litoralis*) and toe lengths ( $I < II < V < III < IV$  in *H. salween* sp. nov. vs.  $I < II < III < V < IV$  in *H. litoralis*). Adult male specimens from Salawin National Park were smaller compared to *H. tigerinus* ( $114.0 \pm 6.52$  mm,  $n = 9$ ), as were the female specimens ( $113.7 \pm 15.43$  mm,  $n = 6$ ). The Salawin specimens could be differentiated from *H. tigerinus* based on the following characteristics: dark supratympanic stripe (present in *H. salween* sp. nov. vs. absent in *H. tigerinus*), thin stripe on the inner side along the tibia (absent in *H. salween* sp. nov. vs. present in *H. tigerinus*), sexual dichromatism (completely yellow except for the eye in *H. salween* sp. nov. vs. lemon yellow in *H. tigerinus*), and vocal sac color (yellow in *H. salween* sp. nov. vs. blue in *H. tigerinus*). The Salawin specimens can be distinguished from *H. crassus* based on their yellow vocal sacs (vs. blackish) and rounded inner metatarsal tubercles (vs. shovel shaped). The specimens from Salawin National Park differed from *H. rugulosus* in the following respects: smaller adult females ( $106.5 \pm 16.4$  mm,  $n = 28$ ), snout shape (pointed in *H. salween* sp. nov. vs. rounded in *H. rugulosus*), snout to anterior eye stripe (indistinct in *H. salween* sp. nov. vs. absent in *H. rugulosus*), supralabial stripe (indistinct in *H. salween* sp. nov. vs. absent in *H. rugulosus*), mid-dorsal stripe (present in *H. salween* sp. nov. vs. absent in *H. rugulosus*), dark supratympanic stripe



**Table 4** Selected measurements (measured in millimeters; mean ± SD) of type series of adult *Hoplobatrachus salween* sp. nov.

Specimen/ Characteristic	KKUC 01173/ THNHM 26829 holotype	KKUC 01175/ THNHM 26830 paratype	THNHM 26827 paratype	THNHM 26828 paratype	Mean ± SD of females	KKUC 01174/ THNHM 26831 paratype	KKUC 01176/ THNHM 26832 paratype
Sex	Female	Female	Female	Female		Male	Male
SVL	93.2	93.4	93.4	96.3	94.1 ± 1.49	94.8	102.4
HW	33.0	29.8	34.5	33.4	32.7 ± 2.03	33.6	34.8
HL	35.3	36.6	37.2	35.7	36.2 ± 0.87	37.4	39.8
MN	29.6	29.4	32.0	30.5	30.4 ± 1.20	31.4	34.7
MFE	23.4	20.9	24.4	24.2	23.2 ± 1.58	23.1	25.1
MBE	14.5	13.2	16.5	15.6	15.0 ± 1.43	15.0	15.9
IFE	13.5	11.6	14.4	11.7	12.8 ± 1.40	12.1	13.0
IBE	20.0	18.2	20.3	19.2	19.4 ± 0.94	19.9	19.9
FTL	20.1	18.4	21.0	21.5	20.2 ± 1.35	21.7	22.3
HAL	18.5	17.7	19.8	16.2	18.0 ± 1.54	19.9	20.7
TFL	9.6	8.1	10.0	8.8	9.1 ± 0.85	9.7	9.8
TL	50.1	47.9	52.8	51.0	50.5 ± 2.03	51.9	54.9
FOL	46.4	45.0	50.3	48.1	47.5 ± 2.27	50.8	51.3
FTL	27.1	26.0	27.2	28.1	27.1 ± 0.86	27.9	28.7
IN	5.4	5.3	4.9	5.5	5.3 ± 0.27	4.8	5.5
EN	8.3	7.7	7.8	8.3	8.0 ± 0.31	8.4	8.9
EL	8.8	7.7	9.3	8.4	8.6 ± 0.65	8.8	9.2
TYD	5.8	6.4	5.9	6.0	6.0 ± 0.23	7.7	8.4
TYE	2.8	3.3	4.4	4.3	3.7 ± 0.80	2.5	3.3
IUE	3.9	5.2	4.2	4.3	4.4 ± 0.56	4.4	4.4
UEW	6.4	6.8	7.2	6.8	6.8 ± 0.35	7.3	7.7
WTF	10.4	9.6	11.3	11.1	10.6 ± 0.78	10.2	11.2
WFF	9.6	7.27	8.9	8.8	8.7 ± 0.99	11.4	8.4
WI	10.2	8.5	10.3	10.3	9.8 ± 0.91	11.3	11.3
WII	6.8	5.8	6.1	7.8	6.6 ± 0.89	9.5	6.5
IMT	4.6	4.2	4.7	4.7	4.5 ± 0.24	5.4	5.0
ITL	11.2	10.0	11.7	11.5	11.1 ± 0.74	11.8	11.7
MTTF	25.9	23.3	27.7	26.0	25.7 ± 1.85	26.9	27.4
MTFF	26.9	22.9	27.5	26.0	25.8 ± 2.03	28.2	26.4
TFTF	17.3	16.2	17.1	17.7	17.1 ± 0.62	18.3	18.7
FFTF	20.2	18.7	21.0	21.3	20.3 ± 1.18	20.5	20.3
FL	46.6	44.7	45.9	47.6	46.2 ± 1.19	49.9	52.2
SL	15.3	15.5	14.6	15.0	15.1 ± 0.40	14.6	15.3
SN	5.3	6.6	5.1	5.7	5.7 ± 0.66	4.7	4.4
TW	17.5	13.0	20.0	18.2	17.2 ± 2.96	14.7	15.1
TFOL	71.3	66.2	70.9	72.5	70.3 ± 2.76	74.0	75.4

SVL = snout-vent length; HW = head width; HL = head length (from the back of the mandible to the tip of the snout); MN = distance from the back of the mandible to the nostril; MFE = distance from the back of the mandible to the front of the eye; MBE = distance from the back of the mandible to the back of the eye; IFE = distance between the fronts of the eyes; IBE = distance between the back of the eyes; IN = internarial space; EN = distance from the front of the eye to the nostril; EL = eye length; SN = distance from the nostril to the tip of the snout; SL = distance from the front of the eye to the tip of the snout; TYD = greatest tympanum diameter; TYE = distance from tympanum to the back of the eye; IUE = minimum distance between upper eyelids; UEW = maximum inter upper eyelid width; HAL = hand length (from the base of the outer palmar tubercle to the tip of the toe); FLL = forelimb length (from the elbow to the base of the outer tubercle); TFL = third finger length (from the base of the first subarticular tubercle); FL = femur length (from vent to knee); TL = tibia length; TW = tibia width; FOL = foot length (from the base of the inner metatarsal tubercle to the tip of the toe); FTL = fourth toe length (from the base of the first subarticular tubercle to the tip of toe 4); TFOL = length of tarsus and foot (from base of tarsus to tip of fourth toe); IMT = length of inner metatarsal tubercle; ITL = inner toe length; MTTF = distance from the distal edge of the metatarsal tubercle to the maximum incurvation of the web between third and fourth toe; TFTF = distance from the maximum incurvation of the web between the third and fourth toes to the tip of the fourth toe; MTFF = distance from the distal edge of the metatarsal tubercle to the maximum incurvation of the web between third and fourth toe; TFF = distance from the maximum incurvation of the web between the fourth and fifth toes; FFTF = distance from the maximum incurvation of the web between the fourth and fifth toes to the tip of the fourth toe; the maximum incurvation of the web between the fourth and fifth toes; WFF = webbing between the fourth and fifth toes (from the base of the first subarticular tubercle); WTF = webbing between the third and fourth toes (from the base of the first subarticular tubercle); WII = webbing between the third and fourth toes when folded along the fourth toe (from the base of the first subarticular tubercle); WII = webbing between the fourth and fifth toes when folded along the fourth toes (from the base of the first subarticular tubercle)



(present in *H. salween* sp. nov. vs. absent in *H. rugulosus*), sexual dichromatism (completely yellow except for the eye in *H. salween* sp. nov. vs. absent in *H. rugulosus*) and relative finger lengths ( $II < IV < I < III$  in *H. salween* sp. nov. vs.  $II < I < IV < III$  in *H. rugulosus*).

### Natural history notes

The type specimens were caught in a rice field during the breeding season (Fig. 7). Six tadpole specimens were collected from the same location with the new species. These tadpoles were assigned as tadpoles of the new species. The six tadpoles showed strong morphological similarities to tadpoles of the genus *Hoplobatrachus*. However, only the new species were found in the type locality. External and buccopharyngeal morphologies of the tadpoles are an ongoing study. Forest types around the sampled areas included dry evergreen, mixed deciduous and dry dipterocarp forests. The males were calling. The female paratypes were caught in a small stream bordering the rice field. The local people (Karen) utilize this frog and tadpoles as food. Currently, *H. salween* sp. nov. is known only from the type locality (Salawin National Park, Mae Sariang district, Mae Hong Son province, Thailand) and Myanmar (Bago and Yangon regions). The distribution map is presented in Fig. 8.

### Discussion

The name *Rana tigerina pantherina* is a synonym of *H. rugulosus* (Stejneger, 1925). However, this name is now invalid (The Integrated Taxonomic Information System, 2022), as *R. rugulosa* was moved to the genus *Hoplobatrachus*. Dubois (1992) classified members of the genus *Hoplobatrachus* into *H. occipitalis* and *H. tigerinus* groups. The *H. tigerinus* group is composed of three species: *H. crassus*, *H. rugulosus* and

*H. tigerinus*. *H. litoralis* (Hasan et al., 2012) and *H. salween* sp. nov., which were separated from *H. tigerinus* plus *H. litoralis*, were placed in this group. Several members of the genus *Hoplobatrachus* have long been mentioned in Thailand and Myanmar (Bourret, 1942; Taylor and Elbel, 1958; Taylor, 1962; Chanda et al., 2000; Khonsue and Thirakhupt, 2001; Schmalz and Zug, 2002; Chan-ard, 2003; Nabhitabhata et al., 2004; Nabhitabhata and Chan-ard, 2005; Chuaynkern and Chuaynkern, 2012; Duengkae et al., 2016; Niyomwan et al., 2019). This species has several synonyms that require further discussion (Frost, 2022). According to Taylor and Elbel (1958) and Taylor (1962), several specimens from northeastern and central Thailand were assigned to *R. tigerina pantherina*. Taylor (1962) recognized *R. tigerina* and *R. rugulosa* from Thailand. Taylor (1962) assigned three specimens from Ubon (Ubon Rachathani province), Bang Saen (Chon Buri province) and Doi Suthep (Chiang Mai province) to *R. rugulosa*. Based on the descriptions of *R. tigerina pantherina* (Taylor and Elbel, 1958; Taylor, 1962) and *R. rugulosa* (Taylor, 1962) specimens, they lack the mid-dorsal stripe. Thus, for the current study, they were assigned to *H. rugulosus*. However, molecular evidence suggested that these two cryptic species may be listed as *H. rugulosus* in Thailand. The results of the current study supported the existence of another species of *Hoplobatrachus* in Thailand, which is described here as a new species. The new species is not one of the two distinct species suggested by Pansook et al. (2012). Waiprom et al. (2017) first reported a new population of *Hoplobatrachus* species in Thailand (reported as *Hoplobatrachus* sp.); this population was intensively studied in the current work and described as a new species, *H. salween* sp. nov.

Annandale (1910) described *Rana burkilli* from Tavoy” (Dawei, southern Myanmar). *R. burkilli* was regarded as a synonym of *R. rugulosa* by Annandale (1917), and Bourret



**Fig. 7** Rice field habitat of *Hoplobatrachus salween* sp. nov. within mountain range in Salawin National Park, Mae Hong Son province, northwestern Thailand



**Fig. 8** Map showing the distribution of *Hoplobatrachus salween* sp. nov. (yellow filled stars) and *H. litoralis* (red filled circles), where map was created using www.simplemappr.net

(1942) regarded it as a synonym of *R. tigerina rugulosa*. At present (see Chanda et al., 2000; Frost, 2022), *R. burkilli* is considered as a subjective synonym of *H. rugulosus*. The description of *R. burkilli* provided by Annandale (1910) is more similar to *H. rugulosus* than *H. salween* sp. nov. The snout of *R. burkilli* is much less pointed and does not project as far beyond the mouth, compared to that of *H. salween* sp. nov. (Fig. 3), and projects beyond the lower jaw (Fig. 4). The inner metatarsal tubercle is somewhat feebly developed in *R. burkilli* but is prominent and distinct in *H. salween* sp. nov. The skin of the back and dorsal surface of the thighs is more warty in *R. burkilli* than *H. salween* sp. nov. The mid-dorsal stripe is absent in *R. burkilli* but present in *H. salween* sp. nov. The current molecular analyses of the 16S + Cytb and Cytb genes indicated that *H. rugulosus* from Phangnga province, southern Thailand formed clades with *H. rugulosus* (Figs. 1 and 2).

Zug et al. (1998) listed *H. rugulosus* (as *R. rugulosa*) and *H. tigerinus* (as *R. tigerina*) from Chatthin Wildlife Sanctuary, central Myanmar. Recent work by Mulcahy et al. (2018), who sequenced three *H. tigerinus* specimens from Yangon and Bago, indicated that they are *H. litoralis*. However, these three 16S sequences were analyzed based on specimens from Thailand, which the current study reassigned to *H. salween* sp. nov. In the 16S + Cytb analysis, the three sequences from Myanmar and *H. salween* sp. nov. formed a clade with a high posterior probability value (1.00). Furthermore, the *H. salween* sp. nov. clade was separated from the *H. litoralis* clade with high posterior probability (1.00). These results supported that the three sequences from Myanmar were *H. salween* sp. nov. and distinguished them from *H. litoralis* of Bangladesh.

Based on the molecular evidence, *H. salween* sp. nov. clearly formed a clade separated from other related species. *H. salween* sp. nov. and *H. litoralis* from Cox's Bazar (Bangladesh) are very similar in external morphology; however, the average genetic distance between the species was 3.1% for 16S and 11.5% for Cytb, similar to the distance separating *H. litoralis* from *H. tigerinus* (Hasan et al., 2012) supported by high posterior probability of BI. *H. salween* sp. nov. formed the same clade with *H. litoralis* from Bago and Yangon, Myanmar, supported by the posterior probability of 1.00. In addition, the sequence divergence between the populations was very low (0.5% of 16S). However, the sequence data of *H. litoralis* from Bago and Yangon used in the current study were derived from the 16S only. Therefore, the current study included the Bago and Yangon populations as members of *H. salween* sp. nov.

According to Vences et al. (2005), 3% 16S divergence between populations is a threshold value that might warrant new species status. Analyses of mitochondrial Cytb, 12S, and 16S rRNA genes of *Hoplobatrachus* and *Euphlyctis* from Bangladesh and other Asian countries have identified 13 major clades consistent with various cryptic species and the monophyly of Asian *Hoplobatrachus* species; however, the position of *H. occipitalis* has not been clarified (Alam et al., 2008). In addition, the current results indicated monophyly of *Hoplobatrachus*, and so *H. occipitalis* from Tanzania was included in the analyses. Sultana et al. (2016) provided a phylogenetic tree of Asian *Hoplobatrachus* based on the Cytb and 21 microsatellite markers. The tree revealed monophyly of each *Hoplobatrachus* species and the authors suggested that the environment specific to the river system (riverine delta region) has sustained the *H. tigerinus* population structure in this region. Kundu et al. (2020) provided an updated distribution of *H. litoralis* in the Dampa Tiger Reserve, Mizoram state, northeast India, based on morphological and molecular data, where the BI phylogenetic tree revealed monophyly of five *Hoplobatrachus* species (*H. crassus*, *H. tigerinus*, *H. litoralis*, *H. rugulosus*, *H. occipitalis*) and a sister relationship was presented between *H. litoralis* and *H. tigerinus*. Nevertheless, *Hoplobatrachus* specimens from Myanmar (Bago and Yangon in Mulcahy et al., 2018) and Thailand were not included in the work of Kundu et al. (2020). The current results showed that *H. salween* sp. nov. was more closely related to *H. litoralis* from Bangladesh than *H. tigerinus*, with a sister relationship demonstrated between the clades. Furthermore, examination of the distribution and natural history of *H. salween* sp. nov. indicated that the preferred habitat of the new species was rice fields at an elevation of approximately 86–106 m a.s.l., whereas *H. litoralis* prefers vegetated, marshy ditches/ponds beside wetlands created by hill streams at an elevation of > 3 m a.s.l. and occasionally, the species can be found at the mountain base, which has a different soil texture from mainland Bangladesh (Hasan et al., 2012).

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### Conflict of Interest

The authors declare that there are no conflicts of interest.

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### Acknowledgements

The following institutes facilitated this work: Khon Kaen University (Faculty of Science, Department of Biology), National

Science Museum (Thailand Natural History Museum), Kasetsart University, Bangkok, Thailand (Faculty of Forestry, Department of Forest Biology), and Department of National Parks, Wildlife and Plant Conservation (Salawin National Park). The Department of National Parks, Wildlife and Plant Conservation granted access to the study area and approved the collection of specimens. Dr. George R. Zug, Prof. Dr. Arunrat Chaveerach and Dr. Unchaleeporn Ameamsri provided useful input. Special thanks go to all staff members of the Salawin National Park for assistance during the field surveys. This work was financially supported by the Research and Academic Services Research Fund of Khon Kaen University for Lecturer Students with High Potential (2019), awarded to Miss Prapaiporn Thongproh and Asst. Prof. Chantip Chuaynkern and the Research Capability Enhancement Program/Graduate Student Scholarships of the Faculty of Science, Khon Kaen University for Miss Jidapa Chunskul and Asst. Prof. Yodchaiy Chuaynkern.

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