



Identification of *Hylarana tytleri* (Theobald, 1868): elements for the systematics of the genus *Hylarana* Tschudi, 1838 (Anura, Ranidae)

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For more than a century, *Hylarana tytleri* (Theobald, 1868) has been confused with two congeneric species, *H. taipehensis* and *H. erythraea*, in Bangladesh and neighboring countries, due to phenetic similarities as well as a lack of sufficient molecular and morphometric data. To resolve these problems, we conducted molecular and morphological surveys of *Hylarana* species throughout Bangladesh and examined *H. taipehensis* from Taiwan and Vietnam, “*H. erythraea*” from Vietnam, Thailand, Myanmar and Malaysia, and *H. macrodactyla* from China and Vietnam. Based on mitochondrial DNA sequence data, *H. tytleri* was shown to be genetically divergent from these relatives (7.4, 11.8 and 12.3 % for the 16S rRNA gene and 15.8, 19.8 and 20.9 % for

the Cyt b gene in relation to *H. macrodactyla*, *H. taipehensis* and “*H. erythraea*”, respectively). Morphologically, *H. tytleri* could also be distinguished from these congeners by the following: snout-vent length (SVL): 28.7–41.8 mm, rounded snout, moderately elongated head and off-white dorsolateral folds. Although *H. tytleri* showed the closest affinity with *H. macrodactyla* in the molecular phylogeny, the former is strikingly different in the absence of a middorsal stripe. In the future, these data may be useful as a reference to avoid erroneous species identification of *Hylarana* species in these regions.

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INTRODUCTION

The family *RANIDAE* Batsch, 1796 is one of the most diverse and speciose amphibian groups in the world, consisting of about 400 currently recognized species in 23 genera and representing about 5 % of all anurans (Anonymous 2018). Among these genera, the taxonomy of the genus *Hylarana* Tschudi, 1838 has been revised numerous times, with a series of taxonomic rearrangements having been suggested based on morphological data (Dubois 1987, 1992) and molecular phylogenetic studies (e.g., Che *et al.* 2007; Pyron & Wiens 2011). Most recently, Oliver *et al.* (2015) redelimited *Hylarana* and proposed that members of this genus be split into 10 distinct genera based on their morphology, multi-locus molecular phylogeny and geographic distribution. As a result, only four species—*Hylarana erythraea* (Schlegel, 1837), *H. macrodactyla* (Günther, 1858), *H. taipehensis* (Van Denburgh, 1909) and *H. tytleri* (Theobald, 1868)—were retained under *Hylarana s. s.* (Oliver *et al.* 2015), thus coming back to the narrow extension of this taxon of Dubois (1992). The redelimited *Hylarana*, now restricted in range to Southeast Asia, can be identified from closely related genera such as *Humerana* Dubois, 1992 by the first finger, which is subequal to the second, and disc expansion of 1.2 to 1.7 times the width of the finger (Dubois 1992; Oliver *et al.* 2015).

Previously, five species, allocated to *Hylarana* were thought to exist in Bangladesh (Kabir *et al.* 2009): “*H. erythraea*”, *H. taipehensis*, *H. tytleri*, *H. leptoglossa* and *H. nigrovittata*; the latter two have been allocated to *Hydrophylax leptoglossa* and *Sylvirana nigrovittata* respectively (Oliver *et al.* 2015). However, descriptions and identification of *Hylarana* species remain limited. Although the identification of *H. macrodactyla* (Günther, 1858) is clear (long body, slim elongated legs and specially foot and toes, presence of a striped dorsal pattern, including a middorsal line), for more than a century *H. tytleri* (Theobald, 1868) has been confused with *H. taipehensis* (Van Denburgh, 1909) and “*H. erythraea*” (Schlegel, 1837). This confusion has occurred due to the very similar color patterns of these frogs, overlapping body length, a lack of sufficient molecular and morphological data and local research aimed at identification of *Hylarana* species based on only pictures and/or sporadic information.

Hylarana tytleri was first described based on a single specimen from “Dacca” (presently written as “Dhaka”, 23°71'N, 90°36'E), Bangladesh, by Theobald (1868), the holotype (ZSI 10035) of which is still extant in the Zoological Survey of India, Kolkata.

However, this specimen is in a bad state of preservation, making it of limited use in comparative studies (Ohler & Mallick 2002). Moreover, the original description is inadequate and not informative enough for identification of other members of this species. Consequently, various authors have queried its identity and taxonomic placement. For example, Boulenger (1882, 1890) listed *Rana tytleri* as a valid species. Sclater (1892a) made an attempt to allocate specimens named *Rana tytleri* and concluded that the holotype of *Hylorana tytleri* could be identified as *Rana erythraea* whereas other specimens studied by Boulenger should be identified as *Rana nigrovittata* based on variation of width of the dorsolateral folds. These identifications were maintained by Sclater (1892b) and by Boulenger (1920) who separated *Rana leptoglossa* (Cope, 1868) from *Rana nigrovittata* (Blyth, 1856). Pillai & Chanda (1981) described a new species *Rana bilineata* from the Garo Hills (elevation about 400 m), Meghalaya, India, based on morphology. This name was preoccupied in the genus *Rana* by *Rana bilineata* Shaw, 1802 (currently considered a junior synonym of *Dryophytes cinereus* [Schneider, 1799]) and was replaced as a junior primary homonym by the *nomen novum* *Rana* (*Hylarana*) *albolineata* by Dubois (1987), and later put to the synonymy of *Rana* (*Hylarana*) *taipehensis* by Dubois (1992). Ohler & Mallick (2002) identified the specimens described by Pillai & Chanda (1981) as *R. (Hylarana) tytleri*. Therefore *R. (Hylarana) bilineata* Pillai & Chanda, 1981 and *R. (Hylarana) albolineata* Dubois, 1987 are removed from the synonymy of *R. (Hylarana) taipehensis* and referred as junior subjective synonyms of *R. tytleri*. Iskandar (1998) treated *Rana tytleri* as a synonym of *R. (Hylarana) chalconota* (Schlegel, 1837) without proper discussion, while Mahony *et al.* (2009) treated a few specimens from Jahangirnagar University, Bangladesh, as *Hylarana tytleri* based on the work of Ohler & Mallick (2002). Similarly, Kabir *et al.* (2009) reported *H. tytleri*, *H. taipehensis* and “*H. erythraea*” in Bangladesh without proper delineation, while Hasan *et al.* (2012a) denoted *Hylarana* species (morphologically similar to *H. taipehensis*) from Bangladesh as corresponding to *H. cf. taipehensis* based on mitochondrial DNA (mtDNA) data. Later, Biju *et al.* 2014 in Genbank denoted a specimen from Tripura, India (adjacent to eastern Bangladesh), as *H. cf. tytleri*, whereas recently Oliver *et al.* (2015) identified a few specimens from Myanmar as *H. tytleri*. Very recently, Mulcahy *et al.* (2018) commented that these *H. tytleri* are similar to “*H. erythraea*” from the Yangon region in Myanmar.

Mitochondrial DNA is a useful tool for delineating tentative species, particularly the phenetically similar frogs in the genus *Hylarana* and closely related groups (Matsui 2011; Matsui *et al.* 2012; Biju *et al.* 2014; Hasan *et al.* 2014a; Oliver *et al.* 2015). In amphibians, the 16S rRNA gene (*16S*) is often considered an indicator for the taxonomic status of frog species (Vences *et al.* 2005), whereas the protein-encoding Cytochrome b gene (*Cytb*), which exhibits more rapid nucleotide substitutions than ribosomal RNA genes, is considered more phylogenetically informative at a more terminal level (Koike & Matsui 2003).

In this study, we provide detailed molecular and morphological data for *H. tytleri* from across Bangladesh and compare the data with that of *H. taipehensis* from Taiwan (topotypic area) and Vietnam, “*H. erythraea*” from Malaysia, Vietnam, Myanmar and Thailand, and *H. macrodactyla* from China and Vietnam (fig. 1). Then we provide new usable molecular and morphological data to avoid ambiguous species identification of *Hylarana* species in Bangladesh and adjacent areas.

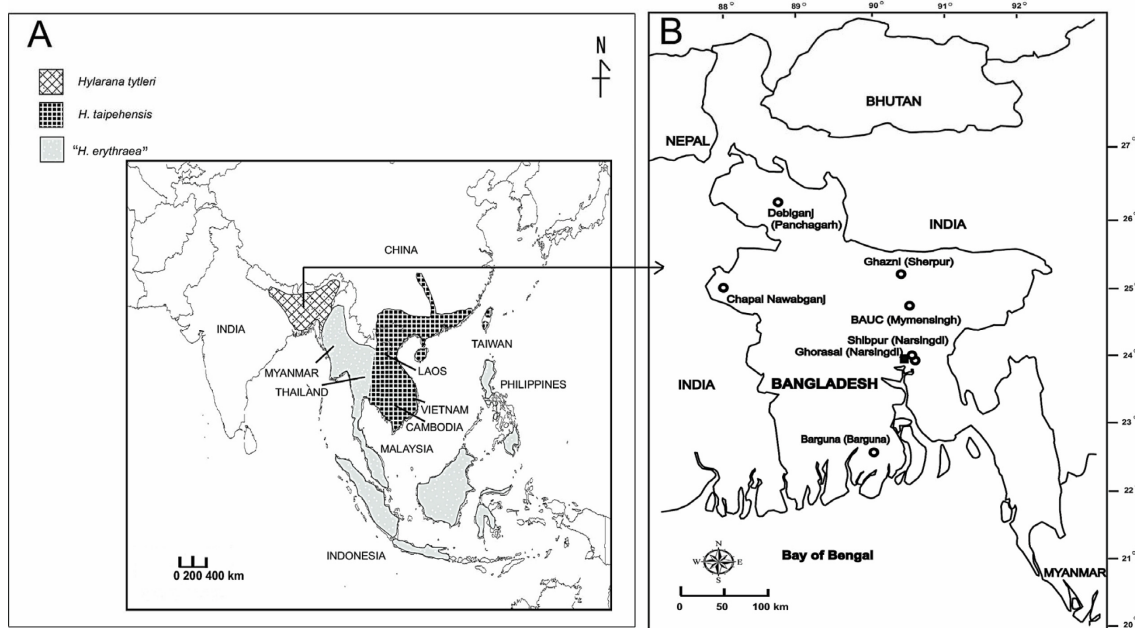


Figure 1. (A) Map showing the approximate distribution areas of three *Hylarana* species in Southeast Asia. (B) Map of Bangladesh showing the collecting localities of *H. tytleri*. Closed square indicates the original type locality of *H. tytleri* stated by Theobald (1868).

MATERIALS AND METHODS

Specimens

Specimens of *Hylarana* were collected from seven localities in Bangladesh (fig. 1B), two localities in Taiwan, one locality in both China and Malaysia, and eight localities in Vietnam (not shown in the Figure). Two specimens of "*H. erythraea*" (CAS 229614 and 247465) from Myanmar were also examined for morphological purposes. The sequences of other closely related species were retrieved from GenBank (see Tab. 1). Specimens and/or tissue samples were stored in the Amphibian Research Center, Hiroshima University (IABHU), Department of Fisheries Biology & Genetics, Bangladesh Agricultural University (DFGBAU), Department of Life Science, National Taiwan Normal University, Taiwan (NTNU), Zoological Museum of Moscow University (Moscow, Russia) (ZMMU) and the California Academy of Sciences in San Francisco (CAS).

Table 1. Samples of *Hylarana* used in this study and GenBank accession numbers. AMNH: American Museum of Natural History; BNHS: Bombay Natural History Society Museum; CAS: California Academy of Sciences; DFBGBAU: Department of Fisheries Biology and Genetics, Bangladesh Agricultural University; DZ: Department of Zoology, University of Peradeniya; FMNH: Field Museum of Natural History; IABHU: Institute for Amphibian Biology (Currently named as Amphibian Research Center), Hiroshima University; NTNU: National Taiwan Normal University; SDBDU: Systematics Lab, University of Delhi; USNM: National Museum of Natural History; ZMMU: Zoological Museum of Moscow University and NA: Not available.

Species	Locality	Voucher	Accession No.	
			16S	Cytb
<i>Hylarana tytleri</i>	BAUC, Mymensingh, Bangladesh	IABHU 4219	LC061444	LC061597
<i>Hylarana tytleri</i>	BAUC, Mymensingh, Bangladesh	DFBGBAUHtai 228	AB530523	
<i>Hylarana tytleri</i>	Ghazni, Sherpur, Bangladesh	DFBGBAUHtai 216	AB530522	
<i>Hylarana tytleri</i>	Ghorasal, Narsingdi, Bangladesh	IABHU 3893	AB530524	LC061598
<i>Hylarana tytleri</i>	Ghorasal, Narsingdi, Bangladesh	IABHU 3895	AB530525	
<i>Hylarana tytleri</i>	Raipura, Narsingdi, Bangladesh	IABHU 4154	LC061445	
<i>Hylarana tytleri</i>	Shibpur, Narsingdi, Bangladesh	Release	LC061446	LC061599
<i>Hylarana tytleri</i>	Shibpur, Narsingdi, Bangladesh	Release	LC061447	LC061600
<i>Hylarana tytleri</i>	Barguna, Bangladesh	IABHU 3892	AB543603	
<i>Hylarana tytleri</i>	Chapai Nawabganj, Bangladesh	IABHU 3889	LC061448	LC061601
<i>Hylarana tytleri</i>	Panchagarh, Bangladesh	IABHU 4124	LC061449	
<i>Hylarana tytleri</i>	Panchagarh, Bangladesh	IABHU 4125	LC061450	
<i>Hylarana taipehensis</i>	Shimen District, New Taipei City, Taiwan	NTNU 202470	LC061451	LC061602
<i>Hylarana taipehensis</i>	Shimen District, New Taipei City, Taiwan	NTNU 202471	LC061452	
<i>Hylarana taipehensis</i>	Shimen District, New Taipei City, Taiwan	NTNU 202473	LC061453	
<i>Hylarana taipehensis</i>	Shimen District, New Taipei City, Taiwan	NTNU 202474	LC061454	
<i>Hylarana taipehensis</i>	Sanzhi Township, New Taipei City, Taiwan	NTNU 202428	LC061455	
<i>Hylarana taipehensis</i>	Sanzhi Township, New Taipei City, Taiwan	NTNU 202429	LC061456	LC061603
<i>Hylarana taipehensis</i>	Sanzhi Township, New Taipei City, Taiwan	NTNU 202431	LC061457	
<i>Hylarana taipehensis</i>	Sanzhi Township, New Taipei City, Taiwan	NTNU 202432	LC061458	
<i>Hylarana taipehensis</i>	Cam Ranh, Khanh Hoa, Vietnam	ZMMU NAP-03217	MH503784	
<i>Hylarana taipehensis</i>	Nha Trang, Khanh Hoa, Vietnam	NA	MH503785	
<i>Hylarana taipehensis</i>	Cat Tien N.P., Dong Nai, Vietnam	ZMMU NAP-01505	MH503786	
<i>Hylarana taipehensis</i>	Cat Tien N.P., Dong Nai, Vietnam	ZMMU NAP-01506	MH503787	
<i>Hylarana taipehensis</i>	Cat Tien N.P., Dong Nai, Vietnam	ZMMU NAP-01507	MH503788	
<i>Hylarana taipehensis</i>	Lo Go-Xa Mat N.P., Tay Ninh, Vietnam	ZMMU NAP-03663	MH503789	
<i>Hylarana taipehensis</i>	Lo Go-Xa Mat N.P., Tay Ninh, Vietnam	ZMMU NAP-03664	MH503790	
<i>Hylarana taipehensis</i>	Lo Go-Xa Mat N.P., Tay Ninh, Vietnam	ZMMU NAP-03665	MH503791	
<i>Hylarana taipehensis</i>	Phu Quoc N.P., Kien Giang, Vietnam	ZMMU NAP-03769	MH503792	
<i>Hylarana taipehensis</i>	Phu Quoc N.P., Kien Giang, Vietnam	ZMMU NAP-03770	MH503793	
<i>Hylarana taipehensis</i>	Phu Quoc N.P., Kien Giang, Vietnam	ZMMU NAP-03771	MH503794	
<i>Hylarana taipehensis</i>	Binh Chau-Phuok Buu N.P., Ba Ria-Vung Tau, Vietnam	ZMMU NAP-03837	MH503795	
<i>Hylarana taipehensis</i>	Binh Chau-Phuok Buu N.P., Ba Ria-Vung Tau, Vietnam	ZMMU NAP-03838	MH503796	
<i>Hylarana taipehensis</i>	Binh Chau-Phuok Buu N.P., Ba Ria-Vung Tau, Vietnam	ZMMU NAP-03839	MH503797	

Species	Locality	Voucher	Accession No.	
			16S	Cytb
<i>Hylarana taipehensis</i>	Yok Don N.P., Dak Lak, Vietnam	ZMMU NAP-04276	MH503798	
<i>Hylarana taipehensis</i>	Yok Don N.P., Dak Lak, Vietnam	ZMMU NAP-04277	MH503799	
<i>Hylarana taipehensis</i>	Binh Chau-Phuok Buu N.P., Ba Ria-Vung Tau, Vietnam	ZMMU NAP-03063	MH503800	
<i>Hylarana taipehensis</i>	Binh Chau-Phuok Buu N.P., Ba Ria-Vung Tau, Vietnam	ZMMU NAP-03064	MH503801	
<i>Hylarana taipehensis</i>	Binh Chau-Phuok Buu N.P., Ba Ria-Vung Tau, Vietnam	ZMMU NAP-03065	MH503802	
<i>Hylarana taipehensis</i>	Loc Bao, Lam Dong, Vietnam	ZMMU NAP-02801	MH503803	
<i>Hylarana taipehensis</i>	Loc Bao, Lam Dong, Vietnam	ZMMU NAP-02802	MH503804	
<i>Hylarana taipehensis</i>	Loc Bao, Lam Dong, Vietnam	ZMMU NAP-02803	MH503805	
<i>Hylarana taipehensis</i>	Kon Ka Kinh N.P., Gia Lai, Vietnam	ZMMU NAP-07454	MH503806	
<i>Hylarana taipehensis</i>	Kon Ka Kinh N.P., Gia Lai, Vietnam	ZMMU NAP-07453	MH503807	
<i>Hylarana taipehensis</i>	Taoyuan, Taiwan	NA	MH503808	
<i>Hylarana taipehensis</i>	Taoyuan, Taiwan	NA	MH503809	
<i>Hylarana taipehensis</i>	Taoyuan, Taiwan	NA	MH503810	
<i>Hylarana macrodactyla</i>	Hainan, China	NTNU 241912	LC061459	LC061604
<i>Hylarana macrodactyla</i>	Phu Quoc N.P., Kien Giang, Vietnam	ZMMU NAP-3764	MH503781	
<i>Hylarana macrodactyla</i>	Phu Quoc N.P., Kien Giang, Vietnam	ZMMU NAP-3765	MH503782	
<i>Hylarana macrodactyla</i>	Phu Quoc N.P., Kien Giang, Vietnam	ZMMU NAP-3766	MH503783	
“ <i>Hylarana erythraea</i> ”	Langkawi Island, Malaysia	IABHU 21105	AB530584	LC061605
“ <i>Hylarana erythraea</i> ”	Chantaburi, Thailand	NA	AB530580	LC061606
“ <i>Hylarana erythraea</i> ”	Koh Kong Province, Cambodia	FMNH 263289	KR264075	KR264165
“ <i>Hylarana erythraea</i> ”	Con Son Isl., Con Dao N.P., Ba Ria-Vung Tau, Vietnam	ZMMU NAP-00561	MH503777	
“ <i>Hylarana erythraea</i> ”	Con Son Isl., Con Dao N.P., Ba Ria-Vung Tau, Vietnam	ZMMU NAP-00562	MH503778	
“ <i>Hylarana erythraea</i> ”	Con Son Isl., Con Dao N.P., Ba Ria-Vung Tau, Vietnam	ZMMU NAP-00563	MH503779	
“ <i>Hylarana erythraea</i> ”	Bin Chau, Phuok Buu N.P., Ba Ria-Vung Tau, Vietnam	ZMMU NAP-03062	MH503780	
<i>Hydrophylax leptoglossa</i>	Golapganj, Sylhet, Bangladesh	IABHU 3784	AB530528	LC061607
<i>Amnirana</i> cf. <i>nicobariensis</i>	Muara Siberut, Indonesia	IABHU 20707	AB530581	LC061608
“ <i>Hylarana erythraea</i> ”	Myanmar	USNM 583188	KR264119	KR264206
“ <i>Hylarana erythraea</i> ”	Tanintharyi Division, Myanmar	CAS 247465	KR264066	KR264156
“ <i>Hylarana tytleri</i> ”	Tripura, India	SDBDU 2009.421	KM069012	KM069225
<i>Humerana</i> cf. <i>humeralis</i>	Assam, India	SDBDU 2009.1094	KM069010	KM069223
<i>Sylvirana guentheri</i>	Ha Giang, Vietnam	AMNH 163940	KR264039	KR264130
<i>Hydrophylax gracilis</i>	Hiyare, India	DZ1164	KM068933	KM069148
<i>Hydrophylax malabarica</i>	Meladoor, India	BNHS 5879	KM068966	KM069180
<i>Indosylvirana aurantiaca</i>	Chathankod, India	SDBDU 2011.520	KM068909	KM069124
<i>Indosylvirana flavescens</i>	Settukunnu, India	BNHS 5844	KM068930	KM069145
<i>Indosylvirana temporalis</i>	Panwila, India	DZ 1153	KM068998	KM069212
<i>Pelophylax nigromaculatus</i>	Hiroshima, Japan	NA	AB043889	AB043889

Morphological measurements

For morphological comparisons, the following 30 measurements were taken using digital calipers to the nearest 0.1 mm: SVL, snout-vent length; HL, head length; HW, head width; S-N, snout to nostril distance; N-N, inter-nostril distance; N-E, nostril to eye distance; ED, longitudinal eye diameter; E-E, inter-orbital distance between inner borders of upper eyelids; ELW, eyelid width; TD, Tympanum diameter; FLL, forelimb length; FHL, forearm and hand length; FAW, forearm width; HAL, hand length; F1–F4, lengths of 1st to 4th finger; HLL, hind limb length; FEL, femur length; TIL, tibia length; TFL, tarsus and foot length; FOL, foot length; T1–T5, lengths of 1st to 5th toes; IMT, inner metatarsal tubercle length; and OMT, outer metatarsal tubercle length. Original measurement data of *Hylarana* species from Bangladesh and other Asian countries are shown in Appendix Table S1. Statistical analysis was performed using SPSS (22.0) software (IBM, USA). Webbing formula follows Savage & Heyer (1967) as modified by Myers & Duellman (1982).

Morphometry

In order to minimize the effect of allometric growth, measurements were transformed into the natural logarithm and then the mean of all ln-transformed measurements was subtracted from this value. These variables are presented by adding the letter L before the measurement abbreviation. For all species we tested sexual dimorphism using Mann-Whitney *U* test on size-corrected variables. The presence of sexual dimorphism in various measurements obliged to perform further analysis for males and females separately. As individual specimens were allocated to clades by our molecular data, we used these specimens and individuals from the same populations to perform discriminant analysis using measurements corrected for size. Significant level used throughout was $P \leq 0.05$. As for *Hylarana macrodactyla* and *H. tytleri* sample size was small, a $P \leq 0.1$ was adopted.

DNA extraction, PCR, sequencing and phylogenetic analyses

Methods of DNA extraction, PCR amplification and sequencing of mtDNA fragments (*16S* and *Cytb*) were as reported by Hasan *et al.* (2012a–b) and Nasrin *et al.* (2014). The obtained *16S* and *Cytb* sequences were deposited in the DDBJ/EMBL/GenBank database (accession numbers: LC061444–LC061459 and MH503777–MH503810 for *16S*, and LC061597–LC061608 for *Cytb*).

The resulting *16S* sequences were aligned using ClustalW (Thompson *et al.* 1994). Sequence divergence of *16S* (uncorrected *P* values) was calculated using MEGA Ver. 6.0 (Tamura *et al.* 2013) with the pairwise-deletion option, in which all alignable sites were used for calibration, but indel sites were not counted. Gaps and ambiguous sites were excluded using Gblocks Ver. 0.91b (Castresana 2000) with default parameters. Gap sites in the alignments were treated as missing data. The initial two alignments (*16S* and *Cytb*) were combined into one concatenated data set containing a total of 1876 sites (1255 for *16S* and 621 for *Cytb*), 488 of which were parsimoniously informative.

Phylogenetic analyses were performed using *16S* data for our *Hylarana* specimens as well as specimens of related species. The sequence length and total number of operational taxonomic units (OTUs) determined from the alignment data was 512 sites of 31 OTUs.

Phylogenetic analyses were performed using maximum likelihood (ML), maximum parsimony (MP) and Bayesian inference (BI) methods. Nucleotide substitution models for ML and BI analyses were selected based on the Akaike information criterion and Bayesian information criterion, respectively, which are implemented in the Kakusan 3.0 program (Tanabe 2007). ML analysis was performed using Treefinder (Jobb 2008) and the resultant tree was evaluated by bootstrap (BS) analysis with 1000 replicates. BI analysis was performed using MrBayes Ver. 3.1.2 (Ronquist & Huelsenbeck 2003) with the following settings: number of Markov chain Monte Carlo (MCMC) generations = 10 million, sampling frequency = 100, with the first 1 million generations discarded as burn-in. The number of MCMC generations and burn-in size was determined by checking the convergence of -log likelihood (-lnL) values and tree length against generation number using Tracer ver. 1.4 (Rambaut & Drummond 2007). Statistical support of the BI tree was evaluated by Bayesian posterior probability (BPP). MP was performed with 1000 BS replicates using PAUP* 4.0b10 (Swofford 2003).

RESULTS

Morphometry

Sexual dimorphism

Males are smaller than females in all species of *Hylarana* (Tab. 2) and show several size corrected measurements with significant differences between males and females (Tab. 3). “*H. erythraea*” shows significant differences in distance between eyes (E-D) (relatively larger in females), tympanum diameter (TD) (relatively larger in females), length of first finger (F1) (relatively shorter in males) and length of toes (relatively shorter in males). In *H. taipehensis*, size is significantly smaller in males, as is relative width of head, length of first two fingers and first toe, as well as length of femur (FEL), whereas tympanum is relatively larger in females, as the inner and outer metatarsal tubercles. In *H. tytleri*, males differ from females significantly in two measurements: they show relatively shorter femur length and relatively shorter first toe. In *H. macrodactyla*, males are significantly smaller ($P < 0.1$) and have relatively shorter toes than females, but have a relatively wider distance between nostrils, larger tympanum; and females have relatively longer and larger forelimbs (HAL, FAW). The raw values of the measurements for males and females are given in Appendix Table S1.

Table 2. Mean values, standard deviation, minimum and maximum values of measurements of males and females of “*Hylarana erythraea*”, *H. taipehensis*, *H. tyleri* and *H. macrodactyla*. Abbreviations for measurements are given in the *Material and methods* section; N, sample size; ♂, males; ♀, females.

	“ <i>Hylarana erythraea</i> ”		<i>Hylarana taipehensis</i>		<i>Hylarana tyleri</i>		<i>Hylarana macrodactyla</i>	
	♂ N = 6	♀ N = 12	♂ N = 36	♀ N = 12	♂ N = 8	♀ N = 2	♂ N = 3	♀ N = 3
SVL	31.4±4.46 26.7–35.4	58.0±4.29 51.4–64.9	29.0±1.8 26.3–33.6	42.8±3.17 34.9–46.8	31.45±1.641 28.7–34.7	41.51±0.417 41.2–41.8	26.3±0.88 25.4–27.1	33.2±4.45 28.1–36.1
HL	12.8±1.81 10.6–14.6	23.7±1.73 21.3–26.9	11.91±0.86 10.5–14.5	16.9±1.56 13.8–20.2	11.69±1.582 9.4–15	16.25±3.606 13.7–18.8	10.3±0.13 10.2–10.4	12.0±2.04 9.9–14.0
HW	9.47±1.37 7.3–10.9	18.1±1.27 15.9–20.4	8.39±0.74 7.2–10	12.5±1.06 10.3–14.9	8.35±1.016 7.8–10.8	11.65±1.626 10.5–12.8	7.31±0.55 6.7–7.6	9.07±1.67 7.2–10.4
S-N	2.05±0.38 1.7–2.6	3.87±0.69 2.9–5.5	1.91±0.2 1.4–2.6	2.58±0.27 2.2–3.1	1.86±0.393 1.2–2.3	2.45±0.212 2.3–2.6	1.74±0.12 1.6–1.9	2.04±0.08 2.0–2.1
N-N	2.57±0.4 2.0–2.9	4.8±1.02 3.2–7.0	2.38±0.22 1.9–2.8	4.05±2.85 2.6–13.1	3.31±0.304 2.9–3.7	4.15±0.071 4.1–4.2	2.43±0.06 2.4–2.5	2.29±0.09 2.2–2.4
N-E	3.14±0.46 2.7–3.6	5.73±0.66 4.6–6.8	2.81±0.28 2.2–3.4	3.84±0.32 3.2–4.5	3.21±0.36 2.5–3.5	4.4±0.283 4.2–4.6	2.17±0.05 2.1–2.2	2.79±0.47 2.4–3.3
ED	3.98±0.33 3.6–4.4	6.73±0.58 5.7–8.0	3.48±0.44 2.2–4.3	4.91±0.54 3.8–5.7	3.11±0.562 2.2–3.9	4.1±0.424 3.8–4.4	3.44±0.12 3.3–3.6	3.77±0.37 3.5–4.2
E-E	2.97±0.23 2.7–3.3	5.32±1.48 4.0–9.0	2.52±0.22 2.2–3.1	3.42±0.31 2.8–3.8	3.2±0.385 2.5–3.6	3.3±0.849 2.7–3.9	2.06±0.06 2.0–2.1	2.31±0.46 1.8–2.7
ELW	2.71±0.39 2.2–3.1	4.71±0.89 3.8–6.7	2.21±0.23 1.7–2.9	2.97±0.22 2.6–3.3	2.08±0.423 1.5–2.7	2.65±0.636 2.2–3.1	2.09±0.09 2.0–2.2	2.26±0.09 2.2–2.4
TD	3.45±0.72 2.6–4.2	4.9±0.34 4.4–5.5	3.37±0.32 2.6–4.1	3.79±0.41 3.1–4.4	2.89±0.702 1.5–3.7	3.05±0.071 3–3.1	3.29±0.11 3.2–3.4	2.88±0.32 2.6–3.2
FLL	18.8±2.57 16.2–21.4	35.5±3.79 28.6–40.6	18.51±1.04 15.1–20.5	25.9±2.4 20.8–31.4	19.14±1.501 17.1–21.6	24.95±1.909 23.6–26.3	14±0.7 13.4–14.8	18.2±2.09 15.8–19.5
FHL	15.1±2.54 12.5–17.9	28.2±2.43 24.0–32.1	13.42±1 11.8–16.8	18.9±1.79 16.1–23.2	13.9±0.938 12.9–15.6	17.5±1.131 16.7–18.3	11.5±0.15 11.4–11.7	14.3±1.97 12.0–15.6
FAW	1.87±0.22 1.7–2.2	3.85±0.9 3.0–5.6	1.8±0.14 1.5–2	2.4±0.17 1.9–2.6	1.85±0.457 1.2–2.7	2.15±0.212 2–2.3	1.85±0.08 1.8–1.9	1.97±0.03 1.9–2.0
HAL	9.5±1.82 7.6–11.2	17.2±0.77 15.9–18.1	8.29±0.54 7.4–10.2	11.9±1.24 10.3–15.3	8.34±0.573 7.2–9.1	11.7±0.141 11.6–11.8	7.47±0.08 7.4–7.5	8.53±0.57 7.9–9.0
F1	4.31±0.76 3.4–5.1	8.86±1.14 7.2–11.3	3.34±0.46 2.4–4.8	5.05±0.73 4.0–6.9	3.34±0.256 3.1–3.9	4.65±0.071 4.6–4.7	2.72±0.16 2.6–2.9	3.3±0.36 2.9–3.6
F2	4.18±0.44 3.5–4.8	8.63±1.26 6.7–10.5	3.51±0.47 3.1–4.9	5.1±0.65 4.5–7.0	4.19±0.577 3.3–4.9	5.75±0.919 5.1–6.4	3.28±0.03 3.2–3.3	3.69±0.35 3.5–4.1
F3	6.59±1.28 5.2–8.2	12.7±1.45 10.6–14.7	5.72±0.54 4.5–6.9	8.1±0.91 6.2–9.9	6.53±0.56 5.8–7.5	9.45±1.202 8.6–10.3	5.19±0.04 5.2–5.2	5.94±0.41 5.6–6.4
F4	4.46±0.7 3.7–5.2	8.7±1.23 6.8–10.4	3.52±0.41 2.9–4.8	4.79±0.54 4.4–6.1	4.14±0.441 3.3–4.8	5.75±0.495 5.4–6.1	3.46±0.14 3.3–3.6	3.35±0.47 2.9–3.8
HLL	51.8±9.07 41.9–62.3	97.5±9.72 82.3–111.8	50.67±2.64 45.5–59.6	71.5±5.13 61.5–78.6	52.81±3.091 48.6–58.6	67.4±5.798 63.3–71.5	45.89±1.07 45.0–47.1	57.4±12.16 43.6–66.5
FEL	14.8±2.83 11.8–18.7	27.93±2.29 24.2–31.2	13.82±3.01 12–30.5	19.6±1.73 16.9–22.6	13.48±1.374 11.8–15.4	17.85±0.778 17.3–18.4	12.0±0.41 11.5–12.2	15.4±1.85 13.3–16.8
TIL	16.9±2.77 14.1–19.7	31.7±2.54 27.6–35.6	16.3±1.07 14.4–18.5	23.6±1.81 19.1–25.8	17.3±1.085 15.7–19.3	22.05±2.333 20.4–23.7	14.1±0.4 13.7–14.5	19.1±2.19 16.7–21.0
TFL	24.5±4.31 19.6–28.7	46.6±3.64 40.5–50.4	24.03±1.22 20.5–27	34.3±1.98 31.6–38.7	25.33±1.809 23.1–29	31.25±0.495 30.9–31.6	22.6±0.72 22.2–23.4	29.5±2.54 26.6–31.4
FOL	17.3±2.62 14.1–19.9	32.6±2.74 27.2–35.9	16.48±0.83 14.6–18.6	23.5±1.58 20.0–26.6	17.33±0.848 16.5–19.3	21.2±0.141 21.1–21.3	15.8±0.34 15.4–16.1	20.5±1.75 18.6–22.0
T1	3.31±0.43 2.9–3.9	7.79±1.63 6.1–10.9	2.94±0.52 2–4.3	4.61±0.49 4.1–5.9	3.71±0.482 3–4.4	4.95±0.071 4.9–5	2.03±0.08 1.9–2.1	3.53±0.72 2.7–4.0

	<i>"Hylarana erythraea"</i>		<i>Hylarana taipehensis</i>		<i>Hylarana tytleri</i>		<i>Hylarana macrodactyla</i>	
T2	5.8±0.96 4.5–6.8	12.1±1.98 9.7–15.8	5.19±0.67 4.1–7	7.45±0.65 6.7–9.1	6.04±0.907 5–7.8	9±0.424 8.7–9.3	3.58±0.08 3.5–3.6	5.99±1.39 4.4–6.9
T3	8.1±1.06 7.0–9.3	16.8±3.92 7.7–22.7	7.96±0.73 6.8–9.6	11.3±0.94 10.1–13.6	8.94±0.98 7.9–10.9	12.1±1.131 11.3–12.9	5.62±0.47 5.2–6.1	9.94±1.52 8.2–11.0
T4	12.1±1.6 10.1–14.5	25.8±3.14 21.9–32.1	12.69±0.76 11–14.2	18.2±1.25 17.0–21.2	13.18±1.057 12–15.1	17.7±0.707 17.2–18.2	10.0±1.13 8.9–11.2	15.7±1.7 13.7–16.7
T5	8.28±1.29 7.3–10.2	19.8±2.99 16.0–25.0	8.36±1.21 6.4–10.8	11.9±1.47 9.7–14.4	9.83±0.997 8.2–10.7	14.75±0.636 14.3–15.2	5.34±1 4.7–6.5	8.98±1.47 7.3–10.0
IMT	1.08±0.12 0.9–1.2	2.19±0.57 1.4–3.3	1.24±0.31 0.9–2	1.37±0.3 1.2–2.3	1.36±0.22 1–1.7	1.4±0.141 1.3–1.5	1.05±0.04 1.0–1.1	1.23±0.41 1.0–1.7
OMT	0.7±0.08 1.0–1.0	1.45±0.78 1.0–4.0	0.82±0.35 1.0–2.0	0.86±0.29 1.0–2.0	1.31±0.23 1–1.7	1.3±0.566 0.9–1.7	0.58±0.04 1.0–1.0	0.72±0.07 1.0–1.0

Discriminant analysis

The four groups can be morphologically defined by three functions as given in Table 4. The differences observed in sexual dimorphism are reflected by the quite distinct appearance of the two plots (fig. 2A–B). Males of *H. tytleri* are separated by the variables of Function 2 that concern measurements of head and hind limbs. *H. macrodactyla* males are separated from the other males by variables of Function 1 (eye distance, hand length). In females, Function 1 separates *H. macrodactyla* from the other species by measurements of tympanum size and eye-distance (Tab. 4). The other species are differentiated by Function 2 grouping a series of measurements on head and hind limbs.

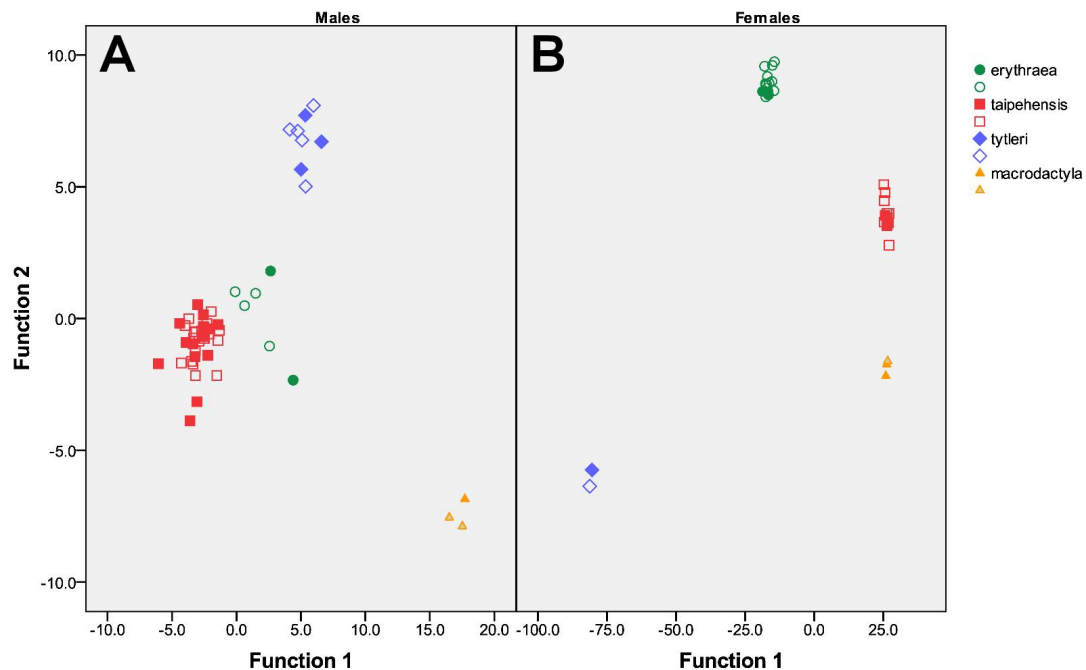


Figure 2. Scatterplot of individual discriminant scores on the first and second canonical axes for *"Hylarana erythraea"* (circle), *H. taipehensis* (rectangle), *H. tytleri* (rhombus), and *H. macrodactyla* (triangle). (A) Males; (B) females. In each species, specimens identified by molecular markers were marked as solid and those identified by morphology only as open icons, respectively.

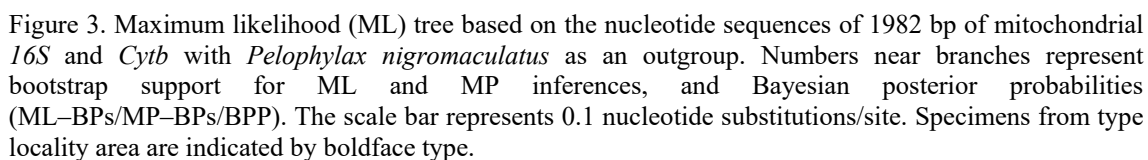
Molecular phylogeny

In the ML tree based on both *16S* and *Cytb* data (fig. 3), all individuals of *H. tytleri* from the seven localities in Bangladesh formed a clade with high bootstrap support (BS: 100 ML and MP, 1.0 BPP) and minor sequence divergence (0.6 % for *16S* and 2.0 % for *Cytb*) within the clade. The Bangladeshi *H. tytleri* was sister to the Chinese *H. macrodactyla* (BS: 100 ML and 96 MP, 1.0 BPP; sequence divergence: 7.4 % for *16S* and 15.8 % for *Cytb*) and together both species showed a sister relationship with the topotypic *H. taipehensis* with high bootstrap support (BS: 98 ML and 95 MP, 1.0 BPP) and high sequence divergence (11.8 % for *16S* and 19.8 % for *Cytb*). “*Hylarana erythraea*” from Thailand and Malaysia formed a single clade (BS: 98 ML and 100 MP, 1.0 BPP) and was sister to all other sequenced *Hylarana* species (BS: 98 ML and 100 MP, 1.0 BPP), showing high sequence divergence (12.3 % for *16S* and 20.9 % for *Cytb*) (see *16S* & *Cytb* divergence in Appendix Tab. S2).

To increase robustness of our phylogenetic dataset and to potentially increase support for the observed relationships among the examined *Hylarana* taxa, we incorporated several sequences (*16S*) from the DNA database (Tab. 1) and performed further phylogenetic analyses. The resultant ML tree (fig. 4) showed that Bangladeshi *H. tytleri* formed a single clade containing the haplotype of *H. cf. tytleri* from Tripura, India, with little sequence divergence within the clade (*16S* divergence 0.6 %). They were also distantly related to Myanmarian “*H. erythraea*” (sequence divergence 8.8 % for *16S*). “*H. erythraea*” specimens from the four countries examined, Thailand, Malaysia, Myanmar and Vietnam, formed a clade, but sequence divergence between specimens was large (3.7 % [range: 6.1–0.2 %] for *16S*) (see *16S* divergence in Appendix Tab. S3).

Morphological comparisons

Of the *Hylarana* species examined, “*H. erythraea*” can be distinguished by its large body size (SVL = 31.4 ± 4.46 mm ♂, 58.0 ± 4.29 mm ♀), cream-white or yellow dorsolateral folds, the frequent presence of a red dot on the tympanum, tibiotarsal articulation reaching the anterior corner of the eye, both tips of fingers and toes dilated into small discs with well-developed grooves, not more than two phalanges of the fourth toe free of webbing, prominent subarticular tubercles and the presence of metatarsal tubercles. In contrast, *H. taipehensis* is easily distinguishable from other *Hylarana* species by its body size (SVL = 29.0 ± 1.8 mm ♂, 42.8 ± 3.17 mm ♀), pure white dorsolateral folds, tibiotarsal articulation reaching the nostrils, moderate webbing, three phalanges of the fourth toe free of webbing and the presence of two small metatarsal tubercles. *Hylarana tytleri* can be separated from other *Hylarana* species by a combination of the following characteristics: SVL: 31.45 ± 1.64 mm ♂, 41.51 ± 0.41 mm ♀), snout rounded, head moderately elongated, two off-white dorsolateral folds, tibiotarsal articulation reaching beyond the eye to the nostrils and two and half phalanges of the fourth toe free of webbing. Thus, although, *H. tytleri* showed close affinity with *H. macrodactyla* in the molecular phylogeny, it was strikingly different in terms of morphological traits (elongated limbs and the absence of a middorsal stripe).



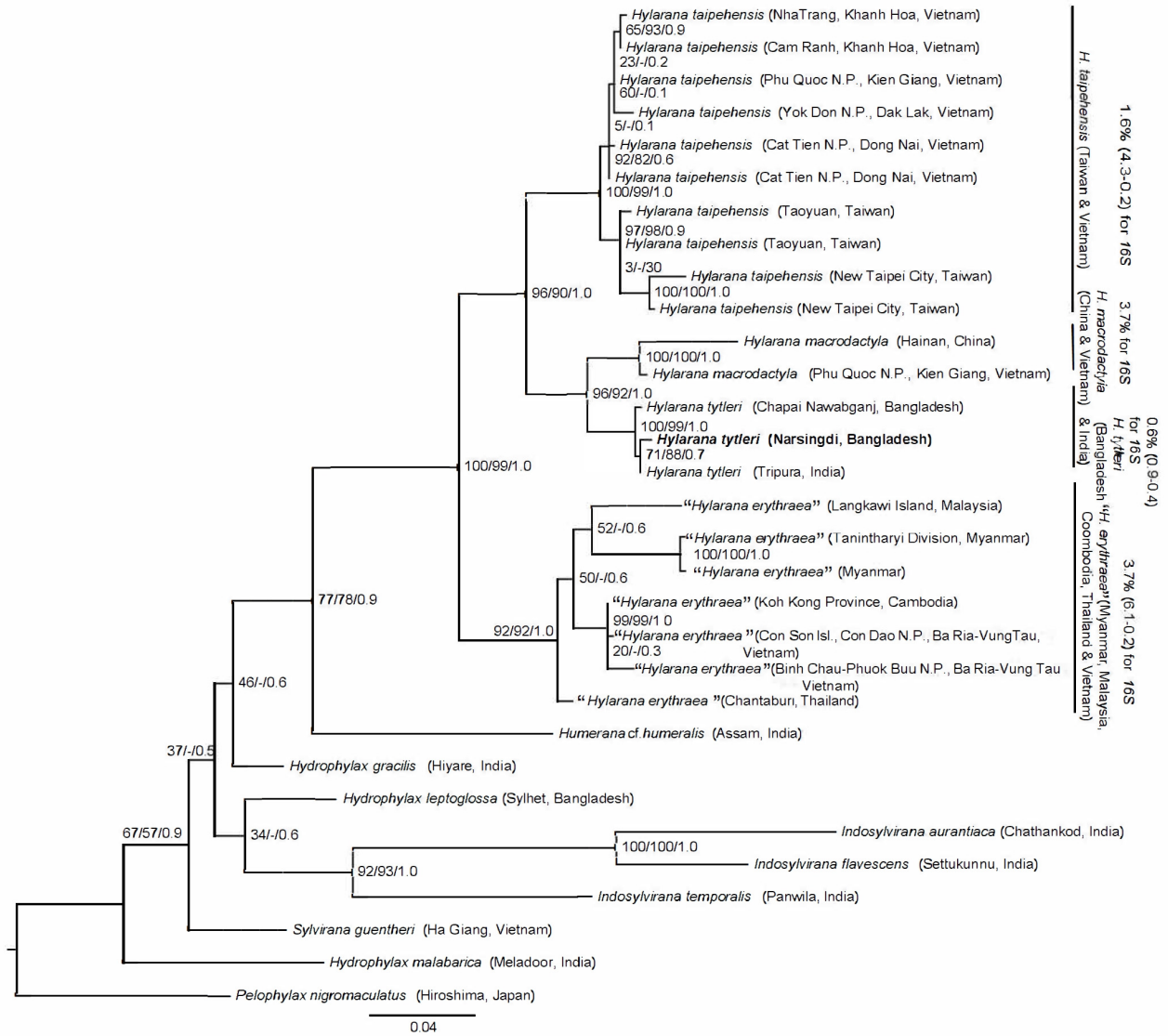


Figure 4. Maximum likelihood (ML) tree of *Hylarana* frogs, with the incorporating data from GenBank. Numbers near branches represent bootstrap support for ML and MP inferences, and Bayesian posterior probabilities (ML-BPs/MP-BPs/BPP). The scale bar represents 0.1 nucleotide substitutions/site. Specimens from type locality area are indicated by boldface type.

Morphometric comparisons

We performed detailed morphological comparisons between *H. tytleri*, *H. taipehensis*, “*H. erythraea*” and *H. macrodactyla*. Measurements of the 30 body parts in *H. tytleri*, *H. taipehensis* and “*H. erythraea*” are summarized in Table 2. *Hylarana tytleri* is slightly larger in size (SVL: 31.45 ± 1.64 mm ♂, 41.51 ± 0.41 mm ♀) than *H. macrodactyla* (SVL: 26.3 ± 0.88 mm ♂, 33.2 ± 4.45 mm ♀) but smaller than female “*H. erythraea*” (SVL: 58.0 ± 4.29 mm ♀).

The four species could be clearly separated by canonical discriminant analysis (DA) both in males and females (fig. 2A–B). In case of males, “*H. erythraea*” and *H. taipehensis* are very similar whereas *H. tytleri* and *H. macrodactyla* are more dissimilar

from each other (fig. 2A). In case of females, the four species of *Hylarana* (*H. tytleri*, “*H. erythraea*”, *H. taipehensis* and *H. macrodactyla*) are equally dissimilar (fig. 2B).

Comparison using Mann-Whitney *U* test of males and females of “*Hylarana erythraea*”, *H. taipehensis*, *H. tytleri* and *H. macrodactyla* based on raw measurement (for SVL) and on measurements corrected for size are shown in Table 3, and discriminant functions 1 to 3 for males and females based on size-corrected measurements are shown in Table 4.

Advertisement call

One advertisement call was recorded in the area of the type locality of *H. taipehensis* at an air temperature of 28.3°C at around 22:00 h on July 31, 2014, by M. Sumida. The recording was composed of 13 notes and lasted for 5.1 s (fig. 5A). Each note was emitted at a gap (between the beginnings of two successive notes) of 0.39 ± 0.04 s. The note repetition rate was 2.84/s and the note length was 0.04 ± 0.01 s. Each note was composed of several indistinct pulses and marked frequency modulation was observed within a single note (fig. 5B). The number of harmonic bands observed was 3 to 4 with the dominant frequency occurring at 3.5 or 4.5 kHz.

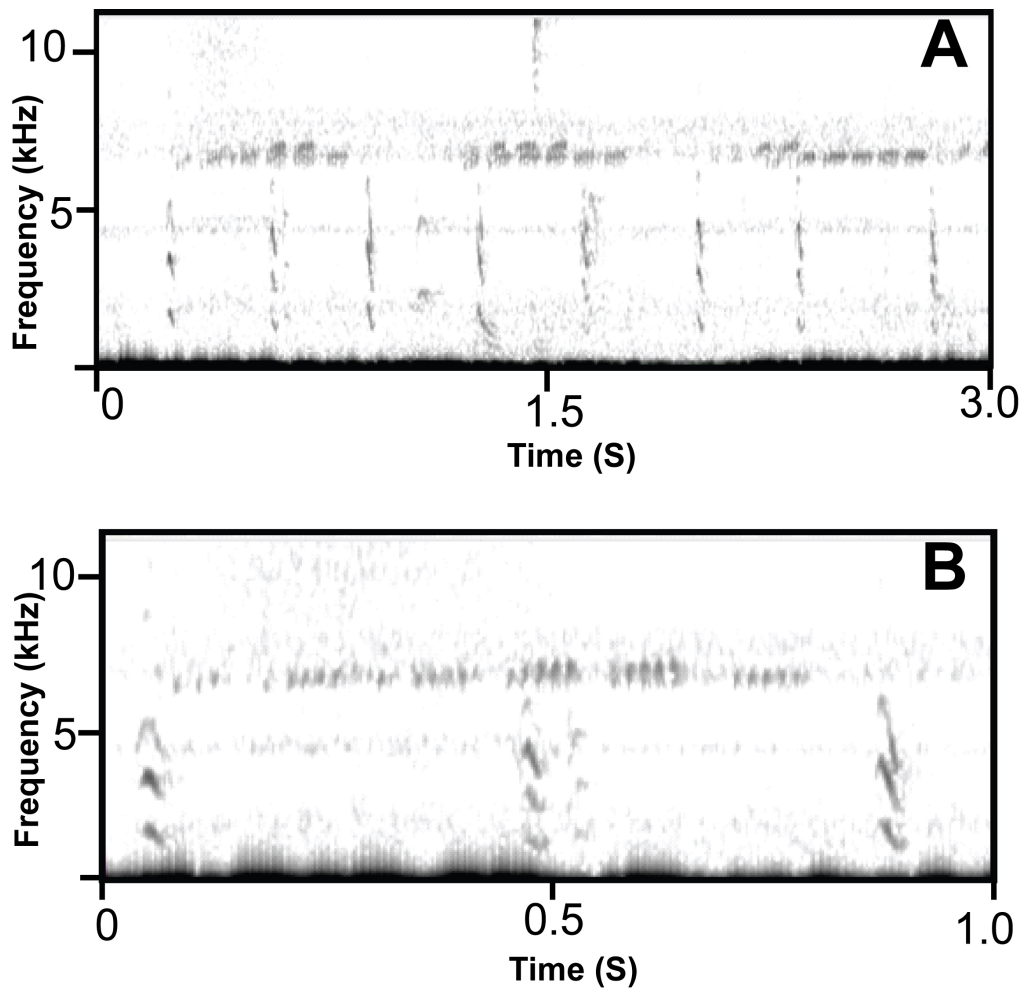


Fig. 5. Advertisement call of *Hylarana taipehensis* from Taiwan.

Table 3. Comparison using Mann-Whittney U test of males and females of “*Hylarana erythraea*”, *H. taipehensis*, *H. tytleri* and *H. macrodactyla* based on raw measurement (for SVL) and on measurements corrected for size. Abbreviations for measurements are given in the *Material and methods* section; n_1 , sample size of males; n_2 , sample size of females; U, Mann-Whittney U; p, probability; *, $p < 0.05$; +, $p < 0.100$.

Species	<i>“Hylarana erythraea”</i> $n_1 = 6, n_2 = 12$		<i>Hylarana taipehensis</i> $n_1 = 36, n_2 = 12$		<i>Hylarana tytleri</i> $n_1 = 8, n_2 = 2$		<i>Hylarana macrodactyla</i> $n_1 = 3, n_2 = 3$	
	U	p	U	p	U	p	U	p
SVL	0	0.000 *	0	0.000 *	5	0.533	0	0.100 +
LHL	33	0.494	192	0.568	7	0.889	3	0.700
LHW	36	0.659	124	0.028 *	5	0.533	4	1
LS-N	38	0.779	162	0.199	7	0.889	4	1
LN-N	38	0.779	197	0.651	8	1	0	0.100 +
LN-E	39	0.841	170	0.273	5	0.533	4	1
LED	8	0.003 *	212	0.924	8	1	3	0.700
LEE	25	0.179	186	0.475	4	0.4 +	3	0.700
LTD	0	0.000 *	37	0.000 *	3	0.267	0	0.100 +
LFLL	38	0.779	214	0.962	7	0.889	2	0.400
LFHL	31	0.397	202	0.739	6	0.711	3	0.700
LFAW	41	0.968	154	0.140	5	0.533	0	0.100 +
LHAL	28	0.274	156	0.153	1	0.089	0	0.100 +
LF1	12	0.012 *	100	0.006 *	2	0.178	4	1
LF2	21	0.091	108	0.010 *	6	0.711	3	0.700
LF3	34	0.547	170	0.273	4	0.4 +	3	0.700
LF4	32	0.444	180	0.391	5	0.533	0	0.100 +
LHLL	36	0.659	198	0.668	7	0.889	3	0.700
LFEL	39	0.841	124	0.028 *	4	0.4 +	3	0.700
LTIL	32	0.444	151	0.122	7	0.889	1	0.200
LTFL	41	0.968	139	0.067	4	0.4 +	1	0.200
LFOL	33	0.494	151	0.122	0	0.044	2	0.400
LT1	0	0.000 *	61	0.000 *	5	0.533	0	0.100 +
LT2	15	0.026 *	137	0.060	1	0.089	0	0.100 +
LT3	13	0.015 *	159	0.175	6	0.711	0	0.100 +
LT4	8	0.003 *	160	0.182	4	0.4 +	0	0.100 +
LT5	0	0.000 *	192	0.568	0	0.044 +	1	0.200
LIMT	40	0.904	61	0.000 *	2	0.178	3	0.700
LOMT	22	0.109	94	0.004 *	5	0.533	4	1

Table 4. Discriminant functions 1 to 3 for males and females of *Hylarana* based on size-corrected measurements. Abbreviations of measurements are given in the *Material and methods* section. *, largest absolute correlation between each variable and any discriminant function.

Sex	Males			Females		
Function	1	2	3	1	2	3
LFLL	-0.114*	0.006	0.113	0.012	-0.026	-0.118*
LTD	-0.025	-0.213*	0.021	0.023*	-0.020	-0.019
LE-E	0.040	0.197*	-0.132	-0.001	0.023	-0.032*
LT1	-0.018	0.188*	-0.062	-0.013	0.037*	0.022
LT3	-0.103	0.176*	0.121	0.001	-0.024	0.030*
LT2	-0.061	0.175*	-0.100	-0.018	-0.003	0.023*
LT5	-0.077	0.174*	0.103	0.043	0.132*	-0.088
LE-D	0.000	-0.171*	-0.129	0.019*	-0.004	0.001
LFEL	-0.009	-0.144*	-0.086	0.018	-0.010	0.026*
LHL	-0.037	-0.136*	-0.039	0.009	0.038*	0.003
LFAW	0.037	-0.131*	0.070	0.004	0.030	0.068*
LN-E	-0.023	0.118*	-0.085	-0.008	-0.012	-0.042*
LS-N	-0.012	-0.110*	-0.017	0.004	0.003	0.026*
LSVL	0.032	-0.033*	0.024	0.015	-0.054*	-0.032
LF1	-0.029	-0.055	-0.413*	-0.014	0.118*	-0.013
LT4	-0.092	-0.014	0.322*	-0.075	0.055	-0.160*
LTFL	0.041	-0.143	0.296*	0.038	-0.095	0.097*
LF4	0.099	0.030	-0.262*	-0.039	0.092*	-0.007
LHLL	0.004	-0.122	0.235*	0.018	-0.049*	-0.005
LN-N	0.174	0.223	0.224*	0.000	-0.018	-0.060*
LFOL	0.070	-0.190	0.216*	0.036	-0.075	0.138*
LIMT	-0.001	0.013	0.207*	-0.001	0.000	0.049*
LHAL	0.000	-0.141	-0.189*	0.003	0.006	-0.070*
LOMT	0.023	0.170	0.185*	0.004	-0.077	0.090*
LHW	-0.021	-0.106	-0.143*	0.006	0.016	-0.039*
LF2	0.069	0.060	-0.136*	-0.024	0.057*	0.001
LFHL	-0.016	-0.065	-0.133*	0.007	-0.002	-0.067*
LTIL	-0.010	-0.035	0.122*	0.025	-0.068*	0.007
LF3	0.055	0.040	-0.094*	-0.021	0.007	-0.032*

KEY FEATURES OF *HYLARANA* SPECIES*Hylarana tytleri* (Theobald, 1868)*Examined materials (15 specimens)*

BANGLADESH: Chapai Nawabganj: IABHU 3889–3890, males; Barguna: IABHU 3891, female; IABHU 3892, male; IABHU 4141–4143, males; IABHU 4147, male; Narsingdi: IABHU 3893, male; IABHU 3894, female; IABHU 4154, female; IABHU 4155, male; Panchagarh: IABHU 4214–4215, males; Mymensingh: IABHU 4219, female.

Descriptive characters

Small-sized frog with a SVL of 28.7–34.7 mm in males and 41.2–41.8 mm in females. Snout rounded and head moderately elongated. White or off-white dorsolateral folds running from behind the eye to the groin, usually (14 of 15 specimens here observed) with a dark brown band following on dorsal side of folds, but no middorsal line (fig. 6A). Tympanum distinct and slightly smaller than the eye. Limbs slender (fig. 6C). Tips of fingers dilated into small discs. Fingers free; relative finger length $F1 < F2 < F4 < F3$ (fig. 7I). Tips of toes more prominently dilated into small discs with ventrolateral grooves; relative toe length $T1 < T2 < T3 < T5 < T4$ (fig. 7M). Two and half phalanges of the fourth toe free of webbing (fig. 6E; 7M). Dorsal and ventral skin smooth with granular zone in the thigh region (fig. 6C).

Color in life

Body surface varying in color from brown to slightly greenish. Dorsolateral folds usually bordered by a brown band on dorsal side: numerous specimens from Bangladesh examined show brown bands, but the specimen presented in fig. 6A (reared in captive condition in the Amphibian Research Center, Hiroshima University, Japan) has a particular color pattern, with green back showing white dorsolateral fold but no brown bands. Surface of the limbs slightly yellowish with few scattered small dark spots (fig. 6A). Underside creamy white (fig. 6C).

Color in alcohol

Body surface slightly brownish or greyish, but gradually becoming faint near the vent (fig. 6A). In general, dorsolateral folds bordered with dense, deep brown bands. Dorsal surface of the femur, tibia and tarsus region showing a few longitudinal discontinuous, scattered black lines; rear of the thigh whitish with a few indistinct black spots; ventral body surface whitish; hands and feet also ventrally white to slightly yellow (fig. 7E).

Variation

Dorsal pattern variable among individuals. Of the 15 examined specimens, one (IABHU 3893) lacked a brown borderline around off-white dorsolateral folds. Sporadically, some brown dots present on the dorsal regions of the thigh, several connected dots forming short irregular lines. Two (IABHU 3889, 4154) specimens had indistinct dots; one (IABHU 3893) completely lacked dots. Ventral side milky white, except in one individual (IABHU 4219).

Habitat

Hylarana tytleri is sympatric with *Fejervarya* sp., *Microhyla* sp. and *Hoplobatrachus tigerinus* (Daudin, 1802). It was found in marshes, paddy fields and beels (confined water reservoirs) in low-altitude foothills, sometimes near human settlements. In winter, it remains hidden in loose soil, probably for hibernation, at the junction between river banks and flat paddy land.

Known occurrence of H. tytleri

The known occurrence of *H. tytleri* includes the Sherpur, Mymensingh, Narsingdi ('Dacca', now Dhaka, original type locality), Barguna, Chapai Nawabganj and Panchagarh districts, Bangladesh (fig. 1B). Also found in Natore, Nilphamary and Dinajpur districts, Bangladesh (pers. comm. to M.A.R. Sarkar; Sarkar & Howlader 2012; Selim *et al.* 2013). Sampling sites ranged from north to south Bangladesh, suggesting that it also occurs in other areas beyond the study sites (fig. 1B) (see below).

The species is also present in adjacent Bhutan (Wangyal 2013), India (Arunachal Pradesh, Assam, Meghalaya, Nagaland, Tripura, West Bengal) (Ohler & Mallick 2002; Ao *et al.* 2003; Das 2008; Ohler *et al.* 2018; Biju unpublished data), Myanmar (Bago) (Mulcahy *et al.* 2018) and Nepal (Dubois 1974, 1981, as *Rana taipehensis*; Ohler & Mallick 2002). Its presence east of Bago in Myanmar cannot be confirmed by identified specimens. No record of a small-sized green-backed *Hylarana* is known from Bago to the Bangkok region, forming thus a large distribution gap.

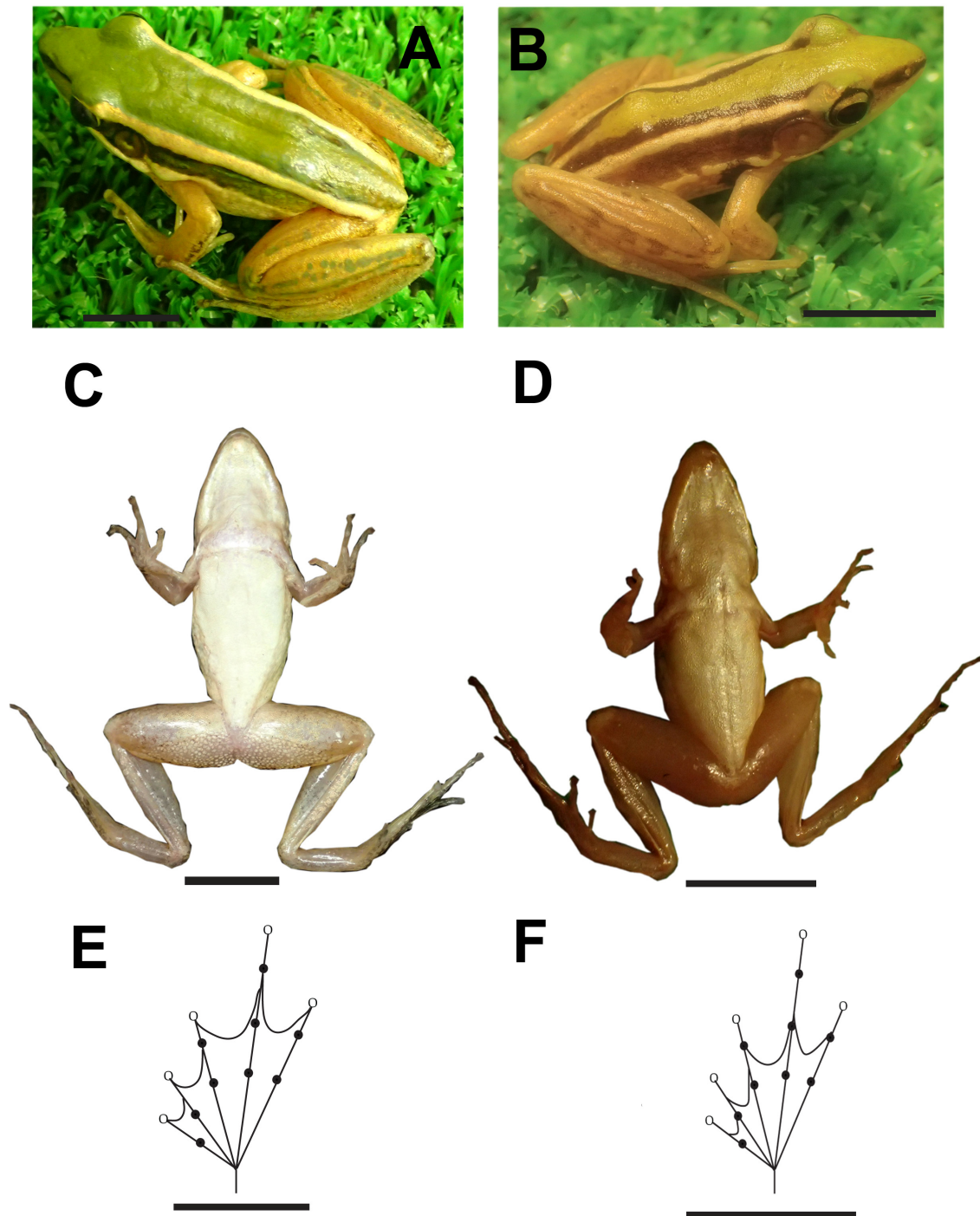


Figure 6. Comparisons of live *H. tytleri* (IABHU 4219) and *H. taipehensis* (NTNU 202474) from Bangladesh and Taiwan, respectively. (A, B) Dorsolateral, (C, D) ventral views and (E, F) schematic illustrations of webbing on foot of *H. tytleri* (IABHU 4219) and *H. taipehensis* (NTNU 202474), respectively. Scale bar = 10 mm.

Hylarana taipehensis (Van Denburgh, 1909)*Examined materials (48 specimens)*

TAIWAN: New Taipei City, Sanzhi Township: NTNU 202428–202429, females; NTNU 202430–202432, males; Shimen district: NTNU 202468–201475, males. VIETNAM: Dong Nai Province, Cat Tien N.P.: ZMMU NAP 1505–1507, males; Lam Dong Province, Loc Bao: ZMMU NAP 2801–2803, males; Ba Ria-Vung Tau Province, Binh Chau-Phuok Buu N.P.: ZMMU NAP 3063–3065, males; Khanh Hoa, Cam Ranh: ZMMU NAP 3217, male; Tay Ninh Province, Lo Go-Xa Mat N.P.: ZMMU NAP 3663, female; ZMMU NAP 3664–3665, males; Kien Giang Province, Phu Quoc N.P.: ZMMU NAP 3769, male; ZMMU NAP 3770, female; ZMMU NAP 3771, male; Dak Lak province, Yok Don N.P.: ZMMU NAP 4276–4277, males. Localities of the following samples are not available: ZMMU NAP 1133, male; ZMMU NAP 1675, female; ZMMU NAP 1694, male; ZMMU NAP 2804, male; ZMMU NAP 2821, female; ZMMU NAP 2936, female; ZMMU NAP 3066, male; ZMMU NAP 3069, male; ZMMU NAP 3423, female; ZMMU NAP 3666, male; ZMMU NAP 3772, male; ZMMU NAP 3773, female; ZMMU NAP 3774, male; ZMMU NAP 3807, female; ZMMU NAP 3808, male; ZMMU NAP 3809, female; ZMMU NAP 6053, female.

Descriptive characters

Small-sized frogs with a SVL of 26.3–33.6 mm in males and 34.9–46.8 mm in females. White dorsolateral folds running from the posterior corner of the eye to the groin and often a short white vertebral line above the anus (fig. 6B). Tympanum distinct and large. Limbs slender (fig. 6D). Tips of both fingers and toes slightly swollen. Fingers free; relative finger length $F1 < F4 < F2 < F3$ (fig. 7J). Three phalanges of the fourth toe free of webbing (fig. 6F); relative toe length $T1 < T2 < T3 < T5 < T4$ (fig. 7N). Dorsal and ventral surface of body smooth.

Color in life

Body surface greenish in color. Inner sides of dorsolateral folds potentially bordered by a brown line. A blackish to yellowish, large, continuous band running from behind the tympanum to the groin. A few, irregular black spots on the surface of limbs (fig. 6B). Underside white (fig. 6D), but some indistinct black spots on the pectoral region.

Color in alcohol

Body surface tan in color. Dorsolateral folds usually bordered by dense, deep, brown color lines (fig. 7B). Dark brown lines distinct along the dorsolateral folds. Surface of the tibia slightly yellowish. Crossbars distinct on the surface of tibia. Ventral side whitish (fig. 7F).

Variation

Color of the dorsum varying from slightly yellowish to greenish. Of the examined 48 specimens, there was no large black marking at the base of the forelimbs, except in one individual (NTNU 202474). Surface of the tibia lacking spots, except for a few spots in one individual (NTNU 202474).

Habitat

At the vicinity of its type locality near Taipei, Taiwan, *Hylarana taipehensis* was found on lily flowers in a small body of water (from 140 to 225 m asl). It was generally found in areas of thick vegetation. It was sympatric with *Sylvirana guentheri* (Boulenger, 1882) in Shimen District, Taiwan.

Known occurrence of H. taipehensis

Hylarana taipehensis has been found in Shimen District, Sanzhi Township, New Taipei City and Longtan Township, Taiwan. However, the distribution may cover the entire northwest and southwest region of the country (fig. 1A, see above). The species has been reported from Cambodia (Pursat Province) (Daltry & Traeholt 2003; Goldberg *et al.* 2017), China (Fujian, Guangdong, Guangxi, Guizhou, Hainan, Hong Kong, Taiwan, Yunnan) (Fei *et al.* 1999), Laos (Centre, Champasak Province, Kammouan Province, Mondolkiri Province, Ventiane Prefecture) (Duckworth *et al.* 1999; Teynié *et al.* 2010; Stuart 2005; Stuart *et al.* 2006; Manthey & Manthey 2017), Thailand (Chantaburi Province, Nakhon Ratchasima Province, Udon Thani Province) (Ohler & Mallick 2002; Sun & Narins 2005; Danaisawadi 2009; Kaensa *et al.* 2014) and Vietnam (most of the country, in particular there are recently published references for Bac Giang Province, Dong Nai Province, Gia Lai Province, Ha Gian Province, Kien Giang Province, Lam Dong Province; Lang Son Province, Lao Cai Province, Quang Binh Province) (Furey *et al.* 2002; Bain & Nguyen 2005; Chen *et al.* 2005; Grismer *et al.* 2011; Hecht *et al.* 2013).

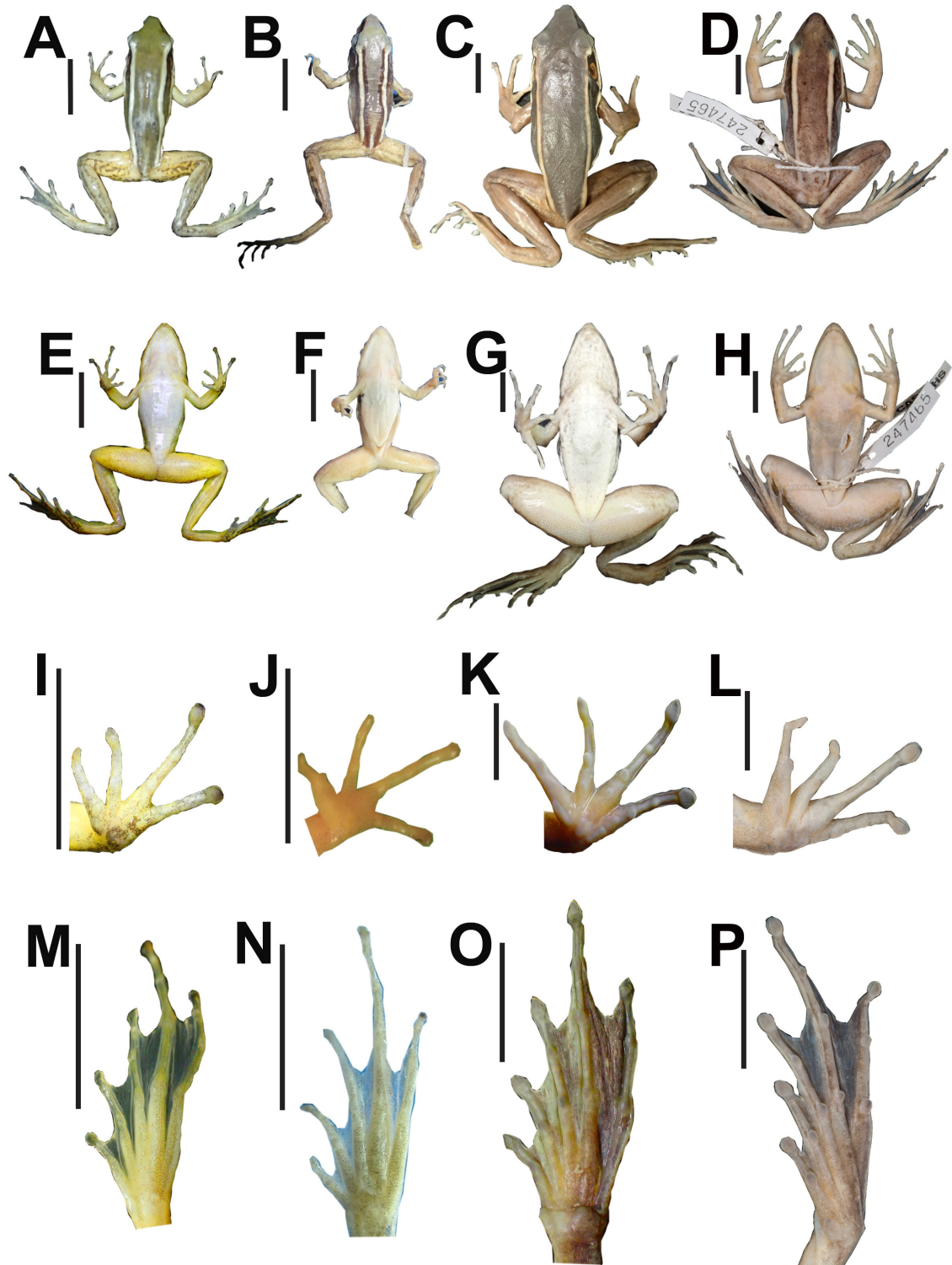


Figure 7. Comparisons of specimens after preservation in alcohol. (A, B, C, D) Dorsal aspects and (E, F, G, H) ventral aspects of *H. tytleri* (IABHU 4219), *H. taipehensis* (NTNU 202474), “*H. erythraea*” (IABHU 21106) and “*H. erythraea*” from Myanmar (CAS 247465), respectively. (I, J [alive condition], K, L) Ventral views of left hand of *H. tytleri* (IABHU 4219), *H. taipehensis* (NTNU 202474), “*H. erythraea*” (IABHU 21106) and “*H. erythraea*” from Myanmar (CAS 247465), respectively. (M, N, O, P) Ventral views of left foot of *H. tytleri* (IABHU 3893), *H. taipehensis* (NTNU 202472), “*H. erythraea*” (IABHU 21105) and “*H. erythraea*” from Myanmar (CAS 247465), respectively.

Hylarana erythraea (Schlegel, 1837)*Examined materials (18 specimens)*

MALAYSIA: Langkawi Island: IABHU 21105–21107, females. Vietnam. Ba Ria-Vung Tau Province, Con Son Isl., Con Dao N.P.: ZMMU NAP 0562–0563, males; Binh Chau-Phuok Buu N.P.: ZMMU NAP 3062, female. Localities of the following samples are not available: ZMMU NAP 0598–0599, females; ZMMU NAP 02547, female; ZMMU NAP 0600, male; ZMMU ABV 01151, female; ZMMU NAP 01152–01153, males ZMMU A 5237 1–2, males; ZMMU A 3521 (24), female; ZMMU A 3521 (19), female; ZMMU A 4523, female.

Descriptive characters

Large-sized frogs with a SVL of 51.4–64.9 mm in females and 26.7–35.4 mm in males, showing an important dimorphism in body size. Cream-white dorsolateral folds prominent and running from the posterior corner of the eye to the groin (fig. 7C). Head longer than wide. Eye diameter larger than tympanum diameter. Fingers free of webbing. Tips of fingers dilated into small discs with well-developed ventro-lateral grooves; relative finger length $F1 < F4 < F2 < F3$ (fig. 7K). Tips of toes dilated into ogival-shaped discs with distinct ventro-lateral grooves; relative toe length $T1 < T2 < T3 < T5 < T4$ (fig. 7O). No more than two phalanges of the fourth toe free of webbing. Subarticular tubercles moderately large and prominent. Inner metatarsal tubercles elongated and outer metatarsal tubercles oval. Both distinct. Tibiotarsal articulation reaching the anterior corner of the eye. Numerous granules present on the posterior region of the femur just above the anus.

Color in alcohol

Dorsum greyish brown in color (fig. 7C). Ventral side of the throat slightly whitish with numerous irregular black spots. Surface of hind limbs brown becoming gradually faint. Poorly distinct blackish bands present on the surface of the hind limbs, but absent on the ventral surface (fig. 7G).

Variation

In the examined specimens, ventral color is variable: in a first frog numerous greyish spots present on the ventral surface of the throat, ranging from the base of the lower lip to the middle of the belly (IABHU 21107). The black spots are poorly distinct in a second individual (IABHU 21106) and completely absent in the third (IABHU 21105). Color variation of remaining 15 specimens is not available.

Remarks

We examined two specimens (CAS 229614 and 247465) of “*H. tyleri*” (Oliver *et al.* 2015) from Tanintharyi Division, Myanmar. These two specimens, currently considered as “*H. erythraea*” (Mulcahy *et al.* 2018), are not adult despite their relatively

large body size (SVL 54.0–57.5 mm). Their dorsum is light brownish with a few black scattered dots (fig. 7D) and dorsolateral folds are present. The ventral surface is whitish (fig. 7H). Fingers tips are dilated into small discs. The first finger is shorter than the forth, which is shorter than the second (fig. 7L). Tips of toes form small discs with well-developed latero-ventral grooves. The third toe is shorter than the fifth (fig. 7P). Subarticular tubercles are moderately large and prominent.

DISCUSSION

The original description of *Hylarana tytleri* by Theobald (1868) is very short: “In size equal to *H. erythraea*, but with a much more pointed snout, though not so narrow a muzzle as *H. macrodactyla*. Upper lip and beneath white. Back reddish brown. No pale lateral stripes”. These data do not allow reliable allocation of other members to the species. Adding to this problem, many researchers (e.g., Hasan *et al.* 2012a; Biju *et al.* 2014; Oliver *et al.* 2015) misidentified the *Hylarana tytleri* from Bangladesh and adjacent areas. Ohler & Mallick (2002) provided morphological characters to identify the species but they did not include topotypic specimens in their study.

Phylogenetic analysis of our data confirms the results of other studies (Oliver *et al.* 2015). All specimens of *H. tytleri* from Bangladesh and India form a single clade with high bootstrap and posterior probability support and show a sister relationship with *H. macrodactyla*. Together, they show a sister relationship with the topotypic *H. taipehensis* from Taiwan and Vietnam. “*Hylarana erythraea*” from Malaysia and Myanmar formed sister taxon to the other *Hylarana* species (fig. 4). As long as only considering mitochondrial DNA, *16S* is considered an indicator for outlining the taxonomic status of frog species (Vences *et al.* 2005), with more than 3 % *16S* divergence used as the rough species threshold (Fouquet *et al.* 2007), the *16S* divergence of more than 3 % observed among the examined “*H. erythraea*” specimens (0.2–6.1 %) suggests that several unrecognized *Hylarana* species exist inside their range of distribution from Philippine to Myanmar including Cambodia. To clarify the taxonomic status of these populations, further surveys that include the topotypic population of *H. erythraea* (Java and Sumatra, Indonesia) and populations of potential contact zones of these clades are necessary; these studies should also include more DNA data, in particular including nuclear genes to resolve the taxonomic status of these clades.

In DNA analyses, *H. tytleri* was separated from its congeners, *H. taipehensis* and “*H. erythraea*”. Body size of *H. tytleri* and *H. taipehensis* overlap, but both, *H. tytleri* and *H. taipehensis*, are much smaller than “*H. erythraea*”. “*Hylarana erythraea*” is a large frog, particular for females, ranging in size from 51.4 to 64.9 mm, but size of adult males (26.7–35.4 mm) falls within the size range of adult specimens of the other *Hylarana* species considered and might thus be source of errors. Oliver *et al.* (2015) treated two specimens (e.g., CAS 229614 and 247465) from Myanmar as “*H. tytleri*”, but later Mulcahy *et al.* (2018) noted that these specimens are related with “*H. erythraea*”. Although we did not include the morphometric data of these two specimens in our DA analyses, their SVL (54.0–57.5 mm), relative finger length and pattern of foot webbing clearly indicates that they belong to what is now considered “*H. erythraea*” (fig. 7L,P).

Generally, it is suggested that during uplift of the Himalayas, the Bengal basin (present Bangladesh and West Bengal) was formed between 20 and 14 Mya (Alam *et al.* 2003; Uddin & Lundberg 2004). A recent study (Oliver *et al.* 2015) proposed that both *H. tytleri* and *H. macrodactyla* split from *H. taipehensis* at 5.6 Mya, after which the former two separated further at 3.9 Mya. These speciation events therefore appear to have occurred after the land formation of the Bengal basin. The common ancestor of these species therefore seems to have evolved somewhere in East or Southeast Asia before formation of the Bengal landmass, with *H. tytleri* colonizing the Bengal basin from this area at 3.9 Mya. A similar age of divergence was predicted for two new microhylid frogs from Bangladesh by Hasan *et al.* (2014b). In the present study (fig. 4), the *H. tytleri* specimen from Tripura, India, was found to be embedded within the same clade as *H. tytleri* from Bangladesh. The sequences of these specimens were obtained from Genbank (deposited by Biju *et al.* 2014) and the data retrieved from DDBJ. Historical Bengal was split into West Bengal (India) and Bangladesh; before 1947 it was part of British India, politically the “Bengal Presidency”. It is a large homogenous geographical space formed by the Ganges-Brahmaputra delta. There is no specific geographical boundary between Bangladesh and India that would prevent the movement of anuran taxa between the two countries. As a result, many species (e.g., *Hoplobatrachus tigerinus*) sympatrically occur in Bangladesh and India (Hasan *et al.* 2012b) and therefore the presence on morphological evidence of *H. tytleri* to adjacent areas can be considered as granted (e.g., West Bengal to Mizoram, Orissa, Nepal and to the northern part up to Meghalaya and Assam) (see references above and fig. 1A). This view is consistent with the conclusions of Ohler & Mallick (2002).

The type locality of *Hylarana taipehensis* is Taipei, Taiwan. The occurrence of this species in central Taiwan is unconfirmed. Specimens of this species are found in northern Taiwan (New Taipei City [Shimen and Sanzhi] and Taoyuan county [Longtan and Yangmei]) and southern Taiwan (Tainan City, Kaoshiung City and Pintung City) (Lue *et al.* 1999). Recently, their distribution range inside Taipei was found to be shrinking due to anthropological affects and deforestation. Therefore, the Taipei Zoo attempted to conserve this species in collaboration with local farmers by making artificial habitats in foothill areas. Confirmed presence in Vietnam is based on specimens from northern, central and southern parts of the country. The species occurs also in Cambodia. There are confirmed records from eastern Thailand. The presence from more western parts of south-east Asia (central, north-western and southern Thailand, eastern Myanmar) must be re-evaluated with care and should be confirmed by voucher specimens. There is no evidence of its presence in Bangladesh, India or Nepal as all documented records using the name *taipehensis* are in fact wrongly identified *tytleri*. Published evidence as available on databases hold numerous outdated distribution data that might be based on wrongly identified “*H. erythraea*” or *H. tytleri*. As already indicated by Ohler & Mallick (2002) it would be interesting to know if the two species, *H. tytleri* and *H. taipehensis*, have a contact zone or if the distribution areas are disjunct as the present data suppose (fig. 1A).

The type locality of *H. erythraea* is Java and Sumatra, Indonesia, but the species is found in Cambodia, Laos, Indonesia, Sulawesi, Brunei and Sabah (Borneo), West and East Malaysia, Singapore, Thailand and Vietnam and the species has been introduced to the Philippines (Negros and Panay) (Diesmos *et al.* 2002). The sympatric distribution of *H. taipehensis* and “*H. erythraea*” ranges from Southern Laos including southern part of

Vietnam and Cambodia through eastern Thailand. Considering the large range, it is possible therefore that unknown taxa are hidden in the currently known species *H. taipehensis* and “*H. erythraea*”. Based on *16S* divergence between two specimens of *H. macrodactyla* from Hainan, China and Phu Quoc National Park, Kien Giang Province, Vietnam indicates that more than one species within *H. macrodactyla* complex might exist in this region which need further investigation including the specimens from entire distribution area of this species.

KEY PROPOSED TO IDENTIFY *HYLARANA* SPECIES

- 1 A complete middorsal line present *H. macrodactyla*
No middorsal line or only a short line in posterior back 2
- 2 Three phalanges of the forth toe free of webbing *H. taipehensis*
Less than three phalanges free of webbing 3
- 3 Two and half phalanges of the forth toe free of webbing; tibiotarsal articulation
reaching beyond the eye *H. tytlteri*
Two phalanges of the forth toe free of webbing; tibiotarsal articulation reaching
anterior border of eye *H. erythraea*

More diagnostic morphological characters of these species are given in Table 5.

Both molecular and morphological data confirmed that *H. tytlteri* is a distinct species that occurs in Bangladesh and adjacent areas. There is no confirmed evidence for presence of “*H. erythraea*” and *H. taipehensis* in India, Nepal and Bangladesh. Basic information is in need of confirmation, such as distribution ranges of *Hylarana* species; such work might be facilitated for field herpetologists and collection managers by the morphological characters published here and by Ohler & Mallick (2002). Further genetic studies need a much finer geographic scale that might include zones of sympatry of the different potential species and should incorporate samples of *H. taipehensis* and “*H. erythraea*” from their entire distribution to accurately clarify their taxonomic status.

Table 5. Characters to identify the members of the genus *Hylarana*.

Characters	<i>H. tytlteri</i>	<i>H. taipehensis</i>	“ <i>H. erythraea</i> ”	<i>H. macrodactyla</i>
SVL (adult male) mm	28.7–34.7	26.3–33.6	26.7–35.4	25.4–27.1
SVL (adult female) mm	41.2–41.8	34.9–46.8	51.4–64.9	28.1–36.1
Dorsolateral fold	White or off-white	Pure white	Cream-white or yellow	White with golden shades
Middorsal line	Absent	Absent	Absent	Present
Tibiotarsal articulation	Reaches to beyond the eye or to the nostrils	Reaches the nostrils	Reaches the anterior corner of the eye	Reaches the tip of snout or a little beyond
Webbing on feet	Two and half phalanges of the fourth toe free of webbing	Three phalanges of the fourth toe free of webbing.	Not more than two phalanges of the fourth toe free of webbing	Three phalanges of the fourth toe free of webbing

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SUPPLEMENTARY MATERIAL

Appendix Table S1: <https://drive.google.com/file/d/1Zqu7BY95OYC47O8OjvO0XkkMpHRL81A3/view>

Appendix Table S2: https://drive.google.com/file/d/1vSRYh3PIvBqead5r7AT90_VLkJZ8IUA/view

Appendix Table S3: <https://drive.google.com/file/d/1PN81ySp80JTCWAXsG3dngfCcZFxAIjBJ/view>

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