

Limnonectes from Enggano Island (Indonesia) with comments on its taxonomic status

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Abstract. Gonggoli AD, Atmajaya VY, Kadafi AM, Arida EA, Hamidy A. 2023. *Limnonectes* from Enggano Island (Indonesia) with comments on its taxonomic status. *Biodiversitas* 24: 473-480. *Limnonectes shompenorum* is a fanged frog known to be distributed in the satellite islands of the western coast of Sumatra and some parts of mainland Sumatra. This frog was also recorded in Enggano Island, but the taxonomic status and morphological data information still need to be studied. Herein, we examined *L. shompenorum* from Enggano Island based on molecular methods using the 16S rRNA gene and redescribed its morphological characters. The phylogenetic trees were constructed using Neighbor-Joining (NJ), Maximum Likelihood (ML), and Bayesian Inference (BI) analysis, and morphological measurements were taken using 23 characters. The results demonstrate that *L. shompenorum* is a monophyletic group and a sister taxon of *L. macrodon*. The monophyletic group of *L. shompenorum* consists of two clades (A and B) with relatively large genetic distances within the population (0.00-5.10%), representing distinct interspecific lineages. Clade A consists of the Enggano population with a genetic distance within the population of 0.00-0.48%, and clade B consists of the Mentawai Islands and Mainland Sumatra with a genetic distance within the population of 1.48-2.74%. The Enggano population is closer to the Mentawai population (2.47-2.74%) than the Sumatra population (3.52-5.10%). Our genetic data aligns with previous studies that indicate the Enggano population possibly comes from the Mentawai population. Morphologically, *L. shompenorum* from Enggano Island is similar to the original description, however it has consistent, distinctive characteristics by having the fourth finger longer than the second finger in females. Further research using broader molecular data, including the holotype population, is needed to confirm the taxonomic status of *L. shompenorum*.

Keywords: Enggano Island, fanged frog, mitochondrial DNA, morphology, taxonomic

INTRODUCTION

The Fanged Frog, *Limnonectes* Fitzinger 1843, is a genus of Dicroglossidae that currently comprises 78 species (Frost 2022). This genus is characterized by having an odontoid process (tooth-like projections) on the lower jaw, sexual dimorphism, several male species having voicelessness, and parental care (Koch 2012). This genus also has many character variations among the species, including size, skin texture, webbing, and breeding habits (Inger et al. 2017). Genus *Limnonectes* has several lineages with unclear taxonomic status (Emerson et al. 2000; Evans et al. 2003; Matsui et al. 2016) and is widely distributed in southern Asia, southern and eastern China, Indochina, Sundaland, Timor, New Guinea, Philippines, and south Japan (Frost 2022).

Limnonectes shompenorum is one of the *Limnonectes* species named after the Shompen tribe, i.e., the indigenous people of the Great Nicobar Island (the species type locality). *Limnonectes shompenorum* is remarkably similar

to the variety of *Rana macrodon* described by Boulenger (1920) named *R. macrodon* var. *blythii*. Based on a single specimen, Sarkar (1990) reported *R. macrodon* var. *blythii* presence in Great Nicobar, India. Then, Das (1996) assessed and described it as a new species based on four adult female specimens. In additional study, Chandramouli (2020) described the morphology of a topotypical adult male from Campbell Bay, Great Nicobar, that was previously unknown. The following morphological characteristics of *L. shompenorum* are (1) Toes completely webbed; (2) Head larger than the body; (3) Interorbital distance greater than the upper eyelid; (4) Fingers with moveable dermal fringe; (5) Tips of fingers weakly swollen; (6) Absence of the inverted V-shaped fold on the scapular region; and (7) Female with pigmented eggs (Das 1996). This frog can be found on riverbanks or non-riparian habitats in primary or secondary forests (Das 1996; Hari Krishnan and Vasudevan 2018; Chandramouli 2020). This frog is known to be distributed in the Nicobar Islands (Great Nicobar and Little Nicobar) and mainland Sumatra

(Aceh, North Sumatra, South Sumatra, Riau, and Bengkulu) (Das 1996; Inger and Iskandar 2005; Teynié et al. 2010; Chandramouli 2020). In addition, this species is also recorded from satellite islands on the western coast of Sumatra, viz. Simeulue Island, Banyak Islands, Nias Island, Batu Island, Mentawai Islands, and including Enggano Island (Che et al. 2009; Lawalata 2011; Maryanto et al. 2017; GBIF.org 2022).

Enggano is one of the satellite islands with a unique diversity of herpetofauna. However, only reptiles are currently known to have an endemic species, such as *Cnemaspis modiglianii*, *Hemiphyllodactylus engganoensis*, *Coelognathus enganensis*, and *Draco modiglianii*. In amphibians, there are only three species recorded on this island. Vinciguerra (1892) was the first to explore this island's reptiles and amphibians. He reported two species of Anura on Enggano Island: *Rana nicobariensis* (*Indosylvirana nicobariensis*) and *R. macrodon* (*Limnonectes macrodon*). In addition, Brongersma (1933) recorded *Rana chalconota* (*Chalcorana chalconota*) from this island.

However, records of *Limnonectes* on the Enggano island were first identified as *L. macrodon* by Vinciguerra (1892). Then, more than a century later, Che et al. (2009) published a report sequence of *Limnonectes* from this island and identified it as *L. shompenorum* without morphological description, followed by Hasan et al. (2014) that identified *Limnonectes* from the Mentawai Islands as the same species. These previous publications possibly followed Inger and Iskandar (2005), that identified a population of "smooth dorsum form" from West Sumatra

concordant with Das (1996) in the description of *L. shompenorum* from Nicobar Island. Then, Lawalata (2011) identified *Limnonectes* from Enggano island as *L. cf. shompenorum* without explaining the morphological characteristics in detail. All these prior studies provided limited information on both morphology and molecular data. As a result, the general morphological characteristics of the genus *Limnonectes* are look-alike among species causing some difficulties and mistakes in species identification. In the case of *L. shompenorum* from Enggano Island, the taxonomic status of this species still needs to be clarified, and more detailed morphological descriptions should be provided. Therefore, this paper aims to examine the taxonomic status of *L. shompenorum* from Enggano Island based on molecular methods using the 16S rRNA gene and redescribe its morphology.

MATERIALS AND METHODS

Field survey

A field survey was conducted on April 19-23, 2015, at two sampling points in Enggano Island (Figure 1). A total of 17 samples have been collected. First, liver tissue was stored in 95% ethanol for molecular analysis. Then, the samples were fixed using 10% formalin. Finally, voucher specimens and liver tissue were deposited at the Museum Zoologicum Bogoriense (MZB), Research Center for Biosystematics and Evolution, National Research and Innovation Agency.

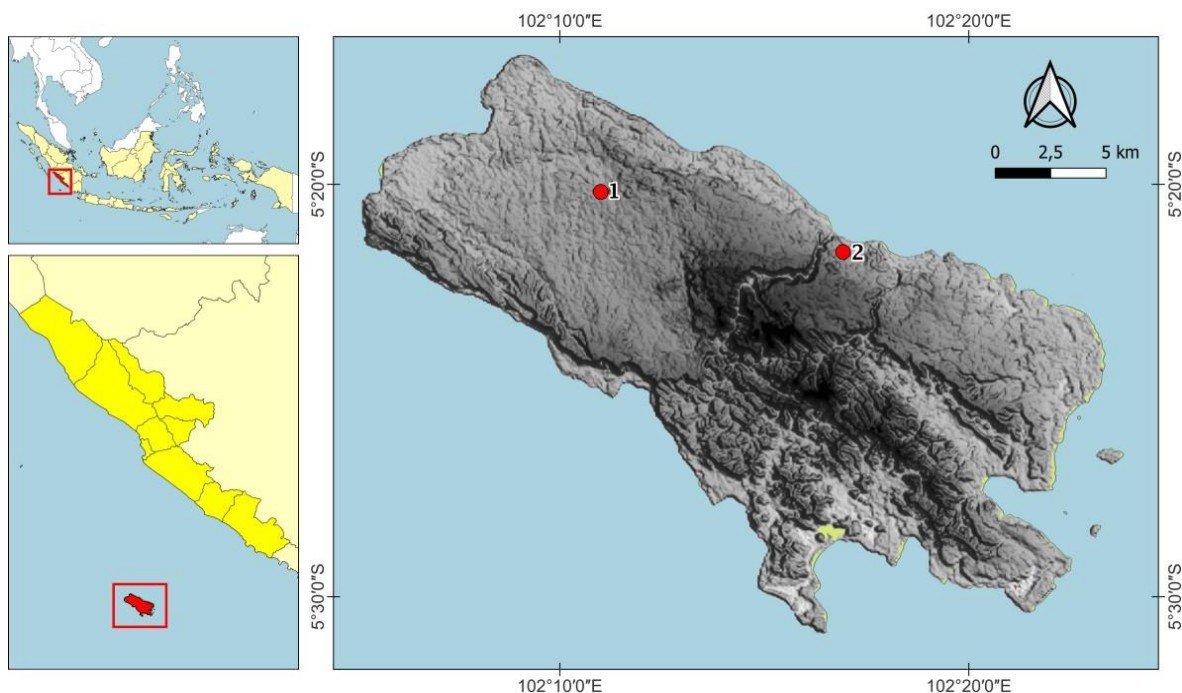


Figure 1. Map of Enggano island showing two sampling points of *Limnonectes shompenorum*: point 1 at Camp malakoni (S:05°20'08,7"; E:102°11'02,1") and point 2 at Kampung Bendung (S:05°21'35,9"; E:102°16'57,8"). Land topography using Shuttle Radar Topography Mission (SRTM) 90m database from CGIAR CSL (<https://srtm.csi.cgiar.org>) and created using QGIS version 3.22 Białowieża

Molecular analysis

Total DNA from liver tissue was extracted using the standard phenol-chloroform procedure (Sambrook et al. 1989). The partial sequences of the 16S rRNA gene were amplified using primers H3056 5'–CTCCGGTCTGAACTCAGATCACGTAGG–3', L2606 5'–CTGACCGTGCAAAGGTAGCGTAATCACT–3'. The PCR procedure followed the method by Matsui et al. (2016): initial denaturation for 4 min at 94°C and 33 cycles of denaturation for 15 s at 94°C, primer annealing for 15 s at 53°C, and extension for 2 min 40 s at 72°C. The purified PCR product was sequenced by 1st Base Asia (Singapore) using BigDye® Terminator.

DNA sequences were edited using Chromas Pro (Technelysium Pty Ltd., Tewantin, Queensland, Australia). Our sequences, including additional sequences from Genbank (Table 1), were aligned using the ClustalW in MEGA11 (Tamura et al. 2021) with *Fejervarya limnocharis*, *Occidozyga martensii*, and *Ingerana tenasserimensis* as outgroup members. The phylogenetic trees were constructed using Neighbor-Joining (NJ), Maximum Likelihood (ML), and Bayesian Inference (BI) analysis. NJ analysis was constructed using Kimura 2 Parameter Model and 1000 bootstrap replicated in MEGA11 (Tamura et al. 2021). The evolutionary model chosen for ML and BI is Akaike Information Criterion (AIC) with the GTR+G4 model based on the calculation results from Kakusan4 (Tanabe 2011). We constructed a phylogeny tree for ML analysis with 1000 ultrafast bootstraps (Hoang et al. 2017) and 1000 maximum iterations using the IQTREE web server (Trifinopoulos et al. 2016). BI analysis was constructed using MrBayes 3.2.7a (Ronquist et al. 2012) on the CIPRES Science Gateway (Miller et al. 2010), replication up to 10 million generations, sampled every 1000 generations using Markov Chain Monte Carlo (MCMC) simulation and 25% burn-in. The analysis results were visualized using Figtree v1.4.3 (Rambaut 2016). Genetic distance was calculated using the pairwise distance method in MEGA11 (Tamura et al. 2021). The genetic distance reference used as a species delimitation is three percent (Fouquet et al. 2007). Values that are highly supported in the ML analysis are >70% (Maximum Likelihood Bootstrap Support/MLBS) (Huelsenbeck and Hillis 1993) and 0.95 or more in the BI analysis (Bayesian Posterior Probabilities/BPP) (Leache and Reeder 2002).

Morphological measurement

Adult specimens were measured (Table 2) using a digital caliper to the nearest 0.1 mm. Abbreviations are presented here, following the method employed by Matsui (2015) for 23 morphological characters: snout-vent length (SVL, from tip of snout to cloaca); head width (HW, maximum width of the head from ventral view); head length (HL, from back of jaw to tip of snout); interorbital distance (IOD, distance of medial edge of eyeballs); internarial distance (IND); upper eyelid width (UEW); eye diameter (ED, from anterior to posterior angle of eye); tympanum diameter (TD, tympanum diameter-horizontal); snout-eye length (S-EL, from tip of snout to eye); nostril-

eyelid length (N-EL, from eye to nostril); snout-nostril length (SL, from tip of snout to nostril); forelimb length (FLL, from tip of third finger to base of forelimb); lower arm length (LAL, from flexed elbow to the tip of third finger); first finger length (FFL, from base of inner palmar tubercle to tip of first finger); hand length (HAL, from base of middle palmar tubercle to tip of third finger); inner palmar tubercle length (IPTL, maximal length of inner palmar tubercle); third finger disk width (3FDW); hindlimb length (HLL, length of straightened hindlimb from cloaca to tip of fourth toes); tibia length (TL, from base of knee to tibiotarsal articulation); thigh length (THL); foot length (FL, from base of inner metatarsal to tip of fourth toes); first toe length (1TOEL, from base of inner metatarsal to tip of first toes); inner metatarsal tubercle length (IMTL, maximal length of inner metatarsal tubercle); and fourth toe disk width (4TDW). We follow Savage and Heyer (1997) for webbing formulation.

RESULTS AND DISCUSSION

Molecular diagnostic

The results of DNA analysis showed a total of 442 base sites from 19 sequences, with 244 conserved sites and 181 variable sites. ML analysis results in a phylogeny tree with nucleotide frequency A: 0.325; C: 0.227; G: 0.194; T: 0.253, with a gamma shape of 0.328. BI analysis results in the nucleotide frequency A: 0.337; C: 0.210; G: 0.187; T: 0.264, with a gamma shape of 0.344.

The phylogenetic tree (Figure 2) shows that the sequence from Enggano Island is nested in the genus *Limnonectes* and formed a monophyletic group with *L. shompenorum* from mainland Sumatra (Padang, Malibou Anay) and Mentawai islands (Muara Siberut) (NJ: 95, MLBS: 81, BPP: 0.95) with *L. macrodon* as its sister clade (6.40–8.58%). *Limnonectes shompenorum* formed two clades, the clade A consisted of *L. shompenorum* from Enggano with a genetic distance range from 0.00 to 0.48% (NJ: 99, MLBS: 80, BPP: 0.99). Clade B comprised *L. shompenorum* from mainland Sumatra and Mentawai Islands, with a genetic distance ranging from 1.48 to 2.74% (NJ: 98, MLBS: 95, BPP: 1). The genetic distance between these two clades (A and B) of 2.47–5.10%. The sequence from Emerson and Ward (1998) with accession number U66131 is registered as *Rana blythi* in the Genbank database. From the analysis, we suggested the sequence as *L. shompenorum* based on its position nested into the *L. shompenorum* group.

Morphological description

Limnonectes shompenorum Das, 1996

Synonym: *Limnonectes macrodon* (Vinciguerra 1892).

Examined material. Nine males, twelve females, and three juveniles (n: 24) (Table 2).

Morphological description. The adult males and females have a robust body (SVL 59.03–81.5 mm in males, 47.29–79.65 mm in females); head length (HL 25.35–36.73 mm in males, 20.17–33.52 mm in females) longer than head wide (HW 22.50–32.20 mm in males, 17.88–29.58 mm in

females); mandible with two odontoids, obtuse; choanae oval; snout projection, slightly rounded; nostril nearer to snout (SL 3.83-5.32 mm in males, 2.83-5.27 mm in females) than to eye (N-EL 6.12-8.11 mm in males, 5.00-7.98 mm in females); canthus rostralis concave; a weak skin fold between interorbitals; eye diameter larger (ED 6.17-9.40 mm in males, 5.56-7.79 mm in females) than upper eyelid width (5.18-6.98 mm in males, 4.80-7.01-mm in females); tympanum smaller (TD 4.15-5.77 mm in males, 3.16-5.40 mm in females) than eye (ED 6.17-9.40 mm in males, 5.56-7.79 mm in females), semi-circular, almost reach lips; supratympanic fold curved; vomerine teeth oblique, separated with one individual vomerine.

Forelimbs are relatively short (FLL 39.33, 55.12%SVL in males, 37.22, 56.63%SVL in females); the fingers moderately slender; tips are rounded and swollen (disk diameter of the third finger, 3FDW 0.92-1.43 mm in males, 0.79-1.53 mm in females); dermal fringes on the second and third fingers; no webbing between fingers; relative finger lengths III>I>IV>II; outer metacarpal distinct and oval elongated connected with middle metacarpal; middle metacarpal low, inner metacarpal oval elongated; supernumerary metacarpal absent; proximal subarticular tubercle rounded and greater than middle subarticular tubercle; distal subarticular tubercle less prominent.

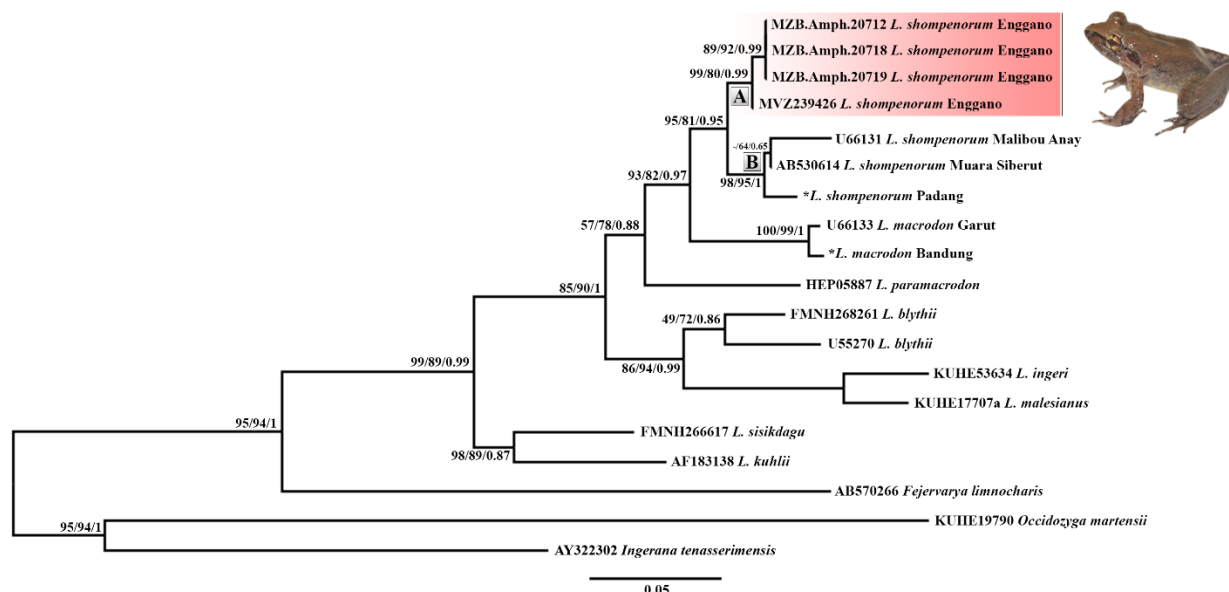
Table 1. This study used mtDNA analysis samples with species identification, locality, museum voucher, GenBank accession number, and reference

Species	Locality	Voucher	Accession Number	Reference
<i>L. shompenorum</i>	Indonesia, North Bengkulu, Enggano Island, Malakoni Village, Camp Malakoni	MZB.Amph.20712	OQ301806	This study
<i>L. shompenorum</i>	Indonesia, North Bengkulu, Enggano Island, Banjar Sari Village, Kampung Bendung	MZB.Amph.20724	OQ301807	This study
<i>L. shompenorum</i>	Indonesia, North Bengkulu, Enggano Island, Banjar Sari Village, Kampung Bendung	MZB.Amph.20718	OQ301808	This study
<i>L. shompenorum</i>	Indonesia, North Bengkulu, Malakoni Village	MVZ239426	EU979794	Che et al. (2009)
<i>L. shompenorum</i>	Indonesia, Malibou Anay, Anay Valley, 60 km from Padang	-	U66131	Emerson and Ward (1998)
<i>L. shompenorum</i>	Indonesia, West Sumatra, Padang	-	-	Tjong et al. (2010)
<i>L. shompenorum</i>	Indonesia, Mentawai Islands, Muara Siberut	IABHU 20716-20719	AB530614	Hasan et al. (2014)
<i>L. blythii</i>	Thailand, Prachuap Kirikan Prov, Huay Yang National Park	FMNH268261	GU934328	Inger and Stuart (2010)
<i>L. blythii</i>	Malaysia, West Malaysia	-	U55270	Emerson and Ward (1998)
<i>L. ingeri</i>	Malaysia, Borneo, Sarawak, Mulu	KUHE 53634	KP318721	Matsui et al. (2015)
<i>L. macrodon</i>	Indonesia, West Java, Bandung	-	-	Tjong et al. (2010)
<i>L. macrodon</i>	Indonesia, West Java, Tarogong, Garut	-	U66133	Emerson and Ward (1998)
<i>L. malesianus</i>	Malaysia, Sarawak, Matang	KUHE17707	KP318722	Matsui et al. (2015)
<i>L. paramcrodon</i>	Malaysia, Sabah, Maliau Basin Conservation Area	HEP05887	MH537635	Haas et al. (2018)
<i>L. sisikdagu</i>	Indonesia, West Jawa, Sukabumi	FMNH266617	JF836880	McLeod et al. (2011)
<i>L. kuhlii</i>	Indonesia, Jawa, Cibodas, Mt. Gede	Deposited in MZB	AF183138	Emerson et al. (2000)
<i>F. limnocharis</i>	Indonesia, Sumatera, Bengkulu, Alas Baru	-	AB570266	Kurniawan et al. (2014)
<i>O. martensii</i>	Thailand, Chachoengsao	KUHE19790	KP318725	Matsui et al. (2015)
<i>I. tenasserimensis</i>	Myanmar	CAS 205064	AY322302	Roelants et al. (2004)

Note: Voucher abbreviation: MZB: Museum Zoologicum Bogoriense; KUHE: Graduate School of Human and Environmental Studies, Kyoto University; MVZ: Museum of Vertebrate Zoology, University of California; FMNH: Field Museum of Natural History; USNM: US National Museum, Smithsonian Institution; IABHU: Institute for Amphibian Biology, Hiroshima University; LSUMZ: Louisiana State University Museum of Zoology; HEP: BORNEENSIS Herpetological Collection at Institute for Tropical Biology and Conservation. Universiti Malaysia Sabah, Malaysia

Table 2. Specimens examined of *Limnonectes shompenorum* (n: 24)

- Camp Malakoni, Malakoni Village, Enggano Island, North Bengkulu, Sumatra (S:05°20'08.7"; E:102°11'02.1"): two males (MZB.Amph.20712, MZB.Amph.20714) and one female (MZB.Amph.20713);
- Mangrove forest, Banjar Sari Village, Enggano Island, North Bengkulu, Sumatra (S:05°21'25.2"; E:102°16'55.1"): one male (MZB.Amph.20716), one female (MZB.Amph.20717), and one juvenile (MZB.Amph.20715);
- Jangkar River, Malakoni Village, Enggano Island, North Bengkulu, Sumatra (S:05°21'35.9", E:102°16'57.8"): two males (MZB.Amph.20719, MZB.Amph.20721), and three females (MZB.Amph.20718, MZB.Amph.20720, MZB.Amph.20722);
- Kampung Bendung, Banjar Sari Village, Enggano Island, North Bengkulu, Sumatra (S:05°21'35.9", E:102°16'57.8"): two males (MZB.Amph.20725, MZB.Amph.20727), and four females (MZB.Amph.20723-20724, MZB.Amph.20726, MZB.Amph.20728). All these specimens were collected by Amir Hamidy and Evy Ayu Arida in 19-21 April 2015.
- Malakoni Village, Enggano Island, North Bengkulu: two males (MZB.Amph.26324-26325), three females (MZB.Amph.26320, MZB.Amph.26323, MZB.Amph.26326), and two juveniles MZB.Amph.26321-26322, collected by Oscar in 19-23 October 2015.



Eggs. The diameter of twenty eggs from a female (MZB.Amph.20724) ranged from 1.66-1.88 mm (mean $\pm$ SD = 1.77 $\pm$ 0.07). Black and orange colors pigment the eggs.

Distribution. In Enggano Island, this species is distributed in Malakoni Village (Jangkar River), Banjar Sari Village (Kampung Bendung and Mangrove Forest), Chifaiuc, and Bua-Bua.



Figure 3. An adult male of *Limnonectes shompenorum* (MZB.Amph.20712) from Enggano Island

Table 3. Measurement of *Limnonectes shompenorum* from Enggano Island (in mm)

Characters	9 males		12 females	
	Mean±SD	Range	Mean±SD	Range
SVL	72.73±7.80	59.03-81.50	65.98±11.83	47.29-79.65
HW	28.10±3.45	22.50-32.20	25.20±4.09	17.88-29.58
HL	31.38±3.84	25.35-36.73	27.31±5.03	20.17-33.52
IOD	6.14±0.74	5.08-7.31	5.48±1.17	3.84-7.44
IND	6.18±0.72	4.98-7.03	5.69±0.79	4.43-6.95
UEW	6.33±0.55	5.18-6.98	5.93±0.71	4.80-7.01
ED	7.69±0.98	6.17-9.40	6.97±0.75	5.56-7.79
TD	5.09±0.50	4.15-5.77	4.44±0.72	3.16-5.40
S-EL	12.14±1.20	10.14-13.34	11.03±1.78	7.83-13.56
N-EL	7.27±0.75	6.12-8.11	6.58±0.92	5.00-7.98
SL	4.80±0.45	3.83-5.32	4.35±0.77	2.83-5.27
FLL	39.93±2.95	33.80-43.13	37.22±5.99	26.44-44.42
LAL	33.09±2.88	27.82-36.67	30.39±5.30	21.75-36.36
FFL	14.08±1.34	11.61-15.65	13.42±2.29	9.42-16.06
HAL	18.44±1.87	14.86-20.38	16.97±2.68	12.23-19.96
IPTL	4.50±0.59	3.85-5.69	4.23±0.68	3.09-5.23
3FDW	1.20±0.17	0.92-1.43	1.15±0.24	0.79-1.53
HLL	118.29±9.89	98.43-127.82	107.79±17.51	76.64-127.82
TL	37.52±3.59	30.65-41.78	34.17±5.91	24.33-40.57
THL	34.63±3.58	27.57-38.55	32.38±5.6	23.05-39.88
FL	37.53±3.72	29.93-40.99	34.21±5.46	25.19-40.65
1TOEL	14.67±1.54	11.68-16.63	13.24±2.06	9.77-15.63
IMTL	4.62±0.41	3.80-5.05	4.20±0.65	3.11-5.35
4TDW	1.37±0.20	1.10-1.67	1.36±0.24	0.96-1.78

Discussion

Our molecular data show the existence of two distinct clades with relatively large genetic distances (0.00-5.10%) within the population of the *L. shompenorum* group. These genetic distances are greater than three percent, representing the differentiation at the species level (Vences

et al. 2005; Fouquet et al. 2007). The result also indicating *L. shompenorum* from the Enggano population is likely to be a distinct lineage from the Mentawai and mainland Sumatra population. However, the taxonomic status between this group and the Nicobar population is still unknown.

Enggano Island is separated from the main Sumatra and Mentawai Islands by the sea, which has a depth of about >1000 m (Barber 2000). Sea fluctuations and glaciations have an important role in land bridges between islands, thus affecting the genetic differentiation and endemism of an island's fauna (Voris 2000). Based on geographic history, Mentawai Islands were once merged with mainland Sumatra in the Pliocene period and caused a land bridge between the islands (Voris 2000). This event will likely cause the ancestral invasion of the mainland Sumatra population to the Mentawai Islands. While between the Enggano and Mentawai Islands, there was no land bridge in the Pliocene period based on bathymetry studies (Sathiamurthy and Voris 2006), thus the invasion time of the Mentawai population to Enggano Island is still unknown.

However, the genetic distance between the Enggano and Mentawai population is relatively closer (2.47%) than the Sumatra population (3.52-5.10%) (Table 4). This phenome also occurs in other taxa in previous studies. For example, according to Lawalata (2011), *Draco sumatranus* from Enggano formed a monophyletic group with the Mentawai, Simalue and Nias population than Sumatra Population. This study is in line with our genetic data that the Enggano population is closest to the Mentawai population rather than the Sumatra population, which indicates that the Mentawai population possibly invaded the population from Enggano.



Figure 4. An adult male (A-E, MZB.Amph.20728) and adult female (F-J, MZB.Amph. 20724) of *Limnonectes shompenorum* from Enggano island. Dorsal and ventral view of male (A) and female (F); ventral view of hand and foot on male (E) and female (J); lateral and front views of head male (B and C) and female (G and H); tibia view on male specimen (D) and female bicolored eggs (I)

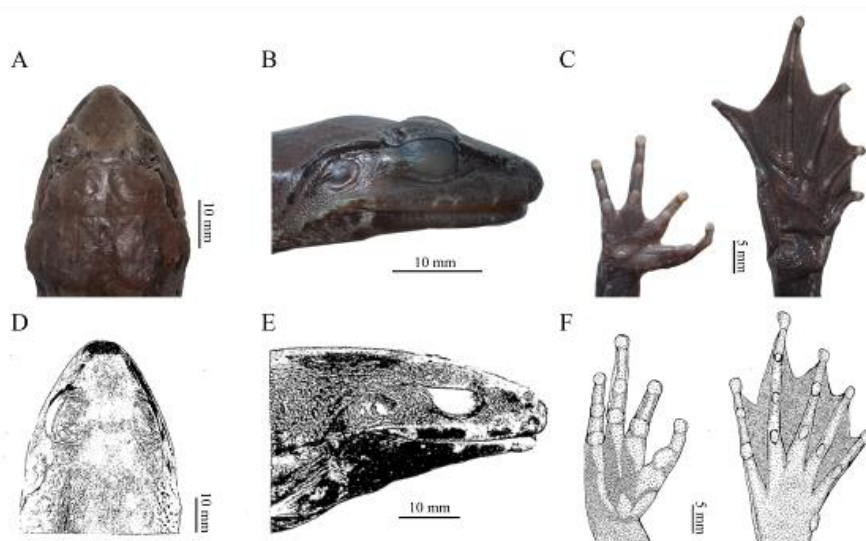


Figure 5. An adult female of *Limnonectes shompenorum* from Enggano Island (A-C, MZB.Amph. 20724) compared with an adult female from Nicobar Island (D-F, modified from Das (1996)). Head dorsal view of Enggano (A) and Nicobar (D); Head lateral view of Enggano (B) and Nicobar (E); palmar views of hand and foot of Enggano (C) and Nicobar (F)

Table 4. Uncorrected p-distance (%) among *Limnonectes shompenorum* from Enggano Island with related species using 442 bp of 16S rRNA gene

Species	1	2	3	4	5	6	7	8	9	10	11	12	13
1 Enggano Island (Clade A)	0.00-0.48												
2 Sumatra (Malibou Anay) (Clade B)	3.52-3.81	-											
3 Sumatra (Padang) (Clade B)	4.79-5.10	2.74	-										
4 Mentawai Island (Clade B)	2.47-2.74	1.48	2.21	-									
5 <i>L. blythii</i>	8.27-9.03	9.56-9.98	11.49-11.62	8.89-9.29	5.64								
6 <i>L. macrodon</i>	6.40-7.07	7.05-7.10	8.38-8.58	6.99-8.38	10.32-11.60	0.97							
7 <i>L. ingeri</i>	12.23-12.65	13.36	15.06	12.32	9.62-10.33	12.51-12.57	-						
8 <i>L. malesianus</i>	12.84-13.27	14.02	15.39	13.25	8.81-9.21	12.91-13.46	5.04	-					
9 <i>L. paramacrodon</i>	8.41-9.06	10.3	11.7	9.02	9.42-10.72	10.11-10.17	11.09	13.22	-				
10 <i>L. sisikdagu</i>	7.05-12.99	13.48	14.87	13.37	10.42-13.53	12.74-13.29	15.62	15.69	12.43	-			
11 <i>L. kuhlii</i>	6.99-13.02	13.82	15.23	13.39	11.31-12.60	14.36-14.93	15.6	15.35	13.58	8.32	-		
12 <i>Fejervarya limnocharis</i>	19.64-20.20	23.27	24.47	22.32	21.86-23.00	22.40-22.83	24.21	25.33	22.83	20.59	20.92	-	
13 <i>Occidozyga martensii</i>	28.58-29.64	32.75	33.45	31.55	31.57-32.61	31.45-31.98	33.6	34.69	33.13	33.77	30.89	30.25	-
14 <i>Ingerana tenasserimensis</i>	25.78-25.97	28	28.8	27.26	24.66-24.81	24.66-24.81	26.46	27.92	24.59	26.63	25.01	28.58	26.95

Meanwhile, morphologically, *Limnonectes* from Enggano is similar to the original description by Das (1996), including head length greater than head width, fingers with moveable dermal fringe on the third and fourth, full webbed of toes between nostril and eyes having a solid black stripe, tips of fingers weakly swollen, and the female eggs are pigmented. We have consistent, distinctive characteristics of the Enggano population to the Nicobar population, by having the fourth finger longer than the second finger in females (Figure 5). However, the description of finger lengths in the holotype population is unclear. Further studies involving extensive samples from the holotype population are needed to confirm the taxonomic status of *L. shompenorum*.

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REFERENCES

- Barber AJ, Crow MJ, Milsom J. 2005. Sumatra: Geology, Resources and Tectonic Evolution. Geological Society, London.
- Boulenger GA. 1920. A monograph of the south Asian, Papuan, Melanesian, and Australian frogs of the genus *Rana*. Rec Indian Mus 20: 1-226.
- Brongersma LD. 1933. Herpetological Notes: III Snakes from the Mentawai Islands. Zoologische Mededeelingen, Leiden.
- Chandramouli SR. 2020. First description of a male shompen frog, *Limnonectes shompenorum* Das 1996, with notes on distribution. Reptiles Amphibians 27: 235-236. DOI: 10.17161/randa.v27i2.14189.
- Che J, Hu J, Zhou W, Murphy RW, Papenfuss TJ, Chen M, Rao D, Li P, Zhang Y. 2009. Phylogeny of the Asian spiny frog tribe Painei (Family Dicroglossidae) sensu Dubois. Mol Phylogenet Evol 50: 59-73. DOI: 10.1016/j.ympev.2008.10.007.
- Das I. 1996. *Limnonectes shompenorum*, a new species of ranid frog of the *Rana macronotus* complex from Great Nicobar, India. J South Asian Nat Hist 2: 127-134.
- Emerson SB, Inger RF, Iskandar D. 2000. Molecular systematics and biogeography of the fanged frogs of Southeast Asia. Mol Phylogenet Evol 16: 131-142. DOI: 10.1006/mpev.2000.0778.
- Emerson SB, Ward R. 1998. Male secondary sexual characteristics, sexual selection, and molecular divergence in fanged ranid frogs of Southeast Asia. Zool J Linn Soc 122: 537-553. DOI: 10.1006/mpev.2000.0778.
- Evans BJ, Brown RM, McGuire JA, Supriatna J, Andayani N, Diesmos A, Iskandar D, Melnick DJ, Cannatella D. 2003. Phylogenetics of fanged frogs: Testing biogeographical hypotheses at the interface of the Asian and Australian faunal zones. Syst Biol 52: 794-819. DOI: 10.1080/10635150390251063.
- Fouquet A, Gilles A, Vences M, Marty C, Blanc M, Gemmell NJ. 2007. Underestimation of species richness in neotropical frogs revealed by mtDNA analyses. Plos One 2 (10): e1109. DOI: 10.1371/journal.pone.0001109.
- Frost DR. 2022. Amphibian Species of the World: an Online Reference. Version 6.1. Electronic Database. American Museum of Natural History, New York, USA. <https://amphibiansoftheworld.amnh.org/index.php>.
- GBIF.org. 2022. GBIF Occurrence Download. DOI: 10.15468/dl.khqi8p.
- Haas A, Boon-Hee K, Joseph A, Asri Mb, Das I, Hagmann R, Schwander L, Hertwig ST. 2018. An updated checklist of the amphibian diversity of Maliau Basin Conservation Area, Sabah, Malaysia. Evol Syst 2: 89-114. DOI: 10.3897/evolsyst.2.27020.
- Harikrishnan S, Vasudevan K. 2018. Amphibians of the Andaman & Nicobar Islands: Distribution, natural history, and notes on taxonomy. Alytes 36: 238-265.
- Hasan M, Islam MM, Khan MMR, Igawa T, Alam MS, Djong HT, Kurniawan N, Joshy H, Sen YH, Belabut DM, Kurabayashi A, Kuramoto M, Sumida M. 2014. Genetic divergences of South and Southeast Asian frogs: A case study of several taxa based on 16S ribosomal RNA gene data with notes on the generic name *Fejervarya*. Turk J Zool 38: 389-411. DOI: 10.3906/zoo-1308-36.
- Hoang DT, Chernomor O, Haeseler AV, Minh BQ, Vinh LS. 2017. UFBoot2 : Improving the ultrafast bootstrap approximation. Mol Biol Evol 35: 518-522. DOI: 10.1093/molbev/msx281.
- Huelsenbeck JPP, Hillis DM. 1993. Success of phylogenetic methods in the four-taxon case. Syst Biol 42: 247-264. DOI: 10.1093/sysbio/42.3.247.
- Inger RF, Iskandar DT. 2005. A collection of amphibians from west Sumatra, with description of a new species of *Megophrys* (Amphibia: Anura). Raffles Bull Zool 53: 133-142.
- Inger RF, Stuart BL. 2010. Systematics of *Limnonectes* (Taylorana) Dubois. Curr Herpetol 29: 51-68. DOI: 10.3105/018.029.0201.
- Inger RF, Stuebing RB, Grafe TU, Dehling JM. 2017. A Field Guide to the Frogs of Borneo (Third Edition). Natural History Publications Borneo, Sarawak.
- Koch A. 2012. Discovery, Diversity, and Distribution of the Amphibians and Reptiles of Sulawesi and its Offshore Islands. Chimaira, Frankfurt am Main.
- Kurniawan N, Djong TH, Maideliza T, Hamidy A, Hasan M, Igawa T, Sumida M. 2014. Genetic divergence and geographic distribution of frogs in genus *Fejervarya* from Indonesia inferred from mitochondrial 16s rRNA gene analysis. Treubia 41: 1-16. DOI: 10.14203/treubia.v41i0.361.
- Lawalata SZS. 2011. Historical Biogeography of Sumatra and Western Archipelago, Indonesia: Insights from the FLYING LIZARDS in the GENus *Draco* (Iguania: Agamidae). [Dissertation]. University of California, Berkeley.
- Leache A, Reeder TW. 2002. Molecular systematics of the eastern fence lizard (*Sceloporus undulatus*): A comparison of parsimony, likelihood, and bayesian approaches. Syst Biol 51: 44-68. DOI: 10.1080/106351502753475871.
- Maryanto I, Hamidy A, Keim AP, Sihotang VBL, Lupiyaningdyah P, Irfham M. 2017. Ekspedisi Pulau Enggano. LIPI Press, Jakarta.
- Matsui M, Kuraishi N, Eto K, Hamidy A, Nishikawa K, Shimada T, Yambun P, Vairappan CS, Hossman MYB. 2016. Unusually high genetic diversity in the Bornean *Limnonectes kuhlii*-like fanged frogs (Anura: Dicroglossidae). Mol Phylogenet Evol 102: 305-319. DOI: 10.1016/j.ympev.2016.06.009.
- Matsui M, Nishimura T, Eto K, Kuraishi N, Hamidy A, Nishikawa K, Sudin A. 2015. Genetic divergence in a Bornean fanged frog *Limnonectes leporinus* (Dicroglossidae, Anura). Curr Herpetol 34: 28-37. DOI: 10.5358/hsj.34.28.
- Matsui M. 2015. A new species of *Limnonectes* from the border of East Kalimantan and Sarawak, Borneo Island (Anura, Dicroglossidae). Curr Herpetol 34: 120-127. DOI: 10.5358/hsj.34.120.
- McLeod D, Horner SJ, Husted C, Barley A, Iskandar DT. 2011. Same-same, but different: An unusual new species of the *Limnonectes kuhlii* Complex from West Sumatra (Anura: Dicroglossidae). Zootaxa 2883: 52-64. DOI: 10.11646/zootaxa.2883.1.4.
- Miller MA, Pfeiffer W, Schwartz T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Proceedings of the Gateway Computing Environments Workshop (GCE). New Orleans, USA, 14-14 November 2010.
- Rambaut A. 2016. FigTree-version 1.4.3, A Graphical Viewer of Phylogenetic Trees. www.tree.bio.ed.ac.uk/software/figtree.
- Roelants K, Jiang J, Bossuyt F. 2004. Endemic ranid (Amphibia: Anura) genera in southern mountain ranges of the Indian subcontinent represent ancient frog lineages: Evidence from molecular data. Mol Phylogenet Evol 31: 730-740. DOI: 10.1016/j.ympev.2003.09.011.
- Ronquist F, Teslenko M, Mark PVD, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol 61: 539-542. DOI: 10.1093/sysbio/sys029.
- Sambrook JF, Fritsch EF, Maniatis T. 1989. Molecular Cloning: A Laboratory Manual. 2nd edition. Cold Spring Harbor Laboratory Press, New York.
- Sarkar AK. 1990. Taxonomic and ecological studies on the amphibians of Andaman and Nicobar Islands, India. Rec Zool Surv India 86: 103-117. DOI: 10.26515/rzsi/v86/i1/1990/161682.
- Sathiamurthy E, Voris HK. 2006. Maps of holocene sea level transgression and submerged lakes on the sunda shelf. Nat Hist Chulalongkorn Univ 2: 1-43.
- Savage JM, Heyer WR. 1997. Digital webbing formula for Anurans: A refinement. Herpetol Rev 3: 113-144.
- Tamura K, Stecher G, Kumar S. 2021. MEGA11: Molecular Evolutionary Genetics Analysis Version 11. Mol Biol Evol 38: 3022-3027. DOI: 10.1093/molbev/msab120.
- Tanabe AS. 2011. Kakusan4 and Aminosan: Two programs for comparing nonpartitioned, proportional and separate models for combined molecular phylogenetic analyses of multilocus sequence data. Mol Ecol Resour 11: 914-921. DOI: 10.1111/j.1755-0998.2011.03021.x.
- Teynié A, David P, Ohler A. 2010. Note on a collection of Amphibians and Reptiles from Western Sumatra. Zootaxa 2416: 1-43. DOI: 10.5281/zenodo.194395.
- Tjong DH, Iskandar DT, Gusman D. 2010. Hubungan filogenetik spesies *Limnonectes* (Ranidae: Amphibia) asal Sumatra Barat dan asal Asia Tenggara berdasarkan gen 16S ribosomal RNA. Makara Sains 14: 79-87. DOI: 10.7454/mss.v14i1.463. [Indonesia]
- Trifinopoulos J, Nguyen L, Haeseler Av, Minh BQ. 2016. W-IQ-TREE: A fast online phylogenetic tool for maximum likelihood analysis. Nucleic Acids Res 14: W222-W234. DOI: 10.1093/nar/gkw256.
- Vences M, Thomas M, van der Meijden A, Chiari Y, Vieites DR. 2005. Comparative performance of the 16S rRNA gene in DNA barcoding of amphibians. Front Zool 2: 1-12. DOI: 10.1186/1742-9994-2-5.
- Vinciguerra D. 1892. Annali del museo civico di storia naturale. In Annali Del Museo Civico Di Storia Naturale 12: 517-526. [Italia]
- Voris HK. 2000. Maps of Pleistocene sea levels in Southeast Asia: shorelines, river systems and time durations. J Biogeogr 27: 1153-1167. DOI: 10.1046/j.1365-2699.2000.00489.x.