Morphological Variation of *Ingerophrynus parvus* (Boulenger, 1887) in Peninsular Thailand

L Srion*1*, S Wangkulangkul1 and A Aowphol2

1Department of Biology, Faculty of Science, Prince of Songkla University, Hat Yai campus, Songkhla 90110 Thailand
2Department of Zoology, Faculty of Science, Kasetsart University, Bangkok, 10900 Thailand

*E-mail: lalita.psu@gmail.com*

**Abstract.** *Ingerophrynus parvus* (Boulenger, 1887) or Stream Toad is widely distributed in Southeast Asia region including Thailand. 41 samples from 3 localities, i.e., Ngao Waterfall National Park, Khao Chong Wildlife Development and Conservation Promotion Station and Hala-Bala Wildlife Sanctuary in peninsular Thailand were examined the morphological variation by external morphology observation (Binomial test) and morphometric analysis (ANOVA analysis). There are 5 from 15 morphological characters show significantly different among localities. Moreover, the morphometric analyses showed 8 significant characters from 16 morphometric characters which emphasize the shape variation among localities. Furthermore, the discriminant analysis shows samples from Khao Chong Wildlife Development and Conservation Promotion Station and Hala-Bala Wildlife Sanctuary are high homogeneity than samples from Ngao waterfall National park. Then, as the variation in morphology of *Ingerophrynus parvus* occur, the sharing morphological characters are still remaining among populations in the same time. Therefore, the requirement of specifically in microhabitat and the poor ability of dispersion among the toad populations, cooperate with the isolated mountain ranges might act as the barriers and disrupted sharing gene pool among localities, which cause diversity in morphological characters through time.

**Keywords:** Stream toad, morphological variation, mountain range, geographical barrier, southern Thailand

1. **Introduction**

Thai-Malay peninsula is the transition zone of flora and fauna between Indochinese and Sudanic subregions [1]. The influence of monsoon climate and the complexity of topography, latitude, longitude and altitude, cause high biodiversity of many animals including amphibians [2]. There are two main mountain ranges that run through Southern Thailand which are Tenasserim Mountain Range in the northern part and Nakhon Si Thammarat Mountain Range in the central part. Additionally, there is another mountain range at Thailand - Malaysia border, named Sankarakiri Mountain Range. These three mountain ranges prevent many terrestrial animals from migration. This assumption is supported by the distribution patterns of *Amolops panhai* and *Amolops larutensis*, *Rana nigrovittata* (sensu), and *Hoplobatrachus rugulosus* in Southern Thailand [3-5]. *Ingerophrynus parvus* [6] or stream toad is distributed from Southern Myanmar, Eastern through Southern Thailand, Peninsular Malaysia, Cambodia and Indonesia [7,9]. This small toad lives in specific habitats in primary and secondary
rainforests with slow moving streams, and breeds in small ponds close to streams or swampy areas [6,7,9]. The low dispersal ability, together with geographic barriers in this area, lead to the question of morphological variation in *Ingerophrynus parvus* among the isolated mountain ranges. Then, this work aimed to investigate morphological variation *Ingerophrynus parvus* in peninsular Thailand using morphological characters and morphometric measurement.

2. Study sites and methods

Based on geography, three main mountain ranges in peninsular Thailand were surveyed. These 3 mountain ranges are arranged from northern part to southern part of the Thai peninsula, i.e., Tenasserim Mountain Range, Nakhon Si Thammarat Mountain Range and Sankarakiri Mountain Range; these 3 mountain ranges are representative of different geographical areas. In total, 41 specimens were collected; 11 from Ngao Waterfall National Park, Ranong Province (Tenasserim Mountain Range), 17 specimens from Khao Chong Wildlife Development and Conservation Promotion Station, Trang Province (Nakhon Si Thammarat Mountain Range) and 13 specimens from Hala-Bala Wildlife Sanctuary, Narathiwat Province (Sankarakiri Mountain Range) (figure 1). Voucher specimens were then deposited at the Princess Maha Chakri Sirindhorn Natural History Museum, Songkhla, Thailand.

In this study, both qualitative and quantitative data were examined, using external morphology observation and morphometric analysis. For non-parametric analysis, fifteen morphological characters were examined and classified as present=1 and absent=0. The morphological characters included a curved nose, a distinct pair of ridges on frontal head, the end of ridges curved, spiny warts above parotid glands, black and white stripes around mouth, oval tympanum, a pair of tubercles behind parotid glands, white ventral, pairs of warts on the middle area of back line, forelimbs with dark cross bands, spiny warts under forelimbs, hindlimbs with dark cross bands, outer metacarpal tubercle larger
than inner metacarpal tubercle, outer metatarsal tubercle larger than inner metatarsal tubercle and tip of tibiofibular reach tip of snout when fold leg toward head. For data analysis, Binomial test (Crosstab method) were took place using SPSS program version 17.0. Moreover, sixteen morphometric characters were examined, and the data was transformed into ratios with body length (snout to vent length; SVL) for preventing body size bias. The morphometric characters are as follow: internarial length (IN), snout length (SNL), snout width (SW), interjaws width (IJW), head width (HW), head length (HL), head depth (HD), eye diameter (ED), eye width (EW), interorbital length (IO), ridge length (RL), parotid gland width (PGW), parotid gland length (PGL), hand length (HDL), tibia length (TB) and foot length (FL). For statistical analysis, One-way ANOVA analyses (p < 0.05) with multiple comparison analysis (Post Hoc test) were performed using SPSS program version 17.0.

3. Results

3.1. Morphological characters

The comparison of body size (SVL) indicated that females of Ingerophrynus parvus were larger than males on average. Females from Khao Chong Wildlife Development and Conservation Promotion Station and males from the Hala-Bala Wildlife Sanctuary were the largest in body size (42.60±4.24 mm and 34.37±2.69 mm), respectively.

There were five morphological characters exhibited significantly differences of occurrence frequency among localities (p<0.05), which were white ventral, spiny warts under forelimbs, spiny warts above parotid gland, curved ridge and tip of tibiofibular reach tip of snout when fold leg toward head. In Khao Chong Wildlife Development and Conservation Promotion Station population, the occurrence frequency of white ventral, spiny warts under forelimbs and spiny warts above parotid glands characters were found significantly more often than in Hala-Bala Wildlife Sanctuary and Ngao Waterfall National Park populations. The population in Hala-Bala Wildlife Sanctuary showed 46.2% presence of white ventral, 61.5% presence of spiny warts above parotid glands and 23.1% presence of spiny wart under forelimbs.

The population in Ngao Waterfall National Park have 90.9% absence of white ventral, spiny warts under forelimbs and spiny warts above parotid glands characters (figure 2A-2C). All specimens from Ngao waterfall National Park and Khao Chong Wildlife Development and Conservation Promotion Station showed 100% presence of curved ridges character, and 53.8% presence in Hala-Bala Wildlife Sanctuary (figure 2D). There are 9.1% of specimens that tip of tibiofibular could reach tip of snout when fold leg toward head in Ngao Waterfall National Park, and 29.4% in Khao Chong Wildlife Development and Conservation Promotion Station. In contrast, 84.6 % of the population in Hala-Bala Wildlife Sanctuary showed tip of tibiofibular could reach tip of snout (figure 2E).

3.2. Morphometric analysis

The eight from sixteen morphometric characters showed significantly different among the localities (table 1). The multiple comparisons (p<0.05) revealed that Tibia length (TB) is a single strongly different character among localities. This character in Khao Chong Wildlife Development and Conservation Promotion Station Specimens were significantly longer than Hala-Bala Wildlife Sanctuary and Ngao Waterfall National park. Eye diameter (ED) showed a grouping variance of specimens from Ngao Waterfall National park and Hala-Bala Wildlife Sanctuary. While the internarial length (IN) and hand length (HDL) revealed coexistence of specimens from Khao Chong Wildlife Development and Conservation Promotion Station and Hala-Bala Wildlife Sanctuary. Eye width (EW), foot length (FL) and head width (HW) showed homogeneity of variance of the specimens from Ngao Waterfall National park and Khao Chong Wildlife Development and Conservation Promotion Station. Moreover, head depth (HD) represented weakness of variance grouping among three localities.
Table 1. The multiple comparison with mean and standard deviation values of *Ingerophrynus parvus* characters from Ngao Waterfall National park, Khao Chong Wildlife Development and Conservation Promotion Station and Hala-Bala Wildlife Sanctuary.

| Variables          | Localities                                      | p-value |
|--------------------|-------------------------------------------------|---------|
|                    | Ngao Waterfall National Park (Mean±SD)           |         |
|                    | Khao Chong Wildlife Development and Conservation Promotion Station (Mean±SD) |         |
|                    | Hala-Bala Wildlife Sanctuary (Mean±SD)          |         |
| Number of specimen | 11                                              | 17      | 13      |         |
| Internarial length | 2.83±0.17<sup>a</sup>                           | 2.85±0.34<sup>b</sup> | 2.63±0.30<sup>b</sup> | 0.000   |
| Eye diameter       | 4.76±0.40<sup>a</sup>                           | 4.95±0.74<sup>b</sup> | 5.08±0.57<sup>a</sup> | 0.001   |
| Eye width          | 3.64±3.23<sup>a</sup>                           | 4.06±0.47<sup>b</sup> | 4.28±0.49<sup>b</sup> | 0.001   |
| Hand length        | 8.02±0.49<sup>a</sup>                           | 11.18±1.63<sup>b</sup> | 10.34±1.23<sup>b</sup> | 0.000   |
| Tibia length       | 13.59±0.70<sup>a</sup>                           | 16.48±2.22<sup>b</sup> | 15.99±1.87<sup>c</sup> | 0.000   |
| Foot length        | 12.08±0.72<sup>a</sup>                           | 14.36±1.85<sup>a</sup> | 14.04±1.80<sup>b</sup> | 0.000   |
| Head width         | 10.19±0.44<sup>a</sup>                           | 11.74±1.36<sup>a</sup> | 11.33±1.13<sup>b</sup> | 0.038   |
| Head depth         | 5.26±0.37<sup>a</sup>                           | 5.93±0.88<sup>a</sup> | 5.77±0.59<sup>a,b</sup> | 0.010   |
| Snout length       | 4.00±0.22                                       | 4.50±0.54 | 4.27±0.51 | 0.539   |
| Snout width        | 4.88±0.21                                       | 5.54±0.79 | 4.93±0.53 | 0.087   |
| Interjaw width     | 11.39±0.70                                      | 13.26±1.66 | 12.75±1.55 | 0.066   |
| Head length        | 10.23±0.55                                       | 11.75±1.28 | 11.31±1.26 | 0.104   |
| Interobital length | 4.00±0.29                                       | 4.64±0.52 | 4.25±0.47 | 0.966   |
| Ridge length       | 5.30±0.39                                       | 6.37±0.58 | 6.03±0.74 | 0.231   |
| parotid gland width| 2.28±0.27                                       | 2.69±0.38 | 2.41±0.44 | 0.759   |
| parotid gland length| 3.62±0.35                                       | 4.20±0.68 | 3.79±0.91 | 0.791   |

*a,b,c* are representative group of variables among localities base on multiple comparison analysis. Asterisk (*) shows a significant different character base on ANOVA analysis (p < 0.05).

3.3. Discriminant function analysis

The discriminant analysis of most significant characters showed all six characters are positively related to the function (p≤0.001). In this study, 2 discriminant functions were produced; the first function can explain 84.1% of variance, and function 2 can explain 15.9% percent of variance, and Wilk’s lambda are 0.063 and 0.458 respectively. Discriminant function 1 discriminates Ngao Waterfall National Park from Khao Chong Wildlife Development and Conservation Promotion Station and Hala-Bala Wildlife Sanctuary populations, meanwhile Hala-Bala Wildlife Sanctuary population discriminates from Ngao Waterfall National Park and Khao Chong Wildlife Development and Conservation Promotion Station in function 2 (table 2).
The canonical discriminant analysis showed Hala-Bala Wildlife Sanctuary and Khao Chong Wildlife Development and Conservation Promotion Station showed more association of morphometric characters than with Ngao Waterfall National Park (figure 3).

![Percentage occurrence of five significant morphological characters of Ingerophrynus parvus](image)

**Figure 2.** Percentage occurrence of five significant morphological characters of *Ingerophrynus parvus* from three localities in Southern Thailand; white ventral (A), spiny warts under forelimbs (B), spiny warts above parotid glands (C), curved ridges (D) and tip of tibiofibular reach tip of snout when fold toward head (E).

| Groups              | Function |
|---------------------|----------|
|                     | 1        | 2        |
| Ngao                | -3.958   | -0.193   |
| Khao Chong          | 1.726    | -0.994   |
| Bala                | 1.092    | 1.462    |
4. Discussion

*Ingerophrynus parvus* is widely distributed in Southeast Asia, ranging from southern Myanmar through western and Peninsular Thailand, Cambodia, Malaysia, and Indonesia [10]. The habitats of this toad are on the ground floor in tropical rainforests [7], and the breeding sites preference of this species are puddle areas close to streams or ponds [11,12]. In general, amphibians are usually poor disperser [13], the dispersal ability of *I. parvus* has been reported at about 30 meters far from streams [7], and the highest elevation is 520 meters above sea level [10]. Natural barriers such as rivers and mountain ranges that can restrict animal distribution, especially in amphibians [14-16]. These barriers might disrupt movement among *I. parvus* populations. The morphology and morphometric studies of *I. parvus* in peninsular Thailand indicated the phenotypic variation among isolated populations, and associate with the disconnection between mountain ranges. It emphasized that specimens from Ngao Waterfall National Park (Tenasserim Mountain Range) showed the most unrelated external morphology with Hala-Bala Wildlife Sanctuary (Sankarakiri Mountain Range) rather than between Hala-Bala Wildlife Sanctuary and Khao Chong Wildlife Development and Conservation Promotion Station (Nakhon Si Thammarat mountain Range) based on the multiple comparison and the discriminant analyses. This incident probably due to the geographical distance and the barriers among the mountain ranges, the connected or closer areas can preserve more genetic relationship than the further isolated areas. On the other hand, the fragmented areas can cause genotypic variation among populations as well [17-19]. Then, the huge lowland between Tenasserim Mountain Range and Nakhon Si Thammarat Mountain Range could act as a barrier between these two mountain ranges. Owing to peninsular Thailand topography and the specific habitat requirement of *Ingerophrynus*
parvus species, resulting in disrupted gene flow among populations [14,20], causing genotypic variation and external morphology and morphometric characters expression. However, homogeneity of morphology is still remaining among populations of I. parvus. Then, sharing in morphological characters are presumed that Ingerophrynus parvus populations in peninsular Thailand used to share same gene pool in the past, but isolated mountain ranges inhibit gene flow among populations cause phenotypic diversity of the populations.

Acknowledgements
This work was done with funding supported from Science Achievement Scholarship of Thailand, faculty of Science, Prince of Songkla University, Songkhla, Thailand. The specimen collections were supported from the Princess Maha Chakri Sirindhorn Natural History Museum, Songkhla, Thailand, and we special thanks to Department of National Parks, Wildlife and Plant Conservation for the corporation, especially the superintendents and staffs from Ngao Waterfall National Park, Khao Chong Wildlife Development and Conservation Promotion Station and Hala-Bala Wildlife Sanctuary.

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