Morphological and Genetic Variations of *Ingerophrynus parvus* (Boulenger, 1887) in Southern Thailand

Lalita Srion

A Thesis Submitted in Partial Fulfillment of the Requirements for the
Degree of Master of Science in Zoology
Prince of Songkla University
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ชื่อวิทยานิพนธ์	ความผันแปรทางสัณฐานวิทยาและพันธุกรรมของคางคกแคระ *Ingerophrynus parvus* (Boulenger, 1887) ในภาคใต้ของประเทศไทย
ผู้เขียน	นางสาวลลิตา ศรีอ่อน
สาขาวิชา	สัตววิทยา
ปีการศึกษา	2560

บทคัดย่อ

คางคกแคระ *Ingerophrynus parvus* เป็นคางคกขนาดเล็กที่มีขอบเขตการแพร่กระจายเฉพาะในแถบเอเชียตะวันตกเฉียงใต้ และมีถิ่นที่อยู่อาศัยที่มีลักษณะจำเพาะโดยลักษณะภูมิประเทศทางภาคใต้ของประเทศไทยอาจส่งผลต่อการแพร่กระจายและติดต่อระหว่างกลุ่มประชากร ในการศึกษาครั้งนี้มีวัตถุประสงค์เพื่อศึกษาความผันแปรทางสัณฐานวิทยาและพันธุกรรมของคางคกแคระ *Ingerophrynus parvus* (Boulenger, 1887) ในภาคใต้ของประเทศไทย โดยศึกษาคางคกแคระจำนวน 166 ตัวอย่างจาก 10 พื้นที่ศึกษา เพื่อเปรียบเทียบลักษณะทางสัณฐานวิทยา (Morphological characters) 15 ลักษณะ แล้ววิเคราะห์ความแตกต่างด้วย chi-squared test และการวิเคราะห์ทางสัณฐานวิทยา (Morphometrics) 18 ลักษณะนำมาวิเคราะห์ความแตกต่างระหว่างเพศด้วยวิธี independent sample t-test และ sexual size dimorphism index รวมถึงเปรียบเทียบความแตกต่างระหว่างพื้นที่ด้วยวิธี one-way ANOVA analysis และ hierarchical cluster analysis จากผลการศึกษาพบว่าลักษณะทางสัณฐานวิทยา 7 ลักษณะจาก 15 ลักษณะแสดงความแตกต่างการปรากฏของลักษณะระหว่างกลุ่มประชากร และเมื่อเปรียบเทียบการวิเคราะห์ทางสัณฐานวิทยาพบว่าคางคกแคระเพศเมียมีความยาวปลายจมูกถึงช่องเปิดทวารร่วม (snout to vent length) มากกว่าเพศผู้อย่างมีนัยสำคัญทางสถิติ ($p<0.05$) อีกทั้งยังมีความสัมพันธ์เชิงบวกกับลักษณะอื่นๆ นอกจากนี้ยังพบว่าอัตราส่วนลักษณะที่วัดต่อความยาวปลายจมูกถึงช่องเปิดทวารร่วม 17 จาก 18 ลักษณะมีความแตกต่างอย่างมีนัยสำคัญทางสถิติ ($p<0.05$) ระหว่างพื้นที่ศึกษา การวิเคราะห์ hierarchical cluster analysis สามารถจัดกลุ่มคางคกแคระจากเกาะตะรุเตาแยกออกจากกลุ่มตัวอย่างบน

คาบสมุทรไทย แต่ยังคงมีลักษณะร่วมทางสัณฐานวิทยากับตัวอย่างบนคาบสมุทรไทย ทั้งนี้อาจเนื่องมาจากเกาะตะรุเตานั้นเคยเชื่อมต่อกับแผ่นดินใหญ่มาก่อนในอดีตจึงนำไปสู่การปรากฏลักษณะร่วมทางสัณฐานวิทยา ในขณะที่ตัวอย่างประชากรคางคกแคระจากบนแผ่นดินไม่สามารถจัดเป็นกลุ่มประชากรย่อยได้ นอกจากนี้ยังได้ศึกษาลักษณะทางพันธุกรรมโดยใช้ยีน 16S rRNA แล้ววิเคราะห์ความสัมพันธ์ทางสายวิวัฒนาการด้วยวิธี maximum likelihood และ network analysis ซึ่งจากการศึกษาความแปรผันของลักษณะทางพันธุกรรมด้วยยีน 16S rRNA ที่มี 518 คู่เบส พบว่าประชากรคางคกแคระแบ่งเป็นสามกลุ่มย่อยสอดคล้องกับแนวเทือกเขาในภาคใต้ของประเทศไทย คือ กลุ่มเทือกเขาตะนาวศรี กลุ่มเทือกเขานครศรีธรรมราช และกลุ่มเทือกเขาสันกาลาคีรี นอกจากนี้ยังพบว่าตัวอย่างคางคกแคระจากเทือกเขาตะนาวศรีมีความใกล้ชิดกับตัวอย่างจากเทือกเขานครศรีธรรมราช แต่แยกกลุ่มจากเทือกเขาสันกาลาคีรี ทั้งนี้อาจเนื่องมาจากพื้นที่ราบต่ำขนาดใหญ่ที่กั้นระหว่างเทือกเขานครศรีธรรมราชและเทือกเขาสันกาลาคีรีอาจมีอิทธิพลต่อการแพร่กระจายของประชากรคางคกแคระ เมื่อเทียบกับพื้นที่ราบระหว่างเทือกเขาตะนาวศรีและเทือกเขานครศรีธรรมราชที่แคบกว่า จึงอาจทำให้ประชากรคางคกแคระระหว่างสองเทือกเขานั้นยังมีความใกล้ชิดกันอยู่ เช่นเดียวกันกับประชากรคางคกแคระจากเทือกเขาสันกาลาคีรีและอุทยานแห่งชาติทะเลบันที่มีความใกล้ชิดกัน การศึกษาครั้งนี้แสดงให้เห็นถึงความแปรผันทางสัณฐานของคางคกแคระระหว่างประชากรในภาคใต้ของประเทศไทย แต่อย่างไรก็ตามผลการศึกษาทางลักษณะทางพันธุกรรมแสดงให้เห็นการแบ่งกลุ่มตัวอย่างประชากรคางคกแคระสอดคล้องกับแนวเทือกเขาในคาบสมุทรไทย

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ABSTRACT

Stream toad *Ingerophrynus parvus* is distributed in Southeast Asia. This species has small body size, and habitat specificity. The topography of southern Thailand might affect its distribution and connection among *Ingerophrynus parvus* populations. This study aimed to compare the morphological and genetic variations of *Ingerophrynus parvus* populations in southern Thailand. Newly collected 166 specimens from 10 study localities were examined for 15 morphological characters, and the data was analyzed using chi-squared test. Sexual size dimorphism were examined base on 18 morphometric characters, and the analysis was performed using independent sample t-test and sexual size dimorphism index. One-way ANOVA analysis and hierarchical cluster analysis were used to compare the differences of morphometric characters among populations. The results show that seven from 15 characters of external morphology were significantly different among localities. The morphometric study exhibited that females of *Ingerophrynus parvus* were significantly larger than that of males, Moreover, morphometric characters of males and females showed positive correlation with snout to vent length (SVL). Besides, 17 from 18 ratios of morphometric characters with SVL showed significantly different characters among localities ($p<0.05$), and hierarchical cluster analysis group specimens from Tarutao Island separated from the mainland populations. It was found that population from Tarutao shared some morphometric characters with population from mainland. This could be results of the connection between Tarutao Island and the mainland in the past, leading to sharing of external morphology between the island and mainland populations. However, morphological characters of the mainland populations did not clearly show the group of *Ingerophrynus parvus* populations.

Molecular study of 16S rRNA gene from 518 nucleotides were analyzed with maximum likelihood and network analysis. The molecular study showed genetic variation followed the isolated mountain ranges in southern Thailand, i.e., Tenasserim Mountain Range, Nakhon Si Thammarat Mountain Range and Sankalakhiri Mountain Range. The specimens from Tenasserim Mountain Range were closely related with populations from Nakhon Si Thammarat Mountain Range, but grouped separated from populations from Sankalakhiri Mountain Range. Explaining by geographical distance that the big lowland area between Nakhon Si Thammarat Mountain Range and Sankalakhiri Mountain Range can influence distribution of *Ingerophrynus parvus* populations. The smaller lowland areas between Tenasserim Mountain Range and Nakhon Si Thammarat Mountain Range, and between Sankalakhiri Mountain Range and Thale Ban National Park had less effect on the distribution of *Ingerophrynus parvus*. This study exhibit variation in external morphology among *Ingerophrynus parvus* populations in southern Thailand, but it's not clearly distinct into subgroup. However, molecular study group *Ingerophrynus parvus* populations follow isolated mountain ranges in southern Thailand.

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Abbreviations

Study localities

NG	Ngao Waterfall National Park
TBK	Than Bok Khorani National Park
KPB	Khao Phanom Bencha National Park
Y	Yong Waterfall National Park
BGT	Thung Khai botanic garden
K	Khao Chong Wildlife Development and Conservation Promotion Station
KHH	Protected area, Kho Hong hill, Prince of Songkla University
TLB	Thale Ban National Park
B	Hala-Bala Wildlife Sanctuary
TRT	Tarutao Island

Morphometric characters

IN	Internarial length
SNL	Snout length
SW	Snout width
IJW	Interjaws width
HW	Head width
HL	Head length
HD	Head depth
ED	Eye diameter
EW	Eye width
IO	Interobital length
LBR	Length between ridges
RL	Ridge length
PGW	Paratoid gland width
PGL	Paratoid gland length
HDL	Hand length
TB	Tibia length
FL	Foot length
RIN	Ratio of internarial length with snout to vent length

morphometric characters (*continued*)

RSNL	Ratio of snout length with snout to vent length
RSW	Ratio of snout width with snout to vent length
RIJW	Ratio of interjaws width with snout to vent length
RHW	Ratio of head width with snout to vent length
RHL	Ratio of head length with snout to vent length
RHD	Ratio of head depth with snout to vent length
RED	Ratio of eye diameter with snout to vent length
REW	Ratio of eye width with snout to vent length
RIO	Ratio of interobital length with snout to vent length
RLBR	Ratio of length between ridges with snout to vent length
RRL	Ratio of ridge length with snout to vent length
RPGW	Ratio of paratoid gland width with snout to vent length
RPGL	Ratio of paratoid gland length with snout to vent length
RHDL	Ratio of hand length with snout to vent length
RTB	Ratio of tibia length with snout to vent length
RFL	Ratio of foot length with snout to vent length

CHAPTER I

INTRODUCTION

1.1. Rationale

Amphibians are recently reported 7,728 species around the world. There are separated into 3 main groups, including frogs and toad group (Order Anura) 6,806 species, newts and salamanders group (Order Caudata) 715 species and caecilians group (Order Gymnophiona) 207 species (Vences and Köhler, 2008; Frost, 2016; Oliveira et al., 2017). From the attendance of these animal studies, the number of amphibian species has been increased more than 60 percent since 1985 (Duellman, 1970; Köhler et al., 2005; Amphibia Web, 2016), however, it still need more information to be clarify.

Bufonid group is widely distributed cosmopolitan except for Australo-Papuan Realm and Madagascar (Duellman and Trueb, 1999). Species numbers in this group has been increased as other amphibian groups from enlarge study, and recently it contains 588 species around the world (Frost, 2016). In Southeast Asia, genus *Bufo* is separated into 3 main groups based on phylogenetic study consist of genus *Duttaphrynus*, genus *Phrynoidis* and genus *Ingerophrynus* (Frost et al., 2006; Matsui et al., 2015). From the study of Inger in 1972, he combined small bufonid toads as one group called "*Bufo biporcatus* group" which are *Bufo biporcatus* (Gravenhorst, 1829), *Bufo claviger* (Peters, 1863), *Bufo divergens* (Peters, 1871), *Bufo parvus* (Boulenger, 1887), *Bufo philippinnicus* (Boulenger, 1887) and *Bufo quadriporcatus* (Boulenger, 1887). Later in 2006, Frost and colleges reclassified these small bufonid toads into a new genus called "*Ingerophrynus*" based on their phylogenetic study. Additionally, *Ingerophrynus gollum* was published as new species in the following year by Lee Grismer (Grismer, 2007). Recently, there are eleven species members in genus *Ingerophrynus* (Frost, 2016), including six species of *Bufo biporcatus* group, plus four more species from the molecular result which are *Ingerophrynus celebensis* (Günther, 1859), *Ingerophrynus galeatus* (Günther, 1864), *Ingerophrynus macrotis* (Boulenger, 1887), *Ingerophrynus kumquat* (Das and Lim, 2001), and *Ingerophrynus gollum* (Grismer, 2007).

Ingerophrynus group can be found only in Southeast Asia region, i.e., southern Myanmar, southwestern Cambodia, Thailand, Peninsular Malaysia and Indonesia (IUCN, 2014). In Thailand, there were three species of *Ingerophrynus* group (Chuaynkern, 2009), consisting of *Ingerophrynus divergens* (crested toad), *Ingerophrynus macrotis* (big-eyed toad) and *Ingerophrynus parvus* (stream toad). However, recently distribution report of *Ingerophrynus* species has been shown only *Ingerophrynus macrotis* and *Ingerophrynus parvus* in Thailand (IUCN, 2017). These small toads have very few studies, and the population status is still unknown (IUCN, 2016). Most of the investigations in this study were related to species checklist, distribution range, and breeding activities (Boulenger, 1912; Das and Lim, 2001; Konsue and Thirakhupt, 2001; Chan-ard, 2003; Chuaynkern and Chuaynkern, 2012; Shahriza et al., 2015). However, this study concentrated only on the morphological variation of *Ingerophrynus parvus* species which is widely distributed in southern Thailand, while *Ingerophrynus macrotis* is hardly find in natural habitats especially in southern Thailand.

Mountains are strongly effect to the distribution ranges of amphibian species especially for amphibian species that live in specific habitat (Bain and Hurley, 2011). The topographical in southern Thailand shows three main isolated mountain ranges laying down from north to south direction. There are Tenasserim Mountain Range, Nakorn Si Thammarat Mountain Range and Sankalakhiri Mountain Range. These three mountain ranges affect the type of forest communities, and provide variety of habitats and microhabitats for animals (Stankovic et al., 2013). Moreover, habitat fragmentation is highly effect to the adaptation of amphibians morphological characters such as the existence of genus *Amolops*, genus *Ansonia* and genus *Hoplobatrachus* that show species discrimination associated with the mountain ranges (Matsui and Nabhitabhata, 2006; Matsui, 2001; Pansook et al., 2012). This study aims to compare the differences in morphological and genetic variation among *Ingerophrynus parvus* populations in southern Thailand by using external morphology comparison, morphometrics and molecular analysis (Polymerase chain reaction: PCR).

1.2. Research question

Do isolated mountain ranges effect to morphological difference and genetic variation of *Ingerophrynus parvus* in southern Thailand?

1.3. Objective

To compare the morphological difference and genetic variation of *Ingerophrynus parvus* in southern Thailand.

CHAPTER II

LITERATURE REVIEW

2.1. Southern Thailand geography

Southern Thailand place in the tropical zone of Southeast Asia region, the western coast face the Andaman Sea, the eastern coast is adjacent to Gulf of Thailand, and the southern part connects with Malaysia’s border. This area is influenced by monsoon climates which are the southwest monsoon and the northeast monsoon that cause rain throughout a year. Combining with the unique topography that divided by barriers such as mountain ranges and rivers. These barriers are supporting factors driving high species diversity of amphibians (Guarnizo, 2014). In southern Thailand, there are two main mountain ranges that place in the north-south direction. The Tenasserim Mountain Range runs through the western part of Thailand to the northern part of southern Thailand. The Nakhon Si Thammarat Moutain Range lies down along the length at the middle part of southern Thailand. Moreover, there is Sankarakiri Mountain Range that located at the border between Thailand and Malaysia countries as Thai-Malay boundaries. These 3 mountain ranges are completely isolated, and act as barriers for migratory animals especially in amphibians that have low distribution capability (Blaustein, 1994).

The two transition zones often used for explaining the limitation of distribution limited of plants and animals in southern Thailand. There are the Isthmus of Kra and the Kangar-Pattani line (Woodruff, 2003; Baltzer et al, 2007). The Isthmus of Kra is at the north end area of southern Thailand that separate two different climate zones. The upper part of the Isthmus of Kra is seasonal evergreen tropical forest, and the lower area is unseasonal evergreen tropical forest (Ashton, 1995). The second transition zone is more southward called Kangar-Pattani line (KPL), this area is dramatically changed in plants species between upper and lower areas of this line (Baltzer et al., 2007). The Kangar-Pattani line is located between Indo-Burma and Sundaland, this line is drawn from Kangar (west coast of Malaysia) to Pattani Province (southern Thailand) at about 7ºN (Whitmore, 1984). This area shows the difference plant composition between upper part and lower part of the line based on climate and soil types. They are three main plant groups occurring in this area; a group of plant i.e., *Parashorea stellata* Kurz (Dipterocarpaceae), *Quercus semiserrata* Roxb. (Fagaceae) which can be found only at the upper part of the line, some plant species in family Dipterocarpaceae (*Shorea lepidota* (Korth) Blume, *Shorea macroptera* Dyer) that dominant below Kangar-Pattani line, and the group of plants that take place along both upper and lower of the line such as *Irvingia malayana* Oliv. ex. Benth, *Millettia atropurpurea* (Wall.) Benth. and *Shorea guiso* (Blanco) Blume. The differences of plant communities usually have an influence on the variable of animal species composition and distribution as are apparent on birds, reptiles and amphibians distribution range (Inger, 1966; Hughes et al., 2003; Woodruff, 2003; Baltzer et al., 2008).

Amphibians have been known as poor disperser, isolated of mountain ranges have more effective to amphibian's distribution comparing with other animals. In Thailand, the taxonomic study of genus *Amolops* species confirmed that the *Amolops* population from Tenasserim Moutain Range is *Amolops panhaii*. Meanwhile, the southern peninsular population was described as *Amolops larutensis* base on the distinct characteristics and localities (Matsui and Nabhitabhata, 2006). Furthermore, the study of genus *Ansonia* in Thai-Malay peninsula showed distribution of three *Ansonia* species are followed isolated mountain ranges (Wood, et. al., 2008). *Ansonia kraensis* has been found at Tenasserim Moutain range, *Ansonia siamensis* has been found in the Nakhon Si Thammarat Mountain Range, and *Ansonia malayana* has been found in Sankarakiri Mountain Range. Moreover, the molecular study of genus *Hoplobatrachus* in Thailand shows similar in result that southern peninsular population grouped separated from the northern peninsular populations (Pansook et. al., 2012).

2.2. Taxonomic part

2.2.1. Genus *Ingerophrynus* history

Genus *Ingerophrynus* is fairly new members of family Bofonidae (Frost et al., 2006), this genus was concealed in genus *Bufo* (Boulenger, 1887) by the general warty on dorsal side, a pair of parotid glands and lacking of webs on forelimbs. Later, small bufonid toads were suggested to separate into new group called "*Bufo biporcatus* group", including *Bufo biporcatus, Bufo claviger, Bufo divergens, Bufo parvus, Bufo philippinicus and Bufo quadriporcatus* (Inger, 1972). From the phylogenetic study of family Bufonidae in Southeast Asia region, they suggested to describe small bufonid toads as the new genus called *Ingerophrynus* (Frost et. al., 2006), and they presumed that this new genus consists of *Bufo biporcatus* group plus 3 more species which are *Ingerophrynus celebensis* (Günther, 1859), *Ingerophrynus galeatus* (Günther, 1864), *Ingerophrynus macrotis* (Boulenger, 1887). Later, the *Ingerophrynus kumquat* (Das and Lim, 2001) and *Ingerophrynus gollum* (Grismer, 2007) were added into genus *Ingerophrynus*. Recently, there are 11 species members in this group base on IUCN data (IUCN red list, 2017). The genus name is named after Robert F. Inger, and the word "Phrynos" means "Toads" in Greek language. The distribution range of genus *Ingerophrynus* are only in Southeast Asia region; from Southern Myanmar to Northern, Eastern, and Southern of Thailand through Malay-peninsular and Sumatra (Taylor, 1962).

2.2.2. Distribution of *Ingerophrynus* group in Thailand

In Thailand, recently there are recently reported two species of *Ingerophrynus* group (IUCN red list, 2017) which are *Ingerophrynus parvus* and *Ingerophrynus macrotis*. However, this study mainly focus on *Ingerophrynus parvus* which is widely distributed in southern Thailand.

2.2.2.1. *Ingerophrynus parvus* (Boulenger, 1887)

Figure 2.1. Photograph of *Ingerophrynus parvus* (Boulenger, 1887) from Than Bok Khorani National Park, Krabi Province.

From the report of IUCN (2014), *Ingerophrynus parvus* has scientific classification as below

Kingdom: Animalia

Phylum: Chordata

Subphylum: Vertebrata

Class: Amphibia

Order: Anura

Family: Bufonidae

Genus: *Ingerophrynus*

Species: *Ingerophrynus parvus*

Description of *Ingerophrynus parvus*

In 1887, Boulenger described *Bufo parvus* as a new species, and reported diagnostic characters of this species (Boulenger, 1887; Boulenger, 1912; van Kampen, 1923). A pair of ridges present on the frontal area of head, blunt and short snout projected beyond mouth, nostril position slightly in the front, the width between eyes equal or little wider than eye diameter, circle tympanum present clearly close to eye with 2/3 to 3/4 of eyelid width, 1 finger of forelimbs is longer than 2 finger, but shorter than 4 finger, 3 toe shorter than 5 toe, webbed between fingers has 1/2 long of finger's length, ventral side of metacarpal and metatarsal present of subarticular tubercles between phalanges, metatarsal present of two big subarticular tubacles with no folded, tarso-metatarsal joint is between eye and tip of nose or a bit toward when fold toward head, rough skin with warts or spiny warts, oval or triangle shape of parotid gland, size of parotid gland equal with tympanum, color of dorsal side is brown with dark spots and maybe with pink scatter dots, forelimbs and hindlimbs with dark cross bands, ventral tubercles are circle and varies in sizes, vocal sac present in male with mottled brown color (Boulenger, 1887; Boulenger, 1912; van Kampen, 1923,).

Distribution of *Ingerophrynus parvus* in Thailand

Ingerophrynus parvus is distributed in Southeast Asia region, from south of Burmar through south of Thailand to Malaysia and Sumatra (Frost, 2014). In Thailand, there are distribution reports in many provinces; Si Sa Ket, Tak, Kanchanaburi, Uthai Thani, Ratchaburi, Phetchaburi, Chachoengsao, Chon Buri, Prachuap Khiri Khan, Chumphon, Ranong, Krabi, Trang, Satun, Surat Thani, Nakhon Si Thammarat, Songkhla, Yala and Narathiwat (Chuaynkhern and Chuaynkhern, 2012) (Figure 2.2).

Figure 2.2. Distribution area of *Ingerophrynus parvus* in south of Burmar, Cambodia, south of Thailand to Malaysia and Sumatra, Indonesia.
(Sited: IUCN SSC Amphibian Specialist Group 2014)

Habitat and ecology

Ingerophrynus parvus is distributed in lowland areas up to 520 meters above sea levels (Frost, 2014), and it can be found on forest floor, temporary pools close to streams, or under twigs and leaves on the ground (Boulenger, 1887).

Breeding period and breeding site of *Ingerophrynus parvus*

Shahriza (2012) studied breeding activities of *Ingerophrynus parvus* in Keddar, Peninsular, Malaysia for 12 months. The results showed rainfall relates calling activity of *Ingerophrynus parvus*, and also eggs clutches and amplexus activities. From this work, this toad produce calls almost every month excepted for March and February, and had high activities in high rainfall months (August and October). For the breeding site, this toad lays eggs in water (aquatic oviposition), but does not lay eggs in streams because the current is too strong and wash eggs away. This toad breeds in temporary puddles that close to streams (Taylor, 1962), swampy areas (Arak, 1984), and pools alongside of small streams (Boulenger, 1887; Inger et al., 1974).

Other reports related to *Ingerophrynus parvus*

Inger (1996) published key to amphibian species in Borneo. For family Bufonidae, he wrote key to genera of family Bufonidae and key to bornean species of all genera in Borneo (genus *Bufo*, genus *Pedostibes*, genus *Pseudobufo*, genus *Ansonia*, genus *Phrylophryne* and genus *Cacophryne*), but in this review focus only on key to bornean species in genus *Bufo*. The main characteristics of genus *Bufo* are stocky body with numerous of warts on its body, no web on forelimbs, tip of fingers rounded, distinct parotid glands behind eyes, terrestrial amphibian, and laying eggs in standing or slow moving water in breeding period. For key to species of genus *bufo*, there is one distinct character that can separated *Bufo biporcatus* group out of other species members (*Bufo menlanostictus*, *Bufo jaxtaster* and *Bufo asper*) which is a pair of ridges between eyes that run to the back around parietal area (Figure 2.3A). Among *Bufo biporcatus* group of the Malay Peninsula, he suggested that *Bufo parvus* should be a subspecies of *Bufo biporcatus* group, and differs from *Bufo biporcatus* and *Bufo divergens* with oval shape of parotid glands. Additionally, he also suggested about the specimens of *Bufo divergens* that reported from Malaya are *Bufo parvus*. Moreover, He also wrote key of *Bufo biporcatus* group that *Bufo parvus* is absent of continuous warts from parotid glands unlike *Bufo divergens* (Figure 2.3C), and cranial crests are not thickened at the end (Figure 2.3B).

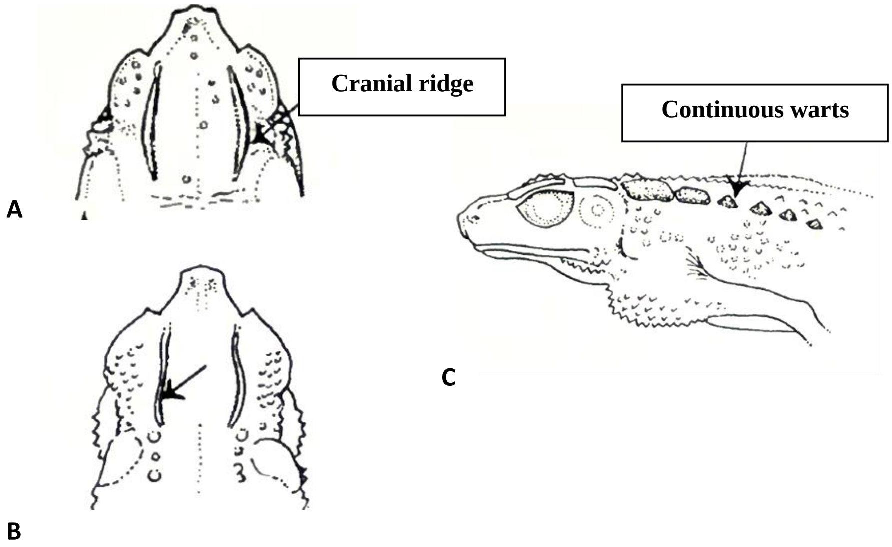

Figure 2.3. The distinct characteristics of *bufo biporcatus* group, cranial ridges present (A), the end of ridge is not thickened in *I. parvus* (B) and Continuous warts on the flanks in *I. divergens* (C) (Inger, 1996)

Grismer (2007) described new species member of genus *Ingerophrynus* which is *Ingerophrynus gollum*. In this work, there was morphological comparison of *Ingerophrynus gollum* with other species members included *Ingerophrynus parvus*. This species has distinct characteristics from others with the slender body, smaller size, absent of dark band across between eyelids, parasphenoid without ridge, long shape of parotid glands, the end of parotid gland pointed shape, ratio of tibia length with body length has longer length when compare with other species in this group, and 1 finger of metacarpal is longer than 2 finger when compare with all members. From the comparison between *Ingerophrynus gollum* and *Ingerophrynus parvus*, *Ingerophrynus parvus* does not has lines of warts continued from parotid gland, and subarticular tubacles are not enlarged as *Ingerophrynus gollum*. Body length of male *Ingerophrynus parvus* is 28.9-37.3 mm, and female 27.0-45.5 mm.

2.2.3. Sexual size dimorphism in frogs

Sexual size dimorphism (SSD) is the difference in morphology between adult males and females within species which is mostly present in animals. There are both male-biased SSD (male’s bigger size than female; birds, mammals) and female-biased SSD (female’s bigger size than male; invertebrate, frogs, snakes) (Nali et al., 2014). For frogs, more than 90 percent are female-biased SSD (Shine, 1979), but male-biased also present in some species. The size biased of male or female within species base on function of use in each sex. Male has bigger size for advantages of high productivity, courtship, male-male competition, and for the benefits of female is higher rate of fecundity.

CHAPTER III

MATERIALS AND METHODS

3.1. Study sites

The field surveys were conducted at eight localities base on previous publications (Taylor, 1962; Konsue and Thirakhupt, 2001; Chan-ard et al, 2003; Chuaynkern and Chuaynkern, 2012), i.e., Ngao waterfall national park, Ranong Province; Than Bok Khorani National Park, Krabi Province; Khao Phanom Bencha national park, Krabi Province; Kaeng Krung National Park, Surat Thani Province; Yong Waterfall National Park, Nakon Si Thammarat Province; Khao Chong Wildlife Development and Conservation Promotion Station, Trang Province; Thung Khai botanic garden, Trang Province; Thale Ban National Park, Satun Province; protected area, Kho Hong hill, Prince of Songkla University, Songkhla Province and Hala-Bala Wildlife Sanctuary, Narathiwat Province. Additionally, specimens from Tarutao Island, and specimens from Princess Maha Chakri Sirindhorn Natural History Museum, Prince of Songkla University were also examined (Figure 3.1).

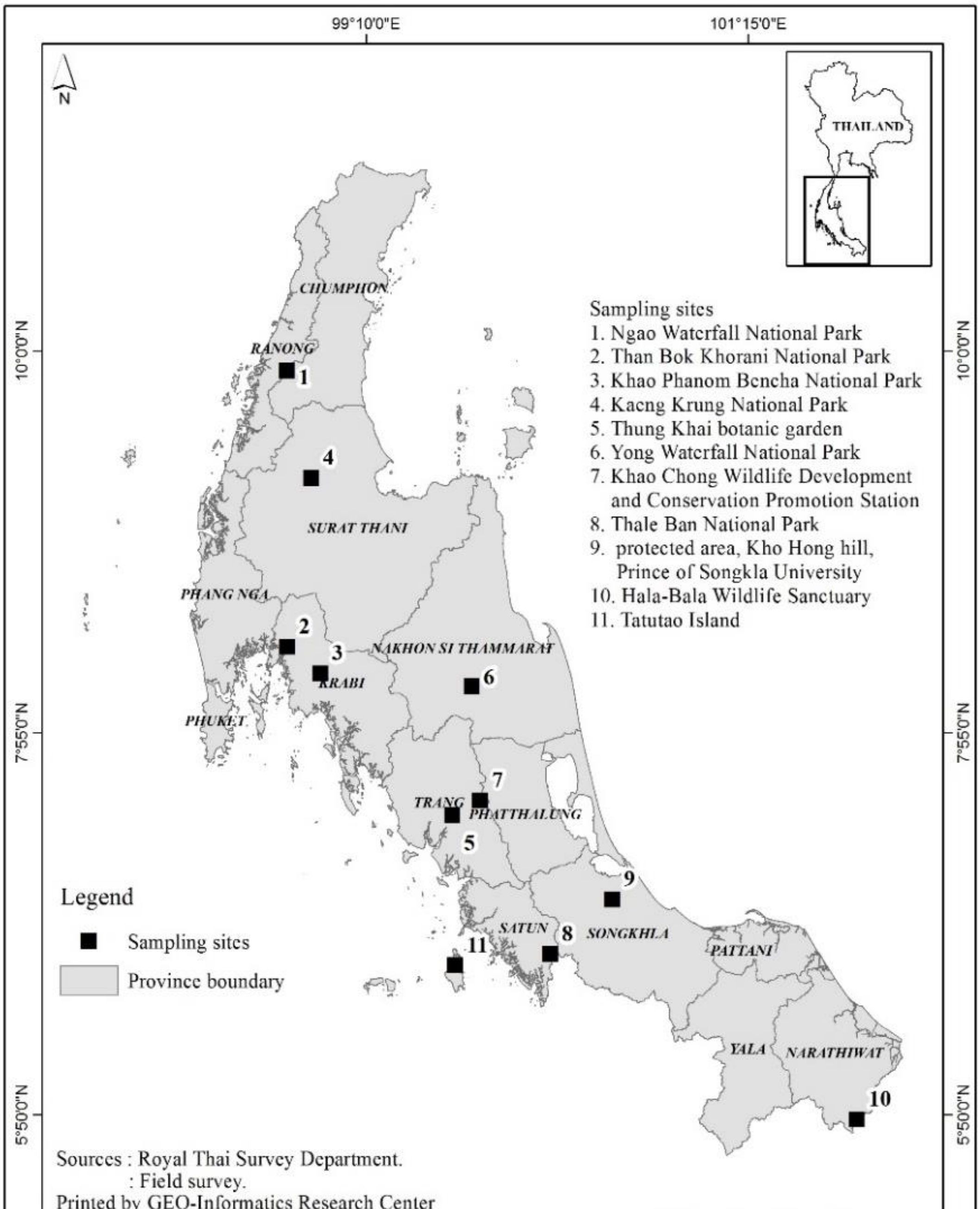

Figure 3.1. Map showing study sites in southern Thailand

3.2. Field Surveys

The field surveys were conducted using line transects method by searching samples along the streams at night time, and specimens were caught by hand capture (Heyer at al., 1994). When samples were found, temperature, humidity and environment around the samples were recorded. After samples were taken from the surveys, the preservation method was done in the field or in the laboratory. Specimens were fixed in 95% ethanol, and preserved in 70% ethanol. Liver tissues were preserved in 95% ethanol. Then, all specimens were labeled with specimen's tag number, and deposited at Princess Maha Chakri Sirindhorn Natural History Museum, Songkla, Thailand.

3.3. Morphological study

3.3.1. External morphology

External morphology was examined as qualitative study. The fifteen external characters were examined as shown below, and some characters such as curved nose (C1), spiny warts present above parotid gland (C5) and warts present on ventral site of forearms (C11) were investigated with JSZ-6 stereo microscope.

1. Curved nose (C1)
2. Pair of ridges present clearly on its head (C2)
3. The end of ridges curved (C3)
4. Oval tympanums (C4)
5. Spiny warts above parotid glands (C5)
6. Distinct tubercle present behind parotid glands (C6)
7. Black and white strips around mouth (C7)
8. White ventral (C8)
9. Pair of wards present in the middle of back (C9)
10. Forelimbs with dark crossed bands (C10)
11. Warts present on ventral side of forearms (C11)
12. Outer metacarpal tubercle bigger than inner metacarpal tubercle (C12)
13. Hindlimbs with dark crossed bands (C13)
14. Tip of tibiofibular reach tip of snout when fold leg toward head (14)
15. Outer metatarsal tubercle bigger than inner metatarsal tubercle (C15)

The data were recorded as “1” for present and “0” for absent. The chi-squared test of independence of categorical variables (Crosstab method) was computed in SPSS program version 16.0.

3.3.2. Morphometrics

Eighteen morphometric characters were measured to the nearest 0.02 mm using Mitutoyo Vernier Caliper (300 mm) model 530-312. The definition of morphometric characters are as follow.

1. Snout to vent length (SVL: length from tip of snout to vent)
2. Internarial length (IN: length between nostrils)
3. Snout length (SNL: length from tip of snout to the beginning of an eye)
4. Snout width (SW: The widest range of snout)
5. Inter-jaws width (IJW: The length between jaws)
6. Head width (HW: The widest part of head)
7. Head length (HL: The length from tip of snout to the end of tympanum)
8. Head depth (HD: The depth from canthus to lower jaw)
9. Eye diameter (ED: The horizontal length of eye)
10. Eye width (EW: The vertical length of eye)
11. Inter-orbital length (IO: The length between eyelids)
12. Length between ridges (LBR: The widest length between ridges)
13. Ridge length (RL: The length of ridge)
14. Parotid gland width (PGW: The widest part of parotid gland)
15. Parotid gland length (PGL: The length of parotid gland)
16. Hand length (HDL: The length from the tip of 3 finger to the end of metacarpal)
17. Tibia length (TB: The length of tibia)
18. Foot length (FL: The length from tip of third toe to the end of metatarsal)

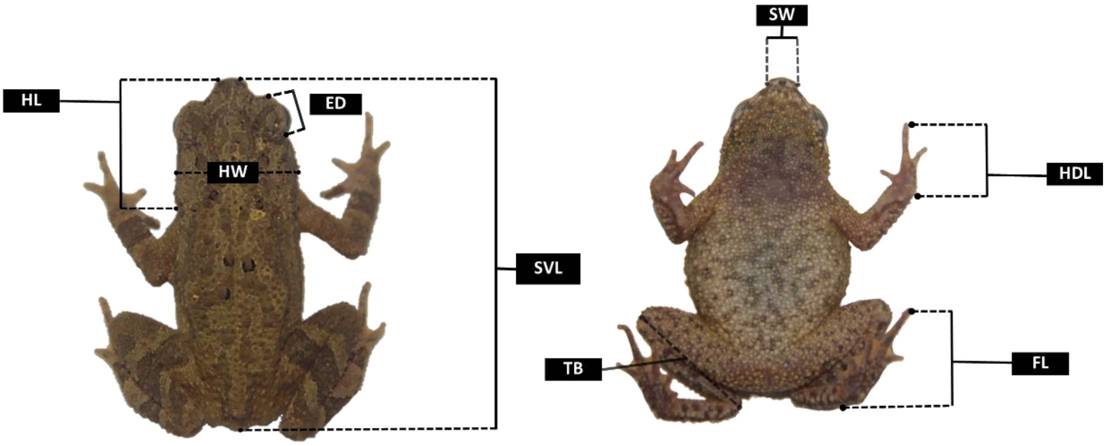

Figure 3.2. Morphometric measurement characters of *Ingerophrynus parvus* specimens **(**SVL, Snout to vent length; HW, Head width; HL, Head length; ED, Eye diameter; HDL, Hand length; TB, Tibia length and FL, Foot length)**.**

Log transformation of measurement characters were computed in all morphometric characters to avoid distortion of measurement data, and sexual size dimorphism (SSD) were investigated. The independent sample T-Test were used for morphometrics comparison of sexual size dimorphism, and sexual size dimorphism index (SDI) (Lovich and Gibbons, 1992) was also used to calculate size different between males and females with the following equation:

$$\text{SDI} = \left[\frac{\text{Mean body size of larger sex}}{\text{Mean body size of smaller sex}}\right] -1$$

If SDI ratio value show positive value, female has larger size than male. In the opposite, if SDI ratio value is negative value, male will have larger size than female.

Allometry and ANOVA analysis

Morphometric characters were transformed with logarithm ($\log_{10}$), and plotted with scatter plot to examined allometry or relative growth correlation between characters and body length. All characters were transformed as ratio data with snout to vent length (SVL) to prevent bias from size variation. The One-way-ANOVA test was used to test morphological differences among localities ($p<0.05$). The relationship among *Ingerophrynus parvus* populations were investigated using hierarchical cluster analysis in SPSS program version 16.0.

3.4. Molecular study

3.4.1 DNA extraction and Polymerase chain reaction (PCR) method

Genomic DNA was extracted using analytik Jena DNA extraction kits by following protocol instructions. A fragment of 16S rRNA was amplified by Polymerase Chain Reaction (PCR) using two primers, i.e. 16Sar, 16Sbr (Palumbi, 1996). The reaction solutions (50 ul) consist of 10x PCR buffer 5 ul, 50 mM MgCl 1.5 ul, 10 mM dNTP 1 ul, 10 ul of primer 1 (16 Sar 5'-CGCCTGTTTATCAAAAACAT-3') 2.5 ul, 10 ul of primer 2 (16 Sbr 5'-CCGGTCTGAACTCAGATCACGT-3') 2.5 ul, Tag DNA polymerase 0.5 ul, and DNA sample 2 ul. For PCR condition, initial denaturation is at 94° for 5 minutes, 35 cycles of annealing at 94° for 30 seconds, 50° for 30 seconds and 72° for 1 minute, and final extension at and 72° for 5 minutes.

3.4.2. Gel electrophoresis and band checking

PCR products were checked amount of mitochondrial DNA with Benchtop UV Transilluminator, UVP following these steps. Agarose gel was prepare from 0.4 grams molecular grade agar with 40 ml TBE Buffer (Tris-borate-EDTA, (1X)). Each DNA sample (4 ul) is mixed with 2 ul of Novel juice DNA strain. Each mixed samples in each band, wait 30-35 minutes (135 Volt).

3.4.3. Sequence analysis

DNA sequences were rechecked with online access GenBank (http://www.ncbi.nlm.nih.gov/Genbank/index.html), edited using Chromas version 2.6.2 and Gene Doc program version 2.7. 2 Sequences (forward, reward) of each sample were combined with GeneDoc program version 2.7.2, and aligned with Mafft program (Kuraku et al., 2013; Katoh et al., 2017). Aligned sequences were removed gaps and rechecked, and checked stop codon with Bioedit program version 7.2.5. DNA tree was built with PhyML program (Guindon & Gascuel., 2003) with the substitution model GTR + I, branch support with bootstrap 1000 replications. Tree was visualized and built with FigTree program version 1.4.3. Haplotype network was built with TCS program version 1.21 and popArt program version 1.7 (Clement et al., 2002).

CHAPTER IV

RESULTS

4.1. Microhabitat of *Ingerophrynus parvus*

Ingerophrynus parvus were found in small streams and swampy areas, but didn’t occurs in the wide and rapid flowing streams. In Ngao Waterfall National Park, the toads were found sitting on leaf litter in the swamp area in March in mating period (Figure 4.1A). The swamp area is at about 56 meters above sea level with shallow waterlogged, covered by herbaceous plants and small trees. In Than Bok Khorani National Park (Figure 4.1B), there were the narrow channels around 0.5 meter width with shallow and slow running water, there were shrubs and small trees occurred nearby. Behind this channel was a big swampy area that connected with the small stream. In this area, toads were found sitting close to the channel and on the timber at altitude around 32 meters above sea level. Moreover, the slow moving water streams with puddle areas along the streams were also suitable for the toad in Khao Phanom Bencha National Park, Khao Chong Wildlife Development and Conservation Promotion Station and Protected area, Kho Hong hill (Figure 4.1C-E). The toads were found sitting along sides of the streams on leaf litter or rocks. Meanwhile, there is strong water current in Hala-Bala wildlife Sanctuary (Figure 4.1F), however, this toad was found in puddle areas not far from the big stream.

Figure 4.1. Microhabitats of *Ingerophrynus parvus* in southern Thailand; (A) Ngao Waterfall National Park, (B) Than Bok Khorani National Park, (C) Khao Phanom Bencha National Park, (D) Khao Chong Wildlife Development and Conservation Promotion Station, (E) Protected area, Kho Hong hill, Prince of Songkla University and (F) Hala-Bala Wildlife Sanctuary.

4.2. Morphological study

Morphological variation were found among study localities, eight characters show non-significant different (Figure 4.2). However, seven from 15 characters showed significant differences by chi-squared test with crosstab method which are curved nose (λ=0.146, p=0.000), pair of ridges present clearly (λ=0.000, p=0.003), the end of ridge curved (λ=0.078, p=0.000), spiny warts above parotid glands (λ=0.039, p=0.000), white ventral (λ=0.087, p=0.000), spiny warts under forearms (λ=0.097, p=0.000), and ankle reach tip of snout (λ=0.000, p=0.000).

Figure 4.2. Occurrence percentage of eight non-significant observed characters among 10 study localities, pale color (■) in column means absence and dark color (■) means presence.

Curved nose (C1)

This character is mostly present in the mainland, all samples from Ngao Waterfall National Park (NG) have curved nose character (100%), the occurrence decrease in Khao Chong Wildlife Development and Conservation Promotion Station to 94.40% present, 92.30% present in Hala-Bala Wildlife Sanctuary, 72.2% present in Thung Khai botanic garden, 71.4% present in Yong Waterfall National Park, 66.7% present in Thale Ban National Park, 55.6% present in Than Bok Khorani National Park, 28.6% present in Khao Phanom Bencha National Park, and especially in protected area, Kho Hong Hill that the occurrence present only 3.2%. In the opposite way from the mainland, samples from Tarutao Island lack of curved nose character (Figure 4.3).

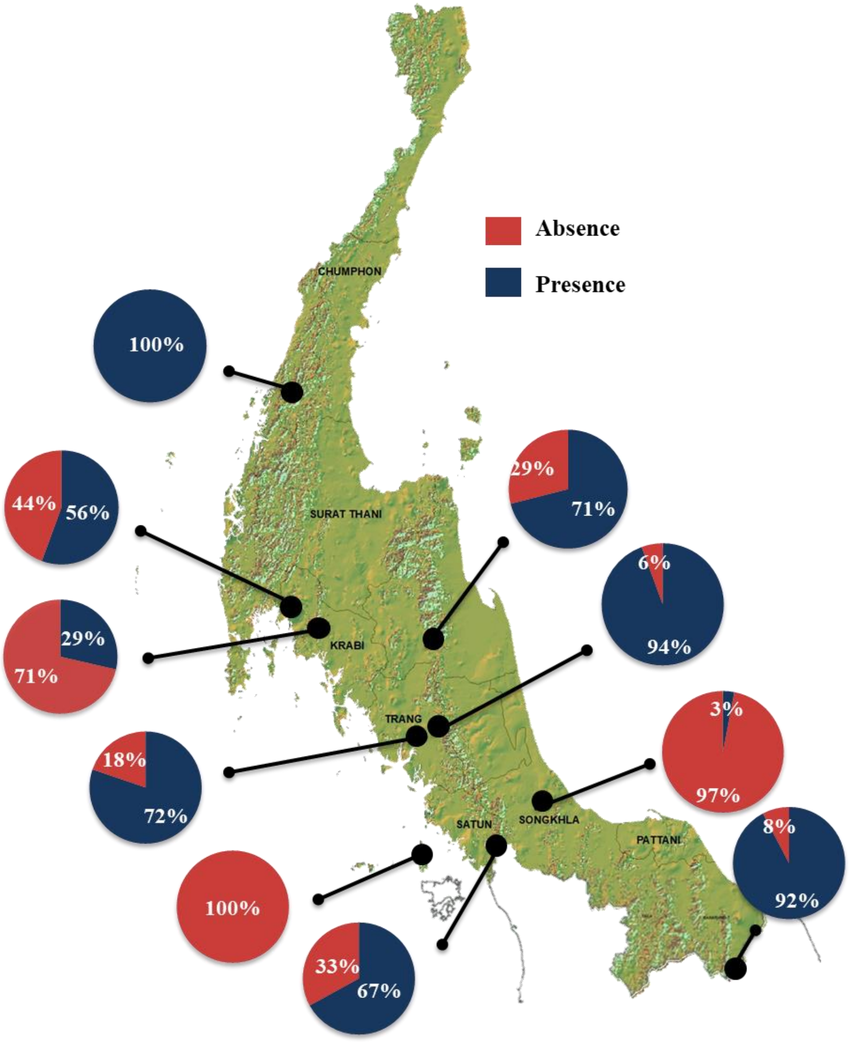

Figure 4.3. Occurrence Percentage of curved nose (C1) character among 10 study localities, blue square (🟦) represent presence in character, and red square (🟥) represent presence in character.

Pair of ridges present clearly (C2)

There are 100% present in pair of ridges character at Ngao Waterfall National Park, Than Bok Khorani National Park, Yong Waterfall National Park, Thung Khai botanic garden, and Khao Chong Wildlife Development and Conservation Promotion Station. The 92.3% present in Hala-Bala Wildlife Sanctuary, following by 91.3% present in Thale Ban National Park, 85.7% present in Khao Phanom Bencha National Park, and 77.8% present in protected area, Kho Hong Hill. There are at least around 80% of samples from the mainland have clearly see the ridges on frontal head, but only 50% of the samples from Tarutao Island can clearly see ridges on frontal area (Figure 4.4).

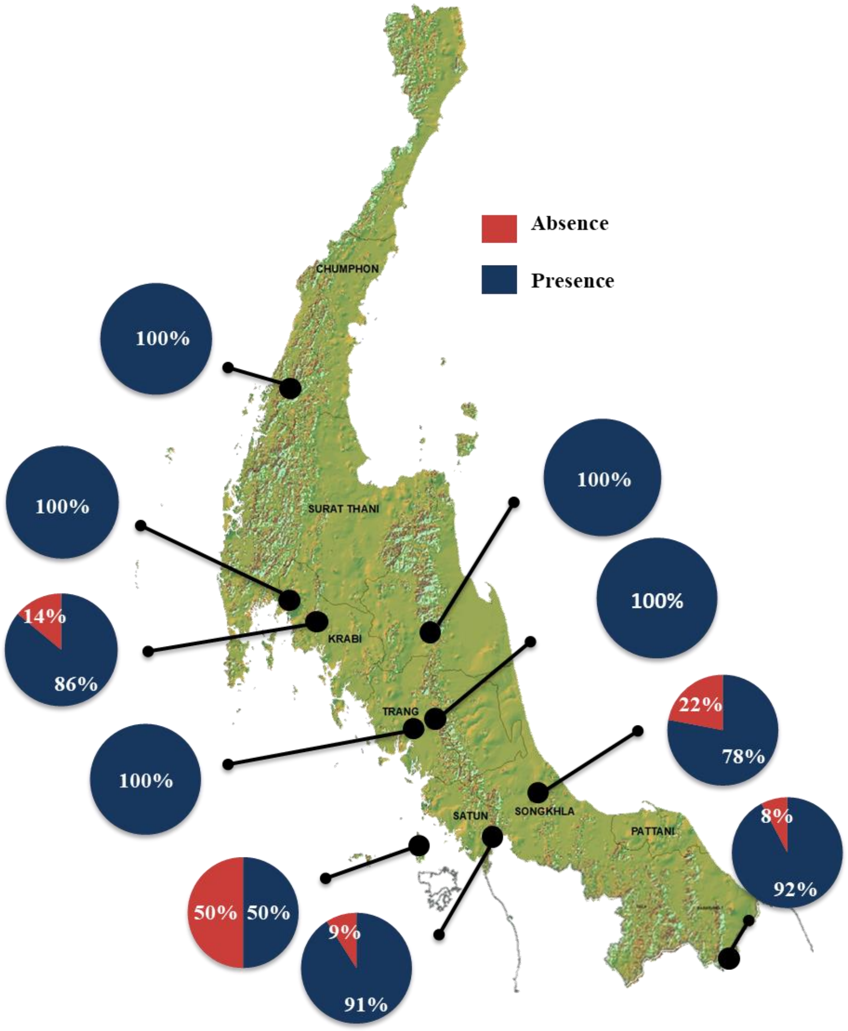

Figure 4.4. Occurrence Percentage of pair of ridges present clearly (C2) character among 10 study localities, blue square (🟦) represent presence in character, and red square (🟥) represent absence in character.

The end of ridges curved (C3)

There are 100% present of curved ridges character in Ngao Waterfall National Park, Than Bok Khorani National Park, and Khao Chong Wildlife Development and Conservation Promotion Station. The 87.5% present in Tarutao National Park, 75% present in Thale Ban National Park, 71.4% present in Khao Phanom Bencha National Park and Yong waterfall National Park, 61.1% present in Thung Khai botanic garden, and 53.8% present in Hala-Bala Wildlife Sanctuary respectively. There are mostly more than 50% of samples have curved ridges characters both in mainland and the island except for the protected area, Kho Hong Hill that has 15.9% present of the end of ridges curved character (Figure 4.5).

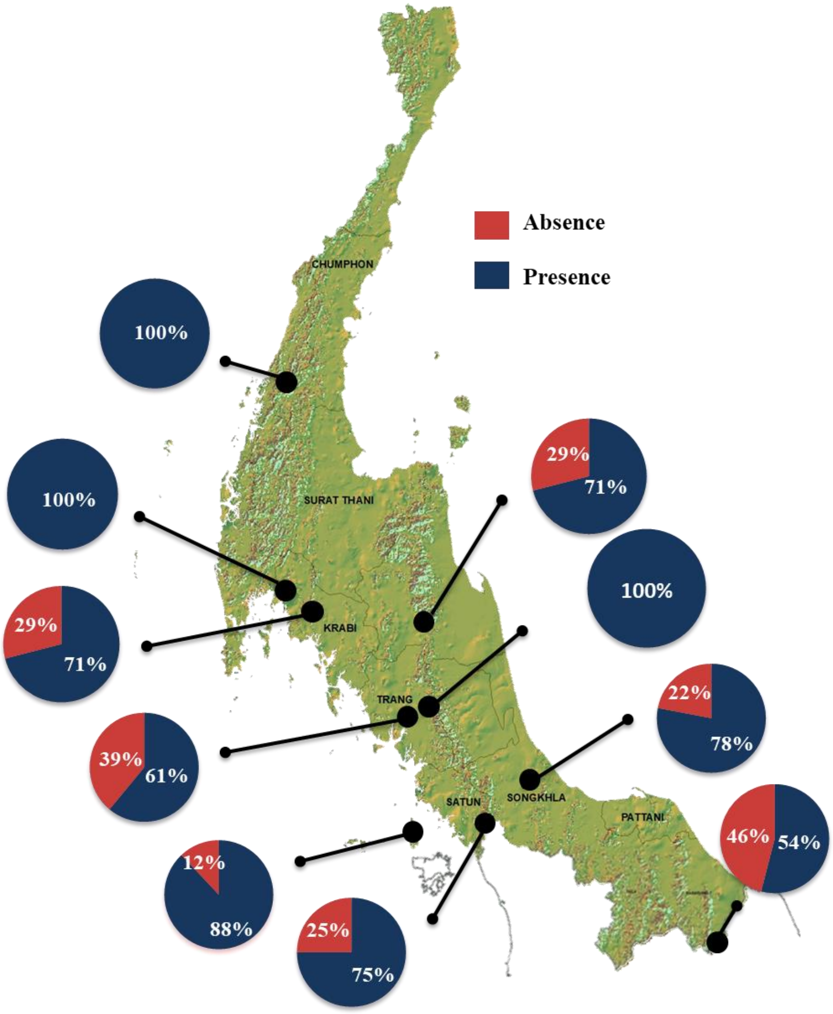

Figure 4.5. Occurrence Percentage of the end of ridges curved (C3) character among 10 study localities, blue square (🟦) represent presence in character, and red square (🟥) represent absence in character.
]

Spiny warts above parotid glands (C5)

There is 100% present of spinose tubercles above parotid glands in the Tarutao Island. In the mainland, meanwhile, there are 88.9% present in Khao Chong Wildlife Development and Conservation Promotion Station, 61.5% present in Hala-Bala Wildlife Sanctuary, 55.6% present in Thung Khai botanic garden. Less than 50% present of spiny warts above parotid glands character in Than Bok Khorani National Park (44.4%), Khao Phanom Bencha National Park (28.6%), protected area, Kho Hong Hill (19%), Thale Ban National Park (16.7%), Yong Waterfall National Park (14.30%), and the less percent present in this character from Ngao Waterfall National Park (9.1%) (Figure 4.6).

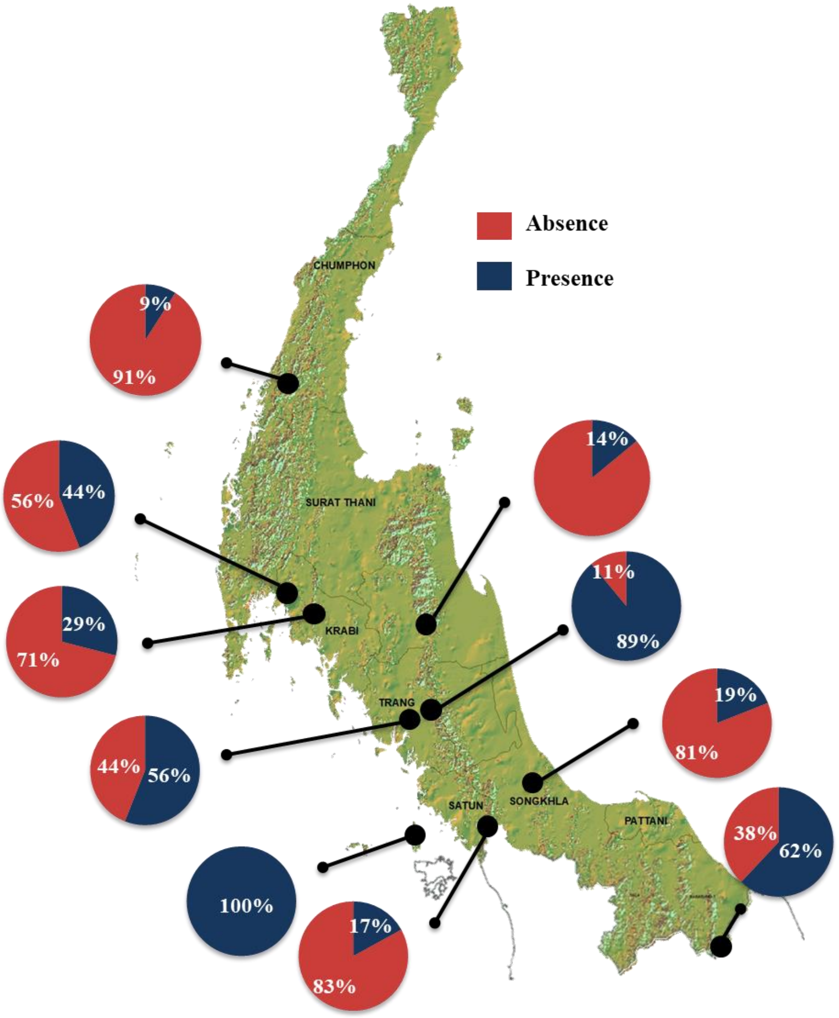

Figure 4.6. Occurrence Percentage of spiny warts above parotid glands (C5) character among 10 study localities, blue square (■) represent presence in character, and red square (■) represent absence in character.

White ventral (C8)

The present of white ventral character shows highest percentage in Khao Chong Wildlife Development and Conservation Promotion Station at 94.4%, follow with 75% present in Thung Khai botanic garden, 75% present in Tarutao National Park, 71.4% present in Yong Waterfall National Park, and 55.60% present in Than Bok Khorani National Park respectively. Although, there are less than half of samples have white ventral character in Hala-bala Wildlife Santuary (46.2%), Khao Phanom Bencha National Park (42.9%), Thale Ban National Park (25%), protected area, Kho Hong Hill (12.7%) and Ngao Waterfall National Park (9.1%) respectively that they have dark mottle dots pattern on ventral side instead of plain white color on ventral area (Figure 4.7).

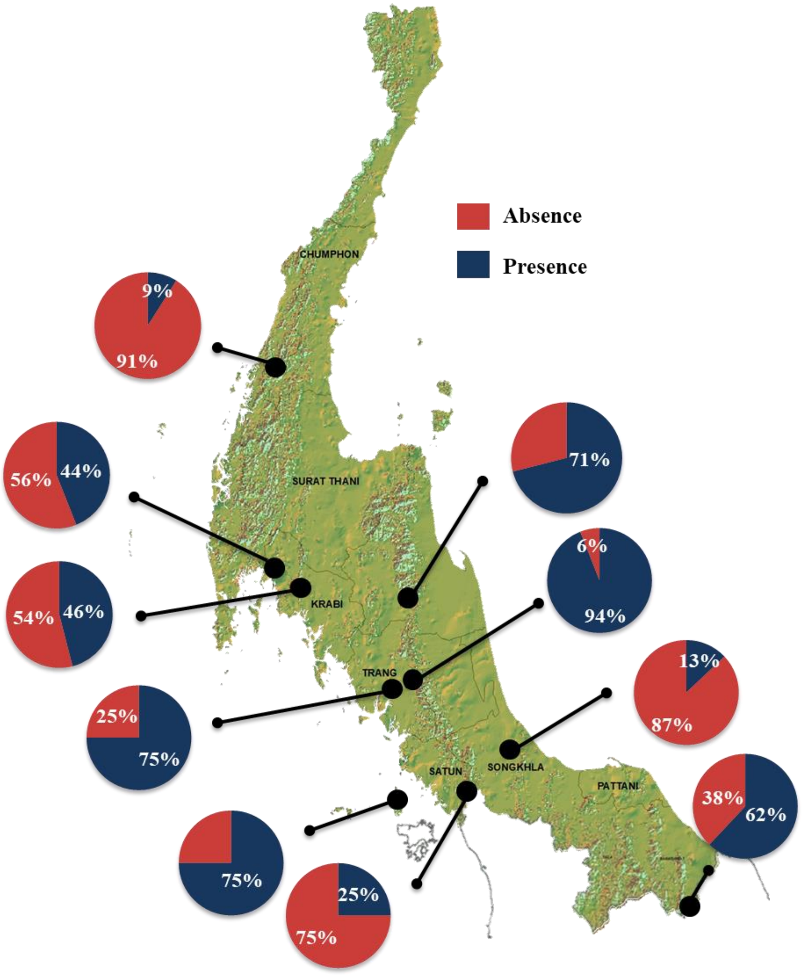

Figure 4.7. Occurrence Percentage of white ventral (C8) character among 10 study localities, blue square (■) represent presence in character, and red square (■) represent absence in character.

Spiny warts under forearms (C11)

In the mainland, mostly the specimens don’t have spinose warts under forelimbs, there are 28.6% present in Khao Phanom Bencha National Park, 23.1% present in Hala-Bala Wildlife Sanctuary, 22.2% present in Than Bok Khorani National Park, 14.3% present in Yong Waterfall National Park, 11.1% present in Thung Khai botanic garden, 8.30% present in Thale Ban National Park, and 3.2% present in protected area, Kho Hong Hill respectively. Excluding the occurrence from Khao Chong Wildlife Development and Conservation Promotion Station samples that present 72.2% in this character. In Tarutao Island, there are 100% present of spiny warts under forearms character (Figure 4.8).

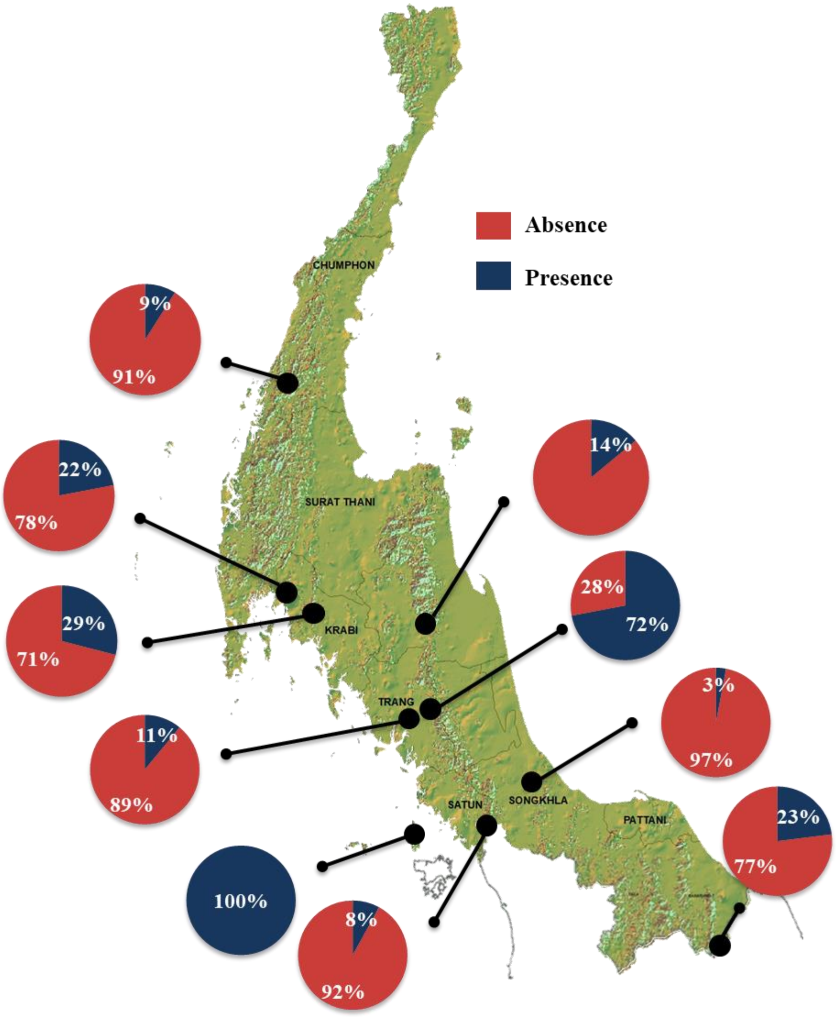

Figure 4.8. Occurrence Percentage of spiny warts under forearms (C11) character among 10 study localities, blue square (■) represent presence in character, and red square (■) represent absence in character.

Tip of tibiofibular reach tip of snout when fold leg toward head (C14)

There are 84.6% present of samples from Hala-Bala Wildlife Santuary, 72.2% present in Thung Khai botanic garden, 71.4 % present in Khao Phanom Bencha National Park, and 55.6% present in protected area, Kho Hong Hill that foot can reach tip of snout respectively. Meanwhile, there are less percentage of present character in Khao Chong Wildlife Development and Conservation Promotion Station (27.8%), Thale Ban National Park (25%), Yong Waterfall National Park (14.3%) and Ngao Waterfall National Park (9.1%) that mostly foot doesn't reach tip of snout. Moreover, none of specimens from Than Bok Khorani National Park foot can reach tip of snout (Figure 4.9).

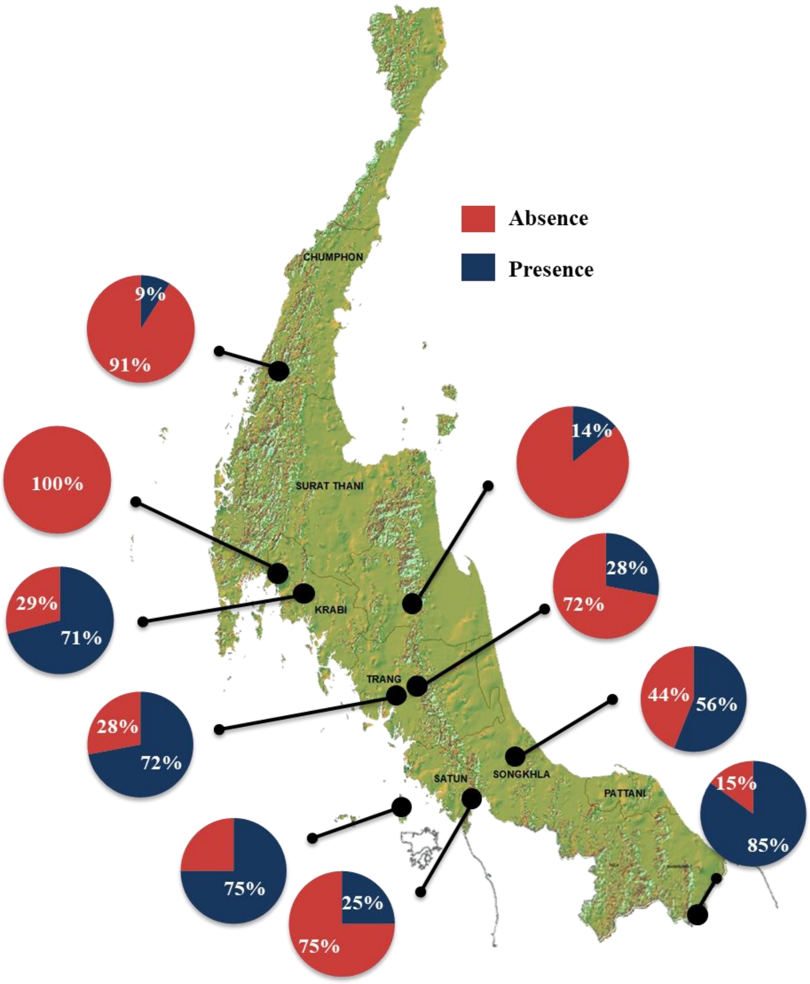

Figure 4.9. Occurrence Percentage of tip of tibiofibular reach tip of snout when fold leg toward head (C14) character among 10 study localities, blue square (■) represent presence in character, and red square (■) represent absence in character.

4.3. External morphology measurements

Morphometric measurements were conducted in 166 adult *Ingerophrynus parvus* (140 males vs 26 females) from 10 localities.

4.3.1. Snout to vent length (SVL)

The snout to vent length (SVL) range of male and female with standard deviation (SD) showed body size different between male and female specimens in all localities (Table 4.1). The SVL range of female has larger size than male ranging from 22.76-48.98 mm. The largest body size was found in population from Khao Chong Wildlife Development and Conservation Promotion Station, and smallest body size was found from Tarutao Island. The rank of male SVL is 21.08-37.60 mm, the largest size was in Khao Chong Wildlife Development and Conservation Promotion Station, and smallest size was in Tarutao Island (Table 4.1)

Table 4.1. Snout to vent length (SVL) range of males and females of *Ingerophrynus parvus* samples with standard deviation (SD) from 10 study localities

Localities	**Male**			**Female**		
	n	Range (mm)	SD	n	Range (mm)	SD
1. Ngao Waterfall National Park	10	31.58-36.42	1.78	1	30.54	-
2. Than Bok Khorani National Park	9	28.40-35.30	2.31	0	-	-
3. Khao Phanom Bencha National Park	7	24.22-37.60	4.88	0	-	-
4. Yong Waterfall National Park	7	29.42-36.66	2.41	0	-	-
5. Thung Khai botanic garden	14	25.90-35.60	2.57	4	28.42-44.02	6.82
6. Khao Chong Wildlife Development and Conservation Promotion Station	8	32.72-36.0	1.05	10	35.24-48.98	4.41
7. Protected area, Kho Hong hill, Prince of Songkla University	59	28.22-34.94	1.55	4	29.22-43.16	6.27
8. Thale Ban National Park	11	30.62-36.90	2.13	0	-	-
9. Hala-Bala Wildlife Sanctuary	10	28.14-37.54	2.69	3	37.32-45.80	4.24
10. Tatutao Island	5	21.08-28.80	3.30	4	22.76-37.64	6.19
Total	140	21.08-37.60	2.86	26	22.76-48.98	7.02

4.3.2. Sexual size dimorphism

The log transformation (log10) of 18 measurement characters were calculated, and independent sample t-test were computed to test the sexual size dimorphism between male and female *Ingerophrynus parvus*. The result from independent sample t-test shows 17 from 18 characters are significant different in mean value between male and female of *Ingerophrynus parvus* specimens except for eye diameter (ED) character. This result exhibit sexual size dimorphism in *Ingerophrynus parvus* that female has significantly larger in measurement characters than male for all significant characters (Table 4.2).

To investigate the body size different between sexes, the sexual size dimorphism index or SDI that proposed by Gibbons and Lovinch (1990) was calculated. The index will be positive value when female has larger size than male, and negative value when male are larger.

$$\text{SDI} = \left[\frac{\text{Mean body size of larger sex}}{\text{Mean body size of smaller sex}}\right] -1$$

$$\text{SDI} = \left[\frac{\text{Mean body size of female}}{\text{Mean body size of male}}\right] -1$$

$$\text{SDI} = \left[\frac{37.9992}{32.2623}\right] -1$$

$$\text{SDI} = 0.178$$

The SSD ratio shows positive value (SDI = 0.178). This result confirm that female *Ingerophrynus parvus* in southern Thailand has larger size than male.

Table 4.2. Independent sample t-test of logarithmic transformed measurement characters with mean, standard deviation (SD), correlation between characters with SVL (r), t values and p values of male and female *Ingerophrynus parvus* samples

Morphological characters	**log Male (n=140)**			**log Female (n=26)**			**t-value**	**p-value**
	Mean	**SD**	**r**	**Mean**	**SD**	**r**		
SVL*	1.51	0.04	-	1.57	0.09	-	-3.78	0.001
IN*	0.40	0.05	0.604	0.45	0.06	0.846	-3.86	0.000
SNL*	0.60	0.04	0.741	0.64	0.07	0.942	-3.44	0.001
SW*	0.67	0.04	0.814	0.72	0.08	0.958	-3.22	0.002
IJW*	1.06	0.05	0.855	1.11	0.09	0.959	-2.68	0.006
HW*	1.01	0.04	0.895	1.06	0.07	0.980	-3.43	0.001
HL*	1.01	0.03	0.866	1.06	0.07	0.926	-3.79	0.001
HD*	0.70	0.04	0.835	0.76	0.07	0.956	-4.08	0.000
ED	0.67	0.05	0.632	0.70	0.07	0.789	-1.53	0.068
EW*	0.59	0.05	0.490	0.61	0.06	0.836	-1.87	0.032
IO*	0.57	0.04	0.608	0.64	0.06	0.860	-5.21	0.000
LBR*	0.62	0.04	0.721	0.69	0.07	0.922	-4.57	0.000
RL*	0.74	0.05	0.663	0.78	0.07	0.862	-2.77	0.005
PGW*	0.35	0.07	0.474	0.42	0.08	0.731	-4.22	0.000
PGL*	0.53	0.07	0.504	0.62	0.09	0.793	-5.44	0.000
HDL*	0.96	0.04	0.734	1.02	0.09	0.959	-3.51	0.001
TB*	1.14	0.08	0.891	1.20	0.09	0.965	-3.20	0.002
FL*	1.08	0.04	0.840	1.14	0.08	0.967	-3.58	0.001

* Significant different characters of logarithmic measurement data between male and female *Ingerophrynus parvus* samples

4.3.3. The allometry in morphometric characters of *Ingerophrynus parvus* populations in southern Thailand

The log transformation was taken for all measurement characters, and linear regression analysis was calculated to compare correlation between morphometric characters with snout to vent length (SVL). Correlation analysis showed all 17 morphometric characters were positive related with the snout to vent length (SVL) as linear ($p<0.05$) for both sexes (Table 4.2). The bigger in body length result to bigger related characters. Additionally, female seems to has more affective in correlation of characters with SVL than male base on the correlation value. Then, to reduce the dependent variable, the morphometrics were calculate as ratio with snout to vent length (SVL) to get rid of body size bias factor. From size variable due to the sex determination, the morphometric study were also separately analyzed to prevent sexual size bias.

4.3.4. Comparison of morphometric characters of male *Ingrophrynus parvus* populations in southern Thailand

Ratio data of 17 morphometric characters to snout to vent length (SVL) were compared using One-way ANOVA analysis (Table 4.3) to compare size difference of morphometric characters among 10 localities (Ngao waterfall national park, Than Bok Khorani National Park, Khao Phanom Bencha national park, Yong Waterfall National Park, Thung Khai botanic garden, Khao Chong Wildlife Development and Conservation Promotion Station, Thale Ban National Park, Protected area, Kho Hong hill, Prince of Songkla University, Hala-Bala Wildlife Sanctuary, and Tarutao Island). Each ratio character was tested normality with Shapiro-Wilk test (Unequal number of samples among localities). Fifteen morphometric characters showed normal distributed data in each study locality ($p>0.05$) with the exception of the ratio of length between ridges (RLBR) that non-normal distribution in protected area, Kho Hong Hill ($p=0.031$) and Ngao Waterfall National Park ($p=0.015$). The ratio of hand length (RHDL) characters is also non-normal distributed in Thung Khai botanic garden ($p=0.019$).

The homogeneity of variances of 15 characters were also calculated with Levene’s statistic test, the variances of 12 morphometric characters were not significant difference except for ratio of snout width (RSW; p=0.008), ratio of eye diameter (RED; 0.005) and ratio of inter-orbital length (RIO; p<0.001). The One-Way ANOVA analysis showed all 15 characters are significant difference among localities (p<0.05) which at least one pair of localities has significant difference in morphometric characters among localities. For the unequal homogeneity of variance characters, Brown-Forsythe test confirmed that ratio of snout width (RSW; p=0.003), ratio of eye diameter (RED; p= 0.020) and ratio of inter-orbital length (RIO; p=0.002) were significant different among localities (p<0.05) respectively.

Table 4.3. One-Way-ANOVA analysis of 17 morphometric characters in male *Ingerophrynus parvus* samples among study localities

Characters		**Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
RIN	Between Groups	0.004	9	0.000	11.164	0.000
	Within Groups	0.005	130	0.000		
	Total	0.009	139			
RSNL	Between Groups	0.002	9	0.000	3.296	0.001
	Within Groups	0.008	130	0.000		
	Total	0.009	139			
RSW	Between Groups	0.002	9	0.000	3.941	0.000
	Within Groups	0.008	130	0.000		
	Total	0.010	139			
RIJW	Between Groups	0.017	9	0.002	6.745	0.000
	Within Groups	0.037	130	0.000		
	Total	0.055	139			
RHW	Between Groups	0.010	9	0.001	8.625	0.000
	Within Groups	0.016	130	0.000		
	Total	0.026	139			
RHL	Between Groups	0.005	9	0.001	2.782	0.005
	Within Groups	0.027	130	0.000		
	Total	0.032	139			
RHD	Between Groups	0.002	9	0.000	4.162	0.000
	Within Groups	0.008	130	0.000		
	Total	0.011	139			
RED	Between Groups	0.004	9	0.000	2.252	0.023
	Within Groups	0.023	130	0.000		
	Total	0.026	139			
REW	Between Groups	0.007	9	0.001	6.012	0.000
	Within Groups	0.017	130	0.000		
	Total	0.025	139			
RIO	Between Groups	0.005	9	0.001	8.176	0.000
	Within Groups	0.010	130	0.000		
	Total	0.015	139			
RLBR	Between Groups	0.005	9	0.001	9.183	0.000
	Within Groups	0.008	130	0.000		
	Total	0.013	139			
RRL	Between Groups	0.006	9	0.001	3.668	0.000
	Within Groups	0.023	130	0.000		
	Total	0.028	139			
RPGW	Between Groups	0.002	9	0.000	2.536	0.010
	Within Groups	0.012	130	0.000		
	Total	0.014	139			
RPGL	Between Groups	0.004	9	0.000	2.144	0.030
	Within Groups	0.027	130	0.000		
	Total	0.031	139			
RHDL	Between Groups	0.036	9	0.004	24.308	0.000
	Within Groups	0.021	130	0.000		
	Total	0.057	139			
RTB	Between Groups	0.022	9	0.002	10.023	0.000
	Within Groups	0.032	130	0.000		
	Total	0.055	139			
RFL	Between Groups	0.020	9	0.002	6.253	0.000
	Within Groups	0.045	130	0.000		
	Total	0.065	139			

The hierarchical cluster analysis with dendrogram was applied to group the morphological relationship among localities of male *Ingerophrynus parvus*. Five significantly difference of morphometric characters ($p<0.01$) from multiple comparison analysis were chose for cluster analysis, including internarial length, snout length, inter-jaws width, interobital length and head depth. The dendrogram showed *Ingerophrynus parvus* specimens were divided into two major clades.

For the first clade, three specimens from Tarutao Island were grouped separate from the mainland localities as one major clade, but two specimens from Khao Phanom Bencha National Park and one specimen from Thung Khai botanic garden were shared morphometric characters within this clade.

The second clade was split into two small clades. The first clade were mostly specimens from protected area, Kho Hong Hill (n=34), Thung Khai botanic garden (n=4), Ngao Waterfall National Park (n=1), Than Bok Khorani National Park (n=1), Khao Phanom Bencha National Park (n=1), Yong Waterfall National Park (n=1), and Tarutao Island (n=1). The second clade were specimens from mainland localities which are unclear grouping in morphometric characters. This clade was sub-divided into 3 groups, including group of Hala Bala Wildlife Sanctuary (n=3), group of Thale Ban National Park (n=4) with Than Bok Khorani National Park (n=1), group of Khao Phanom Bencha National Park (n=3) and Hala- Bala Wildlife Sanctuary (n=2), and the big group of sample from Ngao waterfall National Park (n=7), Than Bok Khorani National Park (n=5), Yong Waterfall National park (n=4), Khao Chong Wildlife Development and Conservation Promotion Station (n=7), Thung Khai botanic garden (n=5), protected area, Kho Hong Hill (n=25), Thale Ban National Park (n=7), and Hala Bala Wildlife Sanctuary (n=4) (Figure 4.10).

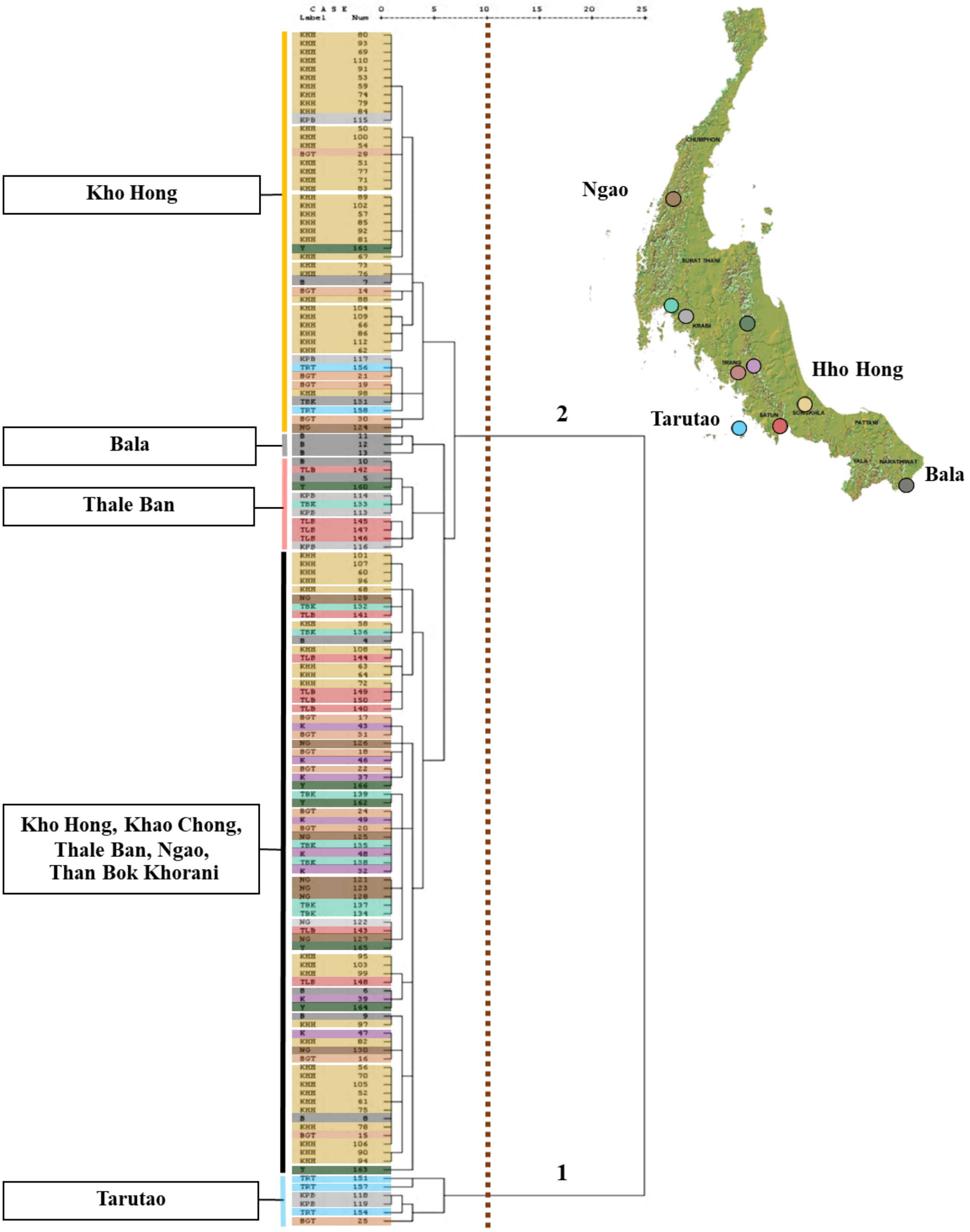

Figure 4.10. Hierarchical cluster analysis of male *Ingerophrynus parvus* shows dendrogram grouping of morphometric characters among study localities.

4.3.5. Comparison of morphometric characters of female *Ingrophrynus parvus* populations in southern Thailand

Ratio data of 25 female specimens from five localities were also analyzed by cluster analysis (Table 4.4). The test of normality shows normal distribution of 15 morphometric character, excluding snout length (SNL) and inter-jaws width (IJW). Then, the characters were tested with One-Way ANOVA analysis, and the result showed 3 significant different characters (Table 4.5) which are length between ridges (LBR, p = 0.006), head depth (HD, p = 0.038) and eye width (EW, p = 0.045).

Table 4.4. Number of female *Ingerophrynus parvus* specimens in southern Thailand

Localities	**Number of female *Ingerophrynus parvus***
1. Khao Chong Wildlife Development and Conservation Promotion Station	10
2. Thung Khai botanic garden	4
3. protected area, Kho Hong Hill	4
4. Tarutao Island	4
5. Hala-Bala Wildlife Sanctuary	3
Total	25

Table 4.5. One-Way-ANOVA analysis of 15 morphometric characters in female *Ingerophrynus parvus* samples among study localities

Characters		**Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
RIN	Between Groups	0.000	4	0.000	2.047	0.126
	Within Groups	0.001	20	0.000		
	Total	0.002	24			
RSW	Between Groups	0.000	4	0.000	1.184	0.348
	Within Groups	0.001	20	0.000		
	Total	0.001	24			
RHW	Between Groups	0.001	4	0.000	1.666	0.197
	Within Groups	0.004	20	0.000		
	Total	0.005	24			
RHL	Between Groups	0.005	4	0.001	2.101	0.119
	Within Groups	0.011	20	0.001		
	Total	0.015	24			
RHD*	Between Groups	0.001	4	0.000	3.125	0.038
	Within Groups	0.001	20	0.000		
	Total	0.002	24			
RED	Between Groups	0.001	4	0.000	0.840	0.516
	Within Groups	0.006	20	0.000		
	Total	0.007	24			
REW*	Between Groups	0.001	4	0.000	2.970	0.045
	Within Groups	0.002	20	0.000		
	Total	0.004	24			
RIO	Between Groups	0.001	4	0.000	2.638	0.064
	Within Groups	0.002	20	0.000		
	Total	0.004	24			
RLBR*	Between Groups	0.001	4	0.000	4.992	0.006
	Within Groups	0.001	20	0.000		
	Total	0.002	24			
RRL	Between Groups	0.001	4	0.001	0.993	0.434
	Within Groups	0.005	20	0.000		
	Total	0.006	24			
RPGW	Between Groups	0.000	4	0.000	0.425	0.789
	Within Groups	0.002	20	0.000		
	Total	0.003	24			
RPGL	Between Groups	0.001	4	0.000	0.941	0.461
	Within Groups	0.005	20	0.000		
	Total	0.005	24			
RHDL	Between Groups	0.000	4	0.000	0.468	0.759
	Within Groups	0.004	20	0.000		
	Total	0.005	24			
RTB	Between Groups	0.004	4	0.001	2.195	0.106
	Within Groups	0.009	20	0.000		
	Total	0.013	24			
RFL	Between Groups	0.002	4	0.001	1.670	0.196
	Within Groups	0.007	20	0.000		
	Total	0.009	24			

* Significant different morphometric character ($p<0.05$) of One-Way ANOVA analysis

The dendrogram of hierarchical cluster analysis was built from ratio data of three significant characters. There are ratio of head depth (RHD), ratio of eye width (REW) and ratio of length between ridges (RLBR). The dendrogram shows female *Ingerophrynus parvus* was grouped into two main clades, even though morphometric characters are not clearly group among mainland localities (Figure 4.11). The first clade was mostly specimens from Tarutao Island (n=3), but there were sharing characters with Thung Khai botanic garden (BGT) and protected area, Kho Hong Hill (KHH). The second clade was separated into two groups. The first group consists of four specimens from Khao Chong Wildlife Development and Conservation Promotion Station, one specimen from Hala-Bala Wildlife Sanctuary and one specimen from Southern Botanic Garden. For the second group, there were six specimens from Khao Chong Wildlife Development and Conservation Promotion Station, two specimens from Southern Botanic Garden, three specimens from protected area, Kho Honh Hill, two specimens from Hala-Bala Wildlife Sanctuary and one specimen from Tarutao Island.

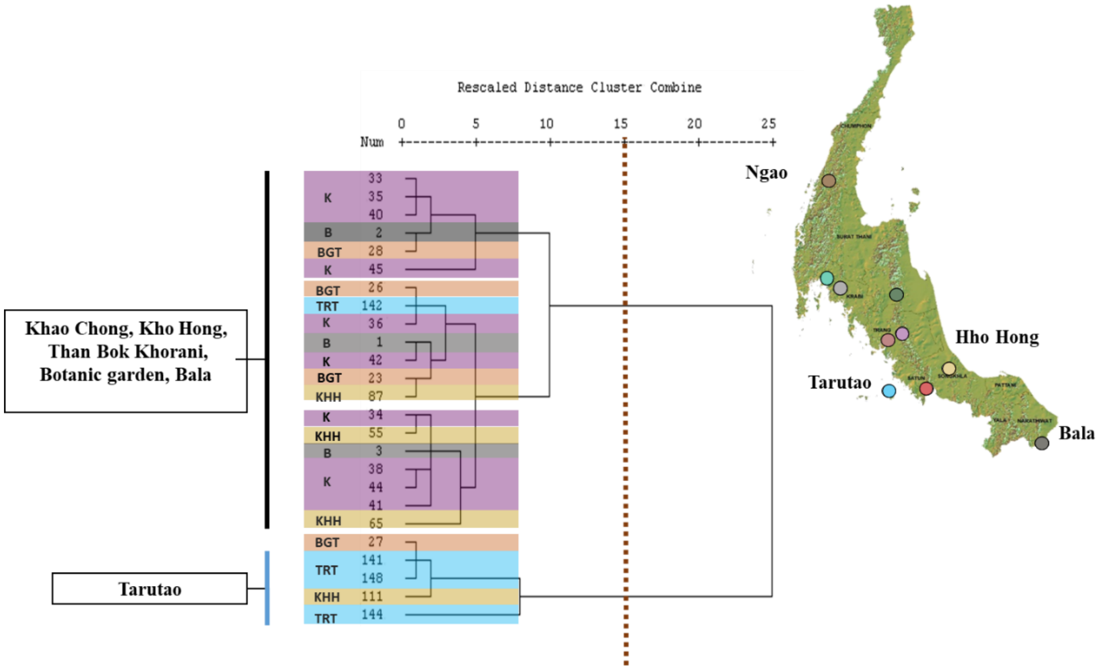

Figure 4.11. The hierarchical cluster analysis of female *Ingerophrynus parvus* shows dendrogram grouping from three significant morphometric characters (RHD, REW, RLBR) among localities.

4.4. Molecular study

4.4.1. Phylogenetic tree

The phylogenetic tree of *Ingerophrynus parvus* in southern Thailand was created from twenty seven sequences of 16S rRNA gene (518 bp) together with two blast sequences from GenBank as output (*Bufo viridis*: AY862558.1, and *Bufo marinus*: AY028498.2). The Maximum likelihood method with bootstrap 1000 replicates was used for sequence analysis by PhyML program. In this study, the most appropriate substitution model is GTR with the gamma shape parameter ($I=0.739$). For the polymorphic sites, the invariable site is 480 with the singleton variable sites equal to 1, and parsimony informative site is 26. The pairwise comparison number (k) is 10.655, and nucleotide diversity (π) is 0.021. There is no insertion and deletion event in the sequences. The Tajima's D value is not significant different ($p=1.904$, $p>0.1$) which means the distribution between π and $S/\alpha1$ are normal distribution (Table 4.6).

Table 4.6. Nucleotide variable of *Ingerophrynus parvus* samples in southern Thailand

Parameters	16S
Number of samples	27
Align position	518
Best fit model	GTR+I
Polymorphic sites	
Invariable (monomorphic) sites	480
Singleton variable sites	1
Parsimony informative sites	26
DNA polymorphism	
Number of pairwise comparison	507
Average number of nucleotide differences (k)	10.655
Nucleotide diversity (π)	0.021
A Frequency	0.314
C Frequency	0.236
G Frequency	0.193
T Frequency	0.256
Number of polymorphic sites (S)	27
Theta-W, per sequence	7.005
Theta-W, per site	0.014
Insertion-Deletion polymorphism	0
Tajima's D test	1.904

For the evolutionary divergent estimated, the rank of uncorrelated pairwise matrix in Ngao Waterfall National Park was 0.0099 to 0.413. There were 0.0020 to 0.0394 in Than Bok Khorani National Park, the 0.0020 to 0.0434 in Thung Khai Botanic Garden, the 0.0098 to 0.0454 in Thale Ban National Park, and 0.0414 in Hala-Bala Wildlife Sanctuary. Among populations, the evolutionary divergent estimate show the highest of uncorrelated pairwise matrix between Thung Khai botanic garden (BGT1) and Thale Ban National Park (TLB2) (0.0454), and the lowest of uncorrelated pairwise matrix is between Than Bok Khorani National Park (TBK1) and Hala-Bala Wildlife Sanctuary (B1) (0.0020), and between specimens from Thung Khai botanic garden (BGT1) and Hala-Bala Wildlife Sanctuary (B1) (0.0020) respectively (Table 4.7).

Table 4.7. The evolutionary divergent estimate of uncorrected pairwise matrix of *Ingerophrynus parvus* populations based on sequences (515 bp) from southern Thailand.

	Bala1	**B2**	**TBK1**	**BGT1**	**NG4**	**NG6**	**TLB2**
Bala1	0						
B2	0.0414	0					
TBK1	0.0020	0.0394	0				
BGT1	0.0020	0.0434	0.0039	0			
NG4	0.0118	0.0374	0.0099	0.0138	0		
NG6	0.0118	0.0413	0.0099	0.0138	0.0039	0	
TLB2	0.0434	0.0098	0.0414	0.0454	0.0394	0.0394	0

The tree of Maximum Likelihood (ML) represents scale bar 0.06 expected substitutions per site in align region. *Ingerophrynus parvus* in southern Thailand defined into two monophyletic clades, and the branch support are strongly support at 100%. The first clade contained populations from north and central area of southern Thailand clade (Ngao Waterfall National park, Khao Phanom Bencha National park, Than Bok Khorani National Park, Yong Waterfall National Park, Thung Khai Botanical Garden and Protected area, Kho Hong Hill). Specimens from Ngao Waterfall National Park group was placed as a sister group population from the central area of southern Thailand localities at branch support 56%. The second clade contain southern part of southern Thailand clade which are the sister group of Thale Ban National Park and

Hala-Bala Wildlife Sanctuary. The most diversity of gene is the clade in the central area of southern Thailand (Figure 4.12).

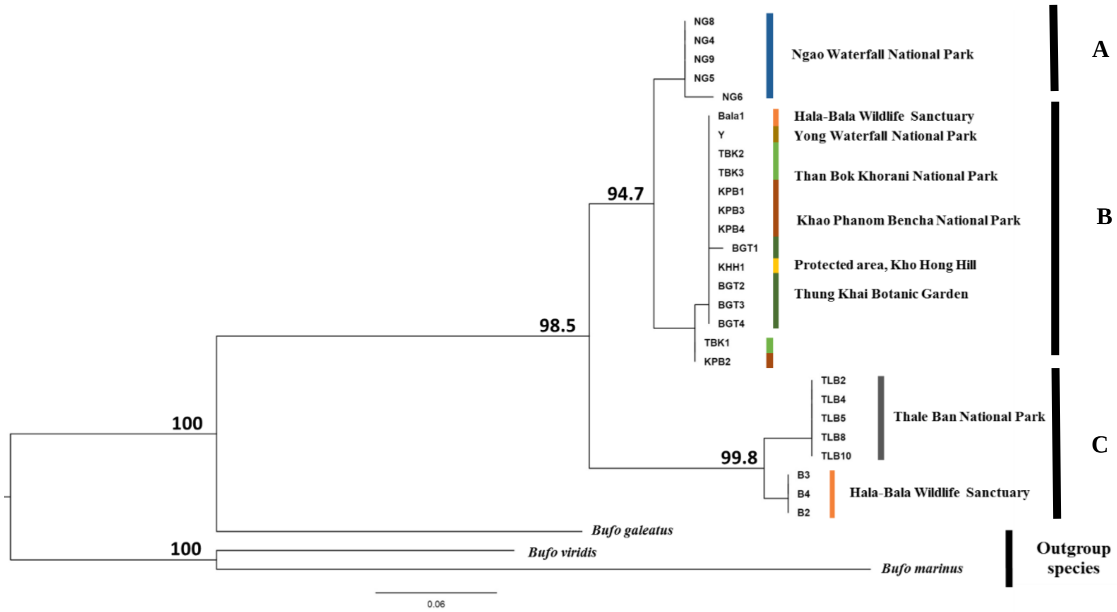

Figure 4.12. The molecular phylogenetic tree by Maximum Likelihood (ML) method from 27 sample sequences of *Ingerophrynus parvus* from southern Thailand.

4.4.2. Haplotype network

According to the network analysis, there were seven haplotypes (h) from twenty seven sequences with the haplotype diversity (Hd) equal 0.786. The highest haplotype diversity was haplotype 1 which contained two sequences from Than Bok Khorani National Park, three sequences from Khao Phanom Bencha National Park, one sequence from Yong waterfall National Park, three sequences from Thung Khai botanic garden, one sequence from protected area, Kho Hong hill, and one sequence from Hala-Bala Wildlife Sanctuary. There were three haplotypes (H4, H5 and H7) that not share locality with others, haplotypes 4 and 5 were from Ngao Waterfall National Park, and haplotype 7 was from Thale Ban National Park. In addition, three sequences from Hala-Bala Wildlife Santuary were representative of haplotype 6, and one sequence was shared with haplotype 1. Haplotype 2 was sharing one sequence from Than Bok Khorani National Park and one sequence from Khao Phanom Bencha national park. Haplotype 3 was contained single sequence from Thung Khai Botanic Garden respectively (Figure 4.13).

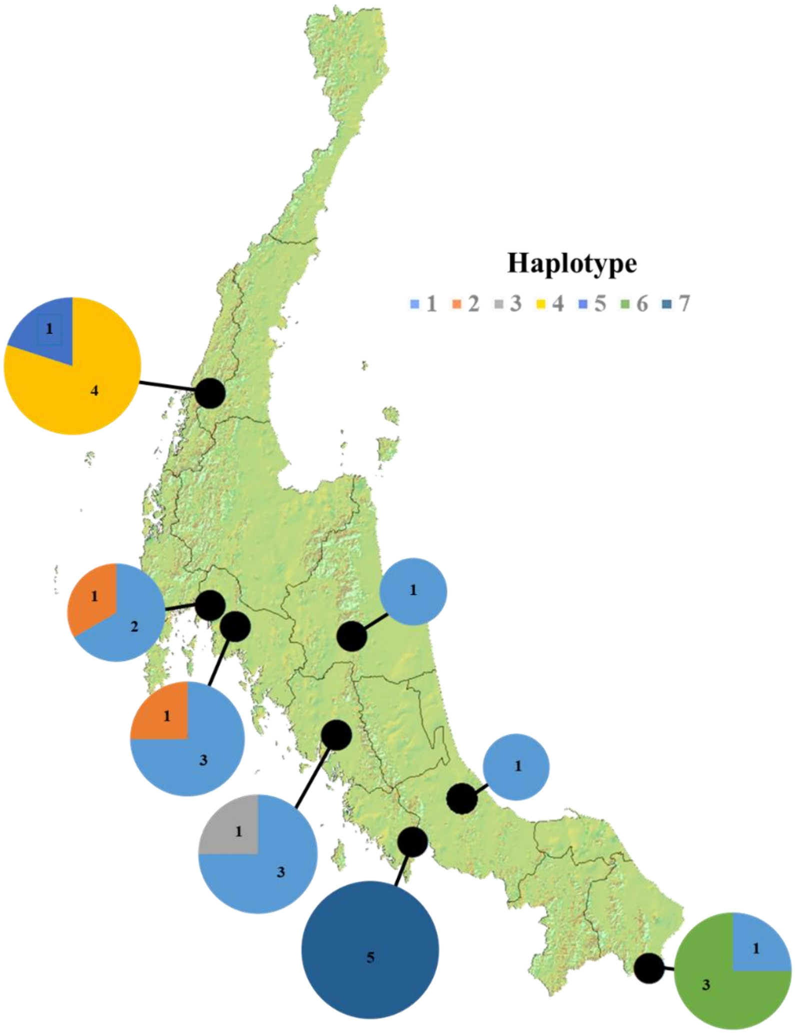

Figure 4.13. Haplotype numbers of 16S rRNA gene with the geographical occurrence in southern Thailand.

From haplotype network, haplotype network was divided into three groups, the first group was from Ngao waterfall national park (H4, H5), and haplotype 4 and haplotype 5 were sister group. The second group was included haplotype 1, 2 and 3 which haplotype 1 was the most diverse in localities when compare with another haplotypes (Than Bok Khorani National Park, Khao Phanom Bencha national park, Yong waterfall National Park, Thung Khai botanic garden, protected area, Kho Hong hill and Hala-Bala Wildlife Sanctuary). The last group was contained haplotype 6 and haplotype 7 from southern part of Thailand which were also sister group (Figure 4.14).

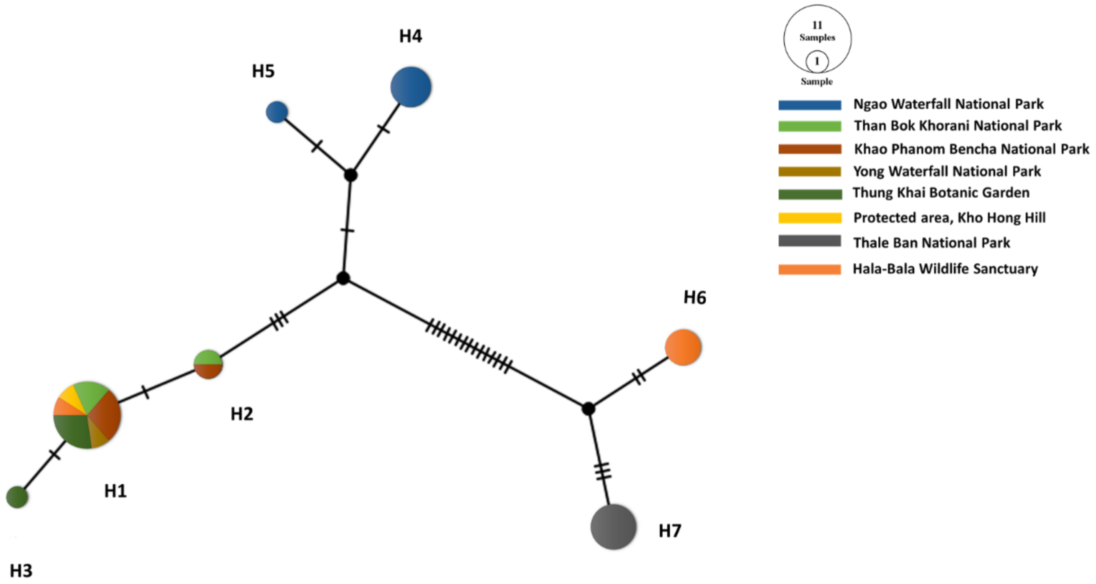

Figure 4.14. Haplotype network indicate distribution of *Ingerophrynus parvus* in southern Thailand, circles sizes are proportional to frequency of haplotype, and the length with lines represent mutation steps proportionally between haplotypes.

Morphological difference and genetic relationship showed concordant pattern in result that the high variation from all study methods were from the central part of southern Thailand. In morphological characters, we compared 15 occurrence characters with total number of specimens in each locality as ratio data. The result was shown ratio variation in six morphological characters which are white ventral, curved nose, spiny warts under radio-ulna, spiny warts above parotid glands, pair of ridges present clearly and curved ridges characters (Table 4.8). The ratio values showed that specimens from Ngao waterfall National Park had opposite occurrence in characters to Tarutao Island except for curved ridges character. Ratio values of white ventral, spiny warts under radio-ulna and spiny warts above parotid glands characters had same ratio values at 0 in Ngao Waterfall National Park, and showing increase in ratio values in Than Bok Khorani National Park. Then, the highest ratio value of each character was in Khao Chong Wildlife Development and Conservation Promotion Station. The ratio values decreased in Southern Botanic Garden, and rapidly decreased in protected area, Kho Hong Hill, but the ratio values raised up again in Thale Ban National Park and Hala-Bala Wildlife Sanctuary. Meanwhile, curved nose, pair of ridges present clearly, and curved ridges characters showed ratio values at 1 in Ngao Waterfall National Park, and decreasing in Than Bok Khorani National Park and Khao Phanom Bencha National Park except for ratio value of character 5. Then, the ratio values raised up in Khao Chong Wildlife Development and Conservation Promotion Station. The value decreased again in Southern Botanic Garden and protected area, Kho Hong Hill, but increasing in Thale Ban National Park and Hala-Bala Wildlife Sanctuary.

Table 4.8. Ratio values of 6 high variation of morphological characters with among study localities.

Localities	Morphological characters					
	White ventral	Curved nose	Spiny warts under radio-ulna	Spiny warts above parotid glands	Pair of ridges present clearly	Curved ridges
NG	0	1	0	0	1	1
TBK	0.56	0.56	0.22	0.33	1	1
KPB	0.50	0.67	0.33	0.33	1	0.67
Y	0.67	0.67	0.17	0	1	0.67
K	1	1	0.50	0.75	1	1
BGT	0.71	0.71	0	0.50	1	0.57
KHH	0.10	0.03	0	0.15	0.76	0.15
TLB	0.18	0.73	0	0.09	1	0.73
B	0.3	0.90	0.10	0.50	0.90	0.70
TRT	0.60	0	1	1	0.40	1

Moreover, the morphometric result exhibited most of specimens from Tarutao Island group separated from the mainland. However, sharing in morphometric characters are still maintain among mainland populations which were not clearly defined as subpopulation. Additionally, supporting by the molecular result that specimens from Ngao Waterfall National Park grouped separately from other populations, and one big group of populations from central part of southern Thailand grouped as sister group with Thale Ban National Park and Hala-Bala Wildlife Sanctuary. However, we lacks of sequence data from Tarutao Island to confirm the grouping of morphological studies. These studies showed differentiation in morphological characters, morphometric characters and genotypes that maintain in Ngao Waterfall National Park and Tarutao Island, and the group of populations from central and southern areas of southern Thailand showed variation in both morphological characters, morphometric and genetics, but still share genotypic characters among population.

CHAPTER V

Discussions

5.1. Microhabitat of *Ingerophrynus parvus*

The microhabitat of *Ingerophrynus parvus* from this study was shown as same as previous studies that this toad prefers smaller streams with slow moving water (Boulenger, 1887; Arak, 1984; Frost, 2014; Iskandar, 2014). It indicates lowland areas might be suitable environment for living and reproducing in *Ingeroprynus parvus* species. In addition, the body size of *Ingerophrynus parvus* is small (21.08-28.80 mm in male and 22.76-48.98 mm in female), living in strong water current is probably not appropriate for this species. This toad lays eggs in small ponds or paddle areas close to streams which water is more stable comparing with strong current streams, and strong water current might wash eggs away from the breeding area cause low in reproductive succession (Shahriza, 2012).

5.2. Morphological variation of *Ingerophrynus parvus*

Ingerophrynus parvus specimens in southern Thailand have similar of snout to vent length (SVL) with the *Ingerophrynus parvus* specimens that reported from peninsular Malaysia (Grismer, 2007). In this study, the snout to vent length (SVL) in range for male is 21.08-37.60 mm, and 22.76-48.98 mm for female. Comparing with male and female specimens that reported from peninsular Malaysia (28.9-37.3 mm for male and 27-45.5 mm for female), the specimens from southern Thailand have similar in body length. In addition, the ratio of tibia length with snout to vent length from southern Thailand (0.39-0.48 mm) also have similar in ratio with the specimens from peninsular Malaysia (0.44-0.46 mm). Moreover, the report of snout to vent length of *Ingerophrynus biporcatus* (55-70 mm for male, 60-80 mm for female), and *Ingerophrynus divergens* (28-45 mm for males, 50-55 mm for female) show longer snout to vent length than *Ingerophrynus parvus* in both male and female. From the previous studies, there were reports of *Ingerophrynus biporcatus* and *Ingerophrynus divergens* from southern Thailand (Chuaynkern and Chuaynkern, 2012; Konsue, 2001; Chan-ard, 2003), however, there are still doubtful about existing of these two species in Thailand. In 1912, Boulenger reported distribution of *Ingerophrynus divergens* in

Boneo, Selangor and Natuna Islands which exclude Thailand. For *Ingerophrynus biporcatus*, this species is restrict to Indonesia country only, and the records of *Ingerophrynus biporcatus* from mainland Southeast Asia (Berry, 1975) are maybe *Ingerophrynus quadriporcatus* (IUCN, 2004). Then, there is maybe only *Ingerophrynus parvus* in southern Thailand.

In southern Thailand, there are intraspecific variation in morphological characters and morphometric characters among *Ingerophrynus parvus* populations while the sharing characters are still maintain among populations. The external morphology characters of *Ingerophrynus parvus* are not clearly defined into group of populations, but some morphological characters from the Tarutao Island seem to unrelated in occurrence frequency comparing with the mainland populations which included curved nose, pair of ridges present clearly, and spiny warts above parotid glands and spiny warts under forearms characters. Furthermore, the morphometric measurements from Tarutao Island also show shape variation among study localities, and the snout to vent length (SVL) of samples from Tarutao Island trend to have smaller in size both male and female when compare with the mainland populations. Additionally, the hierarchical cluster analysis of significant morphometric characters group samples from Tarutao Island separate from the mainland localities both male and female, however, there are some sharing of morphometric characters between the Tarutao and mainland populations.

Tarutao Island samples trend to group separate from the mainland populations of southern Thailand, and it might due to Tarutao Island used to connect with the mainland in Pleistocene period (Inger and Voris, 2001). The island is completely isolated from the mainland at least around 6070 years before present (Sathiamurthy and Voris, 2006); therefore, *Ingerophrynus parvus* population in Tarutao Island is possibly isolated from the mainland populations at least by the time of geographic isolated (Figure 5.1). Then, without sharing gene pool with the mainland populations plus resource limitation in the Tarutao Island, it maybe cause diversity in morphology character between inland and island populations.

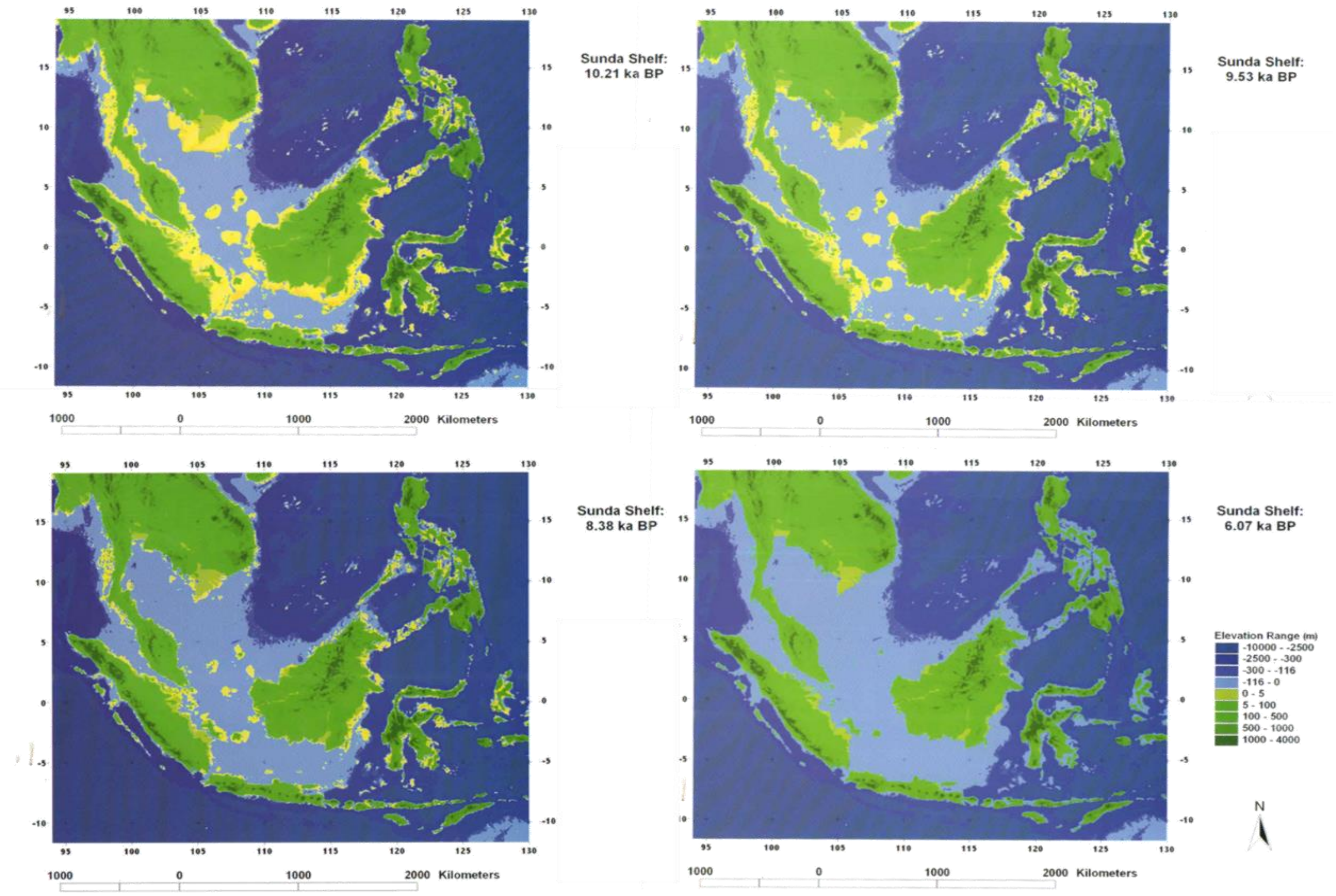

Figure 5.1. Sea level rise in Pleistocene period show Tarutao Island is completely isolated from the mainland of southern Thailand at 6070 years before present. (Modified from Sathiamurthy and Voris, 2006)

For the size different between samples from Tarutao Island and the mainland, the smaller in body size of samples from Tarutao Island are supported by the island rule that size of animals shift to larger or smaller size (gigantism, dwarfism) when animals move to islands. For amphibians, some amphibians have bigger size than insular populations such as green toad (*Bufo viridis*), rice field frog (*Rana limnocharis*) and Brazilian frog (*Phyllodytes luteolus*) (Castellano and Giacoma, 1998; Wu et al, 2006; Mageski et al., 2015), but on the other hand, there is *Rhinella ornata* (Bufonidae) that body size decrease in islands (Montesinos, 2012). In this study, *Ingerophrynus parvus* samples from Tarutao Island tend to respond to dwarfism, and smaller body size maybe due to resource availability, interspecific competition and predation pressure (Wu et al, 2006; Rog et al., 2013; Mageski et al., 2015). The limitation area in Tarutao Island collaborate with the specificity of microhabitats and dispersion ability of *Ingerophrynus parvus* might lead to high interspecific competition with another animals species especially within frog species that use same water source as breeding areas. In Tarutao Island, there are 11 species of frogs contained in the island (Khonsue et al., 2011) which competition among amphibian species maybe occur. Moreover, high in predation pressure may also cause smaller in body size of *Ingerophrynus parvus* due to amphibians are normally pray in ecosystems (Daniel and Kimberly, 2014), however, the interaction of predation pressure in Tarutao Island has not been reported. Therefore, variation of *Ingerophrynus parvus* between the mainland populations in southern Thailand and Tarutao Island are detected.

In the mainland populations, the external morphology characters of *Ingerophrynus parvus* is mixed, but some characters are dominant in particular localities. The curved nose and the end of ridge curved characters have lowest present percentage in protected area, Kho Hong hill, while other localities in mainland have higher present percentage in these characters. In Khao Chong Wildlife Development and Conservation Promotion Station has highest present percentage in spiny warts above parotid gland and spiny warts under forearms which we can see these characters have much lower percentage in another localities in mainland. In addition, there is only specimens from Than Bok Khorani National Park that tip of tibiofibular cannot reach tip of snout. However, these characters are not distinctly defined into subgroup of population as the characters are still maintain among *Ingerophrynus parvus* populations in southern Thailand. Additionally, the hierarchical cluster analysis is also show sharing in morphometric characters, but it’s also not clearly defined to group of populations among study localities.

5.3. Molecular study

In mainland populations, both external morphology and morphometric characters of *Ingerophrynus parvus* were unclear. There were sharing of external morphology among mainland populations. However, the molecular study shows the disconnection in genetic of *Ingerophrynus parvus* among mainland localities, and the grouping conform to isolated mountain ranges in southern Thailand.

Base on Thailand topology, two main mountain ranges run through southern Thailand in north-south direction. The Tenasserim Mountain Range is located at the northern part of southern Thailand, and Nakhon Si Thammarat Mountain Range runs through central part of southern Thailand. Besides, there is Sankalakhiri Mountain Range located at the border between Thailand and Malaysia countries. Ngao Waterfall National park, Ranong Province is only one locality at Tenasserim Mountain range. Than Bok Khorani National Park and Khao Phanom Bancha National Park are isolated hills between Tenasserim Mountain Range and Nakhon Si Thammarat Mountain Range. Yong Waterfall National Park, Thung Khai Botanic Garden and protected area, Kho Hong Hill are representative of Nakhon Si Thammarat Mountain Range. Furthermore, Thale Ban National Park is located at the very end of Nakhon Si Thammarat Mountain Range, and Hala-Bala Wildlife Sanctuary is located at Sankalakhiri Mountain Range.

The phylogenetic tree showed *Ingerophrynus parvus* populations from southern Thailand divided into two major clades with strongly branch support. *Ingerophrynus parvus* populations divided into the north and central group (Ngao Waterfall National Park, Than Bok Khorani National Park, Khao Phanom bancha National Park, Yong Waterfall National Park, Thung Khai Botanic Garden and protected area, Kho Hong Hill), and the southern group (Thale Ban National Park and Hala-Bala Wildlife Sanctuary). The north and central group was composed of sample sequences from Ngao Waterfall National Park that grouped as sister group with sample sequences from Nakhon Si Thammarat Mountain Range. However, haplotype network from Ngao

Waterfall National Park (H6, H7) were differ from the central area of southern Thailand (H1, H3, H5). The southern group (Thale Ban National Park, Hala-Bala Wildlife Sanctuary) shows similar in result that both phylogenetic tree and haplotype network from Thale Ban National Park and Hala-Bala Wildlife Sanctuary are closely relate as sister group (Figure 5.2).

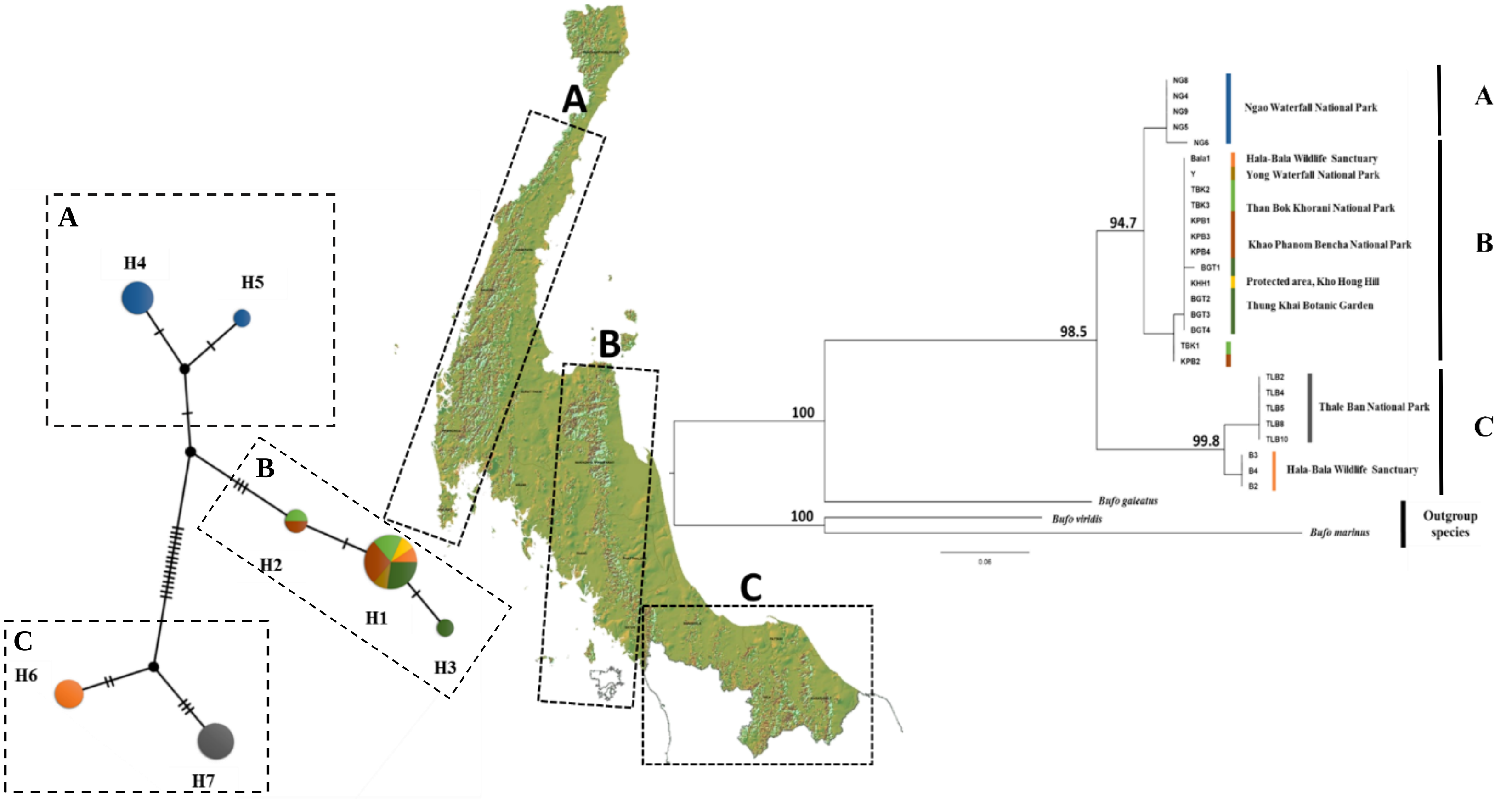

Figure 5.2. Map shows grouping of phylogenetic tree and haplotype network are related with isolated mountain ranges of Thailand topography; Tenasserim Mountain Range (A), Nakhon Si Thammarat Mountain Range (B) and Sankalakhiri Mountain Range (C).

5.4. Geographical topology and genetic difference of *Ingerophrynus parvus* in southern Thailand

From the molecular result, the grouping of *Ingerophrynus* populations follow the pattern of isolated mountain ranges. Three mountain ranges in southern Thailand are completely isolated and surrounded by lowland areas which act as natural barrier for migratory animals, and limited gene flow among populations especially in amphibian species that have low distribution capability (Blaustein, 1994, Storfer, 2007; Savage, 2010; Thomé, 2014). However, population from Ngao Waterfall National Park has more relate to populations from the Nakhon Si Thammarat Mountain Range comparing with the southern group. Supporting by shorter distance of lowland area between Tenasserim Mountain Range and the Nakhon Si Thammarat Mountain Range that not strong geographical barrier as big lowland area between Nakhon Si Thammarat Mountain Range and the Sankalakhiri Mountain Range. Then, closer in geographical distance result in closer in genetic relationship (Hutchinson and Templeton, 1999; Arens et al., 2007; Jang et al, 2011), so the connection between Ngao Water National Park and the Nakhon Si Thammarat Mountain Range populations are probably easier to exchange and share gene pool comparing with the southern populations.

From previous studies, there are some amphibian species in Thailand that also respond to isolated mountain ranges, for example, discovering a new species of genus *Ansonia* (*Ansonia kraensis*) in the Isthmus of Kra (Matsui et al., 2005) which suggested geographical difference is probably cause by geographical topology. Moreover, *Hoplobatrachus* species (Pansook et al., 2012) is also suggested to separate into 2 different species base on molecular study, and divided to the northern and southern Thailand group. Also, *Amolops* species are confirmed by molecular study that northern population from Tenasserim Mountain Range group isolated from southern peninsular population, and described as new species (*Amolops panhai*). However, we found that *Ingerophrynus parvus* specimens from Thale Ban National Park are sister group with Hala-Bala Wildlife Sanctuary specimens instead of grouping with the Nakhon Si Thammarat Mountain Range populations that closer in distance in vertical direction. This phenomenon maybe explain by the lowland barrier between Thale Ban National Park and the end of Nakhon Si Thammarat Mountain Range. Even if there is short distance of lowland area, however, it’s still barrier for distribution of this toad species because of distribution limitation (Inger et al., 1974) and their specific habitat. In addition, Thale Ban National park is the end of the mountain that connect with Malaysia country which possibly used to connect with northern Malaysia mountain ranges and Hala-Bala Wildlife Sanctuary population by horizontal distribution in the past.

Even if external morphology of *Ingerophrynus parvus* populations in southern Thailand are not clearly defined as subpopulations, however, the molecular study can group *Ingerophrynus parvus* into northern-central group and southern group. Then, this study shows genetic differentiation among isolated populations in southern Thailand, and it is possible that this species will be developing to different species in the future.

CHAPTER VI

Conclusion

The morphological variation of *Ingerophrynus parvus* is occurred in species level. The comparative study of external morphology observation exhibit sharing in external morphology characters among *Ingerophrynus parvus* populations, however, some observed characters show dominant characters in particular localities, but there are not clearly defined into subpopulation level by external morphology. Meanwhile, the morphometric measurements show Tarutao samples tend to have smaller body size than the mainland populations which follow insular dwarfism of island’s rule. Moreover, the hierarchical cluster analysis of morphometric characters seem to group samples from Tarutao Island separated from the mainland populations, but still share some morphometric characters with mainland populations. This maybe because once the island used to connect with the mainland population in Pleistocene period, and Tarutao Island population used to share same gene pool with the mainland populations. Meanwhile, in the mainland populations also show unclear grouping in morphometric characters among *Ingerophrynus parvus* populations in southern Thailand. However, the molecular study from 16S rRNA gene support species differentiation, the phylogenetic tree divided *Ingerophrynus parvus* populations in southern Thailand into 2 major clades; the North-central group (Ranong, Krabi, Nakhon Si Thammarat, Trang and Songkhla Province) and the Southern group (Satun and Narathiwat Province) which the geographical barriers in this case are mountain ranges that influence to distribution of *Ingerophrynus parvus*, and disrupted sharing gene pool among populations, and the lowland areas also effect connection among *Ingerophrynus parvus* populations. In the North-central group, sample sequences from Ngao Waterfall National Park (Tenasserim Mountain Range) are grouped as sister group with the central group (Nakhon Si Thammarat Mountain Range), but have unique haplotypes from the central group base on haplotype analysis. In the same way with the Southern group, Thale Ban National Park samples group as sister group with Hala-Bala Wildlife Sanctuary samples, but there is particular haplotype in each locality. Base on the geographical distance, the closer in distance between the northern and central group, and between Thale Ban National Park and Hala-Bala Wildlife Sanctuary cause less genetic different comparing with the big lowland area between the Nakhon Si Thaammarat Mountain Range and Sankarakiri Mountain Range. This study shows genetic differentiation among *Ingerophrynus parvus* populations in southern Thailand, and showing of possibility of changing to different species in the future.

Future study recommendations

1. The lack of sequence samples from Tarutao Island, so sample sequences from the island are required to confirm with the external morphology result.
2. Only one gene was taken for molecular study, using more than one gene would help to confirm and give more confident in molecular result.
3. Collections from other parts of Thailand would be require to compare morphological variation and genetic differentiation of *Ingerophrynus parvus* populations in Thailand.
4. Alternative method such as bioacoustics analysis would help to specify the different among *Ingerophrynus parvus* populations in southern Thailand.

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Appendix I

Table 1. Location of 10 study localities in southern Thailand

Locations	**Province**	**Latitude and Longitude**
1. Ngao Waterfall National Park	Ranong	9.89650° N, 98.73294° E
2. Than Bok Khorani National Park	Krabi	8.38784° N, 98.73521° E
3. Khao Phanom Bencha National Park	Krabi	8.24190° N, 98.91520° E
4. Yong Waterfall National Park	Nakon Si Thammarat	8.17174° N, 99.74227° E
5. Khao Chong Wildlife Development and Conservation Promotion Station	Trang	7.54850° N, 99.78883° E
6. Thung Khai Botanic Gaden	Trang	7.28060° N, 99.38080° E
7. Thale Ban National Park	Satun	6.71038° N, 100.17090° E
8. protected area, Kho Hong hill, Prince of Songkla University	Songkhla	7.00832° N, 100.51089° E
9. Hala-Bala Wildlife Sanctuary	Narathiwat	5.80817° N, 101.84516° E
10. Tatutao Island	Satun	6.64865 N, 99.65186° E

Appendix II

Table 2. List of *Ingerophrynus parvus* specimens in southern Thailand

Locations	Province	Specimen number
1. Ngao Waterfall National Park	Ranong	PSUZC-AMP 1129 PSUZC-AMP 1560-1570
2. Than Bok Khorani National Park	Krabi	PSUZC-AMP 0364 PSUZC-AMP 1571-1578
3. Khao Phanom Bencha National Park	Krabi	PSUZC-AMP 1579-1585
4. Yong Waterfall National Park	Nakon Si Thammarat	PSUZC-AMP 1121-1127
5. Khao Chong Wildlife Development and Conservation Promotion Station	Trang	PSUZC-AMP 0830-0831 PSUZC-AMP 0833 PSUZC-AMP 1303 PSUZC-AMP 1316 PSUZC-AMP 1555 PSUZC-AMP 1601-1609
6. Thung Khai Botanic Gaden	Trang	PSUZC-AMP 1550 PSUZC-AMP 1152-1153 PSUZC-AMP 1555-1158 PSUZC-AMP 1593-1600
7. Thale Ban National Park	Satun	PSUZC-AMP 1684-1694
8. protected area, Kho Hong hill, Prince of Songkla University	Songkhla	PSUZC-AMP 0023 PSUZC-AMP 0026 PSUZC-AMP 0029 PSUZC-AMP 0058 PSUZC-AMP 0161-0162 PSUZC-AMP 0263 PSUZC-AMP 0346-0347 PSUZC-AMP 0349-0350

Table 2. List of *Ingerophrynus parvus* specimens in southern Thailand *continued*

Locations	**Province**	**Specimen number**
8. protected area, Kho Hong hill, Prince of Songkla University	Songkhla	PSUZC-AMP 0354 PSUZC-AMP 0440 PSUZC-AMP 0456 PSUZC-AMP 0880 PSUZC-AMP 1018 PSUZC-AMP 1611-1683
9. Hala-Bala Wildlife Sanctuary	Narathiwat	PSUZC-AMP 0819 PSUZC-AMP 1034 PSUZC-AMP 1193 PSUZC-AMP 1559 PSUZC-AMP 1586-1592
10. Tatutao Island	Satun	PSUZC-AMP 0181-0182 PSUZC-AMP 0186-0187 PSUZC-AMP 0818 PSUZC-AMP 1199 PSUZC-AMP 0189-0193

Appendix III

Table 3. The percentage of 15 occurrence characters (present/absent) among 10 study localities

Characters	**Localities**										
	Occurrence	**B**		**BGT**		**K**		**KHH**		**KPB**	
1. White ventral	Present	7	53.8%	4	22.2%	1	5.6%	55	87.3%	4	57.1%
	Absent	6	46.2%	14	77.8%	17	94.4%	8	12.7%	3	42.9%
2. Curved nose	Present	1	7.7%	5	27.8%	1	5.6%	61	96.8%	5	71.4%
	Absent	12	92.3%	13	72.2%	17	94.4%	2	3.2%	2	28.6%
3. Spiny warts present on ventral side of radio-ulna	Present	10	76.9%	16	88.9%	5	27.8%	60	95.2%	5	71.4%
	Absent	3	23.1%	2	11.1%	13	72.2%	3	4.8%	2	28.6%
4. Spiny warts above paratoid gland	Present	5	38.5%	8	44.4%	2	11.1%	51	81.0%	5	71.4%
	Absent	8	61.5%	10	55.6%	16	88.9%	12	19.0%	2	28.6%
5. Pair of ridges present clearly on it head	Present	1	7.7%	0	0.0%	0	0.0%	14	22.2%	1	14.3%
	Absent	12	92.3%	18	100.0%	18	100.0%	49	77.8%	6	85.7%
6. The end of ridges curved	Present	6	46.2%	7	38.9%	0	0.0%	53	84.1%	2	28.6%
	Absent	7	53.8%	11	61.1%	18	100.0%	10	15.9%	5	71.4%
7. Black and white strips around mouth	Present	0	0.0%	1	5.6%	1	5.6%	0	0.0%	0	0.0%
	Absent	13	100.0%	17	94.4%	17	94.4%	63	100.0%	7	100.0%
8. Outer metacarpal tubercle bigger than inner metacarpal tubercle	Present	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
	Absent	13	100.0%	18	100.0%	18	100.0%	63	100.0%	7	100.0%
9. Outer metatarsal tubercle bigger than inner metatarsal tubercle	Present	13	100.0%	17	94.4%	16	88.9%	63	100.0%	7	100.0%
	Absent	0	0.0%	1	5.6%	2	11.1%	0	0.0%	0	0.0%
10. A tubercle present behind parotid gland	Present	2	15.4%	0	0.0%	1	5.6%	3	4.8%	0	0.0%
	Absent	11	84.6%	18	100.0%	17	94.4%	60	95.2%	7	100.0%
11. Pair of wards in the middle line of back	Present	2	15.4%	2	11.1%	1	5.6%	2	3.2%	0	0.0%
	Absent	11	84.6%	16	88.9%	17	94.4%	61	96.8%	7	100.0%
12. Hindlimbs with dark crossed bands	Present	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
	Absent	13	100.0%	18	100.0%	18	100.0%	63	100.0%	7	100.0%
13. forelimbs with dark crossed bands	Present	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
	Absent	13	100.0%	18	100.0%	18	100.0%	63	100.0%	7	100.0%
14. ankle reach tip of nose when fold leg to the front	Present	2	15.4%	5	27.8%	13	72.2%	28	44.4%	2	28.6%
	Absent	11	84.6%	13	72.2%	5	27.8%	35	55.6%	5	71.4%
15. Oval tympanum	Present	0	0.0%	4	22.2%	1	5.6%	2	3.2%	0	0.0%
	Absent	13	100.0%	14	77.8%	17	94.4%	61	96.8%	7	100.0%

Table 3. The percentage of 15 occurrence characters among 10 study localities *continued*

Characters		**Localities**									
	Occurrence	**NG**		**TBK**		**TLB**		**TRT**		**Y**	
1. White ventral	Present	10	90.9%	4	44.4%	9	75.0%	2	25.0%	2	28.6%
	Absent	1	9.1%	5	55.6%	3	25.0%	6	75.0%	5	71.4
2. Curved nose	Present	0	0.0%	4	44.4%	4	33.3%	8	100.0%	2	28.6%
	Absent	11	100.0%	5	55.6%	8	66.7%	0	0.0%	5	71.4%
3. Spiny warts present on ventral side of radio-ulna	Present	10	90.9%	7	77.8%	11	91.7%	0	0.0%	6	85.7%
	Absent	1	9.1%	2	22.2%	1	8.3%	8	100.0%	1	14.3%
4. Spiny warts above paratoid gland	Present	10	90.9%	5	55.6%	10	83.3%	0	0.0%	6	85.7%
	Absent	1	9.1%	4	44.4%	2	16.7%	8	100.0%	1	14.3%
5. Pair of ridges present clearly on its head	Present	0	0.0%	0	0.0%	1	8.3%	4	50.0%	0	0.0%
	Absent	11	100.0%	9	100.0%	11	91.7%	4	50.0%	7	100.0%
6. The end of ridges curved	Present	0	0.0%	0	0.0%	3	25.0%	1	12.5%	2	28.6%
	Absent	11	100.0%	9	100.0%	9	75.0%	7	87.5%	5	71.4%
7. Black and white strips around mouth	Present	0	0.0%	0	0.0%	0	0.0%	0	0.0%	1	14.3%
	Absent	11	100.0%	9	100.0%	12	100.0%	8	100.0%	6	85.7%
8. Outer metacarpal tubercle bigger than inner metacarpal tubercle	Present	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
	Absent	11	100.0%	9	100.0%	12	100.0%	8	100.0%	7	100.0%
9. Outer metatarsal tubercle bigger than inner metatarsal tubercle	Present	10	90.9%	9	100.0%	12	100.0%	8	100.0%	6	85.7%
	Absent	1	9.1%	0	0.0%	0	0.0%	0	0.0%	1	14.3%
10. A tubercle present behind parotid gland	Present	0	0.0%	0	0.0%	0	0.0%	1	12.5%	0	0.0%
	Absent	11	100.0%	9	100.0%	12	100.0%	7	87.5%	7	100.0%
11. Pair of wards in the middle line of back	Present	0	0.0%	2	22.2%	0	0.0%	0	0.0%	0	0.0%
	Absent	11	100.0%	7	77.8%	12	100.0%	8	100.0%	7	100.0%
12. Hindlimbs with dark crossed bands	Present	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
	Absent	11	100.0%	9	100.0%	12	100.0%	8	100.0%	7	100.0%
13. forelimbs with dark crossed bands	Present	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
	Absent	11	100.0%	9	100.0%	12	100.0%	8	100.0%	7	100.0%
14. ankle reach tip of nose when fold leg to the front	Present	10	90.9%	9	100.0%	9	75.0%	7	87.5%	6	85.7%
	Absent	1	9.1%	0	0.0%	3	25.0%	1	12.5%	1	14.3%
15. Oval tympanum	Present	0	0.0%	0	0.0%	0	0.0%	1	12.5%	0	0.0%
	Absent	11	100.0%	9	100.0%	12	100.0%	7	87.5%	7	100.0%

Appendix IV

Table 4. Descriptive statistics of 17 morphometric characters from 10 study localities

Variables	**Localities**	**N**	**Mean**	**Std. Deviation**	**Minimum**	**Maximum**
RIN	B	10	0.0744	0.00882	0.06	0.09
	BGT	14	0.0774	0.00587	0.07	0.09
	K	8	0.0762	0.00415	0.07	0.08
	KHH	59	0.075	0.00649	0.06	0.09
	KPB	7	0.0841	0.00443	0.08	0.09
	NG	10	0.0847	0.00348	0.08	0.09
	TBK	9	0.0845	0.00423	0.08	0.09
	TLB	11	0.0889	0.0071	0.08	0.1
	TRT	5	0.0898	0.00401	0.08	0.09
	Y	7	0.0777	0.005	0.07	0.09
	Total	140	0.0788	0.00782	0.06	0.1
RSNL	B	10	0.1196	0.01009	0.11	0.14
	BGT	14	0.1246	0.00714	0.11	0.14
	K	8	0.1216	0.00814	0.11	0.13
	KHH	59	0.1219	0.00729	0.11	0.14
	KPB	7	0.1289	0.00888	0.12	0.14
	NG	10	0.1188	0.00549	0.11	0.13
	TBK	9	0.1283	0.00567	0.12	0.14
	TLB	11	0.121	0.01	0.11	0.14
	TRT	5	0.13	0.00229	0.13	0.13
	Y	7	0.1318	0.0089	0.12	0.14
	Total	140	0.1232	0.00821	0.11	0.14
RSW	B	10	0.1388	0.01222	0.12	0.16
	BGT	14	0.1487	0.01009	0.13	0.16
	K	8	0.1439	0.00517	0.14	0.15
	KHH	59	0.1436	0.00586	0.13	0.16
	KPB	7	0.1462	0.00432	0.14	0.15
	NG	10	0.1443	0.00692	0.14	0.16
	TBK	9	0.1484	0.01069	0.14	0.17
	TLB	11	0.1432	0.00816	0.13	0.16
	TRT	5	0.1553	0.00504	0.15	0.16
	Y	7	0.1553	0.01082	0.14	0.17
	Total	140	0.1453	0.00844	0.12	0.17
RIJW	B	10	0.3518	0.01759	0.33	0.38
	BGT	14	0.3458	0.01554	0.32	0.37
	K	8	0.3467	0.01517	0.33	0.38
	KHH	59	0.3664	0.01472	0.33	0.4
	KPB	7	0.3444	0.02126	0.31	0.37
	NG	10	0.3399	0.016	0.32	0.36
	TBK	9	0.3407	0.01902	0.31	0.37
	TLB	11	0.3713	0.02105	0.33	0.4
	TRT	5	0.3486	0.01077	0.33	0.36
	Y	7	0.3636	0.02713	0.33	0.41
	Total	140	0.3571	0.01985	0.31	0.41

Table 4. Descriptive statistics of 17 morphometric characters from 10 study localities *continued*

Variables	Localities	N	Mean	Std. Deviation	Minimum	Maximum
RHW	B	10	0.3165	0.01747	0.29	0.35
	BGT	14	0.3119	0.00705	0.3	0.32
	K	8	0.3069	0.01524	0.29	0.33
	KHH	59	0.3244	0.01081	0.3	0.35
	KPB	7	0.3162	0.00999	0.3	0.33
	NG	10	0.3022	0.00922	0.28	0.31
	TBK	9	0.3129	0.00876	0.3	0.33
	TLB	11	0.3156	0.00726	0.3	0.33
	TRT	5	0.3406	0.01369	0.32	0.35
	Y	7	0.3253	0.01323	0.3	0.34
	Total	140	0.3188	0.0136	0.28	0.35
RHL	B	10	0.3142	0.01435	0.29	0.34
	BGT	14	0.3199	0.01619	0.29	0.35
	K	8	0.3127	0.0106	0.29	0.33
	KHH	59	0.3176	0.01486	0.28	0.35
	KPB	7	0.3146	0.01457	0.3	0.34
	NG	10	0.3054	0.01207	0.29	0.32
	TBK	9	0.3089	0.00937	0.3	0.32
	TLB	11	0.3087	0.01159	0.29	0.33
	TRT	5	0.3369	0.01594	0.31	0.35
	Y	7	0.3225	0.01893	0.29	0.34
	Total	140	0.3159	0.01512	0.28	0.35
RHD	B	10	0.1618	0.00715	0.15	0.17
	BGT	14	0.1505	0.01109	0.13	0.17
	K	8	0.1499	0.00494	0.14	0.16
	KHH	59	0.1569	0.00834	0.14	0.18
	KPB	7	0.1533	0.00504	0.15	0.16
	NG	10	0.1562	0.00897	0.14	0.17
	TBK	9	0.1532	0.00701	0.14	0.16
	TLB	11	0.1565	0.00736	0.14	0.17
	TRT	5	0.1667	0.00449	0.16	0.17
	Y	7	0.1652	0.00321	0.16	0.17
	Total	140	0.1565	0.00873	0.13	0.18
RED	B	10	0.1419	0.01458	0.13	0.16
	BGT	14	0.1528	0.01752	0.13	0.18
	K	8	0.1296	0.00557	0.12	0.14
	KHH	59	0.1476	0.0131	0.12	0.17
	KPB	7	0.1434	0.00765	0.14	0.16
	NG	10	0.1436	0.00648	0.13	0.15
	TBK	9	0.1425	0.00915	0.13	0.16
	TLB	11	0.1501	0.01722	0.12	0.18
	TRT	5	0.1468	0.01204	0.13	0.16
	Y	7	0.1466	0.01647	0.13	0.17
	Total	140	0.146	0.01372	0.12	0.18

Table 4. Descriptive statistics of 17 morphometric characters from 10 study localities *continued*

Variables	Localities	N	Mean	Std. Deviation	Minimum	Maximum
REW	B	10	0.1202	0.01221	0.1	0.14
	BGT	14	0.1208	0.01454	0.09	0.14
	K	8	0.108	0.01091	0.09	0.12
	KHH	59	0.1286	0.01158	0.1	0.16
	KPB	7	0.1132	0.00377	0.11	0.12
	NG	10	0.1076	0.00685	0.1	0.12
	TBK	9	0.1203	0.01245	0.1	0.14
	TLB	11	0.1223	0.00987	0.1	0.14
	TRT	5	0.1307	0.01564	0.11	0.14
	Y	7	0.1213	0.01235	0.11	0.14
	Total	140	0.1225	0.01328	0.09	0.16
RIO	B	10	0.1208	0.01346	0.1	0.15
	BGT	14	0.1245	0.00754	0.11	0.14
	K	8	0.1241	0.00889	0.11	0.14
	KHH	59	0.1117	0.00591	0.1	0.13
	KPB	7	0.1203	0.00673	0.11	0.13
	NG	10	0.1179	0.0106	0.1	0.13
	TBK	9	0.1167	0.00606	0.11	0.13
	TLB	11	0.1124	0.00545	0.1	0.12
	TRT	5	0.1372	0.02246	0.12	0.17
	Y	7	0.1208	0.01122	0.1	0.14
	Total	140	0.117	0.0104	0.1	0.17
RLBR	B	10	0.1319	0.00957	0.12	0.15
	BGT	14	0.1347	0.0081	0.12	0.15
	K	8	0.1383	0.00539	0.13	0.14
	KHH	59	0.1251	0.00771	0.11	0.15
	KPB	7	0.132	0.00955	0.12	0.14
	NG	10	0.1332	0.00703	0.13	0.15
	TBK	9	0.1335	0.00627	0.13	0.15
	TLB	11	0.1299	0.00566	0.12	0.14
	TRT	5	0.1496	0.00443	0.14	0.16
	Y	7	0.1385	0.01018	0.13	0.15
	Total	140	0.1307	0.00949	0.11	0.16
RRL	B	10	0.1716	0.01733	0.14	0.2
	BGT	14	0.1766	0.01199	0.15	0.2
	K	8	0.1739	0.01382	0.15	0.19
	KHH	59	0.1716	0.01516	0.13	0.21
	KPB	7	0.1642	0.00822	0.15	0.18
	NG	10	0.1584	0.00792	0.14	0.17
	TBK	9	0.1787	0.00535	0.17	0.18
	TLB	11	0.1694	0.01059	0.16	0.19
	TRT	5	0.1871	0.01082	0.17	0.2
	Y	7	0.1869	0.00974	0.17	0.2
	Total	140	0.1725	0.01428	0.13	0.21

Table 4. Descriptive statistics of 17 morphometric characters from 10 study localities *continued*

Variables	Localities	N	Mean	Std. Deviation	Minimum	Maximum
RPGW	B	10	0.0652	0.00876	0.06	0.08
	BGT	14	0.0705	0.0131	0.05	0.09
	K	8	0.073	0.00981	0.06	0.09
	KHH	59	0.074	0.00994	0.05	0.09
	KPB	7	0.064	0.00804	0.06	0.08
	NG	10	0.0671	0.00858	0.06	0.08
	TBK	9	0.066	0.00386	0.06	0.07
	TLB	11	0.0647	0.0072	0.06	0.08
	TRT	5	0.0723	0.00619	0.06	0.08
	Y	7	0.0727	0.01084	0.06	0.09
	Total	140	0.0706	0.01002	0.05	0.09
RPGL	B	10	0.1004	0.01595	0.08	0.13
	BGT	14	0.11	0.00955	0.09	0.13
	K	8	0.1091	0.01015	0.1	0.12
	KHH	59	0.1047	0.01544	0.07	0.14
	KPB	7	0.0962	0.00987	0.08	0.11
	NG	10	0.1068	0.01356	0.09	0.13
	TBK	9	0.113	0.02152	0.09	0.15
	TLB	11	0.1026	0.01119	0.08	0.12
	TRT	5	0.1158	0.01362	0.1	0.14
	Y	7	0.121	0.01268	0.1	0.14
	Total	140	0.1065	0.01492	0.07	0.15
RHDL	B	10	0.288	0.00774	0.28	0.3
	BGT	14	0.2894	0.01687	0.24	0.31
	K	8	0.2852	0.01226	0.27	0.3
	KHH	59	0.2945	0.01359	0.27	0.33
	KPB	7	0.2851	0.01241	0.27	0.3
	NG	10	0.2384	0.00464	0.23	0.25
	TBK	9	0.2783	0.01697	0.25	0.3
	TLB	11	0.2585	0.00908	0.24	0.27
	TRT	5	0.2889	0.00569	0.28	0.3
	Y	7	0.2906	0.01182	0.28	0.31
	Total	140	0.2843	0.02024	0.23	0.33
RTB	B	10	0.4416	0.01519	0.42	0.47
	BGT	14	0.4254	0.02094	0.39	0.46
	K	8	0.4255	0.01091	0.41	0.44
	KHH	59	0.4414	0.01616	0.4	0.48
	KPB	7	0.42	0.01693	0.39	0.44
	NG	10	0.4042	0.01425	0.39	0.43
	TBK	9	0.4137	0.01616	0.39	0.44
	TLB	11	0.4368	0.01031	0.41	0.45
	TRT	5	0.4229	0.01369	0.4	0.44
	Y	7	0.4107	0.01384	0.39	0.43
	Total	140	0.4308	0.01986	0.39	0.48

Table 4. Descriptive statistics of 17 morphometric characters from 10 study localities *continued*

Variables	**Localities**	**N**	**Mean**	**Std. Deviation**	**Minimum**	**Maximum**
RFL	B	10	0.3872	0.01575	0.36	0.41
	BGT	14	0.3755	0.02145	0.34	0.41
	K	8	0.371	0.01658	0.35	0.39
	KHH	59	0.3901	0.01897	0.35	0.43
	KPB	7	0.3684	0.02116	0.34	0.4
	NG	10	0.3576	0.01285	0.34	0.38
	TBK	9	0.3579	0.01891	0.34	0.39
	TLB	11	0.3692	0.02341	0.33	0.4
	TRT	5	0.3832	0.01618	0.37	0.4
	Y	7	0.3729	0.00899	0.36	0.38
	Total	140	0.3791	0.02155	0.33	0.43

Appendix V

Table 5. Tests of Normality with Shapiro-Wilk test of morphometric measurements

Variables	**Localities**	**Shapiro-Wilk**	**df**	**Sig.**
RIN	B	0.955	10	0.722
	BGT	0.932	14	0.325
	K	0.944	8	0.649
	KHH	0.981	59	0.486
	KPB	0.884	7	0.247
	NG	0.903	10	0.239
	TBK	0.933	9	0.507
	TLB	0.968	11	0.865
	TRT	0.948	5	0.72
	Y	0.994	7	0.998
RSNL	B	0.941	10	0.561
	BGT	0.933	14	0.333
	K	0.903	8	0.304
	KHH	0.985	59	0.657
	KPB	0.944	7	0.679
	NG	0.978	10	0.952
	TBK	0.988	9	0.993
	TLB	0.923	11	0.345
	TRT	0.895	5	0.381
	Y	0.975	7	0.93
RSW	B	0.974	10	0.926
	BGT	0.907	14	0.145
	K	0.876	8	0.174
	KHH	0.974	59	0.243
	KPB	0.932	7	0.572
	NG	0.916	10	0.323
	TBK	0.897	9	0.238
	TLB	0.94	11	0.515
	TRT	0.933	5	0.617
	Y	0.991	7	0.995
RIJW	B	0.91	10	0.279
	BGT	0.948	14	0.531
	K	0.857	8	0.113
	KHH	0.985	59	0.664
	KPB	0.913	7	0.415
	NG	0.931	10	0.454
	TBK	0.962	9	0.815
	TLB	0.948	11	0.614
	Y	0.926	7	0.517

Table 5. Tests of Normality with Shapiro-Wilk test of morphometric measurements *continued*

Variables	**Localities**	**Shapiro-Wilk**	**df**	**Sig.**
RHW	B	0.99	10	0.997
	BGT	0.899	14	0.108
	K	0.905	8	0.321
	KHH	0.98	59	0.428
	KPB	0.909	7	0.388
	NG	0.889	10	0.166
	TBK	0.97	9	0.899
	TLB	0.945	11	0.581
	TRT	0.907	5	0.448
	Y	0.925	7	0.513
RHL	B	0.963	10	0.817
	BGT	0.944	14	0.475
	K	0.987	8	0.988
	KHH	0.992	59	0.962
	KPB	0.949	7	0.721
	NG	0.9	10	0.218
	TBK	0.958	9	0.776
	TLB	0.97	11	0.885
	TRT	0.904	5	0.431
	Y	0.944	7	0.675
RHD	B	0.881	10	0.133
	BGT	0.957	14	0.677
	K	0.909	8	0.346
	KHH	0.991	59	0.942
	KPB	0.954	7	0.765
	NG	0.943	10	0.583
	TBK	0.944	9	0.622
	TLB	0.987	11	0.993
	TRT	0.968	5	0.863
	Y	0.983	7	0.972
RED	B	0.898	10	0.206
	BGT	0.905	14	0.131
	K	0.902	8	0.299
	KHH	0.986	59	0.746
	KPB	0.893	7	0.289
	NG	0.94	10	0.557
	TBK	0.951	9	0.702
	TLB	0.931	11	0.42
	Y	0.864	7	0.166

Table 5. Tests of Normality with Shapiro-Wilk test of morphometric measurements *continued*

Variables	**Localities**	**Shapiro-Wilk**	**df**	**Sig.**
REW	B	0.94	10	0.554
	BGT	0.975	14	0.939
	K	0.932	8	0.538
	KHH	0.985	59	0.699
	KPB	0.866	7	0.171
	NG	0.946	10	0.621
	TBK	0.916	9	0.36
	TLB	0.948	11	0.614
	TRT	0.821	5	0.119
	Y	0.975	7	0.932
RIO	B	0.964	10	0.828
	BGT	0.879	14	0.056
	K	0.982	8	0.971
	KHH	0.977	59	0.328
	KPB	0.938	7	0.619
	NG	0.944	10	0.593
	TBK	0.958	9	0.779
	TLB	0.937	11	0.486
	TRT	0.876	5	0.291
	Y	0.985	7	0.98
RLBR	B	0.987	10	0.991
	BGT	0.984	14	0.992
	K	0.885	8	0.21
	KHH	0.956	59	0.031
	KPB	0.917	7	0.445
	NG	0.802	10	0.015
	TBK	0.896	9	0.227
	TLB	0.917	11	0.297
	TRT	0.972	5	0.89
	Y	0.863	7	0.161
RRL	B	0.929	10	0.443
	BGT	0.981	14	0.982
	K	0.951	8	0.726
	KHH	0.982	59	0.535
	KPB	0.971	7	0.905
	NG	0.879	10	0.126
	TBK	0.924	9	0.427
	TLB	0.922	11	0.333
	TRT	0.975	5	0.905
	Y	0.934	7	0.587

Table 5. Tests of Normality with Shapiro-Wilk test of morphometric measurements *continued*

Variables	**Localities**	**Shapiro-Wilk**	**df**	**Sig.**
RPGW	B	0.925	10	0.403
	BGT	0.959	14	0.705
	K	0.918	8	0.415
	KHH	0.988	59	0.811
	KPB	0.908	7	0.384
	NG	0.906	10	0.256
	TBK	0.968	9	0.874
	TLB	0.953	11	0.688
	TRT	0.916	5	0.502
	Y	0.909	7	0.39
RPGL	B	0.923	10	0.381
	BGT	0.977	14	0.956
	K	0.891	8	0.238
	KHH	0.986	59	0.727
	KPB	0.934	7	0.586
	NG	0.903	10	0.235
	TBK	0.899	9	0.248
	TLB	0.915	11	0.278
	TRT	0.871	5	0.272
	Y	0.96	7	0.818
RHDL	B	0.964	10	0.828
	BGT	0.845	14	0.019
	K	0.886	8	0.213
	KHH	0.988	59	0.835
	KPB	0.962	7	0.832
	NG	0.964	10	0.829
	TBK	0.948	9	0.671
	TLB	0.948	11	0.623
	TRT	0.893	5	0.37
	Y	0.95	7	0.728
RTB	B	0.962	10	0.813
	BGT	0.932	14	0.326
	K	0.951	8	0.725
	KHH	0.991	59	0.942
	KPB	0.908	7	0.385
	NG	0.933	10	0.482
	TBK	0.96	9	0.802
	TLB	0.941	11	0.534
	TRT	0.925	5	0.566
	Y	0.881	7	0.229

Table 5. Tests of Normality with Shapiro-Wilk test of morphometric measurements *continued*

Variables	**Localities**	**Shapiro-Wilk**	**df**	**Sig.**
RFL	B	0.893	10	0.183
	BGT	0.948	14	0.523
	K	0.958	8	0.789
	KHH	0.971	59	0.168
	KPB	0.84	7	0.098
	NG	0.939	10	0.546
	TBK	0.917	9	0.365
	TLB	0.961	11	0.785
	TRT	0.893	5	0.372
	Y	0.963	7	0.846

Appendix VI

Table 6. The haplotype numbers of 16S rRNA gene with the geographical occurrence in southern Thailand

Haplotypes	Number (n)	Location (Number of individual)
1	11	Than Bok Khorani National Park (2) Khao Phanom Bencha National Park (3) Yong waterfall National Park (1) Thung Khai botanic garden (3) Protected area, Kho Hong hill (1) Hala-Bala Wildlife Sanctuary (1)
2	2	Than Bok Khorani National Park (1) Khao Phanom Bencha national park (1)
3	1	Thung Khai botanic garden (1)
4	4	Ngao waterfall national park (4)
5	1	Ngao waterfall national park (1)
6	3	Hala-Bala Wildlife Sanctuary (3)
7	5	Thale Ban National Park (5)
total	27	

Appendix VII

Table 7. Partial sequences of 16S rRNA of *Ingerophrynus parvus* samples

1. Bala1

CAGCCTGCCCAGTGACTTTGTTCAACGGCCGCGGTATCCTAACCGTGCGAAGGTAGCGTAATCACTTGTTCTTTAAATAAGGACTAG
TATGAATGGCACCACGAAGGTTATACTGTCTCCCTTTTCTAATCAGTGAAACTAATCTCCCCGTGAAGAAGCGGGAATAAAAATAT
AAGACGAGAAGACCCTATGGAGCTTCAAACAACATAGCATTTATCATAACCCCCTTAACTCCTGAGTTACACTNAACAGATAATAT
GACTATAAGTTTTCGGTTGGGGTGACCACGGAGCATAACATAACCTCCATGTTGAATCTACCTCTAAACTAAGAACCACGCTTCTAA
GAATCAATACATTGACATTAATTGACCCAATATATTTGACCAACGAACCAAGTTACCCTAGGGATAACAGCGCAATCCACTTCAAG
AGCCCCTATCGACAAGTGGGTTTACGACCTCGATGTTGGATCAGGGTCTCCCAGTGGTGCAGCCGCTACTAAAGGT

2. Bala2

CAGCCTGCCCAGTGACCCTGTTCAACGGCCGCGGTATCCTAACCGTGCGAAGGTAGCGTAATCACTTGTTCTCTAAATAAGGACTA
GTATGAATGGCACCACGAAGGTTACACTGTCTCCCTTTTCTAATCAGTGAAACTAATTTCCCTGTGAAGAAGCAGGGATAAAAATA
TAAGACGAGAAGACCCTATGGAGCTTTAAACAACATAGCATTTATCATAACCTCTTTAACTCCTGAGTTATACTCAATAGATAATAT
GACTATAAGTTTTTGGTTGGGGTGACCACGGAGCATAACATAACCTCCATGTTGAATCCACCTCTAAATTAAGAACCACGCTTCTAA
AAATCAACACCTTGACATTAATTGACCCAATATATTTGATCAACGAACCAAGTTACCCTAGGGATAACAGCGCAATCCACTTCAAG
AGCTCCTATCGACAAGTGGGTTTACGACCTCGATGTTGGATCAGGGTCTCCCAGTGGTGCAGCCGCTACTAAAGGT

3. Bala3

CAGCCTGCCCAGTGACCCTGTTCAACGGCCGCGGTATCCTAACCGTGCGAAGGTAGCGTAATCACTTGTTCTCTAAATAAGGACTA
GTATGAATGGCACCACGAAGGTTACACTGTCTCCCTTTTCTAATCAGTGAAACTAATTTCCCTGTGAAGAAGCAGGGATAAAAATA
TAAGACGAGAAGACCCTATGGAGCTTTAAACAACATAGCATTTATCATAACCTCTTTAACTCCTGAGTTATACTCAATAGATAATAT
GACTATAAGTTTTTGGTTGGGGTGACCACGGAGCATAACATAACCTCCATGTTGAATCCACCTCTAAATTAAGAACCACGCTTCTAA
AAATCAACACCTTGACATTAATTGACCCAATATATTTGATCAACGAACCAAGTTACCCTAGGGATAACAGCGCAATCCACTTCAAG
AGCTCCTATCGACAAGTGGGTTTACGACCTCGATGTTGGATCAGGGTCTCCCAGTGGTGCAGCCGCTACTAAAGGT

Table 7. Partial sequences of 16S rRNA of *Ingerophrynus parvus* samples *continued*

4. B4

CAGCCTGCCCAGTGACCCTGTTCAACGGCCGCGGTATCCTAACCGTGCGAAGGTAGCGTAATCACTTGTTCTCTAAATAAGGACTA
GTATGAATGGCACCACGAAGGTTACACTGTCTCCCTTTTCTAATCAGTGAAACTAATTTCCCTGTGAAGAAGCAGGGATAAAAATA
TAAGACGAGAAGACCCTATGGAGCTTTAAACAACATAGCATTTATCATAACCTCTTTAACTCCTGAGTTATACTCAATAGATAATAT
GACTATAAGTTTTTGGTTGGGGTGACCACGGAGCATAACATAACCTCCATGTTGAATCCACCTCTAAATTAAGAACCACGCTTCTAA
AAATCAACACCTTGACATTAATTGACCCAATATATTTGATCAACGAACCAAGTTACCCTAGGGATAACAGCGCAATCCACTTCAAG
AGCTCCTATCGACAAGTGGGTTTACGACCTCGATGTTGGATCAGGGTCTCCCAGTGGTGCAGCCGCTACTAAAGGT

5. HA01123

CAGCCTGCCCAGTGACTTTGTTCAACGGCCGCGGTATCCTAACCGTGCGAAGGTAGCGTAATCACTTGTTCTTTAAATAAGGACTAG
TATGAATGGCACCACGAAGGTTATACTGTCTCCCTTTTCTAATCAGTGAAACTAATCTCCCCGTGAAGAAGCGGGAATAAAAATAT
AAGACGAGAAGACCCTATGGAGCTTCAAACAACATAGCATTTATCATAACCCCCTTAACTCCTGAGTTACACTNAACAGATAATAT
GACTATAAGTTTTCGGTTGGGGTGACCACGGAGCATAACATAACCTCCATGTTGAATCTACCTCTAAACTAAGAACCACGCTTCTAA
GAATCAATACATTGACATTAATTGACCCAATATATTTGACCAACGAACCAAGTTACCCTAGGGATAACAGCGCAATCCACTTCAAG
AGCCCCTATCGACAAGTGGGTTTACGACCTCGATGTTGGATCAGGGTCTCCCAGTGGTGCAGCCGCTACTAAAGGT

6. LSJ058

CAGCCTGCCCAGTGACTTTGTTCAACGGCCGCGGTATCCTAACCGTGCGAAGGTAGCGTAATCACTTGTTCTTTAAATAAGGACTAG
TATGAATGGCACCACGAAGGTTATACTGTCTCCCTTTTCTAATCAGTGAAACTAATCTCCCCGTGAAGAAGCGGGAATAAAAATAT
AAGACGAGAAGACCCTATGGAGCTTCAAACAACATAGCATTTATCATAACCCCCTTAACTCCTGAGTTATACTNAACAGATAATAT
GACTATAAGTTTTCGGTTGGGGTGACCACGGAGCATAACATAACCTCCATGTTGAATCTACCTCTAAACTAAGAACCACGCTTCTAA
GAATCAATACATTGACATTAATTGACCCAATATATTTGACCAACGAACCAAGTTACCCTAGGGATAACAGCGCAATCCACTTCAAG
AGCCCCTATCGACAAGTGGGTTTACGACCTCGATGTTGGATCAGGGTCTCCCAGTGGTGCAGCCGCTACTAAAGGT

Table 7. Partial sequences of 16S rRNA of *Ingerophrynus parvus* samples *continued*

7. LSJ059

CAGCCTGCCCAGTGACTTTGTTCAACGGCCGCGGTATCCTAACCGTGCGAAGGTAGCGTAATCACTTGTTCTTTAAATAAGGACTAG
TATGAATGGCACCACGAAGGTTATACTGTCTCCCTTTTCTAATCAGTGAAACTAATCTCCCCGTGAAGAAGCGGGAATAAAAATAT
AAGACGAGAAGACCCTATGGAGCTTCAAACAACATAGCATTTATCATAACCCCCTTAACTCCTGAGTTACACTNAACAGATAATAT
GACTATAAGTTTTCGGTTGGGGTGACCACGGAGCATAACATAACCTCCATGTTGAATCTACCTCTAAACTAAGAACCACGCTTCTAA
GAATCAATACATTGACATTAATTGACCCAATATATTTGACCAACGAACCAAGTTACCCTAGGGATAACAGCGCAATCCACTTCAAG
AGCCCCTATCGACAAGTGGGTTTACGACCTCGATGTTGGATCAGGGTCTCCCAGTGGTGCAGCCGCTACTAAAGGT

8. LSJ060

CAGCCTGCCCAGTGACTTTGTTCAACGGCCGCGGTATCCTAACCGTGCGAAGGTAGCGTAATCACTTGTTCTTTAAATAAGGACTAG
TATGAATGGCACCACGAAGGTTATACTGTCTCCCTTTTCTAATCAGTGAAACTAATCTCCCCGTGAAGAAGCGGGAATAAAAATAT
AAGACGAGAAGACCCTATGGAGCTTCAAACAACATAGCATTTATCATAACCCCCTTAACTCCTGAGTTACACTNAACAGATAATAT
GACTATAAGTTTTCGGTTGGGGTGACCACGGAGCATAACATAACCTCCATGTTGAATCTACCTCTAAACTAAGAACCACGCTTCTAA
GAATCAATACATTGACATTAATTGACCCAATATATTTGACCAACGAACCAAGTTACCCTAGGGATAACAGCGCAATCCACTTCAAG
AGCCCCTATCGACAAGTGGGTTTACGACCTCGATGTTGGATCAGGGTCTCCCAGTGGTGCAGCCGCTACTAAAGGT

9. LSJ063

CAGCCTGCCCAGTGACTCTGTTCAACGGCCGCGGTATCCTAACCGTGCGAAGGTAGCGTAATCAATTGTTCTCTAAATAAGGACTA
GTATGAATGGCACCACGAAGGTTATACTGTCTCCCTTTTCTAATCAGTGAAACTAATCTTCCCGTGAAGAAGCGGGAATAAAAATA
TAAGACGAGAAGACCCTATGGAGCTTCAAACAACATAGCATTTATCATAATCCCTTTAGCTCCCGAGCTATACTCAATAGATAATAT
GACTATAAGTTTTCGGTTGGGGTGACCACGGAGCATAACATAACCTCCATGTTGAATTTACCTCTAAACTAAGAACCACGCTTCTAA
GAATCAATACATTGACATTAATTGACCCAATATATTTGACCAACGAACCAAGTTACCCTAGGGATAACAGCGCAATCCACTTCGAG
AGCTCCTATCAACAAGTGGGTTTACGACCTCGATGTTGGATCAGGGTCTCCCAGTGGTGCAGCCGCTACTAAAGGT

Table 7. Partial sequences of 16S rRNA of *Ingerophrynus parvus* samples *continued*

10. LSJ066

CAGCCTGCCCAGTGACTTTGTTCAACGGCCGCGGTATCCTAACCGTGCGAAGGTAGCGTAATCACTTGTTCTTTAAATAAGGACTAG
TATGAATGGCACCACGAAGGTTATACTGTCTCCCTTTTCTAATCAGTGAAACTAATCTCCCCGTGAAGAAGCGGGAATAAAAATAT
AAGACGAGAAGACCCTATGGAGCTTCAAACAACATAGCATTTATCATAACCCCCTTAACTCCTGAGTTACACTNAACAGATAATAT
GACTATAAGTTTTCGGTTGGGGTGACCACGGAGCATAACATAACCTCCATGTTGAATCTACCTCTAAACTAAGAACCACGCTTCTAA
GAATCAATACATTGACATTAATTGACCCAATATATTTGACCAACGAACCAAGTTACCCTAGGGATAACAGCGCAATCCACTTCAAG
AGCCCCTATCGACAAGTGGGTTTACGACCTCGATGTTGGATCAGGGTCTCCCAGTGGTGCAGCCGCTACTAAAGGT

11. LSJ079

CAGCCTGCCCAGTGACTTTGTTCAACGGCCGCGGTATCCTAACCGTGCGAAGGTAGCGTAATCACTTGTTCTTTAAATAAGGACTAG
TATGAATGGCACCACGAAGGTTATACTGTCTCCCTTTTCTAATCAGTGAAACTAATCTCCCCGTGAAGAAGCGGGAATAAAAATAT
AAGACGAGAAGACCCTATGGAGCTTCAAACAACATAGCATTTATCATAACCCCCTTAACTCCTGAGTTATACTNAACAGATAATAT
GACTATAAGTTTTCGGTTGGGGTGACCACGGAGCATAACATAACCTCCATGTTGAATCTACCTCTAAACTAAGAACCACGCTTCTAA
GAATCAATACATTGACATTAATTGACCCAATATATTTGACCAACGAACCAAGTTACCCTAGGGATAACAGCGCAATCCACTTCAAG
AGCCCCTATCGACAAGTGGGTTTACGACCTCGATGTTGGATCAGGGTCTCCCAGTGGTGCAGCCGCTACTAAAGGT

12. LSJ080

CAGCCTGCCCAGTGACTTTGTTCAACGGCCGCGGTATCCTAACCGTGCGAAGGTAGCGTAATCACTTGTTCTTTAAATAAGGACTAG
TATGAATGGCACCACGAAGGTTATACTGTCTCCCTTTTCTAATCAGTGAAACTAATCTCCCCGTGAAGAAGCGGGAATAAAAATAT
AAGACGAGAAGACCCTATGGAGCTTCAAACAACATAGCATTTATCATAACCCCCTTAACTCCTGAGTTACACTNAACAGATAATAT
GACTATAAGTTTTCGGTTGGGGTGACCACGGAGCATAACATAACCTCCATGTTGAATCTACCTCTAAACTAAGAACCACGCTTCTAA
GAATCAATACATTGACATTAATTGACCCAATATATTTGACCAACGAACCAAGTTACCCTAGGGATAACAGCGCAATCCACTTCAAG
AGCCCCTATCGACAAGTGGGTTTACGACCTCGATGTTGGATCAGGGTCTCCCAGTGGTGCAGCCGCTACTAAAGGT

Table 7. Partial sequences of 16S rRNA of *Ingerophrynus parvus* samples *continued*

13. LSJ082

CAGCCTGCCCAGTGACTTTGTTCAACGGCCGCGGTATCCTAACCGTGCGAAGGTAGCGTAATCACTTGTTCTTTAAATAAGGACTAG
TATGAATGGCACCACGAAGGTTATACTGTCTCCCTTTTCTAATCAGTGAAACTAATCTCCCCGTGAAGAAGCGGGAATAAAAATAT
AAGACGAGAAGACCCTATGGAGCTTCAAACAACATAGCATTTATCATAACCCCCTTAACTCCTGAGTTACACTNAACAGATAATAT
GACTATAAGTTTTCGGTTGGGGTGACCACGGAGCATAACATAACCTCCATGTTGAATCTACCTCTAAACTAAGAACCACGCTTCTAA
GAATCAATACATTGACATTAATTGACCCAATATATTTGACCAACGAACCAAGTTACCCTAGGGATAACAGCGCAATCCACTTCAAG
AGCCCCTATCGACAAGTGGGTTTACGACCTCGATGTTGGATCAGGGTCTCCCAGTGGTGCAGCCGCTACTAAAGGT

14. LSJ353

CAGCCTGCCCAGTGACTTTGTTCAACGGCCGCGGTATCCTAACCGTGCGAAGGTAGCGTAATCACTTGTTCTTTAAATAAGGACTAG
TATGAATGGCACCACGAAGGTTATACTGTCTCCCTTTTCTAATCAGTGAAACTAATCTCCCCGTGAAGAAGCGGGAATAAAAATAT
AAGACGAGAAGACCCTATGGAGCTTCAAACAACATAGCATTTATCATAACCCCCTTAACTCCTGAGCTACACTNAACAGATAATAT
GACTATAAGTTTTCGGTTGGGGTGACCACGGAGCATAACATAACCTCCATGTTGAATCTACCTCTAAACTAAGAACCACGCTTCTAA
GAATCAATACATTGACATTAATTGACCCAATATATTTGACCAACGAACCAAGTTACCCTAGGGATAACAGCGCAATCCACTTCAAG
AGCCCCTATCGACAAGTGGGTTTACGACCTCGATGTTGGATCAGGGTCTCCCAGTGGTGCAGCCGCTACTAAAGGT

15. NG4

CAGCCTGCCCAGTGACTTTGTTCAACGGCCGCGGTATCCTAACCGTGCGAAGGTAGCGTAATCACTTGTTCTTTAAATAAGGACTAG
TATGAATGGCACCACGAAGGTTATACTGTCTCCCTTTTCTAATCAGTGAAACTAATCTCCCCGTGAAGAAGCGGGGATAAAAATAT
AAGACGAGAAGACCCTATGGAGCTTCAAACAACATAGCATTTATCATAACCCCTTTAACTCCTGAGTTATACTCAATAGATGATAT
GACTATAAGTTTTCGGTTGGGGTGACCACGGAGCATAACATAACCTCCATGTTGAATCTAACTCTAAACTAAGAACCACGCTTCTAA
GAATCAATACATTGACATTAATTGACCCAATATATTTGACCAACGAACCAAGTTACCCTAGGGATAACAGCGCAATCCACTTCAAG
AGCCCCTATCGACAAGTGGGTTTACGACCTCGATGTTGGATCAGGGTCTCCCAGTGGTGCAGCCGCTACTAAAGGT

Table 7. Partial sequences of 16S rRNA of *Ingerophrynus parvus* samples *continued*

16. NG5

CAGCCTGCCCAGTGACTTTGTTCAACGGCCGCGGTATCCTAACCGTGCGAAGGTAGCGTAATCACTTGTTCTTTAAATAAGGACTAG
TATGAATGGCACCACGAAGGTTATACTGTCTCCCTTTTCTAATCAGTGAAACTAATCTCCCCGTGAAGAAGCGGGGATAAAAATAT
AAGACGAGAAGACCCTATGGAGCTTCAAACAACATAGCATTTATCATAACCCCTTTAACTCCTGAGTTATACTCAATAGATGATAT
GACTATAAGTTTTCGGTTGGGGTGACCACGGAGCATAACATAACCTCCATGTTGAATCTAACTCTAAACTAAGAACCACGCTTCTAA
GAATCAATACATTGACATTAATTGACCCAATATATTTGACCAACGAACCAAGTTACCCTAGGGATAACAGCGCAATCCACTTCAAG
AGCCCCTATCGACAAGTGGGTTTACGACCTCGATGTTGGATCAGGGTCTCCCAGTGGTGCAGCCGCTACTAAAGGT

17. NG6

CAGCCTGCCCAGTGACTTTGTTCAACGGCCGCGGTATCCTAACCGTGCGAAGGTAGCGTAATCACTTGTTCTTTAAATAAGGACTAG
TATGAATGGCACCACGAAGGTTATACTGTCTCCCTTTTCTAATCAGTGAAACTAATCTCCCCGTGAAGAAGCGGGAATAAAAATAT
AAGACGAGAAGACCCTATGGAGCTTCAAACAACATAGCATTTATCATAACCCCTTTAACTCCTGAGTTATACTCAATAGATGATAT
GACTATAAGTTTTCGGTTGGGGTGACCACGGAGCATAACATAACCTCCATGTTGAATTTAACTCTAAACTAAGAACCACGCTTCTAA
GAATCAATACATTGACATTAATTGACCCAATATATTTGACCAACGAACCAAGTTACCCTAGGGATAACAGCGCAATCCACTTCAAG
AGCCCCTATCGACAAGTGGGTTTACGACCTCGATGTTGGATCAGGGTCTCCCAGTGGTGCAGCCGCTACTAAAGGT

18. NG8

CAGCCTGCCCAGTGACTTTGTTCAACGGCCGCGGTATCCTAACCGTGCGAAGGTAGCGTAATCACTTGTTCTTTAAATAAGGACTAG
TATGAATGGCACCACGAAGGTTATACTGTCTCCCTTTTCTAATCAGTGAAACTAATCTCCCCGTGAAGAAGCGGGGATAAAAATAT
AAGACGAGAAGACCCTATGGAGCTTCAAACAACATAGCATTTATCATAACCCCTTTAACTCCTGAGTTATACTCAATAGATGATAT
GACTATAAGTTTTCGGTTGGGGTGACCACGGAGCATAACATAACCTCCATGTTGAATCTAACTCTAAACTAAGAACCACGCTTCTAA
GAATCAATACATTGACATTAATTGACCCAATATATTTGACCAACGAACCAAGTTACCCTAGGGATAACAGCGCAATCCACTTCAAG
AGCCCCTATCGACAAGTGGGTTTACGACCTCGATGTTGGATCAGGGTCTCCCAGTGGTGCAGCCGCTACTAAAGGT

Table 7. Partial sequences of 16S rRNA of *Ingerophrynus parvus* samples *continued*

19. NG9

CAGCCTGCCCAGTGACTTTGTTCAACGGCCGCGGTATCCTAACCGTGCGAAGGTAGCGTAATCACTTGTTCTTTAAATAAGGACTAG
TATGAATGGCACCACGAAGGTTATACTGTCTCCCTTTTCTAATCAGTGAAACTAATCTCCCCGTGAAGAAGCGGGGATAAAAATAT
AAGACGAGAAGACCCTATGGAGCTTCAAACAACATAGCATTTATCATAACCCCTTTAACTCCTGAGTTATACTCAATAGATGATAT
GACTATAAGTTTTCGGTTGGGGTGACCACGGAGCATAACATAACCTCCATGTTGAATCTAACTCTAAACTAAGAACCACGCTTCTAA
GAATCAATACATTGACATTAATTGACCCAATATATTTGACCAACGAACCAAGTTACCCTAGGGATAACAGCGCAATCCACTTCAAG
AGCCCCTATCGACAAGTGGGTTTACGACCTCGATGTTGGATCAGGGTCTCCCAGTGGTGCAGCCGCTACTAAAGGT

20. TLB4

CAGCCTGCCCAGTGACCCTGTTCAACGGCCGCGGTATCCTAACCGTGCGAAGGTAGCGTAATCAATTGTTCTCTAAATAAGGACTA
GTATGAATGGCACCACGAAGGTTATACTGTCTCCCTTTTCTAATCAGTGAAACTAATCTCCCTGTGAAGAAGCAGGGATAAAAATA
TAAGACGAGAAGACCCTATGGAGCTTTAAACAACATAGCATTTATCATAACCTCTTTAACTCCTGAGTTATACTCAATAGATAATAT
GACTATAAGTTTTTGGTTGGGGTGACCACGGAGCATAACATAACCTCCATGTTGAATTCACCTATAAATTAAGAACCACGCTTCTAA
AAATCAACACCTTGACATTAATTGACCCAATATATTTGATCAACGAACCAAGTTACCCTAGGGATAACAGCGCAATCCACTTCAAG
AGCTCCTATCGACAAGTGGGTTTACGACCTCGATGTTGGATCAGGGTCTCCCAGTGGTGCAGCCGCTACTAAAGGT

21. TLB5

CAGCCTGCCCAGTGACCCTGTTCAACGGCCGCGGTATCCTAACCGTGCGAAGGTAGCGTAATCAATTGTTCTCTAAATAAGGACTA
GTATGAATGGCACCACGAAGGTTATACTGTCTCCCTTTTCTAATCAGTGAAACTAATCTCCCTGTGAAGAAGCAGGGATAAAAATA
TAAGACGAGAAGACCCTATGGAGCTTTAAACAACATAGCATTTATCATAACCTCTTTAACTCCTGAGTTATACTCAATAGATAATAT
GACTATAAGTTTTTGGTTGGGGTGACCACGGAGCATAACATAACCTCCATGTTGAATTCACCTATAAATTAAGAACCACGCTTCTAA
AAATCAACACCTTGACATTAATTGACCCAATATATTTGATCAACGAACCAAGTTACCCTAGGGATAACAGCGCAATCCACTTCAAG
AGCTCCTATCGACAAGTGGGTTTACGACCTCGATGTTGGATCAGGGTCTCCCAGTGGTGCAGCCGCTACTAAAGGT

Table 7. Partial sequences of 16S rRNA of *Ingerophrynus parvus* samples *continued*

22. TLB8

CAGCCTGCCCAGTGACCCTGTTCAACGGCCGCGGTATCCTAACCGTGCGAAGGTAGCGTAATCAATTGTTCTCTAAATAAGGACTA
GTATGAATGGCACCACGAAGGTTATACTGTCTCCCTTTTCTAATCAGTGAAACTAATCTCCCTGTGAAGAAGCAGGGATAAAAATA
TAAGACGAGAAGACCCTATGGAGCTTTAAACAACATAGCATTTATCATAACCTCTTTAACTCCTGAGTTATACTCAATAGATAATAT
GACTATAAGTTTTTGGTTGGGGTGACCACGGAGCATAACATAACCTCCATGTTGAATTCACCTATAAATTAAGAACCACGCTTCTAA
AAATCAACACCTTGACATTAATTGACCCAATATATTTGATCAACGAACCAAGTTACCCTAGGGATAACAGCGCAATCCACTTCAAG
AGCTCCTATCGACAAGTGGGTTTACGACCTCGATGTTGGATCAGGGTCTCCCAGTGGTGCAGCCGCTACTAAAGGT

23. TLB10

CAGCCTGCCCAGTGACCCTGTTCAACGGCCGCGGTATCCTAACCGTGCGAAGGTAGCGTAATCAATTGTTCTCTAAATAAGGACTA
GTATGAATGGCACCACGAAGGTTATACTGTCTCCCTTTTCTAATCAGTGAAACTAATCTCCCTGTGAAGAAGCAGGGATAAAAATA
TAAGACGAGAAGACCCTATGGAGCTTTAAACAACATAGCATTTATCATAACCTCTTTAACTCCTGAGTTATACTCAATAGATAATAT
GACTATAAGTTTTTGGTTGGGGTGACCACGGAGCATAACATAACCTCCATGTTGAATTCACCTATAAATTAAGAACCACGCTTCTAA
AAATCAACACCTTGACATTAATTGACCCAATATATTTGATCAACGAACCAAGTTACCCTAGGGATAACAGCGCAATCCACTTCAAG
AGCTCCTATCGACAAGTGGGTTTACGACCTCGATGTTGGATCAGGGTCTCCCAGTGGTGCAGCCGCTACTAAAGGT

24. KHH1

CAGCCTGCCCAGTGACTTTGTTCAACGGCCGCGGTATCCTAACCGTGCGAAGGTAGCGTAATCACTTGTTCTTTAAATAAGGACTAG
TATGAATGGCACCACGAAGGTTATACTGTCTCCCTTTTCTAATCAGTGAAACTAATCTCCCCGTGAAGAAGCGGGAATAAAAATAT
AAGACGAGAAGACCCTATGGAGCTTCAAACAACATAGCATTTATCATAACCCCCTTAACTCCTGAGTTACACTNAACAGATAATAT
GACTATAAGTTTTCGGTTGGGGTGACCACGGAGCATAACATAACCTCCATGTTGAATCTACCTCTAAACTAAGAACCACGCTTCTAA
GAATCAATACATTGACATTAATTGACCCAATATATTTGACCAACGAACCAAGTTACCCTAGGGATAACAGCGCAATCCACTTCAAG
AGCCCCTATCGACAAGTGGGTTTACGACCTCGATGTTGGATCAGGGTCTCCCAGTGGTGCAGCCGCTACTAAAGGT

Table 7. Partial sequences of 16S rRNA of *Ingerophrynus parvus* samples *continued*

25. WTS338

CAGCCTGCCCAGTGACTTTGTTCAACGGCCGCGGTATCCTAACCGTGCGAAGGTAGCGTAATCACTTGTTCTTTAAATAAGGACTAG
TATGAATGGCACCACGAAGGTTATACTGTCTCCCTTTTCTAATCAGTGAAACTAATCTCCCCGTGAAGAAGCGGGAATAAAAATAT
AAGACGAGAAGACCCTATGGAGCTTCAAACAACATAGCATTTATCATAACCCCCTTAACTCCTGAGTTACACTNAACAGATAATAT
GACTATAAGTTTTCGGTTGGGGTGACCACGGAGCATAACATAACCTCCATGTTGAATCTACCTCTAAACTAAGAACCACGCTTCTAA
GAATCAATACATTGACATTAATTGACCCAATATATTTGACCAACGAACCAAGTTACCCTAGGGATAACAGCGCAATCCACTTCAAG
AGCCCCTATCGACAAGTGGGTTTACGACCTCGATGTTGGATCAGGGTCTCCCAGTGGTGCAGCCGCTACTAAAGGT

26. WTS369

CAGCCTGCCCAGTGACTTTGTTCAACGGCCGCGGTATCCTAACCGTGCGAAGGTAGCGTAATCACTTGTTCTTTAAATAAGGACTAG
TATGAATGGCACCACGAAGGTTATACTGTCTCCCTTTTCTAATCAGTGAAACTAATCTCCCCGTGAAGAAGCGGGAATAAAAATAT
AAGACGAGAAGACCCTATGGAGCTTCAAACAACATAGCATTTATCATAACCCCCTTAACTCCTGAGTTACACTNAACAGATAATAT
GACTATAAGTTTTCGGTTGGGGTGACCACGGAGCATAACATAACCTCCATGTTGAATCTACCTCTAAACTAAGAACCACGCTTCTAA
GAATCAATACATTGACATTAATTGACCCAATATATTTGACCAACGAACCAAGTTACCCTAGGGATAACAGCGCAATCCACTTCAAG
AGCCCCTATCGACAAGTGGGTTTACGACCTCGATGTTGGATCAGGGTCTCCCAGTGGTGCAGCCGCTACTAAAGGT

27. WTS370

CAGCCTGCCCAGTGACTTTGTTCAACGGCCGCGGTATCCTAACCGTGCGAAGGTAGCGTAATCACTTGTTCTTTAAATAAGGACTAG
TATGAATGGCACCACGAAGGTTATACTGTCTCCCTTTTCTAATCAGTGAAACTAATCTCCCCGTGAAGAAGCGGGAATAAAAATAT
AAGACGAGAAGACCCTATGGAGCTTCAAACAACATAGCATTTATCATAACCCCCTTAACTCCTGAGTTACACTNAACAGATAATAT
GACTATAAGTTTTCGGTTGGGGTGACCACGGAGCATAACATAACCTCCATGTTGAATCTACCTCTAAACTAAGAACCACGCTTCTAA
GAATCAATACATTGACATTAATTGACCCAATATATTTGACCAACGAACCAAGTTACCCTAGGGATAACAGCGCAATCCACTTCAAG
AGCCCCTATCGACAAGTGGGTTTACGACCTCGATGTTGGATCAGGGTCTCCCAGTGGTGCAGCCGCTACTAAAGGT

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List of Publication and Proceeding (If Possible)

Srion, L., Wangkulangkul, S. and Aowpol, A., 2017. Morphological variation of *Ingerophrynus parvus* (Boulenger, 1887) in peninsular Thailand. Proceedings of the 2nd International Conference on Biosciences (ICoBio), Bogor, Indonesia, 8th – 10th July 2017.