Taxonomic Review of *Rhinolophus pusillus* and *Rhinolophus lepidus* (Chiroptera: Rhinolophidae) in Thailand

Ariya Dejtaradol

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Ecology (International Program)

Prince of Songkla University

2009

Thesis Title	A Taxonomic Review of *Rhinolophus pusillus* and *Rhinolophus lepidus* (Chiroptera: Rhinolophidae) in Thailand
Author	Miss Ariya Dejtaradol
Major Program	Ecology (International Program)

Major Advisor	**Examining Committee:**
……………………………………….	.....…………..………………Chairperson
(Asst. Prof. Dr. Sara Bumrungsri)	(Assoc. Prof. Dr. Kitichate Sridith)
	……..……..…………………………..……
Co-advisor	(Dr. Chavalit Vidthayanon)
……………………………………….	……..……..…………………………..……
(Assoc. Prof. Dr. Chutamas Satasook)	(Asst. Prof. Dr. Sara Bumrungsri)
……………………………………….	……..……..…………………………..……
(Dr. Paul J. J. Bates)	(Assoc. Prof. Dr. Chutamas Satasook)

The Graduate School, Prince of Songkla University, has approved this thesis as partial fulfillment of the requirements for Master of Science Degree in Ecology (International Program)

………………………………………...
(Assoc. Prof. Dr. Krerkchai Thongnoo)
Dean of Graduate School

ชื่อวิทยานิพนธ์ อนุกรมวิธานของค้างคาวมงกุฎเล็กและมงกุฎจมูกแหลมเหนือในประเทศไทย

ผู้เขียน นางสาวอริยา เดชธราดล

สาขาวิชา นิเวศวิทยา (นานาชาติ)

ปีการศึกษา 2551

บทคัดย่อ

ค้างคาวมงกุฎเล็ก (*Rhinolophus pusillus*) และค้างคาวมงกุฎจมูกแหลมเหนือ (*R. lepidus)* เป็นค้างคาวในกลุ่มค้างคาวมงกุฎเล็ก ซึ่งทั้งสองชนิดเป็นค้างคาวมงกุฎที่มีลักษณะทางสัณฐานวิทยาและขนาดทั้งภายนอก และกะโหลกคล้ายคลึงกันมาก การศึกษาด้านอนุกรมวิธานก่อนหน้านี้ได้ชี้ให้เห็นถึงความสับสนของอนุกรมวิธานในค้างคาวทั้ง 2 ชนิดนี้ ซึ่งแต่ละชนิดมีหลายชื่อพ้อง (synonyms) และหลายชนิดย่อย (subspecies) ในการศึกษาอนุกรมวิธานในอดีตมักใช้เทคนิคทั้งเชิงปริมาณและเชิงคุณภาพเพื่อศึกษาลักษณะทางสัณฐานวิทยาภายนอกและกะโหลก แต่ในปัจจุบันมีความก้าวหน้าในการวิเคราะห์มากขึ้นเพื่อใช้ช่วยในการจำแนกชนิด รวมไปถึงการใช้ความถี่คลื่นเสียงของค้างคาวในการช่วยจำแนกชนิดด้วย จากการศึกษาเก็บตัวอย่างค้างคาวกลุ่มค้างคาวมงกุฎเล็กจากทั่วประเทศ เมื่อใช้ลักษณะทางสัณฐานวิทยาช่วยในการจำแนก พบว่าสามารถแบ่งได้เป็น 3 กลุ่ม อย่างชัดเจน ได้แก่ ค้างคาวมงกุฎเล็ก (*Rhinolophus pusillus*) ค้างคาวจมูกแหลมเหนือ (*Rhinolophus lepidus*) ซึ่งเป็นชนิดย่อย *Rhinolophus lepidus refulgens* ที่พบทางภาคใต้ของประเทศไทย และค้างคาวมงกุฎ (*Rhinolophus* sp.) จากอุทยานแห่งชาติภูสวนทราย จังหวัดเลยซึ่งมีความแตกต่างจากค้างคาวชนิดอื่นๆ แต่เมื่อใช้คลื่นความถี่เสียงและขนาดของลักษณะต่างๆ มาช่วยในการจำแนก พบว่ามี 4 กลุ่ม โดยค้างคาวกลุ่มที่สี่ คือ ค้างคาวมงกุฎ (*Rhinolophus* sp.) จากเขาสมอคอน จังหวัดลพบุรี ซึ่งมีความถี่ของคลื่นเสียงสูงสุด จากหลักฐานดังกล่าวแสดงให้เห็นถึงความซับซ้อนของค้างคาวในกลุ่มนี้ และยืนยันได้ว่าในประเทศไทยมีค้างคาวในกลุ่มนี้มากกว่า 2 ชนิด การศึกษาทางด้านชีวโมเลกุลน่าจะเป็นอีกเทคนิคหนึ่งที่จะมาช่วยยืนยันผลการศึกษาในครั้งนี้ได้เป็นอย่างดี

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Major Program	Ecology (International Program)
Academic Year	2008

ABSTRACT

Rhinolophus pusillus and *R. lepidus* are horseshoe bats which have essentially similar external morphology and cranio-dental measurements. Previous taxonomic studies suggested that their taxonomy was confusing as there were number of synonyms and a smaller number of subspecies. In the past, taxonomic studies of the two species based on both qualitative and quantitative characters of external and cranio-dental morphology. Today, morphometric analysis techniques which are more sophisticated and provide better result are commonly applied to discriminate between size-overlapped taxa. Currently, species identification can also be supported with analysis of echolocation calls. From the examination of bats in *R. pusillus* group throughout Thailand, there are possibly at least 3 taxa within this rhinolophid group based on morphological characters: *R. pusillus*, *R. lepidus* which is referred to subspecies *R. l. refulgens* that found in southern Thailand, and *Rhinolophus* sp. from Phu Suan Sai National Park, Loei Province which is significantly different from other taxa in this group. However, they can be divided into four groups based on echolocation frequency and morphometrics: *R. l. refulgens*, *R. pusillus*, *Rhinolophus* sp. from Loei Province and *Rhinolophus* sp. from Khao Samo Khon, Lopburi Province which has highest frequency of echolocation calls among the bats in this group. With the current evidences, it indicated the presence of the species complex in *R. pusillus* group, and there are more than two taxa of this group in Thailand. The further molecular study maybe warrant for taxonomic classification of these bats.

ACKNOWLEDGEMENTS

I am deeply indebted to Assistant Professor Dr. Sara Bumrungsri and Associate Professor Dr. Chutamas Satasook for their advice and recommendation. I am greatly indebted to Dr. Paul J. J. Bates of the Harrison Institute, UK, for his help and outstanding supervision. I would also like to thank Dr. David Harrison and Malcolm Pearch of the Harrison Institute for their help and advice.

I am also grateful to the Thesis Examining Committees, including Associate Professor Dr. Kitichate Sridith of Department of Biology, Faculty of Science, Prince of Songkla University, and Dr. Chavalit Vidthayanon of World Wildlife Fund-Thailand, for their correction and valuable suggestion.

This work was supported by Darwin Initiative (DEFRA) of the UK Government, the TRF/BIOTEC Special Program for Biodiversity Research and Training grant BRT T_151001, and Graduate school of Prince of Songkla University (PSU).

I would like to thank Director-general of the Department of National Park, Wildlife and Plant Conservation (DNP) for research permission, to Prateep Rojanadilok, Siriporn Thong-Aree and Saksit Simcharoen, the officers of DNP for their kind cooperation. My thanks are also due to Dr. Surachit Waengsothorn of Thailand Institute of Scientific and Technological Research (TISTR) who permits me loan specimens from TISTR. I also thank Bounsavane Douangboubpha, Phansamai Phommexay, Phouthone Kingsada, Pipat Soisook, Tuanjit Srithongchuay, and all students at Bat Research Unit for their help in fieldwork and specimens preparation.

I would like to thanks Piyathip Piyapan, Medhi Yokubol, Sunate Karapan and Kwan Nualcharoen for their advice and generous help. Elsewhere, I would like to thank Sebastien Puechmaille of University College Dublin for his advice. I also thank Beatrix Lanzinger for generous help and encouragement when I stayed at Harrison Institute, UK.

I also thank Phannee Sa-ardrit, Bongkot Wichachucherd and Piyapong Petchabun and all staffs of the Princess Maha Chakri Sirindhorn Natural History Museum for their kindly help during a long working with the collections in the museum. I am also grateful to Paula Jenkins and all staff of the mammal department and the libraries of The Natural History Museum, London for their help. I am grateful to all lecturers and staffs of Department of Biology, Faculty of Science, Prince of Songkla University for generous help.

Thanks are also due to Vu Dinh Thong and Pham Duc Tien of Institute of Ecology and Biological Resources (IEBR), Vietnam for their specimens deposited in the Harrison Zoological Museum. I would like to thank Neil Furey for echolocation data of *Rhinolophus pusillus* from Vietnam. I also thank Charles M. Francis for the molecular data from Bats of Southeast Asia Project.

Finally, I would like to thank my father, my mother and my family for their support and encouragement.

Ariya Dejtaradol

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CHAPTER 1
INTRODUCTION

With 119 species, the bat fauna of Thailand is diverse (Bumrungsri *et al.*, 2006). The number publications relating to Thai bats is also relatively large, ranging from the first, simple paper of Finlayson (1826) to the more comprehensive review of Thai mammals by Lekagul and McNeely (1977, 1988). Over the years, most authors have been primarily concerned with simple morphometric taxonomy and the listing of new or interesting species records. There are many examples of such papers in Bumrungsri *et al.* (2006), including Gyldenstope (1917), Shamel (1930 and 1942), Hill and Thonglongya (1972), Thonglongya (1973), Thonglongya and Hill (1974), Hill (1975), Robinson *et al.* (1995 and 1996), Kock and Kovac (2000) and Thong *et al.* (2006).

In addition to taxonomy, there were a number of other topics that were, or have become, of interest to bat scientists. For example, in the 1980s there were several studies of the karyology of Thai bats, with publications such as Harada *et al.* (1982), McBee *et al.* (1986) and Hood *et al.* (1988). Subsequently, there has been a growing interest in the ecology and behaviour of Thai bats. Papers resulting from this research include Miller *et al.* (1988), Duangkhae (1990 and 1991), Bumrungsri (2002) and Boonkird and Wanghongsa (2002 and 2004).

Some topics, such as the study of echolocation and bat genetics that have proved popular elsewhere, for example in Europe, North America and even Malaysia (eg. Kingston *et al.,* 2006), have remained neglected in Thailand. Surlykke *et al.* (1993) published a paper on the echolocation calls of two species of small Thai bats and Thong *et al.* (2006) included data on the calls on two species of vespertilionids. However, little other information is currently available. In genetics, Hulva and Horacek (2002) undertook a molecular study of *Craseonycteris* but currently no other publications are available on this aspect of Thai bats (Bumrungsri *et al.,* 2006).

With a small number of exceptions, identification keys have also been neglected. As part of their overview of the Indo-Malayan region, morphometric keys are available in Corbet and Hill (1992). These are based on external and cranio-dental characters but demand a considerable understanding of taxonomy and anatomy to be used correctly. Csorba *et al.* (2003) had comprehensive keys to the Rhinolophidae, including those from Thailand. Waengsothorn (1995) constructed a field key for the Thai hipposiderid bats and Bumrungsri and Racey (2005) included field keys for fruit bats of the genus *Cynopterus*.

The current study is concerned with two species of rhinolophid, the Least horseshoe bat, *Rhinolophus pusillus* Temminck, 1834, and Blyth's horseshoe bat, *R. lepidus* Blyth, 1844. These two taxa have essentially similar external morphology and the cranio-dental measurements overlap (Bates and Harrison, 1997). Moreover, previous taxonomic studies suggest that their taxonomy is confusing as there are number of synonyms and a smaller number of subspecies, the validity of which are open to question (Csorba *et al.*, 2003; Pearch *et al.*, 2003).

In the past, taxonomic studies of the two species have been of the classical kind with a qualitative and quantitative review of the external and cranio-dental morphology (for example (Andersen, 1905, 1907, 1918; Allen, 1923, 1928; Tate and Archbold, 1939; Sinha, 1973; Hill and Yoshiyuki, 1980; Yoshiyuki, 1990, Corbet and Hill, 1992; Bates and Harrison, 1997). Today, morphometric analysis techniques are more sophisticated and better able to discriminate between taxa. Species identification can also be supported with recordings and analysis of echolocation calls and it has been shown elsewhere that the calls of many bat species are a useful aid to identification at genus and species level (Russo and Jones, 2002).

The current study aims to answer the question of how many species in the *R. pusillus* group (*sensu* Corbet and Hill, 1992) are present in Thailand and can they be identified on the basis of discrete differences in morphometrics and/or echolocation. The objective is to define the species characters of *Rhinolophus lepidus* and *R. pusillus* (and any associated taxa) within Thailand (with regard to intraspecific and interspecific variation). To answer this question, specimens from both within the country and elsewhere from South-East Asia will have to be examined.

Geographical variation in morphology will be compared to geographical variation in echolocation calls. It is not known whether the patterns will overlap. However, it is known that the echolocation calls of bats that live in the different habitats have different duration and frequency (Schnitzler and Kalko, 1998). If the bats have different echolocation call, they should adapt their wing morphology, pattern of flight and other characters to be appropriate with their calls so the external and cranial morphology should relate with echolocation call. Thus morphology and echolocation call could help to identify these bats better.

CHAPTER 2
LITERATURE REVIEW

Bat taxonomy

The classification of bats used today is based on the system developed by Miller in 1907. Miller classified 16 families of Microchiroptera on basis of their bone structure (Neuweiler, 2000). The family usually unites a number of related genera but again some bat families contain only on especially distinctive and isolated genus. Genera are sometimes divided into two or more subgenera, and families similarly into subfamilies (Hill and Smith, 1984).

The genera groups the species together with common similarities but occasionally have only a single, prominently characterised species. They are customarily recognised by more far reaching external, cranial and dental characters, different workers placing a greater or lesser emphasis on different features. The wide range of variation found in bats features and has led to a large number of recognised genera, although sometimes these are based on relatively small differences. Nowadays, the study of bats is resurgence and in their relationships with each other at all levels of classification has led to the application of new and modern techniques and sophisticated equipment. So, the traditional classification might be changed. (Hill and Smith, 1984)

The species is the lowest major category in classification and the many different species of bats are recognized by such features as differences in body or skull size, in colour, in such structures as their noseleaves or ears, or by small differences in cranial or dental morphology. Such features are used by the taxonomist to determine to which species any given bat belongs and to indicate relationships to other species. Often the differences between species are very small and quite subtle, and careful study is required to distinguish them, particularly when closely related species occur together. (Hill and Smith, 1984)

Family Rhinolophidae (Horseshoe bats)

The bats of this family are found throughout the Old World from Europe and Africa to Japan, Philippines and Australia. There is only one modern genus *Rhinolophus* in the family which includes approximately 70 species. They are known from fossils in the late Eocene of Europe (Hill and Smith, 1984).

These bats have a characteristic noseleaf with a horseshoe-shaped cutaneous plate that surrounds and surmounts the nostrils. This noseleaf is very characteristic and complex modifications which consist of an erect posterior lancet that stands erect behind the horseshoe and above the tiny eyes, a lower horizontal horseshoe-shaped expansion surrounding the nostrils which partly or fully covers the upper lip, a perpendicular median sella which it is a flat, strap-like structure that rises from behind the nostrils and stands erect in the middle of the noseleaf and connecting process. The sella is attached to the lancet by a connecting process that acts like a buttress (Figure 1, 2). The noseleaf may be used to identify species as well as groups of species. The noseleaf of rhinolophid superficially resembles that of the closely related family Hipposideridae, yet there are pronounced differences between the two. (Hill and Smith, 1984). In addition to their unique noseleaf, the ears are moderate to large and lack a tragus. The tail is will developed and is completely enclosed in the uropatagium. Beside the two functional mammae on the chest, there are two additional teat-like processes not connect to mammary gland found on the abdominal region of adult females (Csorba *et al.*, 2003).

In the skull the premaxillae are represented by projecting narrow palatal branches only; these two bones are partly cartilaginous and are not fused with each other or with the maxillae. Postorbital processes absent, the palate is deeply incised both anteriorly and posteriorly. The tympanic developed. The skull is always with rostral inflations. The basic dental formula is 1123/2133 but the anterior upper premolars and the middle lower premolars are often missing. The upper incisors are very small but usually well formed; the lower incisors are trifid. The molariform teeth do not show any particular modification, M^1 and M^2 are without hypocone, M^3 almost always with three commissures (Csorba *et al.*, 2003).

Genus *Rhinolophus* Lacépède, 1799

The genus *Rhinolophus* is the only genus in the family Rhinolophidae that belongs to the superfamily Rhinolophoidae Gray, 1985. Andersen (1905, 1918) was the first author who reviewed the Rhinolophidae and was the first to construct a phylogenetic tree for the family.

Many details of the phylogeny and taxonomies *of Rhinolophus* species remain unresolved. Species are distinguished by body size, the position of the anterior upper premolar, and subtle, often difficult to assess differences in the shape and size of nose leaves around the nostrils (Maree and Grant, 1997).

Another study of cranial morphology of the 10 southern African species failed to distinguish some of these species, and it is not clear whether two pairs of morphologically similar allopatric species *(R. denti* and *R. swinnyi, R. capensis* and *R. darlingi)* represent geographical populations or full species (Erasmus and Rautenbach, 1984; Rautenbach, 1986 quoted in Maree and Grant, 1997).

Andersen (1905) found in his taxonomic and phylogenetic arrangement that all *Rhinolophus* groups with Palaeotropical distribution (*R. simplex*, *R. lepidus*, *R. philippinensis*, *R. macrotis*) had the most ancestral species in Asia and the most derived forms in Africa. Bogdanowicz and Owen (1992) studied about phylogenetic analyses of the bat family Rhinolophidae by morphometrics of 62 species. Morphological analyses indicate that they most likely originated in southeastern Asia but which presently inhabits Oriental, Australian, Palaearctic, and Ethiopian zoogeographical provinces. Maree and Grant (1997) studied allozyme variability in bat species occurred in southern Africa, but it is uncertain which species represent dispersals from Eurasia through North Africa and which have resulted from speciation in Africa.

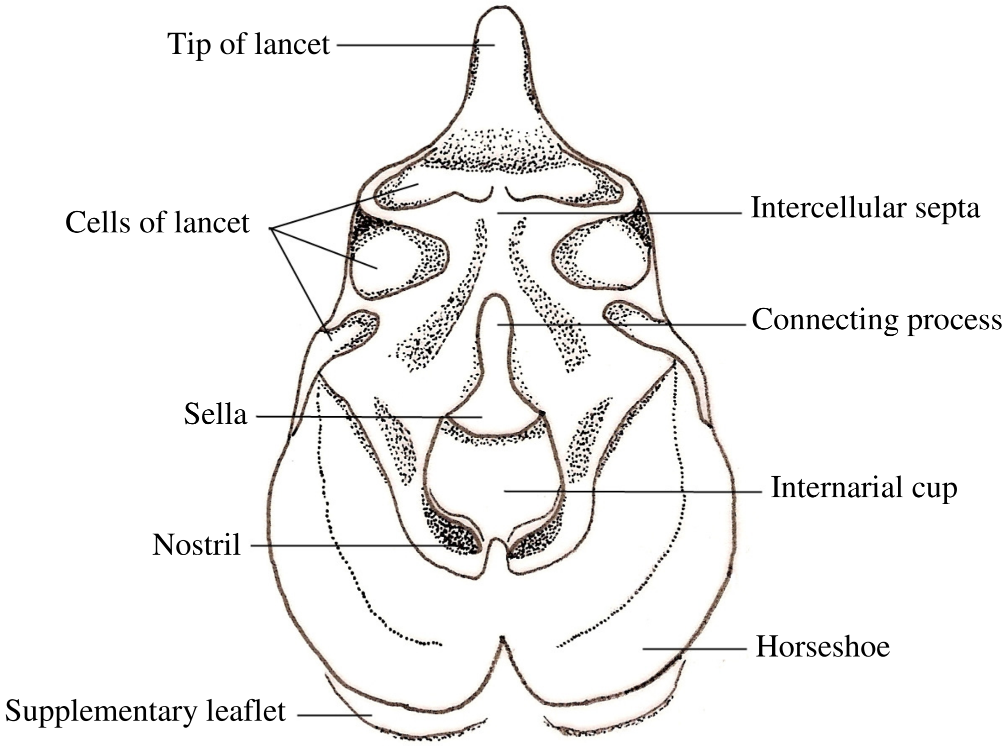


Figure 1. Front view of noseleaf of *Rhinolophus lepidus*

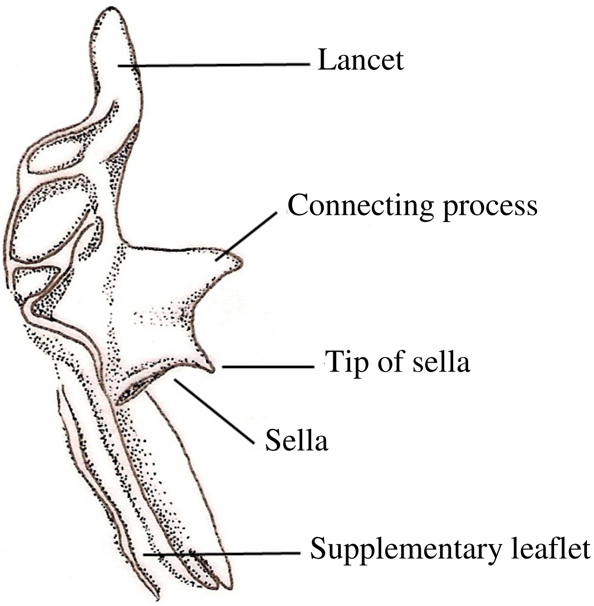


Figure 2. Lateral view of noseleaf of *Rhinolophus lepidus*

Groups of *Rhinolophus*

Andersen (1905) arranged the species of *Rhinolophus* into six groups by external and cranial morphology, named after the species: *R. simplex*, *R. lepidus*, *R. midas*, *R. philippinensis*, *R. macrotis* and *R. arcuatus*. In 1918, He renamed the groups as *R. megaphyllus* (*R. simplex*), *R. pusillus* (*R. lepidus*), *R. luctus* (*R. philippinensis*), *R. euryotis* (*R. arcuatus*) and *R. hipposideros* (*R. midas*). Some groups contain a number of subgroups. Later authors have further changed names of the groups, slightly modified some of them, and incorporated newly described forms into them.

Bogdanowicz (1992) proposed 11 phenetic groups of Rhinolophidae including; *megaphyllus*, *rouxii*, *euryotis*, *pearsonii*, *philippinensis*, *trifoliatus*, *fumigatus*, *ferrumequinum*, *capensis*, *euryale*, and *hipposideros* based on the morphological dispersion analysis.

Servent *et al.* (2003) proposed the arrangement of the groups in family Rhinolophidae based on phylogeny and suggested names for subgenera. There are 6 subgenus groups included; *Aquias*, *Phyllorhina*, *Rhinolophus*, *Indorhinolophus*, *Coelophyllus*, *Rhinophyllotis*. Some of subgenera are split into groups corresponding to smaller lineages in the phylogeny following tradition.

Two species of rhinolophid were selected for these studies are *Rhinolophus lepidus* and *Rhinolophus pusillus*. These species are in the *pusillus* group (Andersen, 1918; Tate and Archbold, 1939; Corbet and Hill, 1992; Servent *et al.*, 2003).

Rhinolophus pusillus group

Andersen (1905) defined the *Rhinolophus lepidus* group as having the basio-occipital between cochleae not usually narrowed and with posterior connecting process of the sella projecting and pointed. He further subdivided the group into 3 ‘types’ based on general characteristics (size of skull, width of braincase and connecting process). The *lepidus*-type included 3 species: *R. lepidus*, *R. monticola* and *R. refulgens*. The *minor*-type included *R. minor*, *R. cornutus*, *R. minutus* and *R. gracilis*. The *subbadius*-type included *R. subedits* and *R. monoceros*. Andersen (1905) supposed that *R. acuminatus* and its synonyms (*R. sumatranus* and *R. calypso*) were scarcely more than giant representatives of the *lepidus*-type.

New species and subspecies of *pusillus* group were described by Andersen (1918) including *R. blythi* from Almora Kumaon, *R. perditus* from Ishigaki southern Liu-Kiu, *R. famulus* from North Central Island, Andamans, *R. lepidus shortridgei* from Myanmar (Burma), *R. refulgens cuneatus* from Sumatra and *R. blythi szechwanus* from Szechuan. He renamed the *lepidus* group as the *R. pusillus* group and renamed the subgroups as *pusillus*, *acuminatus* and *garoensis*. There were 6 species and 4 subspecies in the *pusillus* subgroup including *R. lepidus*, *R. l. shortrigei*, *R. refulgens*, *R. r. cuneatus*, *R. blythi*, *R. b. blythi*, *R. b. szechwanus*, *R. perditus*, *R. pumilus* and *R. cornutus*. In *garoensis*-subgroup, there are 3 species including: *R. garoensis*, *R. famutus* and *R. cognatus* (Andersen, 1918).

Tate and Archbold (1939) mostly followed Andersen (1918) in their arrangement of the species and subspecies. However, in contrast they did not recognise the *R. acuminatus* subgroup, but included *R. acuminatus,* together with *R. cognatus* and *R. famulus,* in the *lepidus* subgroup.

In 1980, Hill and Yoshiyuki described *R. imaizumii* which was a new species of *Rhinolophus* from Iriomote Island, east of Taiwan and referred it to the *pusillus*-group. They also reviewed the eastern and southeastern Asia species of the *pusillus*-group from the collection of the British Museum (Natural History) and suggested that there were eight valid species; *R. acuminatus*, *R. lepidus*, *R. pusillus*, *R. cornutus*, *R. subbadius*, *R. monoceros*, *R. cognatus*, and *R. imaizumii*. This view was followed by Corbet and Hill (1992).

Bogdanowicz (1992) used phenetic analyses to describe the morphometric differences between the species of the family Rhinolophidae and proposed a phenetic classification. He included the *pusillus* group as a subgroup of *megaphyllus* group. Within the *pusillus* subgroup, he listed *R. pusillus*, *R. gracilis*, *R. cornutus*, *R. subbadius*, *R. monoceros*, *R. cognatus*, *R. imaizumii*, *R. lepidus*, *R. feae* and *R. osgoodi*.

Csorba (1997) described a new species from Malaysia. *R. convexus,* which he included in the *pusillus*-group. He included *R. convexus*, *R. osgoodi* and *R. shortridgei* as the members of this group. However, Corbet and Hill (1992) recognized *R. osgoodi* as a synonym of *R. lepidus* and *R. shortridgei* as a subspecies of *R. lepidus*.

According to Corbet and Hill (1992), the *pusillus* group is distributed from southern Europe and North Africa (with three further purely Afrotropical species) in the West to Japan and the Lesser Sunda Islands in the East.

In 2003, *R. pusillus* group was proposed in the subgenus *Rhinophyllotis* by Servent *et al.* based on phylogeny. They included *R. refulgens*, *R. cognatus*, *R. monoceros*, *R. cornutus*, *R. imaizumii*, *R. pusillus*, *R. lepidus*, *R. convexus*, *R. subbadius*, *R. shortridgei* and *R. osgoodi* in this group.

Classification of *Rhinolophus*

Kingdom Animalia Linnaeus, 1758
 Phylum Chordata Bateson, 1885
  Class Mammalia Linnaeus, 1758
   Order Chiroptera Blumenbach, 1779
    Suborder Microchiroptera Dobson, 1875
     Infraorder Yinochiroptera Koopman, 1984
      Superfamily Rhinolophoidea Gray, 1825
       Family Rhinolophidae Gray, 1825
        Subfamily Rhinolophinae Gray, 1825
         Genera *Rhinolophus* Lacépède, 1799

Taxonomic discussion

Rhinolophus lepidus Blyth, 1844

Blyth (1844) described *Rhinolophus lepidus* from the vicinity of Calcutta. *R. lepidus* are similar to *R. subbadius* that it has pointed posterior noseleaf but *R. lepidus* has much paler colour, longer forearm, uppermost and hindmost of lancet being much less angular.

For this discussion, *R. lepidus* is here considered to include the five synonyms *R. lepidus*, *R. monticola*, *R. refulgens*, *R. feae*, *R. refulgens cuneatus.* This follows Csorba *et al*. (2003),

Andersen (1905) distinguished *Rhinolophus lepidus* from *R. minor* (= *pusillus*) by its larger size. He separated *R. monticola* from *R. lepidus* by its narrower nasal swelling, narrower horseshoe, smaller size and shorter metacarpals and separated *R. refugens* from *R. lepidus* by shorter metacapals, shorter tibia and broader horseshoe.

Andersen (1918) divided *pusillus* subgroup into *pusillus* series and *lepidus* series on account of the smaller size of skull and teeth of the former. He described a new subspecies, *R. lepidus shortridgei* from Myanmar (Burma), on account of its larger skull, longer toothrow and longer forearm than *R. lepidus*. He described *R. refulgens cuneatus* from Sumatra on the basis of its sella shape. He noted that the sella was subacute, its tip forming an equilateral triangle when viewed from in front. Sinha (1973) proposed that *Rhinolophus lepidus* has two subspecies; *R. l. lepidus* and *R. l. shortridgei* and considered *R. monticola* and *R. feae* as separate species. He separated *R. monticola* from *R. lepidus* by the longer metacarpals on the third, fourth and fifth fingers. He distinguished *R. feae* on account of its broader horseshoe and shorter metacarpals on the third, fourth and fifth fingers.

According to Hill and Yoshiyuki (1980), *R. lepidus* has six subspecies, namely *lepidus* (North-East India), *monticola* (North West India), *shortridgei* (northern Myanmar), *feae* (southern Myanmar), *refulgens* (southern Thailand and Malaysia), and *cuneatus* (Sumatra). Corbet and Hill (1992) recognized only *R. l. cuneatus* and *R. l. shortrigei* and provisionally included *R. osgoodi* are synonym.

R. shortridgei and *R. osgoodi* are recognized as separate species by Csorba *et al*. (2003). *R. shortridgei* is considered to have large body and skull, whereas *R. osgoodi* has a small skull is closely related to *R. lepidus* (Csorba *et al.*, 2003). Csorba (2002) considered the taxon *shortridgei* as a full species by investigation of the type skull (BM(NH) 18.8.l3.1) and other specimens for the collection of USNM, FMNH, HNHM. It revealed well-defined differences as compared with the other subspecies of *R. lepidus*; upper canines are strong, wide-based; sagittal crest extending posteriorly to the lambda and skull length is over 17 mm. Pearch *et al*. (2003) compared upper toothrow length and skull length of '*R. shortridgei'* from Myanmar with *R. lepidus* from India. Although, the morphometric evidence is not conclusive, there appears to be no distinctive character that clearly discriminates *shortridgei* from *lepidus*. Pearch *et al*. (2003) concluded that all specimens from Myanmar should provisionally be included in the subspecies *R. l. feae*.

Rhinolophus pusillus Temminck, 1834

Horsfield (1823) described *R. minor* briefly from Java but the name *R. minor* was invalidated by *Vespertilio ferrum-equinum minor* Kerr, 1792, which is *R. hipposiderus*, Bechst, 1800 (Andersen, 1907). *R. pusillus* was described by Temminck (1834) from Java. Andersen (1907) renamed the preoccupied *R. minor* as *R. pusillus*.

For this discussion, synonyms of *Rhinolophus pusillus* are here considered to include *R. minor*, *R. pusillus*, *R. minutus*, *R. cornutus pumilus*, *R. gracilis*, *R. minutillus*, *R. blythi*, *R. b. szechuanus*, *R. b. calidus*, *R. b. parcus*, *R. pagi*, *R. p. lakkhanae*. This follows Csorba *et al*. (2003).

Miller (1900) described *R. minutus* as a new species from Anambas Islands (Indonesia) that was similar to *R. minor* Horsfield, 1823 but with shorter ear and tibia. The skull is smaller than that of a specimen of *R. minor* from mainland of India, and the braincase is more narrow, but otherwise no important difference are apparent (Miller, 1900). This name was preoccupied by *Vespertilio minutus*, Montahu, 1808. In 1906, Miller renamed *R. minutus* as *R. minutillus* (Csorba *et al.*, 2003). *R. minutillus* was mentioned as perhaps subspecifically allied to *R. pusillus* (Chasen, 1940).

Andersen (1905) assigned the name *pusillus* to *R. minor*. He separated *R. cornutus pumilus* from *R. pusillus* (*minor*) by the apparently narrower space in *R. minor* between the upper canine and third premolar (P^4); the forearm length of *pumilus* exceeds that of *R. minor*. He described *R. gracilis* as new species from the Malabar coast. The diagnostic characters included the parallel-margined sella, an extremely short tail and small size (FA 36.2 mm).

Andersen (1918) described *R. blythi* as a new species on account of its shorter tibia and smaller foot than *R. cornutus*. Its canines, first upper premolar (P^2)

and second lower premolar (P_3) were 'unmodified' and P_3 is sometimes external but generally half or wholly situated in the toothrow. He described *R. b. szechwanus* as a new subspecies on account of the colour of the fur which is conspicuously darker above and below as compared to *R. b. blythi*.

Allen (1923) described *R. b. calidus* as a new subspecies from Yenping, Fukien provinces, China. It has much brighter fur, more cinnamon coloured. The skull is a little larger than in *szechuanus*. He proposed *R. c. pumilus* as a synonym of this subspecies. Allen (1928) described *R. b. parcus* as a new subspecies from Nodoa, island of Hainan, China. It differs from *szechuanus* and *calidus* in its rich russet or darker brown coloring. Tate and Archbold (1939) described *R. pagi* as a new species from North Pagi, Mentawi Island. It differs from *R. blythi* in the ratio of braincase length to occipito-canine length, which is 44% in *parcus* as against 39% in *blythi*. Sinha (1973) proposed *cornutus*, *blythi* and *gracilis* as subspecies of *R. cornutus.* He was unable to separate *R. gracilis* from *R. cornutus* at a specific level and considered it to be essentially similar to *R. c. blythi. R. c. blythi* differs from *R. c. cornutus* only in having a shorter tibia (length 13.5-16 mm as against 16.5-17 mm).

Corbet (1978) listed 4 synonyms for *R. cornutus*: *miyakonis*, *orii*, *perditus*, *pumilus* (all Ryukyu Islands) and 4 doubtful synonyms: *blythi* (Himalaya). *calidus*, *parcus*, *szechwanus* (S. China).

Hill and Yoshiyuki (1980) proposed 8 subspecies of *R. pusillus* including; *blythi*, *gracilis*, *szechuanus*, *calidus, parcus*, *minutillus*, *pagi* and *pusillus*. Yoshiyuki (1990) described *R. p. lakkhanae* as a new subspecies from Chiang Mai, Thailand on account of its rather short tail and the connecting process, which in side view is erect and sharply point, nearly forming an equilateral triangle. The skull and teeth are smaller in size than the other subspecies. According to measurements of Csorba *et al.* (2003), this taxon fully overlaps with those of the known southern subspecies and even with several Chinese specimens; therefore the geographic limits and the taxonomic validity of this subspecies is highly questionable.

The shape of the rostral profile of *R. pusillus* was described by Corbet and Hill (1992) as being nearly straight, almost horizontal (contrary to rostral profile of *R. lepidus* that it curving upwards near tip, slightly concave behind swelling). This character is not typical or uniform and cannot be used to separate the two species because there is the variability of the lectotype specimen of both species. The development of the posterior median swellings (which influences the shape or the rostral profile) is either a variable feature within both species in question or it has a taxonomic significance not yet fully understood. (Csorba, 2002 and Csorba *et al.*, 2003)

Corbet and Hill (1992) listed five synonyms for *R. pusillus*: *minutus*, *gracilis*, *blythi* and *pagi* and four subspecies: *szechuanus*, *calidus*, *parcus* and *lakkhanae*. Hill and Yoshiyuki (1980) considered *R. cornutus* to be closely similar to *R. pusillus* but with the connecting process narrower, more definitely horn-like – 'varying from subrectangular, its anterior margin straight or even slightly convex, to rather more horn-like and curved, its anterior margin slightly concave'. They go on to suggest that it is highly probable that *cornutus* and *pusillus* are conspecific. However, Corbet and Hill (1992) maintained them as discrete species. Csorba *et al.* (2003) proposed that *R. cornutus pumilus* is a synonym of *R. pusillus*.

Bates and Harrison (1997) noted that there is some overlap in all the external and cranial measurements between *R. lepidus* and *R. pusillus* and minority of specimens from Himalayan region are difficult to assign with confidence to one or other of the species. According to Corbet and Hill (1992), the shape of the rostral profile of *R. pusillus* is nearly straight, almost horizontal, whereas in *R. lepidus*, it curves upwards near tip, and is slightly concave behind the 'swellings' (= nasal inflations) . However, Csorba *et al.* (2003) suggests that the development of posterior median swellings is a variable feature within both species.

In conclusion, although the taxonomy of *Rhinolophus lepidus* and *R. pusillus* in South-East Asia has been studied for over 100 years, different authors have reached different decisions and there is still much detail that is not understood. It is also possible, for example, that a number of sibling species may be hidden within both taxa. This current study seeks to clarify the taxonomic situation through using a combination of morphometric and echolocation data.

Distribution

Rhinolophus lepidus is distributed from Afghanistan, Northwest Pakistan, India, Nepal, Myanmar; Southern China: Sichuan, Yunan, Fujian; Thailand, Malasia including small islands of Tioman, Pemanggil, Aor (Malaysia); Sumatra (Indonesia) (Corbet and Hill, 1992; Simmons, 2005) (Figure 3).

Rhinolophus pusillus is found in India, Nepal, Southern China (Tibet, Sichuan, Guangxi, Anhui, Fujian, Hainan, Hong Kong), Vietnam, Laos, Thailand, Peninsular Malaysia including small islands of langkawi, Penang, Tioman, Aor (Malaysia); Sumatra, Java including Madura Island (Java); Borneo; Northern Pagai (Mentawai Island), Siantan (Anamba Island); Rakarta (Krakatau Island); Lombok (Lesser Sunda Island) (Corbet and Hill, 1992; Simmon, 2005) (Figure 4).

In Thailand, *Rhinolophus lepidus* is found in Chiang Mai: Doi Pui, Doi Inthanon, Wat Tham Tab Tao; Ranong: Ban Bang Non; Phatthaluang: Khuan Kot (Yenbutra and Felten, 1986).

R. pusillus is known from Mae Hong Son: Ban Mae Na, Pai Wildlife Sanctuary; Chiang Mai: Doi Suthep, Doi Inthanon, Wat Thum Tap Tao, Nok Keaw Cave, Tham Sap Sawan; Nakhon Ratchasima: Sakaerat Station; Surin: Ban Hui Sing; Chatnaburi: Khao Sa Bap; Ubon Ratchathani: Ban Dan Kao; Chanthaburi: Khao Sa Bap: Khao Soi Dao Tai Wildlife Sanctuary; Lob Buri: Tham Sap Pong; Phetchaburi: Tham Khao Bin; Luang Ta Pad Cave, Suei Lueng Cave; Surat Thani: Ban Ao Ko; Nakhon Si Thammarat; Ban Khuan Kut, Thale Noi; Phatthaluang: Khuan Kut and Satun: Koh Tarutao (Yenbutra and Felten, 1986; Yoshiyuki, 1990; Wattanakul, 1995).

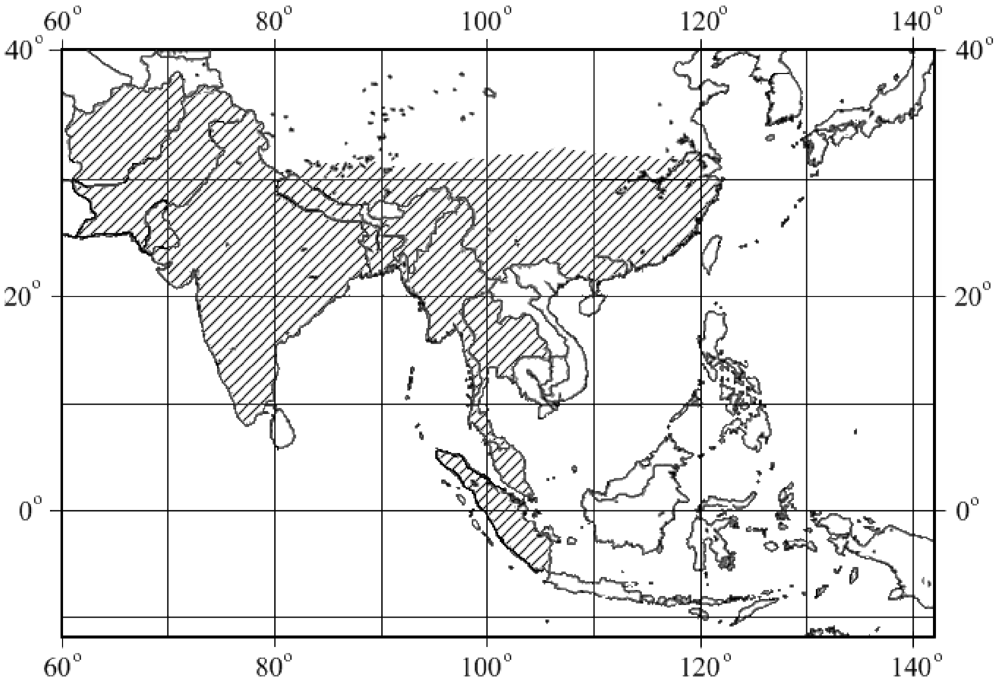


Figure 3. Distribution of *R. lepidus* from the literature review (Backward Diagonal). The limit of distribution is based on the literature.

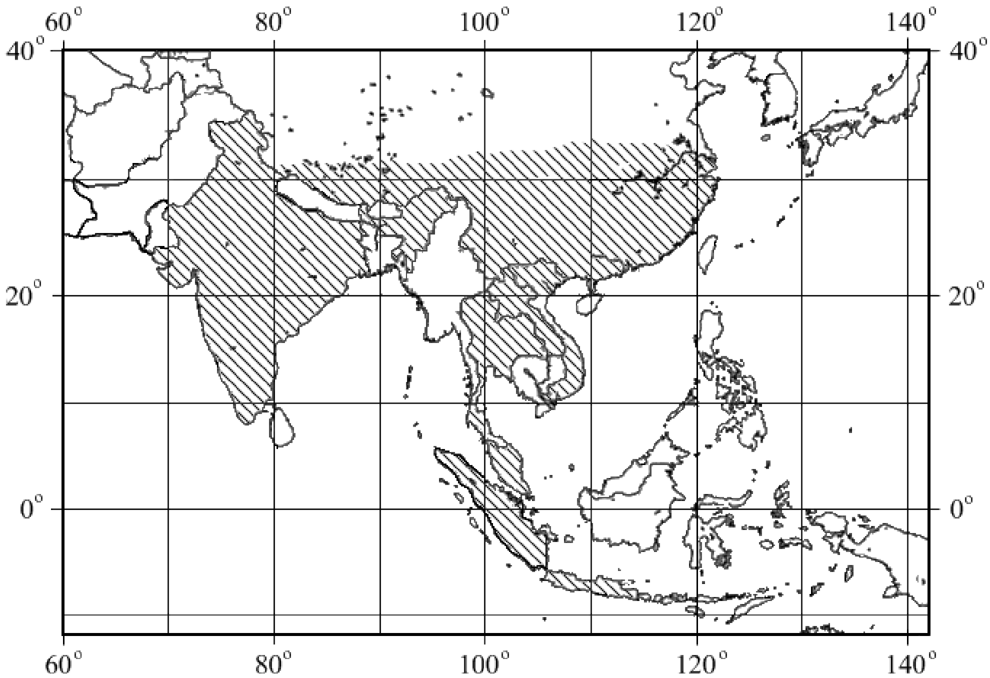


Figure 4. Distribution of *R. pusillus* from the literature review (Forward Diagonal). The limit of distribution is based on the literature.

Status

Status in the IUCN red list of threatened species of *R. lepidus* and *R. pusillus* is LR/lc (lower risk/least concern) (IUCN, 2007).

Echolocation

A simple definition of echolocation is that it is the analysis by and animal of the echoes of its own emitted sound waves, by which it builds a sound-picture of its immediate environment. Echolocation is a complex and highly evolved process which has given bats the ability to exploit an ecological niche closed to all but a few animal groups can exploit niche in the night sky. Echolocation is not unique to bats, but it has arguably reached its evolutionary peak in these mammals (Altringham, 1996). Bats use a wide variety of signal types (Figure 5). The structure of echolocation signals is generally species specific, and each species varies depending on the echolocation task confronting the bat. (Schnitzler and Kalko, 1998)

The echolocation calls of bats are produced by the larynx like the vocalizations of other mammals. The echolocation calls are very short in duration, generally lasting only a few milliseconds. Echolocation calls consist of up to three different types of signal elements, which can be process singly or in combination (Neuweiler, 2000):

1. *Downward FM*. The most common echolocation signal is a downward frequency-modulated (FM) pulse. The type of signal start at a high frequency and sweeps downward to progressively lower frequencies.

2. *CF*. A constant-frequency (CF) signal is a pure tone or a signal that is only slightly modulated in frequency. CF signals typically last for 10-100 ms and are commonly used as search signals. The echolocation calls of horseshoe bats and hipposiderids always contain a CF component.

3. *Upward FM*. Some times the CF component of the echolocation call is preceded by and upward FM component. So far, upward FM signals have only been described in association with CF signals.

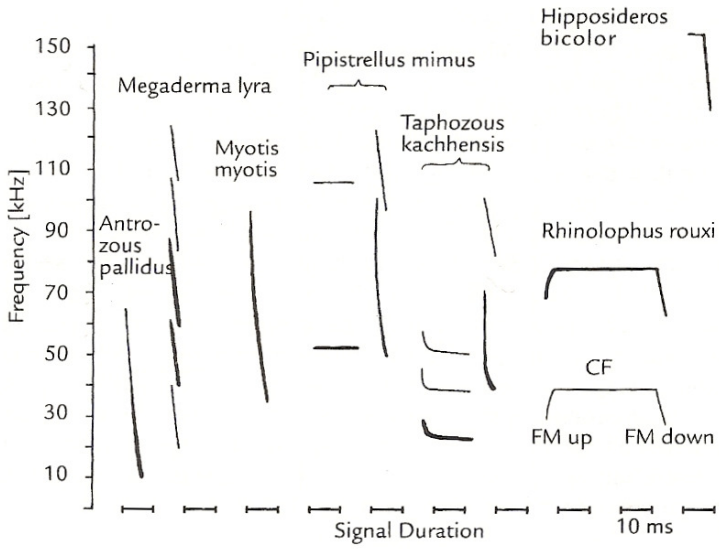


Figure 5. Type of echolocation used by bats (Neuweiler, 2000).

Echolocation calls frequently have a complex harmonic structure, meaning they contain a number of different frequencies that are multiples of a fundamental frequency. For example, 40 kHz, 60 kHz, and 80 kHz are 2nd, 3rd, and 4th harmonics of a fundamental frequency of 20 kHz. The fundamental frequency is also commonly referred to as the first harmonic. The largest amount of energy in the echolocation call is usually in the 2nd or 3rd harmonic, not in the fundamental frequency (Neuweiler, 2000).

All species of horseshoe bats (Rhinolophidae) are CF bats. They are insectivorous and search for fluttering insects in highly cluttered space (Schnitzler and Kalko, 1998). The rhinolophids use echolocation as a primary means of navigating and finding food. Their echolocation calls are characterized by a strong constant frequency (CF) component, usually with a short beginning or terminal frequency-modulated (FM) component (Csorba *et al.*, 2006). Unlike many other microchiropterans, rhinolophids can tolerate considerable overlap between outgoing calls and returning echoes (Hill and Smith, 1984). They have highly specialized auditory systems which are able to exploit Doppler-shifted echoes, and as such, individuals are able to emit and receive signals simultaneously. Their elaborate noseleaves and enlarged nasal cavities are associated with transmission of signals, whilst their large pinnae and cochleae are associated with signal reception (Csorba *et* *al.*, 2003). They emit calls through their nasal passages, which lets them continue calling as they chew. The calls of many species have several harmonics, which increases their frequency range and thus the size distribution of detectable targets (Hill and Smith, 1984).

Feeding behaviour and roosting

Rhinolophids are broad winged bats commonly foraging in cluttered environments feeding by gleaning (taking prey off foliage and the ground) aerial hawking (Altringham, 1996). Their flight is characteristically slow and fluttery; they are also capable of hovering (Hill and Smith, 1984). They feed on flying insects which they detect based on the pattern of their wing beats (Neuweiler, 2000). They often forage near the ground or among leaves and foliage, gleaning insects or other arthropods such as spiders. They may land to capture or consume a prey item. Many species appear to specialize on moths at least during some portions of the year. (Hill and Smith, 1984)

They may be found roosting in caves, hollow trees or on occasion, in human habitation. They may be found in large gregarious colonies, as solitary individuals or small groups. They are frequently found roosting among species of *Hipposideros* which are usually found in the same caves (Hill and Smith, 1984).

CHAPTER 3
MATERIALS AND METHODS

3.1 Specimen examined

The study was conducted between September 2006 and November 2007. Additional data of *R. pusillus* and *R. lepidus* come from specimens held in the collections of the Harrison Institute (HZM), the Natural History Museum (London) and the Princess Maha Chakri Sirindhorn Natural History Museum. Some of specimens from Thailand were loan from Chiang Dao Wildlife Research Station (CDWLRS) and Thailand Institute of Scientific and Technological Research (TISTR). Some of specimens from other countries including India, Myanmar and Vietnam were loan from the collections of the Harrison Institute (HZM). The specimens from Thailand and other countries were compared with the type specimens that deposited in Natural History Museum (London). The new voucher specimens were deposited in Princess Maha Chakri Sirindhorn Natural History Museum.

3.2 Study sites

The study sites were chosen on the basis of previous records of *R. pusillus* and *R. lepidus* (Yenbutra and Felten, 1986; Wattanakul, 1995). There were the new locations that lie within the known distribution range of the species. They were in both protected and non-protected areas. Rhinolophids are mainly cave-dwelling bats, believed to be particularly sensitive to disturbance (Hutson *et al.*, 2001).

There were 8 sites in protected area; Chiang Mai: Chiang Dao Wildlife Sanctuary, Srilanna National Park; Loei: Phu Suan Sai National Park; Phetchabun: Namnao National Park; Kalasin: Phu Si Than Wildlife Sanctuary; Ubon Ratchathani: Pha Taem National Park; Satun: Tarutao National Park; Songkhla: Ton Nga Chang Wildlife Sanctuary.

There were 7 sites in non-protected area; Lopburi: Khao Samo Khon (Tawung District), Khao Don Deung (Ban Mi District); Phetchaburi: Khao Yoi (Khao Yoi District); Ratchaburi Province: Khao Nom Tai (Photharam District), Songkhla:

Khao Tieb (Ratthephum District); Chumphon: Khao Plu (Lamae District), Silawan cave (Patiew District).

Protected Area Sites

These are the descriptions of the areas that the bats were found.

1. Chiang Mai Province, studies were conducted at two sites including:

1.1) Pha Daeng Cave (19º21’N, 99º1’E, 480 m a.s.l.) is located in Srilanna National Park, Chiang Dao District, Chiang Mai Province. This is a limestone outcrop, with caverns inside, and with one large entrance. The cave is surrounded by hill evergreen forest, and rice fields. A harp trap was set at the entrance of the cave.

1.2) Chiang Dao Wildlife Research Station (19°21’N, 99°1’E, 480 a.s.l.) is located in Chiang Dao Wildlife Sanctuary, Chiang Dao District, Chiang Mai Province. The bats live in the concrete conduit that located at the foot of mountain near headquarter of Chiang Dao Wildlife Sanctuary (Figure 6). The mist net was set at the entrance of concrete conduit. The headquarters is surrounded by mixed deciduous forest.

2. Loei Province, studies were conducted at three sites including:

2.1) Kao Liao (17°30’N, 100°57’E, 1300 m a.s.l.) is the junction of the nature trail in Phu Suan Sai National Park, Na Haeo District, Loei Province**.** The harp trap was set across the trail to camping point 1408 near the junction, under canopy of trees which surrounded by hill evergreen forest with banana trees (Figure 7).

2.2) Water tank (17°30’N, 100°57’E, 1004 m a.s.l.) is the starting point of the Kao Liao nature trail in Phu Suan Sai National Park, Na Haeo District, Loei Province, Thailand. A harp trap was set at the foothill across the trail behind the water tank under canopy of trees which surrounded by bamboo forest.

2.3) Pha Kor Waterfall (17°34’N, 101°0’E) is situated in Hueng River which is the Thai-Lao border. This waterfall is located in Phu Suan Sai National Park, Na Haeo District, Loei Province, Thailand, far from the headquarter about 16 km. A harp trap was set near the nature trail which is surrounded by bamboo forest at one side and the other side is the cliff.

3. Phetchabun Province

3.1) Tham Yai Nam Nao (Phu Nam Rin) (16°57'N, 101°30'E, 915 m a.s.l.) is the limestone cave which located in Namnao National Park, Lomkao District, Phetchabun. A harp trap was set perpendicular with the cliff outside the cave. The cave is surrounded by hill evergreen forest.

4. Mukdahan Province

4.1) Phu Pha San (16°39'N, 104°22'E, 415 m a.s.l.) is the limestone mountain which surrounded by mixed deciduous forest in Phu Pha Muang Wildlife Protection Station, Phu Si Than Wildlife Sanctuary, Kamchaee District, Mukdahan Province. Two harp traps were set across the nature trail (Figure 8).

5. Ubon Ratchathani Province

5.1) Wat Tham Partiharn (15°36'N, 105°35'E, 241 m a.s.l.) is the limestone cave in Pha Taem National Parik, Ubon Ratchatani. This is a limestone outcrop, with caverns and small stream inside, and with large entrance. There is the ritual of the temple at the entrance. A harp trap was set across the trail under canopy outside the cave which surrounded by mixed deciduous and deciduous dipterocarp forest.

6. Satun Province, studies were conducted at three sites including:

6.1) km 1-2 road to Talow Wao-Talow Udung, Tarutao National Park: A harp trap was set on side of the km 1-2 road to Talow Wao-Talow Udung, in Tarutao National Park, Satun (6º37'N , 99º40'E, 73 m a.s.l.). The harp trap was set under canopy of trees which lowland evergreen forest, densely covered at ground level.

6.2) Road to Ao Son, Tarutao National Park: A harp trap was set across the stream under canopy of trees which surrounded by lowland evergreen forest set near the road to Ao Son, Tarutao National Park (6º40'N, 99º38'E, 22 m a.s.l.) (Figure 9).

6.3) Boripatra waterfall, Ton Nga Chang Wildlife Sanctuary: A harp trap was set across the natural trail along riverbank near the entrance of Boripatra waterfall, Ton Nga Chang Wildlife Sanctuary, Satun (7°0'N, 100°9'E, 13 m a.s.l.). The harp trap was set under canopy of trees which surrounded by evergreen forest.

Non Protected Area Sites

These are the descriptions of the areas that the bats were found.

1. Lopburi Province, studies were conducted at two sites including:

1.1) Khao Samo Khon (14°55'N, 100°30'E, 32 m a.s.l.) is the limestone outcrop which surrounded by paddy field in Tawung District, Lopburi. There are many caves in this outcrop. Two harp traps were set around. One was set across a small trail from a local road lead to the foot of hill and the vegetation surrounding is *Syzygium cumini*. Another one was set at the small entrance (about 50 cm width) of Tham Ma Tok cave in Wat Tham Ta Ko temple which is the small underground limestone cave behind the temple. It is covered by mixed deciduous forest (Figure 10).

1.2) Khao Don Deung (15°9'N, 100°37'E, 40 m a.s.l.) is the limestone outcrop in Ban Mi District, Lopburi Province. It is covered by mixed deciduous and dipterocarp forest and surrounded by teak plantations, sunflower fields and corn fields. Harp traps were set on trail on the mountain.

2. Phetchaburi Province

2.1) Khao Yoi (13°14'N, 99°50'E, 53 m a.s.l.) is the limestone outcrop in Khao Yoi District, Phetchaburi. A harp trap was set at the foot of the hill under the canopy of trees, one side is the hill and the other side is open area. It is surrounded by villages, temple, rice field and mixed deciduous forest.

3. Ratchaburi Province

3.1) Khao Nom Tai (13°43'N, 99°45'E, 16 m a.s.l.) is located in Photharam District, Ratchaburi Province. A harp trap was set across natural trail near limestone cave which is surrounded by mixed deciduous forest.

4. Songkhla Province

4.1) Khao Tieb (7°4'N, 100°15'E, 100 m a.s.l.) is the limestone outcrop which is located in Ratthaphum District, Songkhla Province. The bats were caught by hand net in the underground limestone cave which has a small entrance (about 1.5 m width). It is surrounded by evergreen forest and rubber plantation.

5. Chumphon Province, studies were conducted at two sites including:

5.1) Khao Plu (9°46' N, 99°6'E, 30 m a.s.l.) is the limestone outcrop which located in Lamae, Patiew District, Chumphon, far from highway 4 about 500 m. There is a small cave in the outcrop surrounded by rubber plantations and cultivated areas. Two harp traps were set across the trail around the outcrop.

5.2) Silawan Cave (10°41'N, 99°14'E, 76 m a.s.l.) is a large limestone cave in a small limestone outcrop which is located in Patiew District, Chumphon Province. One harp trap was set at the small tunnel between chambers in the cave. The other one was set across a small trail beside the cave. This outcrop is surrounded by oil palm plantation, rubber plantation, and villages (Figure 11).

Figure 6. The concrete conduit near headquarter of Chiang Dao Wildlife Sanctuary, Chiang Mai which the bats live in.

Figure 7. The nature trail in Phu Suan Sai National Park which surrounded by hill evergreen forest with banana trees.

Figure 8. The harp trap was set across the nature trail in Phu Pha San, Phu Si Than Wildlife Sanctuary which surrounded by mixed deciduous forest.

Figure 9. The harp trap was set across the stream near the road to Ao Son, Tarutao National Park which surrounded by lowland evergreen forest.

Figure 10. Khao Samo Khon limestone outcrop which covered by mixed deciduous forest and surrounded by paddy field.

Figure 11. Rubber and oil palm plantation around Silawan limestone outcrop, Patiew District, Chumphon Province.

3.3 Field Methods

The specimens from field study were collected from the area that had no data before and that areas were in the range of distribution. Five taken captured bats in each area were collected as vouchers depend on the number of bats captured. These specimens should cover the variation of each population. Pregnant, lactating female and juvenile bats were not collected as the voucher specimens.

Capture methods

There are many kinds of methods for capturing the bats. The methods that used in this study are mist nets, harp traps and hand nets.

Mist nets

Mist nets are the nets that use for capturing bats or birds. These nets made from nylon. This method use for capturing flying bats thus it is difficult to capture flying bats because they can avoid the mist nets. The mist nests have four shelf and 2 m high. They have the range of length from 6-36 m. In this study, the short nets (<12 m) were used because the target bats forage in cluttered area so the suitable placed for setting the nest are narrow and the short nets are easier to handle and change the position of the nets.

The purpose of capturing bats by mist nets is to capture the target bats. The mist nets are lightweight, compact, and easy to transport into the field. The important things that have to consider in this method are when the nets captured the bats, they should be removed from the nets immediately before they cause the damage to the net and they may become injured, or die of stress if they are not remove promptly.

The mist nets are kept by tying the loops at the end and folding the net for taking them into the bags. When a net is prepared for field use, it should be arranged and tied in the appropriate order. All five loops are located at the poles, then top loop should be placed at the top of poles, then the next loop is extended and this procedure is continued until the last loop has been placed in order. The poles that use for setting the nets should be as straight as possible.

The effective sites for capturing the bat are the places where the bats are expected to fly. The sites near the roosts or across trails that are used as flyways are most successful. The places that be chosen to setting the nets can be identified from visual observations of flying bats or listening to bat by using bat detectors.

Harp Traps

Harp traps are the equipment that use for capturing flying bats like the mist nets. The harp trap have four large rectangular frames of aluminum tubing with four bank of fishing lines that thread vertically to the frame, each line spaced 2 cm apart and the frames are 2 m high and 1.8 m wide. The frames are supported by four tubular legs. The trap worked on the principle that the vertical lines could not be easily to detect by echolocation calls of bats and the banks of fishing lines stops the bat that flying and then the bats fall into the bag. A large bag is placed below the frames to catch the bats that fall after they fly into the trap. There is a plastic flap inside the bag. The bag and plastic flap of harp trap can avoid the escape of bats and fighting with other bats.

The harp trap can divide into small pieces for transport to the field and can put them together easily in the field. The best sites for setting the traps are where bats use natural flyways, such as trail, along slowly flowing stream, between trees and rock faces. The harp traps should be placed at cave entrances or in the trails that are broad enough to set the harp traps and have the vegetations or branches at both sides of them. If the upper side of harp traps also have vegetation, that is better. Sometimes they must be block areas around the trap with branches or some other material to funnel the bats into the path of traps.

The harp traps are smaller than mist nets, so they can be placed at the narrower trails or small cave. The harp trap made from fishing lines that can give less injured to the bats. The harp traps can only use at the ground, so they cannot capture the bats that flying higher than the traps. They are smaller that mist net, so they are ineffectiveness at the open areas.

The tension of fishing lines in the harp traps is important factors that influence the capture success of harp traps. The tension should be proportional to the speed of bat. If bats by bouncing off the fishing lines, the tension should be reduced. If bats pass through the fishing lines, the tension should be increased.

Hand nets

Hand nets are the hoop nets that have adjustable-handles. This method use to capture the bats in cave, tree or building. The using hand net is easily method for capturing the bat that hanging on the ceiling of cave. For capturing flying bats, it should have special care to swing nets because it may damage wings of bats and caused injury (Kunz and Kurta, 1988)

Holding devices

Holding devices are used to temporarily house captured bats as they await processing in the field. The bags that were used made from cotton by sewing the fabric into rectangular bags with draw-strings for securing the open end. The size of bag is 22 cm wide and 30 cm deep.

Field Data

The data from field study is recorded in data sheets (Figure 12). In data sheet, there are data includes:

1. Specimen code; this is the number of bats that are captured. The number is used for each individual.

These codes are used for field number. The field number is coded for every bats that be collected in the field each days that are recorded in data sheets and on a label that is attached to the left foot of the specimen. Identity code of each specimen is used by the abbreviations of the collectors, date and the number of specimens that are collected in each day. For example, the second specimen of *Rhinolophus lepidus* that collected by Ariya Dejtaradol on 10th January 2007 is labelled as AD070110.2.

2. Date
3. Time
4. Sex
5. Status

a. age determination

b. reproductive status of adult females

6. Measurement (Figure 13); some measurements are taken by digital vernier caliper including;

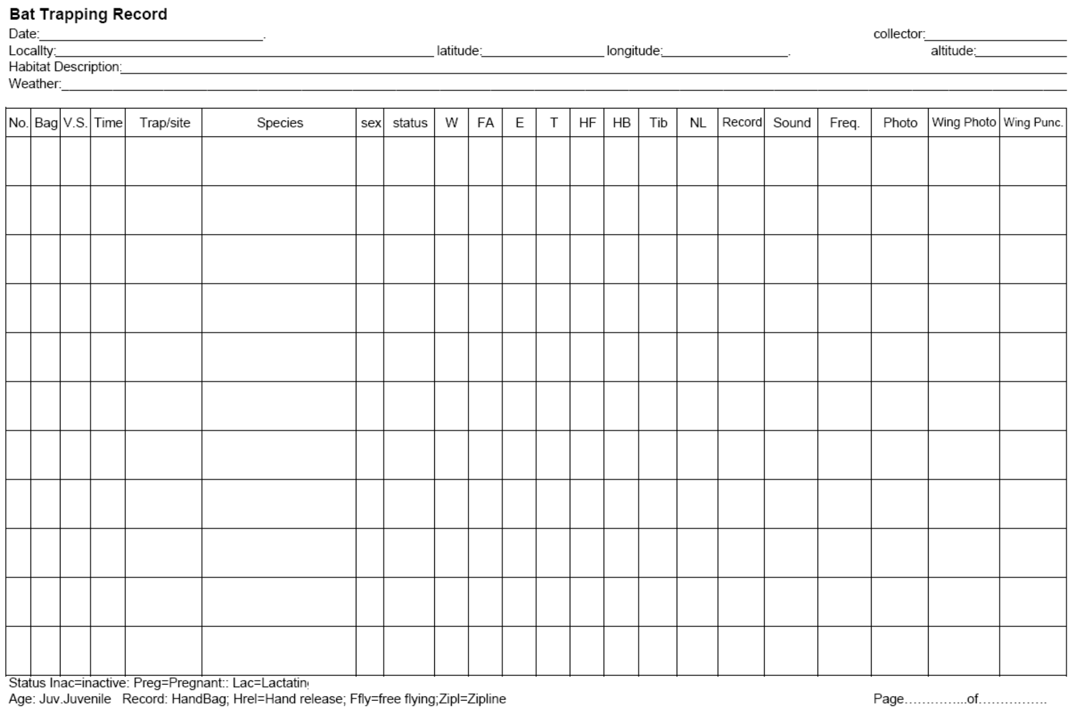
a. W ; Weight (g)
b. FA; forearm –from the extremity of the elbow to the extremity of the carpus with the wings folded.
c. E; Ear –from the lower border of the external auditory meatus to the tip of the pinna, not including any tuft of hair
d. T; Tail length –from the tip of the tail to its base adjacent to the body.
e. HF; foot –from the extremity of the heel behind the os calcis to the extremity of the longest digit, not including the hairs or claws.
f. HB; head and body length –from the tip of the snout to the base of the tail, dorsally.
g. Tibia; length of tibia –from the knee joint to the ankle
h. NL; horseshoe width –greatest width of horseshoe of the noseleaf.

7. Echolocation calls (see below)

a. Record; the method of recording (Hand/Bag/Free flying)
b. Sound; the name of sound file
c. Freq.; peak frequency (kHz)

8. Photo (file name); the sella and connecting process are photographed when they still alive.

9. Wing punc.; wing puncture code. Wing tissues of every specimen are collected for future DNA analysis after they are sacrificed. The tissues have identity label for each specimen and be kept in absolute alcohol.



Bat Trapping Record

Date:______________________________. collector:___________________

Locallty:____ latitude:_______________ longitude:_______________. altitude:____________

Habitat Description:____

Weather:____

No.	Bag	V.S.	Time	Trap/site	Species	sex	status	W	FA	E	T	HF	HB	Tib	NL	Record	Sound	Freq.	Photo	Wing Photo	Wing Punc.

Status Inac=inactive: Preg=Pregnant:: Lac=Lactatin

Age: Juv.Juvenile Record: HandBag; Hrel=Hand release; Ffly=free flying;Zipl=Zipline Page…………..of…………….

Figure 12. Field data sheet

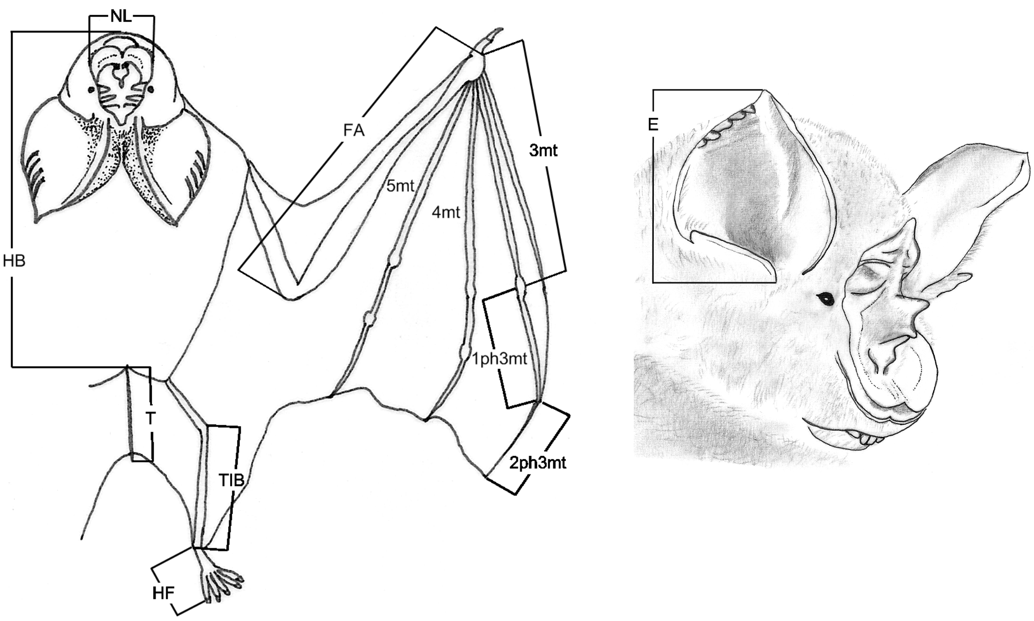


Figure 13. Right wing (on left), head (on right) and some measurements of *Rhinolophus l. refulgens*

Reproductive status of adult female

The adult females were determined the reproductive status; lactating female and pregnant female that were released. The nipples of lactating females become enlarged, and milk can be extruded. Distension of the lower abdomen indicates pregnancy (Racey, 1988).

Age determination

There are some methods that can define the age of bats (Adult and Juvenile) but the exact age is known precisely by marking it at birth at all subsequent observations and can be directly correlated with any anatomical, behavioural or physiological characteristics of interest. In the field work, the techniques that can estimate the age of bat are needed. The purposes of age determination in the current study are defining the status of bats (juvenile/adult) that are caught in each time and for decision of specimens collecting (the juvenile must not be collected).

The method of age determination that is used in this study is epiphyseal-diaphyseal fusion. Following the initial growth phase of long bones, patterns of closure of the cartilaginous epiphyseal growth plates in long bones can be used to extend the period or reliable age estimation in young bats. These plates are readily visible to the unaided eye when a bat's wing is transilluminated; the cartilaginous zones appear lighter than ossified parts of the bones, as lesser mineralization allows more light to pass through. When these cartilaginous plates are no longer grossly visible, the shapes of the finger joints of young bats remain less knobby and more evenly tapered than those of adults, allowing some young bats to be provisionally identified by this characteristic until they are almost a year old. These qualitative criteria are applicable to field situations, as the required observations are quick and easy and require no sophisticated equipment. (Kunz, 1988)

Echolocation data

Echolocation calls of every voucher specimen from field study were recorded by Pettersson ultrasound detector D 240x in time-expansion (10x) mode, connected to sound recorder i-RIVER model: iHP-120 Multi-codec jukebox and recorded in WAV file (Figure 14).

Every specimen was recorded echolocation call in 3 methods (if possible); in hand, in bag and free flying for comparison. The method ‘recorded in hand’ was record the bat calls when hold the bat in hand. The method ‘recorded in bag’ was record the bat calls when the bats are in the cotton bags. The method ‘free flying method’ was record the bat calls when release the bat to fly in room and the size of room depend on the place that the bats are processed. Some juvenile and pregnant female bats were released and recorded the sound when releasing.

Figure 14. Pettersson ultrasound detector D 240x in time-expansion (10x) mode, connected to sound recorder i-RIVER model: iHP-120 Multi-codec jukebox

Preparing wet specimens

Wet specimen

All voucher specimens were sacrificed in the field by conc. chloroform. Specimens from field are stored in 70% ethyl alcohol with the skull. All wet specimens have a field number on a field label attached to the left hind foot before stored in the alcohol. The information that was noted in the field label for each specimen following:

- Field number (see above)
- Locality data
- Sex of specimen
- Provisional species identification

- Basic external measurements including weight, forearm length, ear length, tail, hind foot length, head body length, tibia length and noseleaf width.

Collection number

Each new specimen was deposit at mammal collection in Princess Maha Chakri Sirindhorn Natural History Museum. The wet specimens from field were given the collection number. The collection number is PSUZC-MM(year).(number of specimen in the collection). For example, specimen that was collected in year 2006 and the registration number of year 2006 was 101. The collection number of this specimen is PSUZC-MMM06.101.

The collection number recorded on the label (Figure 15), which was attached to the left foot of the wet specimen and skull. The original field label was not removed and therefore two labels should be attached to each wet specimen.

♂
PSUZC-MM06.101 101.8 kHz
Rhinolophus lepidus
7.10.2006 Boripatra waterfall, Ton Nga-chang WS.,
Songkhla Province, Thailand
7° 0.049'N, 100° 8.534'E

FA: 39.20 Limestone cave
E: 14.00 Alt: 13 m
T: 21.00
HF: 7.59
TIB: 15.89
HB: 46.52 Collector: Ariya Dejtaradol
W: 6.6 Field No: AD070110.2

Figure 15. Label for wet specimen and skull: front of the wet specimen label (above) and back of the wet specimen label (below).

Skull extraction

Extracting a skull

The skull was extracted from the body in laboratory following;

1. The facial skin will be peeled back from the mandible and rostrum using a blunt scalpel carefully.

2. The skin will be cut free from the nasal bones, it is very important to avoid damaging any noseleaf, eyes and zygomatic arches.

3. To free the ears, the auditory meatus must be cut on each side of the skull.

4. Cut the upper cervical spine to free the skull.

5. A temporary skull label with the field number will be immediately attached to the skull to ensure that there is no subsequent confusion between skulls.

Cleaning a skull

The extracted skull was clean by manual dissection following;

1. The extracted skull with its skull label attached will be suspended in water that is brought to the boil

2. The skull will remain in the simmering water for about 15 minutes.

3. The skulls will be left to cool down in the air and then stored temporarily in cold water.

4. The skulls will be cleaned under a dissecting microscope.

5. After completely clean, the skull will be pinned out to dry with its label until it is dry.

Skull storage

After the skull was cleaned and dry, the label was attached to cranium and mandible. The skull with its label was stored in a small plastic pot with a secure lid and supported on cotton wool to minimise any damage during storage.

3.4 Laboratory methods

149 speciemens were conducted. The specimens come from Thailand, India, China, Vietnam, Cambodia, Malaysia, and Indonesia. 66 females 80 males and 1 unknown (only skull and no sex in the label).

Measurements

Measurement and description

Cranial morphology and dental characters (Figure 16, 17, 18) of every specimen was measured and described. All measurement is in millemetres. The abbreviations that were used for measurements are (*sensu* Bates and Harrison, 1997)

L: length –from the tip of snout to the tip of the tail;

HB: head and body length –from the tip of the snout to the base of the tail, dorsally;

T: tail length –from the tip of the tail to its base adjacent to the body;

HF: foot –from the extremity of the heel behind the oscalcis to the extremity of the longest digit, not including the hairs or claws;

TIB: length of tibia –from the knee joint to the ankle;

FA: forearm –from the extremity of the elbow to the extremity of the carpus with the wings folded;

3mt: third metacarpal –from the extremity of the carpus to the distal extremity of the metacarpal;

4mt: fourth metacarpal –from the extremity of the carpus to the distal extremity of the metacarpal;

1ph3mt: first phalanx of the third metacarpal –take from the proximal to the distal extremity of the phalanx;

2ph3mt: second phalanx of the third metacarpal –take from the proximal to the distal extremity of the phalanx;

1ph4mt: first phalanx of the fourth metacarpal –take from the proximal to the distal extremity of the phalanx;

2ph4mt: second phalanx of the fourth metacarpal –take from the proximal to the distal extremity of the phalanx;

1ph5mt: first phalanx of the fifth metacarpal –take from the proximal to the distal extremity of the phalanx;

2ph5mt: second phalanx of the fifth metacarpal –take from the proximal to the distal extremity of the phalanx;

E: ear –from the lower border of the external auditory meatus to the tip of the pinna, not including any tuft of hair;

GTL: greatest length of skull –the greatest anteroposterior diameter of the skull, taken from the most projecting point at each extremity, regardless of what structure forms these points;

CBL: condylo-basal length –from an exoccipital condyle to the alveolus of the anterior incisor;

CCL: condylo-canine length –from the exoccipital condyle to the anterior alveolus of the canine;

SL: skull length –from the greatest posterior point of skull to the anterior alveolus of canine;

ZB: zygomatic breadth –the greatest width of the skull across the zygomatic arches, regardless of where this point is situated on the arches;

BB: breadth of braincase –greatest width of the braincase at the posterior roots of the zygomatic arches;

PC: postorbital constriction –the narrowest width across the constriction posterior to the orbits;

M: mandible length –from the most posterior part of the condyle to the most anterior part of the mandible, including the lower incisors;

C-M^3: maxillary toothrow –from the front of the upper canine to the back of the crown of the last upper molar;

C-M_3: mandibular toothrow –from the front of the lower canine to the back of the crown of the last lower molar;

M^3-M^3: posterior palatal width –taken across the outer borders of the last upper molar;

C^1-C^1: anterior palatal width –taken across the outer borders of the upper canine.

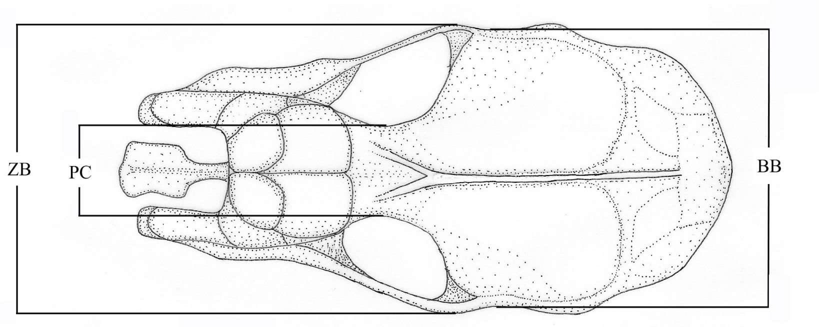


Figure 16. Dorsal view of the skull of *Rhinolophus pusillus*

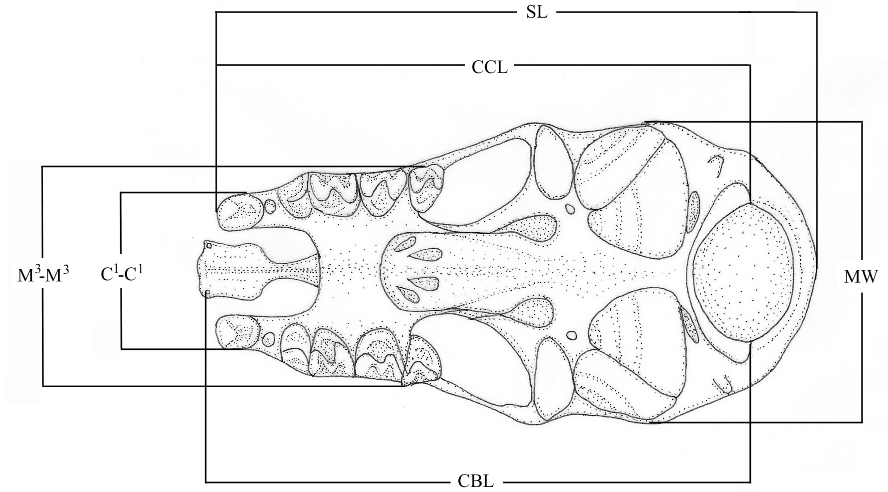


Figure 17. Ventral view of the skull of *Rhinolophus pusillus*

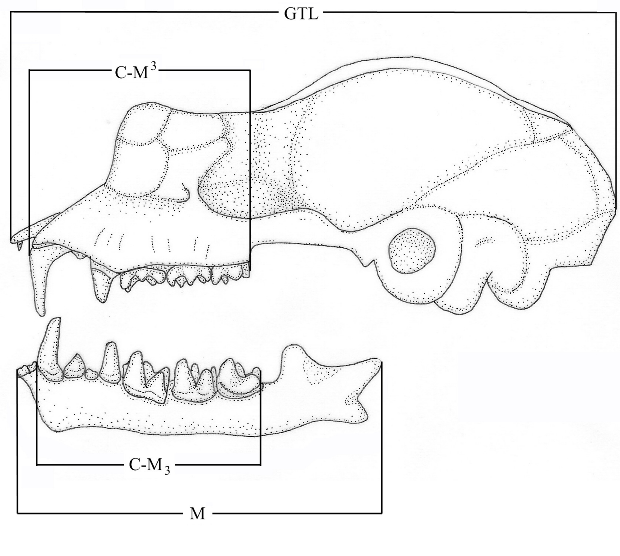


Figure 18. Lateral view of the skull of *Rhinolophus pusillus*

The characters that be described (*sensu* Andersen 1905) were the colour of hair, kinds of hair, tip of connecting process, supplementary leaflet, tip of lancet, ear tip, rostral profile, position of PM_3, second lower premolar, (in, or external to, the tooth-row), position of PM^2, first upper premolar, and PM^4, second upper premolar, (separated or almost or quite in contact).

3.5 Analysis

Morphometric analysis

Univariate and multivariate statistical analysis were run on the linear measurements collected in order to examine patterns in the data.

Sexual dimorphism: in order to determine whether there was sexual dimorphism within the current taxa. A univariate T-test was run on selected external and cranial characters for males compared to females for each taxon at confidence limit of 95% using SPSS 14.0 for Windows. The results obtained determined whether the sexes were treated separately, or the data pooled in subsequent analyses.

The multivariate method that used in this study is Principal Components Analysis (PCA). This method is designed to reduce the number of variables that need to be considered to a small number of indices (called the principal components) that are linear combinations of the original variables. The variables (morphological and cranial measurements of all specimens) are reduced to be the principal components and they are calculated from correlation matrix. The value of the principal components may make the original variables much easier to understand what the data have to say (Manly, 1994). The principal component analysis was calculated by PCORD4 program.

Call analysis

The echolocation calls were analysed with BatSound Pro – Sound Analysis Version: 3.31 program (Pettersson Elektronik AB). Sound format was set as stereo, 16 bit, sampling frequency of 44.1 kHz with 10 time expansion. Six parameters were measured including (*sensu* Preatoni *et al*., 2005);

1. Pulse duration (D, ms; Figure 19a, b), measured automatically by BatSound software (using the Tools/Mark distances function) as the distance between

2 BatSound large measurement cursors, that placed respectively at the beginning and at the end of the signal as plotted in the oscillogram;

2. Frequency at maximum intensity (FINT, kHz; Figure 19c), measured by evaluating the power spectrum maximum on the entire click (i.e., applying the BatSound Power Spectrum function to the selection enclosed between start time and end time cursors;

3. Start frequency (SF, kHz; Figure 19d), measured on the spectrogram via a large measurement cursor placed at the far left end of the spectrogram;

4. End frequency (EF, kHz; Figure 19e), measured on the spectrogram via a large measurement cursor placed at the far right end of the spectrogram;

5. Maximum frequency (FMAX, kHz; Figure 19f), measured on the spectrogram via a large measurement cursor placed at the top end of the spectrogram;

6. Minimum frequency (FMIN, kHz; Figure 19g), measured on the spectrogram via a large measurement cursor placed at the bottom end of the spectrogram.

Each file of each specimen was open and analysed 6 parameters of 5 calls to calculate the average represented for each specimen. Statistical comparison between calls of populations was conducted using a One-way ANOVA. T-test was used to test the sexual dimorphism within population.

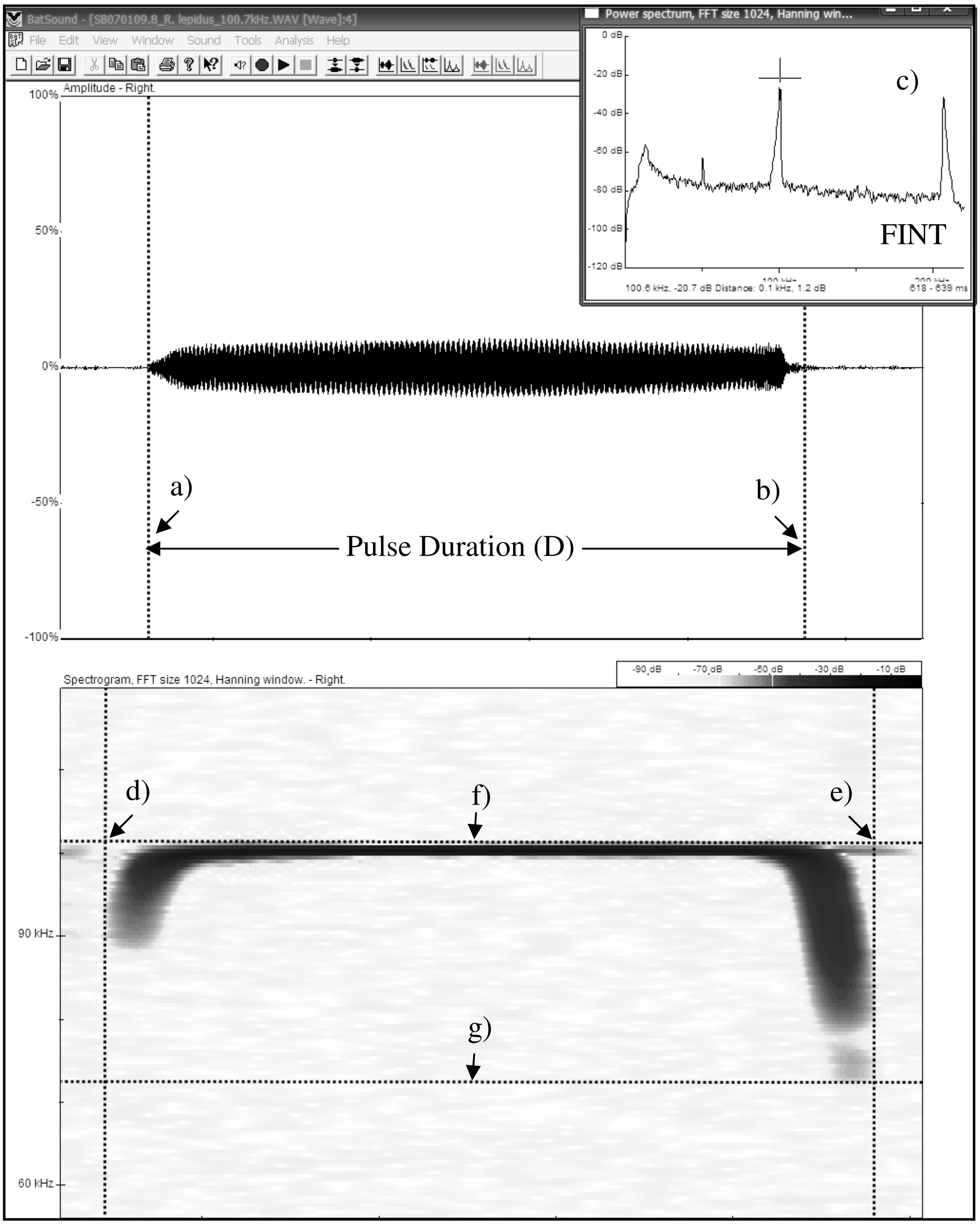


Figure 19. *R. l. refulgens* call and the BatSound software cursors (represented by the dotted lines) and measurement point (indicated by arrows) used for parameter measurements. D (duration) is automatically calculated as the distance (in milliseconds) between a and b. a) start time cursor; b) end time cursor; c) FINT (frequency of maximum intensity); d) SF (start frequency) measurement cursor; e) EF (end frequency) measurement cursor; f) FMAX (maximum frequency) measurement cursor; g) FMIN (minimum frequency) measurement cursor.

CHAPTER 4
RESULTS

4.1 Morphology comparison with other taxa

Comparison with other taxa in *Rhinolophus pusillus* groups

In this section, *R. lepidus lepidus* from India, *R. shortridgei* from Myanmar, *R. cognatus* from Andaman islands, *R. pusillus* from Java, *R. l. refulgens* from Malaysia and all taxa in *R. pusillus* group that found in Thailand were compared.

All taxa in this group have the pointed connecting process. It is nearly impossible to compare the colour between specimens preserved in alcohol because the pelage colour has been changed. Consequently, only cranial characters were compared in this section. For those specimens from Thailand which photograph were available, both external and cranial morphology were compared.

Comparison with the type specimen and specimens from type locality

Based on the morphology of specimens belong to *R. pusillus* groups (complex) that found in Thailand, it can be divided into 3 groups. The first group was the taxon found in central and northern Thailand. This group was similar to the type specimen of *R. pusillus* (see below). The second group was the taxon that found in southern Thailand. This group was similar to *R. lepidus*. And the last group was the taxon from the mountain top in Phu Suan Sai National Park, Loei province.

The taxon that similar to *R. pusillus* from central and northern Thailand was compared to the type specimen from Java. Syntype of *R. minor* (*pusillus*) Horsfield, 1823 from Java is broken at the braincase. The comparison from the drawing picture of type specimens with the taxon from northern Thailand, the shape of rostral inflation of the taxon from northern Thailand was similar to the syntype. The specimens from Thailand were also similar to the *R. pusillus* from Java, Indonesia and East Madura, Malaysia (Figure 20). Since, there was no evidence that *R. pusillus* from Java was different from specimens from northern Thailand and southern China, so the taxon found in central and northern Thailand will be named as *R. pusillus*.

The taxon that similar to *R. lepidus* from southern Thailand was compared to specimens of *R. lepidus lepidus* from India and *R. lepidus refulgens* from Malaysia (*sensu* Hill and Yoshiyuki, 1980). The cranial morphology of *R. lepidus* found in southern Thailand was different from *R. l. lepidus* found in India, but it was similar to the *R. l. refulgens* from Malaysia (Figure 21). The shape of nasal inflation is different between *R. lepidus lepidus* and *R. l. refulgens*. *R. l. lepidus* has the flatter anterior median swellings. In this study, *R. lepidus* that found in Southern Thailand will be referred to *R. l. refulgens* from now.

The third taxon was the specimens found at the mountain top in Phu Suan Sai National Park, Loei Province. These specimens were different from *R. pusillus* and *R. l. refulgens* by size and the shape of lancet. In comparison with the similar taxa, *R. shortridgei* and *R. cognatus*, the cranial of these Thai specimens were more similar to *R. cognatus* than *R. shortridgei* in size, robust zygomatic arch and high sagittal crest. The shape of nasal inflation of *R. shortridgei* was flatter than *R. cognatus* and the taxon from Loei province. The shape of the parietal bones of the taxon from Loei was more elongate than *R. shortridgei*. *R. cognatus* and the specimens from Loei province were very similar in shape (Figure 22). However, there is a difference between them on sizes of the upper anterior median swelling and the anterior lateral swelling. For *R. cognatus*, the upper anterior median swelling were equal to the lower anterior median swelling in height and size in lateral view while in the Loei population, lower median swelling were higher and larger than upper median swelling. So, the taxon from Loei will be referred to Loei population.

There was the other taxon that has the similar morphology to *R. pusillus* but the average size is slightly smaller than normal *R. pusillus*. This taxon has higher frequency and was found at Khao Samo Khon, Lopburi Province. From now, this taxon will be referred to Lopburi population. The details will be explained at the measurement and echolocation section.

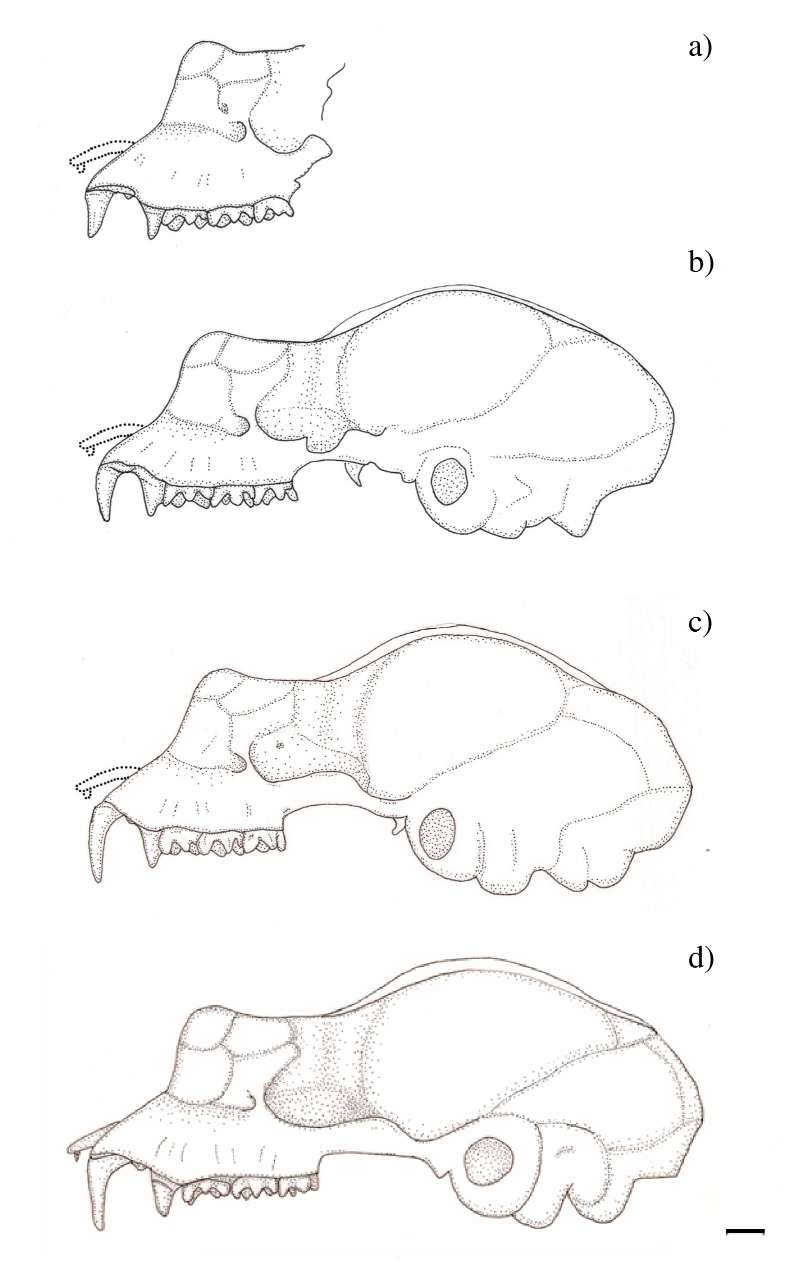


Figure 20. The skulls in lateral view of a) syntype of *R. minor* (*pusillus*) Horsfield, 1823 (BM(NH)1879.11.21.684) from Java, Indonesia; b) Type specimen of *R. gracilis* (*R. pusillus*) (♀ BM(NH) 61.1747) from West Java, Indonesia; c) *R. pusillus* (♂ BM(NH) 10.4.7.8) from East Madura, Malaysia; d) *R. pusillus* (♂ PSUZC-MM07.264) from Mukdaharn, Northeastern Thailand (Scale=1 mm). (Dotted line shows premaxilla that was broken)

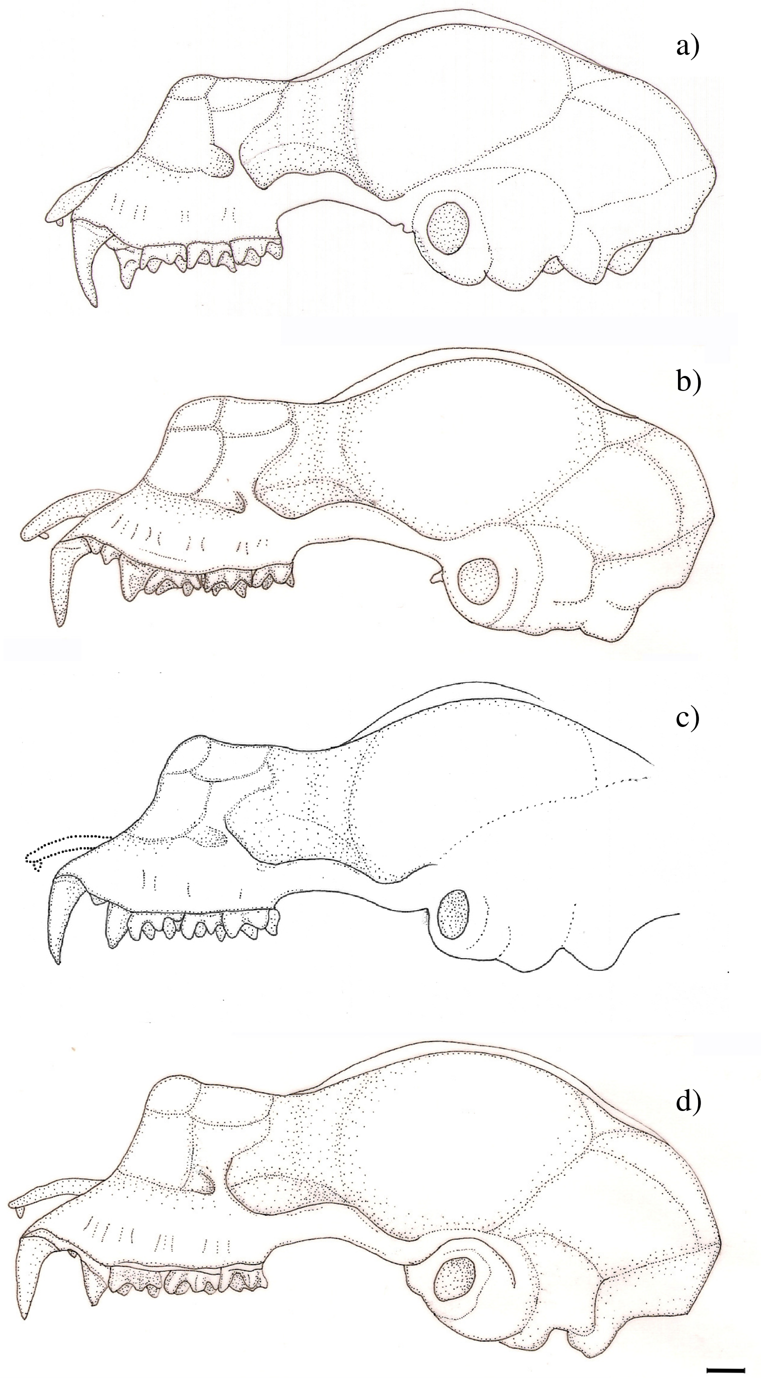


Figure 21. The skulls in lateral view of a) Holotype of *R. monticola* (*R. l. lepidus*) (♂ BM(NH) 1879.11.21.151) from Masuri, Pakistan; b) *R. l. lepidus* (♂ HZM 19.28161) from New Delhi, India; c) *R. l. refulgens* (♂ BM(NH) 67.1572) from Pahang, Malaysia and d) *R. l. refulgens* (♂ PSUZC-MM07.88) from Trang, Southern Thailand (scale = 1 mm). (Dotted line shows premaxilla that was broken)

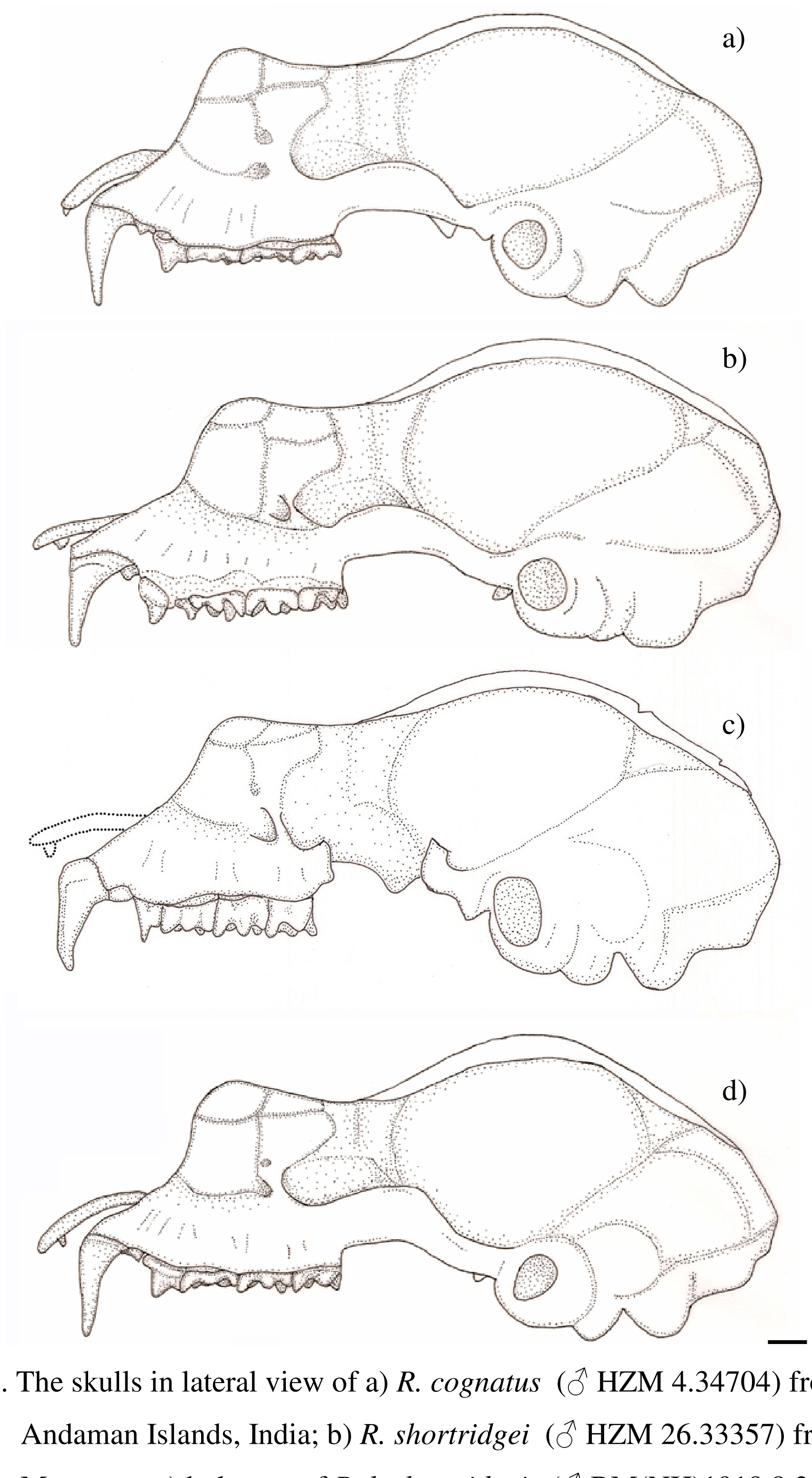


Figure 22. The skulls in lateral view of a) *R. cognatus* (♂ HZM 4.34704) from Andaman Islands, India; b) *R. shortridgei* (♂ HZM 26.33357) from Myanmar; c) holotype of *R. l. shortridgei* (♂ BM(NH)1918.8.3.1) from Pagan, Irrawaddy, Myanmar and d) *Rhinolophus* sp. (♂ PSUZC-MM07.256) from Phu Suan Sai National Park, Loei province, Thailand (scale = 1 mm). (Dotted line shows premaxilla that was broken)

4.2 Variations

There are some variations of the lancet shape, connecting process shape, sella shape and the colour of pelage in *R. l. refulgens* and *R. pusillus*.

The shape of lancet, connecting process, sella and the colour of pelage of Lopburi population (n=10) are comparable to *R. pusillus*. These characters cannot use to distinguish it from *R. pusillus*, so Lopburi population is not included in this section. Loei population (n=4) has no variation in lancet, connecting process and sella. The shape of connecting in Loei population is comparable to *R. l. refulgens*. However, the shape of lancet and sella are different from *R. l. refulgens*, *R. pusillus* and Lopburi population. The description of the shape of lancet and sella of Loei population is in the section of systematics summary (Horseshoe bat from Loei province).

Variation of lancet

Rhinolophus lepidus refulgens (n=20)

The lancet of *R. l. refulgens* is well developed (Figure 23); the tip is variable in shape, in some specimens it is broadly rounded off and in others more pointed; some individuals have deeply concave sides adjacent to apex (Figure 23a, c, d) and in others, the sides of the lancet are almost straight (Figure 23b, e); some specimens have wider horseshoe than others (Figure 23c, d). There are 15%, 10%, 50%, 20% and 5% of specimens that have the shape of lancet in Figure 23a, b. c. d and e orderly (Figure 24).

Rhinolophus pusillus (n=21)

The morphology of the noseleaf is comparable to that of *R. l. refulgens* (Figure 23). There are four shape of lancet (Figure 23a, b, c, d). The normal shape of lancet is in Figure 23c; there are 48% of specimens have this shape (n=21). Nineteen percent of the specimens have the shape in Figure 23a and 23b. Fourteen percent have the shape in Figure 23d (Figure 24).

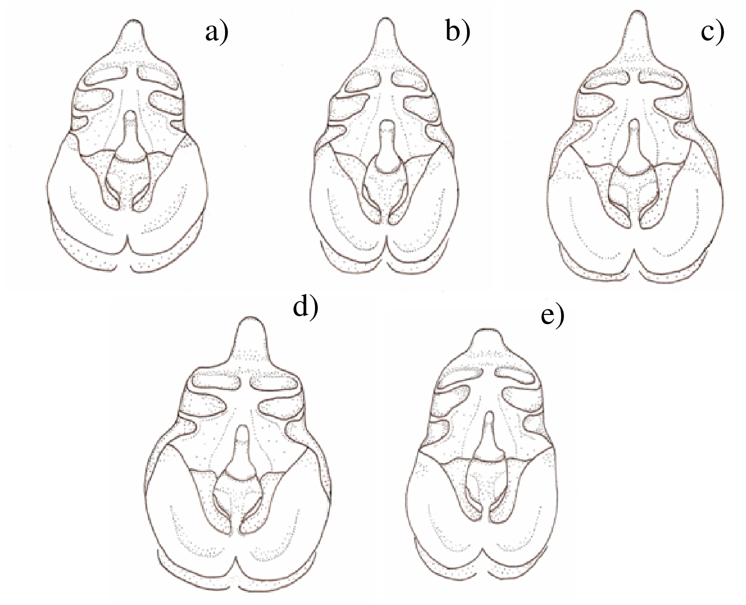


Figure 23. The variations of lancet in *R. pusillus*, *R. l. refulgens* and *Rhinolophus* sp. from Khao Samo Khon, Lopburi. a) *R. l. refulgens* (PSUZC-MM06.107); b) *R. pusillus* (PSUZC-MM07.258); c) *R. l. refulgens* (PSUZC-MM07.107); d) *Rhinolophus* sp. from Khao Samo Khon, Lopburi (PSUZC-MM07.25); e) *R. l. refulgens* (PSUZC-MM07.105).

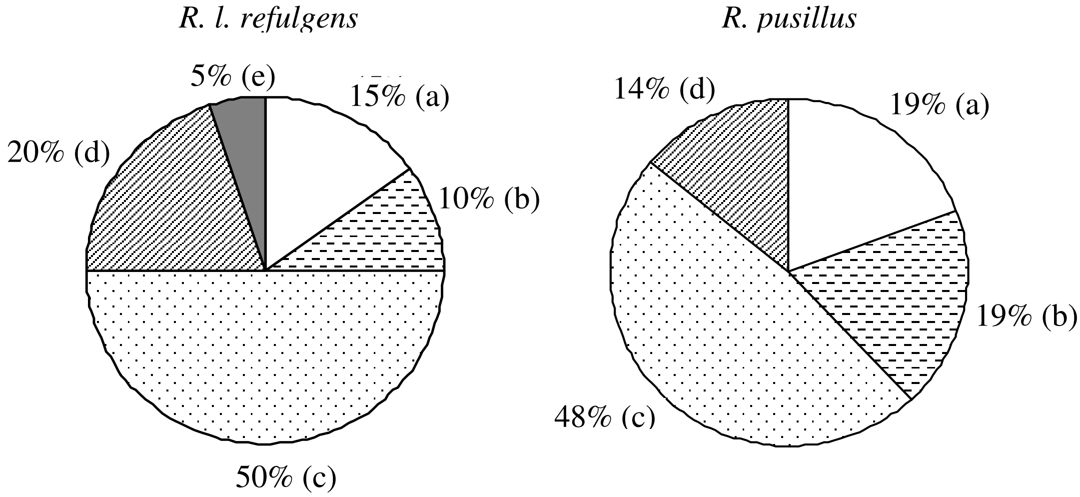


Figure 24. The variation of lancet (percent) in *R. l. refulgens* (n=20), *R. pusillus* (n=21) and showed in the pie chart. The types of lancet from Figure 23 are in parenthesis.

Variation of connecting process

Rhinolophus lepidus refulgens (n=21)

In lateral view, the tip of connecting process is pointed. There are the variations that the tip is blunt but it is not rounded shape like in *R. affinis* group. In *R. l. refulgens*, there are four types of connecting process (Figure 25).

The first type (Figure 25a) is the normal type that the side is parallels the upper and lower tip is point with the triangular shaped at the tip of connecting process; there are 66% from the specimens of *R. l. refulgens* that have this type. The second (Figure 25b), the upper tip is longer than the lower tip with the triangular shaped at the tip; there are 24% from the specimens that have this type. The third type (Figure 25c), this type is similar to first type (Figure 25a) but the upper tip is blunt; there are 5% from the specimens have this type. The last type (Figure 25d), the side is gradually narrow from the base of connecting process, the upper and lower tip are pointed, the shape at the tip of connecting process is semicircle, there was 5% of the specimens was this type (Figure 26).

Rhinolophus pusillus (n=29)

There are four types of connecting process as *R. l. refulgens*. There are 66% of *R. pusillus* specimens that have the connecting process type one (Figure 25a). There are 17% of specimens are type two (Figure 25b). There are 3% are type three (Figure 25c). And there are 14% are type four (Figure 25d) (Figure 26).

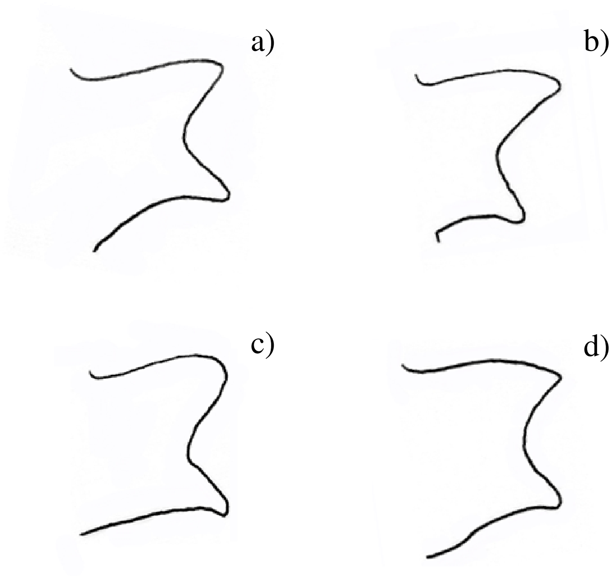


Figure 25. (a-d) Shape variation of connecting process of *R. l. refulgens*, *R. pusillus*, Loei population and Lopburi population.

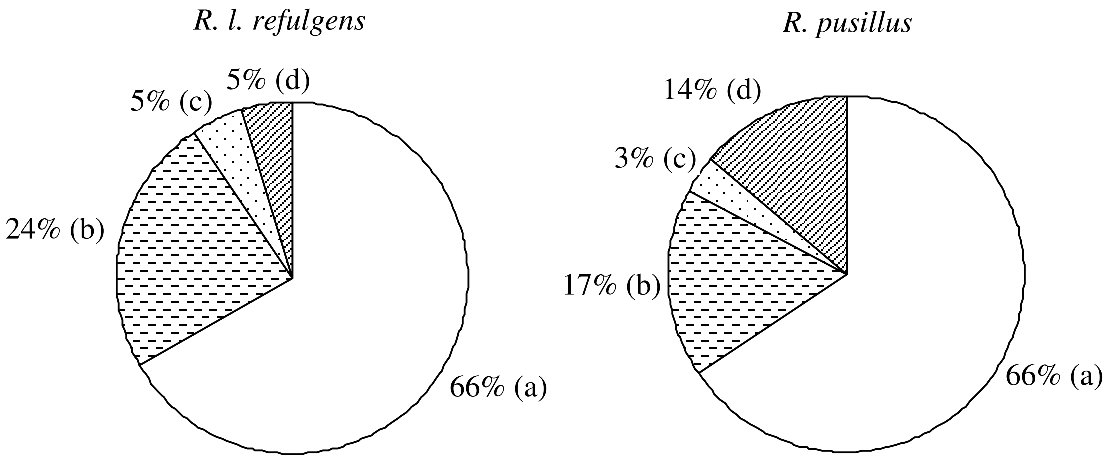


Figure 26. The variation of connecting process (percent) in *R. l. refulgens* (n=21) and *R. pusillus* (n=29) showed in the pie chart. The types of lancet from Figure 23 are in parenthesis.

Variation of sella

Rhinolophus lepidus refulgens (n=13)

The sella of this taxon is narrow; there are two types of sella. The first type (Figure 27a), the base of sella is broader and gradually narrower to the summit, its summit is rounded (46%). The second type (Figure 27b), the sella is parallel sided from base to the summit and its summit is truncated (54%) (Figure 27).

Rhinolophus pusillus (n=24)

There are three types of sella in *R. pusillus*. The first and the second type are comparable to the sella type of *R. l. refulgens*. The first type, the base of sella is broader and gradually narrower to the summit, its summit is rounded (33%) (Figure 28a). The second type, the sella is parallel sided from base to the summit and its summit is truncated (63%) (Figure 28b). The third type, the base and the summit is subeaqully, the summit is subcircular and it has the concave nearly the middle of the sella (4%) (Figure 28c).

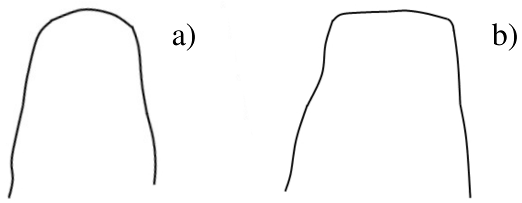


Figure 27. The shape variation of sella in *R. l. refulgens*.

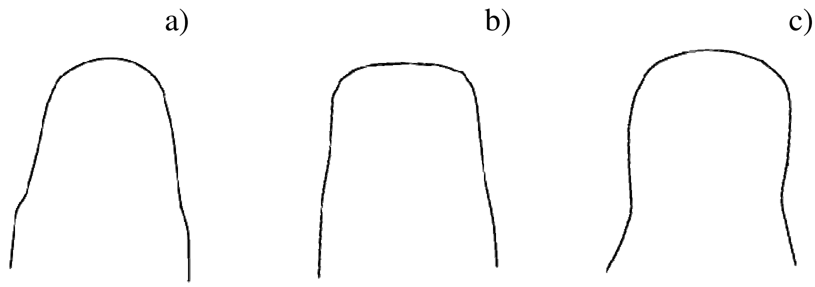


Figure 28. The variation in sella shape of *R. pusillus***.**

Variation in colour of pelage

Rhinolophus lepidus refulgens

The pelage colour is typically grayish brown to dark brown and occasionally bright foxy orange. The pelage on the ventral side is paler than the dorsal. The tip of hairs is paler than base. A minority of individuals are considerably darker and orange-brown. The pelage of *R. l. refulgens* is generally darker than *R. pusillus*. There are two colour patterns, one dark brown and the other reddish brown in the fur. Because these colour patterns appear within one population, they may fall in individual variation.

Rhinolophus pusillus

The pelage colour is typically grayish brown. The pelage is generally paler than *R. l. refulgens*. The pelage at ventral is paler than dorsal. The base of hairs is paler than the tip. A minority of individuals are considerably bright orange brown.

4.3 Echolocation

In the current study, six parameters of echolocation calls of 47 specimens were examined. Four different groups based on frequency at maximum intensity (FINT) of echolocation call were recognised (Table 1). These four populations were: the largest bats from Loei Province found in Phu Suan Sai National Park, bats that found in central and northern Thailand (*R. pusillus*), bats that found below Isthmus of Kra (*R. l. refulgens*) and the smallest bats collected from Khao Samoh-khon, Lopburi Province, Central Thailand. The range of frequency at maximum intensity of each population were 85.16-92.5, 107.82-114.70, 98.80-105.20 and 122-126 kHz respectively. None of them were overlap in FINT (Table 1)

Sexual dimorphism

In each taxon, all parameters of echolocation were tested for sexual dimorphism with T-test except Loei population because there was only one female in its population. There were no significantly different of six parameters between sexes in all groups (Table 2).

Variation in echolocation characters between groups

One-way ANOVA was applied to determine the variation in echolocation characters among these four populations. Pulse duration (D) was the only one character that was not significantly different (P = 0.078). There were significantly different in FINT, SF, EF, FMAX and FMIN between groups (Appendix 1). Tukey HSD post hoc test was used to compare for all parameters between groups. FINT, SF and FMAX were found to be significantly different between each pair within four populations of *Rhinolophus* (Appendix 2).

When forearm length was plotted against frequency at maximum intensity, four groups were recognised. In some cases, there was an overlap in forearm length while the echolocation was completely separated. *Rhinolophus* sp. from Loei province, *R. l. refulgens* and *R. pusillus* are different in morphology but *R. pusillus* and *Rhinolophus* sp. from Lopburi have very similar in morphology. The average of FA, SL and the other characters of *Rhinolophus* sp. from lopburi seem to be smaller than *R. pusillus* but there are some overlaps in all measurement. The scatter plot of peak frequency against FA showed the negative relationship (Figure 29). A similar figure also found between SL and peak frequency (Figure 30).

Latitudinal variation in echolocation

The correlation between latitude and peak frequency were examined with Spearman correlations (Appendix 3). *R. pusillus* was found higher than 12 degree north. There was a negative relationship between latitude and peak frequency ($r = -0.417$, $df = 34$, $P = 0.011$) (Figure 31). For *R. l. refulgens*, it was found lower than 11 degree north and there was no significant correlation between latitude and peak frequency of echolocation calls ($r = 0.334$, $df = 17$, $P = 0.19$ (Figure 32).

Table 1. Descriptive statistics for echolocation call characters of 4 populations of *Rhinolophus*. Table shows mean ± SD and minimum-maximum of parameters: Pulse Duration (D, ms); Frequency at maximum intensity (FINT, kHz); Start frequency (SF, kHz); End frequency (EF, kHz); Maximum frequency (FMAX, kHz) and Minimum frequency (FMIN, kHz).

Frequency parameters	*Rhinolophus* sp. from Loei (n= 3)		*R. l. refulgens* from Southern Thailand (n= 16)		*R. pusillus* from Central and Northern Thailand (n= 18)		*Rhinolophus* sp. from Lopburi (n= 10)	
	Mean ± SD	Min-Max	Mean ± SD	Min-Max	Mean ± SD	Min-Max	Mean ± SD	Min-Max
D (ms)	36.36 ± 11.11	29.28 - 49.17	27.00 ± 8.97	16.38 - 49.00	24.55 ± 7.05	12.55 - 38.26	29.94 ± 6.48	23.75 - 45.80
FINT (kHz)	88.19 ± 3.84	85.16 - 92.50	102.14 ± 1.92	98.80 - 105.20	110.29 ± 1.96	107.82 - 114.70	124.44 ± 1.18	122.00 - 126.30
SF (kHz)	80.49 ± 0.77	79.67 - 81.20	91.93 ± 4.51	84.00 - 100.00	99.28 ± 4.57	91.00 - 105.25	113.27 ± 5.21	103.56 - 122.00
EF (kHz)	76.51 ± 8.56	70.60 - 86.33	83.09 ± 4.79	74.00 - 93.00	89.40 ± 3.27	80.80 - 95.00	104.27 ± 4.38	98.34 - 115.20
FMAX (kHz)	90.07 ± 2.81	87.40 - 93.00	103.64 ± 1.60	101.80 - 107.00	111.66 ± 1.51	109.00 - 115.00	127.21 ± 0.40	126.94 - 128.20
FMIN (kHz)	73.13 ± 8.63	67.00 - 83.00	76.85 ± 6.54	67.60 - 91.00	86.08 ± 3.56	76.60 - 92.40	101.90 ± 4.73	96.82 - 114.40

Table 2. Descriptive statistics and T-test p-value of six echolocation call characters (Pulse Duration (D, ms); Frequency at maximum intensity (FINT, kHz); Start frequency (SF, kHz); End frequency (EF, kHz); Maximum frequency (FMAX, kHz) and Minimum frequency (FMIN, kHz)) in male and female of three populations of *Rhinolophus* in Thailand.

Frequency parameters	*R. l. refulgens* from Southern Thailand					*R. pusillus* from Central and Northern Thailand					*Rhinolophus* sp. from Lopburi				
	Mean ± SD				P	Mean ± SD				P	Mean ± SD				P
	Female	n	Male	n		Female	n	Male	n		Female	n	Male	n	
D (ms)	26.94 ± 5.93	6	27.03 ± 10.70	10	0.986	24.68 ± 8.42	7	24.47 ± 6.48	11	0.955	30.75 ± 8.69	5	29.13 ± 4.15	5	0.717
FINT (kHz)	102.23 ± 1.49	6	102.08 ± 2.21	10	0.882	109.50 ± 1.08	7	110.79 ± 2.27	11	0.127	124.62 ± 1.63	5	124.25 ± 0.61	5	0.642
SF (kHz)	90.73 ± 4.03	6	92.64 ± 4.83	10	0.432	97.66 ± 4.11	7	100.31 ± 4.73	11	0.240	113.28 ± 4.94	5	113.27 ± 6.05	5	0.997
EF (kHz)	82.80 ± 1.33	6	83.27 ± 6.10	10	0.819	90.56 ± 3.17	7	88.67 ± 3.27	11	0.244	105.16 ± 5.68	5	103.38 ± 2.98	5	0.553
FMAX (kHz)	103.33 ± 1.64	6	103.82 ± 1.64	10	0.575	111.46 ± 1.58	13	112.02 ± 1.34	21	0.280	127.28 ± 0.52	5	127.15 ± 0.27	5	0.629
FMIN (kHz)	76.40 ± 2.15	6	77.12 ± 8.28	10	0.799	86.34 ± 3.40	7	85.90 ± 3.82	11	0.808	103.04 ± 6.42	5	100.76 ± 2.43	5	0.480

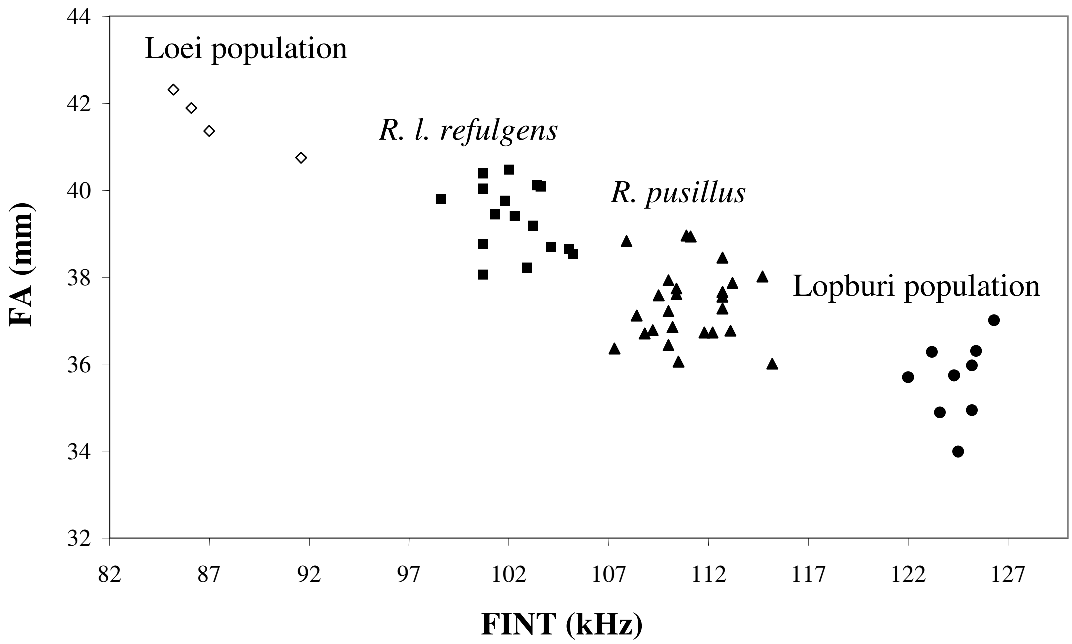


Figure 29. The relationship between frequency at maximum intensity (FINT) and forearm length of each individual of each taxon.

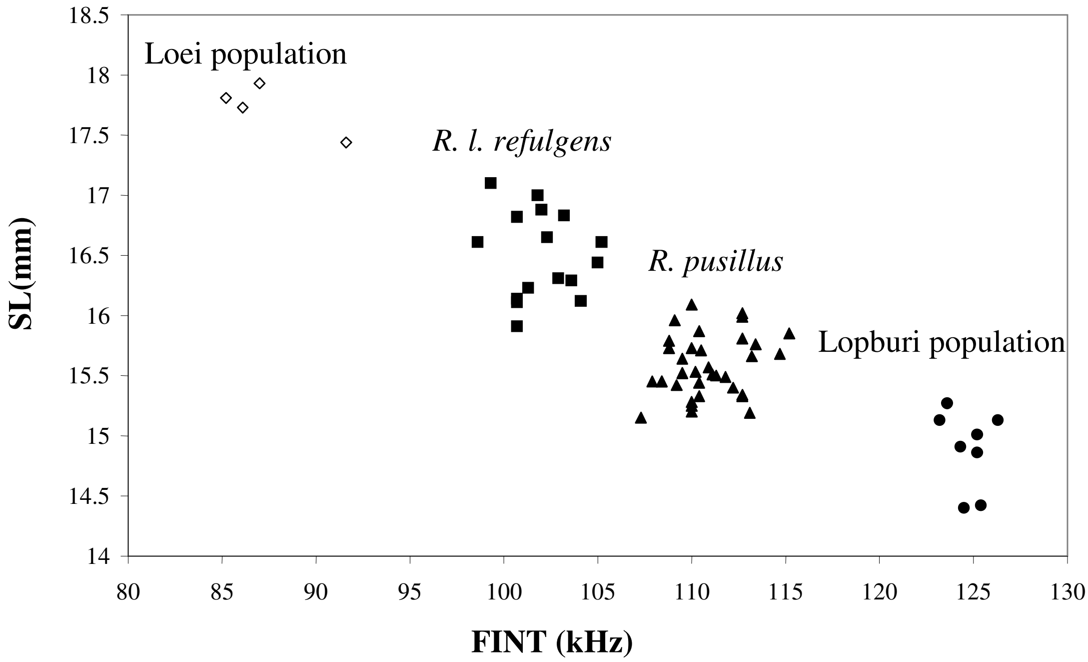


Figure 30. The relationship between frequency at maximum intensity (FINT) and skull length (SL) of each individual of each taxon.

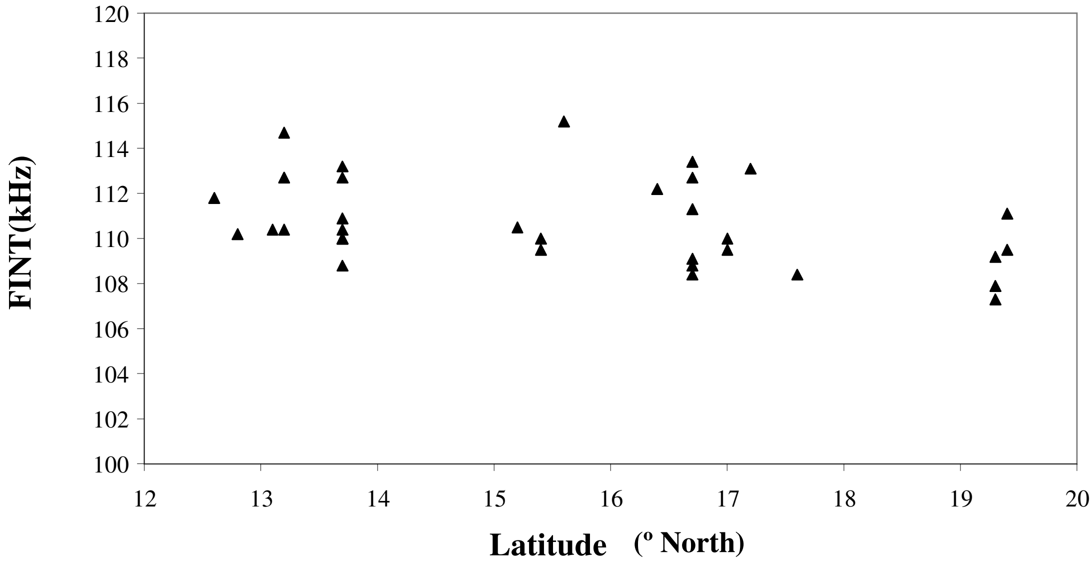


Figure 31. The relationship between latitude (º North) and frequency at maximum intensity (FINT; kHz) of *R. pusillus*.

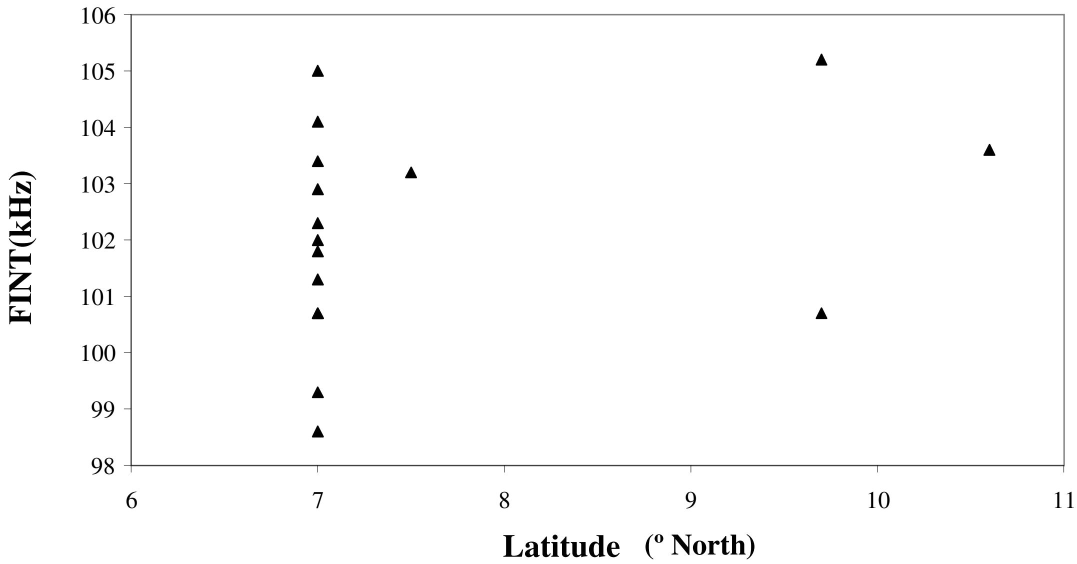


Figure 32. The relationship between latitude (º North) and frequency at maximum intensity (FINT; kHz) of *R. l. refulgens*.

4.4 Measurements

External and cranial morphology measurements

Fifteen external and 15 cranial measurements (see abbreviations from Table 3) of *Rhinolophus* spp. from Thailand, *R. lepidus* from India, *R. l. refulgens* from Malaysia and *R. pusillus* from Java were compared (Table 4). The measurements of *R. lepidus*, *R. l. refulgens* from Peninsular Malaysia and *R. l. refulgens* from southern Thailand are in the same range but *Rhinolophus* sp. from Phu Suan Sai National Park, Loei Province, northeastern Thailand were larger than *R. lepidus* from India and *R. l. refulgens* from southern Thailand. *R. pusillus* from Java and *R. pusillus* from central and northern Thailand were in the same range of all measurement. *R. pusillus* from Khao Samo Khon, Lopburi province is slightly smaller than *R. pusillus* from Java and other *R. pusillus* from Thailand (Table 4). The measurements *of Rhinolophus* sp. from Surin and Tarutao Island, southern Thailand were in the same range as *R. pusillus* but the echolocation was comparable to *R. l. refulgens* found in southern Thailand.

Twenty-seven external and cranial measurements were taken. Descriptive statistic (Mean ± SD amd Minimum – Maximum) of all external and cranial measurements from 4 Thailand populations are showed in Table 5.

Sexual dimorphism

The difference between sex of all group except *Rhinolophus* sp. from Loei were tested by T-test (There is one female from Loei population). There were significant variation between sexes in *R. l. refulgens* and *R. pusillus* in some characters. For *R. l. refulgens* from southern Thailand, there were significant difference in HB, TIB, GTL, CBL, CCL, SL, $C\text{-}M^3$ and $C\text{-}M_3$. For *R. pusillus* from central and northern Thailand, there were significant sexual differences in FA, 5mt, 4mt, SL, $C\text{-}M^3$, $C\text{-}M_3$ and $M_3\text{-}M_3$. Male and female of Lopburi population were not difference (Table 6).

Table 3. List of measurements and their abbreviations.

1. Morphology	
Forearm	FA
Length	L
Head and body length	HB
Tail length	T
Foot	HF
Ear	E
Tibia	TIB
Fifth metacarpal	5mt
First phalanx of the fifth metacarpal	1ph 5mt
Second phalanx of the fifth metacapal	2ph 5mt
Fourth metacarpal	4mt
First phalanx of the fourth metacarpal	1ph 4mt
Second phalanx of the fourth metacarpal	2ph 4mt
Third metacarpal	3mt
First phalanx of the third metacarpal	1ph 3mt
Second phalanx of third metacarpal	2ph 3mt
2. Cranium	
Greatest length of skull	GTL
Condylo-basal length	CBL
Condylo-canine length	CCL
Skull length	SL
Zygomatic breadth	ZB
Breadth of braincase	BB
Mastoid width	MW
Postorbital constriction	PC
Maxillary toothrow	$C\text{-}M^3$
Mandibular toothrow	$C\text{-}M_3$
Anterior palatal width	$C^1\text{-}C^1$
Posterior palatal width	$M^3\text{-}M^3$
Mandible length	M

The measurements were based on Bates and Harrison (1997).

Table 4. Descriptive statistics for morphological measurements of *Rhinolophus* spp.from Thailand, *R. lepidus*, *R. l. refulgens* and *R. pusillus* from type localities. Table shows mean ± SD and minimum-maximum in mm.

Characters	*R. lepidus lepidus*** (India)			*R. lepidus refulgens** (Malaya)			*R. l. refulgens* (Southern Thailand)			*Rhinolophus* sp. from Loei (NE Thailand)		
	Average ± SD	Min - Max	n	Average ± SD	Min - Max	n	Average ± SD	Min - Max	n	Average ± SD	Min - Max	n
FA	40.35 ± 1.04	38.67 - 41.42	6	-	-		39.44 ± 1.13	37.70 - 42.56	20	41.58 ± 0.67	40.75 - 42.31	4
TIB	16.01 ± 0.53	15.08 - 16.60	6	-	-		16.19 ± 0.74	15.00 - 17.99	20	17.23 ± 0.86	16.79 - 18.58	4
SL	16.45 ± 0.46	16.03 - 16.99	6	17.02 ± 0.24	16.85 - 17.19	2	16.49 ± 0.35	15.91 - 17.10	17	17.73 ± 0.21	17.44 - 17.93	4
CBL	14.81 ± 0.62	14.11 - 15.56	6	15.78 ± 0.20	15.64 - 15.92	2	14.98 ± 0.31	14.60 - 15.76	12	15.95 ± 0.23	15.78 - 16.11	2
$C\text{-}M^3$	6.03 ± 0.22	5.74 - 6.34	8	6.20 ± 0.18	6.07 - 6.33	2	6.12 ± 0.18	5.87 - 6.53	17	6.64 ± 0.20	6.36 - 6.81	4
CCL	14.37 ± 0.63	13.76 - 15.04	6	14.95 ± 0.25	14.77 - 15.13	2	14.50 ± 0.34	14.00 - 14.99	17	15.61 ± 0.27	15.30 - 15.79	3
$M^3\text{-}M^3$	5.92 ± 0.11	5.81 - 6.11	7	6.03 ± 0.05	5.99 - 6.06	2	6.07 ± 0.14	5.87 - 6.34	17	6.35 ± 0.10	6.22 - 6.45	4
ZB	8.24 ± 0.24	7.90 - 8.51	7	8.66 ± 0.17	8.54 - 8.78	2	8.33 ± 0.15	8.11 - 8.65	17	8.83 ± 0.21	8.72 - 9.14	4
BB	6.96 ± 0.11	6.81 - 7.07	8	7.42 ± 0.05	7.38 - 7.45	2	7.15 ± 0.26	6.67 - 7.62	17	7.33 ± 0.17	7.09 - 7.50	4
MW	8.08 ± 0.17	7.92 - 8.39	7	8.36 ± 0.08	8.31 - 8.42	2	8.20 ± 0.16	7.95 - 8.57	17	8.57 ± 0.16	8.44 - 8.80	4
PC	2.26 ± 0.10	2.14 - 2.42	8	2.43 ± 0.07	2.38 - 2.48	2	2.31 ± 0.15	2.04 - 2.61	17	2.41 ± 0.13	2.30 - 2.60	4
$C\text{-}M_3$	6.41 ± 0.27	6.01 - 6.79	8	6.68 ± 0.17	6.56 - 6.80	2	6.53 ± 0.22	6.18 - 6.93	17	7.08 ± 0.18	6.90 - 7.26	4
$C^1\text{-}C^1$	3.91 ± 0.33	3.29 - 4.22	8	4.18 ± 0.21	4.03 - 4.32	2	4.23 ± 0.16	4.01 - 4.60	17	4.43 ± 0.15	4.22 - 4.56	4
M	10.96 ± 0.42	10.38 - 11.63	8	11.49 ± 0.23	11.33 - 11.65	2	11.08 ± 0.32	10.70 - 11.64	17	11.46 ± 0.32	10.99 - 11.65	4
RW	4.51 ± 0.16	4.22 - 4.65	6	4.63 ± 0.11	4.55 - 4.70	2	4.54 ± 0.13	4.35 - 4.85	17	4.91 ± 0.16	4.69 - 5.03	4

* specimens from Natural History Museum, London (BMNH)

** specimens from the collections of the Harrison Institute (HZM)

Table 4. Descriptive statistics for morphological measurements of *Rhinolophus* spp.from Thailand, *R. lepidus*, *R. l. refulgens* and *R. pusillus* from type localities. Table shows mean ± SD and minimum-maximum in mm. (continued)

Characters	*Rhinolophus* sp. (Surin Is. and Tarutao Is; southern Thailand.)			*R. pusillus** (Java, Borneo, Tioman Is. And Penang)			*R. pusillus* (Central and northernThailand)			*Rhinolophus sp.* (Lopburi; Central Thailand)		
	Average ± SD	Min - Max	n	Average ± SD	Min - Max	n	Average ± SD	Min - Max	n	Average ± SD	Min - Max	n
FA	37.42 ± 0.65	36.62 - 38.42	5	-	-	-	37.26 ± 0.96	34.92 - 39.42	47	35.73 ± 0.90	33.99 - 37.01	10
TIB	15.28 ± 0.57	14.60 - 16.10	5	-	-	-	15.59 ± 0.78	13.42 - 17.37	47	15.27 ± 0.67	13.83 - 16.41	10
SL	15.74 ± 0.29	15.31 - 16.12	5	15.64 ± 0.19	15.34 - 15.81	6	15.53 ± 0.25	15.10 - 16.09	45	14.89 ± 0.32	14.40 - 15.27	8
CBL	14.69 ± 0.09	14.58 - 14.74	3	14.11 ± 0.04	14.08 - 14.13	2	14.22 ± 0.28	13.45 - 14.72	32	13.51 ± 0.24	13.02 - 13.75	7
$C-M^3$	5.82 ± 0.17	5.57 - 6.05	5	5.72 ± 0.10	5.53 - 5.88	8	6.13 ± 0.15	5.67 - 6.37	45	5.38 ± 0.19	5.13 - 5.66	8
CCL	13.87 ± 0.23	13.51 - 14.11	5	13.66 ± 0.18	13.35 - 13.78	5	13.61 ± 0.26	13.18 - 14.48	45	12.91 ± 0.30	12.30 - 13.19	8
M^3-M^3	5.74 ± 0.08	5.65 - 5.84	5	5.69 ± 0.20	5.31 - 5.98	8	5.64 ± 0.14	5.33 - 5.93	45	5.28 ± 0.08	5.20 - 5.43	8
ZB	7.89 ± 0.12	7.77 - 8.05	5	7.60 ± 0.82	6.00 - 8.23	6	7.74 ± 0.22	7.31 - 8.37	45	7.29 ± 0.15	7.08 - 7.55	8
BB	6.77 ± 0.21	6.50 - 7.00	5	6.90 ± 0.13	6.74 - 7.03	7	6.59 ± 0.21	5.98 - 6.90	45	6.47 ± 0.21	6.16 - 6.82	8
MW	7.82 ± 0.19	7.63 - 8.09	4	7.84 ± 0.11	7.74 - 8.07	7	7.68 ± 0.13	7.40 - 7.93	45	7.34 ± 0.12	7.16 - 7.51	8
PC	2.15 ± 0.12	1.99 - 2.32	5	2.21 ± 0.10	2.09 - 2.38	7	2.11 ± 0.17	1.75 - 2.46	45	2.11 ± 0.19	1.96 - 2.54	8
$C-M_3$	6.24 ± 0.11	6.07 - 6.34	5	6.17 ± 0.17	5.97 - 6.44	9	6.13 ± 0.15	5.67 - 6.37	45	5.73 ± 0.22	5.40 - 6.04	8
C^1-C^1	4.00 ± 0.22	3.65 - 4.18	5	3.75 ± 0.15	3.51 - 3.91	6	3.87 ± 0.14	3.68 - 4.08	44	3.69 ± 0.17	3.45 - 3.96	8
M	10.66 ± 0.10	10.58 - 10.77	4	10.56 ± 0.21	10.26 - 10.87	9	10.29 ± 0.27	9.59 - 10.73	44	9.73 ± 0.21	9.36 - 9.99	8
RW	4.40 ± 0.08	4.29 - 4.46	5	4.34 ± 0.13	4.17 - 4.55	7	4.24 ± 0.12	4.05 - 4.44	40	4.02 ± 0.07	3.93 - 4.13	8

* specimens from Natural History Museum, London (BMNH)

** specimens from the collections of the Harrison Institute (HZM)

Table 5. Descriptive statistics for external measurements of 4 populations of *Rhinolophus* spp. in Thailand. Table shows mean ± SD and minimum-maximum in mm.

Characters	*R. l. refulgens* from Southern Thailand			*R. pusillus* from Central and Northern Thailand			*Rhinolophus* sp. from Lopburi			*Rhinolophus* sp. from Loei		
	Mean ± SD	Min - Max	n	Mean ± SD	Min - Max	n	Mean ± SD	Min - Max	n	Mean ± SD	Min - Max	n
FA	39.44 ± 1.13	37.70 - 42.56	20	37.26 ± 0.96	34.92 - 39.42	47	35.75 ± 0.90	33.99 - 37.01	10	41.58 ± 0.76	40.75 - 42.31	4
L	63.92 ± 5.19	53.58 - 72.79	18	55.58 ± 5.01	45.98 - 64.48	42	55.59 ± 2.04	52.63 - 57.96	10	68.92 ± 4.68	62.44 - 73.60	4
HB	44.28 ± 4.10	37.23 - 51.07	18	38.01 ± 4.10	29.80 - 45.49	42	37.51 ± 1.22	35.89 - 39.87	10	46.13 ± 2.71	42.70 - 48.47	4
E	14.94 ± 1.03	13.67 - 16.77	20	14.23 ± 1.11	11.40 - 17.32	47	12.90 ± 0.97	11.04 - 14.2	10	15.86 ± 0.46	15.49 - 16.53	4
T	19.35 ± 2.13	14.56 - 22.35	20	17.44 ± 1.97	13.35 - 21.26	47	18.08 ± 1.23	16.14 - 20.4	10	22.79 ± 2.80	19.74 - 25.49	4
HF	7.13 ± 0.40	6.18 - 7.91	20	6.41 ± 0.57	4.04 - 7.30	47	6.15 ± 0.30	5.81 - 6.86	10	7.67 ± 0.40	7.44 - 8.27	4
TIB	16.19 ± 0.74	15.00 - 17.99	20	15.59 ± 0.78	13.42 - 17.37	47	15.27 ± 0.67	13.83 - 16.41	10	17.30 ± 0.86	16.79 - 18.58	4
5mt	29.42 ± 0.99	28.18 - 31.74	20	27.92 ± 0.83	26.08 - 29.61	47	26.61 ± 0.77	25.24 - 27.94	9	32.47 ± 0.80	31.91 - 33.64	4
1ph5mt	8.90 ± 0.41	7.94 - 9.50	20	8.50 ± 0.43	7.34 - 9.77	47	8.13 ± 0.43	7.29 - 8.76	9	9.91 ± 0.32	9.57 - 10.23	4
2ph5mt	10.29 ± 1.16	6.29 - 11.96	20	9.83 ± 0.58	8.31 - 11.01	47	9.26 ± 0.53	8.15 - 9.84	9	11.72 ± 0.48	11.09 - 12.20	4
4mt	30.05 ± 0.77	29.10 - 32.19	20	28.28 ± 0.80	26.67 - 30.33	47	27.30 ± 0.77	26.33 - 28.6	9	32.52 ± 0.41	32.03 - 32.92	4
1ph4mt	8.15 ± 0.37	7.33 - 8.74	20	7.77 ± 0.36	6.88 - 8.95	47	7.35 ± 0.32	6.85 - 7.83	9	9.41 ± 0.42	8.85 - 9.85	4
2ph4mt	9.71 ± 0.87	7.03 - 10.86	20	9.40 ± 0.56	7.72 – 10.40	47	8.81 ± 0.52	8.13 - 9.51	9	11.16 ± 0.33	10.87 - 11.62	4
3mt	28.88 ± 0.75	27.86 - 30.68	20	27.21 ± 0.77	25.64 - 29.01	47	26.13 ± 0.87	24.79 - 27.41	9	31.47 ± 0.33	31.15 - 31.93	4
1ph3mt	10.82 ± 0.52	9.97 - 11.78	20	10.32 ± 0.40	9.27 - 11.20	47	9.91 ± 0.53	9.01 - 10.72	9	12.61 ± 0.48	12.18 - 13.08	4
2ph3mt	15.70 ± 0.97	13.87 - 17.62	20	14.27 ± 0.83	12.57 - 16.20	47	13.68 ± 0.75	12.79 - 15.19	9	18.26 ± 0.32	18.00 - 18.69	4

Table 5. Descriptive statistics for external measurements of 4 populations of *Rhinolophus* spp. in Thailand. Table shows mean ± SD and minimum-maximum in mm. (continued)

Characters	*R. l. refulgens* from Southern Thailand			*R. pusillus* from Central and Northern Thailand			*Rhinolophus* sp. from Lopburi			*Rhinolophus* sp. from Loei		
	Mean ± SD	Min - Max	n	Mean ± SD	Min - Max	n	Mean ± SD	Min - Max	n	Mean ± SD	Min - Max	n
GTL	17.12 ± 0.43	16.57 - 17.89	17	16.23 ± 0.27	15.78 - 16.82	38	15.37 ± 0.34	14.87 - 15.79	7	18.48 ± 0.55	17.80 - 18.95	4
CBL	14.98 ± 0.31	14.60 - 15.76	12	14.22 ± 0.28	13.45 - 14.72	32	13.51 ± 0.24	13.02 - 13.75	7	15.95 ± 0.23	15.78 - 16.11	2
CCL	14.47 ± 0.33	14.00 - 14.99	19	13.61 ± 0.26	13.18 - 14.48	45	12.91 ± 0.30	12.30 - 13.19	8	15.61 ± 0.27	15.30 - 15.79	3
SL	16.46 ± 0.35	15.91 - 17.10	19	15.53 ± 0.25	15.10 - 16.09	45	14.89 ± 0.32	14.40 - 15.27	8	17.73 ± 0.21	17.44 - 17.93	4
ZB	8.30 ± 0.17	7.97 - 8.65	19	7.74 ± 0.22	7.31 - 8.37	45	7.29 ± 0.15	7.08 - 7.55	8	8.83 ± 0.21	8.72 - 9.14	4
BB	7.15 ± 0.25	6.67 - 7.62	19	6.59 ± 0.21	5.98 - 6.90	45	6.47 ± 0.21	6.16 - 6.82	8	7.33 ± 0.17	7.09 - 7.50	4
MW	8.17 ± 0.18	7.80 - 8.57	19	7.68 ± 0.13	7.40 - 7.93	45	7.34 ± 0.12	7.16 - 7.51	8	8.57 ± 0.16	8.44 - 8.80	4
PC	2.29 ± 0.15	2.04 - 2.61	19	2.11 ± 0.17	1.75 - 2.46	45	2.11 ± 0.19	1.96 - 2.54	8	2.41 ± 0.13	2.30 - 2.60	4
$C\text{-}M^3$	6.12 ± 0.17	5.87 - 6.53	19	5.75 ± 0.14	5.48 - 6.06	45	5.38 ± 0.19	5.13 - 5.66	8	6.64 ± 0.21	6.36 - 6.81	4
$C\text{-}M_3$	6.52 ± 0.22	6.18 - 6.93	19	6.13 ± 0.15	5.67 - 6.37	45	5.73 ± 0.22	5.40 - 6.04	8	7.08 ± 0.18	6.90 - 7.26	4
$C^1\text{-}C^1$	4.21 ± 0.16	4.01 - 4.60	19	3.87 ± 0.14	3.68 - 4.08	44	3.69 ± 0.17	3.45 - 3.96	8	4.43 ± 0.15	4.22 - 4.56	4
$M^3\text{-}M^3$	6.08 ± 0.14	5.87 - 6.34	19	5.64 ± 0.14	5.33 - 5.93	45	5.28 ± 0.08	5.20 - 5.43	8	6.35 ± 0.10	6.22 - 6.45	4
M	11.07 ± 0.30	10.70 - 11.64	19	10.29 ± 0.27	9.59 - 10.73	44	9.73 ± 0.21	9.36 - 9.99	8	11.46 ± 0.32	10.99 - 11.65	4

Table 6. Descriptive statistics (Mean ± SD; mm) for external and cranial measurements from 3 populations of *Rhinolophus* spp. in Thailand and T-test between male and female.

Characters	*R. l. refulgens* from Southern Thailand					*R. pusillus* from Central and Northern Thailand					*Rhinolophus* sp. from Lopburi				
	Mean ± SD				P	Mean ± SD				P	Mean ± SD				P
	Female	n	Male	n		Female	n	Male	n		Female	n	Male	n	
FA	39.17 ± 0.96	9	39.66 ± 1.26	11	0.347	37.43 ± 1.01	17	37.16 ± 0.94	30	0.376	35.79 ± 1.12	5	35.46 ± 0.67	4	0.621
L	61.48 ± 5.47	8	65.88 ± 4.26	10	0.073	55.22 ± 3.77	17	55.83 ± 5.77	25	0.700	55.43 ± 2.18	5	55.75 ± 2.12	5	0.822
HB	42.02 ± 3.98	8	46.09 ± 3.36	10	0.031*	37.50 ± 3.34	17	38.35 ± 4.58	25	0.513	37.33 ± 1.27	5	37.69 ± 1.29	5	0.665
T	19.04 ± 2.49	9	19.60 ± 1.88	11	0.577	17.72 ± 1.71	17	17.28 ± 2.11	30	0.470	18.10 ± 1.04	5	18.06 ± 1.53	5	0.955
HF	7.00 ± 0.48	9	7.23 ± 0.31	11	0.210	6.54 ± 0.53	17	6.34 ± 0.60	30	0.255	6.15 ± 0.41	5	6.16 ± 0.16	5	0.961
E	15.01 ± 1.14	9	14.88 ± 0.99	11	0.790	14.12 ± 0.84	17	14.29 ± 1.24	30	0.611	12.42 ± 0.93	5	13.39 ± 0.83	5	0.120
TIB	15.84 ± 0.46	9	16.48 ± 0.81	11	0.049*	15.64 ± 0.79	17	15.55 ± 0.78	30	0.708	15.09 ± 0.74	5	15.44 ± 0.61	5	0.436
5mt	29.13 ± 1.01	9	29.65 ± 0.96	11	0.257	28.21 ± 0.79	17	27.76 ± 0.82	30	0.071	26.78 ± 0.97	5	26.41 ± 0.49	4	0.512
1ph5mt	8.84 ± 0.44	9	8.95 ± 0.39	11	0.562	8.62 ± 0.33	17	8.44 ± 0.47	30	0.169	8.05 ± 0.52	5	8.25 ± 0.33	4	0.530
2ph5mt	10.39 ± 0.61	9	10.20 ± 1.50	11	0.714	9.80 ± 0.44	17	9.84 ± 0.65	30	0.820	9.26 ± 0.65	5	9.26 ± 0.45	4	0.997
4mt	29.97 ± 0.57	9	30.12 ± 0.93	11	0.670	28.49 ± 0.88	17	28.16 ± 0.75	30	0.186	27.46 ± 0.87	5	27.10 ± 0.71	4	0.527
1ph4mt	8.11 ± 0.45	9	8.19 ± 0.30	11	0.671	7.78 ± 0.36	17	7.77 ± 0.36	30	0.913	7.48 ± 0.30	5	7.18 ± 0.29	4	0.172
2ph4mt	9.74 ± 0.50	9	9.69 ± 1.12	11	0.908	9.42 ± 0.47	17	9.39 ± 0.62	30	0.865	8.82 ± 0.53	5	8.80 ± 0.58	4	0.964
3mt	28.81 ± 0.67	9	28.94 ± 0.84	11	0.710	27.45 ± 0.71	17	27.08 ± 0.79	30	0.116	26.39 ± 1.03	5	25.81 ± 0.61	4	0.350
1ph3mt	10.67 ± 0.64	9	10.94 ± 0.37	11	0.246	10.39 ± 0.38	17	10.28 ± 0.41	30	0.362	10.06 ± 0.40	5	9.72 ± 0.66	4	0.363
2ph3mt	15.41 ± 0.98	9	15.93 ± 0.94	11	0.245	14.41 ± 0.84	17	14.18 ± 0.83	30	0.362	13.62 ± 0.46	5	13.75 ± 1.09	4	0.809

* The mean difference is significant at the 0.05 level

Table 6. Descriptive statistics (Mean ± SD; mm) for external and cranial measurements from 3 populations of *Rhinolophus* spp. in Thailand and T-test between male and female. (continued)

Characters	*R. l. refulgens* from Southern Thailand					*R. pusillus* from Central and Northern Thailand					*Rhinolophus* sp. from Lopburi				
	Mean ± SD				P	Mean ± SD				P	Mean ± SD				P
	Female	n	Male	n		Female	n	Male	n		Female	n	Male	n	
GTL	16.79 ± 0.19	7	17.36 ± 0.39	10	0.002*	16.11 ± 0.23	14	16.30 ± 0.27	24	0.040	15.28 ± 0.45	4	15.49 ± 0.12	3	0.428
CBL	14.81 ± 0.19	6	15.16 ± 0.31	6	0.040*	14.19 ± 0.27	13	14.25 ± 0.29	19	0.574	13.45 ± 0.33	4	13.59 ± 0.03	3	0.458
CCL	14.22 ± 0.15	9	14.71 ± 0.27	10	<0.001*	13.55 ± 0.33	16	13.64 ± 0.21	29	0.237	12.78 ± 0.40	4	13.03 ± 0.04	4	0.298
SL	16.20 ± 0.15	9	16.70 ± 0.30	10	<0.001*	15.40 ± 0.20	16	15.61 ± 0.25	29	0.007	14.74 ± 0.38	4	15.04 ± 0.19	4	0.225
ZB	8.25 ± 0.11	9	8.35 ± 0.20	10	0.177	7.69 ± 0.17	16	7.76 ± 0.24	29	0.324	7.27 ± 0.20	4	7.31 ± 0.11	4	0.784
BB	7.14 ± 0.23	9	7.16 ± 0.27	10	0.900	6.56 ± 0.23	16	6.61 ± 0.20	29	0.433	6.41 ± 0.07	4	6.54 ± 0.30	4	0.460
MW	8.12 ± 0.18	9	8.21 ± 0.17	10	0.297	7.66 ± 0.10	16	7.70 ± 0.15	29	0.428	7.36 ± 0.14	4	7.31 ± 0.12	4	0.580
PC	2.28 ± 0.17	9	2.30 ± 0.14	10	0.882	2.15 ± 0.18	16	2.08 ± 0.17	29	0.216	2.09 ± 0.07	4	2.13 ± 0.27	4	0.773
$C\text{-}M^3$	5.99 ± 0.09	9	6.23 ± 0.13	10	<0.001*	5.63 ± 0.10	16	5.81 ± 0.11	29	<0.001*	5.28 ± 0.21	4	5.48 ± 0.12	4	0.142
$C\text{-}M_3$	6.36 ± 0.12	9	6.67 ± 0.18	10	<0.001*	6.02 ± 0.14	16	6.19 ± 0.11	29	<0.001*	5.58 ± 0.21	4	5.88 ± 0.12	4	0.045
$C^1\text{-}C^1$	4.14 ± 0.10	9	4.28 ± 0.18	10	0.069	3.81 ± 0.08	16	3.90 ± 0.09	28	0.003*	3.59 ± 0.16	4	3.80 ± 0.11	4	0.080
$M^3\text{-}M^3$	6.07 ± 0.14	9	6.09 ± 0.14	10	0.761	5.58 ± 0.14	16	5.68 ± 0.12	29	0.015*	5.28 ± 0.10	4	5.29 ± 0.06	4	0.906
M	10.84 ± 0.13	9	11.29 ± 0.24	10	<0.001	10.20 ± 0.30	16	10.34 ± 0.24	28	0.093	9.70 ± 0.29	4	9.75 ± 0.13	4	0.783

* The mean difference is significant at the 0.05 level

Principal Component Analysis of 14 characters

The taxa in *R. pusillus* group were different from each other in external and cranial morphology. They were also different in sizes of several characters. However, they cannot be distinguished from each other by only one character. Principal Component analysis was to determine some characters that can distinguish them. Fourteen characters that have less error were chosen to analyse by principal components analysis. The fourteen characters were TIB, FA, 5mt, 4mt, 1ph4mt, 3mt, 1ph3mt, CCL, SL, ZB, C-M^3, C-M_3, C^1-C^1 and M^3-M^3.

Sixty-five specimens from Thailand were included in the analysis. The scatter plot of principal component between the first and second components showed that four populations (Loei population, *R. l. refulgens*, *R. pusillus* and Lopburi population) completely separate from each other in the same way as the results from echolocation analysis (Figure 33).

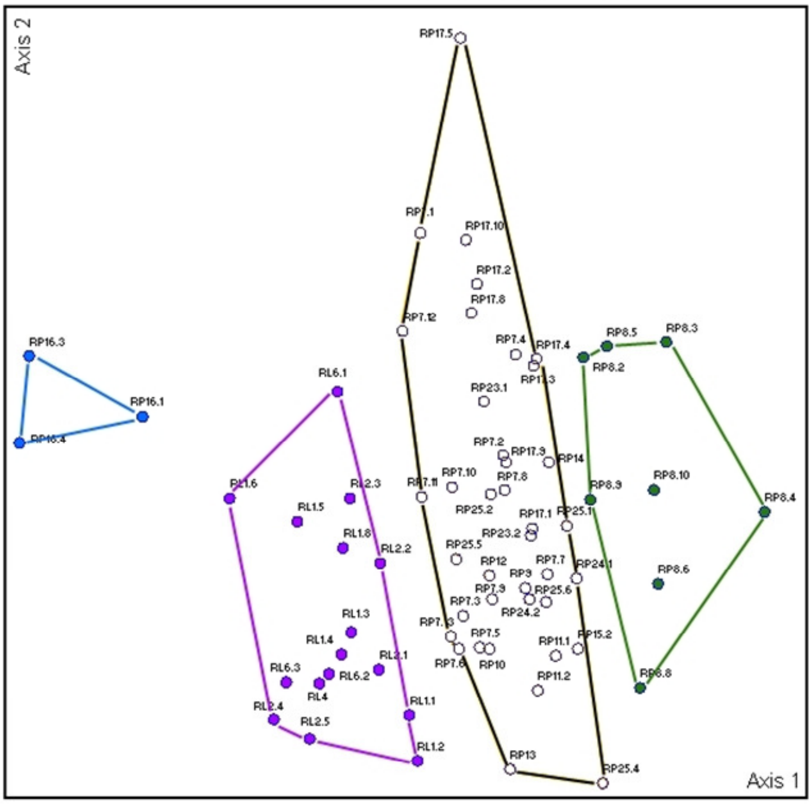


Figure 33. The principal component chart between first and second components from 14 characters of 65 specimens from Thailand (Loei population: blue, *R. l. refulgens*: purple, *R. pusillus*: open circle with black line, Lopburi population: green). (see Appendix 4 for variance explained)

Analysis of variance (ANOVA) test between groups

One-way ANOVA were applied to examine the variation in the measurements of these four groups. There were significantly different in all characters. These results supported the result from principal component analysis (Table 7). Tukey HSD post hoc test was applied for all characters between groups. Four populations were significantly different in all characters except TIB and 1ph3mt of *R. l. refulgens* and *R. pusillus* in (Table 8).

Table 7. One-way ANOVA of the measurements in 14 characters that used in PCA between 4 populations. Abbrevation and indices as Table 3.

Characters	df	MS	F	P
TIB	3	5.685	9.890	<0.001*
FA	3	55.339	56.133	<0.001*
5mt	3	42.115	56.287	<0.001*
4mt	3	39.825	65.298	<0.001*
1ph4mt	3	4.639	36.046	<0.001*
3mt	3	39.376	66.812	<0.001*
1ph3mt	3	8.138	40.452	<0.001*
CCL	3	8.820	110.937	<0.001*
SL	3	11.004	136.611	<0.001*
ZB	3	3.575	88.700	<0.001*
$C\text{-}M^3$	3	2.032	86.039	<0.001*
$C\text{-}M_3$	3	2.325	75.495	<0.001*
$C^1\text{-}C^1$	3	1.019	65.847	<0.001*
$M^3\text{-}M^3$	3	1.911	109.342	<0.001*

* The mean difference is significant at the 0.05 level

Table 8. The result from Tukey HSD post hoc test for the measurements in 14 characters that used in PCA. Abbrevation and indices were as Table 3 (1= Loei population, 2=*R. l. refulgens*, 3=*R. pusillus* and 4=Lopburi population)

Characters	Comparison between population of *Rhinolophus* spp. in Thailand					
	1 versus 2	1 versus 3	1 versus 4	2 versus 3	2 versus 4	3 versus 4
TIB	0.018*	0.012*	0.046*	0.628	<0.001*	<0.001*
FA	<0.001*	<0.001*	0.001*	<0.001*	<0.001*	<0.001*
5mt	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
4mt	<0.001*	<0.001*	<0.001*	0.005*	<0.001*	<0.001*
1ph4mt	0.001*	<0.001*	<0.001*	0.009*	<0.001*	<0.001*
3mt	<0.001*	<0.001*	<0.001*	0.001*	<0.001*	<0.001*
1ph3mt	<0.001*	<0.001*	<0.001*	0.067	<0.001*	<0.001*
CCL	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
SL	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
ZB	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
$C\text{-}M^3$	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
$C\text{-}M_3$	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
$C^1\text{-}C^1$	<0.001*	<0.001*	0.015*	0.003*	<0.001*	<0.001*
$M^3\text{-}M^3$	<0.001*	<0.001*	0.002*	<0.001*	<0.001*	<0.001*

* The mean difference is significant at the 0.05 level

Variations in some measurements

R. lepidus and *R. pusillus* can be distinguished from each other by size (Andersen, 1905). Variations in the measurements of FA, 3mt, SL and $C\text{-}M^3$ of samples in 4 populations were shown in Figure 34-37. The Loei population was the largest in such measurements among the populations found in Thailand and predominantly larger than the other taxa. There was one outlier in FA and 3mt of *R. l. refulgens* that collected from Songkhla province. This specimen overlapped in forearm length with the range of Loei population. Comparison between *R. l. refulgens* and *R. pusillus* and Lopburi population, their median were different but they were overlapped in range (Min-Max) of these characters.

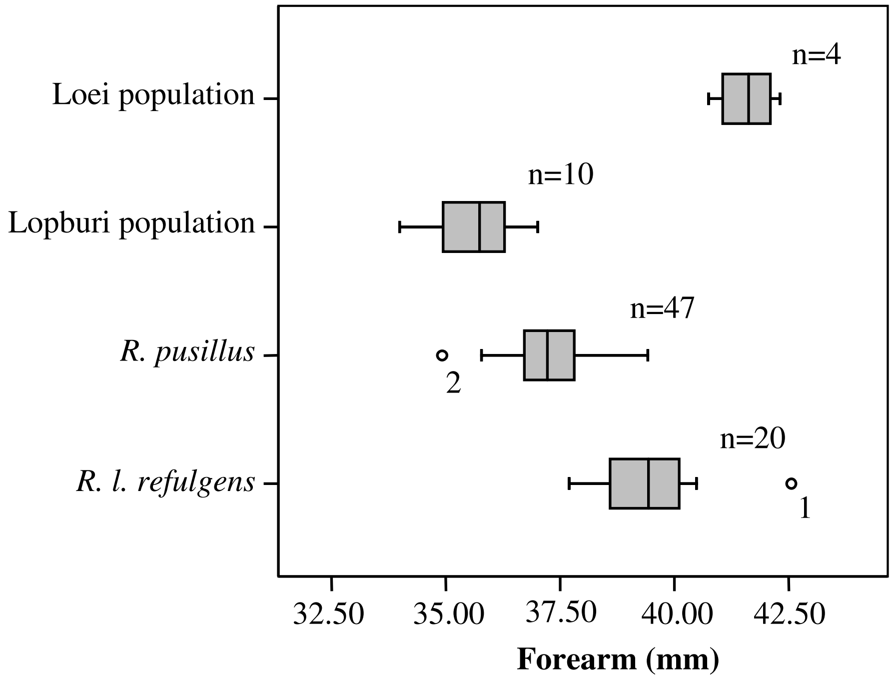


Figure 34. Variation of forearm (FA) in 4 populations is showed by box plot. (1: PSUZC-MM06.208 from Songkhla, southern Thailand; 2: PSUZC-MM07.263 from Mukdahan, northeastern Thailand)

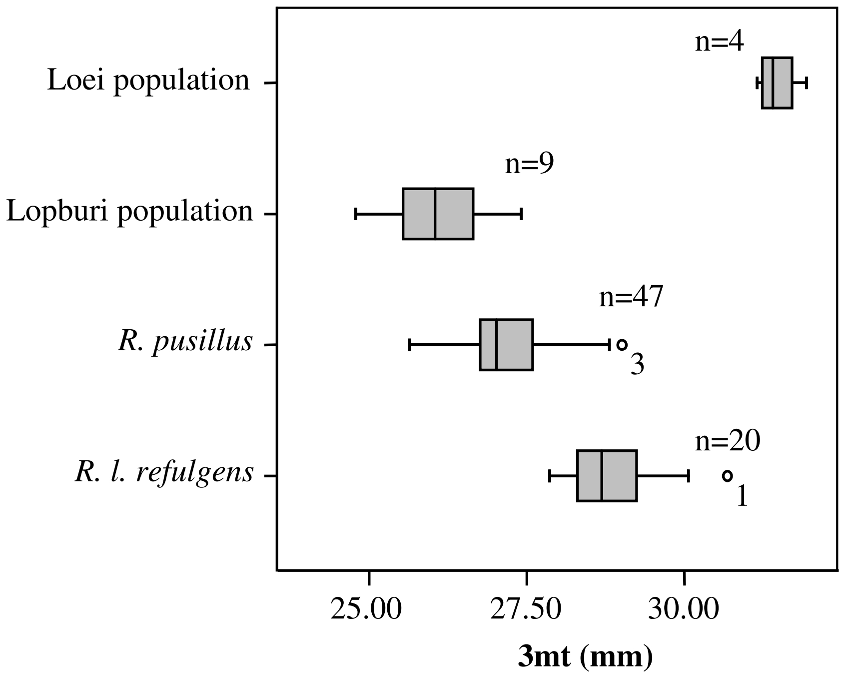


Figure 35. Variation of third metacarpal (3mt) in 4 populations is showed by box plot. (1: PSUZC-MM06.208 from Songkhla, southern Thailand; 3: PSUZC-MM07.93 from Ratchaburi, central Thailand)

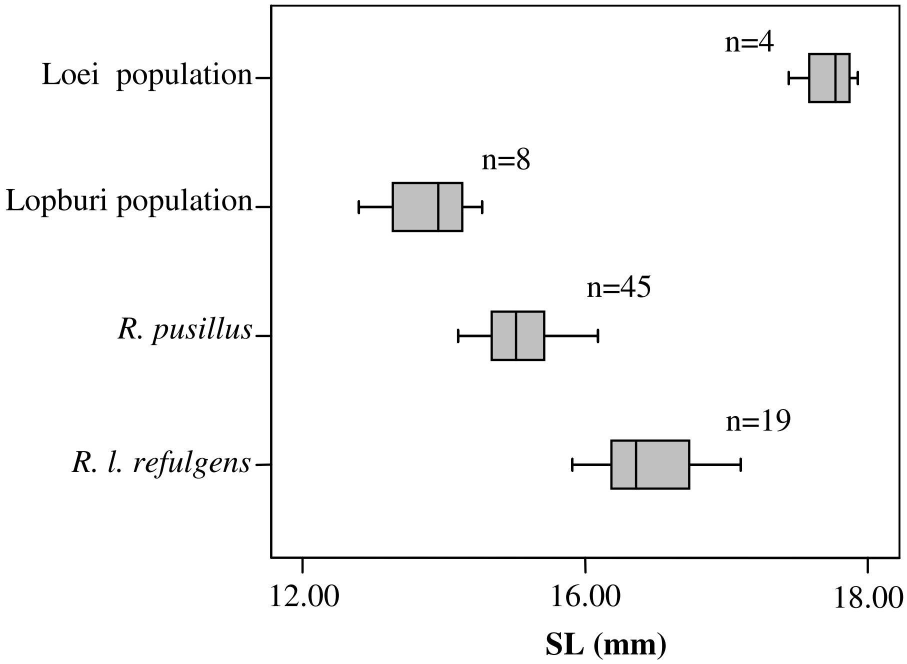


Figure 36. Variation of skull length (SL) in 4 populations is showed by box plot.

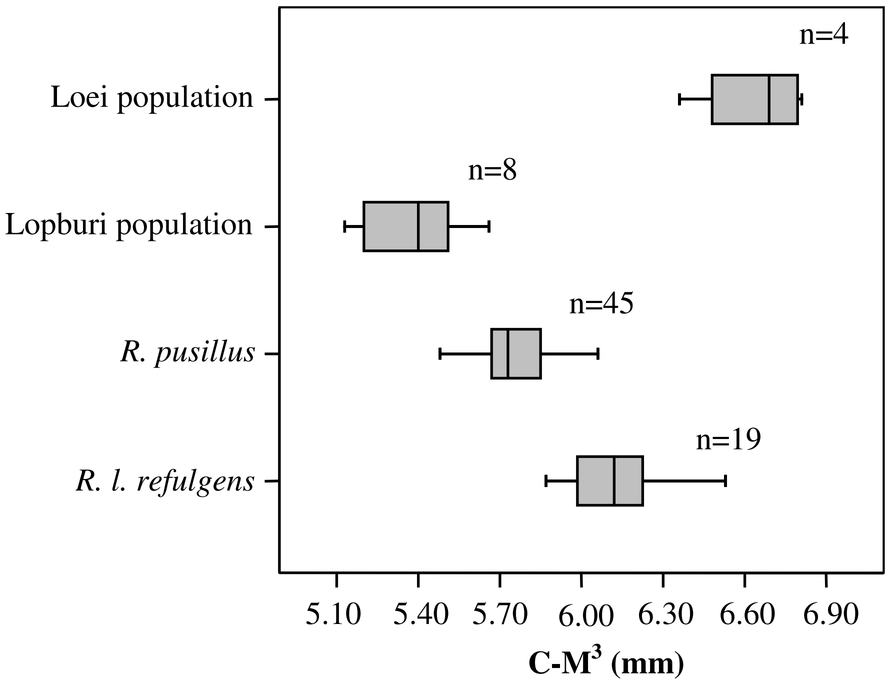


Figure 37. Variation of maxillary toothrow (C-M^3) in 4 populations is showed by box plot.

Latitudinal variation in skull length

The correlation between latitude and peak frequency were examined with Spearman correlations. *R. pusillus* was found higher than 12 degree north. There was a negative relationship between latitude and skull length (r = -0.438, df = 45, P = 0.003) (Figure 38, Appendix 3). For *R. l. refulgens*, it was found lower than 11 degree north and there was no significant correlation between latitude and skull length (r = 0.171, df = 11, P = 0.512) (Figure 39, Appendix 5).

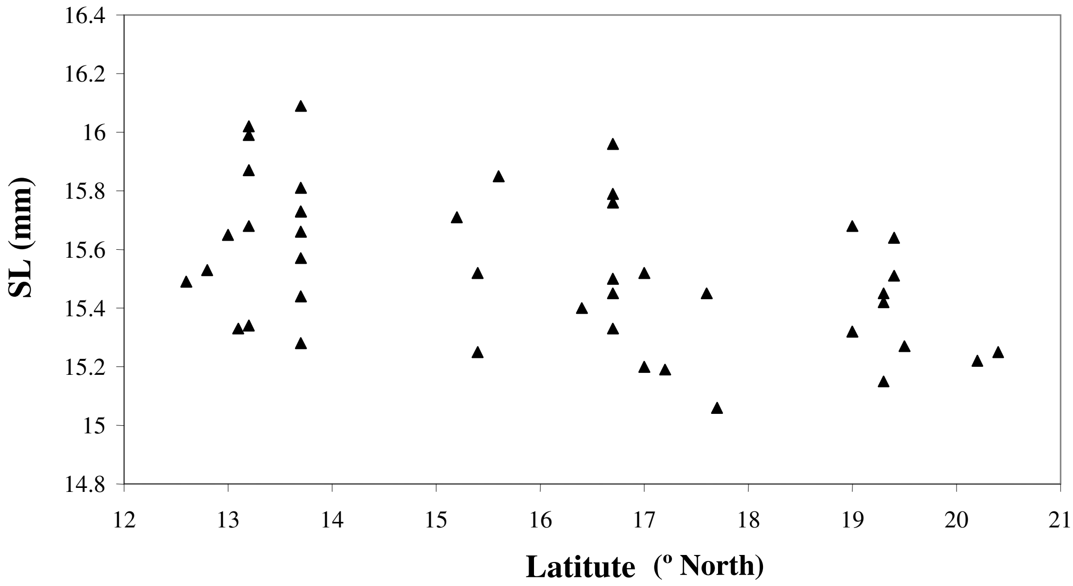


Figure 38. The relationship between latitude (º North) and skull length (mm) of *R. pusillus*

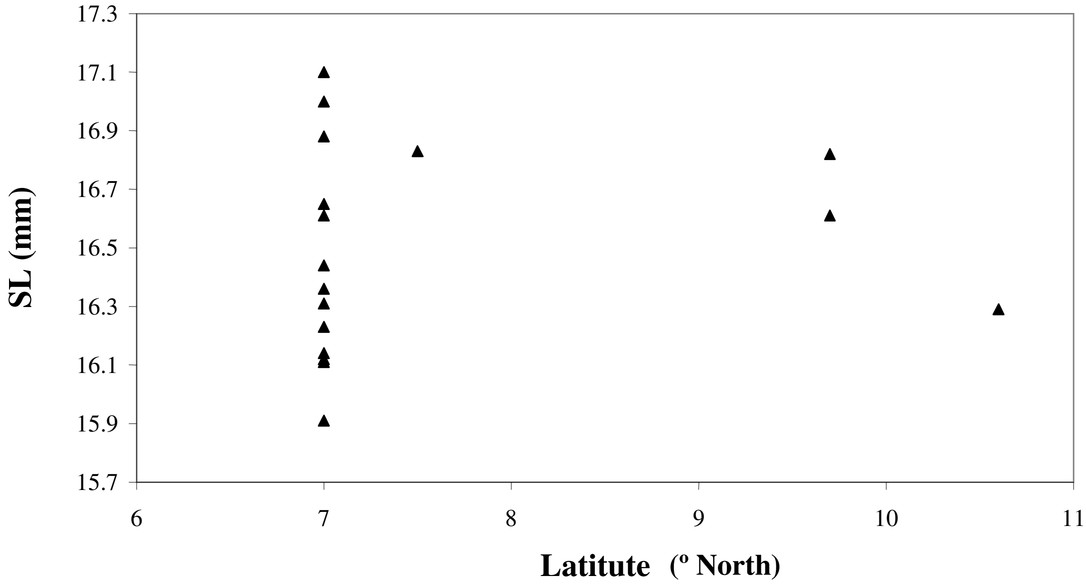


Figure 39. The relationship between latitude (º North) and skull length (mm) of *R. l. refulgens*.

Measurements comparison with other taxa

R. lepidus from India

There were 14 external and cranial characters of 65 specimens from Thailand and 5 *specimens* of *R. lepidus* from India [dark brown] were analysed. The scatter plot of principal component between first and second components showed that all populations from Thailand and India can be separated from each other by the second axis (Figure 40).

R. shortridgei from Myanmar

When 5 specimens of *R. shortridgei* from Myanmar were included in the analysis, the scatter plot of principal component between first and second components shows that all populations from Thailand, India and Myanmar separated from each other. *R. lepidus* [dark brown] and *R. l. refulgens* [purple] from southern Thailand are likely to be the same group (Figure 41).

R. cognatus from Andaman islands, India

65 specimens from Thailand, 3 specimens of *R. cognatus* [grey] are analysed from 14 external and cranial characters. The scatter plot of principal component between first and second components shows that *R. cognatus* did not completely separate from Loei population (Figure 42).

R. pusillus from Java; Indonesia, *R. lepidus* from India and *R. l. refulgens* from Malaysia

From 65 specimens from Thailand, 4 specimens of *R. pusillus* [red] from Java; Indonesia, *R. lepidus* [dark brown] from India and *R. l. refulgens* [light green] from Malaysia were analysed from 7 cranial characters with PCA. The scatter plot of principal component between first and second components shows that *R. pusillus* from Java; Indonesia was in the same group with *R. pusillus* [yellow] from central and northern Thailand. *R. lepidus* [dark brown] from India and *R. l. refulgens* [light green] are in the same group with *R. l. refulgens* [purple] from southern Thailand (Figure 43).

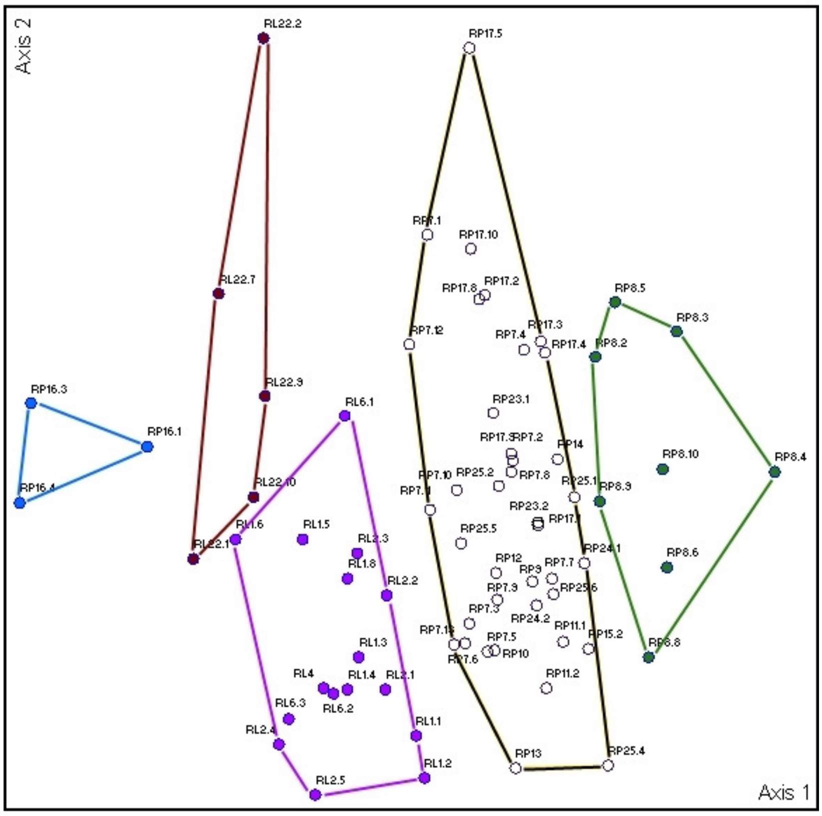


Figure 40. The principal component chart between first and second components from 14 characters of 65 specimens from Thailand and 5 specimens from India (dark brown) (see Appendix 6 for variance explained).

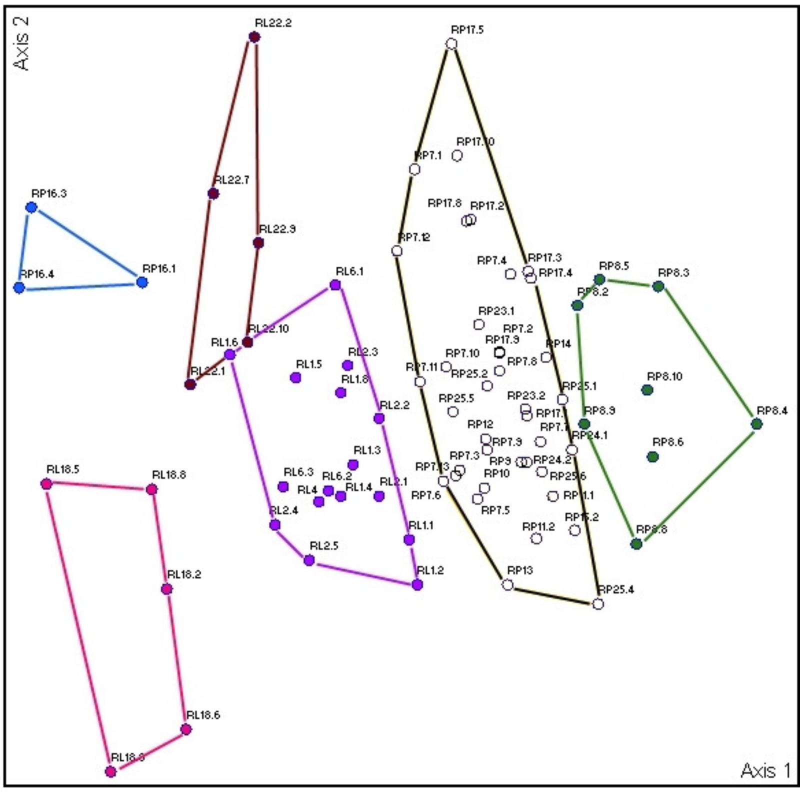


Figure 41**.** The principal component chart between first and second components from 14 characters of 65 specimens from Thailand, 5 specimens from India (*R. lepidus*; dark brown) and 5 specimens from Myanmar (*R. shortridgei*; pink) (see Appendix 7 for variance explained).

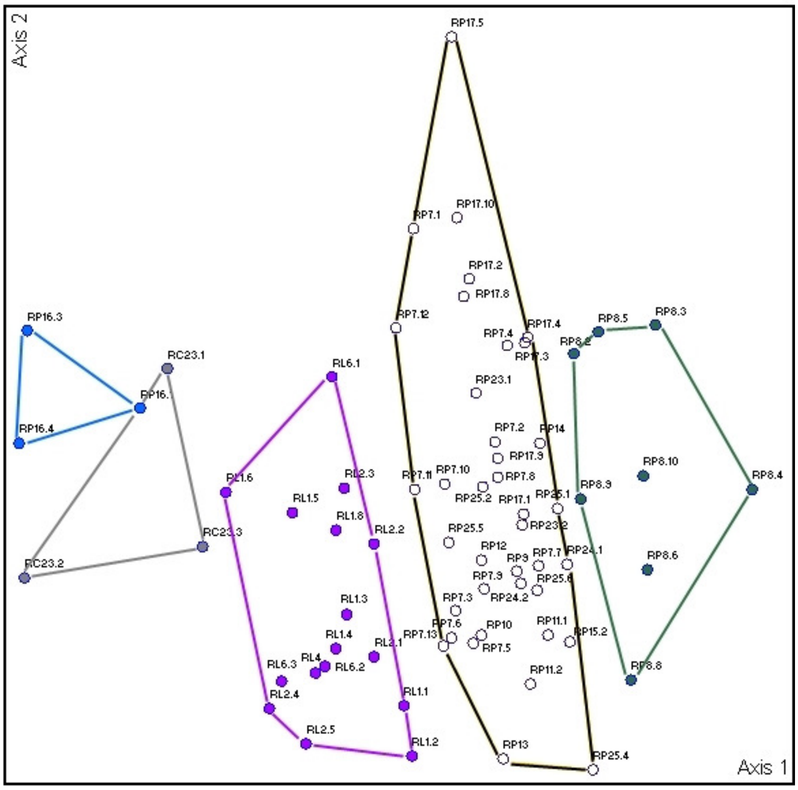


Figure 42. The principal component chart between first and second components from 14 characters of 65 specimens from Thailand, 3 specimens from Andaman islands, India (*R. cognatus*; grey) (see Appendix 8 for variance explained).

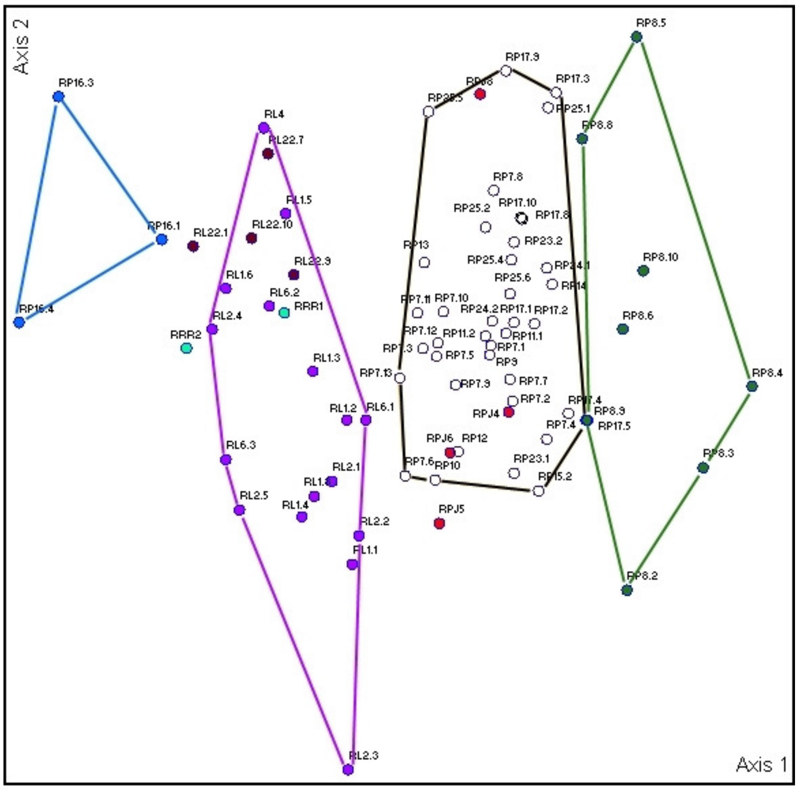


Figure 43. The principal component chart between first and second components from 7 cranial characters of 65 specimens from Thailand, 4 specimens from Java (*R. pusillus*; red), 2 specimens from Malaysia (*R. l. refulgens*; light green) and 4 specimens from India (*R. lepidus*; dark brown) (see Appendix 9 for variance explained).

4.5 Systematics summary

All measurement and description are based on species listed in the measurements section unless otherwise stated.

Blyth's Horseshoe bat

Rhinolophus lepidus refulgens Blyth, 1844 (Figure 44a, b, c, 45)

External characters: This is a small sized horseshoe bat with and average forearm length of 39.48 mm (38.06-42.56 mm); it is usually larger than *R. pusillus* in size. The horseshoe does not cover the whole muzzle. The lower lip has three grooves. The lancet is well developed; the tip is variable in shape (see in the variations). The pelage colour is typically grayish brown to dark brown and occasionally bright foxy orange.

Cranial characters: The skull is small, the zygomatic width is slightly greater or usually subequal to mastoid width, although small, usually exceeds that of *R. pusillus* in size, especially the condylo-canine length which averages 14.5 mm (14.0-15.0 mm). The average of palatal width (M^3-M^3) is 6.1 mm (5.9-6.3 mm). The rostral inflations are more developed than *R. l. lepidus* from peninsular India. The anterior median swellings are medium and equal to the posterior median swelling. The anterior ones are subcircular in outline the posterior ones are relatively well inflated, the rostral profile slightly slopes backwards. The sagittal crest is moderatedly strong and flattened posteriorly.

Dentition characters: Upper toothrow length (C-M^3) average 6.1 mm (5.8-6.5 mm). The upper canine is well developed; it usually greatly exceeds the height of the second upper premolar (PM^4). The first premolar (PM^2) is a functional tooth that lies within toothrow. The second lower premolar (PM_3) is small; it is usually, but not always situated externally to the toothrow. The dentition characters are overlapped between *R. l. refulgens* and *R. pusillus*. The shape of the teeth of *R. l. refulgens* is similar to *R. pusillus*.

Echolocation: Based on this study, the frequency at maximum intensity averaged 102.1 kHz (98.8-105.2 kHz) in Thailand. Other studies had reported 98 kHz in Malaysia (Heller and Helversen, 1989). There is no sexual variation in their echolocation calls.

Ecological notes: It was collected in the areas of evergreen forest and limestone karst. In Malaysia, Kingston *et al.* (2006) recorded that they are primarily found lowland evergreen forest, but also found in hill forest. Their roosting is gregariously in crevices of rock boulders and caves, particularly in association with *Rhinolophus stheno*. Kingston *et al.* (2006) recorded pregnant individuals in the following months: January, March, April, May, August, September, October, November, and December. Medway (1983) reported all female collected from Krau Wildlife Reserve (Malaysia) between February and April 1967 was pregnant.

Distribution and conservation status: From the current study, *R. l. refulgens* is known from southern Thailand, south of Isthmus of Kra (12°N) (Figure 52), Sumatra (Indonesia), Cambodia, southern Vietnam, and Malaysia (Figure 53). In Malaysia, its distribution is widespread and locally common, ranging up to the summit of Kedah Peak and to 3,400 ft on Maxwell's Hill, Petak; also on the islands of Langkawi, Tioman, Pemanggil and Aur (Medway, 1983). Status in the IUCN red list of threatened species of *R. lepidus refulgens* is included in *R. lepidus* which the status is LR/lc (lower risk/least concern) (IUCN, 2007).

Figure 44. *Rhinolophus l. refulgens* (♀ PSUZC-MM06.151); a) connecting process; b) lancet; c) sella.

Figure 45. The skull of *R. l. refulgens* (♂ PSUZC-MM07.88) from Khao Chong, Trang; Southern Thailand (scale = 5 mm).

Least Horseshoe bat

Rhinolophus pusillus Temminck, 1834 (Figure 46a, b, c, 47)

External characters: This is a very small species with an average forearm measurement of 37.3 mm (34.9-39.4 mm). It is usually smaller than *R. l. refulgens* in size but there are some specimens that overlapped to this species. The horseshoe does not cover the whole muzzle. The lower lip has three grooves. The morphology of the noseleaf is comparable to that of *R. l. refulgens*. The lancet is well developed; the tip is variable in shape (see in the variations). In lateral view, the tip of connecting process is pointed. It is similar to the connecting process of *R. l. refulgens*. The sella is narrow. The pelage colour is typically grayish brown.

Cranial Characters: The skull is small, it differ from the skull of *R. l. refulgens* by it average smaller size especially the condylo-canine length which averages 13.6 mm (13.2-14.5 mm). The average of palatal with width (M^3-M^3) is 5.6 mm (5.3-5.9 mm); these characters can be the diagnostic characters for *R. pusillus* and *R. l. refulgens*. The shape of rostral inflations is similar to *R. l. refulgens* that have the small curving upwards at the anterior median swellings. The rostrum and palate, as measured across the canines (C^1-C^1) and maxillary molars (M^3-M^3) are narrower than *R. l. refulgens*. The average of canine length (C^1-C^1) is 3.9 mm (3.7-4.1 mm). The average of the palatal width is 6.1 mm (5.9-6.3 mm). The sagittal crest is small and flattened posteriorly.

Dentition: Upper toothrow length (C-M^3) average 5.8 mm (5.5-6.0 mm). The upper anterior premolar (PM^2) is well developed and situated in the toothrow. The canine and the posterior upper premolar (PM^4) are always widely separated. The second lower premolar (PM_3) is small and usually situated externally to the toothrow.

Echolocation: Based on this study, the frequency at maximum intensity averaged 110.3 kHz (107.8-114.7 kHz) in Thailand. There is no sexual variation in their echolocation calls.

Ecological notes: In this study, it was collected in the areas of limestone karst, mixed deciduous forest, dry evergreen forest and hill evergreen forest. In India and Nepal, it has been collected in high altitudes, higher than 100 metres (Bates and Harrison, 1997). In Myanmar, the roost was situated in a forest clearing in primary forest (Csorba *et al.*, 2003).

Distribution and conservation status: From the current study, *R. pusillus* is known from north of Isthmus of Kra (12°N) in Thailand (Figure 52), Myanmar, Vietnam, Malaysia and Indonesia (Figure 53). It is also known from India, Nepal, Southern China (Tibet, Sichuan, Guangxi, Anhui, Fujian, Hainan, Hong Kong), Vietnam, Laos, Peninsular Malaysia including small islands of langkawi, Penang, Tioman, Aor (Malaysia); Sumatra, Java including Madura Island (Java); Borneo; Northern Pagai (Mentawai Island), Siantan (Anamba Island); Rakarta (Krakatau Island); Lombok (Lesser Sunda Island) (Corbet and Hill, 1992; Simmon, 2005). Status in the IUCN red list of threatened species of *R. pusillus* is LR/lc (lower risk/least concern) (IUCN, 2007).

Figure 46. *Rhinolophus pusillus* ♂ (PSUZC-MM07.88); a) connecting process; b) lancet; c) sella.

Figure 47. The skull of *R. pusillus* ♂ (PSUZC-MM07.264) from Phu Pha San, Mukdaharn; Northeastern Thailand (scale = 5 mm).

Horseshoe bat from Loei province

Rhinolophus sp. (Figure 50, 51)

External characters: Although, this is a small sized horseshoe bat with an average forearm length of 41.6 mm (40.8-42.3 mm); its average is significantly larger than *R. l. refulgens* and *R. pusillus* in size. The horseshoe does not cover the whole muzzle. The lower lip has three grooves. The tip of connecting process is pointed comparable to *R. l. refulgens*.

The lancet is different from *R. l. refulgens* and *R. pusillus* in shape. The lancet is like triangular shape (Figure 48). The sella is parallel sided from base to the summit and its summit is truncated (Figure 49). The skin of the lancet and the face is orange. The pelage colour is grayish brown to dark brown. The tip of hairs is paler than base.

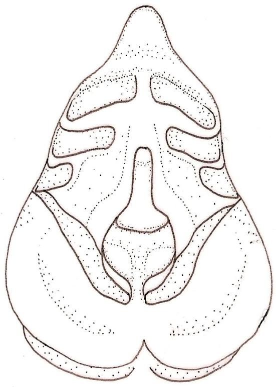


Figure 48. The shape of lancet in *Rhinolophus* sp. ♂ (PSUZC-MM07.256) from Loei Province.

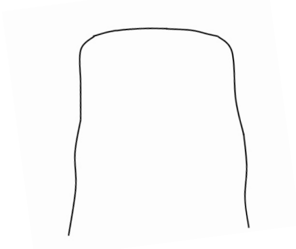


Figure 49. The shape of sella of *Rhinolophus* sp. ♂ (PSUZC-MM07.256) from Loei Province.

Cranial characters: Although, the skull is small, it is larger than *R. l. refulgens* in size. The zygomatic width is slightly greater to mastoid width, The average of condylo-canine length is 15.6 mm (15.3-15.8 mm). The average of palatal width (M^3-M^3) is 6.4 mm (6.2-6.5 mm). The anterior median swellings of the rostrum are well developed. The anterior ones are subcircular in outline the posterior ones are relatively well inflated, the rostral profile slightly slopes backwards. The sagittal crest is strong.

Dentition characters: Upper toothrow length (C-M^3) average 6.6 mm (6.4-6.8 mm). The upper canine is well developed; it greatly exceeds the height of the second upper premolar (PM^4). The first premolar (PM^2) is a functional tooth that lies within toothrow. The dentition characters are overlapped to *R. l. refulgens*. The averages of dentition characters are greater than that *R. l. refulgens*.

Echolocation: The frequency at maximum intensity averaged 88.2 kHz (85.2-92.5 kHz).

Ecological notes: It was collected from hill evergreen forest, Phu Suan Sai National Park, Loei Province (for further details see Chapter 3) (Figure 52).

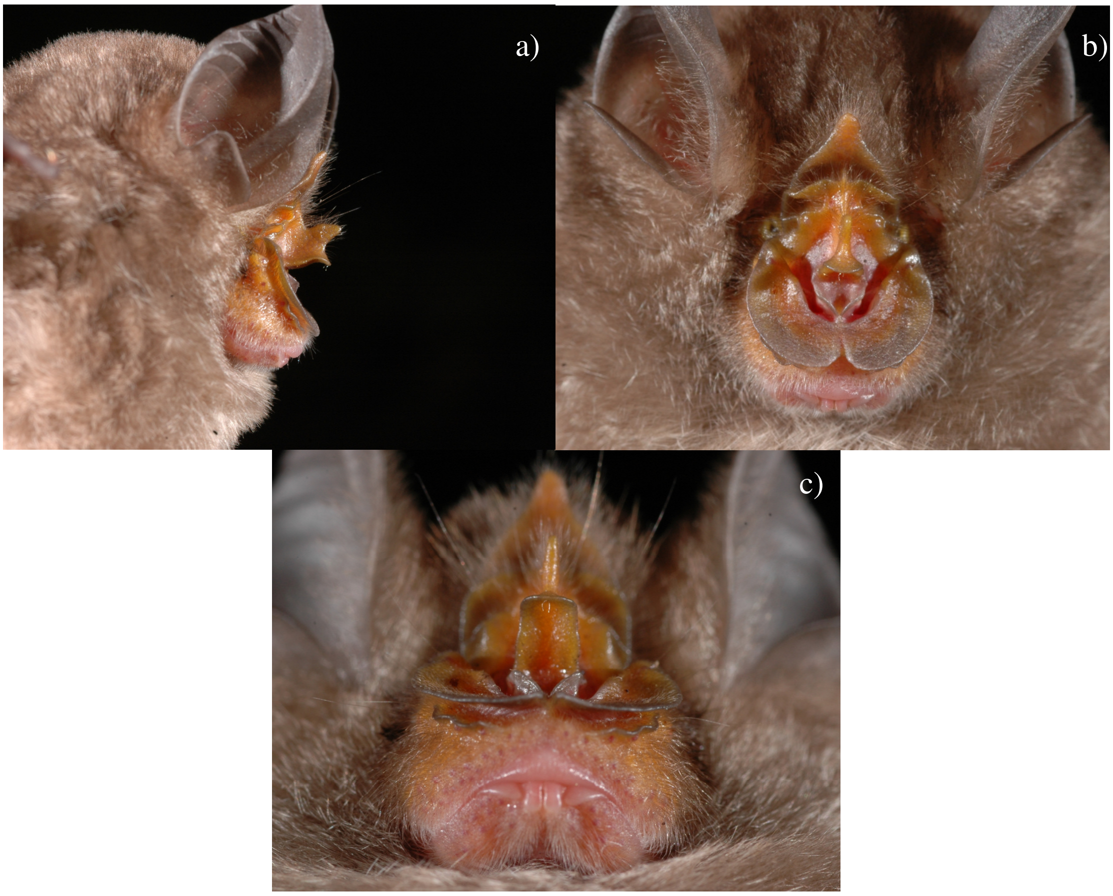


Figure 50. *Rhinolophus* sp.♂ (PSUZC-MM07.256); a) connecting process; b) lancet; c) sella.

Figure 51. The skull of *Rhinolophus* sp.♂ (PSUZC-MM07.256) from Phu Suan Sai National Park, Loei; Northeastern Thailand (scale = 5 mm).

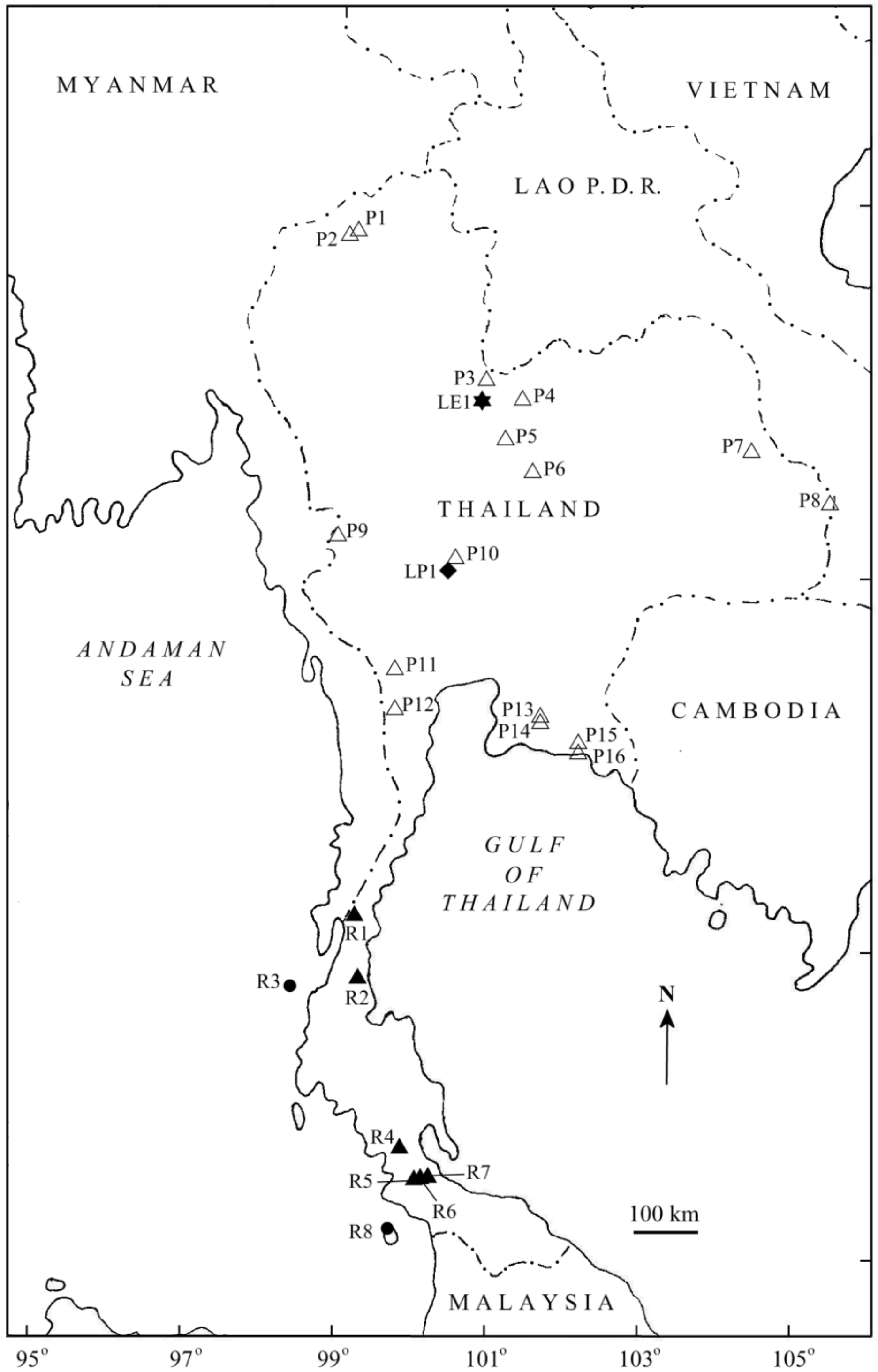


Figure 52. Localities of specimens in *Rhinolophus pusillus* group in Thailand; △: *R. pusillus*; ▲: *R. l. refulgens*; ●: *R. l. refulgens* from the islands; ◆: Lopburi population; ✸: Loei population. Locality information and specimen numbers are included in Appendix 10.

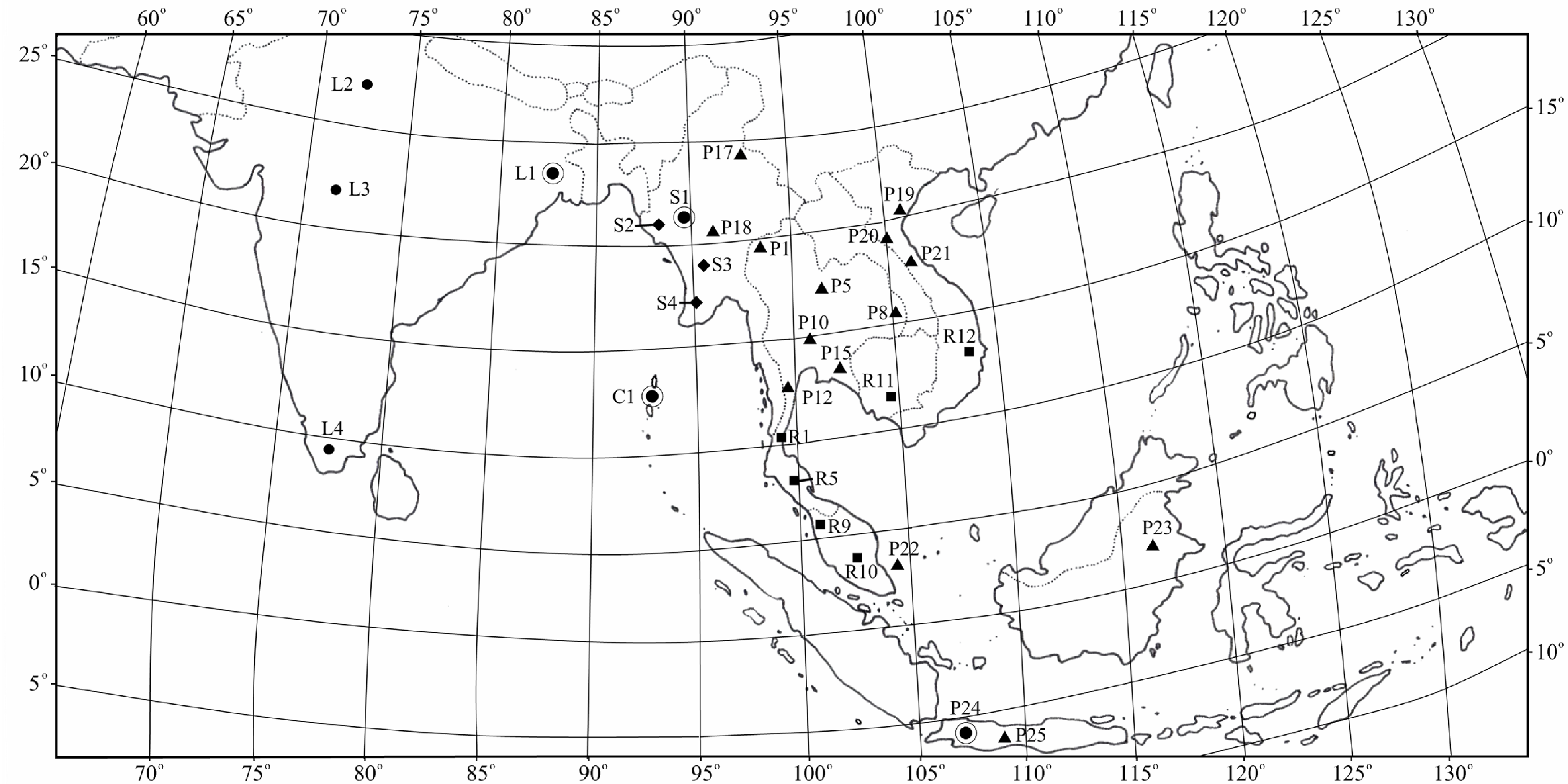


Figure 53. Localities of specimens in *Rhinolophus pusillus* group (▲: *R. pusillus*; ■: *R. l. refulgens*; ●: *R. l. lepidus*; ◉: Type locality). Locality information and specimen numbers are included in Appendix 10

CHAPTER 5
DISCUSSION

In this study, *R. pusillus* was distinguished from *R. l. refulgens* by the morphometrics and echolocation, although there is an overlap in size between *R. pusillus* and *R. l. refulgens*. The external morphology cannot be used to distinguish them because there are high variations in noseleaf and sella shape within species. Therefore, the combination of morphometric and acoustic data can be used to discriminate between these two species. They do not overlap in their distributions. The distribution of these allopatric species is divided by the Isthmus of Kra. The molecular data from Bats of Southeast Asia Project (2007) indicated that *R. lepidus* (*R. l. refulgens*) from southern Thailand was in the different group from *R. pusillus* from northern Thailand, Lao, Vietnam and China, (see Appendix 11). *R. pusillus* and *R. lepidus* also were belong to different group in supertree by Jones *et al*. (2002). These evidences supported that *R. l. refulgens* and *R. pusillus* are different species.

R. lepidus from Southern Thailand was referred to the subspecies *R. l. refulgens* in terms of geographical races. From the results, principal components analysis showed that *R. l. lepidus* and *R. l. refulgens* are likely to be the same group. However, *R. l. lepidus* from India and *R. l. refulgens* were different in the shape of nasal inflation. *R. l. lepidus* has the flatter anterior median swellings. The peak frequency of *R. refulgens* from Malaysia was reported 98 kHz (Heller and Helversen, 1989) which is in the range of peak frequency of *R. l. refulgens* that found in southern Thailand. Pottie *et al.* (2005) studied the microchiropteran bat fauna of Singapore reported average frequency with most energy of *R. lepidus* was 97.8 ± 0.07 kHz (24 bats, 240 calls). *R. lepidus* in Singapore should be *R. l. refulgens* as in Malaysia and Thailand. The distributions of different subspecies of *R. lepidus* were discrete. *R. l. refulgens* is distributed from southern Thailand (this study), Malaysia (Medway, 1978; Heller and Helversen, 1989; Kingston, 2006) to Singapore (Pottie *et al.*, 2005). *R. l. lepidus* was found in India and Pakistan. The distributions of *R. lepidus* from India and southern Thailand are disjunct. Previous study reported a similar disjunct distribution of birds in the genus *Batrachostomus*. These birds are found in the wet evergreen forests of southwest India and Sri Lanka but not in the rest of Indian subcontinent which has low rainfall (50-100 cm per year) and found in the wet evergreen forests of northeast India and throughout Southeast Asia (Karanth, 2003). Fossil evidence suggests that during mid-Miocene times, much of India was cover with humid forest that was continuous with the forests of Southeast Asia that has recently been broken into isolated patches, mainly due to climatic changes (Karanth, 2003). From the evidence of this study, the taxonomic status of these taxa could not be decided yet. The *R. lepidus* from India and southern Thailand might be the different species and vice versa. The morphological similarity might be a by-product of independent adaptation by different species to very similar ecological conditions in widely separated area or they used to be the same species. The present day distributions would have been affected by the biogeographical history. More information on gene exchange between these taxa is needed to clarify their taxonomic status.

There was no evidence that *R. pusillus* in Thailand differ from the nominate species so in this study it was treated as *R. pusillus*. There are some doubts about distribution of these bats. *R. pusillus* was described from Java, Indonesia but in Thailand, only found from central to northern parts of the country. From literature, *R. pusillus* is distributed from India and southern China to Java but in Malaysia known only from the islands of Langawi, Tioman and Aur (FA 37-40 mm) (Medway, 1978). It has not been found in southern Thailand. The specimens from Java are similar with the specimens from central and northern Thailand but only a few numbers of specimens from type locality were examined, so the conclusion cannot be made. In this study, there were lack of the information about external morphology and measurement of Javan specimens. Thus, further investigation based on more specimens from Indonesia and Malaysia is needed. Glacio-eustatic depression of sea level by ~120m at the Last Glacial Maximum (20,000 years ago) fully exposed the Sunda shelf joining mainland Southeast Asia to Sumatra, Java and Borneo (Bird *et al.*, 2005). This exposed continent often referred to as Sundaland. It would have been a corridor of low rainfall passing through the center of the Sunda Shelf, extending in an arc from southern Thailand to eastern Java. Vegetation in this corridor would perhaps have been seasonal forest and savannah (Heaney, 1991). *R. pusillus* from central and northern Thailand and *R. pusillus* from Java and Borneo might be the same taxon in that period.

There were specimens of *Rhinolophus* sp. in *R. pusillus* group from the islands in Andaman Sea (Surin islands, Phang Nga; Similan islands, Phang Nga; Petra islands, Satun and Tarutao islands, Satun) which smaller than average size of *R. l. refulgens* collected from mainland of southern Thailand. From principal component analysis, they were within the group of *R. pusillus* from central and northern Thailand. Their echolocation calls were within the range of *R. l. refulgens* peak frequency from mainland of southern Thailand. It is still the question that it was *R. l. refulgens* which found in the mainland or it was *R. pusillus* from the nominate species. In terms of biogeography, this case is very interesting. It seems to be the effect of isolation. The theory of island biogeography may be applied to explain this situation.

Rhinolophus sp. from Loei Province was the one that different from other taxa of *R. pusillus* group in morphology, echolocation and measurement. The diagnostic characters are the triangular shape of noseleaf and the orange face skin. This taxon is the largest bat of *R. pusillus* group in Thailand. It might be the new species but it still requires more data about echolocation and molecular to support. *R. pusillus* and *Rhinolophus* sp. from Loei Province were in the same group in the molecular data using COI gene (DNA barcodes) from Bats of Southeast Asia Project (2007) however it is significantly difference in morphometric and acoustic data between them. So this molecular data cannot explain the difference of all taxa in *R. pusillus* group.

For *Rhinolophus* sp. from Lopburi Province, it was very similar to *R. pusillus* but it was smaller, and separated from *R. pusillus* from central and northern Thailand in principal component analysis. The peak frequency in their echolocation was also different from the other taxa in *R. pusillus* group. It has very restricted distribution, found only at Khao Samo Khon, Lopburi Province. The landscape around this limestone outcrop is the paddy field dominated floodplain. *R. pusillus* was found in the limestone outcrop that 30 km far from this area. Rhinolophid bats are typically forest-interior specialists, foraging in the cluttered area, characterized by an ecomorphological trait that is unsuitable to open habitats (Chen *et. al.*, 2006). This trait is limited their dispersal ability. It is likely that these taxa cannot fly across the open area in the long way. Restricted contemporary gene flow is typically considered a consequence of isolation by distance. This isolation might effect to this population. In addition, the echolocation frequency of *Rhinolophus* sp. from Lopburi Province is significantly different from *R. pusillus*. The echolocations of these bats are characterized by a strong constant frequency (CF) component with a short beginning and terminal frequency-modulated (FM) component that much of energy of their call is contained within the CF component. The cochleae of rhinolophid bats are finely tuned to the frequency emitted by the bat when it is stationary (Hill and Smith, 1984; Francis and Harbersetzer, 1998). For bats, even 10 kHz difference in minimum frequency of the echolocation call may be sufficient to separate auditory space (Gannon *et al.*, 2001). The echolocation calls are also important for the communication in bats. Vocal communication in bats involves social calls that serve only in communication, as well as echolocation calls that influence the behaviour of conspecifics and others (Fenton, 2003). Thus, the great difference in echolocation frequency of *Rhinolophus* sp. from Lopburi Province suggests that this taxon possibly is the different species from *R. pusillus*. It is interesting to study more about the history of the isolation and the differences in its biology, ecology and also the genetic to the nearby population of *R. pusillus*.

The echolocation frequency of four taxa in Thailand showed the negative relationship with body size: forarm length and condylo-canine length. This is reported by many researches on interspecific comparisons of resting frequency in Rhinolophidae and Hipposideridae which demonstrated a negative correlation between call frequency and body size (as represented by forearm length) (Francis and Habersetzer 1998; Heller and Helversen 1989; Zhang *et. al.*, 2000, Feng *et. al.*, 2002). Anyway, in case of *Rhinolophus* sp. in *R. pusillus* group from the islands in Andaman Sea, it cannot be explained by this correlation. The negative correlation was not very strong, presumably a reflection of the influenced factors such as ecological competition, morphological and physiological features rather than body size in echolocation (Feng *et. al.*, 2002). Previous studies showed that the acoustic data can be used to distinguish the cryptic sibling species such as in *Pipistrellus pipistrellus* and *P. pygmaeus* (Jones and van Parijs, 1993), *Hipposideros lavatus* (Thabah *et. al.*, 2006), and also support the difference in morphological characters in *Rhinolophus* *stheno* and *R. microglobosus* (Soisook, 2008). Call frequency in closely related bat species may also diverge to facilitate intraspecific communication, rather than to facilitate resource partitioning (Jones and Barlow, 2004 quoted in Jones and Holderied, 2007).

The distribution patterns of *R. l. refulgens* and *R pusillus* in Thailand are interesting in the aspect of biogeography. If the distributions in Thailand are considered, *R. pusillus* is restricted to the Indochinese subregion and *R. refulgens* is restricted to Sundaic subregion. It seems that the Isthmus of Kra is the boundary of their distributions. There are many kinds of fauna that the distributions are restricted by the Isthmus of Kra (Corbet and Hill, 1992; Hughes *et al.*, 2003). It might be associated with a change from tropical evergreen forest in south of Isthmus of Kra to mixed deciduous forest in north of Isthmus of Kra (Hughes *et al.*, 2003) or possibly associated with Neogene seaways separating the two regions in Miocene and early Pliocene times (Hughes *et al.*, 2003, Woodruff, 2003; Bruyn *et al.*, 2005). However, the horseshoe bat that similar to *R. l. refulgens* from southern Thailand was found in Cambodia and southern Vietnam (Figure 53) but the specimens from Cambodia and Vietnam do not have the echolocation data so it requires the echolocation data to confirm whether they are the same species. The possibly presence of *R. l. refulgens* in Cambodia and southern Vietnam parallel to the distribution of *R. stheno* (Soisook, 2008) and the freshwater prawn *Macrobrachium rosenbergii* (Bruyn *et al.*, 2005). It is interesting in the history of the isolation and the evolutionary process of these species.

CHAPTER 6
CONCLUSION

Rhinolophus pusillus and *R. lepidus* are the horseshoe bats in *R. pusillus* group. They have essentially similar external morphology and the cranio-dental measurements overlap. Their distributions from the literatures were recorded throughout Thailand. Their taxonomy is confusing as there are number of synonyms and a smaller number of subspecies. The question of this study is how many species in the *R. pusillus* group is present in Thailand and can they be identified on the basis of discrete differences in morphometrics and/or echolocation. Morphometric analysis techniques and echolocation calls were used in this study to answer the question. The specimens of the bats in *R. pusillus* group within Thailand and elsewhere from South-East Asia were examined. Echolocation data was collected from the field work that have done throughout Thailand.

From the results, there are at least four taxa of *Rhinolophus pusillus* group that found in Thailand. *R. pusillus* and *R. lepidus refulgens* are distinct species which have non-overlap distributions. They are distinguished from each other by the combination of morphometric and echolocation. If the distribution of *R. pusillus* and *R. l. refugens* in Thailand are considered, *R. pusillus* is restricted to central and northern Thailand whilst *R. l. refulgens* is restricted to southern Thailand. It seems that the Isthmus of Kra is the boundary of their distributions. The new finding taxa from this study are *Rhinolophus* sp. from Loei Province and *Rhinolophus* sp. from Lopburi Province. *Rhinolophus* sp. from Loei Province can be distinguished from the other taxa by the shape of noseleaf, forearm length, and echolocation calls. It was found only in Phu Suan Sai National Park, Loei Province. *Rhinolophus* sp. from Lopburi province can be distinguished from the others by the combination of morphometric and echolocation calls. The distribution of the last taxon is restricted to Khao Samo Khon which is the isolated limestone outcrop in Tawung District, Lopburi Province. It might be the endemic species to this area. The two latter taxa might be the new species of *Rhinolophus pusillus* group.

Biogeography and ecology of these taxa are interesting for further study. However, morphological and acoustic data as well as gene exchange are necessary to clarify their taxonomic status.

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APPENDIXES

Appendix 1. One-way ANOVA of 6 echolocation parameters between 4 populations.

Characters	df	MS	F	P
Pulse Duration (D, ms)	3	151.170	2.428	0.078
Frequency at maximum intensity (FINT, kHz)	3	1,488.456	393.664	<0.001
Start frequency (SF, kHz)	3	1,286.143	61.114	<0.001
End frequency (EF, kHz)	3	1,114.878	56.707	<0.001
Maximum frequency (FMAX, kHz)	3	1,605.882	729.528	<0.001
Minimum frequency (FMIN, kHz)	3	1,450.714	51.648	<0.001

Appendix 2. Tukey HSD comparisons for all echolocation parameters and populations. Abbrevation and indices as Table 3 (1= Loei population, 2=*R. l. refulgens*, 3=*R. pusillus* and 4=Lopburi population).

Index	Comparison between population of *Rhinolophus* spp. in Thailand					
	1 versus 2	1 versus 3	1 versus 4	2 versus 3	2 versus 4	3 versus 4
D	0.249	0.092	0.608	0.804	0.792	0.320
FINT	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
SF	0.002*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
EF	0.101	<0.001*	<0.001*	0.001*	<0.001*	<0.001*
FMAX	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
FMIN	0.683	0.002*	<0.001*	<0.001*	<0.001*	<0.001*

Appendix 3. Spearman correlations between latitude and Frequency at maximum intensity (FINT; kHz) of *R. pusillus* and *R. l. refulgens*

	R. pusillus	*R. l. refulgens*
Correlation Coefficient	-0.417*	0.334
Sig. (2-tailed)	0.011	0.190
N	36	17

* Correlation is significant at the 0.05 level (2-tailed)

Appendix 4. The eigenvalues and eigenvectors of the correlation matrix for 14 morphological characters on 65 bats from Thailand (Figure 33).

Coefficient of	Eigenvector				
Components	1	2	3	4	5
TIB	-0.223	0.309	0.734	0.430	0.016
FA	-0.275	0.194	0.254	-0.196	-0.109
5mt	-0.276	0.268	-0.004	-0.123	0.216
4mt	-0.276	0.202	-0.026	-0.382	0.281
1ph 4mt	-0.235	0.375	-0.421	0.187	-0.639
3Mt	-0.279	0.186	-0.094	-0.388	0.263
1ph 3mt	-0.244	0.357	-0.280	0.243	0.049
CCL	-0.287	-0.193	-0.108	0.089	0.089
SL	-0.288	-0.198	-0.085	0.132	0.160
ZB	-0.274	-0.226	0.113	-0.222	-0.356
$C\text{-}M^3$	-0.277	-0.253	-0.147	0.304	0.179
$C\text{-}M_3$	-0.272	-0.271	-0.128	0.376	0.222
$C^1\text{-}C^1$	-0.260	-0.293	0.250	-0.098	-0.343
$M^3\text{-}M^3$	-0.267	-0.318	0.014	-0.231	-0.153
Eigenvalue	11.036	1.199	0.478	0.355	0.219

Appendix 5. Spearman correlations between skull length and frequency at maximum intensity (FINT; kHz) of *R. pusillus* and *R. l. refulgens*

	R. pusillus	*R. l. refulgens*
Correlation Coefficient	-0.438*	0.171
Sig. (2-tailed)	0.003	0.512
N	45	11

* Correlation is significant at the 0.05 level (2-tailed)

Appendix 6. The eigenvalues and eigenvectors of the correlation matrix for 14 characters of 65 specimens from Thailand and 5 specimens from India (*R. lepidus*). (Figure 40)

Coefficient of	Eigenvector				
Components	1	2	3	4	5
TIB	-0.2218	0.2583	0.7488	0.1917	-0.4121
FA	-0.2774	0.166	0.173	0.2731	0.1373
5mt	-0.2793	0.2718	-0.1354	0.1796	-0.005
4mt	-0.2784	0.213	-0.2083	0.3008	0.0852
1ph 4mt	-0.2459	0.3547	-0.08	-0.3904	0.3171
3Mt	-0.2744	0.2046	-0.3487	0.3309	0.0571
1ph 3mt	-0.2421	0.324	0.1558	-0.5943	0.1866
CCL	-0.2921	-0.1498	-0.1379	-0.0734	-0.116
SL	-0.2927	-0.1421	-0.1389	-0.0565	-0.2221
ZB	-0.2786	-0.1874	-0.0136	0.1527	0.038
$C\text{-}M^3$	-0.2831	-0.1966	-0.1134	-0.2256	-0.3572
$C\text{-}M_3$	-0.2764	-0.2482	-0.0619	-0.25	-0.3615
$C^1\text{-}C^1$	-0.228	-0.4287	0.3731	0.0373	0.5407
$M^3\text{-}M^3$	-0.2585	-0.3899	0.0317	0.0647	0.225
Eigenvalue	10.815	1.206	0.555	0.382	0.298

Appendix 7. The eigenvalues and eigenvectors of the correlation matrix for 14 characters of 65 specimens from Thailand, 5 specimens from India (*R. lepidus*) and 5 specimens from Myanmar (*R. shortridgei*) (Figure 41)

Coefficient of	Eigenvector				
Components	1	2	3	4	5
TIB	-0.2237	0.2654	0.8061	-0.0822	-0.1546
FA	-0.2683	0.2432	0.1857	-0.1948	0.3682
5mt	-0.2786	0.2445	-0.1234	-0.2266	-0.1026
4mt	-0.282	0.1527	-0.1369	-0.3545	-0.0824
1ph 4mt	-0.2165	0.4639	-0.2792	0.4952	0.4826
3Mt	-0.2751	0.1887	-0.2707	-0.4314	-0.028
1ph 3mt	-0.2488	0.2855	0.055	0.5343	-0.4202
CCL	-0.291	-0.1593	-0.1221	0.0297	-0.0979
SL	-0.2908	-0.1597	-0.1191	0.0024	-0.1616
ZB	-0.2817	-0.1794	-0.0162	-0.0623	0.1592
$C\text{-}M^3$	-0.2826	-0.2247	-0.083	0.1258	-0.2546
$C\text{-}M_3$	-0.279	-0.2457	-0.045	0.1515	-0.2446
$C^1\text{-}C^1$	-0.2442	-0.3742	0.2985	0.127	0.402
$M^3\text{-}M^3$	-0.2655	-0.348	0.0072	0.0437	0.2563
Eigenvalue	10.96	1.273	0.501	0.347	0.249

Appendix 8. The eigenvalues and eigenvectors of the correlation matrix for 14 characters of 65 specimens from Thailand, 3 specimens from Andaman Islands, India (*R. cognatus*) (Figure 42)

Coefficient of Components	Eigenvector 1	2	3	4	5
TIB	-0.2365	0.2663	0.7201	0.4778	0.0503
FA	-0.2684	0.1967	0.2261	-0.2925	0.2519
5mt	-0.2759	0.2581	0.0419	-0.095	-0.171
4mt	-0.2752	0.2045	0.0246	-0.3531	-0.3124
1ph 4mt	-0.2358	0.4014	-0.4876	0.1659	0.6431
3Mt	-0.2779	0.1857	-0.0451	-0.353	-0.273
1ph 3mt	-0.25	0.3625	-0.2238	0.2377	-0.2652
CCL	-0.2828	-0.1946	-0.1406	0.0488	-0.0646
SL	-0.2832	-0.2044	-0.1143	0.0983	-0.0855
ZB	-0.2723	-0.2359	0.085	-0.2073	0.2941
$C\text{-}M^3$	-0.2768	-0.2457	-0.1494	0.299	-0.1678
$C\text{-}M_3$	-0.2721	-0.2683	-0.1356	0.3737	-0.1574
$C^1\text{-}C^1$	-0.2611	-0.3002	0.2392	-0.0709	0.3074
$M^3\text{-}M^3$	-0.268	-0.3118	-0.0039	-0.234	0.0581
Eigenvalue	11.447	1.005	0.399	0.296	0.203

Appendix 9. The eigenvalues and eigenvectors of the correlation matrix for 7 cranial characters of 65 specimens from Thailand, 4 specimens from Java (*R. pusillus*), 2 specimens from Malaysia (*R. l. refulgens*) and 4 specimens from India (*R. lepidus*). (Figure 43)

Coefficient of Components	Eigenvector 1	2	3	4	5
CCL	-0.387	0.1814	-0.1231	-0.3356	-0.5989
SL	-0.3888	0.2215	-0.0457	-0.2887	-0.2461
ZB	-0.3716	-0.4667	-0.4194	-0.4565	0.4976
$C\text{-}M^3$	-0.3838	0.3757	0.0289	0.212	0.1458
$C\text{-}M_3$	-0.3789	0.4623	0.1487	0.1785	0.5055
$C^1\text{-}C^1$	-0.362	-0.4659	0.8049	-0.0161	-0.0512
$M^3\text{-}M^3$	-0.373	-0.3582	-0.3689	0.7201	-0.2318
Eigenvalue	6.342	0.271	0.169	0.109	0.05

Appendix 10. Localities and specimens number**.**

Localities and specimens number in Thailand (Figure 52).

Rhinolophus pusillus

P1 Chiang Dao Wildlife Research Station, Chiangdao, Chiang Mai, Thailand (19°22′N, 98°55′E): PSUZC-MM05.58, PSUZC-MM06.146, PSUZC-MM06.147, PSUZC-MM06.148, PSUZC-MM06.149, PSUZC-MM06.150

P2 Khimee Cave, Chiangdao, Chiang Mai, Thailand (19°21′N, 98°50′E): CDWLRS-B-0004, CDWLRS-B-0005, CDWLRS-B-0009, CDWLRS-B-0010, CDWLRS-B-0017

P3 Pha Kor Waterfall, Phu Suan Sai NP., Loei, Thailand (17°34′N, 101°00′E): PSUZC-MM06.25

P4 Hui Nam Chan, Phu Luang, Loei, Thailand (17°21′N, 101°34′E): PSUZC-MM05.57

P5 Yai Nam Nao Cave, Nam Nao, Phetchabun, Thailand (16°57′N, 101°30′E): PSUZC-MM07.258, PSUZC-MM07.259

P6 Phu Keaw, Chaiyaphum, Thailand (16°23′N, 101°34′E): PSUZC-MM06.9

P7 Phu Pha San, Phu Si Than WS., Mukdaharn, Thailnad (16°39′N, 104°22′E): PSUZC-MM07.262, PSUZC-MM07.263, PSUZC-MM07.264, PSUZC-MM07.265, PSUZC-MM07.266, PSUZC-MM07.267

P8 Pha Taem NP., Ubon Ratchathani, Thailand (15°36′N, 105°35′E): PSUZC-MM07.261, PSUZC-MM05.45, PSUZC-MM07.260

P9 East Thung Yai, Tak, Thailand (15°42′N, 99°01′E): AD080217.7

P10 Khao Don Dueng, Ban Mi, Lopburi, Thailand (15°08′N, 100°36′E): PSUZC-MM07.171

P11 Khao Nom Tai, Photharam, Ratchaburi, Thailand (13°43′N, 99°45′E): PSUZC-MM07.92, PSUZC-MM07.93, PSUZC-MM07.94, PSUZC-MM07.95, PSUZC-MM07.96, PSUZC-MM07.97, PSUZC-MM07.98, PSUZC-MM07.99

P12 Khao Yoi, Phetchaburi, Thailand (13°14′N, 99°46′E): PSUZC-MM07.100, PSUZC-MM07.101, PSUZC-MM07.102, PSUZC-MM07.103, PSUZC-MM07.104

P13 Bo-tong, Ang Runai, Chacherngsao, Thailand (7°01′N, 100°36′E): PSUZC-

MM05.93

P14 Klong Plu, Khao Chamao NP., Rayong Thailand (12°58′N, 101°43′E): PSUZC-MM05.53

P15 Wang Kaprae, Khao Soi Dao, Chanthaburi (12°51′N, 102°16′E): PSUZC-MM05.60

P16 Klong Makok, Phliu, Chantaburi, Thailand (12°35′N, 102°15′E): PSUZC-MM05.43

Rhinolophus lepidus refulgens

R1 Silawan Cave, Chumphon, Thailand (10°42′N, 99°14′E): PSUZC-MM07.107

R2 Khao Plu, Lamae, Chumphon, Thailand (9°44′N, 99°07′E): PSUZC-MM07.105, PSUZC-MM07.106

R3 Surin Island, Phang Nga, Thailand (9°26′N, 97°52′E): PSUZC-MM06.5

R4 Khao Chong, Trang, Thailand (7°33′, 99°47′E): PSUZC-MM07.88

R5 Boripatr waterfall, Satun, Thailand (7°01′N, 100°09′E): PSUZC-MM06.26, PSUZC-MM06.100, PSUZC-MM06.101, PSUZC-MM06.102, PSUZC-MM06.103, PSUZC-MM06.104

R6 Ton Nga Chang, Hatyai, Songkhla, Thailand (6°55′N, 100°17′E): PSUZC-MM06.11

R7 Khao Tieb Cave, Rattaphum, Songkla (7°04′N, 100°15′E): PSUZC-MM06.205 PSUZC-MM06.206, PSUZC-MM06.207, PSUZC-MM06.208, PSUZC-MM06.209, PSUZC-MM06.145, PSUZC-MM06.151

R8 Tarutao Island, Satun, Thailand (6°37′N. 99°40′E): PSUZC-MM05.48, PSUZC-MM07.89, PSUZC-MM07.90, PSUZC-MM07.91

Loei population

LE1 Phu Suan Sai NP., Na Haeo, Loei, Thailand (17°30′N, 100°57′E): PSUZC-MM06.7, PSUZC-MM06.22, PSUZC-MM06.23, PSUZC-MM07.256

Lopburi population

LP1 Khao Samo Khon, Tawung, Lopburi, Thailand (14°55′N, 100°30′E): PSUZC-MM07.25, PSUZC-MM07.26, PSUZC-MM07.27, PSUZC-MM07.28,

PSUZC-MM07.29, PSUZC-MM07.30, PSUZC-MM07.31, PSUZC-MM07.32, PSUZC-MM07.33, PSUZC-MM07.34

Localities and specimens number (Figure 53).

Rhinolophus lepidus lepidus

India

L1 Culcutta (Kolkata), West Bengal, India (22°34'N, 88°20'E): (Type locality of *R. lepidus lepidus*)

L2 New Delhi, India (28°37'N, 77°13'E): HZM.20.28162, HZM.19.28161

L3 Mandu, Madhya Pradesh, India (22°22'N, 75°24'E): HZM.17.25685, HZM.18.25686, HZM.16.25684

L4 Tamil Nadu, India (8°52'N, 77°22'E): HZM.30.36208, HZM.29.35005, HZM.27.35090, HZM.31.36209

Rhinolophus lepidus refulgens

Thailand

R1 Silawan Cave, Chumphon, Thailand (10°41'N, 99°14'): PSUZC-MM07.107

R5 Ton-Nga Chang, Songkhla, Thailand (7°4'N, 100°15'E): PSUZC-MM06.11

Malaysia

R9 Pasang Kemunting, Penang, Malaysia (5°42'N, 100°37'E): BM(NH) 73.608

R10 Pahang, Malaysia (3°39'N, 102°29'E): BM(NH) 67.1578, BM(NH) 67.1571

Cambodia

R11 Talai Lieu, Cambodia (11°49'N, 103°32'E): HZM.31.36495

Vietnam

R12 Kon Ka Kinh Nature Reserve, Gai Lai, Vietnam (14°17'N, 108°25'E): HZM.23.32311, HZM.4.32309, HZM.22.32310

Rhinolophus shortridgei

Myanmar

S1 Pagan, Irrawaddy (Type locality): BM(NH) 18.8.3.1

S1 Naung-Oo (Nyaung-U), Mandalay Division, Myanmar (21°11'N, 94°54'E): HZM.25.32583, HZM.24.32582

S2 Bar Min Gu, Mrauk-u, Rakhine State, Myanmar (20°31'N, 93°11'E): HZM.28.35294

S3 Pyay, Bago Division, Myanmar (18°49'N, 95°13'E): HZM.26.33357, HZM.27.33358

S4 Mayan Haung, Gwa, Rakhine State, Myanmar (17°35'N, 94°40'E): HZM.28.33359

Rhinolophus pusillus

Thailand

P1 Chiang Dao, Chiang Mai, Thailand (19°22'N, 98°55'E): PSUZC-MM05.58, PSUZC-MM06.146, CDWLRS-B-0004, CDWLRS-B-0005, CDWLRS-B-0009, CDWLRS-B-0010, CDWLRS-B-0017, PSUZC-MM06.147

P5 Nam Nao, Phetchabun, Thailand (16°57'N, 101°30'E): PSUZC-MM07.258, PSUZC-MM07.259

P8 Pha Taem, Ubon Ratchathani, Thailand (13°9'N, 100°37'E): PSUZC-MM07.261, PSUZC-MM05.45, PSUZC-MM07.260

P10 Ban Mi, Lopburi, Thailand (15°9'N, 100°37'): PSUZC-MM07.171

P12 Khao Yoi, Phetchaburi, Thailand (10°14'N, 99°50'E): PSUZC-MM07.100, PSUZC-MM07.101, PSUZC-MM07.102, PSUZC-MM07.103, PSUZC-MM07.104

P15 Ang Ru Nai, Chachoengsao, Thailand (13°11'N, 101°44'E): PSUZC-MM05.93

Myanmar

P17 Tong Khan Village, Muse, Shan State, Myanmar (23°59'N, 97°58'E): HZM.6.34947, HZM.5.34946

P18 Taung Pauk Village, Shan State, Myanmar (20°21'N, 96°11'E): HZM.7.35118

Vietnam

P19 Cuc-Phung National Park, Vietnam (20°9'N, 105°37'E): HZM.1.30543

P20 Phu Mat Nature Reserve, Vietnam (18°58'N, 104°46'E): HZM.4.32307, HZM.2.32308

P21 Pu Ru Cave, Phong Nha-Ke Bang National Park, Vietnam (17°39'N, 105°59'E): HZM.3.32306

Malaysia

P22 Tioman Island, Malaysia (2°48'N, 104°10'E): BM(NH) 61.1682

Indonesia

P23 25 km inland from Sangkulirang, a small port at the mouth of the R Baa, East Kalimantan, Indonesia (0°59'N, 117°55'E): BM(NH) 78.24.91

P24 Klapanunggal, West Java, Indonesia (Type locality) (6°31'S, 106°52'E): BM(NH) 61.1747

P25 Tasikmalaya, Java, Indonesia (7°20'S, 108°12'E): BM(NH) 9.1.5.178

Rhinolophus cognatus

India

C1 Andaman Islands, India (12°40'N, 77°20'E): HZM 3.34703, HZM 1.34701, HZM 4.34704, HZM 2.34702

Appendix 11. A dendrogram, built using the Kimura 2 Parameter distance model, indicating the amount of genetic difference between species of *Rhinolophus pusillus* group from Southeast Asia. (Modified from Bat of Southeast Asia Project, 2007)

VITAE

Name Miss Ariya Dejtaradol

Student ID 4910220099

Educational Attainment

Degree	Name of Institution	Year of Graduation
B.Sc. (Biology)	Prince of Songkla University	2006

Work Position and Address

Address: Department of Biology, Faculty of Science, Prince of Songkla University, Hatyai, Songkhla 90112

E-mail address: sciariya@gmail.com

List of Publication and Proceedings

Dejtaradol, A., S. Bumrungsri and P. J. J. Bates. 2007. A taxonomic review of *Rhinolophus pusillus* and *Rhinolophus lepidus* (Chiroptera: Rhinolophidae) in Thailand. Proceeding of the First International South-East Asian Bat Conference. Club Andaman Resort Beach Hotel, Patong, Phuket, Thailand, 7-10 May 2007. p 80.