A Taxonomic Review of *Rhinolophus malayanus* Bonhote, 1903 and *Rhinolophus stheno* Andersen, 1905 (Chiroptera: Rhinolophidae) in Thailand

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ชื่อวิทยานิพนธ์	อนุกรมวิธานของค้างคาวมงกุฎมลายูและค้างคาวมงกุฎเลียนมลายูหางสั้นในประเทศไทย
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บทคัดย่อ

การศึกษาอนุกรมวิธานของค้างคาวสองชนิดในวงศ์ Rhinolophidae คือ ค้างคาวมงกุฎมลายู (*Rhinolophus malayanus* Bonhote, 1903) และค้างคาวมงกุฎเลียนมลายูหางสั้น (*R. stheno* Andersen, 1905) ซึ่งเป็นค้างคาวที่มีลักษณะคล้ายคลึงกันและยากต่อการจำแนกชนิด ได้ดำเนินการศึกษาระหว่างเดือนพฤษภาคม พ.ศ. 2549 ถึงเดือนกุมภาพันธ์ พ.ศ. 2551 จากการศึกษาลักษณะทางสัณฐานวิทยาของตัวอย่างค้างคาว และการวิเคราะห์ความถี่ของคลื่นเสียงของค้างคาวทั้งสองชนิดที่ทำการเก็บมาจากทั่วประเทศ รวมทั้งตัวอย่างจากประเทศอื่นๆ ในเอเชียตะวันออกเฉียงใต้ ค้างคาวชนิดย่อย *R. stheno microglobosus* ซึ่งกระจายอยู่ในเขตสัตวภูมิศาสตร์ย่อย Indochinese ได้รับการยกฐานะขึ้นเป็นชนิด คือ *R. microglobosus* แยกออกจากค้างคาวมงกุฎเลียนมลายูหางสั้น *R. stheno* ด้วยความแตกต่างอย่างชัดเจนทางสัณฐานวิทยาและความถี่ของคลื่นเสียง ค้างคาวชนิดนี้ได้รับการรายงานการพบอย่างเป็นทางการในประเทศกัมพูชาเป็นครั้งแรกจากการศึกษาครั้งนี้อีกด้วย ส่วนในค้างคาวมงกุฎมลายูพบว่ามีความผันแปรของความถี่ของคลื่นเสียง โดยประชากรที่พบในภาคเหนือมีความถี่ของคลื่นเสียงต่ำกว่าประชากรที่พบในภาคใต้ ซึ่งมีความถี่ของคลื่นเสียงสูงกว่า แต่อย่างไรก็ตาม ไม่พบว่าความผันแปรนี้ส่งผลต่อลักษณะทางสัณฐานวิทยาระหว่างทั้งสองประชากรนี้ และสถานภาพทางอนุกรมวิธานของทั้งสองประชากรนี้สมควรมีการศึกษาอย่างละเอียดต่อไป ลักษณะทางสัณฐานวิทยาและความแตกต่างของค้างคาวมงกุฎทั้งสามชนิดได้รับการบรรยาย พร้อมด้วยข้อมูลการแพร่กระจายและนิเวศวิทยาของแต่ละชนิด ประโยชน์ของการใช้ความถี่ของคลื่นเสียงในการช่วยจำแนกค้างคาวที่มีลักษณะคล้ายคลึงกัน รวมไปถึงความสำคัญของเขตสัตวภูมิศาสตร์ต่อขอบเขตการแพร่กระจายของค้างคาวทั้งสามชนิด

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ABSTRACT

The study of taxonomic revision of the two cryptic species; Malayan horseshoe bat *Rhinolophus malayanus* Bonhote, 1903 and the Lesser brown horseshoe bat *R. stheno* Andersen, 1905 (Chiroptera: Rhinolophidae) in Thailand was undertaken between May 2006 and February 2008. External, cranial and dental characters from a large sample size of specimens and echolocation calls of *R. malayanus* and *R. stheno* (*Sensu* Csorba *et al.*, 2003) collected from throughout Southeast Asia were examined. The taxon *R. stheno microglobosus*, which restricted to the Indochinese subregion, is elevated to specific rank on the basis of clearly defined morphometric and acoustic characters which differentiate it from *R. stheno.* It is also recorded from Cambodia for the first time. *R. malayanus* exhibits considerable geographical variation in echolocation calls, with apparently two phonic types: a northern population with lower frequency calls and a predominantly southern population with higher frequencies. However, this acoustic divergence is not reflected in any morphometric divergence, and the taxonomic status of the two phonic populations remains unclear. Character descriptions of all three species are given, together with distribution data and ecological summaries. The value of echolocation as an indicator of cryptic species and the zoogeographical implications of the study are briefly discussed.

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Pipat Soisook

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CHAPTER 1

INTRODUCTION

The Malayan horseshoe bat, *Rhinolophus malayanus* was firstly described from Biserat, Jalor, peninsula Thailand by Bonhote (1903). It was included in the *R. ferrumequinum* group (Lekagul and McNeely, 1977; Corbet and Hill, 1992) and currently included in *megaphyllus* group (Csorba *et al.,* 2003). Except the type locality of *R. malayanus*, its distribution has been known from Myanmar (Bates *et al.,* 2000), Lao PDR (McFarlane and Blood, 1986; Robinson and Webber, 1998), Vietnam (Hendrichsen *et al.*, 2001b), Cambodia (Hendrichsen *et al.,* 2001a) and peninsula Malaysia (Hill, 1972; Csorba and Jenkins, 1998). The Lesser brown horseshoe bat, *R. stheno* Andersen (1905) was firstly described from Selangor, Malay Peninsula. It was included in the *R. borneensis* subgroup of *R. simplex* group (Andersen, 1905) and currently listed in the same group with *R. malayanus,* in *R. megaphyllus* group (Csorba *et al.,* 2003). Hill (1975) reported *R. stheno* from Thailand for the first time with specimens collected by Kitti Thonglongya, and mentioned that they seem to smaller than those Malaysian specimens and also suggested that it might represent a distinct subspecies. Subsequently, in 1998, Csorba and Jenkins described a new distinct subspecies; *R. stheno microglobosus* from Vietnam, separated from the nominate subspecies *R. stheno stheno* from Malaysia. It was distinguished from *stheno* by smaller median anterior rostral swellings and generally small body size. Some specimens from Thailand were examined and those were proved to belong this new subspecies. Recently, Csorba *et al.,* 2003 published a most comprehensive book of world's currently known *Rhinolophus* and they also mentioned that the specimens from Thailand north of Isthmus of Kra belong to *R. s. microglobosus* as same as Lao and Vietnam specimens, while specimens from Thailand south of Isthmus of Kra have been considered to be the nominate race. Nowadays, it has been currently recorded throughout the mainland South-East Asia, from Thailand (Hill, 1975; Lekagul and McNeely, 1977) Myanmar (Bates *et al.,* 2000), Lao PDR (Robinson and Webber, 1998), Vietnam (Csorba and Jenkins, 1998), to peninsula Malaysia, Sumatra and Java (Corbet and Hill, 1992; Csorba *et al.,* 2003).

The two species, *R. malayanus* and *R. stheno* have been a long time known as cryptic species and hardly distinguishable morphologically (e. g. Csorba *et al.*, 2003; Hendrichsen *et al.*, 2001b; Lekagul and McNeely, 1977; McFarlane and Blood, 1986), even though Bogdanowicz (1992) provisionally considered their relationship to be more distant. Hendrichsen *et al.*, (2001b) listed the two species from Vietnam and noted that they can be distinguished externally by the proportion of the first and second phalanges of the third digit. In the skull, the differences of them have been discussed by Bates *et al.*, (2000, 2004) who suggested that they can be distinguished on the basis of the inflation of the anterior median compartments of the rostrum which much more inflated in *R. stheno.* Moreover, the difference of the post orbital constriction between the two species has been reported. In *R. malayanus,* the post orbital constriction was not markedly narrow, but it was very narrow in *R. stheno* (Bates *et al.*, 2004; Csorba *et al.*, 2003).

The acoustic characters of the two taxa are also virtually unknown, although Robinson (1996) reported that the call frequency of *R. stheno* from Western Thailand is 85-95 kHz, whereas the call frequency of 86 kHz was recorded from Malaysia (Francis and Habersetzer, 1998; Kingston *et al.*, 2000). The idea of this study came from observed differences of up to 10 kHz in the resting frequencies of acoustic calls of both *R. stheno* (*sensu* Csorba *et al.*, 2003) and *R. malayanus* from northern and southern Thailand. 86 kHz of the call frequency of *R. stheno* was recorded from the south contrastingly to the north, where 95 kHz of the call frequency was observed. It seems to be consistent with the morphological difference in this species which mentioned by Csorba and Jenkins (1998) and Csorba *et al.*, (2003). In the case of *R. malayanus* which is known only from the nominate form, resting frequency of 75 kHz of echolocation call in the north and broadly increased to 90 kHz in the south were observed. Previous studies in Asia had suggested that acoustic data are a useful tool to identify cryptic species (Francis and Habersetzer, 1998; Francis *et al.*, 1999; Kingston *et al.*, 2001; Thabah *et al.*, 2006). Recent observed echolocation data suggested the presence of the cryptic species and their taxonomic status need to be revised.

This study sought to determine if differences in echolocation were consistent, had distinctive geographical patterns and was congruent with observable, discriminating, morphometric characters.

CHAPTER 2

LITERATURE REVIEW

The Chiroptera

Bat, order Chiroptera, has been known as only mammalian order that capable to fly. It has been divided into two suborders; the Megachiroptera (megabats or fruit eating bats) and the Microchiroptera (microbats or insectivorous bats) (Altringham, 1996). Over 1100 species of bats (Neuweiler, 2000) promoted themselves as one of the most successful mammal. Only the rodents (order Rodentia) are the mammalian order to outnumber bats, by approximately 1700 species (Altringham, 1996). The Megabats are belong to the same family that is, Pteropodidae- the old world fruit bats with 42 genera and approximately 175 species. The Microbats which are distributed worldwide are belong to 16 families, 744 genera and 782 species (Simmons, 2005). The power of flight enabled bats distributed over all continents (except the polar region), and in many habitats. But, as same as all other forms of life, the number of species declines away from the equator (Altringham, 1996, Neuweiler, 2000). The widespread of them led to a variety of feeding behaviour. The megachiropterans feeding on fruit, nectar and pollen while many microchiropterans are mainly insectivorous (such as Emballonuridae, Hipposideridae, Rhinolophidae and Vespertilionidae), some are carnivorous bats such as megadermatids, nycterids and phyllostomids. In addition, a small number of them are also fish-eating bats (Noctilionidae) and blood-feeding bats (Vampires bats, subfamily Desmodontinae) (Bates and Harrison, 1997).

Echolocating bats

A simple definition of the echolocation is the orientation by analysis of the echoes from emitted sound pulses (Altringham, 1996). Insectivorous bats commonly used echolocation for orientation and find their prey. This extraordinary, high frequencies sound, beyond the range of humans hearing, are produced in the larynx which is close to the auditory bullae and emitted from the facial region (open mouth or nostrils) (Fenton, 1995). Megabats, only the genus *Rousettus* are known to echolocate within their roost caves by clicking the tongue (Altringham, 1996).

The echolocation calls can be characterized by their frequency, duration and intensity (Fenton, 1988), which are different among species, or occasionally found highly variable even within species, especially, in the bats that emitted frequency modulated (FM) components (such as Vespertilionids). The echolocation calls of bats in family Hipposideridae and Rhinolophidae are more easily characterized by the long narrow band constant frequency (CF) components. Some bats have more than one foraging strategy and feeding habitat, so there are highly variable in the echolocation behaviour (Fenton, 1990). Even though the relatively invariable CF calls of Hipposiderid and Rhinolophid bats can be established species-specific calls, which can help in identification of the cryptic species (e. g. Francis *et al.*, 1999; Kingston *et al.*, 2001), the variation of call frequency among populations within species also could be found (see 'geographic variation' below).

Relationship between morphology and echolocation calls

The echolocation calls are commonly used in microchiropterans. The calls of bats in the family Rhinolophidae and Hipposideridae are dominated by constant frequency with high duty cycles (Jones, 1999). The study of correlation between morphology and echolocation calls is one of the most interesting subjects because understanding the relationship between echolocation calls and morphological features could provide further insight into the evolution of the particular morphology and echolocation of each species (Francis and Habersetzer, 1998).

Sales and Pye (1974) reported that most of rhinolophoid bats concentrate nearly all their energy into the second harmonic and then emit higher frequencies than most other taxa. In addition, the relationship between body size and higher call frequency is often found in hipposiderids, than rhinolophids (Heller and von Helversen, 1989; Francis and Habersetzer, 1998). The body size (represented by Forearm length, FA) was also regarded by Bogdanowicz *et al.*, (1999) as a good predictor of frequency. Moreover, the body mass is negatively relate to the call frequency in at least five bat families; Vespertilionidae, Rhinolophidae, Hipposideridae, Emballonuridae and Molossidae (Jones, 1996, 1999).

Previous studies reported the correlation between the call frequencies with some other morphological features: nostril spacing (Sales and Pye, 1974; Pye, 1988), noseleaf (or horseshoe) width (Robinson, 1996) external ear size (Obrist *et al.*, 1993) cochlear width, (Francis and Habersetzer, 1998). Recently, Zhao *et al.*, (2003) reported negative relationships between call frequency and ear length in Rhinolophidae and Hipposideridae. Ear length was proved to be a more important morphological parameter influencing the call frequency in rhinolophids. The enlarged ears of rhinolophids correlated with the lower frequencies that rhinolophids used relative to larger body size. The closely relationship between echolocation call and body size (HB, FA and Body mass) in *Hipposideros armiger* and *H. larvatus* were supported by Zhang *et al.*, (2000). Additionally, others reflect the association of calls with flight constraints, e.g. wing morphology (Kingston *et al.*, 2000) diet composition, e.g. teeth, jaws, crania (Bogdanowicz *et al.*, 1999) and perhaps resource partitioning (Heller and von Helversen, 1989; Kingston *et al.*, 2000).

The ecological constraints affecting the morphology are still unclear. Guillén *et al.*, (2000) suggested that variation in echolocation call frequency among populations in *H. ruber* (approx. 133-148 kHz) was correlated with body size, body condition, the presence of ecologically similar species, and with environmental factors (especially, humidity). Lower frequencies were associated with higher humidity.

The Rhinolophidae-Horseshoe bats

The genus *Rhinolophus* has been known as a single modern genus belongs to the family Rhinolophidae which is the large family of insectivorous bat, comprising 71 species (Csorba *et al.*, 2003). They distributed throughout the Old World, from Europe and Africa, to South-east Asia and Japan, Philippines, New Guinea and Australia (Corbet and Hill, 1992). The earliest fossil record that referred to the genus *Rhinolophus* are known from the Eocene of Europe, the Miocene of Australia and Africa (Savage and Russell, 1983).

At the beginning, the genus *Rhinolophus* was introduced by Lacépède (1799) but was still associated with the Vespertilionidae until the family 'Rhinolophidae' was firstly named by Bell (1836) (Corbet and Hill, 1992). In 1876, Dobson recognised the genus *Rhinolophus* as a distinct taxon within the Rhinolophidae and it was promoted to subfamily status; Rhinolophidae, separated from *Hipposideros* in another distinct subfamily; Phyllorhininae. Subsequently, Miller (1907) placed the other taxa in the Hipposideridae and also elevated the subfamily Rhinolophidae to family rank.

The genus *Rhinolophus* was initially reviewed by Andersen (1905) and it was divided into six groups, named after the species: *R. simplex*, *R. lepidus*, *R. midas*, *R. philippinensis*, *R. macrotis* and *R. arcuatus*. Then in 1918, they were renamed as *R. megaphyllus*, *R. pusillus*, *R. hipposideros*, *R. luctus* and *R. euryotis* (Andersen, 1918). The *Rhinolophus* species groups were reviewed again by Tate and Archbold (1939) they renamed the *R. megaphyllus* group to *R. ferrumequinum* group. Subsequently, Tate (1943) merged the *R. macrotis* group into the *R. philippinensis*.

The systematic arrangement of the Indomalayan *Rhinolophus* was made by Hill (1992), who followed Andersen (1905) and Tate (1943). He moved *R. pearsoni* and *R. yunanensis* into the *R. fumigutus* group. In 1992, the work on systematic of the family Rhinolophidae was undertaken by Bogdanowicz, who organized the genus into groups and subgroups that mostly similar to Andersen's arrangements. *R. stheno* was added into the *R. euryotis* group and a new group *R. rouxii* which including *R. affinis* was proposed (Bogdanowicz, 1992).

Recently, Csorba *et al.*, (2003) published a comprehensive work on systematic of the genus *Rhinolophus*. They included the new group *R. adami, R. maclaudi*. In addition, they elevated the *R. megaphyllus* and the *R. pusillus* from subgroups level of Bogdanowicz (1992) to distinct groups and also moved *R. stheno* and *R. affinis* backed to the *R. megaphyllus* group. According to this paper, the *R. megaphyllus* group is including *R. affinis, R. borneensis, R. celebensis, R. malayanus, R. megaphyllus, R. neresis, R. stheno, R. virgo* (Csorba *et al.*, 2003).

Classification

Bats are mammal in the Phylum Chordata, Subphylum Vertebrata, Class Mammalia, Order Chiroptera. According to Corbet and Hill (1992); Csorba *et al.*, (2003); Lekagul and McNeely (1977) and Neuweiler (2000), *R. malayanus* and *R. stheno* can be classified as below;

Class Mammalia

Order Chiroptera

Suborder Microchiroptera

Superfamily Rhinolophoidae

Family Rhinolophidae

Genus *Rhinolophus*

Species *R. malayanus* Bonhote, 1903

R. stheno Andersen, 1905

As above, *R. malayanus* and *R. stheno* are members of the *R. megaphyllus* group. This group can be characterized and distinguished from another similar group; *R. rouxii* by the triangular shape of lancet and the lateral margins are more or less straight, but it is strongly concave in *R. rouxii*. Moreover, it can be distinguished from *R. ferrumequinum* by lowered and rounded connecting process, large anterior upper premolar (P^2) which usually in row or slightly extruded (Csorba *et al.*, 2003).

R. malayanus and *R. stheno* can be distinguished from the other members of the *R. megaphyllus* group and other similar species on the basis of morphological features. *R. malayanus* is differs from *R. affinis* and *R. rouxii* by the smaller body size; from *R. stheno* and *R. thomasi* in the shape of lancet and sella (Borissenko and Kruskop, 2003). *R. borneensis* and *R. celebensis* are differ from *R. malayanus* in the form of the anterior nasal swelling which is large, much inflated and extending laterally in *R. malayanus*. In *R. borneensis* and *R. celebensis* the anterior median swelling are less developed, with the lateral swelling conspicuously larger in *R. malayanus* (Csorba *et al.*, 2003; Hill and Thonglongya, 1972).

Although *R. stheno* has similar body size with *R. borneensis* and *R. rouxii*. It may be recognised by parallel sided sella (while in *R. borneensis* and *R. rouxii* are pandurate) and shorter tail. Differs from *R. thomasi* by long lancet, straight side and cuneate tip (not reduced lancet tip like *R. thomasi*) (Borissenko and Kruskop, 2003; Csorba *et al.*, 2003).

Taxonomic Backgrounds

It was mentioned by many authors that *R. malayanus* is hardly distinguishable from *R. stheno.* Lekagul and McNeely (1977) reported that they can be distinguished by the body size, lancet shape and the relative proportion of the first and second phalanges of the third digit. However, McFarlane and Blood (1986) suggested that there were no reliable external characters that can be used in distinguishing them. Subsequently, Robinson (1996) supported the suggestion of McFarlane and Blood (1986) but pointed that the two species can be distinguished by the horseshoe or noseleaf width and the difference in echolocation call frequencies. He reported that *R. malayanus* has wider horseshoe width (7.9-9.0 mm. while in *R. stheno* is 7.2-7.6 mm.) and the call frequency of *R. malayanus* was lower (75 kHz) than *R. stheno* (85-95 kHz) based on using the QMC Mini Bat detector. The clearly distinction between them in the basis of the shape of the rostrum and nasal swelling was established by Corbet and Hill (1992).

The difference in rostral morphology between *R. malayanus* and *R. stheno* was also discussed and illustrated by Bates *et al.*, (2001, 2004), which the anterior median compartments of the rostrum of *R. malayanus* were less inflated than that of *R. stheno* whilst the posterior compartments were more highly inflated. The basis of difference on proportion of the first and second phalanges of the third digit between them was supported by Hendrichsen *et al.*, (2001), which in *R. malayanus* is shorter than *R. stheno.*

Csorba *et al.*, (2003) also noted about the difference between these cryptic species. As same as Bates *et al.*, (2001), they noted that the post orbital constriction of *R. stheno* is apparently narrower (1.49-2.00 mm.) than *R. malayanus* (2.13-2.67 mm.). In addition, the difference in their median septum width, which is much wider in *R. stheno*, was firstly mentioned.

In 1998, a new subspecies of *R. stheno*; *R. s. microglobosus* was described from northern Vietnam (Csorba and Jenkins, 1998). It was distinguished from the nominate subspecies on the basis of the apparently smaller anterior median chambers of the rostrum and its generally smaller size. This new subspecies appears to be present in mainland South East Asia north of the Isthmus of Kra, whilst the nominate subspecies presents in the south of the Isthmus of Kra, from Thai-Malay peninsula to Java (Csorba *et al.*, 2003).

Acoustic divergence and Geographic variation in echolocation call

Acoustic divergence in cryptic species bat was usually occurs in species using narrowband constant frequency of echolocation calls (e.g. in Rhinolophidae and Hipposideridae). Jones and Barlow (2004) suggested that the acoustic signatures were reliable badges of species identity. Communication among bats of the same species would then be facilitated through each species echolocating within its own bandwidth of frequencies.

After the discovery of two phonic types of *Hipposideros commersoni* (56 and 66 kHz call frequencies) in a cave in Kenya (Pye, 1972), the further study on the intraspecific and interspecific variation in echolocation call frequency of bats were more interested from scientists. *Hipposideros caffer* and *H. ruber*, which are sympatric in West Africa, are similar morphologically but readily separated by echolocation call frequency (Jones *et al.*, 1993). Similarly in Southeast Asia, the new species *Hipposideros orbiculus* was recognised. and was discriminated from *Hipposideros ridleyi* by a 19-kHz difference in call frequency (Francis *et al.*, 1999). Francis and Habersetzer (1998) published the differences in echolocation and also body size of *H. cervinus* from peninsular Malaysia and Borneo (which emitted call frequency at 128.8 and 115.8 kHz, respectively). Both phonic types were identified as *H. cervinus labuanensis* but they suggested that these populations may prove to represent distinct subspecies.

Another example in validity of using the call frequencies as the species identity was documented by Kingston *et al.*, (2001). They described two cryptic species of *Hipposideros bicolor* (call frequencies 131 and 142 kHz) in Malaysia, which were ~7% sequence divergence at the cytochrome *b* gene.

Kingston and Rossiter (2004) recently proposed that divergence among sympatric three size morphs of the Wallacea's bat *Rhinolophus philippinensis* might be facilitated by disruptive selection on diet, resulting from large-scale harmonic shifts at low frequencies. These morphs were associated with different call frequencies; large (FA 56.1 mm) 27.2 kHz, small (FA 47.0 mm) 53.6 kHz, Buton

Island intermediate (FA 50.6 mm) and Kabaena Island intermediate (FA 48.4 mm) 41.7 kHz. There were genetic divergence among them and currently recognised as subspecies. Nevertheless, they suggested that more than one species might be present.

Taylor *et al.*, (2005) reported highly significant differences in peak frequency of calls and cryptic speciation of *R.* cf. *hildebrandtii* (37, 42 and 46 kHz) in Africa. Even though Jones *et al.*, (1993) reported the variation in call frequency of the West African *H. caffer* (128-153 kHz), Taylor and his colleagues suggested that it was due to geographical variation and sexual dimorphism (Taylor *et al.,* 2005). Recently, Thabah *et al.*, (2006) proposed a new species; *Hipposideros khasiana* which was a cryptic species of *Hipposideros larvatus* after they found the sympatrically genetic divergence and slightly morphological differences in the two phonic types; 85 kHz and 98 kHz *Hipposideros larvatus* from India.

Jones and Barlow (2004) concluded that the major factor promoting acoustic divergence in cryptic pipistrelle species was associated with facilitating communication with conspecifics, rather than resource partitioning. Therefore, where frequency drift is sufficient to alter communication, reproductive isolation may be maintained on secondary contact.

CHAPTER 3

MATERIALS AND METHODS

Specimens of *Rhinolophus malayanus* and *R. stheno* were collected from 29 sites throughout Thailand. Sites selection was based on previous reports of *R. malayanus* and/or *R. stheno*. In addition, since most *Rhinolophus* commonly roost in cave, priority of further survey sites was given to limestone area which comprised of limestone caves.

133 specimens of *R. stheno* (*sensu* Csorba *et al.,* 2003) were examined, including 102 specimens from Thailand held in the mammal section of the zoological collections of the Princess Maha Chakri Sirindhorn Natural History Museum (formerly PSU-Natural History Museum) Prince of Songkla University (PSUZC-MM); the Thailand Institute of Scientific and Technological Research (TISTR); Chiangdao Wildlife Research Station (CD) and the Thailand National Museum of Natural History (THNHM). To investigate the whole intraspecific and interspecific variation, it is necessary to examine the holotype and specimens those collected from other countries. So, in addition, five specimens from Myanmar (Harrison Institute, HZM); two from Cambodia (HZM); 14 from Vietnam (Vu Dinh Thong's collection and the Natural History Museum, London (BMNH)) and eight from peninsular Malaysia (BMNH and Senckenberg Forschungsinstitut und Naturmuseum (SMF)) were also examined.

106 specimens of *R. malayanus* were examined including 81 from Thailand (PSUZC-MM and BMNH); 15 from Myanmar (HZM); one from Lao PDR (HZM); four from Vietnam (HZM and BMNH) and five from Malaysia (BMNH).

Specimen localities are shown in Fig. 1 and 2 and the list of specimens examined is included in Appendix 1.

3.1 Study Areas

During June 2006 and September 2007, bat surveys in Thailand were conducted in the following localities:

Chiang Mai Province: field surveys took place in October 2006 at two sites, Mae Ja cave in Pha Daeng National Park (formerly Chiangdao National Park) and Pha Daeng cave in Si Lanna National Park. They are situated in Chiangdao district. The area is characterised by rugged mountain ranges, which are the source of various tributaries of Ping River, Mae Tang River and Mae Ngat River. Most of the area is limestone mountain, including flood plains and scattered limestone outcrops. It is covered by hill evergreen forest, mixed deciduous forest and cultivated area. The climate of the area is tropical monsoon with annual precipitation averages 1839 mm. (National Park Office, 2006). Bats were captured from two sites:

(1) **Mae Ja cave** (19°31'N, 98°50'E) [loc. G5, Fig. 1], located in a small isolated limestone outcrop surrounded by cultivated area, nearby a small stream. The cave entrance is 4x3 m with three large chambers and a subterranean stream. Bats were captured in cave by using hand net and 2 mist nets set at the entrance of the second and the third chamber. Five bat species were found: *R. stheno microglobosus*, *R. affinis*, *R. pusillus*, *R. coelophyllus* and *Miniopterus magnator*.

(2) **Pha Daeng cave** (19°20'N, 99°01'E) [loc. M16, Fig. 2], set in a limestone mountain, which was surrounded by rice fields and tens of villages. The cave entrance was a steeply large hole with two major chambers. The first chamber measured about 20x20 m wide and 15 m high which *Eonycteris spelaea* were seen. The second chamber is much narrower, approximately 40 m deep by 4 m wide and 3 m high. Bats were captured by two harp traps set at the cave entrance. Four bat species were captured: *R. malayanus*, *R. pusillus*, *H. cineraceus* and *H. halophyllus*.

Tak Province: a survey took place on 14 November 2006 at Manora cave (16°46'N, 98°39'E) [loc. G7, Fig. 1], in Mae Sot district. The area is characterised

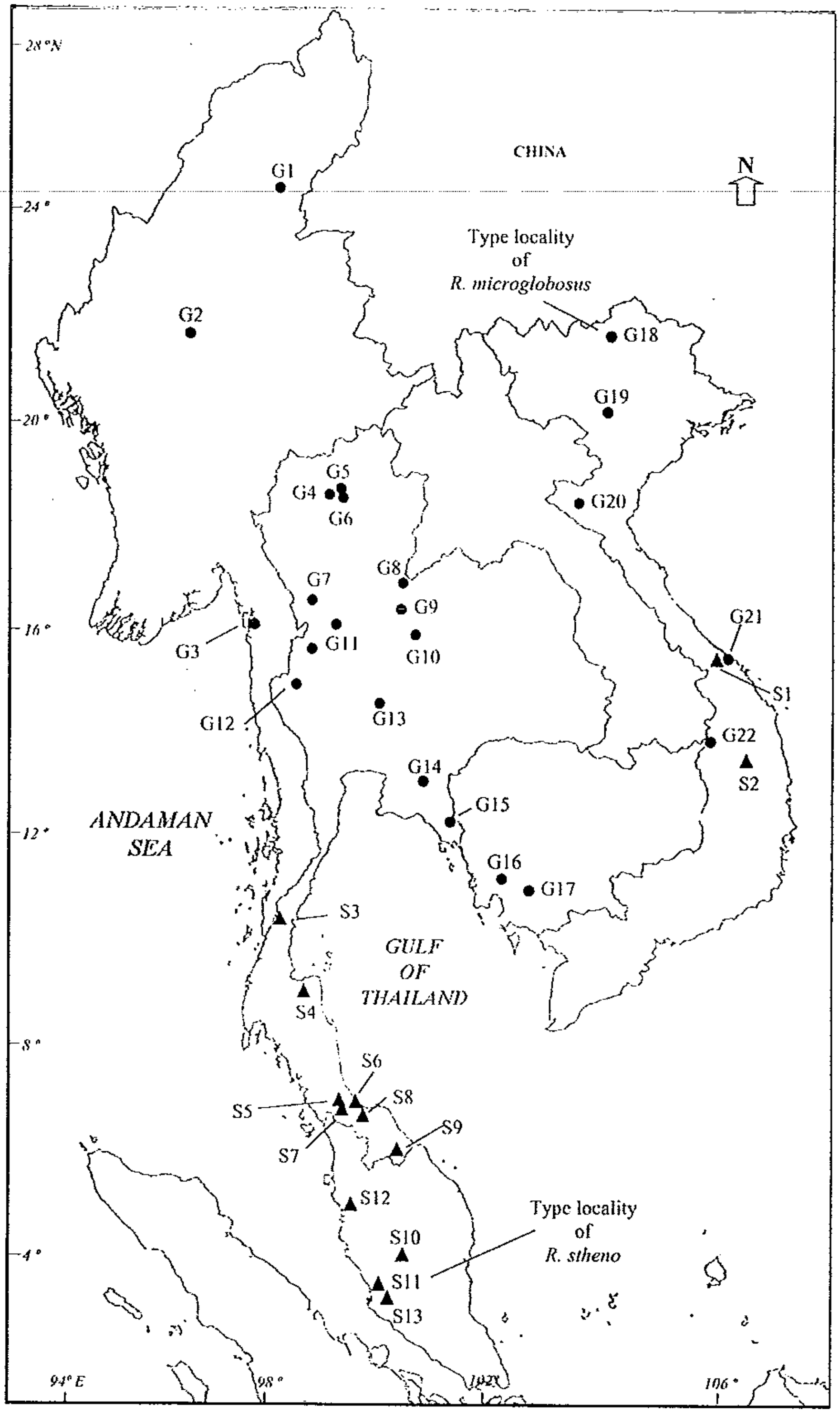

Fig. 1. Localities of *R. microglobosus* (dots) and *R. stheno* (triangles). Locality information and specimen numbers are included in Appendix 1.

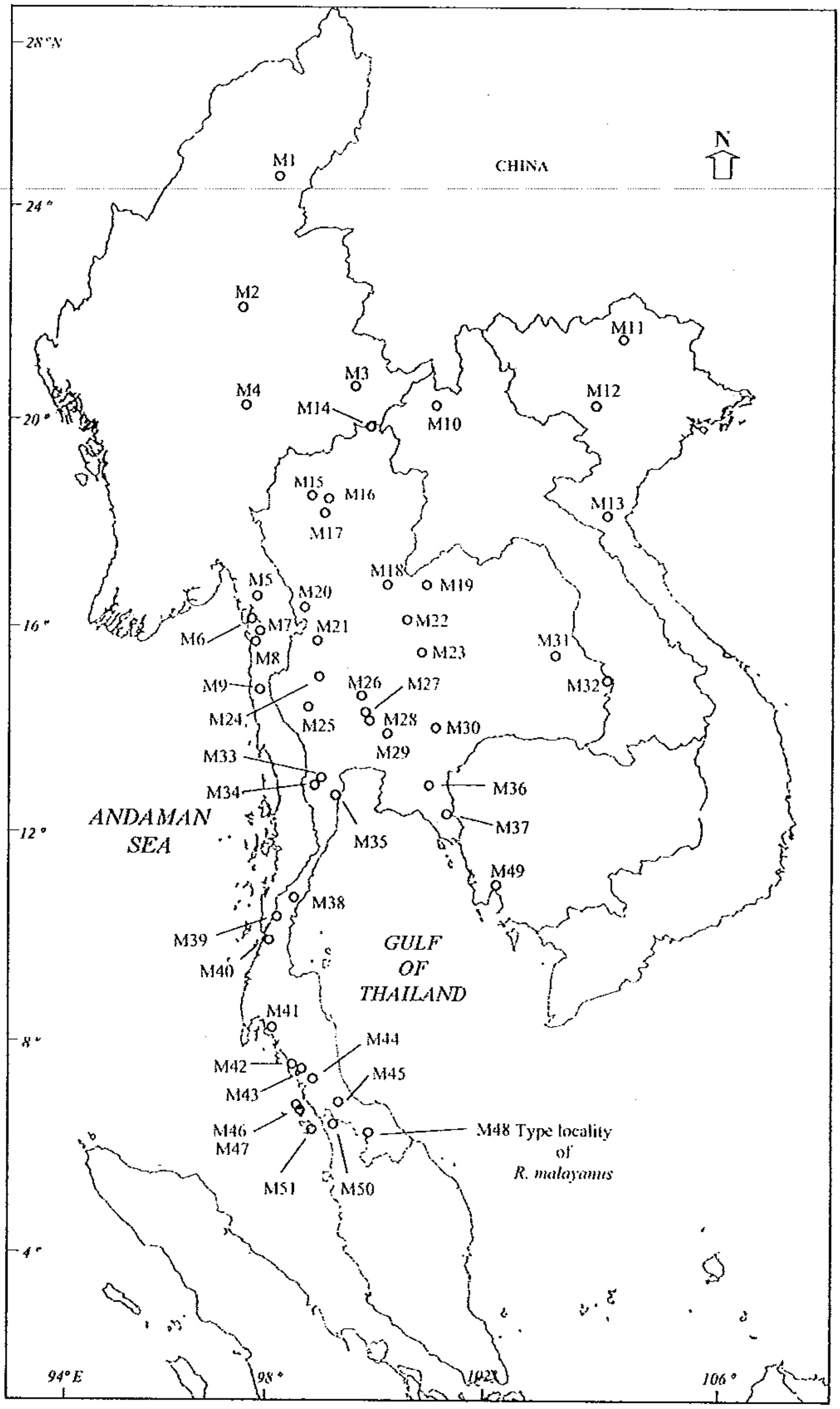

Fig. 2. Localities of *R. malayanus*. Locality information and specimen numbers are included in Appendix 1.

by rugged limestone mountain range. The vegetation in the area covered by mixed measured deciduous forest and deciduous dipterocarp forest. It rains heavily between August and October. The temperature averages 22 °C and could drop to 6 °C between November and January (National Park Office, 2006). The cave set in a limestone hill at 604 m a.s.l. surrounded by bamboo forest. The cave had two chambers, the outer measured approximately 20x30 m wide and 20 m high. The entrance to the second chamber is very narrow. Bats were caught by hand net in the second chamber which approximately 50 m deep by 30 m wide and 20 m high. Approximately 10000 individuals of bat were found roosting in this chamber. Four bat species were found in the cave: *R. stheno microglobosus, R. coelophyllus, H. larvatus* and the most species in the cave is *Min. medius.*

Loei Province: the survey took place on September 2007 in Phu Suan Sai National Park [loc. G8, Fig. 1]. The national park is situated in the north of Na Haeo district, covers total area of 117.16 km^2 (centre on 17° 31'N, 101° 30'E). It is mainly mountainous with a highest peak of 1408 m. a. s. l. The vegetation comprises lower montane, moist evergreen, dry evergreen, mixed deciduous and some dry dipterocarp forest (National Park, Wildlife and Plant Conservation Department, 2006a). The climate is tropical monsoon, with an annual precipitation of about 1200 mm. Most of the rains occur from May to October. The dry season lasts for 6 months. Mean monthly temperatures range from 20-24 °C in the cool-dry season (November-January) to 25-29 °C in the hot-dry season (February-April) (WorldClimate, 2005). The geology of the area is characterised by non-marine Cretaceous rocks (Department of Mineral Resources, 2006a). Bats were captured by using harp traps set across the nature trails in the mountain. Totally 72 harp trap-hours were made around the top of the mountain (17°30'N, 100°57'E), where covered mainly by lower montane forest, and other 36 trap-hour were made around the head quarter office (17°30'N, 100°56'E). Eleven species of bat were captured: *R. stheno microglobosus, R. affinis, R. lepidus, R. pusillus, H. larvatus, H. pomona, Min. pusillus, Tylonycteris pachypus, Kerivoula hardwickii, K. kachinensis* and *K. titania.*

Petchabun Province: field survey took place in September 2007 in Nam Nao National Park. The national park is situated in the Petchabun Range, in eastern Petchabun Province and northern Chaiyaphum Province in north-central Thailand (c.o. 16° 44'N, 101° 34'E). The geology of the mountain range is mainly sedimentary and igneous rocks (Department of Mineral Resources, 2006b). The national park covers a total area of 966 km^2 and comprises deciduous dipterocarp, mixed deciduous, dry evergreen, pine forests and grassland. Annual temperature averages 22.7 °C and annual precipitation is between 1300 and 1500 mm (National Park Research Division, 2004). The rainy season is between July and October. The coldest month is November, when temperatures may fall to 0 °C (National Park, Wildlife and Plant Conservation Department, 2006b). Trapping were made at these two following sites:

(1) Nam Nao cave (16°57'N, 101°30'E) [Loc. M22, Fig. 2 and Loc. G10, Fig. 1] is situated in a limestone mountain of 955 m peak. The large complex of cavern, with subterranean stream, is home to large amount of bat. The cave entrance was surrounded by dry evergreen forest, a stream which runs through the cave, and a small shrine. The cave is one of the most attractive sites of the national park and is under protection of the national park's ranger station. A harp trap was set at the cave entrance and another two were set close to the cliff, about 30 m far from cave entrance. Thirteen bat species were found: *R. malayanus, R. stheno microglobosus, R. thomasi, R. affinis, R. pusillus, R. marshalli, R. luctus, R. pearsoni, R. pearsoni, Aselliscus stoliczkanus, H. pomona, H. armiger*, and *H. lylei*.

(2) Phu Kor (16°45'N, 101°34'E) is one of a best view point of the national park. There is an about 12 km long nature trail from the view point to the head quarter office. Two harp traps were set across the nature trail, which surrounded by bamboo groves. One was set at the starting point of the trail and another one was set 300 m away. In the evening, traps were opened from dust (18:30 h) until 21:00 h and were checked again at dawn. . Four bat species, which were captured at dawn only, were found: *H. armiger, T. robustula, K. hardwickii* and *K. kachinensis*.

(3) Hiking trail (16°44'N, 101°35'E) is situated in the area of head quarter office. The trail, with a stream runs along, is surrounded by mixed deciduous forest and some bamboo groves. Two harp traps were set across the trail on both side of the stream. Traps were opened until 21:00 h in the evening and then were left overnight. Two species of bat were captured in the morning: *K. hardwickii* and *K. titania.*

Kalasin and Mukdahan Province: surveys were taken place in September 2007 at Phu Sithan Wildlife Sanctuary (between 16°30'N to 16°45'N and 104°0.5'E to 104°25'E). The wildlife sanctuary is located on the southern part of Phu Pan Range. The area is characterised by sandstone mountain with plains on the top. A series of limestone outcrop was presented. The mountain is the source of various streams that served thousands of people who live in Kalasin and Mukdahan. The wildlife sanctuary covers a total area of 250 km^2, with an elevation between 200 and 592 m a.s.l. The sanctuary is surrounded by rice fields and hundreds of villages. The vegetation comprises deciduous dipterocarp, mixed deciduous, dry evergreen forest and grassland. The underlying geology is characterised by non-marine Cretaceous sedimentary rocks. The climate is tropical monsoon with annual precipitation averages of 1364.7 mm. Annual temperatures between 22 and 29 °C (National Park, Wildlife and Plant Conservation Department, 2003). Bat surveys were conducted in three sites:

(1) Tham Bing (=bat cave, 16°35'N, 104°09'E) located in the area of head quarter office, which surrounded by deciduous dipterocarp forest and rice fields. A reservoir was presented nearby. The cavern set in sandstone hillock with two narrow entrances, approximately 1x1 m wide. The first chamber, which was the larger, measured about 20 m deep by 25 m wide and 2 m high. The second chamber with subterranean stream was much narrower, only 5 m deep by 3 m wide and 0.8 m high. A harp trap was set at one of the cave entrance from 21:00-06:30 h. Three species of bats were caught in the morning: *Megaderma spasma, H. pomona* and *H. galeritus.*

(2) Pha Peung cave (16°33'N, 104°08'E) is a sandstone cavern situated on the top of the hill, at 299 m a.s.l. The area features various rock creeks and holes. It is covered by dry evergreen forest including some mixed deciduous forest. The cave had one major chamber which approximately 25 m deep by 10 m wide and 5 m high. A harp trap was set at the main entrance of the cave. Another one was set across a trail. Five bat species were captured: *Taphozous melanopogon, R. shameli, H. larvatus, H. galeritus* and *H. pomona.*

(3) Phu Pha San [loc. M31, Fig. 2], located in the east of the wildlife sanctuary, in Kham Cha-I district, Mukdahan. The area is under protected of Phu Pha Muang ranger station. The area is characterised by steeply scarped sandstone mountain, with plains on the top. The foothill is covered by mixed deciduous forest whereas the top covered by dry evergreen forest and deciduous dipterocarp forest. Two harp traps were set across a forest trail around the top of the hill, at 417 m a.s.l. (16°38.6'N, 104°22'E). *R. malayanus* and *R. pusillus* were captured from this site. Another trap was set across a forest trail in mixed deciduous forest, near bamboo (16°38.5'N, 104°22'E, 270 m a.s.l.). Totally, 36 harp trap-hours were made at this site and two bat species were caught: *R. pusillus* and *H. larvatus.*

Ubon Ratchathani Province: survey took place in September 2007 at Pha Taem National Park. The national park is located in the north east of Ubon Ratchathani Province, on the west bank of Mekong River that borders Thailand and Lao PDR. The national park covers a total area 340 km^2. The area is characterised by rugged sandstone mountain. The vegetation type comprises mainly deciduous dipterocarp forest, and some patches of dry evergreen forest occupies around creeks and streams. The climate is tropical monsoon with big difference among each season. It is very wet in rainy season between June and September, cold and dry winter from October to February, and hot summer during March to May (National Park Office, 2006). Bats were captured from two sites:

(1) Patihan cave (15°35'N, 105°34'E) [loc. M32, Fig. 2], is a large sandstone cavern situated in a temple. The temple covered by a small patch of dry evergreen forest and surrounded by dwarf deciduous dipterocarp forest. A seasonal stream was presented. The cave entrance, which approximately 10 m wide by 5 m high, and the first chamber, which about 30 m deep by 15 m wide and 8 m high, were modified to be a shrine. There were two more major chambers inside where is home of a large amount of bats. The second chamber considered equally as the first but only 2 m high. The inner chamber, with a subterranean stream, is about 100 m deep by 15 m wide and 3 m high. *R. shameli, R. malayanus, H. pomona, H.* cf. *larvatus* and *H.*

galeritus were seen in the cave. Three harp traps were set across the trail around the temple in the evening and were opened overnight. All the same species found in cave were captured in harp traps.

(2) Camp ground (15°24'N, 105°30'E) [also loc. M32, Fig. 2], situated 400 m east of the head quarter office. The area was covered by deciduous dipterocarp forest with bamboo groves. A stream was present in the area. Three harp traps were set across the nature trail, which surrounded by bamboo. Five species of bat were captured: *R. malayanus, R. shameli, R. pusillus, H. larvatus* and *H. pomona.*

Nakhon Ratchasima Province: survey took place in January 2007 at the Wang Nam Khieo Forestry Training Camp, Pak Thong Chai district. The area is located in the sandstone mountain, south of the Korat Plateau with elevation between 280-762 m a.s.l. Underlying geology is characterised by Triassic-Cretaceous sandstone. The vegetation comprises dry dipterocarp, mixed deciduous and dry evergreen forest. The climate is tropical monsoon with annual precipitation averages 1082 mm. Annual temperature averages between 21.72 °C and 28.85 °C (TISTR, 2005). Bats were captured by two harp traps set across a forest trail in dry evergreen forest, near a botanical garden (14°28'N, 101°56'E) [loc. M30, Fig. 2]. Seven bat species were captured: *R. malayanus, R. coelophyllus, H. bicolor, H. larvatus, Myotis muricola, Cynopterus sphinx* and *Macroglossus sobrinus.*

Lop Buri Province: a survey took place in January 2007. The province is situated in east bank of the Chao Phraya River, and characterised by flood plains, alluvial plains and series of isolated limestone outcrops. The climate is tropical monsoon with annual precipitation averages between 850 and 1401 mm. The annual temperature averages of 27.9 to 29 °C (Ministry of the Interior, 2000). Bats were captured from two sites:

(1) Ma Tok cave (14°54'N, 100°29'E) [loc. M27, Fig. 2] set in Khao Samorkhon limestone outcrop, Ta Wung district. The outcrop is covered by secondary forest and surrounded by rice field, tens of villages. The cave is situated in the area of a temple, where is home of hundred Long-tailed Macaques (*Macaca fascicularis*). As same as most of the caves in this outcrop, the cave is characterised by a sink hole with very narrow entrance (0.5 by 0.3 m wide). A harp trap was set above the cave entrance for 3 hours in the evening (18:00-21:00 h). Two bat species were captured from this cave: *R. malayanus* and *R. pusillus.* Another harp trap was set across a trail which runs between foothill and rice field, approximately 1.5 km far from the first trap (14°54'N, 100°30'E). Four species were captured: *R. malayanus, R. coelophyllus, R. pusillus* and *H. halophyllus.*

(2) Wat Tham Suea Leung (14°49'N, 100°47'E) [loc. M28, Fig. 2], in Koktum, Muang Lop Buri. The area is characterised by rugged limestone mountain, which covered by mixed deciduous forest and bamboo groves. It is surrounded by rice fields, cornfields and villages. A harp trap was set between bamboo groves at the foothill. Two bat species were captured from this site: *R. malayanus* and *H. larvatus.*

(3) Khao Don Dueng (15°09'N, 100°37'E) [loc. G13, Fig. 1 and loc. M26, Fig. 2], Ban Mi district. The area is characterised by large limestone outcrop which surrounded by mixed deciduous forest and villages. Three harp traps were set across trails around the outcrop, near bamboo. Traps were opened in the evening, from 18:00-20:00 h. Eight species of bat were captured: *R. malayanus, R. stheno microglobosus, R. pusillus, R. coelophyllus, H. pomona, H. halophyllus, H. cineraceus, H. larvatus.*

Sara Buri Province: survey took place in January 2007 at Wat Tham Phra Phothisat, Tab Kwang (14°34'N, 101°08'E) [loc. M29, Fig. 2]. The province, which located on the east bank of Chao Phraya River and on the west of Korat Plateau, is characterised by flood plains and a series of limestone outcrops. The climate is tropical monsoon with annual temperature between 21 and 38 °C. Most of the rains occur in September. Annual precipitation averages of 1308 mm (Ministry of the Interior, 2000). The Wat Tham Phra Phothisat is a temple, which situated in a large limestone outcrop. The outcrop features a complex of limestone caves and caverns. It is surrounded by a patch of mixed deciduous forest with bamboo and a stream. Two harp traps were set at the foothill across forest trail that led to the caves. Traps were opened from 18:00-21:00 h. Six bat species were found: *R. malayanus, R. pearsoni, R. coelophyllus, R. thomasi, M. siligorensis* and *T. pachypus.*

Ratchaburi province: research in February 2007 took place at Khao Bin cave, Chom Bueng district and Khao Nom Tai, Photharam district. The area is characterised by plains and a series of limestone outcrops. The climate is tropical monsoon with annual precipitation averages of 1486 mm (Leelapaibul *et al.* 2005), and the average temperature between 21 and 38 °C (Ratchaburi Provincial Office, 2007).

(1) Khao Bin cave (13°35'N, 99°40'E) [loc. M34, Fig. 2], a complex of cavern situated in a large limestone outcrop, which surrounded by mixed deciduous forest and townships. The cave has been decorated for tourism and hardly seen any bat in the cave. The main entrance of cave had a gate which was shut at night. There is, however, a narrow entrance, about 1x1 m wide, besides the main entrance. A number of bats used this entrance at dusk and dawn, while most of bats in this cave used another entrance on the top of the outcrop. Two harp traps were set in the evening. One was set at main entrance of the cave and another one was set between groves of bamboo, about 200 m far away. Traps were opened for three hours in the evening, from 18:00-21:00 h. Four bat species were captured: *R. malayanus, R. thomasi, H. cineraceus* and *H. pomona.*

(2) Khao Nom Tai (13°42'N, 99°45'E) [loc. M33, Fig. 2], a limestone outcrop located in a township, which approximately 100 km west of Bangkok. The outcrop was surrounded by a narrow strip of mixed deciduous forest. There is a large limestone cavern which had two entrances in the outcrop. Two harp traps were set across trail which runs around the outcrop, near bamboo groves. Traps were opened from 18:00-21:00 h. Five species of bats were found: *R. malayanus, R. pusillus, R. coelophyllus, R. thomasi* and *H. larvatus.*

Pet Buri Province: survey took place in February 2007 at Khao Yoi limestone outcrop (13°14'N, 99°49'E) [loc. M35, Fig. 2], which located in township of Khao Yoi district. The area is characterised by plains and rugged mountain range on the west of the province, which is the sources of various tributaries of Pet Buri River. The climate is tropical monsoon. Annual precipitation is between 799 and 1136 mm and the annual temperature varies between 8 and 28 °C (Ministry of the Interior, 2000). Khao Yoi is a large-steeply scarped limestone outcrop surrounded by local roads and patches of mixed deciduous forest. The outcrop contains a complex of caves, which was modified to be a tourist attraction. There were hundreds of Long-tailed Macaques, that fed by tourists, lived around the outcrop. Traps were opened from 18:00-21:00 h. A trap was set under canopy of a tree on the hillside, whereas another one was set in the mixed deciduous forest at the foothill. Bats were also caught by a hand net at the entrance of a sink hole, where a number of *R. pusillus* was found. Other seven species were captured in harp traps include *R. malayanus, R. coelophyllus, H. bicolor, H. halophyllus, H. cineraceus, H. larvatus* and *M. siligorensis.*

Chumporn Province: survey took place in October 2006 and January 2007 at two sites in Pathiu district. The area is characterised by plains and series of limestone outcrops. Most of the area is covered by agricultural lands; rubber, palms plantations and various kinds of orchards. The climate is tropical monsoon with annual precipitation between 1741 and 2215 mm. The annual temperature averages of 26.8 °C (Ministry of the Interior, 2000). Surveys were taken place in the following sites:

(1) Silawan cave (10°41'N, 99°14'E) [loc. M38, Fig. 2], situated in a limestone outcrop surrounded by a patch of mixed deciduous forest, and agricultural areas which include pineapples and rubber plantations. The cave had two major chambers with a large entrance, about 8 m wide by 8 m high. The outer chamber, which *Emballonura monticola* was found, was approximately 20 m deep by 6 m wide and 8 m high. The inner chamber was much shorter, approximately 10 m deep by 10 m wide and 8 m high. At least three species of *Hipposideros* and a *Rhinolophus* were found roosting in the inner chamber. Two harp traps were set in the evening of 11 January 2007; a trap was set in cave between the outer and inner chambers from 18:00-21:00 h. And another, which was opened overnight, set across forest trail besides the outcrop. Nine bat species were captured: *R. malayanus, R. lepidus, H. armiger, H. cineraceus, H. bicolor, H. larvatus, H. galeritus, M. siligorensis* and *T. pachypus.*

(2) Khao Kram cave (10°55'N, 99°22'E), a limestone cave set in a small limestone outcrop, which surrounded by rubber plantation. From the cave entrance, which approximately 5 m wide and 3 m high, the cave was separated to the left and right chambers. The left chamber was approximately 8 m deep by 3 m wide and 2.5 m high. Whereas the right chamber, which was much deeper, measured approximately 40 m deep by 10 m wide and 3 m high. Harp traps were set across the narrowest part of each chamber, near the cave entrance. Four species of bat were captured: *R. affinis, H. armiger, H. larvatus, H. bicolor* and *H. cineraceus.*

Ranong Province: survey took place in January 2007 in La Aun and Kra Buri district. The province is located on the narrowest part of the Peninsular Thailand and known as "Isthmus of Kra". 86% of the area is characterised by rugged limestone mountain and only 14% are plains. Ranong is the province which had highest annual precipitation in Thailand, between 3553 and 5330 mm a year. Annual temperature averages of 27 to 28.9 °C (Ministry of the Interior, 2000). Surveys were conducted from the following sites:

(1) Knaddai cave (19°21'N, 98°43'E) [loc. S2, Fig. 1 and loc. M39, Fig. 2], a large cavern complex set in a large limestone outcrop near the Nai Wong Neua township, La Aun district. The outcrop was surrounded by mixed deciduous forest, a public park and a stream was presented. The main entrance was measured approximately 4 m wide by 8 m high. The cave had many chambers and was home of a large number of bats, but most of bats were seen roosting in the first two chambers. *Eonycteris spelaea* and *Megaderma spasma* were seen in the second chamber. Three harp traps were set in the evening from 18:00- 21:00 h. A trap was set inside the cave, between the first chamber and the main entrance; another was set at a smaller entrance on the back side of the outcrop and the other one was set between the outcrop and a tree. Six species of bat were captured: *R. malayanus, R. stheno, R. affinis, H. galeritus, H. armiger, H. lekaguli* and *M. siligorensis.*

(2) Phra Khayang cave (10°19.5'N, 98°46'E) located in Kra Buri district. The large cavern set in a limestone outcrop which was surrounded by mangrove forest, nature trail and a stream that runs to the sea. The cave had 3 major chambers with the cave entrance, which was measured approximately 6 m wide by 8 m high. The outer, which was the largest chamber, measured about 50 m deep by 15 m wide and 20 m high. The inner two chambers were much smaller, approximately half size of the outer. Hand net was used in capturing bats that roosting in the cave by day and two harp traps were set across nature trail in the evening. Traps were opened from 18:00-21:00 h. Eight species of bat were found from this site: *E. spelaea, T. melanopogon, R. affinis, H. lekaguli, H. cineraceus, H. galeritus, Min. medius* and *M. horsfieldi.*

***Krabi Province*:** a short survey was conducted in Khao Phanom district during September 2006. The province located on the west coast of the Peninsular Thailand. The area is characterised by rugged limestone mountains, coastal and islands. Most of the plains are agricultural areas; palm and rubber plantation. The climate is tropical monsoon with averages precipitation between 2069 and 2309 mm and the averages temperature between 27 and 32 °C (Ministry of the Interior, 2000). Bats were captured from the area of Sang Phet cave (8°09'N, 98°53'E) [loc. M41, Fig. 2], which set in limestone outcrop which was surrounded by a stream, rubber plantations and mixed deciduous forest. Most of the area was occupied by monks and shrines. The cave had two major chambers with their own entrance in the opposite side of the outcrop. The chambers were connected to each other by a very narrow cavity and inaccessible. Approximately 80 individuals of *H. turpis* were seen roosting in a chamber. Three harp traps were set in the evening from 18:00-22:00 h. A trap was set at an entrance of cave, whereas another two were set across a forest trail. Four species of bat were captured: *R. malayanus, R. coelophyllus, H. bicolor* and *H. turpis.*

***Satun Province*:** located on the west coast of the Peninsular Thailand and connect to the north of Perlis, Malaysia. A survey took place in June 2006 at two sites in Manang district. The area is characterised by steeply scarped limestone mountains. The vegetation types comprise of lowland moist evergreen forest and rubber plantations. The climate is tropical monsoon with annual average precipitation between 1822 and 2627 mm a year. Annual average temperature between 27-28 °C (Ministry of the Interior, 2000). Bats were captured from the following sites:

(1) Chet Kot cave (7°05'N, 99°53'E) [loc. M43, Fig. 2], situated in a limestone outcrop which was surrounded by a township and rubber plantations. A harp trap was set across a local road which led to the cave entrance. Five species of bat were captured: *R. malayanus, R. affinis, H. bicolor, H. armiger* and *H., larvatus.*

(2) **Wang Saithong waterfall** (7°05'N, 99°54'E) [loc. M44, Fig. 2], set in a limestone mountain which was surrounded by moist evergreen forest and a township. A series of small crevices was presented in the area. Two harp traps were set across forest trail which runs in front of the waterfall. Traps were opened from 18:00-22:00 h. Four species of bat were captured: *R. malayanus, H. bicolor, H. armiger* and *Nycteris tragata.*

Songkla Province: surveys took place between March and December 2006 at three sites in Hat Yai and Rattaphum district. The province located on the east coast of Thai-Malay peninsula. The area is characterised by rugged mountain range and coastal plains. The covered vegetations comprise; seasonal evergreen, mixed deciduous forest and rubber plantations. The climate is tropical monsoon with average temperature between 20 and 36 °C. Annual precipitation varies between 1698 and 2963 mm (Ministry of the Interior, 2000). Bats were captured from the following sites:

(1) **Khao Rak Kiat** (07°04'N, 100° 15'E) [loc. S4, Fig. 1], located in Rattaphum district. The cave complex set in a limestone outcrop, which was situated in a temple, and surrounded by township and paddy fields. There was a narrow strip of secondary forest, dominated by *Cassia siamensis,* along the local road side, which runs around the foothill. Two harp traps were sets across the road in the evening, and were opened from 18:00-20:30 h. Five species of bat were captured: *R. stheno, R. affinis, H. bicolor* and *Min. medius.*

(2) **Boripat waterfall** (7°01'N, 100°08'E) [loc. S5, Fig. 1], located in Rattaphum district, which approximately 10 km east of Khao Rak Kiat. The area is covered by lowland evergreen forest including some mixed deciduous forest, fruit orchards and a village. Two harp traps were set in the evening from 18:00 to 20:30 h. A trap was set across a forest trail which runs along the stream side, and another was set next to a bridge that crosses over the stream. Nine species were found: *R. stheno, R. affinis, R. pusillus, R. robinsoni, H. larvatus, H. bicolor, H. cineraceus, M. horsfieldi* and *Hesperopternus blanfordi.*

(3) **Khao Tieb cave** (6°60'N, 100°17'E) [loc. S6, Fig. 1], situated in a small limestone outcrop which was surrounded by rubber plantation, approximately

10 km west of Hat Yai. The cave was an underground cave with the entrance measured 1.5 m by 1 m wide. The cave had one major chamber which measured approximately 15 m deep by 4 m wide and 3 m high. Bats were captured by a hand net in the cave. Four species of bat were found in this cave: *R. stheno, R. affinis, R. lepidus* and *Min. pusillus.*

(4) Ma Kling waterfall (7°02'N, 100°12'E) [loc. M45, Fig. 2] set in a limestone area. Two harp traps were set across forest trails and a mist net in the transition zone between mixed deciduous forest and a rubber plantation. Eight species were captured: *Cynopterus sphinx, C. brachyotis, Megaerops ecaudatus, R. malayanus, R. stheno, R. robinsoni, H. larvatus* and *K. hardwickii.*

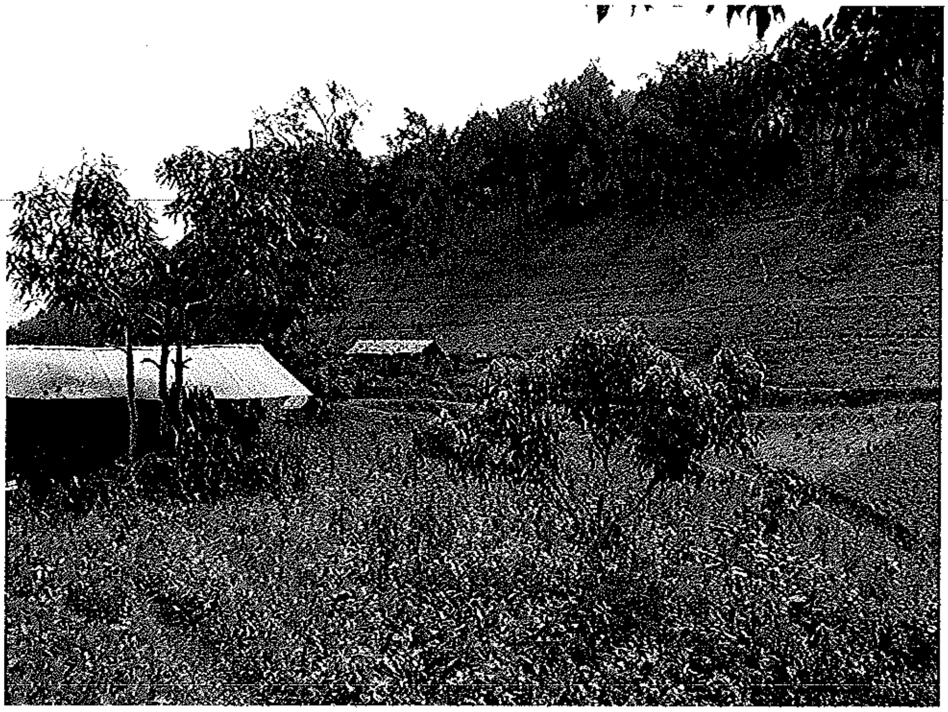

Fig. 3. Agricultural area which surrounds an isolated limestone outcrop in Chiang Mai [loc. G5, Fig 1].

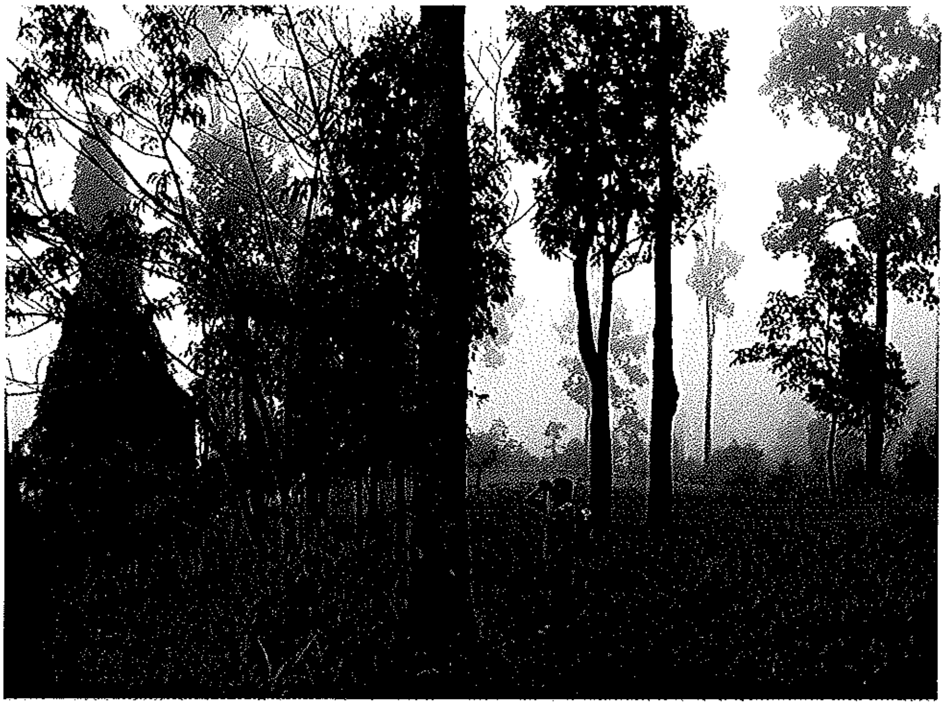

Fig. 4. Hill evergreen forest in Phu Suan Sai NP, Loei [loc. G8, Fig. 1].

Fig. 5. Khao Samorkhon limestone outcrop which surrounded by paddy fields in Lop Buri [loc. M27, Fig 2].

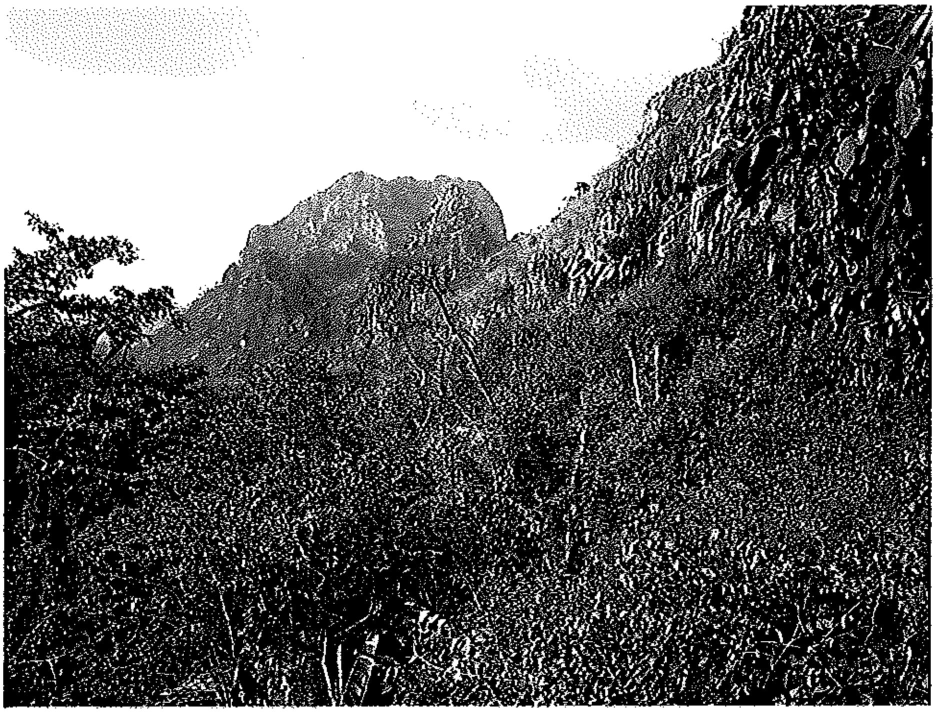

Fig. 6. Series of limestone mountain in the central plain of Thailand [loc. M29, Fig 2].

Fig. 7. Dwarf-deciduous dipterocarp forest on sandstone mountain of Pha Taem National Park, Ubon Ratchathani [loc. M32, Fig. 2].

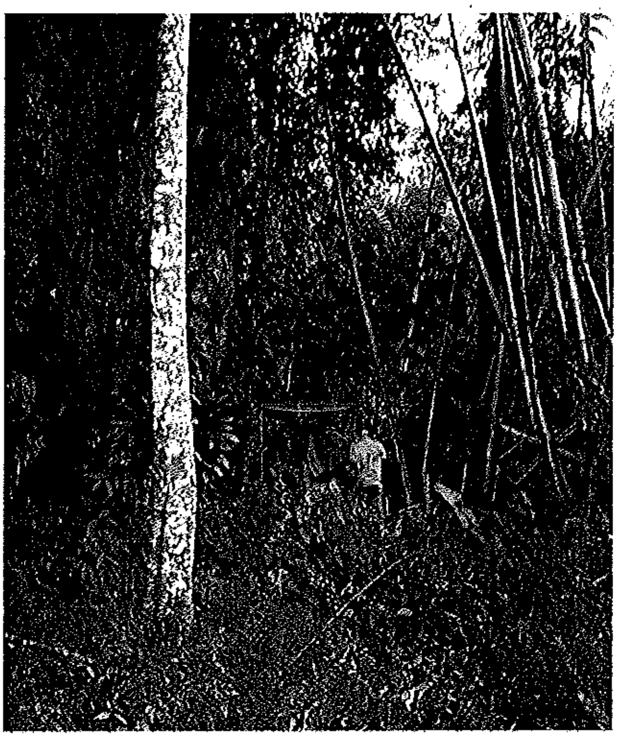

Fig. 8. Limestone outcrop which surrounded by mixed deciduous forest in Ranong, peninsula Thailand [loc. S2, Fig. 1 and loc. M39, Fig. 2].

3.2 Data Collections

3.2.1 Field data recording

The field data recording of all specimens and site description are needed for taxonomic studies. All field data of this study was recorded in strong field notebooks. The vital informations recorded included:

Field number: the identity code of specimens which prefixed with letters referred to specimen collector. The following numbers date and order of collection. These field numbers were recorded both in the field notebook and on the field label (Fig. 9) which attached to the right hind foot of the specimens.

Example of field number in this study; the first specimen collected by Pipat Soisook on 20 October, 2006 were '**PS061020.1**'.

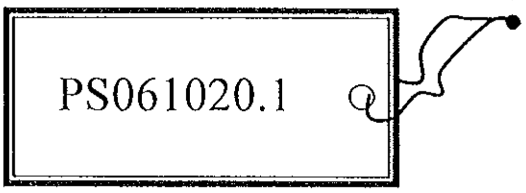

Fig. 9. Field number written in pencil on a field label made of 2.5 x 4.5 cm. durable card and has a thin thread for attaching to the right hind foot of the specimen.

Date of collection: in 'dd.mm.yyyy' system, e. g. 20.10.2006.

Locality: the nearest point of reference, e. g. village, stream, or hill where the field surveys was taken place, district, province, country, elevation and geographical co-ordinates in degrees and minutes system.

Habitat description: a description for each trapping site was noted in the field notebook. The description included, e. g. trapping across forest trail or stream, the type of vegetation or non-vegetation. And if the trapping sites were caves, coexisting bats and habitat surrounding the caves were described.

Sex, reproductive status and age of specimens: male (♂) or female (♀), their reproductive status and adult or juvenile (see specimens examination below).

Provisional species identification: species of bat identified in the field; *Rhinolophus malayanus* or *Rhinolophus stheno* were provisionally identified based on previous studies (Bates *et. al.* (2004): Corbet and Hill (1992): Lekagul and McNeely (1988).

External measurements: all of external morphological characteristics were measured from fresh specimens after sacrificed (see specimens examination for definitions of measurements).

Call files: echolocation calls of bats were recorded as digital files (see call recording below), and then the numbers file names were noted in field notebook, e. g. AUDIO012. Each file was renamed again to the same as the field number of each specimen when calls were analyzed.

Photographs: identity numbers of photograph taken from each live specimen were recorded in field notebooks, e. g. DSC3403.

3.2.2 Trapping Methods

Trapping or bats capturing is an important part of taxonomic study. Lack of data may sometimes because of choosing a wrong method. Capture of bats requires experience, skill and knowledge of biology of bats. There are so many trapping methods for bats, which some methods are more suitable than others in a typical situation, thus several methods can be compiled to capture bat.

Some of trapping methods were used to collect bat specimen in this study. Generally, they are the most commonly used methods in capturing bat. Bat specimens were collected from study sites as described above using these following equipments:

Harp traps

Harp traps are effectively device or capturing method to capture bats flying in a narrow flyway, e. g. forest trails, cave entrance or flyways near cave entrance (Francis, 1989). These harp traps work on the principle that lines could not be easily detected by echolocating bats (Kunz and Kurta, 1990). Harp traps were set mostly at cave entrances and forest trails nearby or in the limestone areas.

These harp traps is 1.5 m wide and 2.2 m high, and have four frames of vertical monofilament fishing lines. The distance between frames is 7.5 cm and lines are 2.5 cm apart. Bats are stopped by these frames of lines and slide down along the line to a large bag beneath the trap. The tension of lines is an important factor influence the capture success, the tension should be appropriated to flight speed of bats which are the target species. If several bats bounce off, the line tension should be reduced. But if bats pass through all of four frames, then the tension of lines should be increased. The tension of the lines of our harp traps can be adjusted by stretching or reducing the length of the trap poles.

Harp traps were usually set at cave entrances in the evening and across forest trail (Fig. 10) and were checked once an hour. Captured bats were carefully removed from harp trap by hand. Bats were held from the back by the elbows and then were placed into the 17 x 25 cm soft cloth bag individually.

Mist Nets

Mist nets are the most widely used to captured flying bats, especially in the tropics. This kind of device is easily transported because of their lightweight, compact and the most advantage of mist net is cheapness. Mist nets that are the most commonly used generally, and which were used in this study have mesh size of 1.5 inches (36 mm) and have four shelves. Nylon mist nets were 6 and 9 m long and 2.6 m high and accompanied with the aluminum tube poles were used to capture bats in this study.

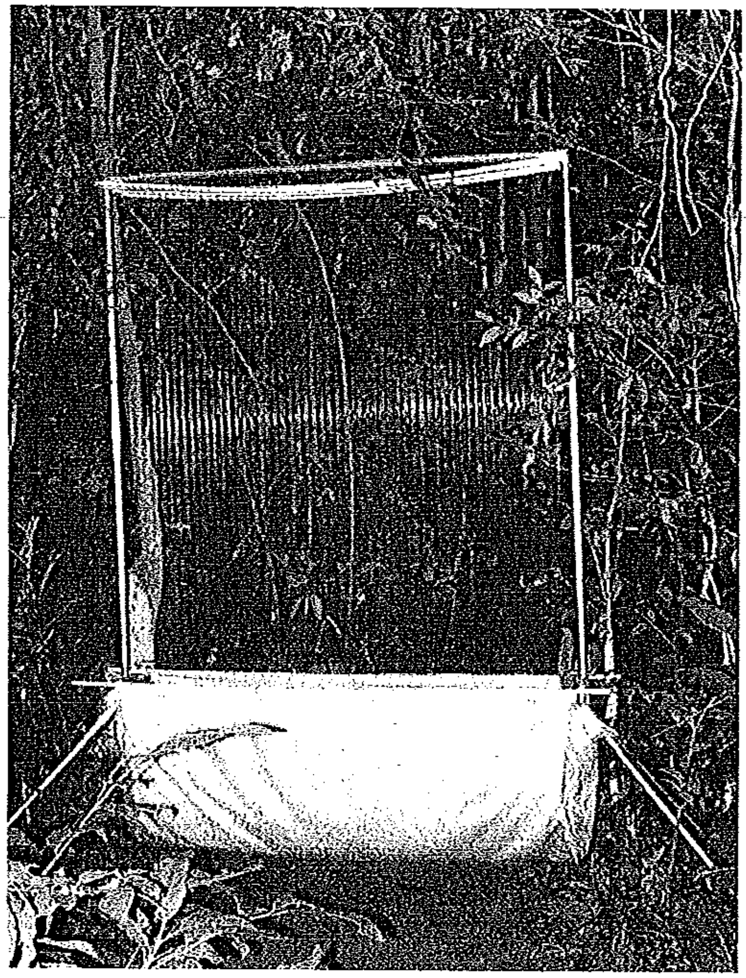

Fig. 10. A harp trap set across a forest trail- the flyway of bats. The spaces between harp trap and the edge of the trail were closed by a few branches of tree.

The nets were placed mostly near caves; over streams, across large trails and sometimes were set together with the harp trap. Nets were checked every 15 minutes from 1830 h until 2300 h. In case of cave entrance which has a large opening and harp trap cannot close these openings, mist nets also were used. Bats entangled in the mist nets were gently removed from the nets and transferred into the 17 x 25 cm soft cloth bag individually.

Hand Nets

Hand nets are most commonly used to capture roosting bats and also provided high successful in capturing flying bats from small openings and caves. Hand nets with adjustable angle of the hoop and length of pole were used in this study. It was made of heavy duty black wire with some pieces of aluminum tube poles. It was 60 cm in diameters of the hoop and 100 cm deep, which can prevent bats from escaping. Using hand nets to capture flying bats was taken with special care to avoid the damage or injury to captured bats.

3.2.3 Echolocation Calls Recording

There are several types of ultrasound detectors used in bat acoustic study, e. g. heterodyne, frequency division and time expansion. The bat detector commonly used for species identification is the time expansion system which most call characters are retained.

The target species of this study, *Rhinolophus malayanus* and *R. stheno* emit long narrow band constant frequency call (CF). To avoid the variation of call frequency from different recording conditions, calls of *R. malayanus* and *R. stheno* were recorded in hand held (resting frequency). There were, however, some populations that the calls were also recorded in another situation; from hand-released bat, to compare the difference of call frequencies.

Before recording calls, the bat detector and recorder were prepared. After finish recording the call, sound files were transferred to notebook computer to be analysed with BatSound Pro 3.31 (Pettersson Elektronik AB).

Bat detector and recorder preparation

The Pettersson D-240X time expansion bat detector was used in this study. The batteries were checked for day by day of each trip. The bat detector was set to the mode of time expansion and headphones were set; left ear set to listen to heterodyne, right ear set to listen to time expansion. The relay output was set to 10 times expansion and the trigger of start/stop recording was set to manual mode.

The recorder which was used in this study was the digital iRiver iHP-120 Multi-Codec Jukebox which can record sound as WAV file which were ready for sound analysis. The bat detector was connected to the recorder by line in port.

Call recording

The echolocation calls of each *R. malayanus* and *R. stheno* were recorded in hand held. The start/stop button was pressed and red light was "on" that meant the bat detector was recording. When a series of the call in heterodyne system was heard through headphones, the start/stop was pressed again then the output sound from time expansion system was played back and audible sound was heard. This play back sound was recorded and saved to the digital recorder, and then, the identity of call files were noted in the field notebook.

3.2.4 Specimens Preparation and Storage

Specimens from each site provide valuable data on the geographical distribution and intraspecific variation. A number of captured bats were prepared as fluid or wet specimens and skull specimens. They were deposited at the Princess Maha Chakri Sirindhorn Natural History Museum where the specimens were maintained, catalogued and available for further studies.

Sacrifice bats

Sacrifice bats for specimen collection is an important part and this process should be as fast as possible. One thing should be aware that do not leave the bats too long. In this study, a peace of cotton soaked with chloroform in a jar was used for killing bats. Bat was placed in the jar and left for a few minutes. After ensured that the bat was aethesized, then it was taken out from the jar for external measurements (see below).

Wet specimens

After bats were aethesized by chloroform, body mass and external morphological measurements of specimens were taken. The abdomens of a series of bats were opened to collect the liver for further DNA analysis, and allow better fixation. Wet specimens were preserved in 70% alcohol in the air-tight jars that stored in the references room of the Princess Maha Chakri Sirindhorn Natural History Museum. The size of these jars is as same as the coffee jars and they were not overfilled with specimens because crowding can damage the noseleaves of horseshoe bats.

Skull preparation

Skull extraction and cleaning require special materials and skills from collector, as well as time and labor consuming. However, skull specimens provide a valuable data for taxonomic study, especially for the cryptic species which hardly distinguishable by only external characters.

According to Bates (in litt.), skull extraction are described below.

Skull extraction

Skull extraction needs a set of dissection tools which included forceps, small blunt scalpel and small sharp scissors. The steps of extraction are followed;

- A cut were made by a scissors from the corner of the mouth on one cheek to enlarge the hole through which the skull will be extracted.

- Using a combination of dissection tools as above, the facial skin was peeled back from the mandible and rostrum.

- When cutting the skin free from the nasal bones, it was important to avoid damaging of noseleaf.

- The skin at the part of zygomatic arches was peeled carefully.

- Care was taken when cutting around the eyelids to ensure that the skin did not have unnaturally large opening.

- To free the ears, the auditory meatus was cut on each side of the skull, at a point as near the surface of the skull as possible to avoid making an unnecessary hole in the head skin.

- When cutting the skull free, it was important to cut the upper cervical spine rather than risk cutting the occipital part of the skull.

- A temporary skull label with the field number which as same as the field label, and linked to the wet specimen was immediately attached to the skull to ensure that there was no confusion between skulls.

Skull cleaning – manual dissection

Skulls were cleaned both by hand and using the dermestid beetles (*Dermestes maculatus*). Firstly, the manual cleaning steps are followed;

- The skull with their temporary labels were suspended in water that was gently brought to the boil (instant immersion in boiling water may cause the skull to crack)

- These medium skulls of *R. malayanus* and *R. stheno* were left in the simmering water for 20 minutes. The length of time varies slightly on the method of preservation and number of years that the specimen has been preserved.

- The skulls were left to cool down in the air and then stored temporarily in cold water for half a day before they were cleaned.

- The skulls were cleaned under a stereo microscope using a set of dissection tools as above.

- The tongue was removed by cutting around the inside of the mandible and extracted with pair of forceps.

- The mandible was teased away from the braincase carefully.

- The muscle on the braincase and all parts of the skull were removed.

- The brain was removed through the foramen magnum using cotton wool and forceps.

After these steps, the skulls were almost completely cleaned. The final cleaning was made by using dermestid beetles.

Skull cleaning – Dermestid beetles

A colony of *Dermestes maculatus* DeGeer, 1774 used in this study was maintained in the plastic box in the laboratory. The almost cleaned skulls with their temporary labels were placed into the dermestid beetles box for a day. Then the skulls were taken out, removed the beetles off the skulls and then soaked the skulls again in warm water for final cleaning and ensure that no eggs or larvae of the beetles were left on the skulls.

Skull storage

The cleaned skulls were immediately attached to their skull labels. The label thread was pass either side of the posterior constriction, and it was tied that allowed to ride easily up and down. The mandible was tied on to the other end of the thread, so the label, cranium and mandible were all attached to each other. Then, the skulls were kept in a small plastic pot which contained with cotton wool to avoid cracking damage during transportation.

Wet specimens and skull labeling

The 2.5 x 7.5 cm durable but thin cards were used as wet specimens and skull labels in this study. Each label had a thin polyester thread for attaching to the right foot of each wet specimen, and attaching to the mandible and cranium of each skull. The data written on the wet specimens labels were similar to the label of skulls (fig. 11.). All these informations were made in a neat handwriting by 2B pencil on both sides of the labels. The following informations were written on the label:

On the front side of label included;

- Museum collection number, which is referred to the specimen database.
- Species name.
- Sex.
- Frequency of call.
- Locality in full.
- Geographical co-ordinates.

On the back side of the label included;

- External measurements and weight.
- Notes on habitat, elevation, behaviour
- Original field number.
- Collector(s) – if known.

PSUZC-MM.06.14	
Rhinolophus malayanus sex♀ freq. 87	
16.06.2006 Chet Kot Cave, Ma Nang district,	
Satun Province, S. Thailand	
07° 05.415'N, 099° 54.586' E. Alt. 50 m	

FA 41.32	Forest trail lead to a limestone
HB 37.00	cave.
T 22.07	
HF 8.11	Field No. SB060616.18
W 5.3 g.	Coll. P. Soisook

Fig. 11. Example of specimen labels in this study. Front of the specimen label (above), and back of the specimen label (below).

Museum collection number

Each bat specimen in Princess Maha Chakri Sirindhorn Natural History Museum has its own unique collection number to ensure that the specimens can be accessed through the database by other researchers or person interested. In addition, these collection numbers also link to other parts of the original specimen, e. g. body and skull, so that parts can be referred to in the publication. Specimens of the Princess Maha Chakri Sirindhorn Natural History Museum have a coding system that links the particular specimens with their order and year of registration to the museum, e. g. PSUZC-MM06.14, which means that the 14th specimen registered in the year 2006 in the mammal section of the Princess Maha Chakri Sirindhorn Natural History Museum.

3.2.5 Specimens Examination

Determining Reproductive Condition

Age Determination

There are many criteria to determining age of bats. Practically, the main qualitative criteria which were used in this study were the characteristics of bones. Age of bat can be estimated by pattern of closure of the cartilaginous epiphyseal growth plates in the bones. Juvenile bats can be distinguished from adults by the clearly presence of cartilaginous epiphyseal plates in finger bones. These plates can be easily seen when bat's wing is transiluminated; the cartilaginous zone which allow more light to pass through appear lighter than ossified parts of bones. If these cartilaginous plates were not clearly visible, the shapes of the finger joints can be used. Shapes of the finger joints of young bats appear knobby and more tapered than adults. These criteria are easy to use in the field and in laboratory with require no sophisticated equipment (Anthony, 1990).

Sex Determination

Sex of bats can be determined by the presence of an enlargement of testis and the conspicuous penis in male bats, whereas females have a single pair of mammary glands and nipples in subaxillary (Racey, 1990). In males *Rhinolophus*, the penes are erected and could be clearly seen. Females; *Nulliparous* females can be identified by the small nipples and concealed with fur, whereas in *Parous* females, nipples are enlarged and usually flattened or surrounded with relative hairless skin. Late pregnancy of *Pregnant* females shows a distended abdomen and swollen mammary glands. This state may be confused with the recently fed bats which stomach may remain inflated for several hours. *Lactating* females, the mammary are much swollen and surround by large hairless areas (Borissenko and Kruskop, 2003). All mature female *Rhinolophus* has pubic or false nipples which sometimes may be confused with penis of male and are not associated with mammary glands but appear to function for the attachment of young.

Weighing and Measurements

Weighing

Weight of bats is an useful indicator of individual condition, such as maturity and reproductive state. In some cases or conditions, it can also be used for discrimination of related species (Borissenko and Kruskop, 2003).

There are many procedures to taking weight, such as using small spring balances or electronic balances with the precision of 0.1-0.5 g. In this study a

50 g. Pesola spring balances was used with the precision of 0.5 g. Live bats were aethesized by chloroform before weighing (and also other external measurement, see below). Importantly, balances must be calibrated before weighing.

Measurements

External, cranial and dental measurements and description are very important information which usefully used to identify species of bats based on qualitative characters. Measurements in this study were taken with FAITHFULL's digital vernier caliper to the nearest 0.01 mm after bats were sacrificed by chloroform. All of measurements data were entered to the morphometrics for data analysis.

A variety of external, cranial and dental measurements (applied from Bates and Harrison (1997) and Csorba *et al.*, (2003) in this study are including:

External Measurements

HB: head and body length – from the tip of the snout to the base of the tail, dorsally (Fig. 12).

T: tail length – from the tip of tail to its base adjacent to the body (Fig. 12).

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.

TIB: length of tibia – from the knee joint to the ankle (Fig. 12).

FA: forearm – from the extremity of the elbow to extremity of the carpus with the wing folded (Fig. 12).

3mt (MET): third metacarpal – from the extremity of the carpus to the distal extremity of the metacarpal (Fig. 12).

4mt (MET); 5mt (MET): as above but for the fourth and fifth metacarpals respectively (Fig. 12).

1ph3mt: first phalanx of the third metacarpal – taken from the proximal to the distal extremity of the phalanx (Fig. 12).

2ph3mt: second phalanx of the third metacarpal – taken from the proximal to the distal extremity of the phalanx (Fig. 12).

E: ear – from the lower border of the external auditory meatus to the tip of the pinna, not including any tuft of hair (Fig. 13).

NL: noseleaf width – taken from outer border of each end of noseleaf (Fig. 14).

Cranial and Dental measurements

GTL: greatest length of skull - the greatest antero-posterior diameter of the skull, taken from the occiput to the most projecting point of the premaxilla (Fig. 15, 16).

SL: skull length – taken from the occiput to the anterior of canine (usefully for the skull which the premaxilla was broken) (Fig. 16).

CBL: condylo-basal length – from an exoccipital condyle to the alveolus of the anterior incisor (Fig. 16).

CCL: condylo-canine length – from the exoccipital condyle to the anterior alveolus of the canine (Fig. 16).

ZB: zygomatic breadth – the greatest width of the skull across the zygomatic arches, regardless of where this point is situated on the arches (Fig. 17).

MB: mastoid breadth – the greatest distance across the mastoid region (Fig. 16).

BB: breadth of braincase – greatest width of the braincase at the posterior roots of the zygomatic arches (Fig. 17).

PC: postorbital constriction – the narrowest width across the constriction posterior to the orbits (Fig. 17).

M: mandible length – from the most posterior part of the condyle to the most anterior part of the mandible, including the lower incisors (Fig. 15).

C-M^3: maxillary toothrow – from the front of the upper canine to the back of the crown of the last upper molar (Fig. 15).

C-M_3: mandibular toothrow – from the front of the lower canine to the back of the crown of the last lower molar (Fig. 15).

M^3-M^3: posterior palatal width – taken across the outer borders of the last upper molar (Fig. 16).

C^1-C^1: anterior palatal width – taken across the outer borders of the upper canine (Fig. 16).

AMSW: anterior median swelling width – from the outer borders of the anterior median swelling in dorsal view (Fig. 17).

ALSW: anterior lateral swelling width – the greatest width of the anterior lateral swelling in dorsal view (Fig. 17).

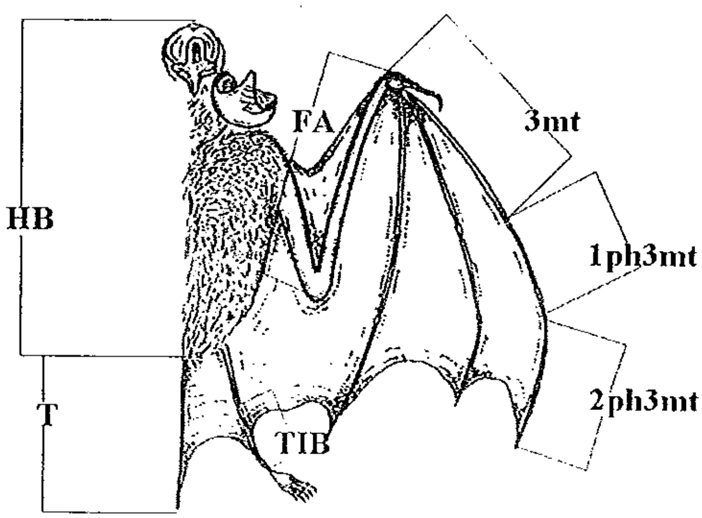

Fig. 12. Right wing and body of *Rhinolophus* showing external measurements.

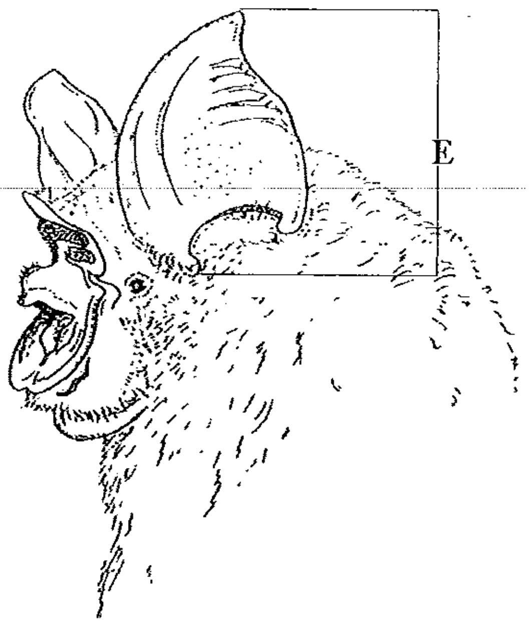

Fig. 13. Left ear and face of *Rhinolophus malayanus.*

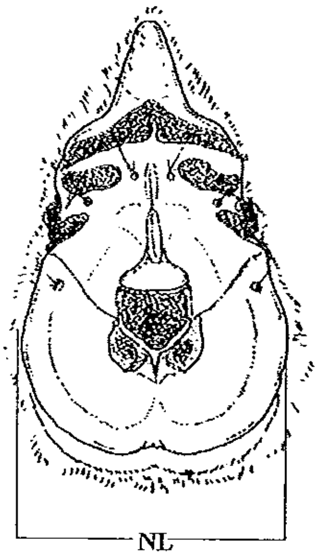

Fig. 14. Noseleaf of *Rhinolophus.*

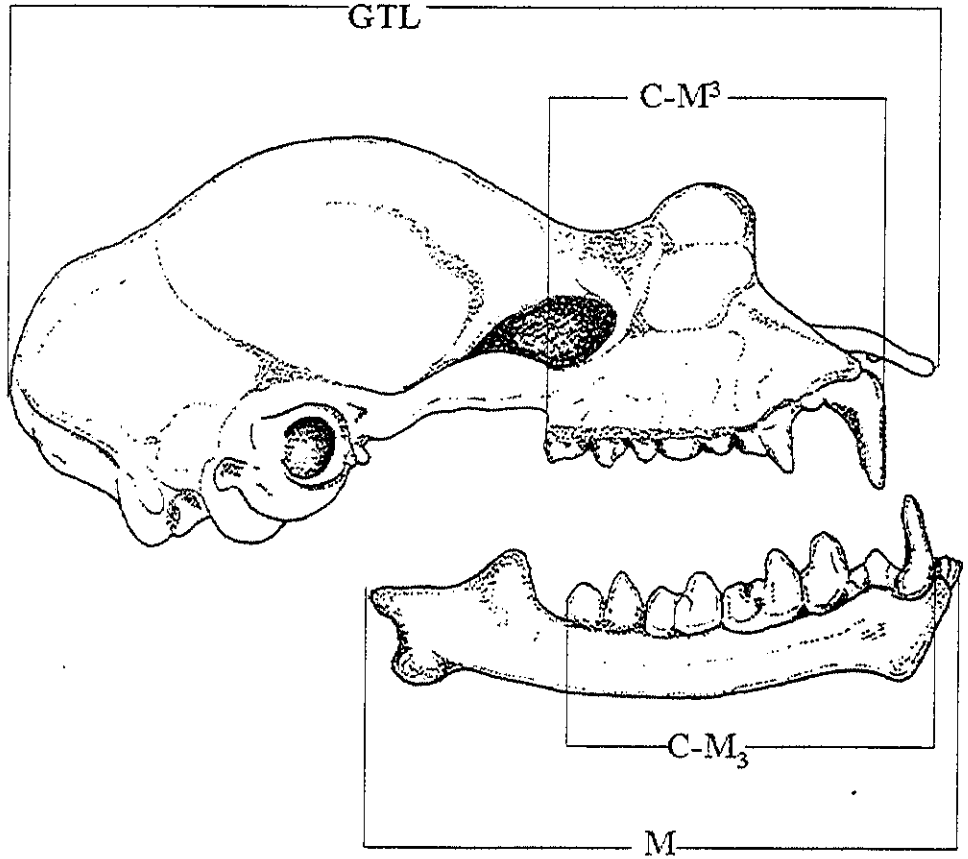

Fig. 15. Lateral view of the skull of *Rhinolophus*.

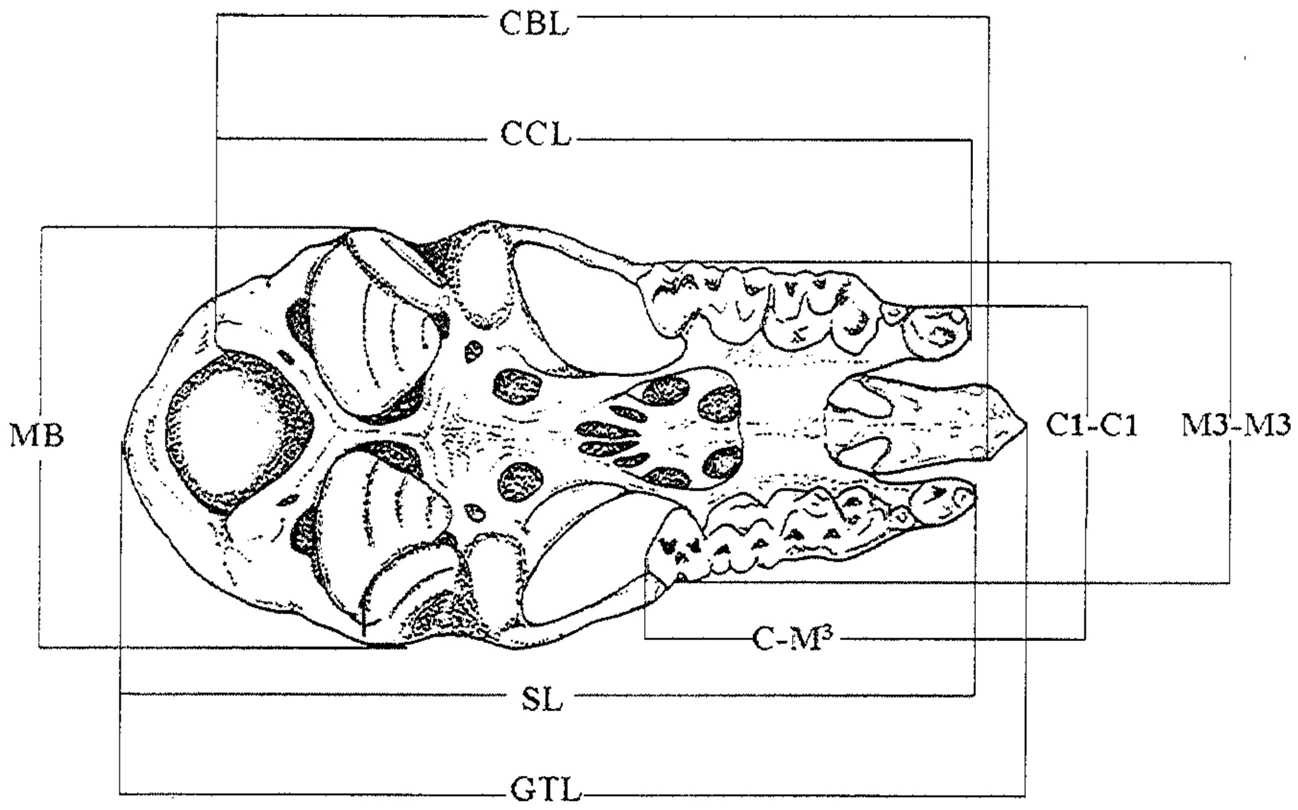

Fig. 16. Ventral view of the skull of *Rhinolophus*.

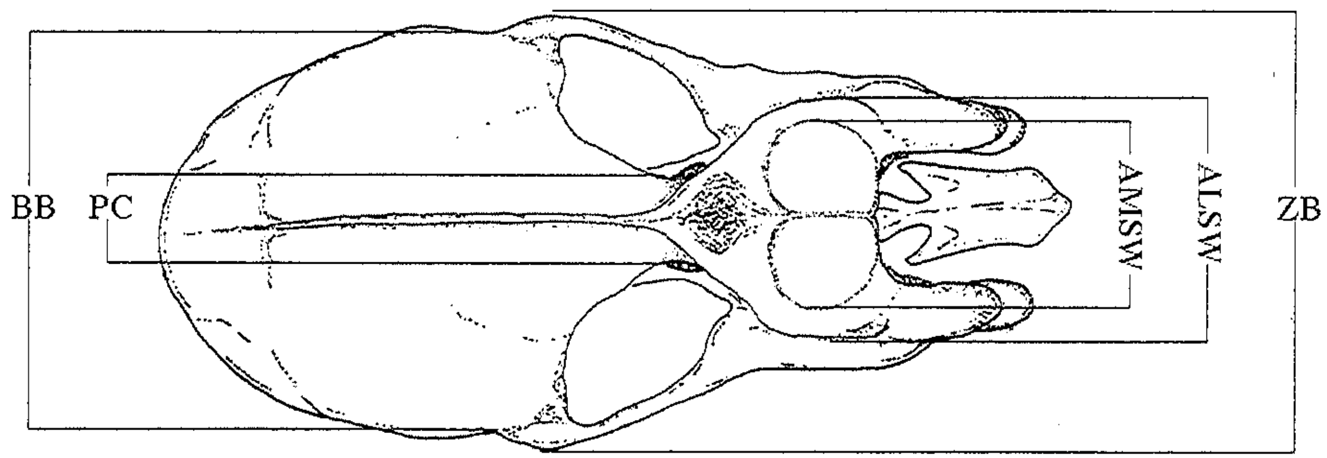

Fig. 17. Dorsal view of the skull of *Rhinolophus*.

3.3 Data Analysis

3.3.1 Call Analysis

The digital sound files of echolocation calls recorded by iRiver iHP-120 were transferred to a notebook computer by the USB port. Then the program BatSound Pro-Sound Analysis version 3.31 (Pettersson Elektronik AB) was opened. The sound format was set as stereo, 16 bits and 44100 samples per second with 10 time expansion. The spectrogram file was set using a Hanning window for the Fast Fourier Transformation (FFT). Each file of each specimen was opened and analyzed one by one. The only one factor; peak frequency or the frequency of maximum energy (FmaxE) was measured from the power spectrum window (Fig. 18). These peak frequencies were noted in field notebook, specimen labels and also recorded in the database. The completely analysed calls were renamed and saved as new files which referable to the specimens, including field number, provisional species identification, sex and recording method. For example, "SB060616.18_R. malayanus_female_in hand". The CF portions of 10 calls from each individual were analysed.

The echolocation calls of *R. malayanus* were recorded from throughout Thailand (87 specimens) and Cambodia (four specimens). In addition, the echolocation data of three specimens from Myanmar were received from Sebastien Puechmaille. In *R. stheno* (*sensu* Csorba *et al.*, 2003), the echolocation calls were also recorded from throughout Thailand (66 specimens). Additional data from Myanmar (four specimens) was also received from Sebastién Puechmaille. The intraspecific and interspecific variation of echolocation call frequency were examined

3.3.2 Statistical Analysis

The morphological difference between taxon was determined by the multivariate analysis; the Mann-Whitney *U*-test was used to compare means of morphological characteristics between taxa. Statistical comparison between the frequency of calls for male and female specimens was conducted using a two way ANOVA. For intraspecific comparison, Pearson Correlation was used to investigate the relationship between call frequency and morphological characters. These tests were run in SPSS 15.0 for Windows (SPSS Inc.). The cluster analysis and the principal component analysis were run in PC-ORD for Windows version 4.17 (McCune and Mefford, 1999).

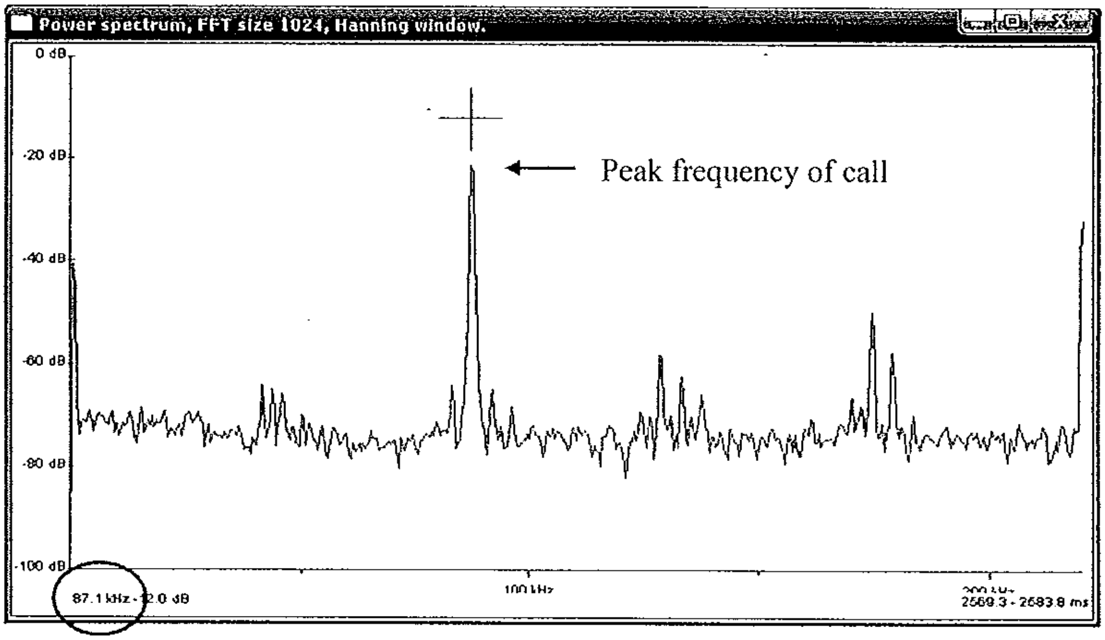

Fig. 18. The power spectrum window or spectrogram. Peak frequency can be measured by pointing cursor mark on the top of the spectrogram and the peak frequency was showed at bottom left corner.

CHAPTER 4

RESULT

4.1 Echolocation

Echolocation calls were recorded at forty-two localities in Thailand and Cambodia for three taxa, comprising six localities for *R. stheno stheno,* nine localities for *R. stheno microglobosus* and twenty-nine localities for *R. malayanus.* At ten localities, the resting frequency of males and females were compared. Despite the relatively low sample sizes, the results indicate that there is no significant variation in frequency between sexes in all three taxa ($p=0.725$). In contrast, with a very low sample size at six localities, the comparison between two different recording procedures, the resting frequency and hand releasing frequency, were also undertaken. Hand released bats were found to have slightly lower frequencies (Table 1).

In *R. stheno stheno*, there was well marked geographical variation (Fig. 19). All specimens from five localities in peninsular Thailand have a resting frequency of between 85 and 88 kHz. In *R. s. microglobosus*, north of the peninsular Thailand, the frequency was 92 to 101 kHz. There was no overlap in the frequency between the two geographically separated populations. In *R. malayanus*, there was also geographical variation in call frequency (Fig. 20). All individuals in peninsular Thailand have a resting frequency of between 83 and 91 kHz. In northern Thailand, north of latitude 16° N, all frequencies were between 73 and 80 kHz. In the zone between 12° and 16° N., two distinct populations, with two different phonic types, appeared to be present. One population has a resting frequency of 75 to 82 kHz and another of 82 to 86 kHz. Although individuals exhibiting these different frequencies were not found at the same site, they were found in populations that were in relatively proximity for example M25 (75 kHz) and M26 (84-86 kHz), which are 166 km apart. As a diagnostic character, echolocation calls cannot differentiate between *R. stheno* and *R. malayanus* (Figs 19 and 20) in peninsular Thailand. However, in Thailand north of the peninsula and potentially in southeast Myanmar and Cambodia (although data in these latter areas is still scarce), it is apparent that both phonic types of *R. malayanus* are distinguishable from *R. s. microglobosus* (*sensu* Csorba *et al.*, 2003).

4.2 Morphometrics

As with echolocation, metric data generally supported the division of *R. stheno* (*sensu* Csorba *et al.*, 2003) into two populations. Specimens from peninsular Thailand and Malaysia averaged larger in 28 of the 29 external and cranio-dental measurements as compared to *R. s. microglobosus* from north of latitude 12°N (Table 2 and 3). The exception was tibia length (Tables 2 and 4). The cluster dendrogram based on 21 external and craniodental characters (Fig. 21), and principal components analysis (PCA), based on 17 external and cranio-dental characters, also clearly supports this division (Fig. 22). Differences in size between the larger southern and smaller northern populations were also reflected in a difference in the morphology of the rostrum. All specimens of *R. stheno* in peninsular Thailand and Malaysia have well developed anterior rostral compartments, contrasting strongly with the less inflated posterior compartments (Fig. 23a). In 54 of 56 specimens examined from north of the Isthmus of Kra, the rostrum is less well developed anteriorly (Fig. 23b). The exceptions to this general rule are three specimens from two localities in central Vietnam. At Bach Ma National Park (loc. G21, S1, Fig. 1), specimens corresponding to both forms were observed. At Kon Ka Kinh (loc. S2, Fig. 1), a specimen corresponding to the peninsular form were observed. In terms of morphology, it is apparent that within the study area, the range of *R. s. stheno* is disjunct with the primary population in southern Thailand and peninsular Malaysia and a secondary, apparently isolated population in central Vietnam. Distribution of this latter population overlaps with that of *R. s. microglobosus* (*sensu* Csorba *et al.*, 2003).

As with the variation in echolocation call frequency of *R. malayanus*, the morphological characters which were significantly correlated with the call frequency are include; head and body length (HB, r=0.31, p<0.05, n=71), greatest length of skull (GTL, r=0.40, p<0.01, n=69), skull length (SL, r=0.32, n=71), mastoid width (MW, r=0.33, p<0.01, n=71), maxillary toothrow length (C-M^3, r=0.32, p<0.01, n=72) mandibular toothrow (C-M_3, r=0.31, p<0.01, n=71), mandible length (M, r=0.32, p<0.01, n=71), anterior lateral swelling width (ALSW, r=0.29, p<0.05, n=72) and anterior median swelling width (AMSW, r=0.38, p<0.01, n=72). The most significantly correlated characters are noseleaf width (NL, r=0.51, p< 0.001, n=68) and condylo-canine length (CCL, r=0.40, p<0.001, n=72) (Fig. 24). Size, both external and cranio-dental, did show some variation and larger specimens were seen at Nagamuak cave (loc. M8, Fig. 2) in southeast Myanmar, at Pha Daeng cave (loc. M16, Fig. 2) in northwest Thailand and at Lub Lae cave (loc. M24, Fig. 2) in southwest Thailand. Furthermore, there was some variation in rostral morphology, with for example, specimens from Ranong, southern Thailand (loc. M39, Fig. 2) have relatively uninflated anterior rostral chambers (Fig. 23d). However, as well as the result of the principal components analysis (PCA) (Fig. 25), acoustic, metric and morphological data are not congruent and there is no obvious geographical pattern.

In terms of interspecific variation, the data highlighted a number of morphometric characters that can distinguish *R. malayanus* from the other two taxa. The sella of *R. malayanus* is larger with a squared off, rather than rounded tip (Fig. 26). The median septum is noticeably narrower than that of *R. s. stheno* and *R. s. microglobosus* (Fig. 27, 28 and 29). In the wing, the second phalanx of the third digit is relatively short, usually less than 1.5 times the length of the first phalanx (Table 2). In *R. stheno* (*sensu* Csorba *et al.*, 2003, which includes *microglobosus*), it is equal to or exceeds 1.5. The tail of *R. malayanus* is longer than those of *stheno* and *microglobosus* but the tibia is shorter and the foot averages smaller. The skull of *R. malayanus* averages smaller than both the other taxa. It is both shorter and narrower and with shorter upper and lower toothrows. However, the postorbital constriction is distinctly broader. The rostrum has a more developed posterior compartment which leads to a less stepped rostral profile, when viewed laterally (Fig. 23).

The Mann-Whitney U test based on external and craniodental measurements supports the division among the three taxa. Highly significant differences ($p \leq 0.001$) between *R. malayanus* and *R. s. stheno*, and between *R. malayanus* and *R. s. microglobosus* were found in almost measurements. The exceptions are only condylo-canine length (CCL) and anterior lateral median swelling width (ALSW) to between *R. malayanus* and *R. s. stheno*, and noseleaf width (NL), skull length (SL) and condylo-canine length (CCL) to between *R. malayanus* and *R. s. microglobosus* (Table 4). In the comparison between *R. s. stheno* and *R. s. microglobosus*, the highly significant differences ($p \leq 0.001$) were found in forearm length (FA), noseleaf width (NL), skull length (SL), and rostrum width (ALSW, AMSW) (Table 4).

4.3 Systematic Section

The results indicate that there are at least three distinct taxa present within the study area. Specimens of *R. stheno* from south-central Vietnam and the Thai-Malay peninsula compare favourably with the holotype of *R. stheno* Andersen, 1905, which was described from western peninsular Malaysia. They are therefore referred to that species. Specimens from Thailand north of the peninsula, southeastern and eastern Myanmar, Cambodia and northern and central Vietnam agree closely with the holotype of *R. s. microglobosus* Csorba and Jenkins, 1998 (described from northern Vietnam) and are referred to that taxon. Since there are morphometric characters that are constant throughout their respective ranges that clearly discriminate between *stheno* and *microglobosus*, including in the apparent zone of overlap in central Vietnam and since, where known, there is also a significant difference in the echolocation call, the taxon *microglobosus*, previously treated as a subspecies, is here recognised as a distinct species, *R. microglobosus*.

In the case of *R. malayanus*, the taxonomic conclusions are less clear cut. In terms of echolocation, there is circumstantial evidence that there are two separate, phonic populations present in continental Southeast Asia between the latitudes of 12° and 16° N. However, as noted above, in terms of morphometrics there is no strong evidence to support this conclusion. In the absence of any supporting molecular data, it is here considered that *R. malayanus* should continue to be considered as a monotypic species, albeit one that exhibits two distinct phonic types.

All information provided below is based on data collected for this current project unless otherwise stated.

Table 1. Comparison of acoustic calls between males and females of *R. malayanus*, *R. stheno* and *R. microglobosus*. The mean, standard deviation, minimum and maximum are given. Sample sizes are in parentheses.

Species/Population	Resting frequencies (kHz)		Hand releasing frequencies (kHz)	
	male	female	male	female
R. malayanus	76.8, 76.8	78.4±1.0	-	76.9
(Nam Nao NP., Petchabun)	[2]	(77.7-79.6) (3)	-	[1]
R. malayanus	82.7±0.7	83.2±0.6	81.3±0.5	81.3±0.9
(Pha Taem NP., Ubon Ratchathani)	(82.0-83.3) (4)	(82.3-84.2) (13)	(80.7-81.7) (3)	(79.9-83.2) (12)
R. malayanus	80.4	80.4	-	-
(Phra Phothisat cave, Sara Buri)	(78.6-81.6) (7)	(80.0-80.7) (3)	-	-
R. malayanus	88.1±0.6	89.3±1.2	-	-
(Knaddai cave, Ranong)	(87.7-88.8) (5)	(87.7-90.7) (4)	-	-
R. malayanus	87.2±0.9	88±0.4	85.3±1.1	85.9±0.7
(Makling waterfall, Songkla)	(85.4-88.2) (7)	(87.5-88.3) (3)	(83.7-86.6) (6)	(85.1-86.3) (3)
R. stheno	88.1±0.2	88.2, 88.2	85.9	-
(Knaddai cave, Ranong)	(87.7-88.2) (7)	[2]	[1]	-
R. stheno	86.5±0.4	86.1±0.7	-	-
(Boripat Waterfall, Songkla)	(85.9-86.8) (4)	(85.4-86.3) (5)	-	-
R. stheno	86.3	86.3, 86.6	84.6	86.1, 86.2
(Makling waterfall, Songkla)	[1]	[2]	[1]	[2]
R. microglobosus	94.7±0.4	94.6±0.2	-	-
(Mae Ja cave, Chiang Mai)	(94.1-95.0) (5)	(94.5-95.0) (5)	-	-
R. microglobosus	100.3	98.9±1.2	-	98.8
(Phu Suan Sai NP., Loei)	[1]	(97.7-99.6) (5)	-	[1]

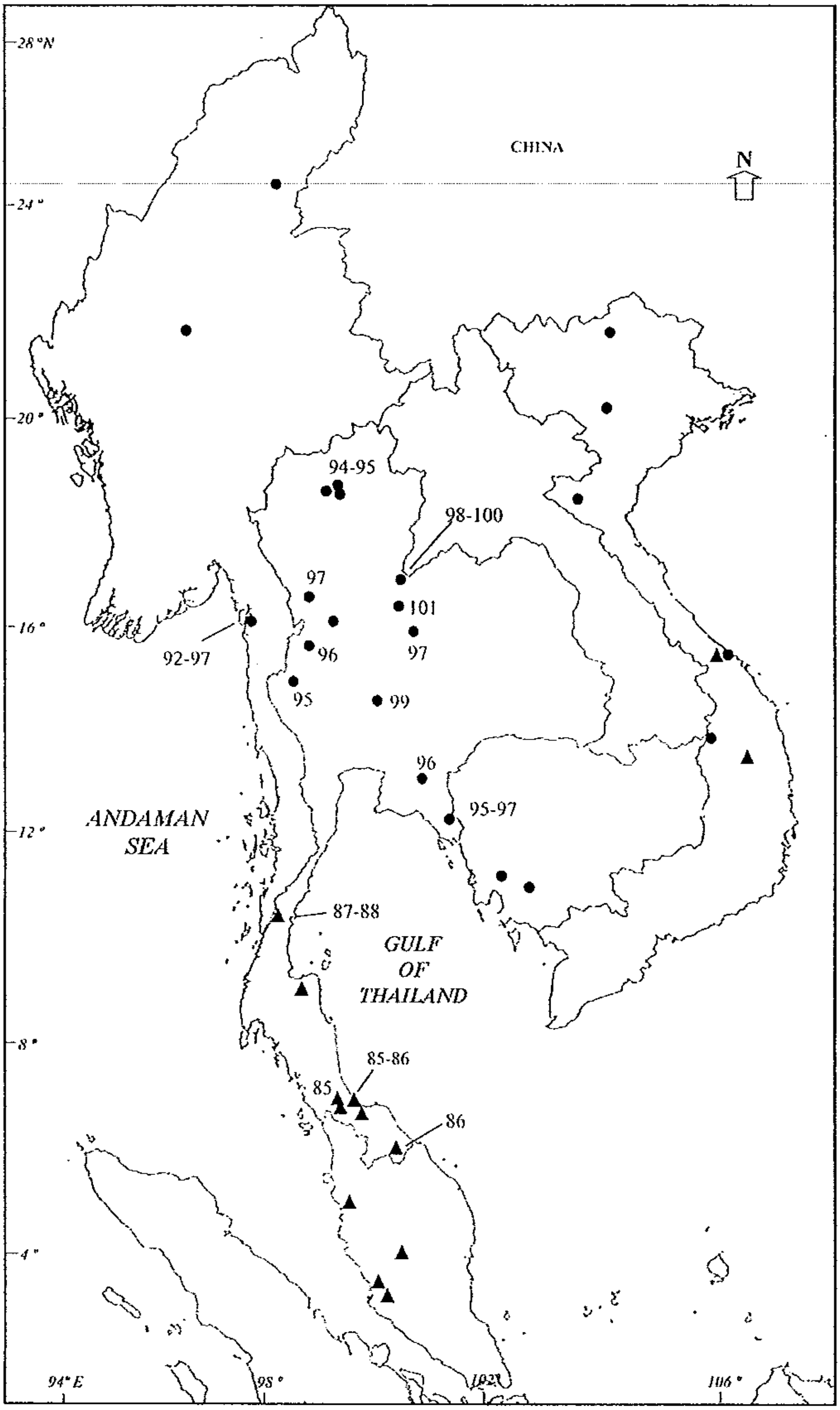

Fig. 19. Echolocation call frequencies (in kHz) of *R. microglobosus* (dots) and *R. stheno* (triangles). Locality information is included in Appendix 1.

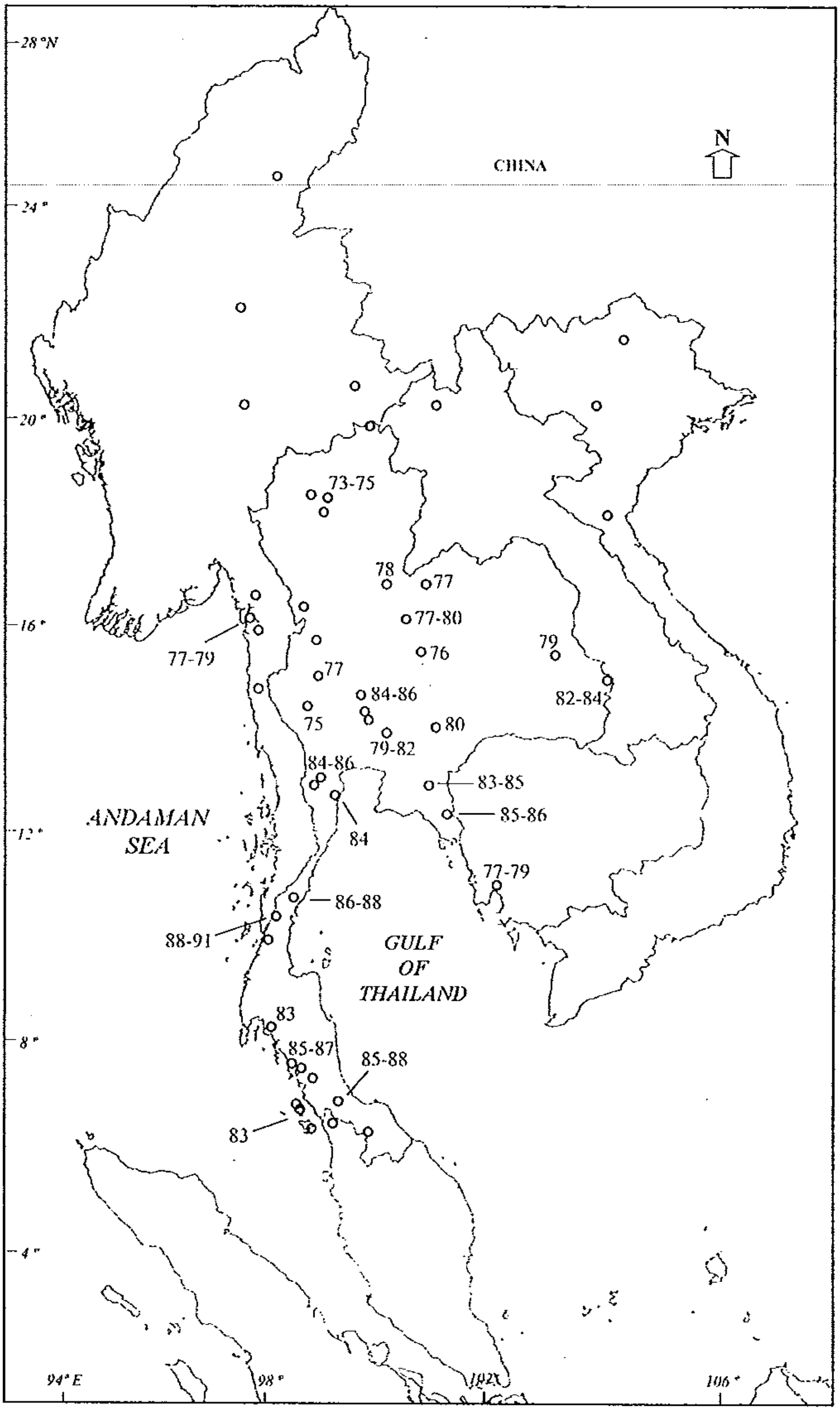

Fig. 20. Echolocation call frequencies (in kHz) of *R. malayanus*. Locality information is included in Appendix 1.

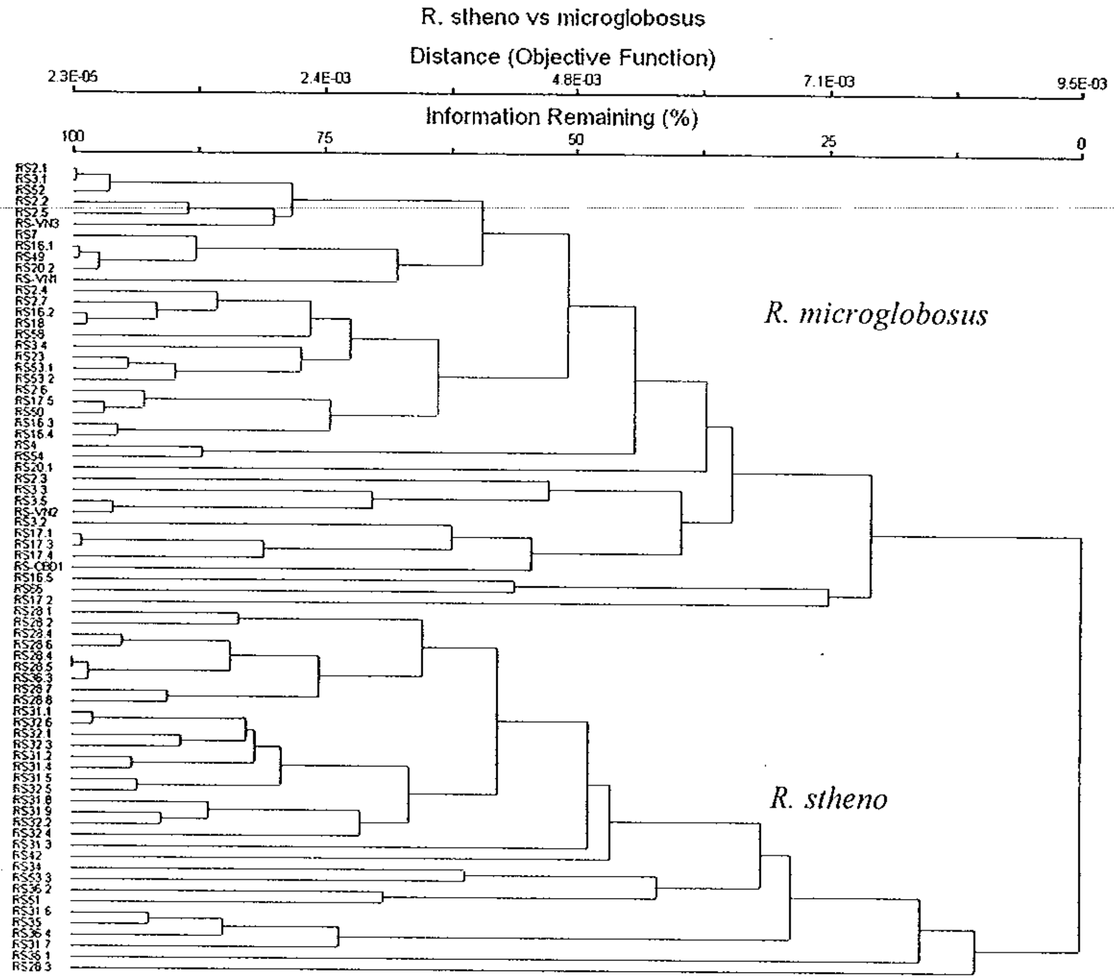

Fig. 21. Cluster dendrogram of the two taxa *R. stheno* and *R. microglobosus* based on 21 external and craniodental characters (n=73).

Rhinolophus stheno Andersen, 1905

Lesser brown horseshoe bat

R. stheno Andersen 1905a: 91; Selangor, Malaysia

Description and taxonomic notes

***External*:** This is a medium size horseshoe bat with a forearm length of 43.2-48.1 mm (Table 2). The tail is short (12.9-21.4 mm) and averages shorter than the tibia (19.8-22.7 mm). In the wing, the fifth metacarpal is subequal or slightly longer (33.3-37.5 mm) than the fourth (33.2-36.5 mm) and the third is the shortest (31.9-35.7 mm). The ratio of the first to the second phalanx of the third digit is 1.7 (1.6-1.9 mm). The wings are usually attached between 1 and 3 mm above the ankles. The ears are not greatly enlarged (Fig. 27a). The horseshoe is 7.4-9.0 mm in width and a supplementary noseleaf is clearly present. The outer ring of the horseshoe is dark in comparison to the paler area around the nostrils. The connecting process is rounded. The sella is narrow, parallel sided or slightly concave in the mid-part and with a rounded tip (Fig. 26a). The lancet is long and broadly triangular. The median septum is broad (Fig. 27b). The hairs on the upperpart of the body are greyish to reddish-brown, the underparts essentially the same but usually much paler.

***Cranial and dental*:** The skull has a condylo-canine length of 16.5-18.3 mm, averaging larger *R. microglobosus* and *R. malayanus* (Table 3, Fig. 30). The zygomatic width is equal to or only slightly exceeds the mastoid width (Table 3). The post orbital constriction is narrow, usually less than 2.0 mm (1.4-2.0 mm). The anterior median rostral chambers are distinctly elevated above the posterior ones (Fig. 23a). The small first upper premolar (P^2) is situated in the toothrow. The first (P_2) and the third (P_4) lower premolars are in contact, the second (P_3) is extruded.

Echolocation: Based on this study, the resting frequency of individuals from peninsular Thailand is between 85-88 kHz (Fig. 19). According to Kingston *et al.*, (2000), in Malaysia, it is 86 kHz.

Ecological notes: In Thailand, *R. stheno* was found in limestone karst areas, although this could reflect collecting bias. Within these areas it was collected in caves, mixed deciduous and lowland evergreen forest, agricultural areas and rubber plantations. In peninsular Malaysia, it was netted in hill forest (Kingston *et al.*, 2006). Pregnant females were collected in March in Thailand, and from March to May in Malaysia, where lactating females were found from May to July (Kingston *et al.*, 2006). In Vietnam, it co-exists with *R. microglobosus* in Bach Ma National Park in the Annamite Mountains and was found at 1700 m.a.s.l. on a mountain ridge in pristine montane forest at Kon Ka Kinh. In Thailand, it shares its roosts with a range of species (see Method section).

Distribution and conservation status: As here understood, it is restricted to peninsular Thailand; central Vietnam and peninsular Malaysia, for details of localities see Appendix 1. In addition, specimens from Sumatra and Java listed in Csorba *et al.*, (2003) are also probably referable to this species. It is listed as 'Lower Risk: least concern' by Hutson *et al.*, (2001) and Boitani *et al.* (2006) based on the taxonomic understanding of Corbet and Hill (1992).

Rhinolophus microglobosus Csorba and Jenkins, 1998

Indo-Chinese lesser brown horseshoe bat

R. stheno microglobosus Csorba and Jenkins 1998): 208; Na Hang Nature Reserve, Tuyen Quang Province, Vietnam

Diagnosis

The skull, with a condylo-canine length of 15.1-16.8 mm, is narrow, with a zygomatic breadth of 8.5-9.3 mm (Table 3). The anterior median chambers of the rostrum are narrow (3.4-4.0 mm) and elevated in comparison to the posterior chambers but not greatly inflated (Fig. 23b).

Description and taxonomic notes

External: This species generally resembles *R. stheno* but averages smaller, with a forearm length of 40.9-46.3 mm (Table 2). The tail is short (14.5-22.4 mm) and the tibia averages longer (18.5-22.8 mm). In the wing, the fifth metacarpal

(31.8-36.2 mm) is subequal with the fourth (31.8-36.0 mm) and the third is the shortest (30.3-34.7 mm). The ratio of the first to the second phalange of the third digit is 1.6 (1.5-1.8). The wings are mostly attached between 1-3 mm above the ankles or occasionally at the ankles. The ears are not greatly enlarged (Fig. 28a). The horseshoe is 6.8-8.4 mm in width, narrower than *R. stheno* and a supplementary noseleaf is clearly present. As with *R. stheno,* the outer ring of the horseshoe is dark in comparison to the paler area around the nostrils. The connecting process is rounded; the lancet tall, triangular and straight-sided. The sella is similar to that of *R. stheno* (Fig 26b). The upperparts are greyish to yellowish-brown, the underparts pale brown.

Cranial and dental: The skull, with a condylo-canine length of 15.1-16.8 mm, averages intermediate between *R. malayanus* and *R. stheno,* although the holotype of *microglobosus* is a particularly large individual (Table 3, Fig. 30). The zygomatic width is about equal or only slightly exceeds the mastoid width. The post orbital constriction is narrow (1.4-2.0 mm). The anterior median swelling width averages smaller than in *R. malayanus* and *R. stheno* (Table 3). The anterior median rostral chambers are elevated in comparison to the posterior chambers, but are less inflated than those of *R. stheno* (Fig. 23b). In consequence, the frontal depression is shallower. The small first upper premolar (P^2) is situated in the toothrow. The first (P_2) and the third (P_4) lower premolars are in contact, the second (P_3) is extruded.

Echolocation: Based on this study, a resting frequency of between 94-101 kHz was recorded from Thailand north of Isthmus of Kra (12°N.). This compares to the report of using heterodyning QMC Mini Bat Detector, 85-95 kHz from western Thailand by Robinson *et al.,* (1995). In Myanmar, frequencies of 92-97 kHz (S. Puechmaille, pers. comm.) and 101.5-101.7 kHz (Khin Mie Mie, 2004) have been recorded, and in Lao PDR, 95 kHz by C. M. Francis (Pers. comm.).

Ecological notes: In Thailand, it was collected in the areas of limestone karst and lowland to hill evergreen forest (for further details see Methods section). Previously, a colony of approximately 1200 individuals was located in western Thailand and torpid individuals were found roosting during the day in July (Robinson *et al.,* 1995). In Myanmar, it was collected from the same caves as *R. malayanus* and *A. stoliczkanus*

(Bates *et al.*, 2004). In Vietnam, it was found in an area of relatively undisturbed forest in Pu Mat (Hendrichsen *et al.*, 2001). In central Thailand, a pregnant female was collected in late February and lactating females in May.

Distribution and conservation status: As here understood, *R. microglobosus* is known from Thailand north of Isthmus of Kra (12°N.), Myanmar, south Cambodia, central Vietnam and Lao PDR (Francis *et al.*, 1999; Csorba *et al.*, 2003) and Yunnan, south-west China (Zhang *et al.*, 2005). This is the first record from Cambodia. A list of specimens is included in Appendix 1. A list of specimens is included in Appendix 1. Its conservation status has not been assessed separately by the IUCN but only in conjunction with *R. stheno.*

Rhinolophus malayanus Bonhote, 1903

Malayan horseshoe bat

R. malayanus (Bonhote 1903): 15; Biserat, Jalor, Thailand

Description and taxonomic notes

External: *R. malayanus* is a small horseshoe bat with the forearm length of 38.2-44.0 mm (Table 2). The tail (15.0-26.9 mm) averages longer than that of *R. stheno* and *R. malayanus* but the tibiae are considerably shorter (15.4-19.2 mm). The wings are usually attached at the ankles or occasionally at the base of the metatarsals. The third, fourth and fifth metacarpals are almost the same length (28.2-33.1 mm, 28.6-33.8 mm and 28.1-33.3 mm respectively). The second phalanx of the third digit is short with a ratio of 1.4 (1.2-1.6) in comparison to the second; it is relatively longer in *R. stheno* and *R. malayanus.* The ears are not greatly enlarged (Fig. 29a). The horseshoe (6.9-8.9 mm) is in intermediate in width between that of *R. stheno* and *R. microglobosus*, and the secondary noseleaf is clearly present. In contrast to *R. stheno* and *R. microglobosus*, the outer ring of the horseshoe is not dark but pale, almost similar to the area around the nostrils (Fig. 29b). The connecting process is broadly rounded. The sella is relatively wide, almost parallel sided and with the tip broadly rounded (Fig. 26c). The lancet is triangular. The pelage is uniformly brown to reddish brown, paler on the belly.

Cranial and dental: The skull, with a condylo-canine length of 14.6-16.7 mm, averages smaller than that of *R. stheno* and *R. microglobosus* (Table 3, Fig. 30). The zygomata slightly exceed the width of the mastoids and the post orbital constriction is broader than that of the other two taxa, usually exceeding 2.0 mm. The nasal swellings are well developed, with the anterior median swelling usually extending down the side of the rostrum (Fig. 23c). The small first upper premolar (P^2) is situated in the toothrow. The second lower premolar (P_3) is slightly extruded or external to the toothrow.

Echolocation: Based on the current study, the resting frequency shows considerable geographical variation. In peninsular Thailand, it was between 83 and 91 kHz (south of 12° N.); in northern Thailand, north of latitude 16° N., all frequencies are between 73 and 80 kHz and in the zone between 12° and 16° N., in Thailand and Cambodia, two distinct populations, with two different phonic types, appear to be present. One population has a resting frequency of 82 to 86 kHz and another of 75 to 82 kHz. Previously Robinson *et al.*, (1995) and Robinson (1996) had recorded 75 kHz from western Thailand. In Myanmar, 76-79 kHz were recorded by Khin Mie Mie (2004) and S. Peuchmaille (Pers. comm.) and in Lao PDR, 75 kHz by C. M. Francis (pers. comm.).

Ecological notes: In Thailand, it was mostly collected in areas of limestone karst, where it roosted in caves and in sandstone mountains. Vegetation types included lowland mixed deciduous forest, hill evergreen forest and highly disturbed dry dipterocarp forest. It was also collected in agricultural areas, including rubber plantations. Its altitudinal range was 5 to 1400 m a.s.l. In western Thailand, approximately 3000 individuals were located roosting in cave. Torpid individuals were found roosting by day in July (Robinson *et al.*, 1995). In Cambodia, pregnant individuals were found in late February in lowland secondary evergreen forest. In Vietnam, it was also collected in limestone karsts areas (V. D. Thong, Pers. comm.). In Myanmar, it was collected in limestone outcrops, agricultural land and forest

(Bates *et al.*, 2001). It was also found in heavily degraded forest and banana plantations in Lao PDR (Robinson and Webber, 1998).

Distribution and conservation status: It is known from Thailand, eastern Myanmar, southern Cambodia, northern Vietnam and the north of Malaysia (Appendix 1). It is also recorded from Lao PDR (Corbet and Hill, 1992; Csorba *et al.*, 2003; Francis *et al.*, 1999; Simmons, 2005). Considered 'Lower risk: least concern' by Hutson *et al.*, (2001) and Boitani *et al.* (2006). It was classified as 'Potentially At Risk in Lao PDR' (Francis *et al.*, 1999).

Table 2. External measurements (in mm) of *R. malayanus*, *R. stheno* and *R. microglobosus* including the length of forearm (FA); ear (E); head and body (HB); tail (TAIL); tibia (TIBIA); hind foot (HF); the fifth, fourth and third metacarpal (5MT, 4MT and 3MT); first and second phalange of the third digit (3D1P, 3D2P) and their ratio (3D1P/3D2P); noseleaf width (NL). Body mass (W) is in g. Peak frequency at maximum energy (FMaxE) in kHz. For male and female specimens, the mean, standard deviation, minimum and maximum are given. Sample sizes are in parentheses.

Sex	FA	E	HB	T	TIB	HF	NL	W	FMaxE
					Rhinolophus malayanus				
♂	41.0.1.1	16.4,1.1	46.4.4.0	21.5.2.0	17.2,0.6	7.2.0.6	7.8.0.5	6.4,0.9	82.9,4.4
	38.8-43.4(64)	12.7-19.3(64)	37.4-55.8(64)	17.7-26.0	15.4-18.6(63)	5.8-9.6(64)	6.9-9.0(51)	5.0-9.7(57)	73.1-88.8(49)
♀	40.9.1.2	16.2,0.8	45.8.4.1	21.4.2.4	16.8,0.8	7.2.0.7	7.6.0.3	5.8,0.6	84.8.3.0
	38.2-44.0(35)	14.1-17.9(35)	36.1-55.2(35)	15.0-26.9(35)	15.5-19.2(35)	6.1-9.6(35)	7.0-8.4(28)	4.5-6.8(28)	79.5-90.7(22)
					Rhinolophus stheno				
♂	45.2.1.1	17.7.1.1	51.8.3.1	17.4.1.9	21.3,0.6	9.2,0.5	8.1.0.5	7.8,0.9	86.8,1.1
	43.2-48.1(21)	15.4-19.5(21)	45.5-56.8(21)	12.9-21.4(21)	20.4-22.7(21)	8.2-9.9(21)	7.4-9.0(19)	6.9-10.0(18)	85.0-88.2(18)
♀	45.2.1.0	17.1.1.0	50.3.3.0	17.3.1.5	20.9,0.6	8.9,0.6	8.1.0.5	7.6,0.4	86.5.1.0
	43.5-46.6(14)	15.1-18.9(14)	45.7-55.4(14)	13.7-19.1(14)	19.8-21.9(14)	7.5-10.0(14)	7.4-8.7(14)	6.9-8.0(12)	85.3-88.2(12)
					Rhinolophus microglobosus				
♂	44.0.1.2	15.1,0.9	44.7.4.3	18.1.1.8	20.8.1.1	8.0.0.6	7.6.0.5	6.1.1.0	96.2, 1.4
	41.4-46.3(37)	13.2-17.3(37)	39.9-60.1(37)	14.5-22.4(37)	17.9-22.8(32)	6.0-9.0(37)	6.8-8.4(29)	5.0-9.0(23)	94.1-100.7(23)
♀	43.8.1.2	15.9,1.0	43.1.2.0	18.7.1.2	20.6.0.7	8.3.0.5	7.6,0.4	6.3,0.6	96.1.1.1
	41.7-45.5(16)	14.5-17.6(16)	39.9-46.4(16)	17.1-22.0(16)	19.4-21.8(15)	7.4-9.5(16)	7.0-8.4(12)	5.1-6.7(6)	94.5-97.4(5)

Table 2. (continued)

Sex	5Mt	4Mt	3Mt	3d1p	3d2p	3d2p/3d1p
			Rhinolophus malayanus			
♂	31.1,0.8	31.7,1.0	31.1,0.8	12.0,0.5	17.2,1.0	1.4,0.1
	29.6-32.9(63)	28.6-33.7(63)	29.2-33.0(63)	11.0-13.3(63)	14.3-19.3(63)	1.2-1.4(63)
♀	31.0,1.1	31.5,1.0	30.7,1.0	11.8,0.5	17.3,0.8	1.5, 0.1
	28.1-33.3(35)	29.4-33.8(35)	28.2-33.0(35)	10.5-12.9(35)	15.5-18.8(35)	1.4-1.6(35)
			Rhinolophus stheno			
♂	35.3,1.0	34.7,0.8	33.5,0.8	13.2,0.5	22.5,0.8	1.7, 0.1
	33.3-37.5(21)	33.2-36.3(21)	32.0-35.7(21)	12.2-14.3(21)	20.9-24.2(21)	1.6-1.9(21)
♀	35.1,0.8	34.8,0.9	33.5,0.8	13.1,0.4	21.9,0.9	1.7,0.1
	33.6-36.4(14)	33.2-36.5(14)	31.9-34.3(14)	12.3-13.6(14)	20.3-23.1(14)	1.6-1.8(14)
			Rhinolophus microglobosus			
♂	34.2,1.0	33.9,1.0	32.5,1.0	13.0,0.6	21.4,1.0	1.7, 0.1
	32.1-36.2(32)	32.4-36.0(32)	30.3-34.7(32)	12.0-14.0(32)	18.8-22.7(32)	1.5-1.8(31)
♀	33.8,1.1	33.5,0.9	32.0,1.0	12.9,0.4	21.2,1.0	1.6,0.1
	32.3-35.8(15)	32.3-35.4(15)	30.3-34.1(15)	12.4-13.6(15)	19.5-22.7(15)	1.5-1.8(15)

Table 3. Cranial and dental measurements (in mm) of *R. malayanus*, *R. stheno* and *R. microglobosus* including greatest length of skull (GTL); condylo-canine length (CCL); skull length (SL); zygomatic breadth (ZB); breadth of braincase (BB); mastoid width (MW); postorbital constriction (PC); upper toothrow length ($C-M^3$); palatal width (M^3-M^3); anterior palatal width (C^1-C^1); lower toothrow length ($C-M_3$); mandible length (M); anterior lateral swelling width (ALSW); anterior median swelling width (AMSW). For male and female specimens, the mean, standard deviation, minimum and maximum are given. Sample sizes in parentheses.

Sex	GTL	CCL	SL	ZB	BB	MW	PC
			Rhinolophus malayanus				
Holotype	17.9	15.4	17.6	8.9	7.7	8.2	2.6
♂	18.8.0.5	15.8.0.4	17.8.0.4	8.8.0.2	7.6.0.2	8.4.0.2	2.5.0.1
	17.9-20.2(57)	15.1-16.7(66)	17.1-19.0(59)	8.2-9.6(59)	6.8-8.3(60)	8.0-8.7(59)	2.1-2.8(60)
♀	18.4.0.6	15.4.0.4	17.5.0.4	8.6.0.3	7.4.0.2	8.3.20.0	2.5.0.1
	17.1-19.5(31)	14.6-16.4(46)	16.6-18.5(36)	8.1-9.2(36)	7.0-7.9(36)	7.8-8.8(35)	2.1-2.7(35)
			Rhinolophus stheno				
Holotype	19.7	16.7	19.4	9.4	8.4	9.4	1.6
♂	20.6.0.4	17.3.0.3	19.7.0.2	9.7.0.2	8.6.0.2	9.4.0.2	1.8.0.1
	19.5-21.3(21)	16.9-18.3(21)	19.3-20.4(21)	9.2-10.0(21)	8.3-8.9(21)	8.8-9.7(21)	1.6-2.0(21)
♀	20.4.0.3	17.1.0.2	19.4.0.3	9.5.0.1	8.4.0.3	9.4.0.2	1.8.0.2
	19.9-20.9(14)	16.7-17.5(14)	19.0-19.9(14)	9.3-9.8(14)	8.0-8.9(14)	9.1-9.6(14)	1.4-2.0(14)
			Rhinolophus microglobosus				
Holotype	20.0	16.8	19.4	9.0	7.9	9.1	1.7
♂	19.4.0.4	16.2.0.3	18.6.0.3	9.0.0.1	7.9.0.1	8.9.0.1	1.7.0.1
	18.5-20.1(38)	15.7-16.8(39)	18.0-19.2(38)	8.6-9.3(38)	7.5-8.2(38)	8.7-9.1(38)	1.4-2.0(38)
♀	19.2.0.4	15.9.0.3	18.3.0.3	8.8.0.2	7.8.0.1	8.8.0.2	1.7.0.1
	18.3-19.8(16)	15.1-16.7(18)	17.8-18.7(16)	8.5-9.1(16)	7.6-8.1(16)	8.6-9.1(16)	1.5-1.9(16)

Table 3. (continued)

Sex	$C\text{-}M^3$	$M^3\text{-}M^3$	$C^1\text{-}C^1$	$C\text{-}M_3$	M	ALSW	AMSW
			Rhinolophus malayanus				
Holotype	6.8	6.9	4.3	7.0	11.6	5.2	4.3
♂	6.9,0.2	6.5,0.2	4.3,0.2	7.3,0.2	12.2,0.3	5.3,0.1	4.2,0.2
	6.5-7.3(60)	5.8-7.0(59)	3.5-4.7(60)	6.6-7.9(59)	11.5-12.9(58)	5.0-5.5(60)	3.9-4.7(60)
♀	6.7,0.2	6.5,0.4	4.2,0.2	7.1,0.2	11.9,0.3	5.1,0.1	4.1,0.2
	6.3-7.1(36)	5.6-8.7(35)	3.7-4.6(33)	6.7-7.5(36)	11.1-12.8(36)	4.8-5.4(36)	3.9-4.5(36)
			Rhinolophus stheno				
Holotype	7.5	7.3	4.9	7.8	12.8	5.0	4.3
♂	7.6,0.1	7.4,0.1	5.0,0.1	8.1,0.2	13.3,0.2	5.3,0.1	4.4,0.2
	7.4-8.1(21)	7.1-7.6(21)	4.7-5.3(21)	7.8-8.3(21)	12.9-13.7(21)	5.1-5.5(21)	4.1-4.6(21)
♀	7.5,0.1	7.3,0.2	4.9,0.2	7.9,0.1	13.1,0.2	5.1,0.1	4.3,0.1
	7.2-7.7(14)	7.0-7.6(14)	4.4-5.1(14)	7.7-8.2(14)	12.8-13.5(14)	4.8-5.3(14)	4.1-4.6(14)
			Rhinolophus microglobosus				
Holotype	7.3	6.7	4.7	7.5	13.0	5.0	3.9
♂	7.1,0.1	6.9,0.2	4.5,0.1	7.5,0.1	12.3,0.2	4.9,0.1	3.8,0.1
	6.8-7.3(38)	6.6-7.1(38)	4.2-4.9(38)	7.2-7.8(38)	11.7-12.7(36)	4.7-5.3(38)	3.4-4.0(38)
♀	6.9,0.1	6.7,0.2	4.5,0.1	7.3,0.1	12.2,0.1	4.8,0.1	3.7,0.1
	6.6-7.2(16)	6.5-7.0(16)	4.3-4.8(16)	7.0-7.5(16)	12.0-12.5(16)	4.7-4.9(16)	3.5-4.0(16)

Table 4. Mann-Whithney U test for comparison of the selected external and craniodental character among three taxa; *R. malayanus*, *R. stheno* and *R. microglobosus*

Characters	Comparison between taxa		
	R. malayanus vs. *R. stheno*	*R. malayanus* vs. *R. microglobosus*	*R. stheno* vs. *R. microglobosus*
Forearm length (FA)	***	***	***
Tail length (T)	***	***	N
Tibia length (TIB)	***	***	N
Noseleaf width (NL)	***	N	***
Ratio of 1st to 2nd phalanges of 3rd digit	***	***	N
Skull length (SL)	***	N	***
Condylo-canine length (CCL)	N	N	N
Post orbital constriction (PC)	***	***	N
Anterior lateral swelling width (ALSW)	N	***	***
Anterior median swelling width (AMSW)	***	***	***

N : not significant difference at p-level ≤ 0.001

*** : significant difference at p-level ≤ 0.001

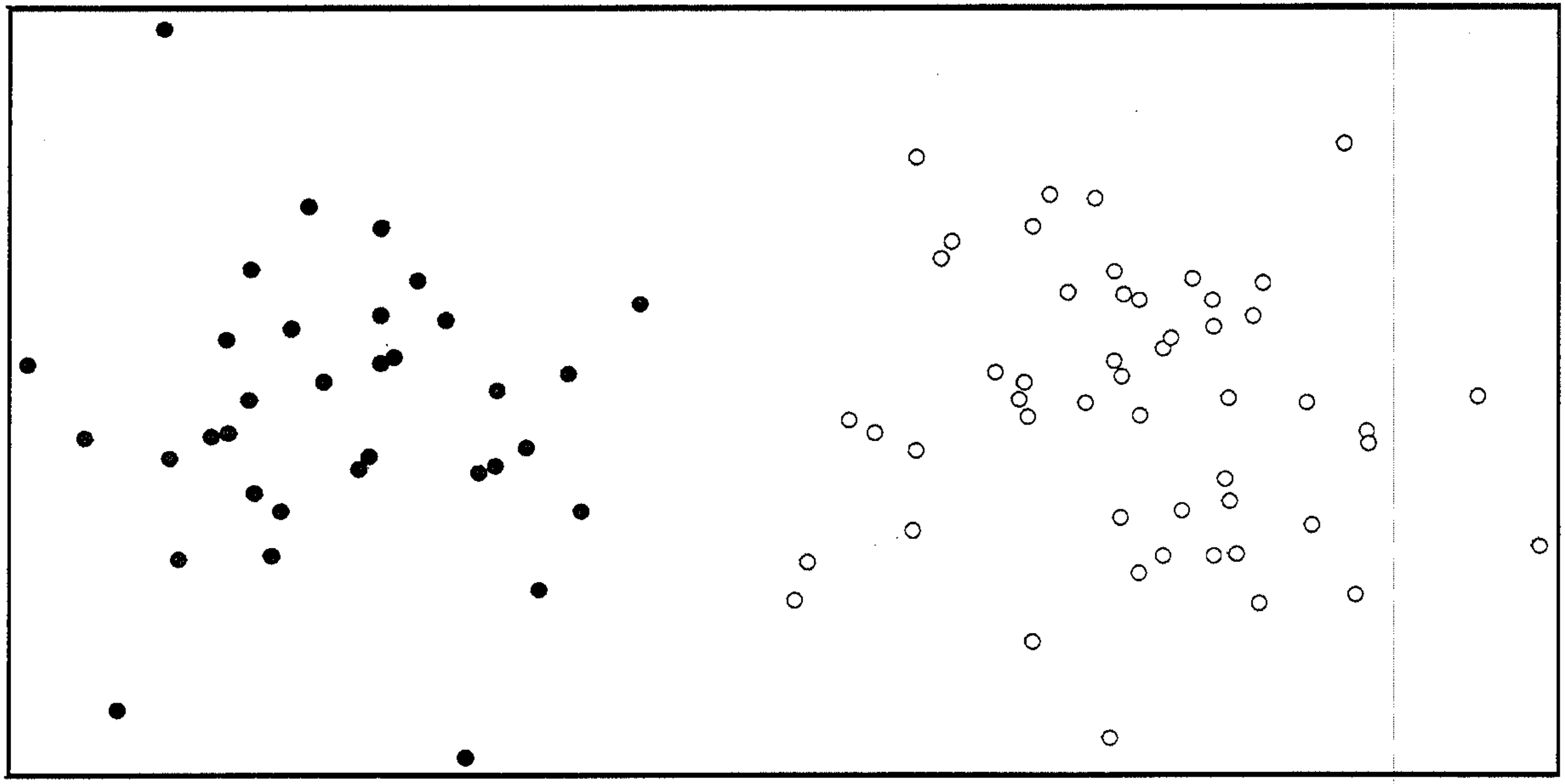

Fig. 22. Principal components analysis (PCA) of *R. stheno* (dots; n=33) and *R. microglobosus* (circles; n=51) based on 17 external and craniodental characters. (See Appendix 2 for variance explained).

a c b d

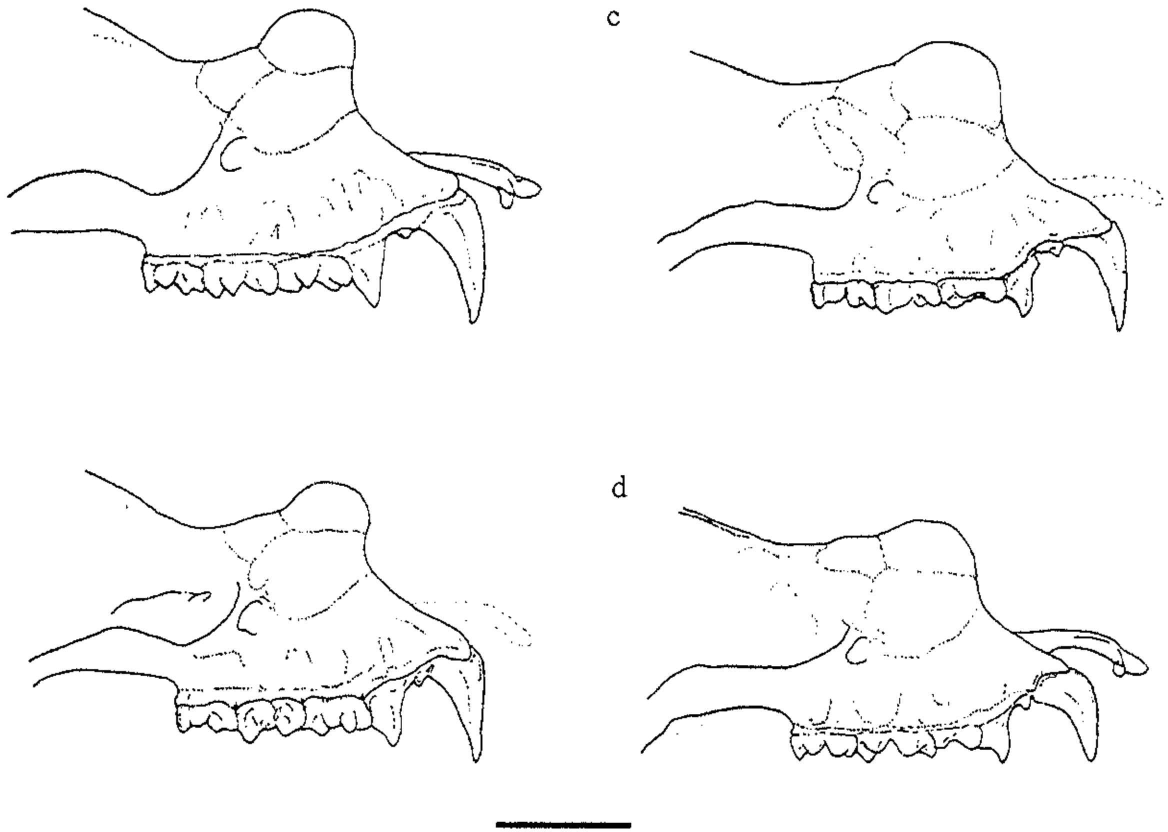

Fig. 23. Lateral view of anterior part of skulls. a: *R. stheno* (♂PSUZC-MM06.44), Khao Tieb cave, Songkla; b: *R. microglobosus* (♂PSUZC-MM06.95), Mae Ja cave, Chiang Mai; c: *R. malayanus* (♂PSUZC-MM06.90), Pha Dang cave, Chiang Mai; d: *R. malayanus* (♂PSUZC-MM07.61) from Knaddai cave, Ranong. Scale = 3 mm.

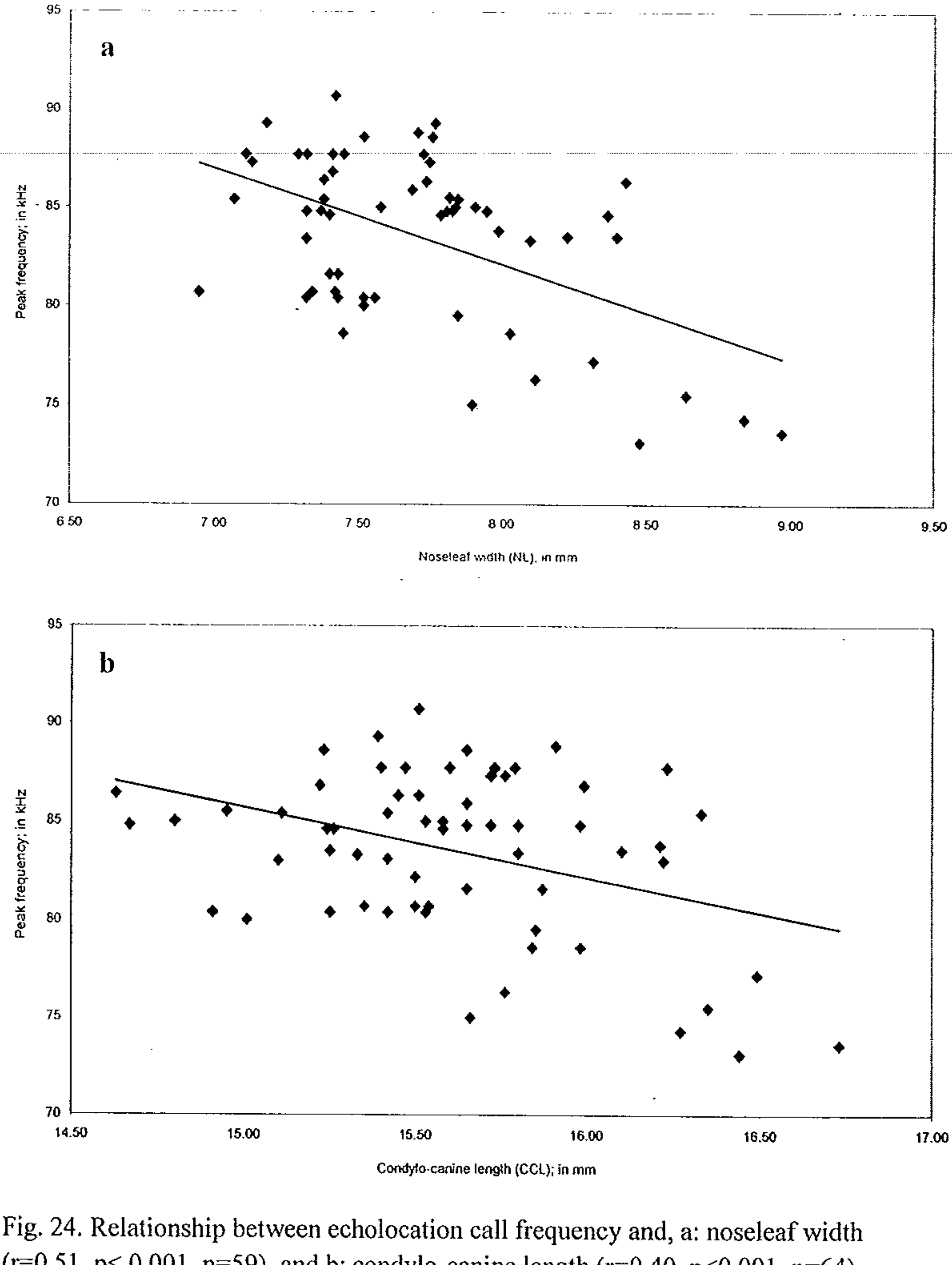

Fig. 24. Relationship between echolocation call frequency and, a: noseleaf width (r=0.51, p< 0.001, n=59), and b: condylo-canine length (r=0.40, p<0.001, n=64).

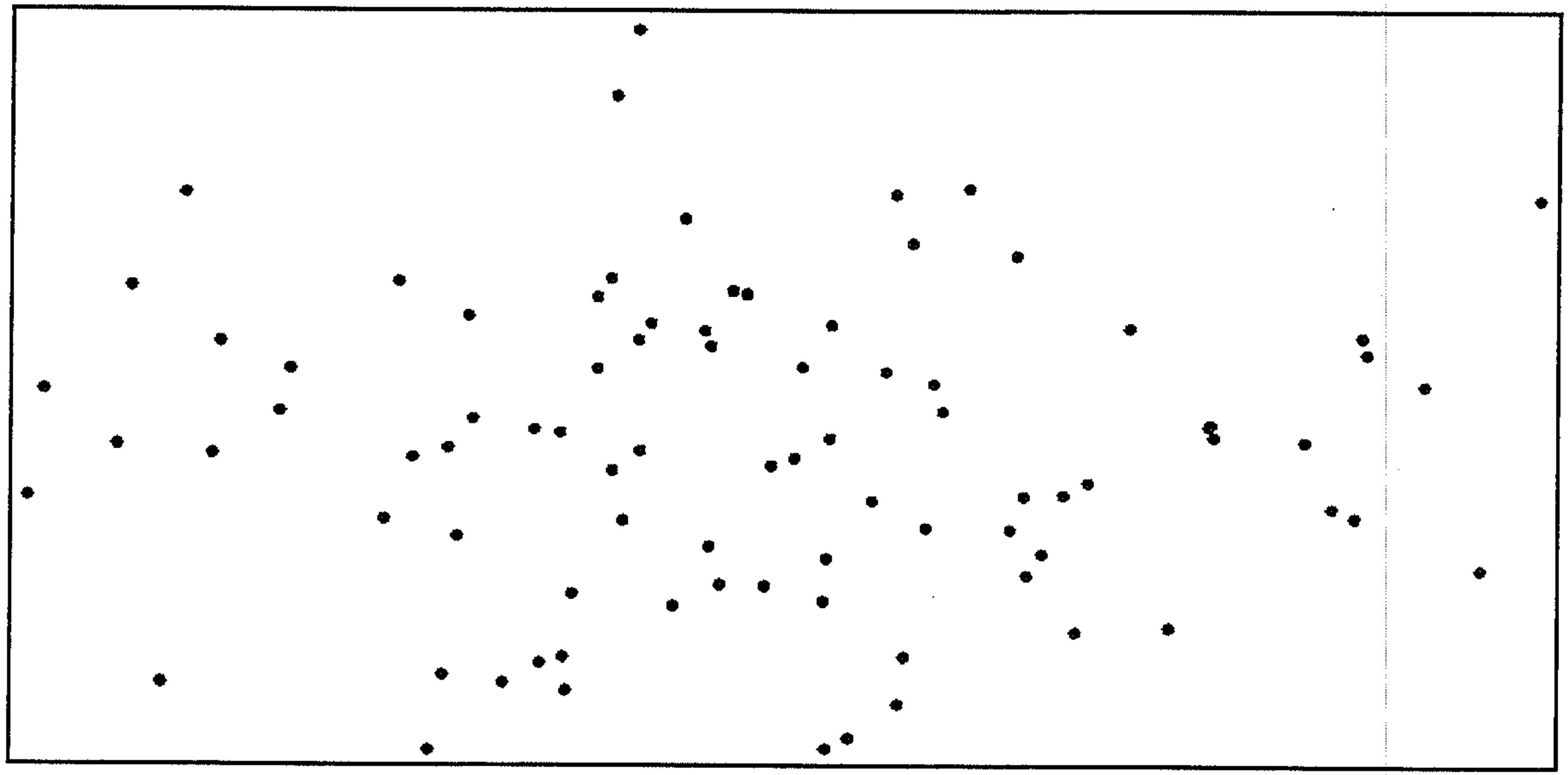

Fig. 25. Principal components analysis (PCA) of *R. malayanus* (n=85) based on 20 external and craniodental characters. (See Appendix 3 for variance explained).

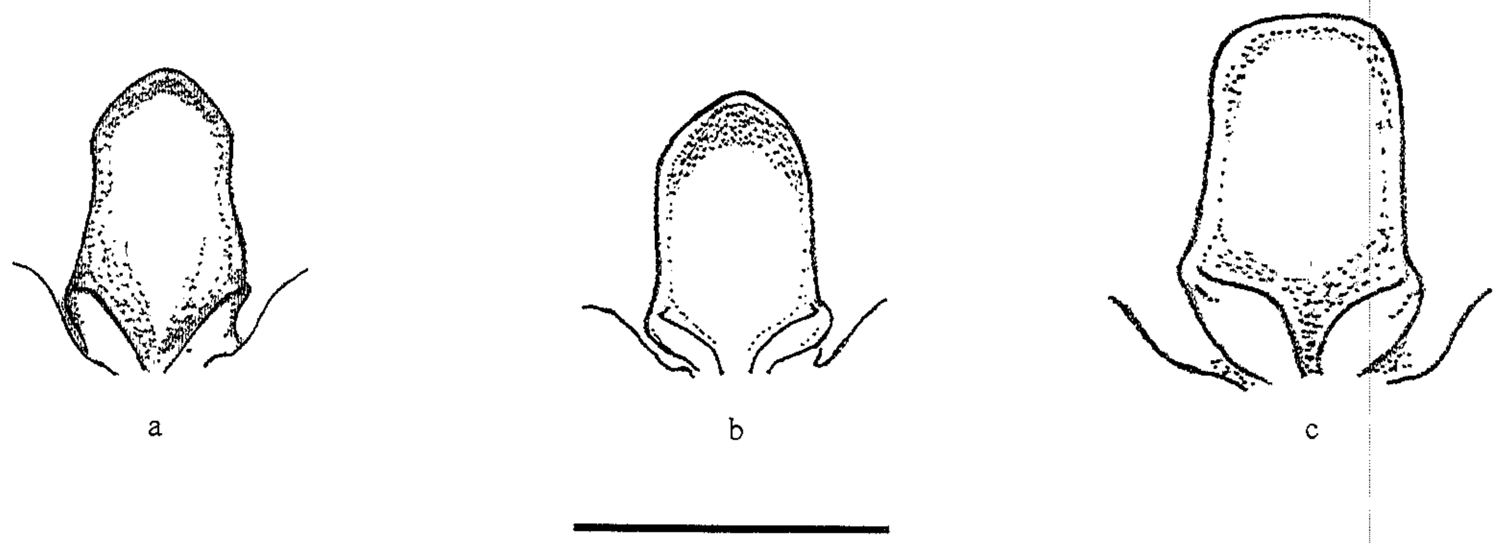

Fig. 26. Shape of sella. a: *R. stheno* (♂PSUZC-MM07.46), Khao Tieb cave, Songkla; b: *R. microglobosus* (♂PSUZC-MM06.27), Thung Slang Luang, Pitsanulok; c: *R. malayanus* (♂PSUZC-MM07.44), Wat Tham Phra Phothisat, Sara Buri. Scale = 3 mm.

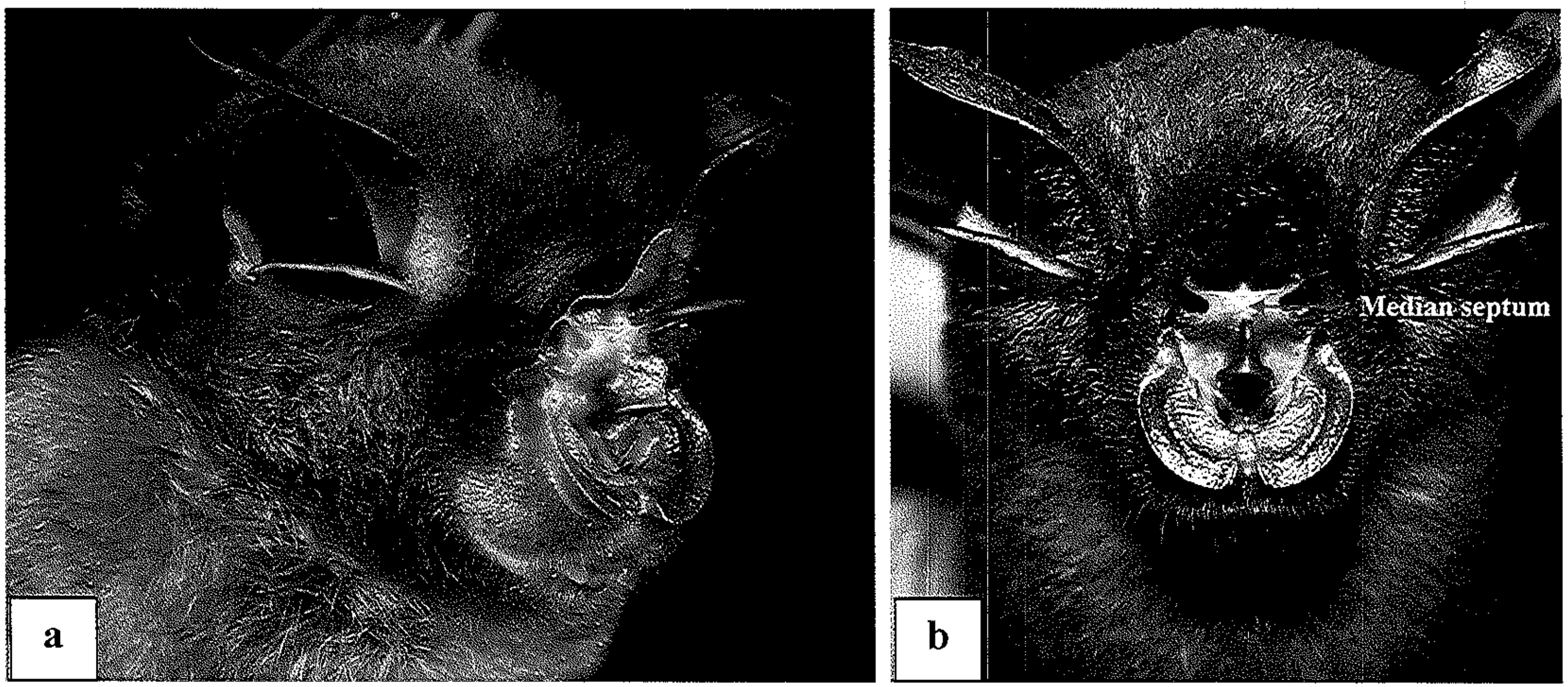

Fig. 27. Face, ears and noseleaf of *R. stheno*.

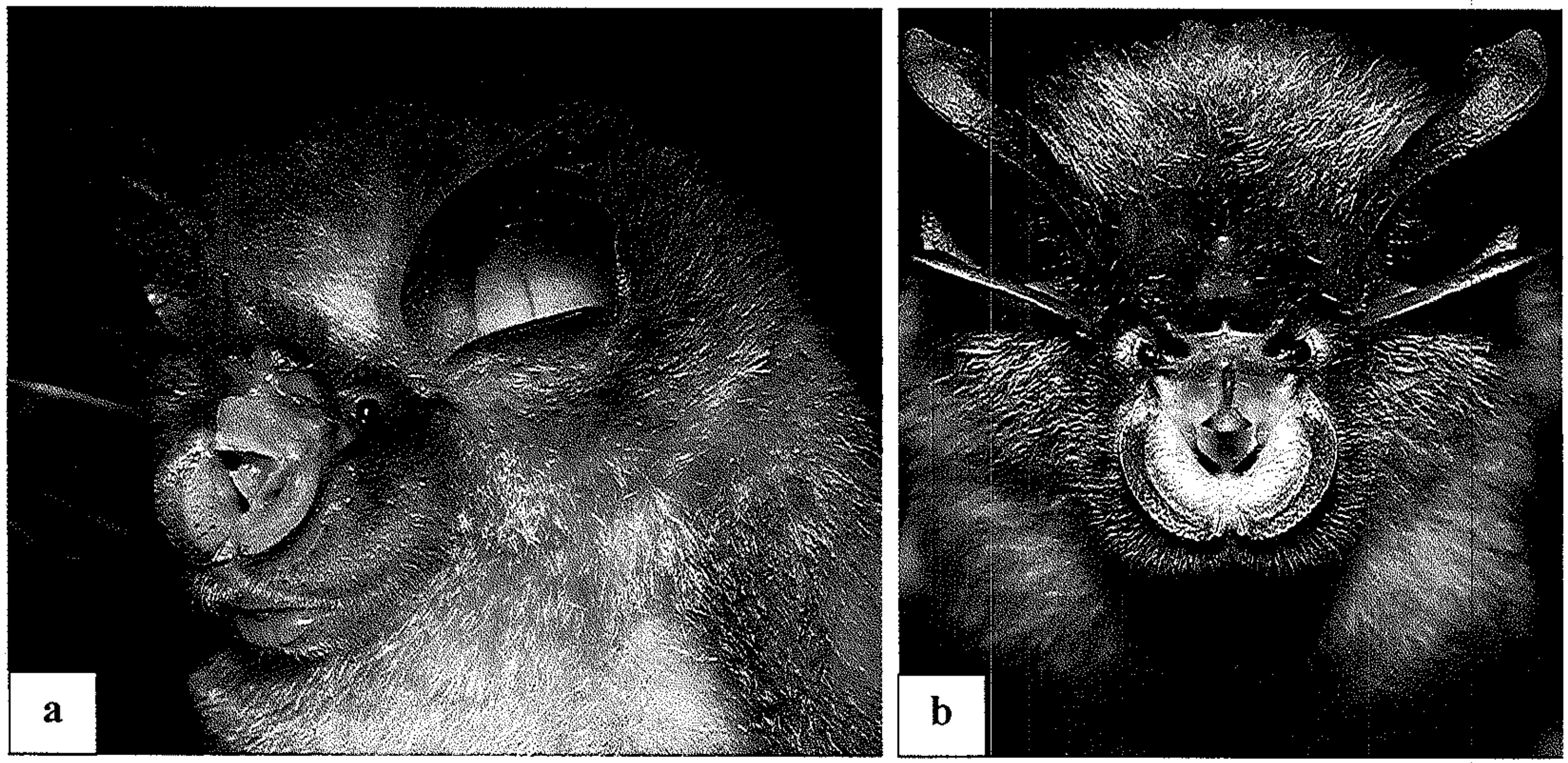

Fig. 28. Face, ears and noseleaf of *R. microglobosus*.

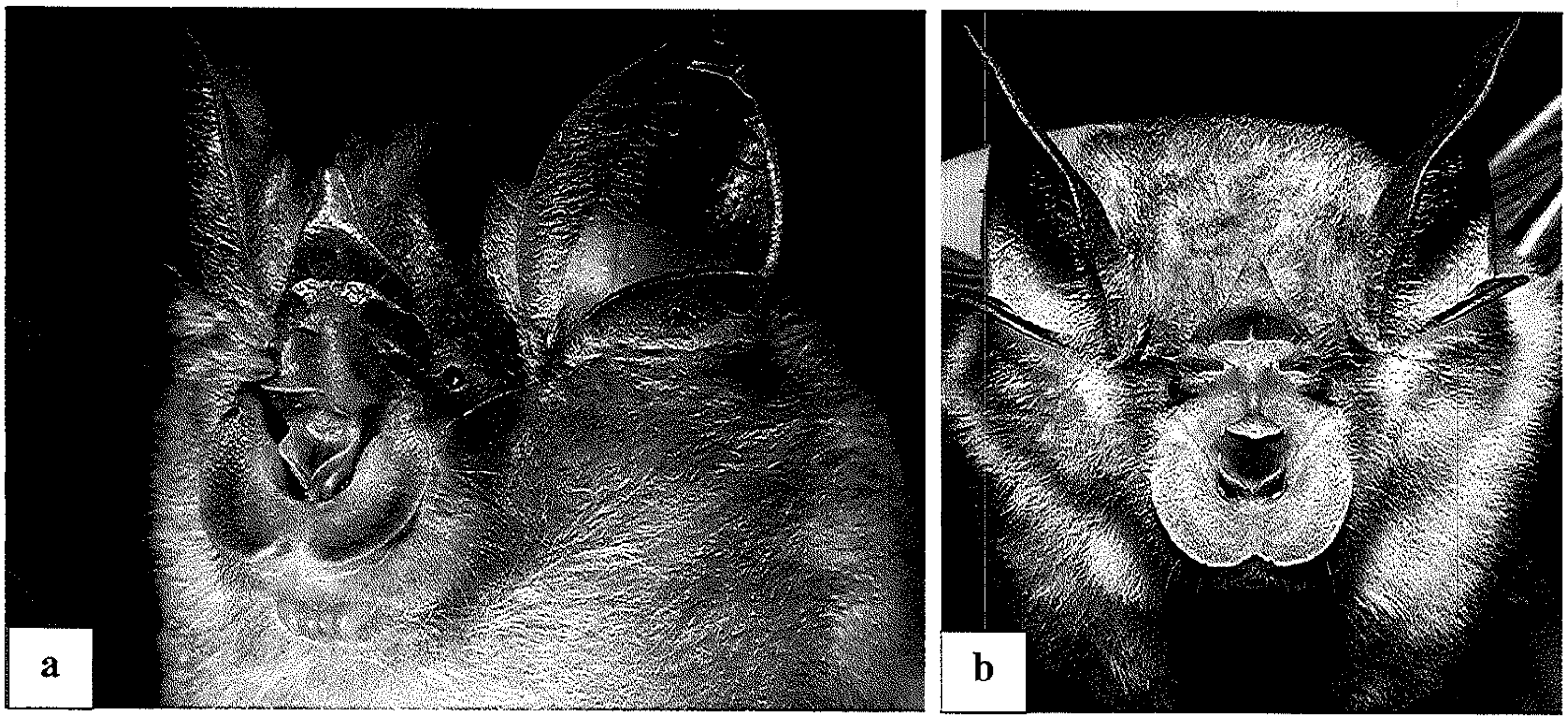

Fig. 29. Face, ears and noseleaf of *R. malayanus*.

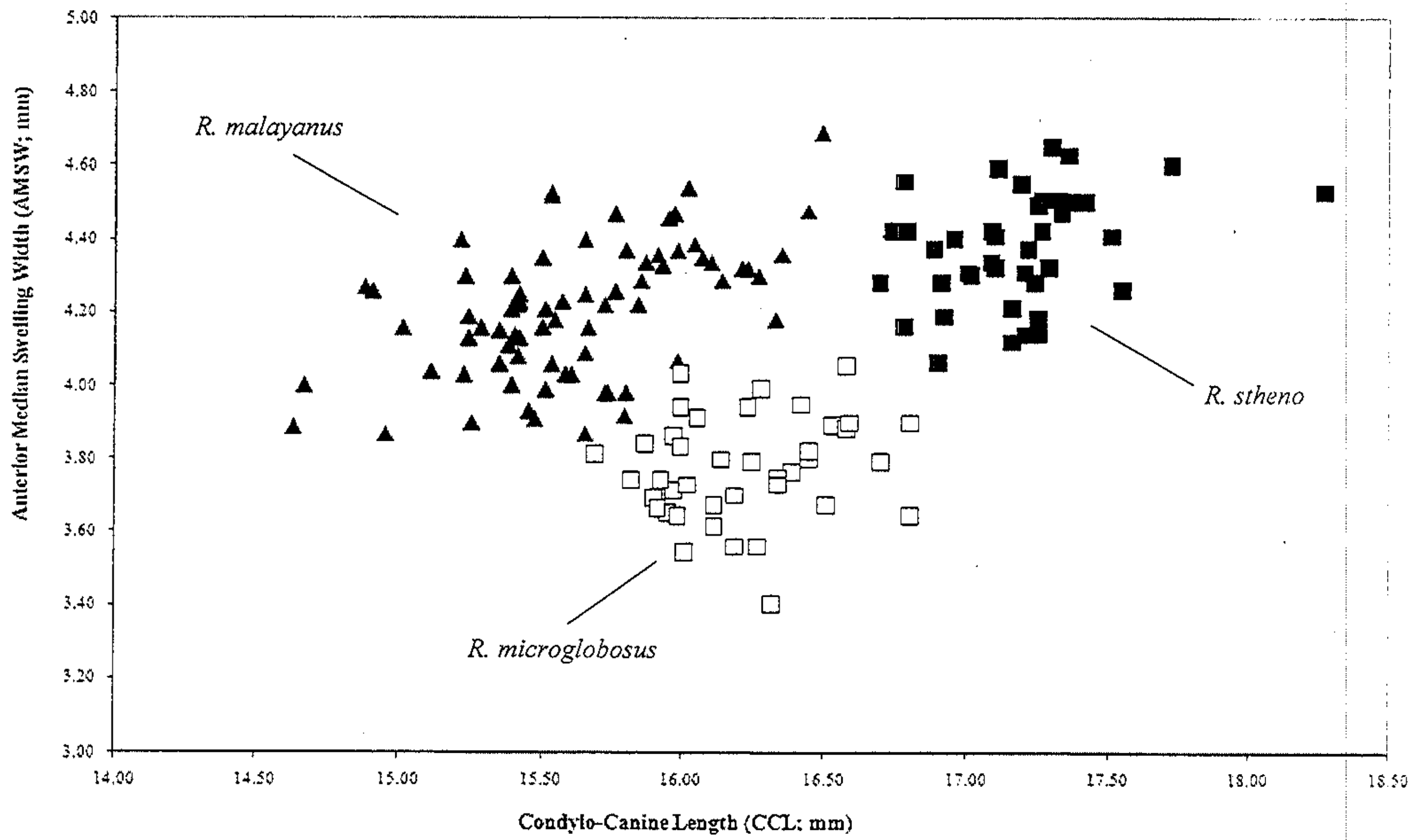

Fig. 30. Anterior median swelling width (in mm) compared to condylo-canine length (in mm) for three taxa: *R. malayanus* (triangles), *R. stheno* (solid squares) and *R. microglobosus* (blank squares).

CHAPTER 5

DISCUSSION

In general, the project gave conflicting signals about the efficacy of using acoustic data alone to identify possible cryptic species. Initial echolocation studies of *R. stheno* (*sensu* Csorba *et al.*, 2003) from throughout continental and peninsular Thailand suggested the possible presence of two species on the basis of an average of 10 kHz difference in resting frequency. In the case of *R. stheno,* this was confirmed by a suite of morphometric characters. However, in the case of *R. malayanus*, a similar divergence of 10 kHz between northern and southern populations is not considered here to represent specific divergence. Morphometric data gave no support to any division and it would appear that phonic differentiation and morphometric differentiation are not congruent in this taxon. This tends to support the view of Thabah *et al.*, (2006) who suggested that drifts in call frequency resulting from geographical separation may theoretically lead to divergence in acoustic communication without concomitant changes in morphology. Subsequent studies of molecular data (already being undertaken at the Prince of Songkla University) will be of great interest to determine the extent of genetic differentiation of the two populations.

No difference was found between the echolocation calls of the two sexes. This is in contrast to the study of *Rhinolophus rouxii* by Neuweiler *et al.* (1987) but is comparable to similar findings observed by Heller and Helversen (1989) for other taxa. As might be predicted from many previous studies (Robinson, 1996; Francis and Habersetzer, 1998; Zhang *et al.,* 2000), *R. stheno,* with its larger body size has a lower resting frequency call than the smaller *R. microglobosus.* There is no such pattern was observed in *R. malayanus*, but modest correlation between animal sizes, including noseleaf width and skull length, and call frequency was found. In contrast to *R. stheno* and *R. microglobosus*, individuals of *R. malayanus* with higher frequency calls were located in the central and southern part of the range and those with lower frequencies in central and northern part. This is contrary to what might be expected since normally, high relative humidity attenuates high frequency sound more than lower frequencies (Griffin, 1971; Hartely, 1989; Guillén *et al.*, 2000) and therefore it would be expected that the lower frequencies should be found in the more humid Thai-Malay peninsula, as is the case with *R. stheno* and *R. microglobosus*. We might suggest that in *R. malayanus*, foraging habitat may be a controlling factor. In less-cluttered microhabitats, bats should use lower frequencies of call to increase their detection ranges, and higher echolocation call frequencies are better suited for foraging in background cluttered and highly cluttered space (Schnitzler and Kalko, 1998; Kingston *et al.*, 2001). As the background cluttered and highly cluttered spaces are major characters of understorey level of tropical rain forest, and therefore it is possible that differences in frequency in *R. malayanus* may reflect microhabitat preferences.

In terms of taxonomic problem, as described in the 'Systematic Section', the three taxa can be distinguished by the combination of morphometric and acoustic data. The separation of the three taxa can be obviously seen in the relationship between the anterior median swelling width (AMSW) and the condylo-canine length (CCL). The results support the differences in rostral inflation between *R. malayanus* and *R. stheno* (*Sensu* Csorba *et al.*, 2003 which includes taxon *microglobosus*) discussed by Bates *et al.*, (2000; 2004). In cranial character, the post orbital constriction (PC) reported in Bates *et al.*, (2004) and Csorba *et al.*, (2003) appeared to be valid as another diagnostic character. Externally, even though the difference in body size and lancet shape among them, that proposed by Lekagul and McNeely (1977) cannot separate them apart, the difference in median septum of the lancet (Csorba *et al.*, 2003) and the ratio of the first and second phalanges of the third digit (Hendrichsen *et al.*, 2001b; Lekagul and McNeely, 1977) can be distinguished *R. malayanus* from *R. stheno* confidentially. Moreover, the hitherto description of the sella shape and its summit (e. g. Corbet and Hill, 1992; Csorba *et al.*, 2003; Lekagul and McNeely, 1977) cannot notify the dissimilarity between *R. malayanus* and *R. stheno*. In fact, their sella shapes are apparently difference.

R. microglobosus, which the results of this study support the original description (Csorba and Jenkins, 1998), can be distinguished from its closely related *R. stheno* on the basis of generally smaller anterior median swellings and external and craniodental measurements. The separation of the two species was strongly supported by statistical comparison and non-overlapped echolocation call frequencies. However, regardless of the acoustic data and distribution pattern, the larger individuals of *R. microglobosus* can be confused with the smaller individuals of *R. stheno.*

It has long been recognized that Thailand is subdivided into two zoogeographic subregions with the Indochinese subregion to the north and the Sundaic subregion to the south (Lekagul and McNeely, 1977; Corbet and Hill, 1992) with a transition zone in the Isthmus of Kra. Distribution patterns corresponding to this division have been observed in a whole range of biota including rodents (Mein and Ginsburg, 1997), insects (Corbet, 1941), reptiles (Inger and Voris, 2001) and plants (Woodruff, 2003). Previously, Wallace (1876) had placed the transition zone at 13-14° N. Whereas Wells (1976) fixed the avifaunal transition zone at about 10° 30' N, in the Isthmus of Kra. Subsequently, Hughes *et al.*, (2003), based on forest birds, found a highly significant transition zone at 11-12° N, at the north of the peninsula.

The distribution patterns of the three species were of considerable interest since in the present study, the patterns of distribution strongly support the existing concepts of a subregional division. *R. microglobosus* is restricted to the Indochinese subregion and is not found further south in the Thai-Malay peninsula. In contrast, *R. stheno* is primarily restricted to the peninsula (and elsewhere in the Sundaic subregion), but also has a small isolated population in a mountain range in south-central Vietnam. This high mountain range, which is part of Annamite Mountains, covered by evergreen forest and runs west-east from Laotian border to the east coast of Vietnam, and forms biogeographical boundary between northern and southern Vietnam, and this area appears to be the wettest part of Vietnam (Tordoff *et al.*, 2004). The presence of this latter population seems analogous to the situation in the freshwater prawn *Macrobrachium rosenbergii*, in which closely related populations are reported from peninsular Thailand and southern Vietnam (de Bruyn *et al.*, 2005). There were such cases of animals that have been elevated from subspecific to specific rank related to the zoogeographical subregion affinity. In small mammal for example, the Northern treeshrew *Tupaia belangeri*, which distributed in north of the Isthmus of Kra has been known as a separated species from the southern species: Common treeshrew *T. glis* (see Corbet and Hill, 1992). In the case of *R. malayanus*, the phonic type with an echolocation call of between 73 and 82 kHz is entirely restricted to the Indochinese subregion, whereas the 82 to 90 kHz phonic type has a Sundaic distribution but also appears to have spread north into south-central Thailand and Cambodia.

Since molecular data are currently in the process of being analysed, it is perhaps inappropriate to speculate about possible vicariance events, and the time scales involved, that may have led to the speciation of *R. stheno* and *R. microglobosus*. However, it is known that frequent changes in global climates during the Pleistocene (and previous to that in the Pliocene and Miocene) had significant effects on both the vegetation and sea levels of the Indo-Malay peninsula. At times, the evergreen rain forest was restricted to a small number of refugia, whilst changes in sea level both exposed vast areas of the Sundaic shelf or led to inundations of the Isthmus of Kra (Woodruff, 2003; de Bruyn, 2005).

Anyway, the distribution of *R. stheno* in south-central Vietnam is very disjunct, and was so far removed from the rest of its range. This strange distribution is considerable similar to the distribution of *Hipposideros turpis* which was found in peninsula Thailand, northern Vietnam and also Japan (Francis, 2008). Further study may prove that these populations represent distinct species.

Today, the transition zone at 11-12° is characterized by a change from seasonal evergreen rain forest to mixed moist deciduous forest (Woodruff, 2003). Vegetation apart, there is no other clearly defined barrier to the movement of bat taxa and yet it seems to represent a barrier to the southern expansion of *R. microglobosus* and to the 73 and 82 kHz phonic type of *R. malayanus*. It would be of considerable interest to determine if other bat taxa have a similar distribution with such clearly defined Indochinese or Sundaic affinities. However, individuals of *R. malayanus* from Knaddai cave, Ranong Province, which is considered the transition zone between the two zoological subregion, had relatively flattened rostral swelling. The result of the molecular study of this population would be very interest. It would also be of interest to collect acoustic data from south-central Vietnam to ascertain the resting frequencies of *R. stheno* and *R. microglobosus* at Bach Ma NP. and ascertain whether they correspond closely to those from these respective taxa elsewhere in their range.

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Geographical Gazeteer

Ao Son-Ao Chak, Tarutao, Thailand	6°39'N, 99°38'E
Ba Be NP., Vietnam	22°32'N, 105°07'E
Bach Ma NP., Vietnam	16°12'N, 07°45'E
Bach Ma NP., Vietnam	16°12'N, 107°45'E
Bala, Hala-Bala, Thailand	5°48'N, 101°49'E
Ban Bang Non, Thailand	approx. 9°60'N, 98°39'E
Ban Huai Kaeo, Thailand	18°51'N, 99°03'E
Ban San Ko Pui, Thailand	20°25'N, 99°53'E
Ban Wang Bla Chan, Thailand	approx. 6°35'N, 100°04'E
Batu Cave	3°14'N, 101°41'E
Bayint Nyi Cave, Myanmar	16°58'N, 97°30'E
Biserat, Thailand (*Type locality of R. malayanus*)	not located
Boripat Waterfall, Thailand	7°01'N, 100°08'E
Bortum Sakor, Cambodia	11°04'N, 103°20'E
Bo-tong, Ang Runai, Thailand	13°11'N, 101°44'E
Bo-tong, Ang Runai, Thailand	7°01'N, 100°08'E
Chet Kot Cave, Thailand	7°05'N, 99° 54'E
Chu Mom Ray NP., Vietnam	14°28'N, 107°47'E
Chumnoap-Areng, Cambodia	11°38'N, 103°34'E
East Thung Yai, Thailand	15°42'N, 99°01'E
East Thung Yai, Thailand	15°42'N, 99°01'E
Gunong Benom, Malaysia	approx. 3°35'N, 102°15'E
Indian Single Rock Temple Cave, Myanmar	16°19'N, 97°43'E
Kao Roi Rai Cave, Thailand	15°07'N, 98°28'E
Khao Bin Cave, Thailand	13°35'N, 99°40'E
Khao Don Dueng, Thailand	15°08'N, 100°36'E
Khao Don Dueng, Thailand	15°08'N, 100°36'E
Khao Nom Tai, Thailand	13°42'N, 99°45'E
Khao Pha Wo, Thailand	approx. 16°47'N, 98°50'E
Khao Rak Kiat, Thailand	7°04'N, 100° 15'E

Geographical Gazeteer (continued)

Khao Tiab Cave, Thailand	6°59'N, 100°17'E
Khao Wong Cave, Thailand	approx. 8°50'N, 98°48'E
Khao Yoi Hill, Thailand	13°14'N, 99°49'E
Khimee Cave, Thailand	19°21'N, 98°43'E
Khimee Cave, Thailand	19°21'N, 98°43'E
Kirirom NP., Cambodia	11°20'N, 104°03'E
Kisap Forest Reserve, Malaysia	6°23'N, 99°52'E
Knaddai Cave, Thailand	10°01'N, 98°50'E
Knaddai Cave, Thailand	10°01'N, 98°50'E
Kon Ka Kinh, Gai Lai, Vietnam	14°19'N, 108°24'E
Lub Lac Cave, Thailand	15°03'N, 99°28'E
Ma Kling Waterfall, Thailand	7°02'N, 100°12'E
Ma Tok Cave, Thailand	14°54'N, 100°29'E
Mae Ja Cave, Thailand	19°31'N, 98°50'E
Mae Pong Cave, Thailand	19°33' N, 98°42'E
Makok , Phliu, Thailand	12°35' N, 102°15'E
Makok, Phliu, Thailand	12°35' N, 102°15'E
Manora Cave, Thailand	16°46'N, 98°39'E
Maxwell's Hill	approx. 4°54'N, 100°42'E
Montawa Cave, Myanmar	20°45'N, 97°01'E
Na Hang, Vietnam (*Type locality of R. microglobosus*)	between 22°16' and 22°31'N and 105°22' and 105°29'E
Nagamuak Cave, Myanmar	16°19'N, 97°42'E
Nam Nao Cave, Thailand	16°57'N, 101°30'E
Nam Nao Cave, Thailand	16°57'N, 101°30'E
Nam Tob, Phu Luang, Thailand	17°15'N, 101°35'E
Nanti Hill Forest, Myanmar	24°34'N, 97°07'E
Nanti Hill Forest, Myanmar	24°34'N, 97°07'E
Patiharn Cave. Thailand	15°35'N, 105°34'E
Pauk Inlay Cave, Myanmar	22°28'N, 96°59'E

Geographical Gazeteer (continued)

Pha Daeng Cave, Thailand	19°20'N, 99°01'E
Phu mieng-Phu thong NP., Thailand	17°19'N, 100°39'E
Phu Pha San, Thailand	16°38'N, 104°22'E
Phu Suan Sai NP., Thailand	17°30'N, 100°57'E
Pisut Cave, Thailand	14°30'N, 99°00'E
Sadden Sin Cave, Myanmar	16°31'N, 97°49'E
Sakaerat, Thailand	14°28'N, 101°56'E
Sang Phet Cave, Thailand	8°09'N, 98°53'E
Sanite cave No.2, Myanmar	22°06'N, 96°37'E
Selangor, Malaysia (*Type locality of R. stheno*)	3°30'N, 101°30'E
Silawan Cave, Thailand	10°41'N, 99°14'E
Suea Luang Cave, Thailand	14°49'N, 100°47'E
Talo-wow, Tarutao, Thailand	6°36'N, 99°41'E
Taung Thit Mine, Myanmar	14°36'N, 97°59'E
Thac Kem Ridge Top, Vietnam	18°58'N, 104°46'E
Thac Kem Ridge Top, Vietnam	18°58'N, 104°46'E
Tham Omar Kok, Lao PDR	20°41'N, 101°11'E
Thung Kamang, Thailand	16°23'N, 101°33'E
Thung Slang Luang, Thailand	16°34'N, 100°52'E
Ton Nga Chang, Thailand	6°55'N, 100°17'E
Tonetar Cave, Myanmar	21°19'N, 99°17'E
Wang Saithong Waterfall, Thailand	7°05'N, 99°54'E
Wang Tangga, Malaya	6°39'N, 100°12'E
Wat Tham Phra Phothisat, Thailand	14°34'N, 101°08'E
Xuan Son NP., Vietnam	21°06'N, 104°57'E
Xuan Son NP., Vietnam	21°06'N, 104°57'E
Yathay Payan Cave, Myanmar	16°32'N, 97°34'E

APPENDIX

Appendix 1. Specimen Localities

1.1 *Rhinolophus stheno*

VIETNAM

S1-Bach Ma NP., (16°12'N, 107.45'E): field no. T44 (IEBR)

S2-Kon Ka Kinh, Gai Lai, (14.19'N, 108.24'E): HZM.1.32327

THAILAND

S3-Knaddai Cave, La Oun, Ranong, (10°01'N, 98°50'E): PSU-M07.79-87

S4-Khao Wong Cave, Klong Panom, Surat Thani, (approx. 8°50'N, 98°48'E): field no. SW00179, 00182 (TISTR)

S5-Khao Rak Kiat, Rattaphum, Songkla, (7°04'N, 100° 15'E): PSU-M06.24, 06.107-109

S6-Boripat, Rattaphum, Songkla, (7°01'N, 100°08'E): PSU-M06.52, 06.54-55, 06.58-59

S7-Khao Tiab Cave, Hat Yai, Songkla, (6°59'N, 100°17'E): PSU-M06.42-47

S8-Ton Nga Chang, Songkla, (6° 55'N, 100°17'E): PSU-M05.44, 06.31

S9-Bala, Hala-Bala, Narathiwat, (5° 48'N, 101° 49'E): PSU-M05.46

MALAYSIA

S10-Gunong Benom, Pahang, (approx 3°35'N, 102°15'E): BMNH 1967.1492, 1967.1533-34

S11-Selangor, (*Type locality of R. stheno*) (3°30'N, 101°30'E): BMNH 1898.3.13.1, SMF 83.843

S12-Maxwell's Hill, Taiping, Perak, (approx. 4°54'N, 100°42'E): SMF 88.436

S13-Batu Cave, Kuala Lumpur (3°14'N, 101°41'E): SMF 91.116, 91.117

1.2 *R. microglobosus*

MYANMAR

G1-Nanti Hill Forest, Bhamo Township, Kachin State, Myanmar (24°34'N, 97°07'E): field no. MS6, HZM.6.35967

G2-Sanite cave No.2, Wetwun Village, Pyin Ooo Lwin, Shan State, Myanmar (22°06'N, 96°37'E): HZM.4.35114

G3-Indian Single Rock Temple Cave, Mon State, Myanmar (16°19'N, 97°43'E): field no. H21 (HZM)

THAILAND

G4-Khimee Cave, Chiangdao, Chiang Mai, Thailand (19°21'N, 98°43'E): CD-B-0047-49, 0054-55, 0057, 0070

G5-Mae Ja Cave, Chiangdao, Chiang Mai, Thailand (19°31'N, 98°50'E): PSUZC-MM05.93, 06.94-98

G6-Mae Pong Cave, Chiang Dao, Chiang Mai, Thailand (19°33' N, 98°42'E): PSUZC-MM05.59

G7-Manora Cave, Mae Sot, Tak, Thailand (16°46'N, 98°39'E): PSUZC-MM06.105-106

G8- Phu Suan Sai NP., Na Haeo, Loei, Thailand (17°30'N, 100°57'E): PSUZC-MM07.295-296

G9- Thung Slang Luang, Nhong Mae Na, Pitsanulok, Thailand (16°34'N, 100°52'E): PSUZC-MM06.27

G10-Nam Nao Cave, Nam Nao, Petchabun, Thailand (16°57'N, 101°30'E): PSUZC-MM07.297

G11-East Thung Yai, Tak, Thailand (15°42N, 99°01E): THNHM 12-14

G12-Kao Roi Rai Cave, Sangkla Buri, Kanchanaburi, Thailand (15°07'N, 98°28'E): PSUZC-MM05.127

G13-Khao Don Dueng, Ban Mi, Lop Buri, Thailand (15°08'N, 100°36'E): PSUZC-MM07.163

G14-Bo-tong, Ang Runai, Chacherngsao, Thailand (7°01'N, 100°08'E): PSUZC-MM05.128-137

G15-Makok, Phliu, Chantaburi, Thailand (12°35' N, 102°15'E): PSUZC-MM05.138-143

CAMBODIA

G16-Chumnoap-Areng, Cambodia (11°38'N, 103°34'E): HZM.7.36484

G17-Kirirom NP., Kompong Spev., Cambodia (11°20'N, 104°03'E): HZM.3.34173

VIETNAM

G18-Na Hang, Vietnam (*Type locality of R. microglobosus*) (between 22°16' and 22°31'N, 105°22' and 105°29'E): BMNH 1997.360[holotype], 1997.359, 1997.361

G19-Xuan Son NP., Vietnam (21°06'N, 104°57'E): IEBR35

G20-Thac Kem Ridge Top, Pu Mat, Vietnam (18°58'N, 104°46'E): HZM.4.32376

G21-Bach Ma NP., Vietnam (16°12'N, 07.45'E): field no. B 026(IEBR)

G22-Chu Mom Ray National Park, Vietnam (14°28'N, 107°47'E): field no. T43 (IEBR)

1.3 *Rhinolophus malayanus*

MYANMAR:

M1-Nanti Hill Forest, Bhamo Township, Kachin State, Myanmar (24°34'N, 97°07'E): HZM.17.35969

M2-Pauk Inlay cave, Pyaung Gaung, Shan state, Myanmar (22°28'N, 96°59'E): HZM.12.35122, 14.35124

M3-Tonetar Cave, Tonetar Village, Myanmar (21°19'N, 99°17'E): HZM.19.36073

M4- Montawa Cave, Taunggyi Township, Shan State, Myanmar (20°45'N, 97°01'E): HZM 11.35108

M5-Bayint Nyi Cave, Kayin State, Myanmar (16°58'N, 97°30'E): HZM. 16.35293, H24

M6-Yathay Payan Cave, Hpa-an Township, Myanmar (16°32'N, 97°34'E): HZM 6.34144

M7-Sadden Sin Cave, Myanmar (16°31'N, 97°49'E): HZM .7.34145, 8.34875, 10.34041

M8-Nagamuak Cave, Mon State (16°19'N, 97°42'E): HZM.9.34990

M9-Taung Thit Mine, Ohn Un Kwin, Dawei Dist. Thaninthary, Myanmar (14°36.'N, 97°59'E): HZM.18.35970

LAO PDR:

M10-Tham Omar Kok, Lao PDR (20°41'N, 101°11'E): HZM.20.37754

VIETNAM:

M11-Ba Be NP., Cho Ra District, Crobang, Vietnam (22°32'N, 105°07'E): BMNH 1997.319

M12-Xuan Son NP., Vietnam (21°06'N, 104°57'E): field no. T23 (IEBR)

M13-Thac Kem Ridge Top, Pu Mat, Vietnam (18°58'N, 104°46'E): HZM.12.35122, 14. 35124

THAILAND:

M14-Ban San Ko Pui, Mae Sai, Chiang Rai, Thailand (20°25'N, 99°53'E): TISTR 54-5181

M15-Khimee Cave, Chiangdao, Chiang Mai, Thailand (19°21'N, 98°43'E): CD-B-0039, 0052-53

M16-Pha Daeng Cave, Sri Lan Na, Chiang Mai, Thailand (19°20'N, 99°01'E): PSUZC-MM06.90-93

M17-Ban Huai Kaeo, San Sai, Chiang Mai, Thailand (18°51'N, 99°03'E): field no. 184 (TISTR)

M18-Phu mieng-Phu thong, Pitsanulok, Thailand (17°19'N, 100°39'E): PSUZC-MM06.29

M19-Nam Tob, Phu Luang, Loei, Thailand (17°15'N, 101°35'E): PSUZC-MM05.60

M20-Khao Pha Wo, Mae Sot, Tak, Thailand (approx. 16°47'N, 98°50'E): 54-5436 (TISTR)

M21-East Thung Yai, Tak, Thailand (15°42'N, 99°01'E): PSUZC-MM05.49

M22- Nam Nao Cave, Nam Nao, Petchabun, Thailand (16°57'N, 101°30'E): PSUZC-MM07.290-292, 07.297

M23-Thung Kamang, Chaiyaphum, Thailand (16°23'N, 101°33'E): PSUZC-MM06.28

M24-Lub Lae Cave, Ban Rai, Uthai Thani, Thailand (15°03'N, 99°28'E): PSUZC-MM07.168-169

M25-Pisut cave, Sri Nakarind, Kanchanaburi, Thailand (14°30'N, 99°00'E): PSUZC-MM05.51

M26-Khao Don Dueng, Ban Mi, Lop Buri, Thailand (15°08'N, 100°36'E): PSUZC-MM07.162, 07.64-167

M27-Ma Tok Cave, Khao Samorkhon, Ta Wung, Lop Buri, Thailand (14°54'N, 100°29'E): PSUZC-MM07.52

M28-Suea Luang Cave, Koktum, Muang, Lop Buri, Thailand (14°49'N, 100°47'E): PSUZC-MM07.53-54

M29-Wat Tham Phra Phothisat, Tab Kwang, Kang Koi, Sara Buri, Thailand (14°34'N, 101°08'E): PSUZC-MM07.42-51

M30-Sakaerat, Pak Thong Chai, Nakhonratchasima, Thailand (14°28'N, 101°56'E): 54-5681, 54-5682 (TISTR), PSUZC-MM07.55.

M31- Phu Pha San, Phu Sithan WS., Kham Cha-I, Mukdahan, Thailand (16°38'N, 104°22'E): PSUZC-MM07.293

M32-Patiharn Cave, Pha Taem, Ubon Ratchathani, Thailand (15°35'N, 105°34'E): PSUZC-MM05.16, 05.61

M33-Khao Nom Tai, Photharam, Ratcha Buri (13°42'N, 99°45'E): PSUZC-MM07.70-73

M34-Khao Bin Cave, Chom Bung, Ratcha Buri, Thailand (13°35'N, 99°40'E): PSUZC-MM07.74-75

M35-Khao Yoi Hill, Pet Buri, Thailand (13°14'N, 99°49'E): PSUZC-MM07.76-78

M36-Bo-tong, Ang Runai, Chacherngsao, Thailand (13°11'N, 101°44E): PSUZC-MM05.144, 05.146-148

M37-Makok, Phliu, Chantaburi, Thailand (12°35' N, 102°15'E): PSUZC-MM05.145, 05.149-153

M38-Silawan Cave, Pathiu, Chumporn, Thailand (10°41'N, 99°14'E): PSUZC-MM07.56-60

M39-Knaddai Cave, La Oun, Ranong, Thailand (10°01'N, 98°50'E): PSUZC-MM07.61-69

M40-Ban Bang Non, Ranong, Thailand (approx. 9°60'N, 98°39'E): TISTR 54-5971

M41-Sang Phet Cave, Phanom, Krabi, Thailand (8°09'N, 98°53'E): PSUZC-MM06.41

M42-Ban Wang Bla Chan, Muang, Satun, Thailand (approx. 6°35'N, 100°04'E): BMNH 78.2295

M43-Chet Kot Cave, Manang, Satun, Thailand (07°05'N, 99° 54'E): PSUZC-MM06.13-16

M44-Wang Saithong waterfall, Manang, Satun, Thailand (07°05'N, 99°54'E): PSUZC-MM06.12

M45-Ma Kling waterfall, Rattaphum, Songkla, Thailand (7°02'N, 100°12'E): PSUZC-MM07.155, 07.157

M46-Ao Son-Ao Chak, Tarutao, Satun, Thailand (6°39'N, 99°38'E): PSUZC-MM05.54

M47-Talo-wow, Tarutao, Satun, Thailand (6°36'N, 99°41'E): PSUZC-MM05.50, 05.52, 05.54, 06.30

M48-Biserat, Jalor, Thailand (*Type locality of R. malayanus*) (not located): BMNH 1903.2.6.83[holotype], 1908.2.5.24

CAMBODIA:

M49-Bortum Sakor, Koh Kong, Cambodia (11°04'N, 103°20'E): field no. 3634T, 3635T, 3638T (Frontier Cambodia)

MALAYSIA:

M50-Wang Tangga, Kaki Bukit, Kangkar, Perlis, Malaya (6°39'N, 100°12'E): BMNH 1968.812

M51-Kisap Forest Reserve, Palau, Langkawi, Malaysia (6°23'N, 99°52'E): BMNH 1968.813-816

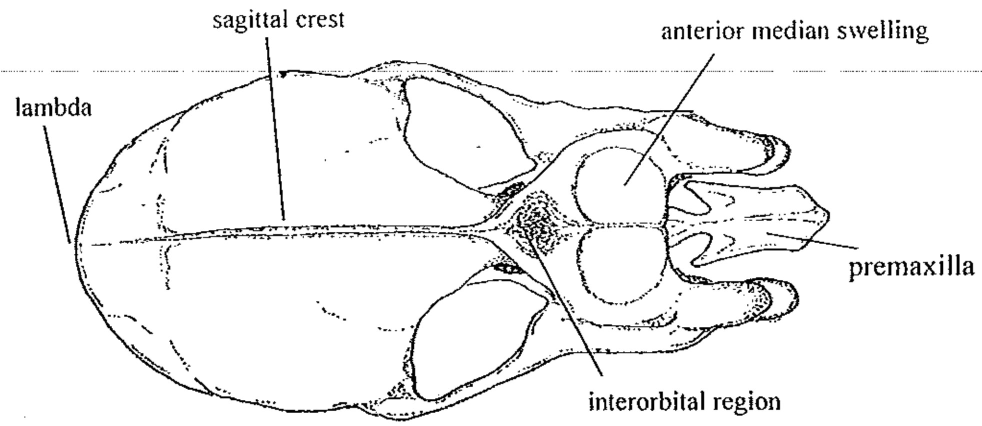
Appendix 2. Eigenvector scores, eigenvalues, and variance explained resulting from the principal component analyses (PCA) of *R. stheno* and *R. microglobosus.*

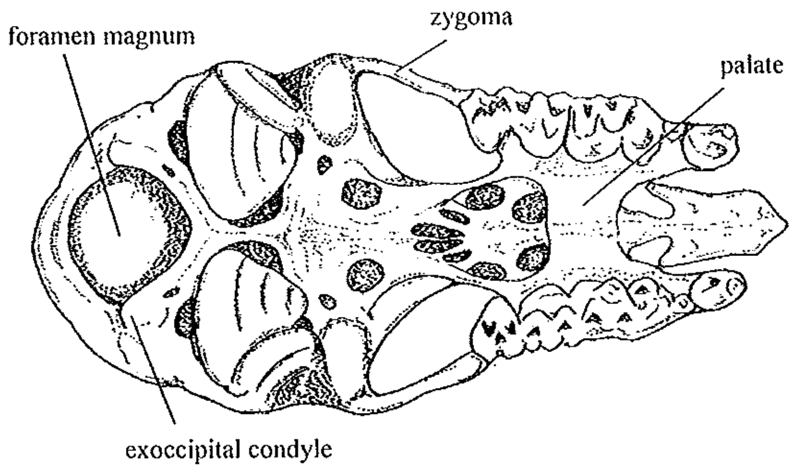
characters	Eigenvector scores	
	Axis 1	Axis 2
Forearm length	-0.168	0.365
Ear length	-0.145	0.381
Head and body length	-0.221	-0.253
Tail length	0.069	0.734
Hindfoot length	-0.212	0.093
Condylo-canine length	-0.282	-0.004
Skull length	-0.281	0.023
Zygomatic breadth	-0.278	0.099
Braincase breadth	-0.274	-0.031
Mastoid width	-0.267	0.015
Post orbital constriction	-0.149	-0.283
Maxillary toothrow length	-0.281	0.019
Posterior palatal width	-0.272	0.082
Anterior palatal width	-0.270	-0.038
Mandibular toothrow length	-0.280	-0.004
Anterior lateral swelling width	-0.268	-0.024
Anterior median swelling width	-0.268	-0.097
Eigenvalue	3.440	2.440
% of variance	67.918	7.217
Cumulative % of variance	67.918	75.135

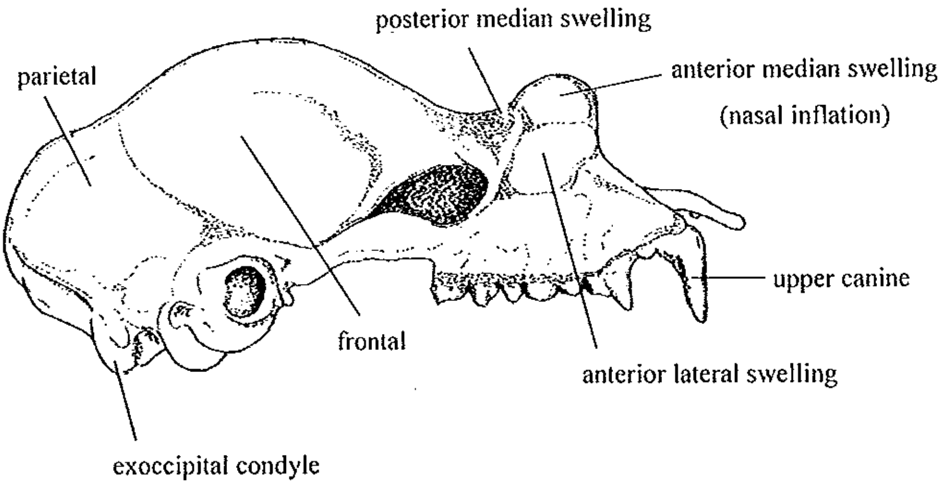
Appendix 3. Eigenvector scores, eigenvalues, and variance explained resulting from the principal component analyses (PCA) of *R. malayanus*.

Characters	Eigenvector scores	
	Axis 1	Axis 2
Forearm length	-0.214	0.367
Ear length	-0.067	-0.056
Tail length	-0.117	0.228
Tibia length	-0.221	0.257
Hindfoot length	-0.121	0.101
Fifth metacarpal length	-0.226	0.373
Fourth metacarpal length	-0.224	0.372
Third metacarpal length	-0.214	0.390
Greatest length of skull	-0.267	-0.169
Condylobasal length	-0.245	-0.119
Condylo-canine length	-0.287	-0.159
Skull length	-0.284	-0.128
Zygomatic breadth	-0.221	-0.002
Braincase breadth	-0.198	-0.020
Mastoid width	-0.267	-0.132
Maxillary toothrow length	-0.253	-0.220
Anterior palatal width	-0.203	-0.158
Mandibular toothrow length	-0.241	-0.233
Mandible length	-0.273	-0.147
Anterior median swelling width	-0.185	-0.231
Eigenvalue	3.598	2.598
% of variance	50.454	9.999
Cumulative % of variance	50.454	60.452

Appendix 4. Dorsal, ventral and lateral views of the skull of *Rhinolophus.*







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List of Publication and Proceeding

- **Soisook, P.**, S. Bumrungsri, A. Dejtaradol, C. M. Francis, G. Csorba, A. Guillén and P. J. J. Bates. 2008. First records of *Kerivoula kachinensis* (Chiroptera: Vespertilionidae) from Cambodia, Lao PDR and Thailand. Acta Chiropterologica, 9(2): 339-345.

- **Soisook, P.**, S. Bumrungsri, C. Satasook, V. D. Thong, S. S. Hla Bu, D. L. Harrison and P. J. J. Bates. A taxonomic review of *Rhinolophus stheno* and *R. malayanus* (Chiroptera: Rhinolophidae) from continental Southeast Asia: an evaluation of echolocation call frequency in discriminating between cryptic species. Acta Chiropterologica (in press.).