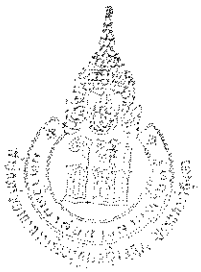

A Taxonomic Evaluation of Four Cryptic Species of *Rhinolophus* (Chiroptera: Rhinolophidae) in Southern India and Southeast-Asia

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

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ABSTRACT

Thailand supports an extremely diverse fauna and flora. Situated in the Indo-Chinese peninsula of the Oriental region, the country has been described as a 'zoogeographical cross roads', which links the biotas of the Sino-Himalayan, Indo-Burmese, Indo-Chinese and Sundaic regions. The bat fauna, with over 120 species, is also very diverse and in this particular project, one widely distributed Rhinolophid species was provided the study subject. It is the Intermediate horseshoe bat, *Rhinolophus affinis*, which has been selected as its taxonomic status is in need of revision and its relationship with a sibling species the Rufous horseshoe bat, *R. rouxii.* In addition, *R. sinicus* and *R. thomasi* found elsewhere in southern India and Southeast-Asia, is taxonomically unclear. The morphometric characters of *R. affinis* and *R. rouxii* currently employed to discriminate between the two taxa are unsatisfactory and in southern Myanmar, specimens were collected recently that shared external and cranial characters with both *R. affinis* and *R. rouxii.* Furthermore, within Thailand, geographical variation between populations of *R. affinis*, especially when measured in terms of echolocation call variability suggest that there may be one or more taxa included within a super-species currently described as *R. affinis.* The important findings in this study as *R. affinis* differed *R. rouxi* by some characters such as rostal depression, zygomatic shapes, forearm length (FA), the second phalanx of the third digit (3met2ph), palatal length (PL) and baculum morphology. *R. sinicus* and *R. thomasi* are clearly different by size and characters. In addition, sexual dimorphism was found in these species, males are larger than females.

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Table Contents

Table Contents (Continued)

Table Contents (Continued)

Table Contents (Continued)

Table Contents (Continued)

Table Contents (Continued)

List of Tables

List of Tables (continued)

List of Figures

List of Figures (Continued)

List of Figures (Continued)

List of Figures (Continued)

List of Figures (Continued)

List of Figures (Continued)

CHAPTER 1
INTRODUCTION

1.1 Evolutionay History of The Chiroptera

Bats are one of the most diverse groups of mammals, with about 18 families, 17 subfamilies, 9 tribes and 1100 species, and are found in the majority of the world's habitats, with the exception of the most extreme desert and Polar Regions (Simmons, 2005). However, they are greatly impoverished in the fossil record (Teeling *et al.*, 2005) since they have small, light skeletons that do not preserve well and many live in tropical forests, where conditions are usually unfavorable for the formation of fossils (Carrol, 1988). Thus at present, we know relatively little about the early evolution of bats, although a range of molecular studies are starting to help us understand the complexities of bat phylogeny (Teeling *et al.*, 2005).

Bats are thought to have originated in the Laurasian land mass (the supercontinent which included North America, Europe and Asia), possibly in North America, during the early Paleocene (over 64 Mya) (Teeling *et al.*, 2005) or possibly the mid/late Cretaceous (70-100 Mya.) (Jepsen, 1970). Some suggested that the evolutionary ancestor of bats was a small, nocturnal, quadrupdal, and arboreal insectivore type creature and some mammal teeth from the Paleocene (65 to 54.8 Mya) of France show characters of both bats and insectivores (the group including the hedgehogs, shrews and moles of today). However, since only the teeth are known, it is not possible to determine the structure of the rest of the animal (Carroll, 1988).

Other recent studies suggest that bats are most closely related to the Dermoptera, a small order of mammals (two species) which includes the colugos or "flying lemurs" of the Philippines. Colugos do not fly, but can move by using a web of skin stretched between their arms and legs, rather like flying squirrels (to which they are not closely related). Perhaps surprisingly, bats are also related to the Primates, the mammal taxon that includes lemurs, monkeys, apes, and humans; and to the Scandentia, the Asian tropical tree shrews.

Proto-bats may have begun by gliding (Jones and Genoways, 1973) or hovering (Jepsen, 1970) from one tree to another tree catching insects, and other activities (Fenton, 1992). Subsequently, they evolved true powered flight and Microchiropterans also evolved a method of acoustic orientation, or echolocation, involving the production of high frequency sounds.

The first true bat fossils appear in deposits of the Eocene epoch (54.8 to 33.7 mya) (Carroll, 1988). Some, such as *Icaronycteris index* (Jepsen, 1966) from the marlstone of the Green River Formation of Wyoming, USA, have unusually complete preservation of the whole skeleton. Others are known from the Messel Shale of Germany (Thomas, 1997). *Icaronycteris index* shows specializations of the auditory region of the skull that suggest that this bat could echolocate. It was assigned to the Microchiroptera on account of anatomical features such as the morphology of the upper molars, the advanced type of shoulder articulation of the humerus and scapula, and the long tail (Thomas, 1997).

Moreover, in the classical taxonomy of bats, there are two distinct suborders assemblage, first, Megachiroptera and second Microchiroptera. The Megachiroptera are represented by one family, the Pteropodidae (fruit bats), which are restricted to the Old World tropics of Africa, Asia and Australasia (Smith, 1977). Megachiropterans usually have a claw on the second finger of the wing and include the largest known bats, such as *Pteropus vampyrus*, which may have a wingspan of nearly two metres. Fruit bats usually have longer muzzles than microbats and have large, light-sensitive eyes for navigation and sensitive noses to determine the presence of ripe fruits (Altringham, 1996). Megachiropterans of the genus *Rousettus* can also use a primitive form of echolocation which is generated by the clicking of their tongues.

In contrast, the Microchiropterans, or "microbats," generally navigate by sending out pulses of high frequency sound and analysing the returning echoes. They tend to have short faces and well-developed tails and lack a claw on the second finger (Altringham, 1996). The modern Microchiroptera are divided into 18 families (Simmons, 2005) and have been grouped into a number of superfamilies by a range of researchers. For example Smith (1977) recognised four superfamilies: the Craseonycteridea; the Rhinolophidea, which includes the families Nycteridae,

Megadermatidae, Rhinolophidae and Hipposideridae; Phyllostomoidae, which includes the families Noctilionidae, Mormoopidae and Phyllostomidae; and the Vespertilionoidae, which includes the families Natalidae, Furlipteridae, Thyropteridae, Myzopodidae, Vespertilionidae, Mystacinidea and Molossidae. A fifth superfamily, the Palaeochiropterygoidae includes three families of fossil microchiropterans (all extinct), the Palaeochiropterygidae, Archaeonycteridae and Icaronycteridae.

Some 52 to 50 Mya, four major microbat (echolocating) lineages [Rhinolophoidea, Emballonuroidea, Noctilionoidea and Vespertilionoidea] appeared within a narrow time frame. This coincided with a period which included a 7°C rise in mean annual temperature, a significant increase in plant diversity and the peak of Tertiary insect diversity. Teeling *et al.*, (2005) suggest that microbat diversification was in response to an increase in prey diversity and that the varied microbat echolocation and flight strategies may have resulted from differential niche exploitation at that time. Today, at least 13 species are known from the Eocene period and have been classified into six families (Fenton, 1992). It is thought that the general composition of chiropteran faunas across the world had been established by the late Miocene to early Pliocene (Smith, 1977).

The oldest megachiropteran (flying fox or fruit bat) is *Archaeopteropus transiens* (Meschinelli, 1903), which is known from an early Oligocene (33.7 to 23.8 mya) site from Italy. However, the dentition of this fossil closely resembles that of the Microchiroptera (Russell and Sigue, 1970; Slaughter and Walton, 1970) and Smith (1976) suggested that these taxa represent an extinct clade of early microchiropterans ("Palaeochiropterygoidea"). In contrast, Van Valen (1979) argued that these fossil forms are representatives of a primitive grade ancestral to both Megachiroptera and Microchiroptera ("Eochiroptera"). Novacek (1987) reanalyzed morphology of *Icaronycteris* and *Palaeochiropterx* and concluded that they are more closely related to Microchroptera than to Megachiroptera. Most recently, Simmons and Geisler (1998) found that *Icaronycteris, Archaeonycteris, Hassianycteris*, and *Palaeochiropteryx* represent a series of consecutive sister-taxa to extant microchiropteran bats. The first true megachiropteran having dentition adapted to a frugivorous diet is thought to be *Propotto leakeyi* from early/middle Miocene (23.8 to

5.3 mya) of Africa (Marshall, 1983). Teeling et al (2003), primarily on the basis of molecular data, suggest that three of the four major microbat lineages are Laurasian in origin. These include all the Old World bats. The fourth lineage is Gondwanan (the supercontinent which included South America, Africa, India, Australasia and Antarctica).

Until the 1970s, the most evolutionary biologists assumed that bats form a monophyletic group. Recently, however, several authors have questioned the monophyly of Chiroptera (Jones and Genoways, 1970; Smith, 1976, 1977; Smith and Madkour, 1980; Hill and Smith, 1984; Pettigrew, 1986, 1991a, 1991b; Pettigrew and Jamieson, 1987; Pettigrew et al., 1989, Teeling et al., 2005) creating what has become known as the "bat monophyly controversy".

Theorems of the hypothesis that bats are diphyletic pointed out that many similarities between Megachiroptera and Microchiroptera involve the flight mechanism. It is therefore possible that convergent evolution of aerial movement, rather than shared ancestry, might explain the similarities found between megachiropteran and microchiropteran bats (Jones and Genoways, 1970). The monophyly versus diphyly debate has flourished, as scientists on both sides of the discussion appear to have strong evidence to corroborate their theories.

A third view has recently been advanced by Teeling et al. (2002, 2005). They also believe in diphyly but in their concept, instead of the Mega and Micro bats forming separate lineages, the fruit bats (Pteropodidae) are included with four families of microbats (the Rhinolophidae, Megadermatidae, Craseonycteridae and Rhinopomatidae) in the Yinpterochiroptera. They postulate that this group diverged from the Yangochiroptera (which includes 12 families of New and Old World bats: Emballonuridae, Nycteridae, Phyllostomidae, Mormoopidae, Noctilionidae, Furipteridae, Thyropteridae, Mystacinidae, Myzopodidae, Vespertilionidae, Molossidae and Natalidae) around 58 Mya. If this theorem is correct then bats either developed echolocation independently in the Yinpterochiroptera and the Yangochiroptera or echolocation was already developed prior to the division but was subsequently lost in the Pteropodidae (Teeling et al., 2005).

1.1.1 Bat Monophyly

The classical bat monophyly hypothesis states that the Megachiroptera and Microchiroptera are each other closest relatives in an evolutionary sense (i.e., they form a clade) (Wible and Novacek, 1988). This has much supporting evidence from morphological, molecular and biochemical studies. If it is true, then the shared characteristics, including the ability to fly, would have been present in their most recent common ancestor (Simmons, 1994; 1995). Simmons (1994) noted that, 'a large number of derived morphological characters indicate that the Chiroptera is a monophyletic taxon'. These characters represent diverse character systems including dentition (Beard, 1993), the skull and cranial vascular system (Wible and Novacek, 1988; Kay et al., 1992; Beard, 1993), the post cranial musculoskeletal system (Wible and Novacek, 1988; Thewissen and Babcock, 1991; Beard, 1993), foetal membranes (Luckett, 1980, 1993; Wible and Novacek, 1988), and the nervous system (Johnson and Kirsch, 1993). It has been argued that 'the case for monophyly of bats is based entirely on the wing' (Pettigrew, 1991a). However, there are over 25 synapomorphies representing many different anatomical systems (Simmons, 1994).

The Microchiroptera are distinguished by the following synapomorphies: a free premaxilla, only two lower premolars on each side of the jaw and a xiphisternum with a medial keel (Simmons and Geisler, 1998). However, many lineages within Microchiroptera have apparently secondarily evolved different conditions. Several other soft-tissue characteristics are found in Microchiroptera, but not in Megachiroptera. These characteristics include the presence of a tragus, a small or absent aquaeductus cochleae, the presence of a sophisticated echolocation system, the membrane of styloglossus originating from the ventral surface of the midpoint of the stylohyal, the clavicle articulating with the coracoids process of the scapula, the membrane of spinotrapesius clearly differentiated from the trapezius complex, the membrane of acromiodeltoideus not originating from the thoracic vertebra, the angle of the spinal cord between the dorsal horns from 0 to 25°, and the inferior colliculus larger than the superior colliculus (Simmons and Geisler, 1998). As it has not so far been possible to examine these features in fossil material collected, it is not known whether they also occurred in fossils which are closely related to Microchiroptera. Thus, these characteristics can only indirectly support microchiropteran monophyly.

In the past, molecular and biochemical analyses tended to support bat monphyly. Such studies include albumin immunological distance data (Cronin and Sarich, 1980), DNA-DNA hybridisation (Kilpartrick and Nunez, 1993), α- and β-globin amino acid sequencing (Stanhope et al., 1993), ε- globin gene nucleotide sequencing (Stanhope et al., 1993), interphotoreceptor retinoid binding protein gene nucleotide sequencing (Stanhope et al., 1992, 1993), cytochrome oxidase subunit II gene (COII) nucleotide sequencing (Adkins and Honeycutt, 1991) and 12 S rDNA gene nucleotide sequencing (Ammerman and Hillis, 1992). In addition, Novacek (1994) carried out the only study combining both morphological and molecular data (COII gene sequencing data), results of which also supported bat monophyly.

1.1.2 Bat Diphyly

The classical diphyly hypothesis states that megachiropteran and microchiropteran bats do not form a monophyletic group but have evolved independently from two different groups of non-flying mammals. The theorems of a diphyletic origin for the Chiroptera believe that mammals evolved flapping powered flight twice (Pettigrew, 1986). It has been suggested that the Megachiroptera are more closely related to Dermoptera and Primates than to the Microchiroptera (Smith and Madkour, 1980; Pettigrew, 1986, 1991a, 1991b, 1995; Pettigrew and Jamieson, 1987; Pettigrew et al., 1989). The Scandentia was also included within the same clade but with the Microchiroptera separated from the other Archontan orders. Fossil evidence (Beard, 1990; Kay et al., 1990) has suggested a sister group relationship between primates and Dermoptera. However, research by Thewissen and Babcock (1991) on the flight muscles of bats has united Dermoptera with a monophyletic Chiroptera, while research by Novacek *et al.* (1988) supported the unity of Dermoptera and Chiroptera, and additionally placed them with Primates and Scandentia.

The flying primate' hypothesis was considered to be highly controversial when it was first proposed by Pettigrew (1986), but does have much supporting evidence and has stimulated fierce debate. The first event is thought to have taken place in the Cretaceous, when an insectivore type mammal lineage evolved from leaping to full flight in pursuit of flying insects, so giving rise to the Microchiroptera. The second event is thought to have occurred in the Tertiary when

an early primate began to glide in search of fruits. Some of these gliders are believed to have evolved into the mammals now referred to the Megachiroptera (Pettigrew and Jamieson, 1987). In this case, the characteristics common to both groups of bats either evolved as a result of convergent evolution or are simply the result of retention of primitive features. If bats are diphyletic, the ability to fly must have evolved once in the Microchiroptera and again in the Megachiroptera. Although Thomas (1997) reviewed of the Linaeus book and stated that originally placed the Megachiroptera in the Order Primates, since that time the more traditional view of a monophyletic origin for the Chiroptera has been followed.

Although there have been numerous of studies using biochemical, molecular, and/or morphological data to analyze the relationship between Megachiroptera, Microchiroptera and other taxa, diphyly has only rarely been supported. However, Pettigrew (1986, 1991a, 1992b), Pettigrew and Jamieson (1987), Pettigrew *et al.* (1989) and Johnson and Kirsch (1993) found that there are features of the nervous system and penis which are shared by the Megachiroptera, primates, and Dermoptera but are absent from the Microchiroptera. These findings support a primate-megachiropteran link (Smith and Madkour, 1980). Pettigrew and his colleagues then reported that research on neural pathways had found several derived features of nervous system which were shared by both Primate and Megachiroptera, but which were also lacking in Microchiroptera (Pettigrew, 1986, 1991a, 1991b; Pettigrew and Jamieson, 1987; Pettigrew *et al.* 1989, and Pettigrew, 1995). These included reduction and specialisation of retinotectal pathway from the eye to the mid-brain, similarity of the cocticspinal motor pathway, the lamination pattern of the lateral genigulate nucleus, microcircuitry in the hipocampus, the threefold representation of the body surface in primary sensory cortices (Pettigrew, 1991a), and similarities in neocortex specialisation's Pettigrew, 1995). In addition, an independent series of investigations on neural pathway by Johnson, Kirsch and colleagues also supported bat diphyly (Switzer *et al.*, 1980; Johnson *et al.,* 1982a, 1982b, 1994; Kirsch, 1983; Kirsch and Johnson, 1983; Kirsch *et al.*, 1983, and Johnson and Kirsch, 1993).

However, biochemical and molecular studies have provided mixed support for the 'flying primate' hypotheses. Analyses of β-globin amino acid

sequence data (Pettigrew *et al.,* 1989; Pettigrew, 1991a) provided inconclusive, as did haemoglobin amino acid sequence data (Pettigrew, 1991a), rDNA restriction site mapping (Baker *et al.,* 1991), and 12S rDNA gene nucleotide sequencing (Springer and Kirsch, 1993). However, Pettigrew (1994), on re-examination of the results obtained by DNA analyses, concluded that the AT bias in both megachiropteran DNA and microchiropteran DNA meant that results which appeared to support bat monophyly could be dubious. De Jong *et al.,* (1993) carried out αA-crytalin amino acid sequencing, results of which at first suggested that bats may be diphyletic, but the authors concluded that the data were 'indecisive as to the monophyletic or biphyletic origin of Microchiroptera and Megachiroptera'. However, research on monoclonal antibodies to serum proteins (Schreiber *et al.,* 1994) has provided support for bat diphyly, showing Megachiroptera and Microchiroptera form a close sister-group relationship. Humans were the only primate representative included in the analysis.

In contrast, monophyly has been supported in studies examining a large and diverse set of morphological features (Smith and Madkour, 1980; Novacek, 1988; Johnson and Kirsch, 1993; Szalay and Lucas, 1993), including those of the nervous and reproductive systems (Luckett, 1980a, 1993; Wible and Novacek, 1988; Kay *et al.,* 1992; Novacek, 992, 1994; Beard, 1993; Simmons, 1993a, 1994, 1995; Simmons and Miyamoto, 1996), DNA-DNA hybridization data (Kirsch *et al.,* 1995 and Kirsch, 1996), and rejects by molecular, biochemical and morphological data (Cronin and Sarich, 1980; Stanhope *et al.,* 1992, 1993; Adkins and Honeycutt, 1993; Honeycutt and Adkins, 1993; Sarich, 1993), and DNA nucleotide sequence data from mitochondrial and nuclear genes (Adkins and Honeycutt, 1991, Ammerman and Hillis, 1992; Stanhope *et al.,* 1992, 1993; Honeycutt and Adkins, 1993; Novacek, 1994, Miyamoto, 1996 and Porter *et al.,* 1996).

1.2 Bat Behaviour

1.2.1. Echolocation

All microbats emit echolocation calls (bisonar), which are vocalizations produced in the larynx. Calls are emitted through the mouth or the nostrils. Bats that emit calls through the nostrils, such as Phyllostomidae and

Rhinolophidae, often have complex folds and/or flaps surrounding the nostrils, which may affect the signal (Simmons and Geisler, 1998).

Returning echolocation calls are analyzed by the bats to learn about the surrounding environment. Microchiropteran bats are not the only animals that use echolocation. Toothed whales, some insectivores (e.g., shrews), oilbirds, and some swiftlets also use various forms of echolocation, and are low infrequency and clearly audible to humans (Fenton, 1983). The higher frequency sounds of bats are covering a range from 10 kHz to more than 200 kHz (Fenton, 1983). Echolocating bats typically emit an ultrasonic pulse with a frequency in excess of 15 kHz, and analyze the returning echo to determine the shape of, and distance to, an object (Fenton, 1992). Most bats alternate between emitting sound and listening for returning sound. The frequency, length of call, intensity, and degree of modulations of the emitted sound differs between species, and there may even be differences between individuals within a species (Fenton, 1992).

The ability to echolocate has allowed many bats to exploit flying nocturnal insects as a food source, as well as to live in dark caves. In neither situation can the bat successfully rely on vision alone to locate objects due to the limited amount of light. Most likely as a result of increased reliance on echolocation, microchiropterans have reduced vision capabilities, having lost some of the complexity found in the eyes and brains of megachiropteran bats. While echolocation has many benefits, it also has costs. The most pronounced is that other animals can often hear the signals emitted by bats. Who are able to hear the sounds include other bats, potential predators and prey? Some moths have evolved complex ears, apparently for listening to bats. When such a moth hears the echolocation calls of an approaching bat, it begins evasive manoeuvres. Some insects actually emit sounds in response to bat calls. This apparently confuses the bat although it does not directly jam the signal.

1.2.2 Dietary System

The widespread distribution of bats has led to an array of eating habits, which is nearly as broad as that found in all mammals. Although, 600 bat species are eatting insects as the main dietary staple (Fenton, 1983). Their diet includes insects,

pollen, fruits, flowers, flesh and blood (Gillette, 1975). Many microchiropterans are exclusively insectivorous, while most megchiropterans are exclusively frugivorous or nectivorous, but some bat family show a wide range of eating habits. The Phylostomidae (Microchiroptera) is divided into six subfamilies comprising 240 species which between them exhibit all the known chiropteran feeding habits, with the exception of piscivory (fish eating) (Gardner, 1976).

Insects are abundant throughout most of the world, and therefore an important source of food for many vertebrates. Bats have learned to exploit dusk and night flying insects, with 79 out of the total 169 genera of bats, including *Rhinolophus, Myotis, Pipistrellus, Eptesicus, Lasiurus, Taradida* and *Eumops* being specialised feeding on insects on the wing. Aerial insectivory and foliage gleaning are common feeding habits in both temperate and tropical regions (Wilson, 1973). Bats can consume between one quarter and one half of their own body weight in insects each night. Small insectivorous bat species eat at least 30 percent of their body weight for each night and usually, most of bats drink water by flying low and dipping their mouths into the water surface of a lake or streams, but some bats live in desert areas, they never drink, and replying on insect prey for their water requirements (Fenton, 1983).

Carnivory is not widespread amongst bats, but certain large size species, such as False Vampires (Megadermatidae), Slit-faced bats (Nycteridae) and New world Leaf-nosed bats (Phyllostomidae) regularly eat other bats, small rodents, birds, frogs and lizards. It would appear that bats use the sounds made by their prey as a method of detection. Carnivory is believed to be an extension of insectivory, as the dentition of carnivorous bats is only slightly modified from that of insectivorous species (Glass, 1970).

A further dietary specialisation of the Chiroptera is piscivory, or fish-eating. Only a small number of bats are known to specialise in this way, these include the Greater Bulldog bat, *Nctilio leporinus* (Noctilionidae) from the Caribbean and Central and South America, and aptly named Fish-eating bat, *Pizonyx vivesi* (Vespertilionidae) from Central America. These species also use echolocation call to detect their prey, which they then scoop from the water with their enlarged, clawed feet (Glass, 1970). They are occasionally insectivorous, and it is though that the fish

eating behaviour may have evolved as a result of catching insects from the surface of the water (Gillett, 1975).

Sangivory, feeding on blood, is practised solely by three species of true vampires in the family Phyllostomidae (subfamily Desmodontinae). These species are virtually restricted to the New World tropical and subtropical regions, with their distribution just reaching the temperate areas of North and South America. They feed exclusively on the blood of mammals, including human and birds, making them a serious agriculture and public health pest. Adaptation to a blood diet has involved specialisation of certain parts, of the body, particularly the dentition (Glass, 1970) and the kidneys (Wimsatt and Guerriere, 1962). It is postulated that sangivory may have involved from bats feeding on the external parasites of mammals, e.g. ticks, mites, with the food source duality of insects and blood (as the removal of ticks usually produces blood) ultimately being relinquished for a solely sangivorous diet (Gillett, 1975).

Frugivory and nectivory, the consumption of fruits, flowers, nectar and pollen, is common amongst bats inhabiting tropical and subtropical regions. Approximately 29% of the known species of bats, the Old world fruit bat (Pteropodidae) and many species of the family Phyllostomidae, are partially or wholly dependant on plants as a source of food (Fleming, 1982). Frugivorous bats have a varied diet, consuming both wild fruits and cultivated cash crops, so pollinating (chiroptergamy) or dispersing (chiropterochory) the seeds of hundreds of species of plants (Fleming, 1993). Through their plant interactions, bats have contributed considerably to the diversity of angiosperms at the generic and specific level (Fleming, 1979), and in turn the increased diversity of plant life has had a diversifying effect on bat population (Fleming, 1982).

A frugivorous diet provides far more carbohydrate than is necessary for body maintenance, and it is believed that this excess may be used as 'fuel' for long foraging flights (Marshall, 1983). Bats that feed primarily on nectar and pollen include members of the subfamily Macroglossinae (Pteropodiade) from the Old World tropics, and subfamilies Glossophaginae and Brachyphyllinae (Phyllostomidae) from the New World tropics. These bats are highly specialised, being delicate and light, with slender muzzles and long, extensible tongues (Glass,

1970). They are able to hover at the mouths of flowers while and feeding much likes their diurnal counterparts, hummingbirds. Many plants rely on bats for pollination, which has led to a change in the morphology of flowers and fruits, such as the timing of flower and fruit production, the size of the flowers or fruit, and the nutritional characteristics of pollen, nectar and fruits (Fleming, 1982).

It is clear the bats are in a position to exploit many of the food sources available to them through the evolution of feeding specialisations. These abilities, combined with the highly sophisticated echolocation system (Microchiropterans), their ability to fly, and their ancient origins, makes the Chiroptera one of the world's most successful orders of mammals.

1.3 Systematics Review

The first comprehensive biological classification of plants and animals was by Aristotle (384 – 322 B.C.E). His observations on the anatomy of octopus, cuttlefish, crustaceans and many other marine invertebrates are remarkably accurate, and could only have been made from first-hand experience with dissection. Aristotle described the embryological development of a chick; he distinguished whales and dolphins from fish (Thompson, 1994, 2000).

Aristotle's classification of animals grouped together animals with similar characters into genera (used in a much broader sense than present-day biologists use the term) and then distinguished the species within the genera. He divided the animals into two types: those with blood, and those without blood (or at least without red blood). These distinctions correspond closely to our distinction between vertebrates and invertebrates. The blooded animals, corresponding to the vertebrates, included five genera: viviparous quadrupeds (mammals), birds, oviparous quadrupeds (reptiles and amphibians), fishes, and whales (which Aristotle did not realize were mammals) (Thompson, 1994, 2000). However, he erected collective categories on the basis of differentiating characters, such as hairy versus feathered, bipedal versus quadrupedal and although he did not devise a fully consistent classification animals (Mayr, 1982; Pellegrin, 1986), his way of thinking was followed by scientists for the next 2000 years.

Plant classification was not revised until between the 16th and 18th centuries with the work of Cesalpino (1519 – 1603) and Linnaeus (1707 – 1778). Linnaeus's plant taxonomy was based solely on the number and arrangement of the reproductive organs; a plant's class was determined by its stamens (male organs), and its order by its pistils (female organs). This resulted in many groupings that seemed unnatural. The sexual basis of Linnaeus's plant classification was controversial in its day; although easy to learn and use, it clearly did not give good results in many cases (Thompson, 1994, 2000).

While animal taxonomy was dominated in the 18th century by the work of Linnaeus and Buffon (1707 – 1788), the method of classification used at that time was called downward classification, and involved the dichotomous splitting of a large group into two smaller groups e.g. animals-with or without blood, animals with blood that were hairy or not hairy (Mayr and Ashlock, 1991).

Zoological and most botanical taxonomic priority begin with Linnaeus: the oldest plant names accepted as valid today are those published in *Species Plantarum* (1753), while the oldest animal names are those in the tenth edition of *Systema Naturae* (1758), which was the first edition to use the binomial system consistently throughout (Thomas, 1997). Although Linnaeus was not the first to use binomials, he was the first to use them consistently, and for this reason, Latin names that naturalists used before Linnaeus are not usually considered valid under the rules of nomenclature. Linnaeus was at the forefront of systematics in the 18th century, and in addition to classifying animals, he devised identification keys, standardised synonymies and invented a system of binominal nomenclature, which meant that a system was applied to taxonomic practice. Nevertheless, Linnaeus's hierarchical classification and binomial nomenclature, in a modified state, have remained standard for over 200 years (Thomas, 1997).

The writings of Linnaeus have been studied by every generation of naturalists, including Erasmus Darwin and Charles Darwin. The search for a "natural system" of classification is still going on. Today however, emphasis is placed on discovering the evolutionary relationships of taxa (Thompson, 1994, 2000).

Buffon (1707 – 1788) was not a taxonomist, but his ideas on classification laid the foundations for the biological species concept and his emphasis

on the importance of characters led to a new approach to taxonomy. One hundred years before Darwin, Buffon, in his *Historie Naturelle,* a 44 volume encyclopedia describing everything known about the natural world, wrestled with the similarities of humans and apes and even talked about common ancestry of Man and apes. Although Buffon believed in organic change, he did not provide a coherent mechanism for such changes (Thompson, 1994, 2000). He thought that the environment acted directly on organisms through what he called "organic particles". Buffon also published *Les Epoques de la Nature* (1788) where he openly suggested that the planet was much older than the 6,000 years proclaimed by the church, and discussed concepts very similar to Charles Lyell's "uniformitarianism" which were formulated 40 years later (Thompson, 1994, 2000). Towards mid 18th century, downward classification was viewed as artificial and was gradually replaced by upward classification. This involved forming a hierarchy by successively assembling taxa into groups of similar or related species rank by rank, and was practised by many posts – Linnaean zoologists (Thomas, 1997).

Many taxonomic progresses were made in the time between Linnaeus and Darwin (1809-1882). Taxonomists became more specialised, working on only one group of plants or animals. Classification became more resolved, with the addition of extra categories including family and phylum, and more empirical. Taxonomists began look for a 'natural' system, which grouped species with common characteristics (Thomas, 1997). In 1859, Darwin published "The origin of species by means of natural selection", which have explained how to members of one taxa were more similar to each other than they were to members of other taxa, a question which had been puzzling to many taxonomists. Darwin's theory of evolution by common descent stated that natural groups existed because members of a natural taxon were descendants of a common ancestor and were therefore more likely to be similar. This theory led to much research into missing links between seemingly unrelated taxa, and the construction of phylogenetic trees, so leading to more work in comparative systematics, comparative morphology and comparative embryology (Thomas, 1997).

The study of intraspecific variation between populations, or population systematics, also contributed to a change in taxonomic thinking. Studies of variation led to the delimitation of lower taxa and categories, a re-evaluation of the species

concept, and a biological approach to taxonomy. Other aspects of species were considered, such as behavioural characters, ecological requirements, physiology and biochemistry, which in turn led to more experimental techniques being used in taxonomic research. Much of this research was concentrated at the species level, while macrotaxonomy received little attention from the 1870's to the 1950's. The development of numerous molecular techniques for testing relationships however, led to a surge in research into higher taxonomy (Mayr and Ashlock, 1991). Although considerable proportions of the world's species have been described there is sill much-undiscovered fauna, particularly from tropical and marine regions. In addition, the higher taxonomy of the most groups of animals is unclear and it is hoped that molecular techniques, such as protein and nucleic acid analysis, combined with morphological studies will play a key role in clarifying some of these relationships.

Many species concepts have been proposed in an attempt to facilitate the assembling of phena into biologically meaningful taxa at the species level. These concepts fall into five groups; the typological species concept, the nominalistic species concept, the biological species concept, the phylogenetic species concept and the evolutionary species concept. The first two have only historical significance, although they are still upheld by some current authors. The typology species concept, or essentialism (Mayr, 1982), relates to the philosophy of Plato, and was followed by Linnaeus (Cain, 1958). It states that species consist of similar individuals sharing the same essence, that each species is separated from all others by a sharp discontinuity, that each species is completely consistent through time, and that there are strict limits to the possible variation within any one species (Mayr and Ashlock, 1991). The shortcomings of this concept are that individual organisms found in nature that are conspecific, can appear to be different due to forms of individual variation, such as sexual dimorphism, age difference or polymorphism. In addition, there are species, known as sibling species, which are vary similar morphologically, but which are proven to be separated species. Therefore, degree of difference cannot be considered decisive in the ranking of taxa as species. The second philosophy, not widely followed today, is the nominalistic species concept, which was particularly popular in France in the 18th century. It states that only individuals exist in nature, while the species is an entity invented by scientists (Mayr and Ashlock, 1991).

As the shortcomings of these two theories were realised in the late 18th century, a new theory, the biological species concept, began to emerge with Jordan (1905) being the first nominalistic concepts in stating that species have independent reality and are typified by populations of individuals, but differed by claiming the importance of genetic cohesion within species and by stressing that species are created by shared information received from the gene pool (Mayr and Ashlock, 1991). Within this concept, members of a species can be seen from three different points of view; they form a reproductive community, ensuring intraspecific reproduction, they form an ecological unit, interacting with the other species in the same environment, and they form a genetic unit, consisting of a large, intercommunicating gene pool. The definition of a species as derived from this concept is "A species is a group of interbreeding natural populations that is reproductively isolated from other such groups" (Mayr, 1942).

In normal taxonomic practice however, it is usually morphological or genetic differences, which define the species, rather than reproductive isolation. This conflict between theoretical and practical application has posed many problems for taxonomists and has been much-discussed (Sokal, 1973; Lovtrup, 1979). However, the biological species concept is employed by many biologists, particularly ecologists, physiologists and behavioural biologists. The strongest arguments against the biological species concept come from phylogeneticists. The concept has been criticised on the grounds of it not necessarily yielding monophyletic species (Donoghue, 1985) and due to relatedness and the ability to reproduce not being as tightly linked as many assumed and possibly varying between major taxa (Baum, 1992). In addition, many botanists due to plant hybridisation have specifically rejected the biological species concept. Another problem with the concept is that it can be applied with ease to sympatric populations but not so easily to genetically isolate allopatric populations (Cracraft, 1982).

Due to the apparent failings of the biological species concept, a new concept, the phylogenetic species concept was supported by (Cracraft, 1983). The phylogenetic species concept and its subsequent numerous modifications, differs from the biological species concept in that it does not consider present biological characters directly but rather the acquisition of defining features during evolution (Donoghue,

1985; Baum, 1992). As such, the following definition was supported: "A species is the smallest possible group of a sexually reproducing organism that possesses at least one diagnostic character which is present in all group members but it is absent from all close relatives of the groups". One of the drawbacks of this concept is that diagnostic character can either be a plesiomorphy (the ancestral states of a character within a group of organisms) or an autapomorphy (derived characters unique to a single taxon). As a result, any species defined by a plesiomorphy would be paraphyletic. It could include a common ancestor whole membership is defined by the possession of a uniquely derived character state, but omits descendants within which the character state has subsequently undergone one or several reversals (Quicke, 1993). Wheeler *et al.* (1990) noted that the application of the phylogenetic species concept would almost certainly give considerably larger estimates of the total number of species than the more traditional biological species concept. Mayr (1992) illustrates this point by reference to work by Rosen (1979) on South American poeciliid fish. Application of the phylogenetic species concept in this case would mean that the distinct populations of many species inhabiting almost every tributary would have to be raised to species rank, entailing a seemingly unnecessary degree of complication.

The evolutionary species concept was supported by Simpson (1961) and as the term suggests, it used"evolutionary" criteria for the definition of species. Simpson's definition was follows: "An evolutionary species is lineage (an ancestral-descendant sequence of populations) evolving separately from others and with its own unitary evolutionary role and tendencies". However, Mayr (1982) noted that this is definition of a phyletic lineage rather than species, and is equally applicable to many isolated population or incipient species. The primary problem with the evolutionary species concept is considered to be that the causation and maintenance of discontinuities between contemporary species is not addressed (Mayr and Ashlock, 1991).

The concept of a subspecies or race (here considered as the same rank) is even more complex. Mayr and Ashlock (1991) proposed the following definition "A subspecies is an aggregate of phenotypically similar populations of species inhabiting a geographic subdivision of the range of that species and differing taxonomically from other populations of that species". Problems with the subspecies

concept with significantly reduce its usefulness include the tendency of different characters to show independent trends of geographic variation, the independence occurrence of similar or phenotypically indistinguishable populations in geographically separated areas, the occurrence of microgeographic races within formally recognised subspecies and arbitrariness of the degree of difference proposed by different scientists as justifying subspecific variation of slightly differentiated local populations (Mayr and Ashlock, 1991). Nevertheless, although the subspecies concept has been widely criticised (Wilson and Brown, 1953; Inger, 1961) it is considered to be valuable tool when applied to allopatric taxta that differ only to a degree commonly found within interbreeding populations and parapatric taxa with a considerable degree of hybridisation (Corbet, 1997). Ideally, subspecies would only be formally recognised where 90% of one population was distinguishable from 90% of the other population (Dadd, 1970), although this is ver difficult to apply in practice. Where few specimens were available or only a few individuals differed from the local race, the differences were commented on, but not recognised at subspecific level. This avoided the proliferation of subspecies of doubtful credibility, which might lead to confusion and an increased workload for future researchers.

In the 1960's the need for a more objective taxonomy led to the independent emergence of two new rigorous areas of systematics. Numerical phenetics was developed and popularised by Sokal and Sneath in their classical text 'Principles of Numerical Taxonomy' (1963). Numerical phenetics is defined as 'the methodology of assembling individuals into taxa on the basic of an estimate of unweighted overall similarity' (Mayr and Ashlock, 1991). The primacy given to similarity assumes that the more similar two taxa are the more closely related they are likely to be. The proposed theory advocated the use of a polythetic (taxa based on the greatest number of shared characters) system for defining groups, as it was not necessary for the numbers of the group to process all the definitive characters. This meant that possession of some minimum number of a set of characters would justify placement of a taxon in that group, but the taxon would not be required to display all of the character states in the polythetic set. Pheneticists therefore proposed that higher taxa could be defined through the use of cluster analysis such that included numbers would on average resemble each other more than they would resemble non-numbers

(Quicke, 1993). However, the results obtained were not intended to depict phylogeny and could be misleading if they were interpreted in this way. The main argument against numerical phenetics is that a method which ignores the necessity of weighting characters and the recognition of monophyletic groups cannot lead to stable and sound classification (Mayr and Ashlock, 1991). As such, phenetic methods lost popularity due to the rise of cladistics.

German entomologist Willi Hennig (1913 - 1976) devised Cladistics in 1950. He asserted that classifications should reflect the evolutionary history of the group and devised a set of principles, which would allow unambiguous genealogical classifications to be established. His theory stated that taxa based exclusively on the possession of shared devised characters (synapomorphies) should be recognised, while ancestral characters (plesiomorphies) should be ignored and that every taxon should be monophyletic thereby taking the emphasis away from similarity (Mayr and Ashlock, 1991). Cladistics allowed the formulation of explicit, potentially testable hypotheses for the origin of morphological, physiological or behavioural character states (Buth, 1984; Patton and Avise, 1983).

Methodology has advanced greatly over the last 25 years with the development of computing in taxonomy, one of the consequences being that far larger data sets could be used in cladistic analysis. However, this also meant that characters might be included, which were not wholly reliable indicators of phylogeny. These incongruences, collectively termed homophasy could lead to conflicting evolutionary hypotheses. One of the basic tenets of much of cladistic analysis is the most likely explanation of a taxonomic data set is the one is requires the least number of evolutionary changes, or character state transitions (Felsentein, 1978; Friday, 1982; Farris, 1983, 1986). The trees derived from the data set which requires the fewest character state transitions are known as the most parsimonious ones and methods used to find these most parsimonious solutions are accordingly referred to parsimony analysis. To date, parsimony is considered to be the best criterion for phylogenetic analysis (Sober, 1988).

Taxonomy, particularly phylogenetic studies is fundamental and underpins other areas of biology. Reliable phylogenetic hypotheses can be used to generate experimentally testable predictions about the evolution of many biological

systems from biochemical and physiological to behavioural studies, but only if the phylogeny is accurate (McLennen *et al.*, 1988). Phylogenetic studies permit the separation of potentially conflicting phylogenetic factors from studies of comparative biology. In addition, phylogenetic hypotheses can help to construct the evolutionary sequence involved in the development of a trait in the behaviours, which can be tested experimentally (McLennen, 1991). This ability of independently constructed phylogenetics to generate hypotheses about evolutionary changes in other character systems is considered to be important feature of cladistic analysis (Quicke, 1993).

In addition to numerical techniques in taxonomy, molecular phylogenetics has become an essential scientific discipline (Avise, 1994). However, prior the late 1980's, broad controversies in evolutionary biology dominated the field molecular evolution and related disciplines. In brief, these concluded the classical - balance debate on the magnitude of genetic variation, the selection – neutrality debate on the adaptive significance of molecular variation, the phenetic – cladistic debate concerning the interpretation of molecular or other data in a systematic context and relative to phylogenetic utility of molecular versus morphological characters (Avise, 1994). While these controversies were under debate, research was carried out which centred on the application of protein and DNA markers to problem in natural history and evolution. In this area of research, new laboratory methods are constantly becoming available and molecular systematics has advanced in tandem with these new techniques. In the mid 1960's, protein electrophoresis as applied to allozymes and isozymes was dominant technique (Buth, 1984; Whitt, 1987) and it is still used for generation molecular markers. In the 1970's and 1980's, the analysis of restriction fragment length polymorphisms (RFLP's) was favoured, particularly using mitochondrial DNA (Harrison, 1989; Wilson *et al.*, 1985), which in turn led to the development of the procedures known as "DNA fingerprinting" (Burke, 1989; Kirby, 1990). Currently, the introduction of polymerase chain reaction (PCR) for the amplification of specific DNA fragments (Erlich and Arnheim, 1992) in conjunction with the development of amplification primers (Kocher *et al.*, 1989) has greatly improved methods for sequence determination (Innis *et al.*, 1988). PCR based methods are valuable as increased access to polygenetic information content of DNA sequences is allowed for both nuclear and cytoplasmic genes. In addition, the PCR

has extended molecular application as DNA segments can be amplified from tiny amounts of starting tissue, including museum – preserved material (Higuchi *et al.*, 1984) and fossil material (Arnheim *et al.*, 1990). Other, less widespread techniques include immunological comparisons of proteins (Wilson *et al.*, 1977) and DNA – DNA hybridisation methods (Britten and Kohne, 1968).

Molecular studies can be integrated into a wide variety of biological disciplines, such as ethnology, field ecology, comparative morphology, systematics and palaeontology. From a systematic viewpoint, molecular and morphology approaches have sometime been viewed in opposition, however more recently it has been recognised that molecular and morphological data can be reciprocally informative (Hillis, 1987, Thomas, 1997, 2000). It is believed that molecular markers are useful most effectively when they address controversial areas or are employed to analyse problems in natural history and evolution, which have proved to be beyond the scope of traditional non-molecular methods. In such cases, the interaction between these alternative lines of evidence is of greater significance than when either data source is considered alone (Avise, 1994).

It is clearly that multiple data classes contain a greater amount of information, and as a result analyses which capitalise on this should pave for more studies combining molecular and morphological based systematics to be undertaken.

1.4 The Rhinolophidae

The family Rhinolophidae comprises a single genus, Lacépède, 1799 (Simmons, 2005). It has an extensive fossil record and is known from the Eocene (54-35 million years approx.), Oligocene (35-24 million years approx.), Miocene (24-5 million years approx.) and Pliocene (5-1 million years approx.) of Europe; the Miocene of Australia and the Miocene and Pliocene of Africa (Savage and Russell, 1983).

It is geographically widespread Rhinolophid bats are found in a variety of biomes throughout the temperate, subtropical and tropical regions of the Old World from Europe to Japan, through to Africa, Southeast Asia, the Philippines, New Guinea and Australia (Corbet and Hill, 1992). They are all insectivorous and hawk for insects in flight from a variety of surfaces as they forage within forests or in open spaces. To

locate insect' bats will swoop low over the surface of lakes, snap them out of the air, and even land on the ground and pursue them on foot, but some of insectivorous bats grab their victims directly in their mouths (Fenton, 1983). Insects may be caught in flight, or taken from vegetation, the ground, or water surfaces in a foraging style referred to as gleaning (Hutson *et al.,* 2001). Their roosts and associated colony sizes are diverse and in some species vary on a seasonal basis (Nowak, 1991a; Nowak, 1991b; Vaughan, *et al.*, 2000). Some species prefer caves, abandoned mines and old houses, others hollow trees and yet others have their diurnal roosts in the open, among the branches of trees (Hill and Smith, 1984; Nowak, 1991a; Nowak, 1991b; Vaughan, *et al.*, 2000).

Rhinolophid bats vary greatly in size from small to moderately large, with a body mass. Echolocation calls are emitted through complex nasal structures, which serve to focus the sound. The ears tend to be large and lack a tragus (Hill and Smith, 1984; Nowak, 1991a; Nowak, 1991b; Vaughan, *et al.*, 2000).

1.4.1 Taxonomic Review of The Rhinolophidae Family

The Family Rhinophidae was named by Gray (1825) Subsequently Dobson (1876) recognised the genus *Rhinolphus* as a distinct taxon within the Rhinolophidae and promoted it to subfamily status. Miller (1907) considered the Rhinolophidae to be monogeneric and other taxa formerly included in the group were placed by him in the Hipposideridae Miller, 1907. This view was followed by Simpson (1945), Hill (1982) and Corbet and Hill (1992) and Simmons (2005). However, others have continued to regard the hipposiderid bats as a subfamily, the Hipposiderinae Lydekker, 1891 of the Rhinolophidae (Ellerman and Morrison-Scott, 1951; Koopman and Jones, 1970; Koopman, 1993).

The first comprehensive review of the genus *Rhinolophus* was published by Andersen in a series of papers (1905a, 1905b, 1918). In Andersen (1905b) the genus was divided into six groups: *simplex, lepidus, midas, philippinensis, macrotis* and *arcuatus.* Later Andersen (1918) renamed them as the *megaphyllus, pusillus, hipposideros, luctus,* and *euryotis* groups respectively. There was no mention of the *macrotis* group.

Tate and Archbold (1939) listed Andersen's synoptic arrangement and updated his subgroups to include species and subspecies described since 1918. The group names were again slightly changed with the *megaphyllus* group becoming the *ferrumequinum* group. Although, few taxonomic changes were made, *macrotis* and its allies were listed within the *philippinensis* group. In Tate (1943), Andersen's arrangement of the *philippinensis* group was considerably altered by dividing it into three, namely the *philippinensis, trifoliatus* and *luctus* subgroups. *R. coelophyllus* was moved from the *arcuatus* group to the *philippinensis* group, while *R. pearsoni* was associated with the *luctus* group. Later than Ellerman and Morrison-Scott (1951), followed the arrangement of Tate (1943) but noted that as *trifoliatus* predated *luctus* the *philippinensis* group should be known as the *trifoliatus* group. However, to avoid taxonomic confusion the name *luctus* was retained.

Topal (1975) reviewed the taxonomy of the Rhinolophidae with particular reference to bacular morphology. Bogdanowicz and Owen (1992) undertook a phylogenetic analysis of the Family and included the findings of an electrophoretic study by Qumsiyeh *et al.* (1988). The result of both studies generally supported prior phenetic classification of the family. Corbet and Hill (1992) reviewed the Rhinolophidae of the Indo-Malayan Region and divided them into the *philippinensis, arcuatus, fumigatus, pusillus, ferrumequinum* and *hipposideros* groups. Bates and Harrison (1997) discussed the taxonomy, distribution and ecology of 16 species of *Rhinolophus* from the Indian Subcontinent. Koopman (1993) carried out a further review of the genus. He included 64 species and listed distributions and synonymies world-wide. Thomas (1997) undertook a taxonomic review of a number of taxa within Corbet and Hill's (1992) *ferrumequinum* group.

Most recently, Csorba et al. (2003) summarised all available information on the Family from throughout its range. He included seventy-one species and 15 groups. The Eurasian species were assigned to 11 groups, namely *euryale, euryotis, ferrumequinum, hipposideros, landeri, megaphyllus, pearsoni, philippinensis, pusillus, rouxi* and *trifoliatus*.

1.4.2 Current Study

For the current study, four species (sensu Csorba et al., 2003) were included. They are *R. affinis* Horsefield, 1823, *R. rouxii* Temminck, 1835, *R. thomasi* Andersen, 1905a, and *R. sinicus* (Andersen, 1905a). Until recently, they have generally been considered to be closely related to one another (all are included in the *R. ferrumequinum* group of Corbet and Hill, 1992) and prior to Thomas (2000), *sinicus* was generally treated as a race of *rouxii*. The geographical range of the study was restricted to the Indian Subcontinent and mainland SE Asia as this area circumscribes the distributions of all four taxa.

Andersen (1905a and 1905b) included all four taxa in the *Rhinolophus simplex* species group. Tate and Archibold (1939) also included them in the *R. simplex* group, with *rouxii, sinicus,* and *thomasi* in the *rouxii* subgroup and *affinis* in the *affinis* subgroup. They were included in the *R. ferrumequinum* group by Corbet and Hill (1992). Bogdanowicz (1992) placed *rouxii, sinicus* and *thomasi* in the *rouxii* subgroup of the *megaphyllus* group. He also included *affinis* in the same subgroup but indicated some element of doubt. The phylogenetic allegiances of the four species were not clearly defined in Bogdanowicz and Owen (1992). However, Csorba et al. (2003) placed *rouxii, sinicus* and *thomasi* in the *rouxii* group but moved *affinis* (together with *stheno*) back to the *megaphyllus* group. They believed that the *rouxii* group was different to such a magnitude that it should be included in a separate subgenus, which they named *Indorhinolophus*, with the type species being *R. rouxii* Temminck, 1835. Based on their molecular studies they suggested that the *affinis* group had diverged from the *rouxii* about 12 MYA. Subsequently, Simmons (2005) followed Csorba et al (2003) but omitted any reference to *Indorhinolophus.*

However, despite the findings of Csorba et al (2003), the four taxa were chosen for study because current morphometric characters employed to discriminate between them remain unsatisfactory and it is difficult to identify the species with certainty. In particular, it is difficult to distinguish *R. affinis* from *R. rouxii* (despite their inclusion in different subgenera) and *R. sinicus* from *R. thomasi.*

1.4.3 Current Taxonomic Problems Within Group

Below are listed some of the taxonomic problems which will be studied in the forthcoming chapters.

1.4.3.1 *Rhinolophus affinis*

(1) Problems in defining *R. affinis* as compared to *R. rouxii.* This was recently illustrated in Tanintharyi Division, southern Myanmar, where specimens were collected that shared some external and cranial characters with both taxa (Paul Bates, pers. comm.).

(2) Echolocation data in Thailand suggests that there may be an additional cryptic species present within the currently defined *R. affinis.*

1.4.3.2 *Rhinolophus rouxii*

(1) Confusion as to whether an additional cryptic species of *R. rouxii* is present in Southern India and Sri Lanka (Thomas, 2000).

(2) Confusion as to whether *R. rouxii* occurs in Myanmar. In Thomas (1997), only one specimen (BMNH. 27.11.18.4) of *R. rouxii* is included from Myanmar but confusingly this same specimen is also referred to *R. affinis.* In Bates et al. (2004), following Thomas (2000), this specimen is referred to *R. rouxii.*

(3) Confusion as to whether *R. rouxii* occurs in Vietnam. None was listed in Thomas (1997; 2000). It was included without comment in the checklist of Hendrichsen et al., (2001) and tentatively included in Borissenko and Kruskop (2003).

1.4.3.3 *Rhinolophus sinicus* and *Rhinolophus thomasi*

(1) The relationships and specific boundaries between *R. sinicus* and *R. rouxii* and *R. thomasi* are not clear (Csorba et al., 2003).

(2) Specimens provisionally referred to *R. sinicus* from Vietnam may prove to be a distinct taxon (Csorba et al., 2003).

(3) *Rhinolophus sinicus* is most easily confused with *R. affinis,* from which it is best distinguished by its straight-sided lancet.

(4) The echolocation call of *R. sinicus* is a long constant frequency signal, with a brief frequency-modulated start and tail. Frequencies with most energy recorded from hand-held bats ranged between 80 and 88.2 kHz (n= 13). Some evidence of males calling at lower frequencies (80-84.2 kHz) than females (84-88.2 kHz), as found in the closely related *R. rouxi* in Sri Lanka which calls at 73.5 - 79 kHz (Neuweiler *et al.* 1987). Call frequencies overlap with those used by *R. sinicus*, and so is not a diagnostic feature for separating these species.

(5) Echolocation call frequencies of *R. sinicus* overlap with those emitted by *R. affinis*. *R. affinis* is also typically a larger species, though overlap occurs with *R. sinicus* at forearm lengths between 50-51 mm.

(6) *Rhinolophus sinicus* is very similar to the smaller *R. thomasi* of Myanmar, Vietnam, Lao PDR and Thailand, to which it is closely related.

(7) Call frequency for *R. thomasi* in Lao PDR is reported as 76 kHz (Francis & Habersetzer 1998), and so the two taxa may use different call frequencies.

To undertake the study, the current taxonomy and the diagnostic characters of each species was reviewed on a species by species basis.

CHAPTER 2
LITERATURE REVIEWS

The current taxonomy of *Rhinolophus affinis, Rhinolophus rouxii, Rhinolophus sinicus* and *Rhinolophus thomasi* has been study for longtime. The synonymies are based on Csorba *et al.*, (2003). They were described and given below:

2.1 *Rhinolophus affinis* Horsfield, 1823

Intermediate horseshoe bat

Rhinolophus affinis Horsefield, 1823: pt. 6; Java.

Rhinolophus andamanensis Dobson, 1872: 337; South Andaman Islands

Rhinolophus affinis himalayanus Andersen, 1905a: 103, pl.3; Mussoorie, Kumaon, north-west India.

Rhinolophus affinis tener Andersen, 1905a: 103, pl 3; Pegu, Myanmar.

Rhinolophus affinis macrurus Andersen, 1905a: 103; Taho, Karenee, Myanmar.

Rhinolophus affinis superans Andersen, 1905a: 104; Pahang, Malaysia.

Rhinolophus affinis nesites Andersen, 1905a: 104; Bunguran Island, north Natuna Islands.

Rhinolophus affinis princes Andersen, 1905a: 106, pl 3; Lombok, Lesser Sunda Islands

Rhinolophus hainanus Allen, 1906: 482; Poutein, Hainan Island, China

Rhinolophus affinis was described from Java (Horsfield, 1823) and the holotype (BMNH.79.11.21.70) deposited in the Natural History Museum, London. Through subsequent years, 8 additional taxa have either been described as geographical races of *affinis* (*himalayanus, tener, macrurus, superans, nesites,* and *princes*) or, in the case of *andamanensis* and *hainanus,* have been referred to this species as junior synonyms by a variety of authors, including Corbet and Hill (1992) and Csorba et al., (2003).

2.1.1 A Review of The Synonyms of *Rhinolophus affinis*

The holotype (BMNH.79.11.21.70) of the typical form of *R. affinis* Horsefield, 1823, which was collected in Java, is damaged. However, according to Andersen (1905a), it is possible to determine that the second phalanx of the third metacarpal is long (15.2 mm), the horseshoe is relatively narrow (8.1 mm) but the tibia (24 mm) is long. Upper toothrow length is 9.0 mm.

R. andamanensis Dobson (1872) was described from the Andaman Islands (no exact locality) and the holotype (15561) is deposited in the Zoological Survey of India, Calcutta, India (Thomas, 1997). It was considered by Dobson to resemble *R. affinis*. The description is brief but suggests that the horizontal horseshoe shaped portion of the noseleaf is broad and flat and conceals the muzzle when viewed from above. The posterior triangular noseleaf is long. Forearm and tail length are given as 2.05 inches (52.3 mm) and 0.9 inches (23.0 mm) respectively. According to Sinha (1973), *R. a. andamanensis* has a larger skull (23.6 - 24 mm), longer ears (22 mm) and broader horseshoe (10 mm).

Rhinolophus affinis himalayanus Andersen, 1905a was collected at Mussoorie in north-west India. The holotype (BMNH. 79.11.21.148) is deposited in the Natural History Museum, London. It was considered by Andersen to be large, with a forearm length of 52 - 56 mm. However, the tail (21.8 - 25 mm) and lower leg (tibia) (22.8 - 23.8 mm) were short, and the horseshoes narrow (8 - 8.8 mm). The greatest length of skull (22.7 - 23.9 mm) and upper toothrow (9.7 - 10.2 mm) were of moderate size and the nasals (15.8-16.5 mm) were narrow. Based on Sinha (1973) who writes 'According to Andersen (1881) this subspecies occurs in Sri Lanka, but this was omitted by Ellerman and Morrison-Scott (1951). There is however, a specimen of this subspecies from Sri Lanka present in our collection, which confirms its occurrence there. Recently, Kurup (1968) recorded it from Meghalaya and Bangladesh.'

Rhinolophus affinis tener Andersen, 1905a was described from Bago (= Pegu), in south, central Myanmar. The holotype (BMNH. 87.3.4.11) is deposited in the Natural History Museum, London. According to Andersen, its size, with a forearm length of 50 mm, was small. The ears (18.8 mm) were also small, the tail short (23 mm) but the lower leg rather long (21 mm). The horseshoe was broad (9.5

mm). The greatest length of skull (21.9 mm) and upper toothrow (8.7 mm) were short and the braincase (9 mm) and nasal swellings (15.5 mm) narrow.

The holotype (BMNH.90.4.4.7) of *Rhinolophus affinis macrurus* Andersen, 1905a was collected from Taho, Karenee (=Kayah State), Myanmar and is deposited in the Natural History Museum, London. Andersen noted that its size, with a forearm length of 51 - 53.8 mm, was moderate. The ears were large (20 - 20.7 mm), the horseshoe (9 - 9.8 mm) broad, the tail (26 - 29.8 mm) and lower leg (tibia) (23.9 - 25.1 mm) long. Total skull length (22.5 - 23.2 mm), width of braincase (11.2 - 11.6 mm) and nasal swellings (15.7 - 16.7 mm) and length of upper toothrow (9.6 - 9.9 mm) were all moderate.

Rhinolophus affinis superans Andersen, 1905a was collected from Pahang, Malaysia and the type (BMNH.0.7.3.2) is the Natural History Museum, London. Externally, it was described as being like *R. a. macrurus* but with a short tail (21.5 - 25.2 mm). The skull is long (22.8 - 23.8 mm) with broad nasal swellings (6.2 - 6.7 mm) and braincase (9.8 - 10.2 mm) and a long upper toothrow (9.0 - 9.7 mm).

The holotype (USNM.104753) of *Rhinolophus affinis nesites* Andersen, 1905a was collected in Bunguran Island, north Natuna Islands of western Sumatra, Indonesia and is deposited in the Smithsonian Institution, Washington. It has large ears (20.2 mm), a broad horseshoe (9.8 mm) and a short tail (22 mm) and tibiae (22.8 mm). The skull is damaged. Upper toothrow length is 9 mm.

Rhinolophus affinis princes Andersen, 1905a was described from Lombok, Lesser Sunda Islands, Indonesia. The holotype (BMNH.97.4.18.13) is in the Natural History Museum, London. The horseshoe (11.1 mm), skull (24.1 mm) and nasal swellings (17.2 mm) are very broad. Upper toothrow length is 9.9 mm.

Rhinolophus hainanus Allen, 1906 was collected at Poutein, Hainan Island, China and the holotype (AMNH.26748) is the American Museum of Natural History, USA. It was described as having large, broad pointed ears and with a short tail. The noseleaf was rather small.

2.1.2 Diagnostic Characters of *Rhinolophus affinis*

Previous authors have included (amongst others) the following diagnosic characters for *R. affinis.*

(1) **Forearm length**, 50.0 - 55.0 mm (Bates and Harrison, 1997).

(2) **Horseshoe** is 8.0 - 11.4 mm, relatively broad but not covering the muzzle (Csorba *et al.,* 2003).

(3) **Sella** is pandurate (lateral margins concave) (Andersen, 1905a). Sella pandurate, its margin at the base arched and broader at the base of its vertical part; a shallow notch present between the top of sella and the connecting process (Sinha, 1973). Sella is pandurate (slightly concave) (Csorba *et al.,* 2003).

(4) **Lancet** is almost cuneate (Andersen, 1905a). Lancet is always straight sided with its tip pointed (Csorba *et al.,* 2003).

(5) **Wing structure** – The second phalanx of third digit is increased in length, always more than, occasionally considerably more than, 1.5 x the length of the first phalanx (Andersen, 1905a). Second phalanx of 3rd and 4th finger much longer (more than 160% of their first phalanx) than the second phalanx of fifth finger (less than 110% of its first phalanx) (Sinha, 1973).

(6) **1ph3met** - 1st phalanx of 3rd digit is considerably less than half length of metacarpal; the 2nd phalanx is long, about 3/4 the length of the metacarpal (66.3 - 80.4%) (Bates and Harrison, 1997).

(7) **Palatal bridge** - extremely short, about 1/4 of maxillary toothrow length or even less (Andersen, 1905a). Palate is exceedingly short, usually less than 1/4 length of the maxillary toothrow; it is emarginated anteriorly to the level of the parastyle of M1 and posteriorly to the mesostyle of M2 (Bates and Harrison, 1997). Palatal bridge relatively short, 23 - 29% of the upper toothrow (Csorba et al., 2003).

(8) **First upper premolar (P^2)** is always in the toothrow, extremely small and the interspaced between the upper canine (C^1) and the second upper premolar (P^4) rather narrow; in 26% of specimens, it is extremely narrow, in most cases almost fine hair (Andersen, 1905a). P^2 is not extruded or only very rarely slightly displaced (Corbet and Hill, 1992). P^2 is smaller, sometimes absent, at most twice upper bulk of upper incisor, tightly compressed in toothrow, slightly extruded or external to row (Corbet and Hill, 1992). P^2 is small but situated in the toothrow (Bates and Harrison, 1997). P^2 is small or medium and in the toothrow or only slightly displaced. The upper canine is usually massive and not in contact with P^4

(Csorba et al., 2003). C^1 and P^4 are closely adjacent or in contact (Corbet and Hill, 1992). C^1 and P^4 are not in contact (Bates and Harrison, 1997).

(9) **Second lower premolar (P_3):** is external and extremely small, rarely within (5%) or partly within toothrow (5%) (Andersen, 1905a). P_3 is usually very small and is situated externally to the toothrow (Bates and Harrison, 1997). P_3 is small or very small, usually fully, rarely partly external (Csorba *et al.*, 2003). P_2 and P_4 are generally quite or almost in contact (74%) and in the remaining more distinctly separated (Andersen, 1905a). P_2 and P_4 are in contact (Bates and Harrison, 1997). P_2 and P_4 are in contact or nearly so (Csorba et al., 2003).

2.2 *Rhinolophus rouxii* Temminck, 1835.

Rufous horseshoe bat

Rhinolophus rouxii Temminck, 1835: 30b; Calcutta and Pondicherry, India (confined to Calcutta by Andersen, 1905a)

Rhinolophus rubidus Kelaart, 1850: 209; Kaduganava, Sri Lanka

Rhinolophus fulvidus Blyth, 1851: 182 (error for *rubidus* Kelaart)

Rhinolophus cinerascens Kelaart, 1852: 13; Fort Frederick, Sri Lanka

Rhinolophus rammanika Kelaart, 1852: 14; Anamapoora Hill, Kaduganava, Sri Lanka

Rhinolophus petersii Dobson, 1872: 337; India "precise locality not known"

2.2.1 A Review of The Synonyms of *Rhinolphus rouxii*

Rhinolophus rouxii Temminck, 1835 was described from Calcutta and Pondicherry, India. The type locality was confined to Calcutta by Andersen (1905a). There are a series of five syntypes held in the Rijksmuseum van Natuurlijke, Leiden, Holland (RMNH.35221 [i]-RMNH.35225 [m]). According to Andersen (1905a), the forearm measurement was 49.5 mm with the general size (excluding the forearm) smaller than that of *R. affinis*. Temminck noted that the small second lower premolar was absent and there were three colour phases: red, dark and intermediate.

Rhinolophus rubidus was described by Kelaart, 1850 from Kaduganava, Sri Lanka. According to Thomas (1997), the holotype is not located. Kelaart (1852) described the taxon as being bright ferruginous brown, the ears pointed

with a deeply emarginated external border. The tip of the 'triangular peak' (= lancet?) is 'emarginated' (= hastate/ narrowed).

Rhinolophus fulvidus Blyth, 1851 is an error for *rubidus* (Kelaart). *Rhinolophus cinerascens* Kelaart, 1852 was named from Fort Frederick, Sri Lanka. The pelage is dusky or ashy brown. The noseleaf is 'as in the last species' (*R. rubidus*). Forearm length is 45.9 mm.

Rhinolophus rammanika Kelaart, 1852: 14 was described on the basis of a single specimen collected from Anamapoora Hill, Kaduganava, Sri Lanka. It was prepared as a dry specimen and this made an assessment of the noseleaf impossible. The lancet was described as 'a small triangular peak', which was 'hairy superiorly'. The tail was 25.5 mm.

Rhinolophus petersii Dobson, 1872 was described without any precise locality. According to Andersen (1905a), the original description was 'meagre and vague' and the 'figures of the head and nose-leaves published four years later are badly drawn'. However, Andersen confidently assigned it to *R. rouxii*, a decision later supported by Sinha (1973).

2.2.2 Diagnostic Characters of *Rhinolophus rouxii*

Previous authors have included (amongst others) the following diagnostic characters for *R. rouxii.*

(1) **Forearm length** > 46 mm (Corbet and Hill, 1992).

(2) **Noseleaf** small, length 11 - 13 mm, width of horseshoe 7.5 - 9.2 mm (Sinha, 1973). According to Thomas (2000), the noseleaf averages 13.6 mm in greatest height and 8.5 mm in greatest width and Csorba *et al.* (2003) considered the horseshoe to be narrow in relation to the muzzle, its breadth is 7.0 - 9.2 mm. A small secondary leaflet is frequently present.

(3) **Sella** is practically parallel-margined from base to summit; occasionally a faint indication of a constriction in the mid-part; the summit is broadly rounded off (Andersen, 1905a). This view is followed by Sinha (1973) who noted that the sella is pandurate but the margins of the horizontal base straight and Csorba *et al.* (2003) who wrote that the sella is practically parallel sided (sometimes with a slight constriction in the middle)

(4) **Lancet** is hastate (abruptly narrowed in the middle), the tip well developed and slender (not abnormally shortened) but in some individuals the lancet is less abruptly narrowed (Andersen, 1905a). This view was followed by Corbet and Hill (1992) who wrote 'Lancet hastate, abruptly narrowed at the centre, lateral margins strongly concave, and tip well developed and slender'. However, Bates and Harrison (1997) noted that the lancet is of variable height, sometimes triangular in shape with straight sides, sometimes with a well developed tip and concave margins below and Thomas (2000) who wrote that the lancet is tall and narrowly pointed with relatively straight sides. A view not followed by Csorba *et al.* (2003) who described the lancet as 'of variable height, hastate, abruptly narrowed in the middle, the tip is well developed and slender.

(5) **Wing structure**: the second phalanx of the third digit is almost always less than 1.5x the length of the first phalanx. However, there are some rare individual exceptions when the second phalanx is equal to or slightly more than 1.5x that of the first (Andersen, 1905a). The first phalanx of the third metacarpal is less than half the length of the metacarpal. The second phalanx is usually less than 66% of the metacarpal (Bates and Harrison, 1997).

(6) **Palatal length** of upper toothrow length is more than 1/4 sometimes almost 1/3 (Andersen, 1905a). According to Bates and Harrison (1997), palatal length is up to 1/3 of upper toothrow length in specimens from India and Sri Lanka and Csorba *et al.* (2003) stated that the palatal bridge is 27-31% of the maxillary toothrow length.

(7) **First upper premolar (P^2)** is usually completely in the toothrow (89%) but occasionally half extruded (11%). The size of P^2 and the space between the canine and the second upper premolar (P^4) are variable (Andersen, 1905a). According to Corbet and Hill (1992), P^2 is more than twice the bulk of the upper incisor, it is not tightly compressed in the toothrow, from which it is slightly extruded; the canine and the posterior upper premolar (P^4) are separated by moderate or wider interspace. Bates and Harrison (1997) stated that P^2 is usually situated in the toothrow, although it may be displaced in specimens from Sri Lanka and Thomas (2000) suggested that P^2 is usually situated in the toothrow. Csorba *et al.* (2003)

noted that it is medium sized and situated in the toothrow or is sometimes half external to it.

(8) **Second lower premolar (P_3)** is most often quite external (63%), sometimes partially in the toothrow (32%) and occasionally absent (5%). The cingula of the first (P_2) and third premolars (P_4) in contact or nearly so (68%) or distinctly separated (32%) (Andersen, 1905a). According to Bates and Harrison (1997), P_2 and P_4 are sometimes in contact; P_3 is usually in the toothrow (Thomas, 2000). Csorba *et al.* (2003) note that P_3 is partly or fully external, rarely missing, P_2 and P_4 are sometimes in contact.

2.3 *Rhinolophus sinicus* Andersen, 1905

Chinese horseshoe bat

Rhinolophus rouxii sinicus Andersen, 1905a: 98; Chinteh, Anhui, China.

Rhinolophus thomasi septentrionalis Sanborn 1939: 40; Nguluko, north of Likiang, Yunnan, China, 27°05'N, 100°15'E.

2.3.1 A Review of The Synonyms of *Rhinolophus sinicus*

Rhinolophus rouxii sinicus was described by Andersen (1905a) from Chinteh, Anhui, China. The holotype (BMNH.99.3.1.6) is the Natural History Museum, London. The forearm is 46 mm. Skull length (19.8 mm) is a little smaller than *R. rouxii*, with slenderer braincase (8.7 mm) and a shorter upper toothrow (7.7 mm). Colour phase darker.

Rhinolophus thomasi septentrionalis was described by Sanborn (1939) from Yunnan, China on account of its larger size and slightly extruded upper premolars. The forearm length is over 50 mm.

2.3.2 Diagnostic Characters of *Rhinolophus sinicus*

Previous authors have included (amongst others) the following diagnosic characters for *R. sinicus.*

(1) **Forearm:** forearm length 44.8 mm - 51.5 mm in Chinese specimens (Csorba *et al.*, 2003).

(2) **Horseshoe** is relatively wide (8.1-8.2 mm) (Csorba *et al.*, 2003). Secondary leaflet is well developed and clearly visible (Csorba *et al.*, 2003).

(3) **Sella** practically parallel-sided and widely rounded off (Csorba *et al.*, 2003).

(4) **Lancet** is hastate (straight-sided) but its tip is variable in length, sometimes very short, in other cases long (Csorba *et al.*, 2003).

(5) **Wings** are relatively large (Csorba *et al.*, 2003).

(6) **Wing structure,** Second phalanx of 3rd digit is very long, 65.0-75.3% of the metacarpal length.

(7) **Palatal bridge** is 26-30% of maxillary toothrow (Csorba *et al.*, 2003).

(8) **Upper canine (C^1)** exceeds P4 in length.

(9) **First upper premolar (P^2)** is medium sized, lying in the toothrow or slightly extruded but the upper canine (C^1) and the second upper premolar (P^4) are widely separated (Csorba *et al.*, 2003).

(10) **Second lower premolar (P_3)** is medium sized or small and partly or fully extruded from the toothrow, the first (P_2) and third premolar (P_4) are in contact or nearly so (Csorba et al., 2003).

2.4 *Rhinolophus thomasi* Andersen, 1905

Thomas's horseshoe bat

Rhinolophus thomasi Andersen, 1905a: 100; Karin Hills, SE Myanmar.

Rhinolophus thomasi latifolius Sanborn, 1939: 39; Muong Moun, Tonkin, Vietnam

2.4.1 A Review of The Synonyms of *Rhinolophus thomasi*

Rhinolophus thomasi was described by Andersen (1905a) from the Karin Hills, SE Myanmar. The holotype (BMNM.90.4.7.10) is the Natural History Museum, London.

Rhinolophus thomasi latifolius was described by Sanborn (1939) from Tonkin, Vietnam.

2.4.2 Diagnostic Characters of *Rhinolophus thomasi*

Previous authors have included (amongst others) the following diagnosic characters for *R. thomasi.*

(1) **Forearm** 44.8 - 45.7 mm (Andersen, 1905a).

(2) **Horseshoe** is considerably narrower than *rouxii* (Andersen, 1905a). Horseshoe is moderately wide (7.2 - 8.9 mm) (Csorba et al., 2003). A well developed secondary leaflet is frequently present (Csorba et al., 2003).

(3) **Sella** is practically parallel sided and broadly rounded off at its apex (Csorba *et al.,* 2003).

(4) **Lancet,** the tip is excessively shortened, almost rudimentary, the hastate lancet of *rouxii* carried to extreme (Andersen, 1905a). The lancet is short, its tip is sometimes almost rudimentary, in other cases better developed (Csorba *et al.,* 2003).

(5) **Palatal bridge** is 30 - 31% of upper toothrow length (Csorba *et al.,* 2003).

(6) **Upper canine (C^1)** only slightly exceeds second upper premolar (P^4) in length; its basal area is usually small.

(7) **First upper premolar (P^2)** external to the toothrow; the canine (C^1) and second upper premolar (P^4) are in contact (Andersen, 1905a). P^2 is small, lying almost in the axis of the tooth row or fully external to it (Csorba *et al.,* 2003).

(8) **Second lower premolar (P_3)** is external and the first (P_2) and third upper premolars (P_4) are in contact (Andersen, 1905a). P_3 is small and external; P_2 and P_4 are in contact or nearly so (Csorba *et al.,* 2003).

CHAPTER 3

MATERIALS AND METHODS

3.1 Specimen Examined

During June 2006 – March 2007, a series of bat survey was mainly conducted throughout northern, central to southern of Thailand based on the distribution of target species especially in forestry and limestone caves. *Rhinolophus affinis, R. rouxii R. sicnicus* and *R. thomasi* were examined.

There are totally 285 adult specimens of Rhinolophus examined for this study, including 170 *R. affinis*, 81 *R. rouxii*, 17 *R. thomasi*, and 17 *R. sinicus,* plus 33 of subspecies of *R. affinis* in 21 localities, there are 15 *R. a. himalayanus*, 12 *R. a. superans,* 3 *R. a. tener,* 2 *R. a. macrurus* and one of *R. a. princeps* (Appendix 1).

Most of the specimens used in this study were primarily wet (with some dry) skins and prepared skulls held in the collections of the Princess Maha Chakri Sirindhorn Natural History Museum (PSUZC), Prince of Songkla University, Hat Yai, Thailand. The Harrison Institute (HZM), Sevenoaks, United Kingdom; The British Natural History Museum (BNHM), London, United Kingdom, and the Institute for Ecology and Biological Resources Vietnamese Academy of Science and technology, Hanoi, Vietnam. Some of specimens were loan from Chiang Dao Wildlife Research Station (CDWLRS), Thailand Institute of scientific and Technological Research (TISTR). Some specimens from India, Sri Lanka, Nepal, and Myanmar were loan from collections of the Harrison Institute (HZM), and from Indonesia, Malaysia and China were loan from collections of The British Natural History Museum (BNHM), London, United Kingdom. In addition, only the thirty-six specimens were collected personally on six field trips to Chiang Mai, Chumporn, Ranong, Songkla, Satun and Tarutao Island in Thailand. The majority were caught in forests and caves. The voucher specimens were deposited in the Princess Maha Chakri Sirindhorn Natural History Museum (PSUZC), Prince of Songkla University,

Hat Yai, Thailand. Lists of species names and specimen locality are presented in Appendices 1.

3.2 Study Areas

This study was based on previous records of *R. affinis* from Thailand, Myanmar, Vietnam, Indonesia and Malaysia. In addition, *R. rouxii*, *R. sinicus* and *R. thomasi* from India, Sri Lanka, Nepal and Vietnam from the collections of the Harrison Institute (HZM) were compared and determined in this study. However, all of subspecies of *R. affinis* were determined and followed previous study and recorded from British Natural History Museum, London.

Each locality and geographical co-ordinates are included in the 'Distribution' section are briefly described and given below, but only the locality from Thailand that I have been visited and collected of specimens. Each locality is given briefly description and following below:

3.2.1 Thailand

My study area is mainly in Thailand and the number of specimens and localities are higher than other country. In Thailand, survey was conducted in 30 localities, covered 11 provinces and 2 Islands.

(1) Northern

1). 1 Chiang Mai Province

The study sites were included two localities, but it was only one locality that *Rhinolophus affinis* was found, six individual of bats were selected for voucher specimens from the Mae Ja cave, in of Chiang Dao Wildlife Sanctuary, Chiang Dao District, Chiang Mai Province (19° 31'915" N 98°50'440"E; 864 m, a.s.l). This is very large limestone cave with a large entrance; it's surrounded by orchards and mixed deciduous forest and bamboo forest. Inside the cave, it has a small stream flowing throughout underground of the cave (Figure 1).

From early records from the collections of British Natural History Museum, London (UK), three subspecies were examined in this study, there are *R. a. macrurus, R. a. superans* and *R. a. tener* from Doi Inthanon, Chomthong district,

Chiang Mai province. These three subspecies have no coordinate and details of locality (collector: Somsak Pantuwatana and J.T.Mashell, November 10, 1964).

1). 2 Tak Province:

Kavackee is located in a Head quarter of East Thung Yai Naresuan wildlife sanctuary, Tak province (15.42'26"N 98.59'28"E, none of a.s.l). Thung Yai Naresuan is wildlife sanctuary, which is much more strictly protected area than a national park, it forms the largest protected area in mainland South-East Asia, covering in total 622,200 ha. It was declared as a World Heritage Site by the United Nations in 1991. There is only one specimen of *R. affinis* and one location that representative for the north-western site, this site was surveyed on March 11, 2003 by Dr. Sara Bumrungsri, the vegetation types and climatic zone in this area is tropical zone or subtropics due to its height and surrounded by dry evergreen forest, mix deciduous forest.

Figure 1: Agricultural land and Orchard surrounded by limestone in Mae Ja cave, Chiang Dao district, Chiang Mai province.

(2) Northeast

In the northeast area, bat surveys were conducted of four sites in four provinces: Loei, Surin, Phetchaboon and Chaiyaphum.

2). 1 Loei Province

This bat study was surveyed from 18 – 20 May 2006 at Phu Suan Sai National Park by Charles Francis and Sara Bumrungsri and PSU team. Three *R. affinis* were found in one locality, Phu Suan Sai National Park, Na Haeo district, Loei province (17° 30'323" N 100°56'295"E, 620 m, 975 m, a.s.l). Harp trap was set across the trail under canopy of trees and bamboo, which surrounded by bamboo forest.

2). 2 Surin Province

Specimens were collected from Ta Muen Thom located in the border of Thailand and Cambodia and Huai Thap Than-Huay Sumran Wildlife Sanctuary, Surin province (14.21'08"N 103. 15'54"E, none of a.s.l), it is a lush jungle between Thailand and Cambodia, there is only one specimen that recorded and stored in the collections of the Natural History Museum, Prince of Songkla University, Hat Yai. This site is surrounded by mix deciduous evergreen forest and dry evergreen forest, bat was collected by Dr. Sara Bumrungsri on January 28, 2000.

2). 3 Phetchaboon Province

On May 16, 2006 Charles Francis and Sara Bumrungsri was surveyed bat in Nhong mae-na, Thung Salang Luang National Park, Khao Kho district, Phetchaboon province (16°34'283"N 100°52'583"E, 730 m a.s.l). One *R. affinis* in this site was selected as a voucher specimen. This location is surrounded dry evergreen forest and mix deciduous dipterocarp forest.

2). 4 Chaiyapum Province

Dr. Sara Bumrungsri was conducted bat series survey on April 8, 2006 in Thung Kamang, Phukieo Wildlife sanctuary, Khon San district, Chaiyapum province (No data record of coordination). Only one *R. affinis* was caught in this site, the climate zone in Thung Kamang is tropical savanna with plant biodiversities; it was

surrounded by hill evergreen forest, dry evergreen forest, mix deciduous and diptercarp species.

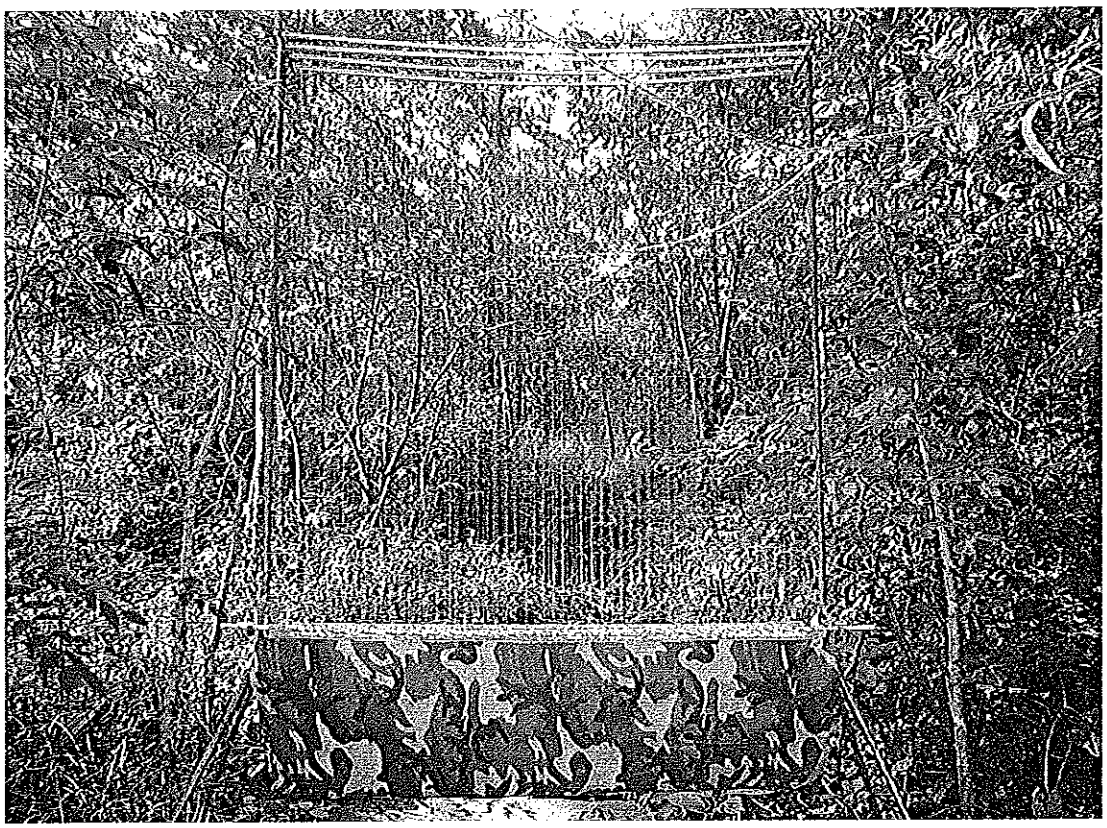


Figure 2: The harp trap was set across the trail in dry dipterocarp forest and Mix deciduous forests exist in Phu Sithan Wildlife Sanctury, Kalasin provice.

(3) Southern

The studies were conducted in eighteen localities which six provinces from southern and peninsula land, there are Chumporn, Ranong, Phang Nga, Satun, Songkhla and Narathiwat provinces. Each locality of this study is shortly described with coordination given for further study.

3).1. ***Chumporn Province*****:** This study was conducted at three sites including:

3). 1.1. Six individuals were selected in the field for voucher specimens from Khao Kram cave, Patiew district, Chumpron province (10° 55'131" N

99°22'440"E, 67 m, a.s.l). This limestone cave was surveyed on October 10, 2006 with one large entrance. The harp trap was set across the entrance of the cave before six o'clock in the evening. This cave is surrounded by a rubber plantation.

3). 1.2. A harp trap was set at the entrance of Huay Wang Cave, this limestone cave is located at Tumbon Khao Talu, Sawi district, Chumporn province (10° 10' 996"N 98°55'183"E, 55 m, a.s.l), one *R. affinis* was taken for bat collection from this cave on January 10, 2007. The location is surrounded by mix deciduous forest and rubber plantation.

3). 1.3. Bat survey was conducted on January 9, 2007 at Khao Plu, a limestone outcrop cave, located in Lamae, Patiew District, Chumporn Province (09° 43' 601" N 99°06'495"E, 30 m, a.s.l). A small cave sits on middle of rubber plantation and cultivation areas. Two harp traps were set along the trail of the outcrop areas and two of *R. affinis* were selected as voucher specimens.

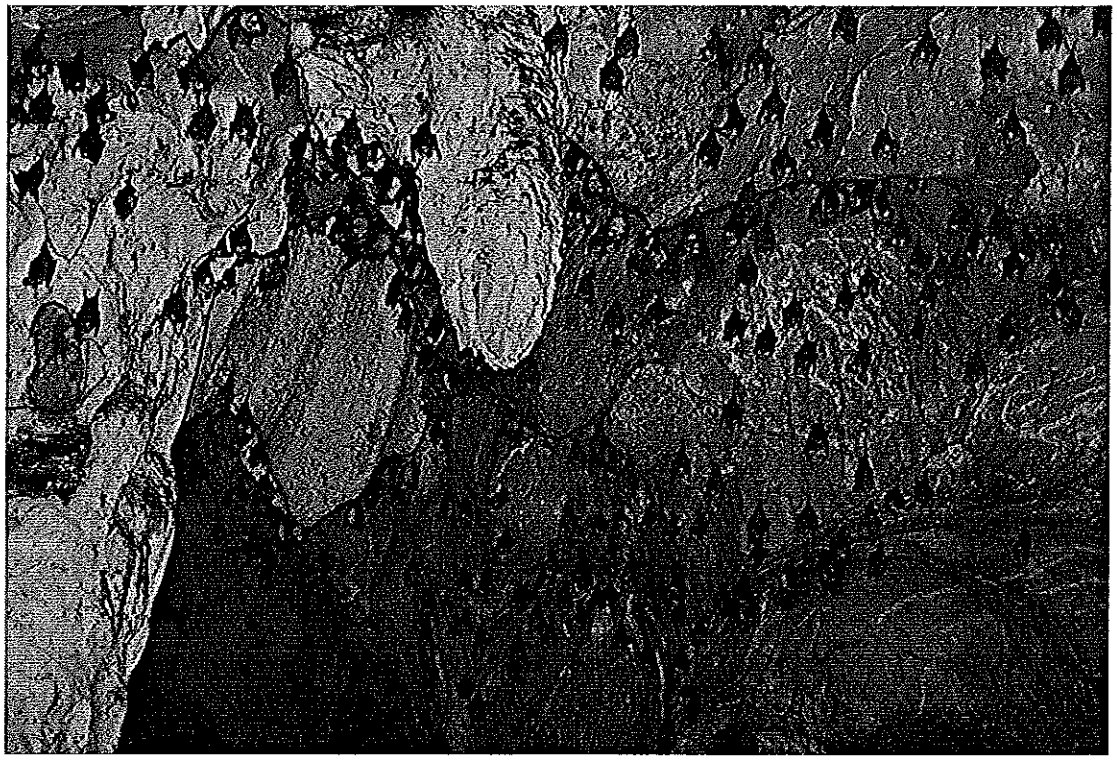


Figure 3: Bats root in the Khao Kram cave, Patew district, Chumporn province.

3).2. Ranong Province: Isthmus of Kra of Thailand

The narrowest part of the Malayan Peninsula is located here at about approximately 60 km north of Ranong Town. The scenery visible from the viewpoint here is the "Kra Buri River", the natural border between Thailand and Myanmar.

During the time period between 12 – 13 January, 2007, two localities were conducted for bat study by bat team, the harp trap and hoop net were used for catching bat and two localities of study sites are given in short description below:

3).2.1. Knad Dai cave is located in La-un district, Ranong province (10° 01'910" N 98°55'183"E, 244 m, a.s.l). This is very large limestone caver which include many chambers in a limestone outcrop close to a stream and villages. In front of the cave is a small local park. Three harp traps were set inside the cave between the first and second chamber, one was set beside the outcrop ~30 m right from the cave entrance and another was set at a small entrance 5 m height from the ground, most of collected specimens were captured from the second harp trap.

One specimen of *R. affinis* was selected from this cave; a bat series survey was conducted on January 12, 2007 by bat team.

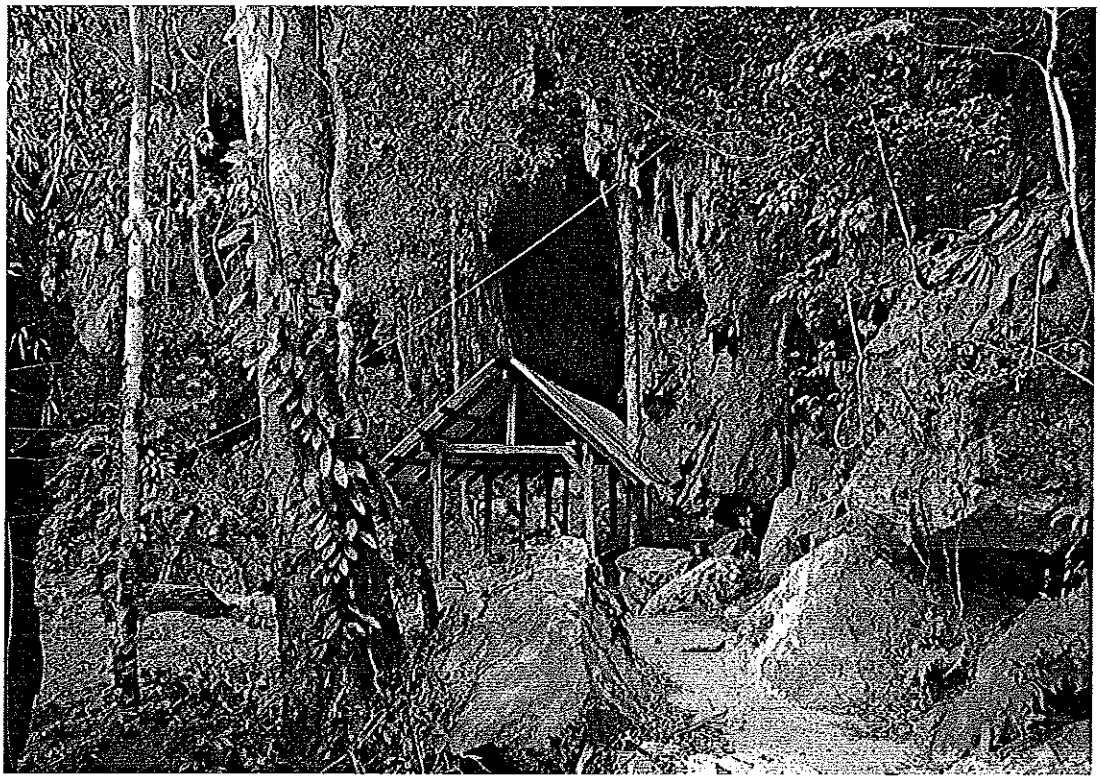


Figure 4: Large entrance of limestone cave with many chambers in Ranong province.

3).2.2. Tham Phra Khayang cave is located at Ban Lum Leang, 12 km off Kra Buri town. It is the northernmost district of Ranong province (10°19.569'N 98°45.923'E, 3 m a.s.l.). This site was surveyed on 13 January 2007. A very large limestone cave in a large isolated limestone outcrop surrounded by Nipah palm plantation (*Nipa fruticans* Wurmb.) and some mangrove forest near by a stream connect to the sea. Two harp traps were set across a small artificial trail surrounded the outcrop opposite the cave entrance. Another one was set at the starting point of the trail under a projected limestone part of the outcrop.

3).2.3. Two specimens of *R. affinis* from Ban Bang Non, Muang district, Ranong province, Thailand were determined also. There is no detail of coordinated and time recorded from this location.

3).3. Phang Nga Province

The province is located on the west side of the Thai Peninsula and includes many islands of the Phang Nga Bay. Situated on the Andaman Sea, Phang-Nga province is famous for its natural beauty in terms of sea, beaches, islands, mountains and forests. The Ao Phang-Nga (Phang-Nga Bay) National Park was established in 1981 to protect the many fascinating islands. Most of Islands are formed limestone rock in the sea. Mu Ko Surin National Park is an archipelago of 5 islands: Ko Surin Nuea (North), Ko Surin Tai (south), Ko Ri, Ko Khai, and Ko Klang. It was declared as a national park on July 9, 1981.

3).3.1. Three *R. affinis* specimens were collected from North Surin Island on Mu Ko Surin National Park on February 2, 2006. The site is surrounded by sandstone forest which has small trees covered by. This study site had no exactly coordination, but just only locality name and date are presented.

3).4. Satun Province

There are a lot of number of localities of bat study are exist in Satun province, including Tarutao Island (Tarutao National Park). Following all localities are briefly describing below:

3).4.1. The river bank is located near the Boripatra waterfall in Ton Nga-chang wildlife sanctuary, Songkla Province (7°00'049" N 100°08'534"E, 13 m,

a.s.l), Harp trap was set beside the road along the river bank that flow across the road near the entrance of the Boripatra waterfall. The site is surrounded by evergreen forest and rubber plantations and orchard. There are nearly a hundred number of bats were caught from this site, but only five specimens of *R. affinis* were collected on October 7, 2006 (Figure 5).

3).4.2. Charge Francis and Sara Bumrungsri collected one *R. affinis* on May 11, 2006 from Boripatra waterfall, Ton Nga-chang Wildlife Sanctuary, Songkhla Province (06° 59' N 100 100°08'E, none of a.s.l). The waterfall is surrounded by

3).4.3. Wang Saithong waterfall (47N 0600469, UTM0783824, none of a.s.l) is located in Manang district of Satun province, which the place is water cascading from the slopes of limestone mountains. The beauty of this waterfall is the limestone in the shape of multi-petalled lotus flowers settling on the bottom of the pools at each of its tiers, this waterfall is surrounded by evergreen forest and mix deciduous forest.

Figure 5: Huge limestone out crop is surrounded by rubber plantation, Satun Province.

3).4. Tarutao Island National Park, Satun Province

Tarutao Island is the largest island of the park, covering an area of 152 km². It is the first marine national park of Thailand. Most of the areas are mountains with a moist evergreen forest, interesting species of plants and wildlife. Some part of the area is a mangrove forest. There are many bays, both small and large, with beautiful beaches. The area was declared a national park on 19 April 1974, and ASEAN Heritage Parks and Reserves by UNESCO in 1982. The localities are following:

3).4.1. Road to Ao Son on Tarutao Island (06° 39'541" N 99° 37'960"E, 22 m, a.s.l). This curve shaped bay has sandy beaches which are interspersed with rocky beaches. It is also an egg-laying ground for sea turtles. Along both sides of Ao son road that the harps trap was set under canopy of trees, which surrounded by lowland evergreen forest, nearest and along the road. Two of *R. affinis* were collected on March 8, 2007.

3).4.2. 2 Ao Son -Ao Chak road (6.39'38"N 99.38'2"E, none of a.s.l), it is the located on Tarutao Island, Satun province. Ao Chak is a small bay next to Ao Phante Melaka. The harp trap was set under canopy of trees in the evergreen forest (lowland), one of *R. affinis* bat were taken for voucher specimens on March 5, 2003 by Dr. Sara Bumrungsri. Three years later, the harp traps were set again close to the last position (6°38'76" N 99°37'4"E, none of a.s.l) by the same collector. This survey was conducted on August 3, 2006 and only one specimen was taken.

3).4.3. Bat survey was conducted on March 9, 2006 by Dr. Sara Bumrungsri. One of *R. affinis* was collected from the km 6th road back to the Tarutao Island National Park office (6° 39'48" N 99°39'4"E, none of a.s.l). This study position is surrounded by lowland evergreen forest, mix deciduous forest with some open space of canopy.

3).4.4. One of my voucher specimens was taken from Tarutao National Park on March 9, 2007 of Satun province, this position (06° 39'292" N 9939 '455"E, 76 m,a.s.l) is the km 7th road from the Head office of Tarutao Island to Tarowao bay. The environment condition areas are lowland evergreen forest and with open space of mix deciduous forest. The harp trap was set cross small stream with the top of secured canopy.

3).4.5. Along the road from Talowao to Taloudang (06° 36'265" N 99°40 '518"E, 9 m, a.s.l), harp trap was set on the trail under the canopy of trees in the lowland dry evergreen forest, many species of bats were caught from this site, but only one of *R. affinis* was selected for voucher specimen. This bat survey was carried out on March 5, 2007.

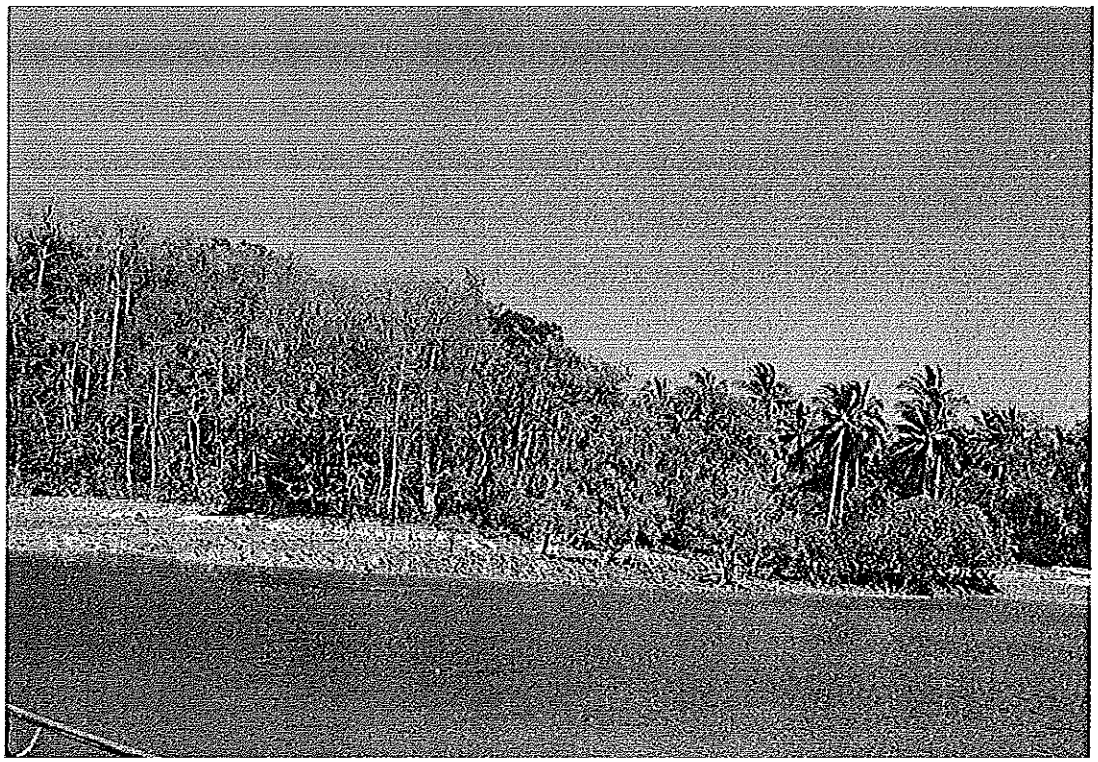


Figure 6: Lowland dry evergreen forest, Tarutao Island National Park, Stun province.

3).5. Songkhla Province

3).5.1. One Klao Rak Kiat is located in Rattaphum district of Songkhla province (6°38.767'N, 99°37.383'E, 100 m a.s.l). This site was surveyed on 16 December 2006. This cave is in a limestone outcrop which is surrounded by disturbed forest and a rubber plantation. The harp traps were set on natural trail under a canopy of the trees and a bat was captured using a hand net in the cave.

3).5.2. Three male and three female specimens were collected from Tham Khao Tieb cave (6°59'975" N 100°17'872"E, 18 m, a.s.l), this cave is limestone outcrop and located in Ratthaphum district of Songkhla province. This cave is underground limestone cave, which has a small hold down 2.5 m depth and 1.5 m

width. This limestone cave was surveyed on October 1, 2006, and is surrounded by evergreen forest and rubber plantation (Fig. 7).

Figure 7: Limestone outcrop and surrounded by rubber plantation

3).6. Narathiwat Province

Narathiwat is the easternmost of four southern provinces that close to Thai-Malaysia border. Most of the area consists of primary rainforest and overgrown mountains.

3).6.1. Hala-Bala Wildlife Sanctuary, Narathiwat, Thailand

Hala-Bala was announced as wildlife sanctuary in 1996. It demarcates the southernmost rain forests in peninsular of Thailand with the vast territories of 434 square kilometers covering Sangalakiri Range in Narathiwat Province, extending to the border of Malaysia. Three specimens of *R. affinis* from Hala - Bala wildlife sanctuary (05° 47'54" N 101°49 '495"E, none of a.s.l) were taken on February 21, 2007 by Amorn Prajukjitr. The harp trap was set under the canopy crossing foot part. The fertile evergreen forest supports a density of huge dipterocarp.

(4). Unknown

Two females of *R. affinis* were collected from Thailand, but there have no details of coordinate location and date of these specimens. These specimens were caught the same time and were conducted on March 21, 2006 by Sara Bumrungsri, Department of Biology, Faculty of science, Prince of Songkla University, Hat Yai, Thailand. For more details of all localities and data information pleases (see in Appendices 1, 2, 3 and 4).

3.3 Capture Methods

3.3.1 Hand Nets or Hoop Nets

Hand nets with adjustable handle lengths are particularly useful for capturing bats in caves, mines, buildings and forests. Hand nets can be made from heavy-duty wire, mosquito netting and almost any type of poles (Kunz, 1990). Hand nets are made from a bag tied to a handle or to sticks (bamboos, wood, etc).

The basket is deep enough to prevent bats from escaping. In the present study, it was about 85 cm deep and 45 cm in diameter. The pole varies in length and is lightweight to ensure the maximum manoeuvrability of the net. The angel of the hoop is adjusted relative to handle and should be made of aluminium if available (Fig. 8).

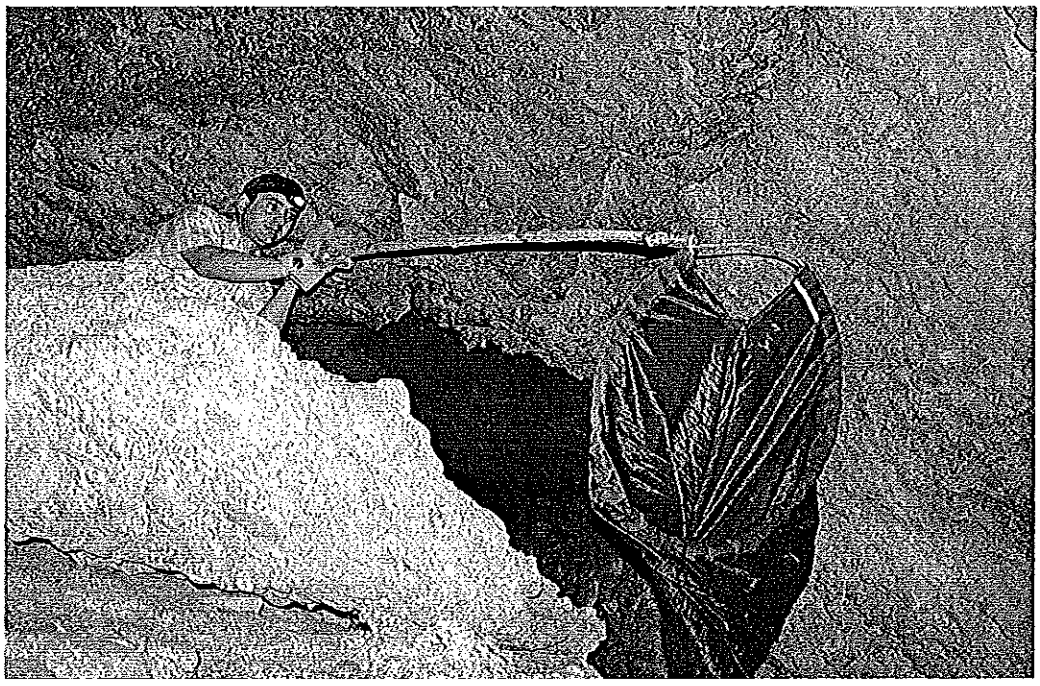


Figure 8: A hand net can be used to collect bats in caves or old building.

3.3.2 Mist Nets

Mist nets are a commonly used tool for capturing flying bats (Kunz, 1988). Generally, in this study, nylon mist nets were used in the field (Figure 9). The mist nets used to capture flying bats are light and if treated correctly can be used repeatedly.

However, they are prone to tearing and may be damaged by rough treatment or by being bitten by the bats. The colour is usually black; it is suitable for capturing bats in dark places. Mist nets are 36 mm, 70-denier/2 ply and have four shelves. They are 2 meters high and range in length from 6 to 12 m. Mist nets should be carefully opened to expose the end loops.

Many nets are supplied with the top loops colour-coded and with different colours at the opposite end. Mist nets were set in early evening in front of cave entrances where bats regularly exit to catch flying insects. In the case of larger cave entrances, two people were hold net poles across the cave entrance. They were also set across forest paths and over streams.

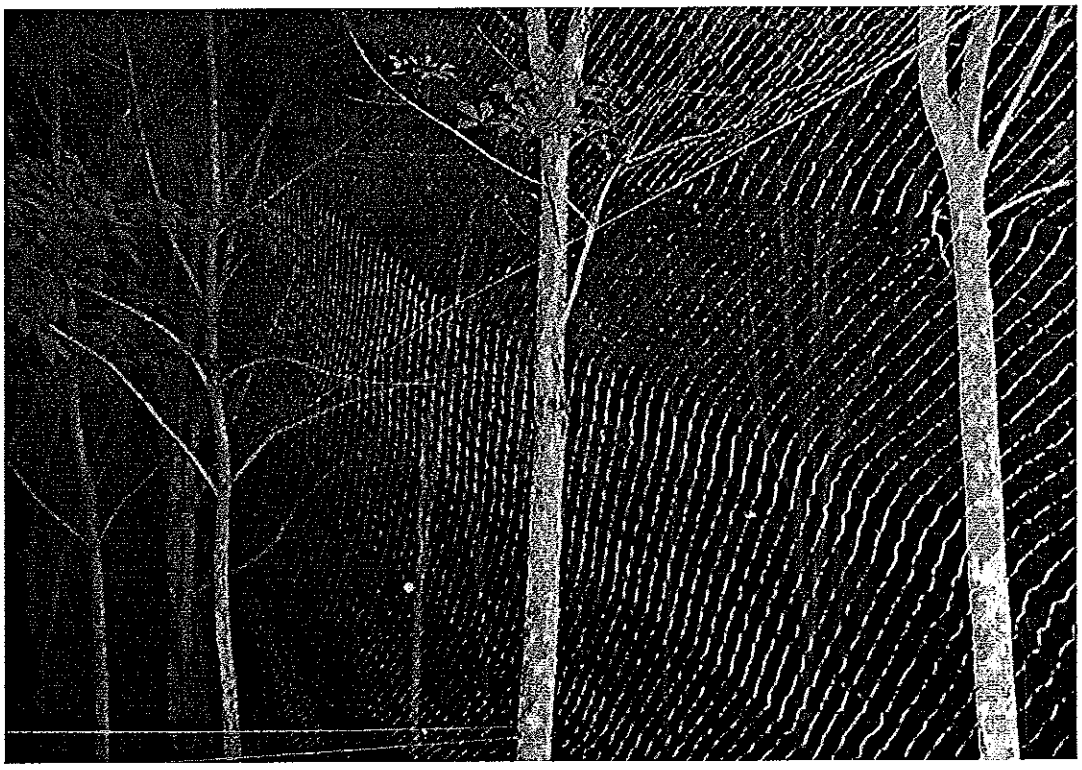


Figure 9: Mist net was set in the rubber plantation or in the forest.

3.3.3 Harp Traps

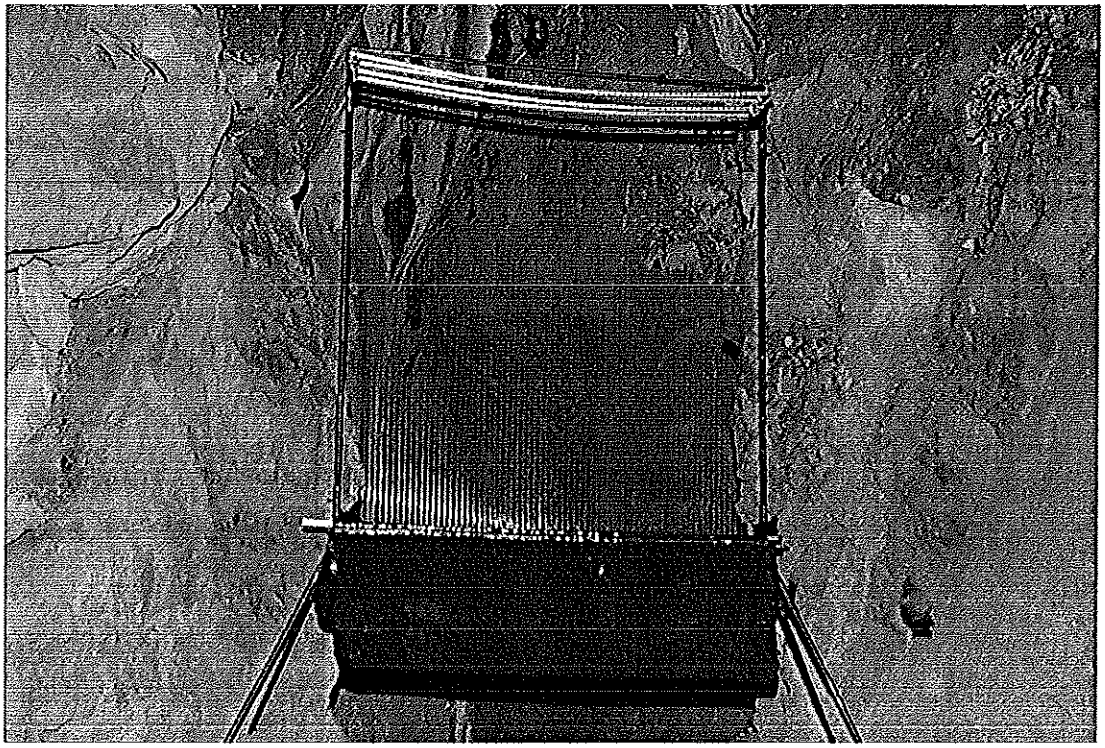
Harp traps are used to catch free flying bats by setting them in constricted areas (e.g. a narrow point in a forest trails). Harp traps have an advantage over mist nets as they are stronger and require less maintenance. In the long term they are also cheaper, as they can be used for many years and are easy to repair.

Harp traps work on the principle that the echolocation calls of bats cannot easily detect the nylon fishing line (= wires) and the tension of the wire banks is sufficient to stop the flight momentum of bats. A harp traps usually have four banks of wires (Francis, 1989), each wire is attached individually to a twirl spring which is spaced approximately 20 mm apart. The frames are 200 cm height and 180 cm width (Fig. 10, A&B).

Four extension legs support the main frame. A cloth bag, partially lined with polyethylene is tied underneath the trap frame to catch bats as they fall after having been intercepted by the trap. A plastic flap is suspended inside the bag to prevent hovering bats and other highly manoeuvrable species from escaping. When bats are captured in harp trap, they are well protected from inclement weather and the possibility of predators (snakes, owls, hawks, bird preys and mammalian carnivores) (Hill and Smith, 1984; Kunz, 1988). However, harp traps have some limitations; the surface area of the traps is limited and is not suitable for collecting bats in open spaces and in some large entrances to caves.

3.3.4 Echolocation Record

Echolocation calls were recorded using a Pettersson 240X bat detector and a Sony cassette tape recorder and digital recorder. Calls can be recorded from bats in various conditions such as hand-held, in bags, and free flying. However, in this study all calls were recorded using a standardised hand held and held inside bag methods. The bat detector was a Pettersson ULTRASOUND DETECTOR D 240x (Fig. 11), which was set at 10x time expansion rate and 17 seconds max storage time. A recorder was connected to the bat detector.



A

B

Figure 10: (A) The harp was set in front of cave's entrance; (B) Harp trap was set across a mall trail at the mountain's hill in dry deciduous forest.

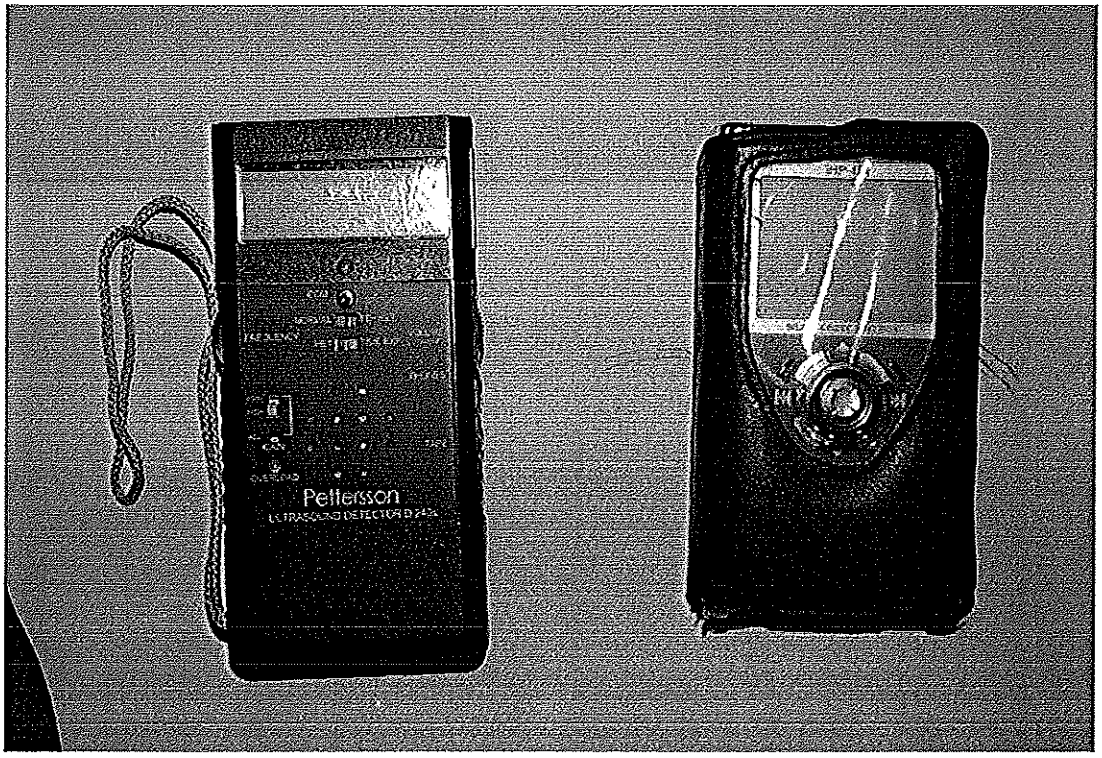


Figure 11: Pettersson Ultrasound Bat detector (left) and iHP-120 Recorder (right).

3.4 Specimen Preparation

3.4.1 Field Data

After bats were captured, species, sex, age status was identified (adult, juvenile) by during filed capture period of the phalanx of metacarpal joints and reproduction condition was identified by females. Body mass of bats were weighed (using a Pesola balance to the nearest ± 0.1g.) and recorded on data sheets in the field.

Basic external measurements (e.g. body length, forearm length, ear length, and tail length) were taken by using the digital caliper and the locations were recordedusing the Global Position system (GPS).

Live bats were taken to the base camp; echolocation calls of each bat were recorded, and photographs taken of characteristic features such as the noseleaf, lancet, and wing morphology. Then the bats selected as voucher specimens were sacrificed using chloroform. In a small number of cases of considerable taxonomic interest, wing punches and fresh livers were collected and kept in 100% alcohol for DNA analysis. The information of field data was sight and given below:

- Date of collection.
- Locality data, especially name of cave or village, district, provincial and geographical of coordination.
- Sexual status
- Specimen number
- Habitat description
- File number of recorded echolocation call.
- File number of bat photograph

An individually numbered label was attached to the right hind foot. In some cases specimens were then stored in 10 % Formalin to keep colour. After one day, specimens are taken out of the formalin. They are rinsed in cold water in order to remove as much formalin as possible. After five minutes, specimens are placed in jars with 70% alcohol, and kept on shelves in a dark place.

However, if this technique is followed no further tissues for DNA analysis can be taken from the specimen and other specimens that come into contact with this material will also be contaminated. Many researchers therefore prefer to put the voucher specimens directly into 70% alcohol.

The wet specimen labels are made from strongly paper that sealed with plastic and tear water proof and alcohol. The wet specimen label must be written by permanent ink or pencil (Figure 12). The details and all information of wet specimen labels are listed below:

- Museum number
- Sex
- Species name
- Call Frequency
- Date of collection
- Locality (briefly describe of habitat collecting)
- Geographical coordination and altitude
- Head and Body Length
- Forearm Length

- Ear Length
- Tail Length
- Hindfoot Length
- Body mass
- Collector
- Field number

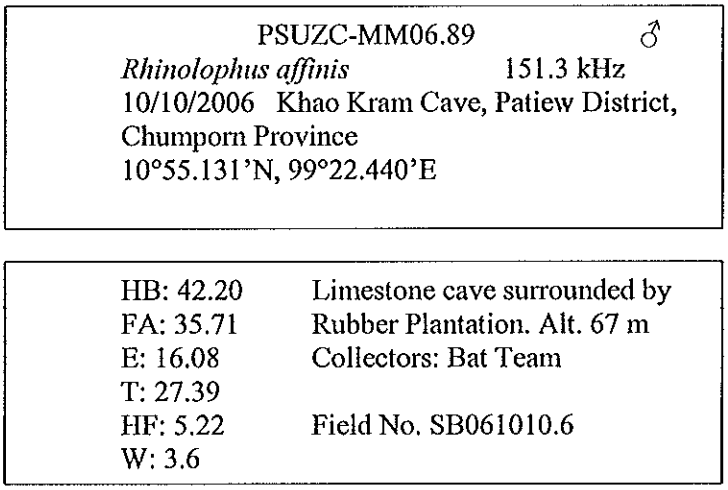


Figure 12: Showing the labels of wet specimens and skulls: front side of label (above) and back side of label (bottom).

3.4.2 Specimen Examined and Taxonomic Measurements

3.4.2.1 External Measurements

The external morphology of each taxon were examined and described in details. Photographs of lancets for each taxon were taken for comparative purpose by using a Nikon digital camera with 100 mm macro lens. The external measurements were taken to nearest 0.01 mm using a digital calliper following Bates and Harrison (1997).

In addition, 285 specimens were measured, which eleven external characters were recorded from each specimen. Lancets were scored and compared of each taxon (Fig. 13). The definitions are as fallow:

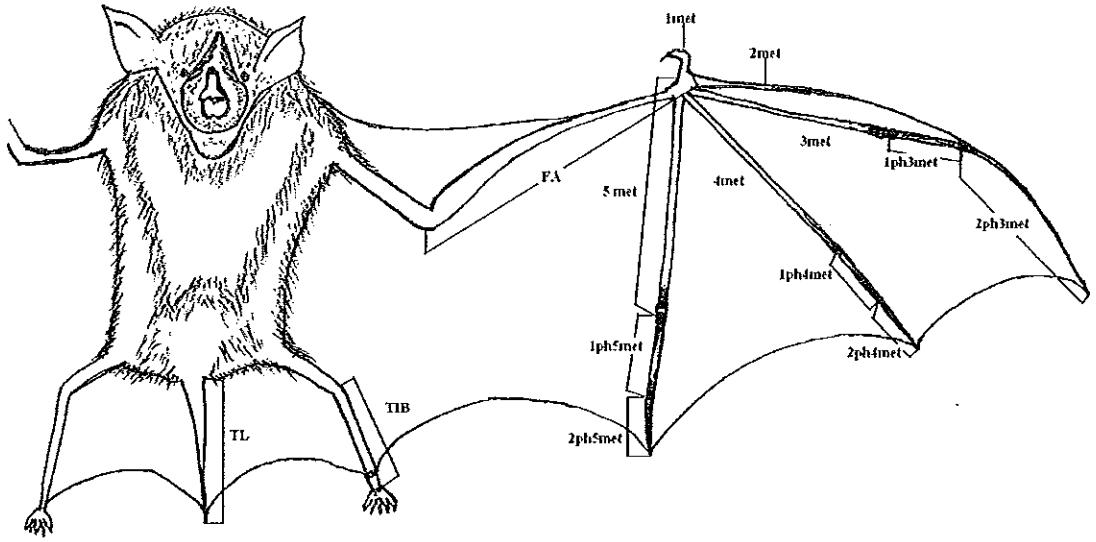


Figure 13: External measurements of *Rhinolophus affinis*

HBL: Head and body length, dorsally, from the tip of snout to the base of the body adjacent to the anus.

TAIL: Tail length, from the tip of the tail to its base adjacent to the anus.

FA: Forearm length, from the extremity of the elbow to the extremity of the carpus with the wings folded.

E: Ear length, from the lower border of the external auditory meatus to the tip of the pinna, not including any tuft of hair.

HF: Foot length, from the extremity of the heel behind the os calcis to the extremity of the longest digit, not including the hairs or claws.

TIBIA: Tibia length, from the knee joint to the ankle.

3 Met (MET): Third metacarpal, from the extremity of the carpus to the distal extremity of metacarpal.

3 Met 1ph: First phalanx of the third metacarpal-taken from the proximal to the distal extremity of the phalanx.

3 Met 2ph: Second phalanx of the third metacarpal-taken from the proximal to the distal extremity of the phalanx.

4 met (MET): Fourth metacarpal as for third metacarpal.

5 met (MET): Fifth metacarpal as for third metacarpal.

3.4.2.2 Wet Specimen Storage

After the skull has been extracted, a cotton wool ball is fitted into the head skin. The mouth is sewn up with a needle and black cotton. Wet specimens are stored in air-tight jars. Each jar only contains one species, but may contain 1-3 specimens. Each specimen is identified and with its data included on a label. The label is attracted to the right foot. The jars contain 70% ethanol. The specimens must stay below the level of the ethanol. The jar is kept on open shelves in a dark place

3.4.2.3 Skull Extraction

The skulls were extracted by hand, which logical method. Although, a small blunt scalpel was used to cut the facial skin on the front of mandible, close to the lower incisor. The facial skin was peeled from the front to the back of the mandible by using a combination of forceps, small blunt scalpel and small sharp scissors. The facial skin on the cranium, nearest to the upper incisors was cut and peeled from front to back. When cutting the skin free from the nasal bone region, it is important to avoid damaging the noseleaf. When removing the skin from the zygomatic arches, it is important to avoid damaging the zygomata. A small blunt scalpel is used to carefully remove the skin on each side of the skull by the ears. When removing the skull from the body, it is necessary to cut the upper cervical spine rather than risk cutting the occipital part of the skull. The tongue is removed by using a pair of forceps. A temporary skull label should be attacked to the skull and the mandible.

3.4.2.4 Cranial and Dental Measurements

Thirteen cranio-dental measurements were taken from each specimen using a digital calliper accurate to the nearest 0.01 mm. However, using a Wild-Heerbrug stereo microscope with attached camera Lucida made line drawing of the skull structure, dentition and palatal.

These measurements are illustrated in (Fig. 14-16) and are defined below:

GTL: greatest length of the skull: the greatest antero-posterior diameter of the skull, taken from the most projecting point at each extremity.

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

CCL: condylo-canine length, from an exoccipital condyle to the alveolus of the anterior canine.

SL: skull length, from the alveolus of the anterior canine to the most posteriorly projecting part of the skull.

ZB: zygomatic breadth, the greatest width of the skull across the zygomatic arches.

BB: breadth of braincase, taken at the posterior roots of the zygomatic arches.

PC: postorbital constriction, the narrowest width across the constriction posterior to the obits.

C^1-M^3: maxillary toothrow length, from the front of the upper canine to the back of the crown of the third upper molar.

M^3-M^3 (Extemal): posterior palatal width, taken across the outer borders of the third upper molar at the widest part.

C_1-M_3: mandibular toothrow length, from the front of the lower canine to the back of the crown of the third lower molar.

C^1-C^1 (External): greatest anterior palatal width taken across the outer borders of the upper canines at the widest part.

ML: mandible length, from the most posterior part of the condyle to the most anterior part of the mandible, including lower incisors.

PL: Palatal length, measured from the median point of the anterior border to the median point of the posterior border.

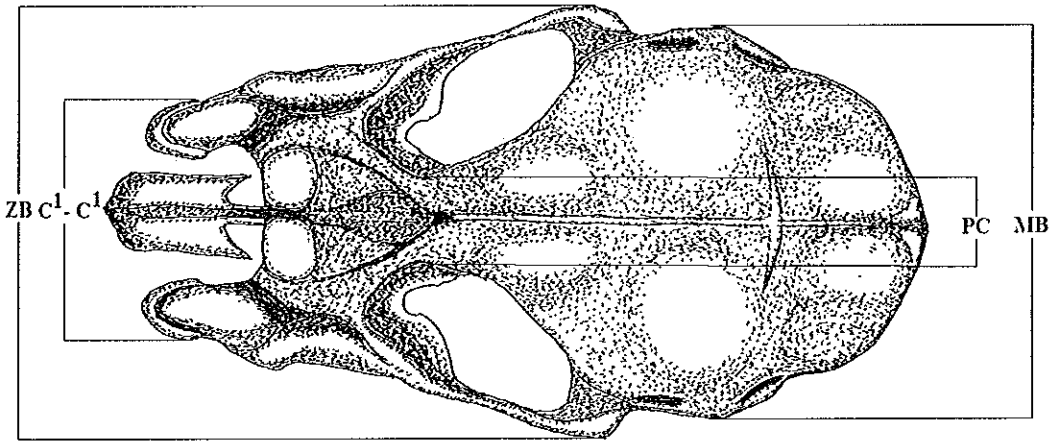


Figure 14: Dorsal view of the skull of *R. affinis* HZM. No.7.28151 from India

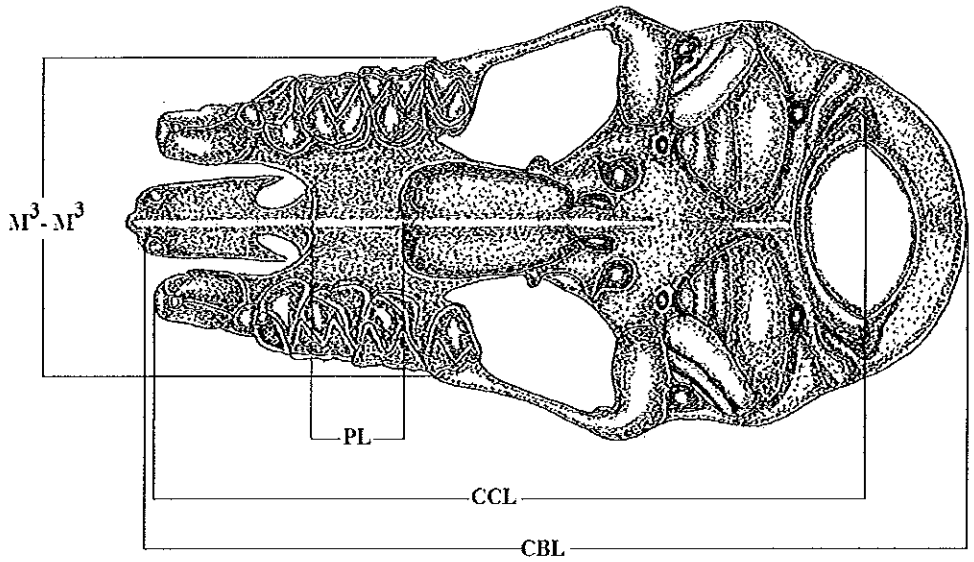


Figure 15: Ventral view of *Rhinolophus rouxii* HZM. No.49.29188 from Sri Lanka

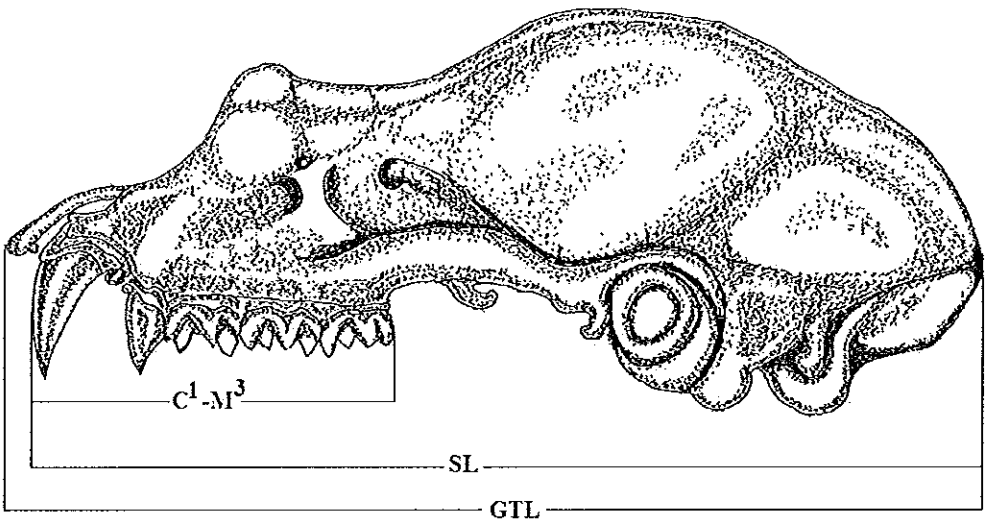


Figure 16: Lateral view of Upper skull (above) and Lower tooth (bottom) of *R. affinis* HZM. No.7.28151 from India

3.4.2.5 Skull Storage

The temporary skull label is replaced by collections skull label (permanently). The skull is stored inside a small plastic pot with a secure lid. The skull label is attached right side of the zygomatic breadth from dorsal view. The label

stays outside of the pot. The skull is supported on cotton wool to minimize any damage during storage. The skull pot is kept in a plastic bag, which is kept in a box.

3.4.2.6 Sella Morphology

The horizontal sella was examined to determine if the sides were straight or parallel sides (A), concave (B) and convex (C) see in (Figure 17).

Some specimens showed an indication of a concavity, without being as convex as a (C) or as straight as a (A), they were assigned to a Type 1.5 or (D), it is not included in the figure below.

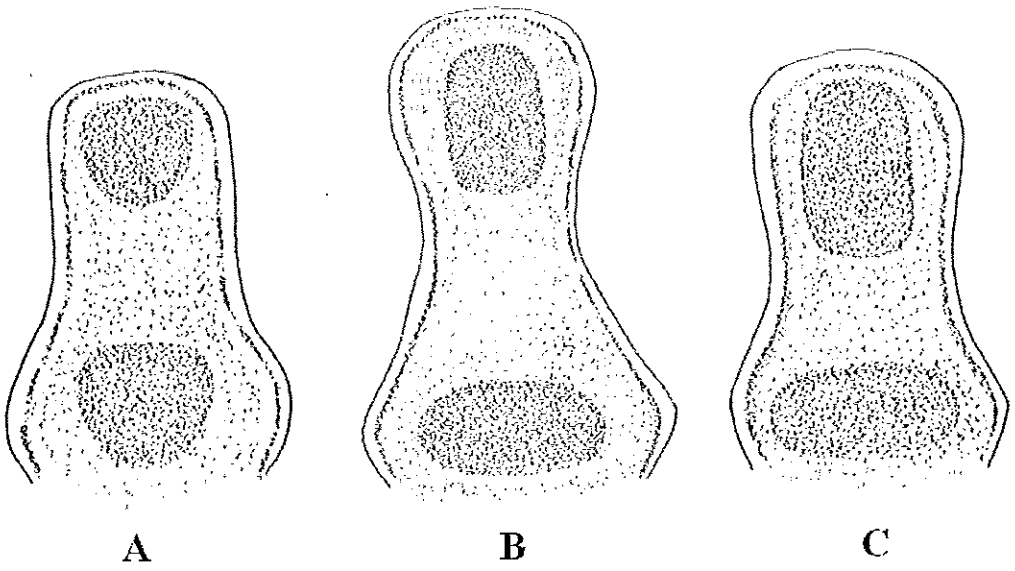


Figure 17: Anterior view of sella variationsin *R. affinis* from mainland Southeast-Asia. (A) The straight or parallel sides of *R. affinis* No.43 from Vietnam, (B) the concave sides of *R. affinis* PSUZC-M05.103 from Thailand and (C) the convex sides of *R. affinis* HZM. No. 22.32195 from Vietnam

3.4.2.7 Lancet Morphology

The noseleaf of each specimen was scored in terms of its lancet morphology since this method allows for objective comparisons between populations within species and for a study of interspecific variation. Lancet shape was divided into four morphotypes (Figure 18). Individuals were allotted to the 'most similar'

morphotype. In some cases, lancet shape was somewhat intermediate between one and another morphotype and in these cases there was element subjectivity in the allocation process.

The four lancet morphotypes are: (A) a triangular shaped lancet with essentially straight sides; (B) a triangular shape lancet but with a slight concavity on each sides below the tip; (C) a small lancet with a domed shape lower part and a short straight sides tip and (D) a lancet with a domed shaped lower part and a clearly defined as elongated pointed tip.

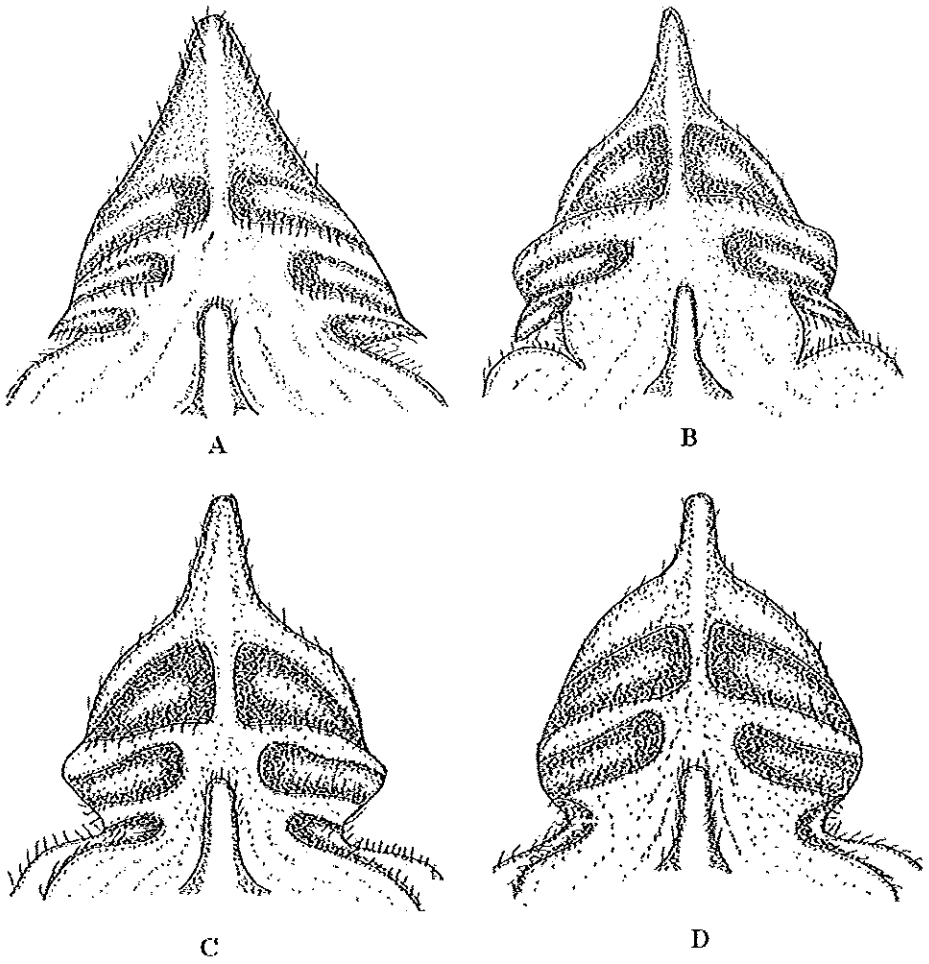


Figure 18: Anterior view of lancet types in *Rhinolophus*. (A) *R. affinis* SB 060518.15 from Thailand; (B) *R. rouxii* HZM.No.55.30958 from Sri Lanka, (C) *R. rouxii* MM120 (BNHM), India and (D) *R. sinicus* HZM. No. 21.28153 from India

3.4.2.8 Internarial Cup

The shape of the internarial cup was examined. Those with rounded sides were scored as Type 1 and the minority with angular sides was assigned to be a Type 2.

3.4.2.9 Attachment of the Secondary Foliole

Most specimens had a single supplementary foliole beneath the anterior horseshoe. In some cases, this foliole was a thickening of the skin but without a free edge. This was scored as a Type 1. In some specimens, the edge of the outside border of the foliole was separate from the muzzle. These were scored as Type 2. In some cases, a small proportion of the outer border was free from the muzzle, while the remaining part of the border was still attached. They were scored as Type 1.5.

3.4.2.10 First upper premolar (P^2) size and position

The size and position of the first upper premolar (P^2) was examined for each individual. Specimens with the smallest premolar were scored as Type 1; intermediate were Type 2 and Type 3 for the largest. The position was scored as 'A' for a premolar extruded from toothrow, 'B' for partially extruded and 'C' for positioned within the toothrow. Therefore, each tooth was given a code which included a number (size) and letter (position). In this figure, the examples include differences in both size and position (Fig. 19). In P^2 positions (toothrow position) are an explanation below:

- md, partly ex = medium sized and partly extruded from the toothrow.
- mt, tr = minute size and situated in the toothrow
- lg, partly ext = large and partly extruded
- sm, partly ext = small and partly extruded
- mt, partly ext = minute and partly extruded
- md, tr = medium sized and situated in the toothrow
- lg, tr = large and situated in the toothrow
- sm, tr = small and situated in the toothrow

3.4.2.11 Second lower premolar (P_3) size and position

The size and position of the second lower premolar (P_3) was examined for each individual. Size was scored as Type 0 for absent, Type 1 for the smaller, Type 2 for larger size. Position was scored as 'A' for a premolar extruded from toothrow, 'B' for partially extruded. Therefore, as with the first upper premolar, each tooth was given a code which included a number (size) and letter (position). In this figure, the examples include differences in size and position (Fig. 20). In P^2 positions (toothrow position) are an explanation below:

- Absent = absent from the toothrow
- mt, partly ext = minute and partly extruded
- mt, ext (P_3) = minute and extruded from the toothrow
- mt, tr = minute size and situated in the toothrow
- md, partly ex = medium sized and partly extruded from the toothrow
- md, ext = medium sized and extruded from the toothrow
- md, tr = medium sized and situated in the toothrow

3.4.2.12 Bacular Extraction

Bacula were prepared for a number of male specimens. The penis was carefully cut from the body of the voucher specimen. It was then heated in a test tube of boiling water for two minutes. This was followed by a period of maceration in a solution of 5% potassium hydroxide mixed with a very small 'pinch' of alizarin red stain. Twenty four hours later, the soft tissue was removed by dissection. The baculum was then ready for storage in glycerol (Thomas *et al.*, 1994 and 1997).

The bacula were then examined under a Wild-Heerbrug stereo microscope and where necessary line drawings were made by using an attached camera Lucida. Measurements were taken using a graticule scale attached within the eyepiece of the microscope. These included the greatest length, greatest width and lateral width of the baculum.

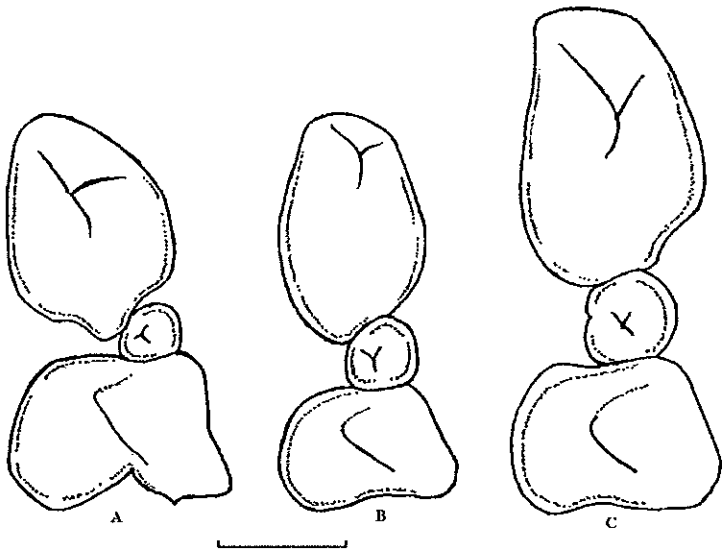


Figure 19: Intraspecific variation in the position of the first upper premolar. (A) Smallest size and extruded from toothrow of *R. sinicus,* HZM.2.16292, ♂, Godavari, Nepal. (B) Intermediate size and partially extruded of *R. rouxii,* HZM.39.28566, ♀, Ingiriya, Western Province, Sri Lanka, and (C) Largest size and within toothrow of *R. rouxii,* HZM.49.29288, ♂, Pussahena Tunnel, near Ruwanwella, Sabaragamuwa, Sri Lanka.

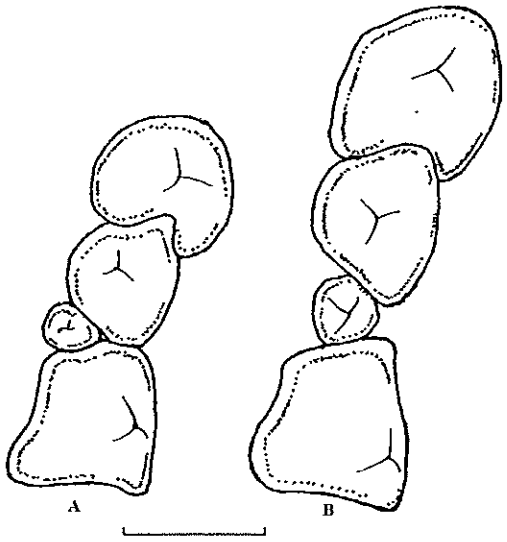


Figure 20: Intraspecific variation in the position of the lower middle premolar. (A) Small size and a premolar extruded from toothrow of *R. rouxii,* HZM.48.29287, ♀, Pallama, Matale District, Sri Lanka. (B) Larger size and partially extruded of *R. rouxii,* HZM.39.28566, ♀, Ingiriya, Western Province, Sri Lanka.

3.5 Data Analysis

3.5.1 Statistic Analysis

A SPSS program was used for multivariate statistical tests in order to examine intra- and interspecific patterns in the data.

Sexual dimorphism, in order to determine whether there was sexual dimorphism within the study taxa, geographically restricted areas were selected for univariate and multivariate analysis. A univariate T-test and a multivariate Hotellings T^2 test were run on selected external, cranial and dental characters for males compared to females for each taxon at a confidence limit of 95% using SYSTAT 6.0 for Windows.

Geographical variation, for the investigation of geographical variation within species, populations and each species, geographical ranges were divided into regions, which had been noted during the morphological examination as appearing to represent possible variation boundaries. The geographical positions of every site were marked on a map. Descriptive statistics were calculated for each region using a set of external, cranial and dental characters.

3.5.2 Echolocation Analysis

Calls of *Rhinolophus* calls were analysed with Batsound programme (Pro-Sound Analysis Version: 3.31 programme) supplied by Pettersson Elektronik. The definitions of the five measurements of Echolocation Calls are following.

1. Pulse duration (D): The duration of a single pulse, obtained by measuring the pulse envelope from the spectrogram and oscillogram (Fig. 21.A).

2. Start at maximum frequency (SF, fmax, kHz): The start or maximum frequency, measured from the spectrogram and power spectrum (Fig. 21.B).

3. End frequency (EF, fmin, kHz): The end or minimum frequency, measured from the spectrogram and power spectrum (Fig. 21.C).

4. Maximum frequency (FMAX, kHz), measured a single pulse from the spectrogram via a large measurement cursor placed at the top end of the spectrogram (Fig. 21.D).

5. Minimum frequency (FMIN, kHz), measured a single pulse from the spectrogram via a large measurement cursor placed at the bottom end of the spectrogram (Fig.21.E).

6. Frequency at maximum intensity (FINT, kHz); or Peak frequency (PF, FmaxE, kHz): The frequency containing maximum energy, obtained from the spectrogram and power spectrum (Fig. 21.F).

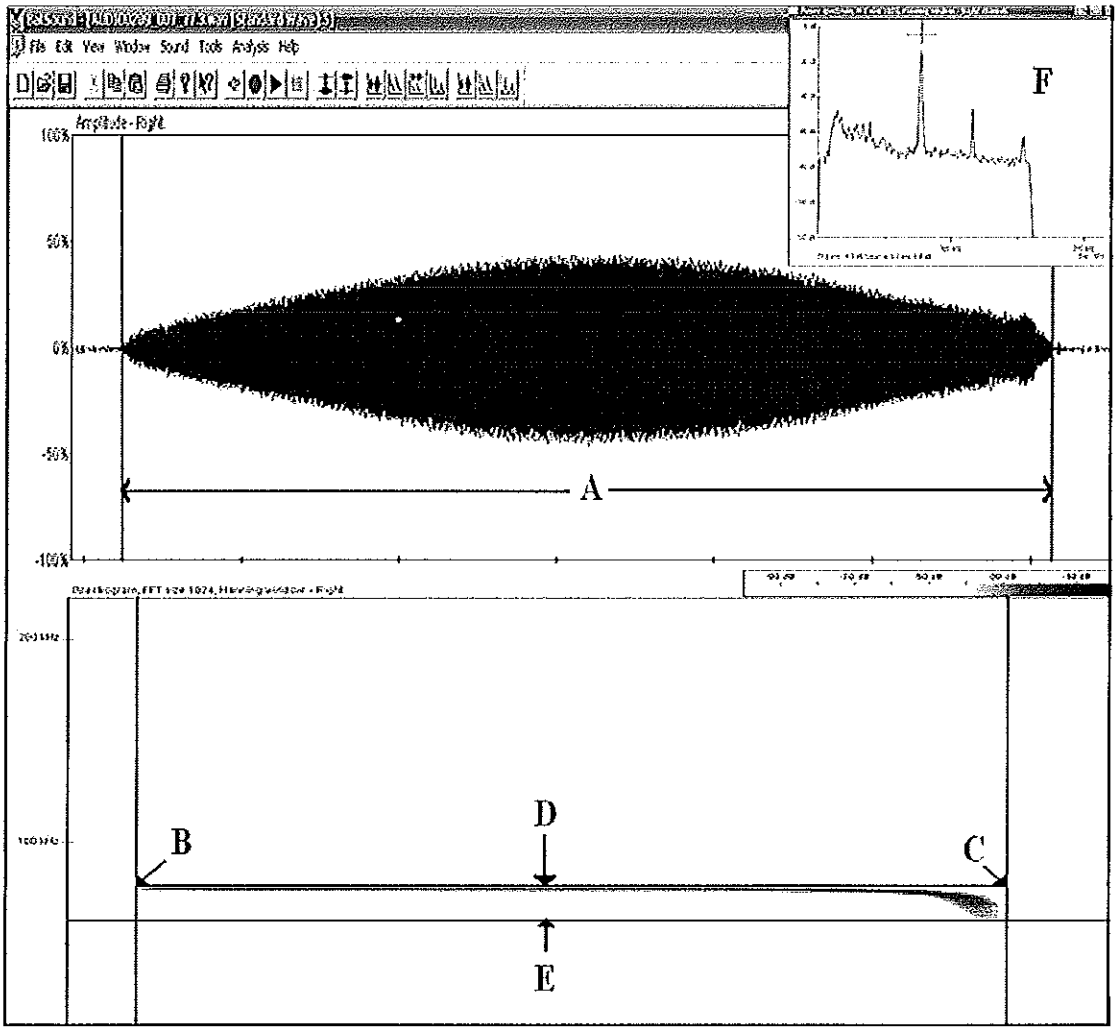


Figure 21: *R. affinis* call and the BatSound software cursors (represented by the lines) and measurement point (indicated by arrows) used for parameter measurements. (A) pulse duration of call (in milliseconds), (B) SF start frequency measurement cursor; (C) end frequency measurement cursor; (D) FMAX (maximum frequency) measurement cursor; (E) FMIN (minimum frequency) measurement cursor and (F) FINT (frequency of maximum intensity).

CHAPTER 4
RESULTS

4.1 *Rhinolophus sinicus* and *Rhinolophus thomasi*

4.1.1 External Morphology Comparison

4.1.1.1 Sella Variation

The sella shape of *R. sinicus* is typically parallel-margined with a widely obtuse apex (100%) (Fig.25), the majorities (55%) of the sella shapes of *R. thomasi* are straight or parallel margined with a blunt tip. However, a sizeable minority have convex sides (18%) or an indication of a concavity (27%) see in Fig.22.

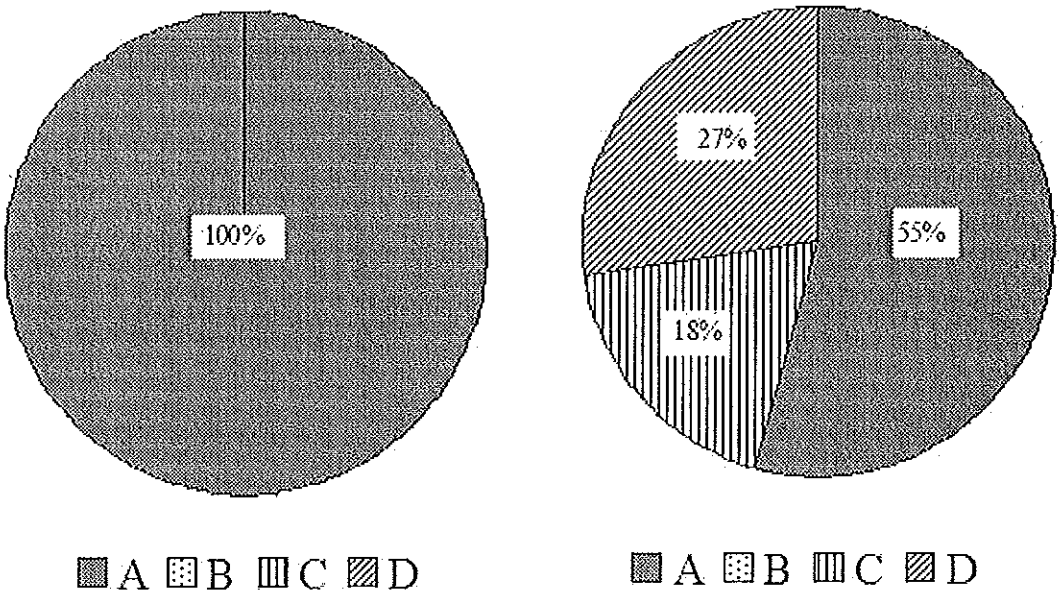


Figure 22: The percentage of sella variation in *R. sinicus* (left, n= 15) and *R. thomasi* (right, n= 11). The sella shape with parallel margined (A), concave sides (B), convex sides (C), and undefined (D) see in Fig 17, p 62.

4.1.1.2 Lancet Variation

In *R. sinicus,* the lancet is typically elongate-margined and with a blunt tip or sometimes straight foreword-tip. It is always very short/or short in *R. sinicus,* there are the dome shape with elongated sides (94%) and the straight sides (6%). In *R. thomasi,* the lancet is short, the tip is almost simple or straightforward with elongated sides, is completely shown the dome shape with elongated sides of lancet (100%) (Fig. 23)

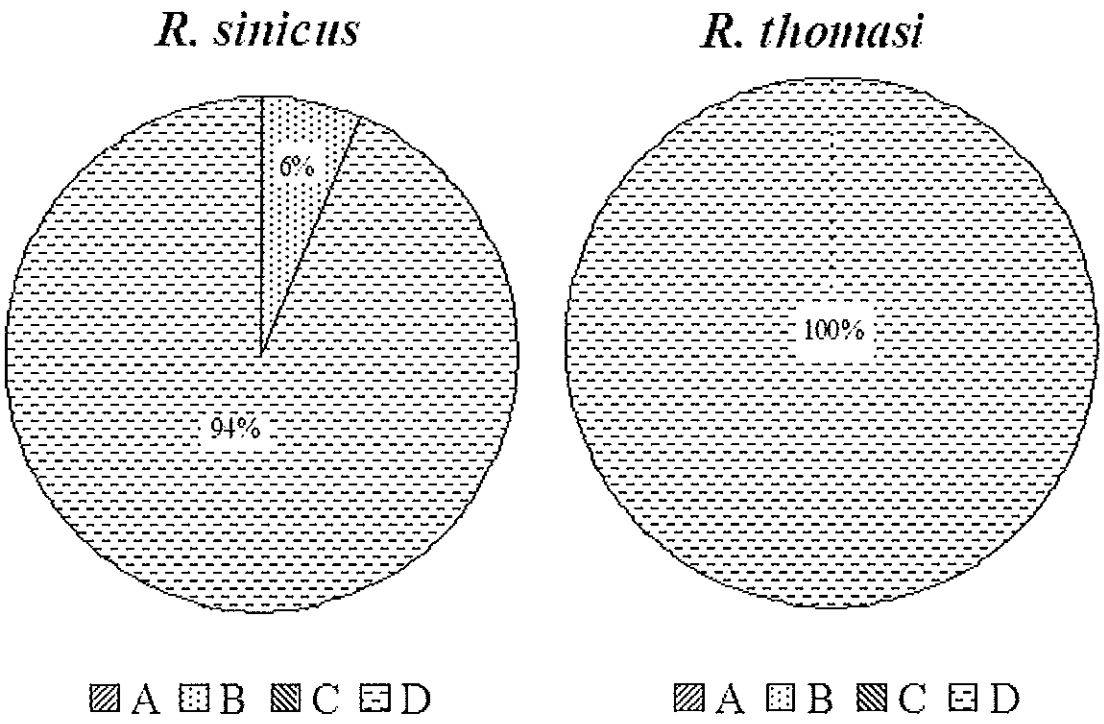


Figure 23: High percentages of dome shape with elongated sides of lancet are dominated in both species *R. sinicus* (n= 16) and *R. thomasi* (n= 10). Triangular shaped with straight sides (A), the triangular shaped with concave sides (B), the dome shaped with straight sides tip (C) and the dome shaped with elongate sides tip (D), see in Fig. 18, p 63.

4.1.2 Skull Morphology

The skull of *R. sinicus* is bigger than *R. thomasi*, with CCL: 17.81 mm in average. The rostral depression profile is narrowly convex (Fig. 49 c). The zygoma is well developed and concave in shape, with high jugal projection (curve) (Fig 50 c).

The palatal bridge is 1.62 – 2.46 mm or 27.04% in average of the maxillary toothrow length (C-M^3). In *R. thomasi*, the skull is a small and robust, with CCL: 16.20 mm. The nasal swelling is high and rostal depression profile is deeper and slopping rearward than *R. sinicus* (Fig. 50 d). The zygoma is slightly strong with low jugal projection (convex) (Fig 50 d). The palatal bridge is 1.45 – 2.50 mm/27.84 % in average of the maxillary toothrow length (C-M^3).

4.1.3 Morphometrics

Eleven external and ten cranial and dental character measurements of *Rhinolophus sinicus* and *R. thomasi* were taken and compared. *R. sinicus* and *R. thomasi* are significantly different in body sizes, external and internal characters, accept ears and the palatal bridge. The Mann-Whitney samples test and the descriptive statistics (Mean ± SD, Min, Max and number) are presented in Table 1.

4.1.3.1 Sexual Dimorphism

Males and females of *Rhinolophus sinicus* and *R. thomasi* species are considerable different in some characters within each species ($P<0.05$), but most of them are slightly similar in morphology ($P>0.05$). A summary of the measurements and statistics test are included in Table 2 and Table 3.

4.1.3.2 Wing Structure Measurements

The first phalanx of the third metacarpal of *R. sinicus* is less than half (43%) the average length of the third digit. The second phalanx of the third digit is relatively short compared to *R. affinis*; it averages 68.9% of the length of the third metacarpal. In addition, the average length of the second phalanx of the third digit is mostly less than 1.60 mm the length of the first phalanx of the third metacarpal.

Usually, the first phalanx of the third metacarpal of *R. thomasi* is less than half of the third metacarpal; it is 44.5% of average length. The second phalanx of the third digit is gradually more than 70.33% length in average of the third metacarpal (Fig. 24, 25).

Table 1: Two independent samples test (Mann-Whitney) and the descriptive statistics showing the mean ± SD, minimum-maximum (mm) for external, cranial and dental measurements of *Rhinolophus sinicus* and *R. thomasi*.

Characters	Mann-Whitney Samples test 95% Confidence Interval of the Difference								
	Rhinolophus sinicus				*Rhinolophus thomasi*				Both
	Mean ± SD	Min	Max	n	Mean ± SD	Min	Max	n	Sig (P)
HBL	52.31 ± 2.65	49.00	58.49	16	46.26 ± 1.82	43.77	49.20	13	.001*
E	17.63 ± 1.53	14.38	20.00	17	16.87 ± 1.74	14.45	19.30	13	.478
TL	25.07 ± 1.62	21.50	28.00	17	20.20 ± 3.42	14.80	26.00	13	.001*
HF	8.53 ± 0.65	7.50	9.44	17	7.00 ± 0.94	5.20	9.20	13	.001*
TIB	19.64 ± 0.68	18.59	21.19	17	17.43 ± 0.66	15.74	18.32	12	.001*
FA	47.35 ± 1.27	43.63	48.80	17	43.79 ± 1.05	41.50	45.10	13	.001*
5Met	37.84 ± 1.09	36.14	40.22	17	33.53 ± 1.55	29.31	35.17	12	.001*
4Met	36.47 ± 1.03	34.13	38.22	17	32.85 ± 1.64	28.23	34.36	12	.001*
3Met	35.49 ± 1.14	33.18	37.28	17	31.48 ± 1.66	26.83	33.17	12	.001*
3Met1ph	15.21 ± 0.68	13.39	16.17	17	14.03 ± 0.46	13.41	15.11	12	.001*
3Met2ph	24.36 ± 1.26	20.31	25.78	17	22.46 ± 1.48	18.71	24.43	12	.001*
GTL	20.78 ± 0.53	20.14	21.93	13	19.04 ± 0.58	18.38	19.95	15	.001*
CCL	17.81 ± 0.45	17.29	18.87	13	16.20 ± 0.37	15.59	16.83	17	.001*
SL	19.89 ± 0.45	19.48	21.08	14	18.34 ± 0.42	17.74	19.23	17	.001*
ZB	10.38 ± 0.32	9.68	10.89	16	9.53 ± 0.35	8.83	10.03	17	.001*
BB	9.54 ± 0.15	9.33	9.77	14	8.93 ± 0.23	8.60	9.35	17	.001*
C-M^3	7.80 ± 0.25	7.34	8.31	17	7.01 ± 0.26	6.61	7.45	17	.001*
M3-M3	8.03 ± 0.28	7.40	8.44	17	7.28 ± 0.20	6.98	7.73	17	.001*
PL	2.11 ± 0.19	1.62	2.46	16	1.95 ± 0.30	1.45	2.50	17	.220
C-M_3	8.34 ± 0.26	7.80	8.86	17	7.75 ± 1.37	7.13	12.98	17	.001*
ML	13.89 ± 0.49	12.55	14.76	17	12.65 ± 0.34	11.96	13.13	17	.001*

* The mean difference is significant at the 0.05 level

Table 2: Two independent samples test (Mann-Whitney) and the descriptive statistics (Mean ± SD, mm) for eleven external and ten cranial and dental characters of *R. sinicus* between males and females.

Characters	Mann-Whitney Samples test 95% Confidence Interval of the Difference				
	Rhinolophus sinicus Mean ± SD				
	Males	n	Females	n	Sig (P)
HB	52.85 ± 2.71	8	51.76 ± 2.66	8	**.342**
E	17.86 ± 1.86	8	17.43 ± 1.25	9	**.499**
TL	25.12 ± 1.42	8	25.03 ± 1.87	9	**.923**
HF	8.83 ± 0.48	8	8.26 ± 0.68	9	**.099**
TIB	20.07 ± 0.58	8	19.26 ± 0.55	9	**.007***
FA	47.29 ± 1.55	8	47.40 ± 1.04	9	**.630**
5MET	37.95 ± 1.02	8	37.75 ± 1.20	9	**.700**
4MET	36.78 ± 0.71	8	36.19 ± 1.21	9	**.268**
3MET	35.94 ± 0.69	8	35.09 ± 1.34	9	**.336**
3MET1PH	15.45 ± 0.59	8	15.00 ± 0.72	9	**.248**
3MET2PH	24.95 ± 0.76	8	23.84 ± 1.42	9	**.027***
GTL	21.05 ± 0.59	6	20.55 ± 0.38	7	**.086**
CCL	18.23 ± 0.43	5	17.54 ± 0.15	8	**.003***
SL	20.18 ± 0.57	6	19.67 ± 0.15	8	**.053**
ZB	10.46 ± 0.39	8	10.30 ± 0.25	8	**.293**
BB	9.68 ± 0.07	6	9.43 ± 0.09	8	**.037***
C-M^3	7.90 ± 0.31	8	7.72 ± 0.15	9	**.112**
M3-M3	8.04 ± 0.23	8	8.01 ± 0.32	9	**.247**
PL	2.14 ± 0.25	8	2.08 ± 0.11	8	**.847**
C-M_3	8.45 ± 0.32	8	8.24 ± 0.15	9	**.038***
ML	14.00 ± 0.65	8	13.79 ± 0.28	9	**.060**

* The mean difference is significant at the 0.05 level

Table 3: Two Independent samples test (Mann-Whitney) and the descriptive statistics (Mean ± SD, mm) for eleven external and ten cranial and dental characters of *R. thomasi* and between males and females.

Characters	Mann-Whitney Samples test 95% Confidence Interval of the Difference				
	Rhinolophus thomasi Mean ± SD				
	Males	n	Females	n	Sig (P)
HBL	46.84 ± 1.36	7	45.59 ± 2.17	6	**.291**
E	17.91 ± 1.38	7	15.65 ± 1.28	6	**.042***
TL	21.34 ± 1.98	7	18.87 ± 4.40	6	**.415**
HF	6.79 ± 1.20	7	7.25 ± 0.51	6	**.744**
TIB	17.34 ± 0.81	7	17.55 ± 0.41	5	**.850**
FA	43.46 ± 1.28	7	44.18 ± 0.60	6	**.193**
5MET	33.66 ± 0.84	7	33.35 ± 2.34	5	**.705**
4MET	33.23 ± 0.78	7	32.31 ± 2.42	5	**1.00**
3MET	31.64 ± 0.87	7	31.24 ± 2.52	5	**.571**
3MET1PH	14.16 ± 0.54	7	13.86 ± 0.28	5	**.257**
3MET2PH	22.82 ± 1.19	7	21.95 ± 1.83	5	**.571**
GTL	19.44 ± 0.53	6	18.78 ± 0.47	9	**.078**
CCL	16.46 ± 0.34	7	16.01 ± 0.27	10	**.047***
SL	18.62 ± 0.43	7	18.14 ± 0.29	10	**.035***
ZB	9.79 ± 0.18	7	9.34 ± 0.32	10	**.025***
BB	9.10 ± 0.16	7	8.81 ± 0.19	10	**.317**
C-M^3	7.18 ± 0.18	7	6.89 ± 0.25	10	**.084**
M3-M3	7.34 ± 0.16	7	7.25 ± 0.23	10	**.249**
PL	2.17 ± 0.19	7	1.79 ± 0.26	10	**.015***
C-M_3	7.64 ± 0.22	7	7.84 ± 1.81	10	**.063**
ML	12.81 ± 0.29	7	12.53 ± 0.35	10	**.110**

* The mean difference is significant at the 0.05 level

4.1.3.3 Palatal Bridge

In *R. sinicus,* the average length of the bony palate is about 27.40% of maxillary upper toothrow length (25.72 - 31.30%) or even more and sometime up to one third of maxillary toothrow length. Palate is emarginated posterioly to the level of the mesostyle of the first upper molar (M^1) and anterioly to the level metacone of the second upper molar (M^2). In *R. thomasi* the palatal bridge averages 29.75% of maxillary toothrow length but with a wide variation (21.9% - 34.4%). The palate is emarginated posteriorly to the level of the mesostyle of the first upper molar (M^1) and posteriorly to the middle level commissures of the second upper molar (M^2) see in Fig. 26.

4.1.3.4 First Upper Premolars (P^2)

In *R. sinicus,* the first upper premolar is small to fairly sized, but even though extremely tiny in some specimens and usually lying in the toothrow (88.24%), but with a minority (11.76%) slightly extruded from the toothrow (Fig. 19, p 68). The upper canine (C^1) and the second upper premolar (P^4) are greatly separated, nevertheless the cingular are both relatively close together, but not in contact.

Generally, the first upper premolar of *R. thomasi* is medium sized, and is situated in the toothrow. The upper canine (C^1) and second upper premolar (P^4) are not in contact (Fig. 19, p 65).

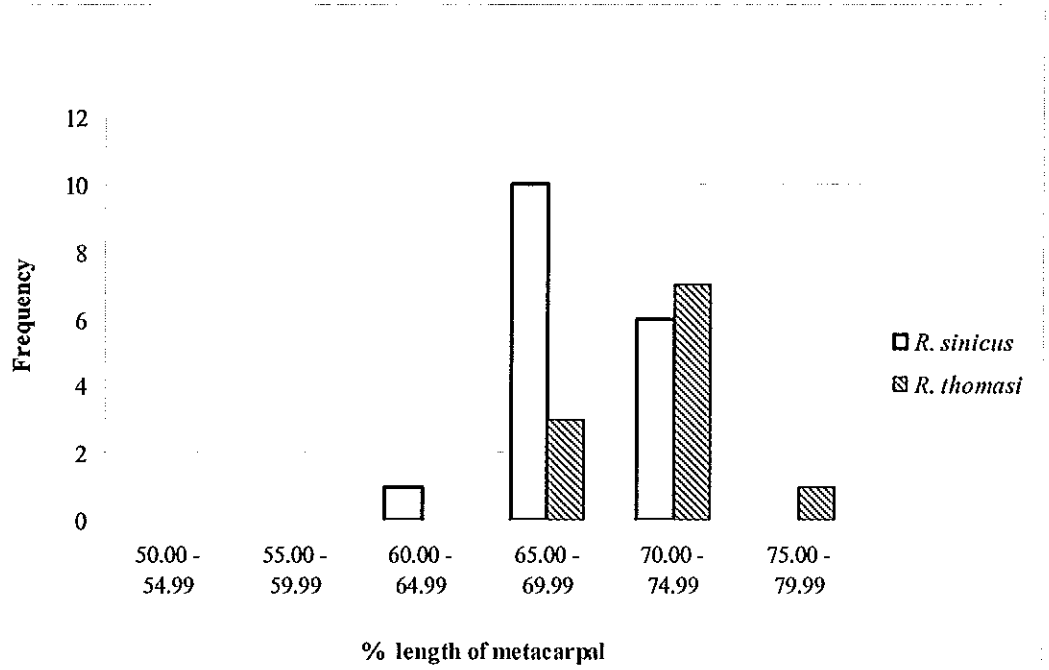


Figure 24: The ratio of the length of the second phalanx of the third digit to the length of metacarpal between *R. sinicus* and *R. thomasi*

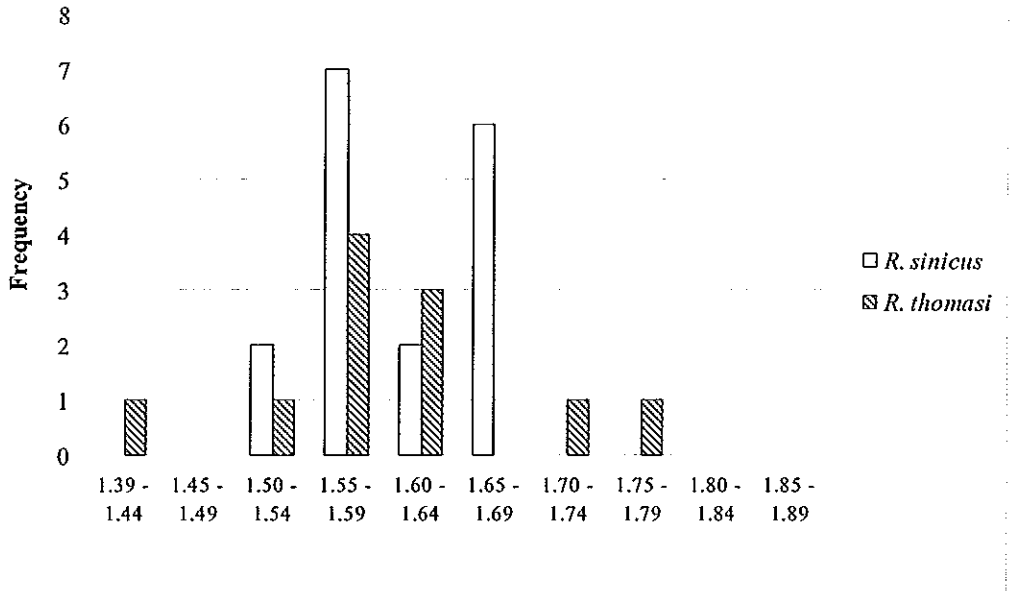


Figure 25: The ratio of the second phalanx of the third digit and the first phalanx of the third digit between *R. sinicus* and *R. thomasi*.

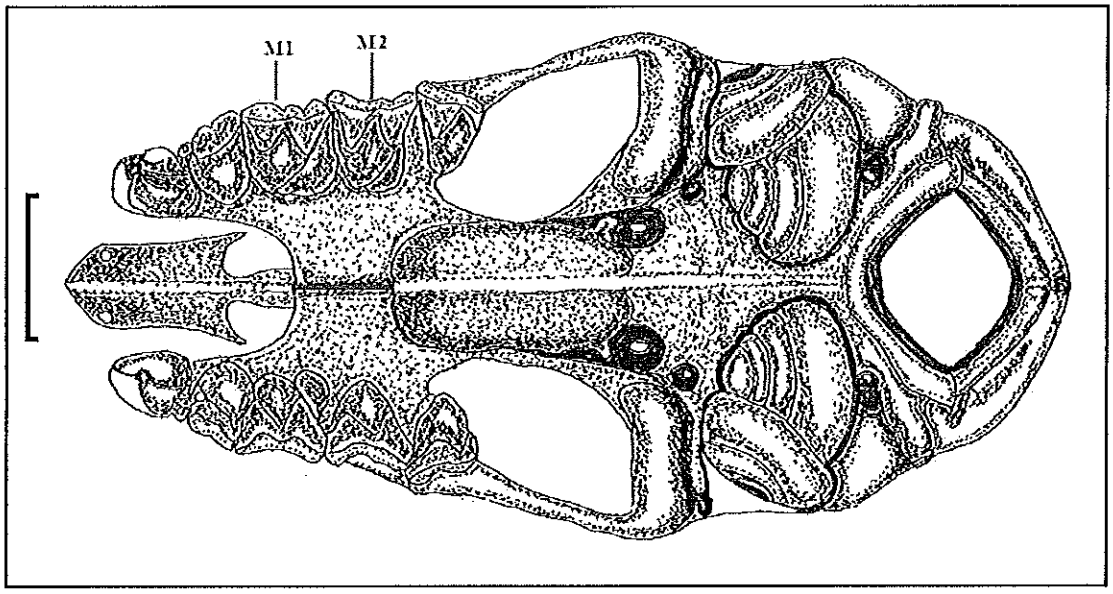


Figure 26: Palatal length (red line) of *R. sinicus* MM 86. HZM (BMZS) from India. Scale = 1 mm.

4.1.3.5 Second Lower Premolars (P_3)

In *R. sinicus,* the second lower premolar (P_3) is small or very tiny. It is partially or fully extruded from the toothrow. The first lower premolar (P_2) and third lower premolar (P_4) are in contact. The second lower premolar of *R. thomasi* is very small and usually partly or completely extruded from the toothrow. The first lower premolar (P_2) and the third lower premolars (P_4) are in contact (Fig. 20, p 65).

4.1.3.6 Bacular Morphology

Only the baculum of *R. thomasi* was extracted and there is not baculum of *R. sinicus* from this study because it's relevant to the rare specimens of males of *R. sinicus*. In lateral view, the baculum has a simple shaft and is usually slightly curved towards the bluntly pointed tip. The basal cone is expanded and flattened. Average baculum length is 1.81 mm (1.67 – 1.95 mm). The baculum of specimen from Vietnam had a slightly more robust tip (Fig. 27).

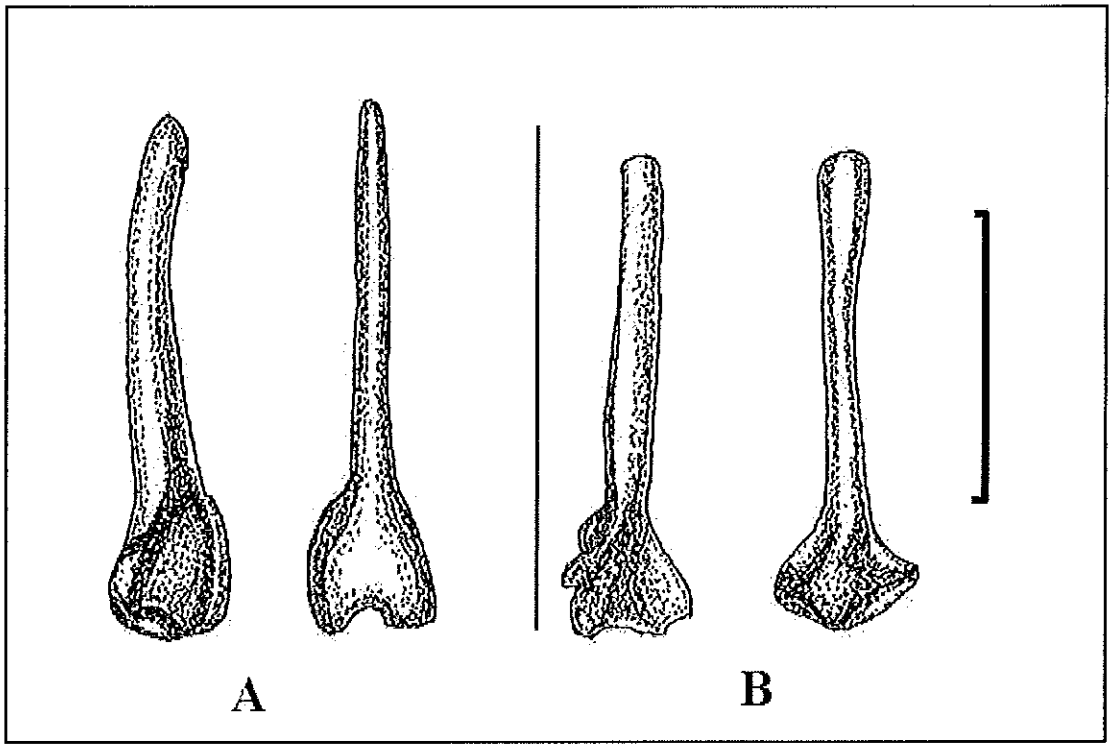


Figure 27: Baculum shape: Dorsal (left of each pair) and Lateral (right of each pair) views of A: *R. thomasi* HZM.No.8.32320 from Vietnam and B: *R. thomasi* HZM.No.10.35115 from Vietnam. Scale = 1 mm.

4.1.4 Principal Component Analysis (PCA) of fifteen characters

Principal Component Analysis (PCA) clearly differentiated *R. sinicus* from *R. thomasi* (Fig 28). The analysis was based on eleven specimens of *R. thomasi* and thirteen of *R. sinicus* and included fifteen characters: FA, 5 Met, 4Met, 3Met, 3Met1ph, 3Met2ph, CCL, SL, ZB, BB, PC, C-M^3, M^3-M^3, PL and ML (for abbreviations see in the pp 56 – 59).

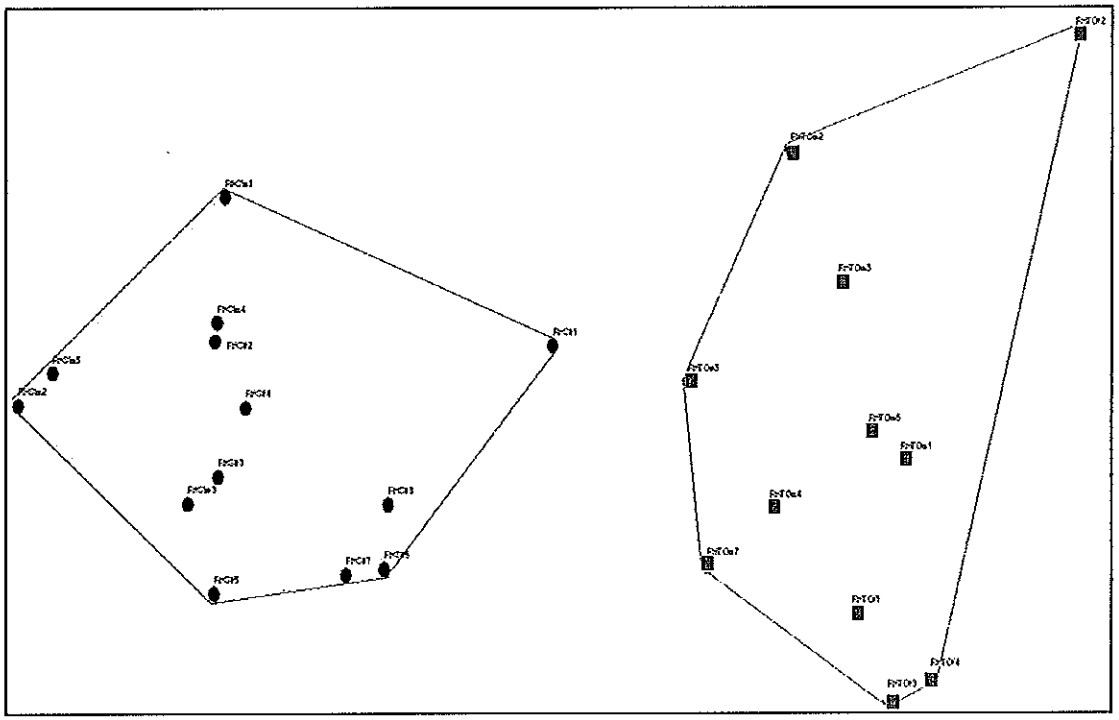


Figure 28: The principal component analysis (PCA) of twenty-five specimens of *R. sinicus* (blue) and *R. thomasi* (red) based on fifteen metric characters.

4.1.5 Echolocation

Currently, no echolocation data are available for *Rhinolophus sinicus* and *R. thomasi* since the study was based on existing voucher specimens held in the collections of the Harrison Institute and the Natural History Museum (London).

4.2 *Rhinolophus rouxii* from India and *Rhinolophus rouxii* from Sri Lanka

4.2.1 External Morphology Comparison

4.2.1.1 Sella Morphology and Variation

Sella is particularly straight-sided or parallel-margined (59%) from the base to the apex; the apex is broadly rounded off. Sella of *R. rouxii* is varying in shapes and side, sella with concave-margined of 18% of *R. rouxii* and occasionally, a 3% of sella with a slightly constriction at the middle part. Besides, there is other sella shape that obtainable in *R. rouxii,* it is indicated to be concavity, but without being as convex or as straight sides (Fig. 29). In addition, the sella shapes of *R. rouxii* from India are more stable line with parallel sides (73%) than *R. rouxii* from Sri Lanka (55%). Furthermore, the *R. rouxii* from Sri Lanka are having more variation of sella shapes than *R. rouxii* from India (Fig.30).

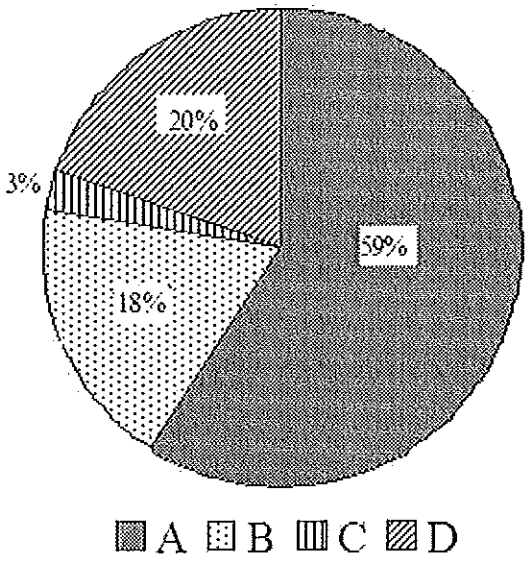


Figure 29: The percentage of different sella shapes in *Rhinolophus rouxii* from Southern India and Sri Lanka (n= 77). Sella shape with parallel margined (A), concave sides (B) and convex sides (C) see in Fig 17, p 62.

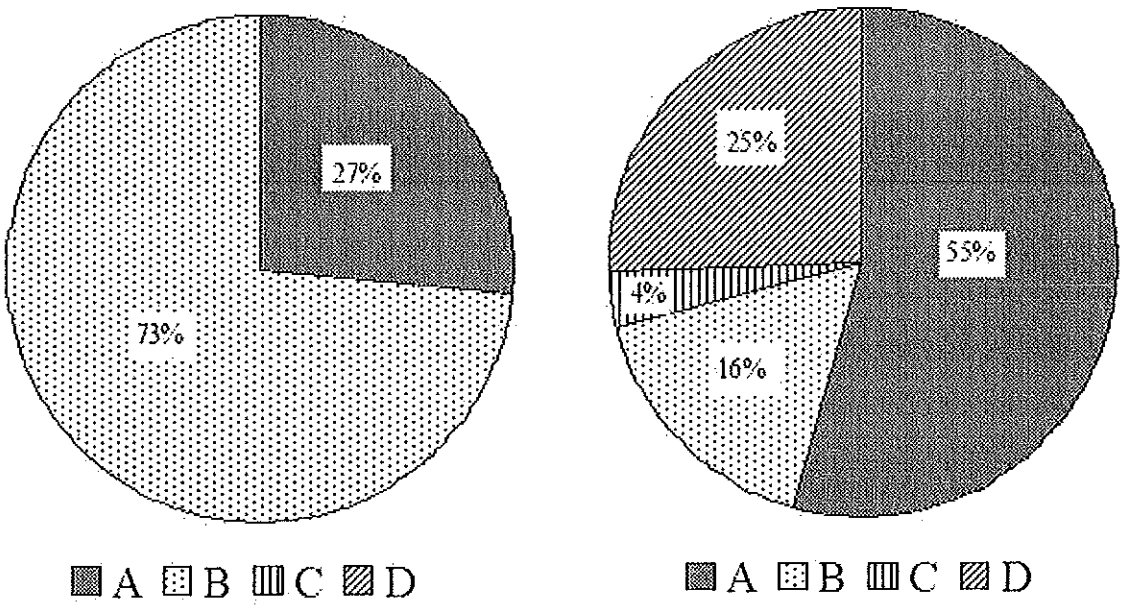


Figure 30: Comparison of sella shapes between *Rhinolophus rouxii* from India (left) and Sri Lanka (right). Sella shape with parallel margined (A), concave sides (B) and convex sides (C) see in Fig 17, p 62.

4.2.1.2 Lancet Morphology and Variation

In general, *R. rouxii* have had a high variation of lancet morphology. In this study, *R. rouxii* showed a discrepancy in lancet shapes (lancet-margin). There are three types of lancet, 36% of straight-margined or cuneate shape with the tip with rounded off (A), 46% of hastate shape with slender tip as concave sided (pagoda-shaped or B), the 14% of dome shapes with straight sided (C) and 4% of the last lancet margins are parallel with dome shape (D) see in Fig. 31.

Indeed, both *R. rouxii* from India and Sri Lanka typically vary in lancet shapes and the higher percentage of concave sides of lancet was found in the specimens from Sri Lanka. In contrast, the specimens from India have higher percentage of dome shape with straight side's lancet (Fig. 32).

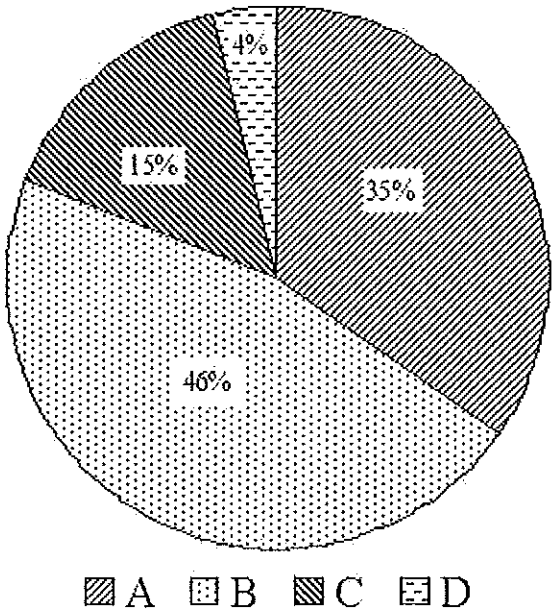


Figure 31: The percentage of different lancet shapes of *R. rouxii* from India and Sri Lanka (n= 77). Straight sides with triangular shaped (A), the triangular shaped with concave sides (B), the dome shaped with straight sides tip (C) and the dome shaped with elongate sides tip (D), see in Fig. 18, p 63.

4.2.2 Morphometrics

Eleven external and ten cranial and dental character measurements of *Rhinolophus rouxii* were compared and determined. The Descriptive statistic (Mean ± SD, Minimum, Maximum and Number) were presented in Table 4.

4.2.2.1 Sexual Dimorphism

Males and females of *R. rouxii* were tested for variation in size of eleven external and ten cranial and dental characters. From the results, there was significantly different in size between males and females in both countries (India and Sri Lanka). Some characters were significantly different between males and females such as the external characters, forearm length (FA), tibia length (TIB) and all of the cranial and dental characters (Table 4).

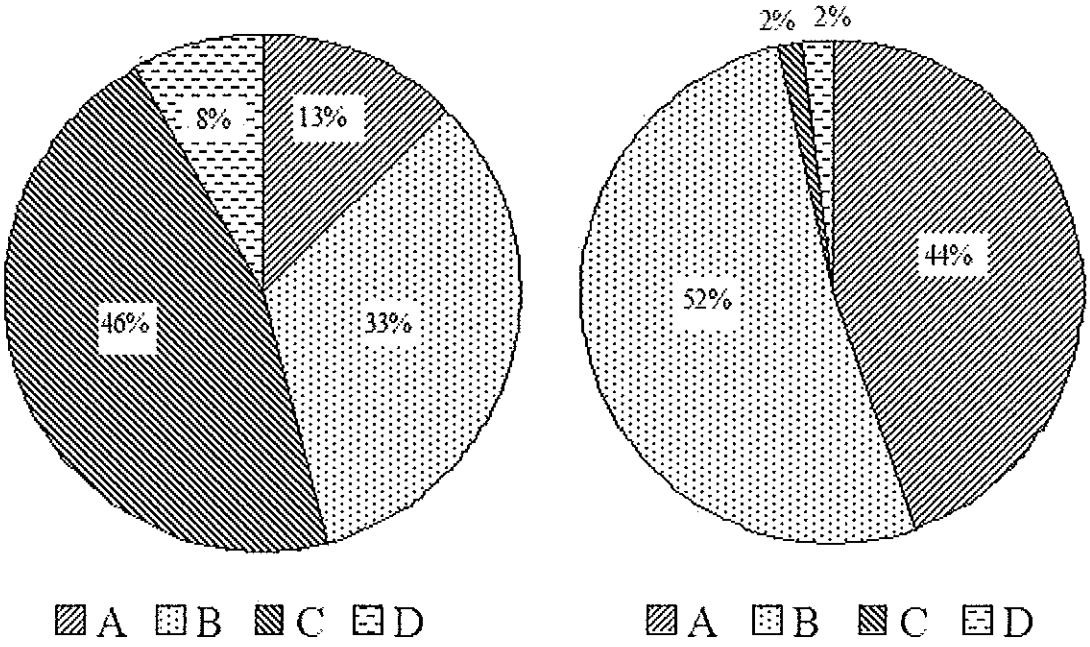


Figure 32: Comparison of lancet shape in *R. rouxii* from India (left) and *R. rouxii* from Sri Lanka (right). Straight sides with triangular shaped (A), the triangular shaped with concave sides (B), the dome shaped with straight sides tip (C) and the dome shaped with elongate sides tip (D), see in Fig. 18, p 63.

4.2.2.2 Wing Structure Measurement

The second phalanx of the third digit is more than 1.50 length of the first phalanx of third digit (in average length 1.532; N= 65). The first phalanx of the third metacarpal is always less than half the length of the third metacarpal, it is about 40%, and the second phalanx of the third metacarpal is on average less than 61% in length of the third metacarpal (Fig. 33).

4.2.2.3 Palatal Bridge

Palatal length is rather long, up to 1/3 of maxillary toothrow length or even more. Average length of palate is 2.30 mm, which is especially longer than *R. affinis* and *R. sinicus*, it is emarginated anteriorly to the level of the parastyle of the

first upper molar (M^1) and posteriorly to the metacone of second upper molar (M^2). Palatal bridge is relatively long, 26.94% - 28.11% of the upper toothrow.

4.2.2.4 First Upper Premolars (P^2)

First upper premolar is usually medium sized and completely in the toothrow (81.49%) but occasionally partly extruded (18.51%). P^2 of specimens from Sri Lanka and four specimens from Tamil Nadu, south India are partly extruded from the toothrow (Fig. 19, A, B, C; p 66).The size of P^2 and the gap between the canine (C^1) and the second upper premolar (P^4) are unstable.

4.2.2.5 Second Lower Premolars (P_3)

The second lower premolar is mostly external (66.68%), sometimes partially in the toothrow (25.92%), and occasionally is absent (1.23%). Additionally, a few specimens (6.17%) have second lower premolar within toothrow. The cingular of the first lower premolar (P_2) and third lower premolars (P_4) are mostly in contacted, but some specimens are nearly so or distinctly separated (Fig. 20, A, B; p 66).

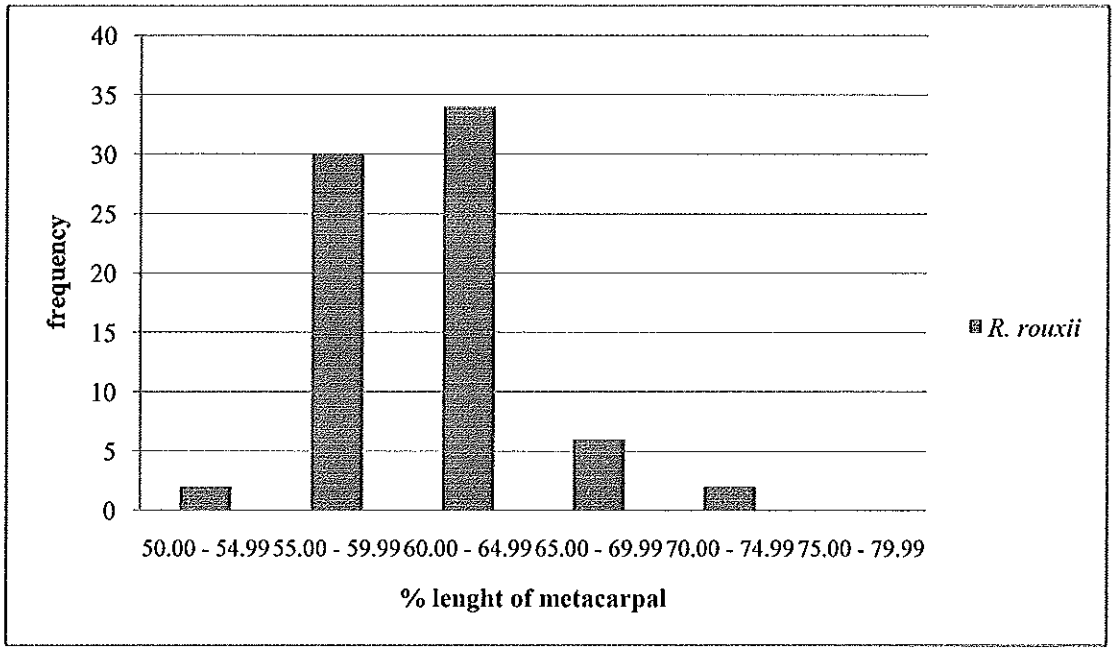
4.2.2.6 Bacular Morphology

Baculum is parallel-sided; the basal is expanded and near the tip is bent. The lengths of bacular are 2.07 – 2.70 mm, and average length is 2.25 mm. The greatest widths are 0.42 – 0.85 mm, and the mean of greatest width 0.58 mm. However, the baculum of *R. rouxii* (HZM. No.13.27453) from Sri Lanka is especially longer (2.70 mm) than other specimens of *R. rouxii* and the shape is different (slender). It is very similar in length to bacular of *R. affinis*, which is bigger and heavier than those Sri Lanka and India. These measurements and shape characters are new evidences to propose that *R. affinis* might be existed in Sri Lanka (Fig. 34).

Table 4: Comparison of matrix characters between *Rhinolophus rouxii* from India and Sri Lanka

Characters	*Rhinolophus rouxii* from India							*Rhinolophus rouxii* from Sri Lanka						
	Males			Females				Males			Females			
	Mean ± SD	Max	Min	Mean ± SD	Max	Min	Sig (P)	Mean ± SD	Max	Min	Mean ± SD	Max	Min	Sig (P)
HBL	56.63 ± 4.62	63.63	49.78	57.42 ± 7.77	66.00	50.68	**1.00**	53.54 ± 2.38	58.50	50.30	52.47 ± 2.64	57.50	48.17	**0.12**
E	17.30 ± 1.44	19.80	14.50	18.20 ± 0.72	18.90	17.20	**0.14**	18.08 ± 1.05	19.80	15.40	17.87 ± 1.13	19.93	16.00	**0.46**
TL	24.50 ± 1.91	28.50	22.00	26.50 ± 1.29	28.00	25.00	**0.06**	23.26 ± 2.46	29.00	17.10	21.77 ± 3.16	26.38	12.80	**0.60**
HF	10.40 ± 1.56	12.80	7.20	9.98 ± 1.61	11.50	8.30	**0.90**	9.71 ± 0.67	10.50	8.00	9.49 ± 0.62	10.50	7.90	**0.22**
TIB	22.65 ± 0.95	23.66	20.91	23.24 ± 0.72	24.10	22.60	**0.33**	**21.88 ± 0.56***	22.80	20.47	**20.86 ± 0.73***	22.07	19.05	**0.01**
FA	50.39 ± 1.32	52.30	48.03	50.56 ± 1.15	51.80	49.00	**0.78**	**48.50 ± 1.13***	50.99	46.40	**47.71 ± 1.06***	49.60	45.09	**0.09**
5Met	39.79 ± 0.96	41.18	37.80	39.70 ± 0.91	40.94	38.42	**0.85**	38.68 ± 0.94	40.90	37.00	38.36 ± 0.98	39.84	35.55	**0.21**
4Met	39.01 ± 0.98	40.64	37.22	39.11 ± 1.62	41.59	37.37	**0.92**	38.53 ± 0.95	40.99	36.69	38.27 ± 1.21	40.55	34.69	**0.39**
3Met	37.96 ± 1.05	39.49	35.58	37.92 ± 1.18	39.74	36.86	**0.78**	37.20 ± 1.20	39.50	34.30	36.88 ± 1.20	38.60	33.48	**0.31**
3Met1ph	15.35 ± 0.54	16.18	14.21	15.56 ± 0.76	16.45	14.44	**0.35**	14.59 ± 0.60	15.59	13.42	14.64 ± 0.65	15.84	12.93	**0.79**
3Met2ph	24.23 ± 1.26	25.87	21.90	24.09 ± 1.91	26.18	21.10	**1.00**	22.10 ± 0.79	23.77	20.48	22.09 ± 1.01	23.68	18.67	**0.98**
GTL	22.76 ± 0.60	23.61	21.60	22.36 ± 0.94	23.21	21.54	**0.43**	**22.62 ± 0.45***	23.33	21.39	**21.69 ± 0.44***	22.89	20.93	**0.01**
CCL	19.45 ± 0.81	20.33	17.08	19.10 ± 0.88	20.25	17.92	**0.26**	**19.38 ± 0.27***	19.80	18.77	**18.38 ± 0.44***	19.82	17.79	**0.01**
SL	22.06 ± 0.56	22.81	20.73	21.49 ± 0.87	22.75	20.59	**0.20**	**21.82 ± 0.30***	22.32	21.12	**20.74 ± 0.50***	22.31	19.85	**0.01**
ZB	11.40 ± 0.25	11.68	10.96	11.08 ± 0.54	11.76	10.34	**0.20**	**11.01 ± 0.20***	11.39	10.65	**10.59 ± 0.29***	11.35	10.14	**0.01**
BB	10.38 ± 0.16	10.68	10.15	10.15 ± 0.30	10.61	9.74	**0.69**	**10.26 ± 0.16***	10.53	10.00	**9.96 ± 0.20***	10.37	9.60	**0.01**
C-M^3	8.89 ± 0.28	9.35	8.42	8.71 ± 0.49	9.33	8.12	**0.37**	**8.81 ± 0.18***	9.18	8.46	**8.38 ± 0.22***	9.06	7.99	**0.01**
M3-M3	8.37 ± 0.20	8.79	8.04	8.22 ± 0.24	8.55	7.83	**0.17**	**7.97 ± 0.14***	8.30	7.70	**7.86 ± 0.19***	8.32	7.45	**0.02**
PL	2.52 ± 0.14	2.76	2.21	2.43 ± 0.17	2.65	2.18	**0.19**	2.44 ± 0.18	2.90	2.07	2.35 ± 0.12	2.65	2.11	**0.57**
C-M_3	9.47 ± 0.35	10.06	9.08	9.28 ± 0.53	10.00	8.62	**0.23**	**9.52 ± 0.16***	9.77	9.10	**9.00 ± 0.30***	9.93	8.48	**0.01**
ML	**15.66 ± 0.44***	16.35	14.89	**14.91 ± 0.76***	15.93	13.88	**0.02**	**15.38 ± 0.25***	16.09	14.87	**14.63 ± 0.45***	15.95	23.65	**0.01**

*The mean difference is significant at the 0.05 level



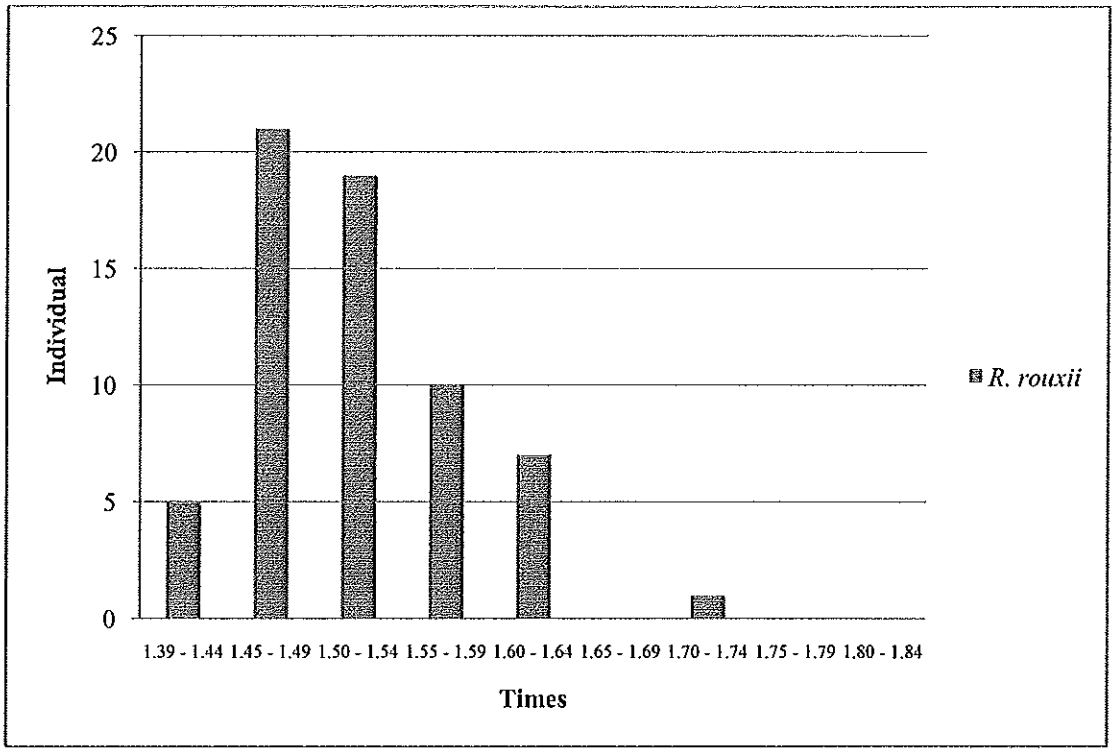


Figure 33: Wing structure comparing (% length) between the second phalanxes of the third digit divided by the first phalanx of the third digit (above) and the ratio length of the second phalanxes of the third digit (bottom).

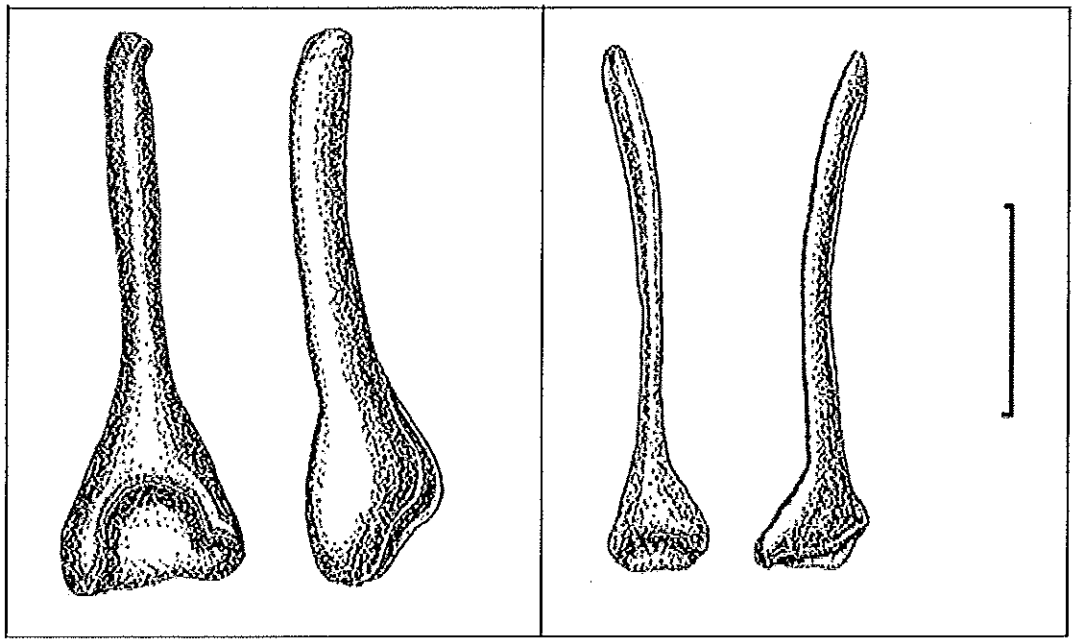


Figure 34: Baculum morphologies of *R. rouxii*: (A) Dorsal (left of each pair) and lateral (right of each pair) views of *R. rouxii* HZM. 13. 27453 from Sri Lanka and (B) Dorsal (left) and lateral (right) views of *R. rouxii* HZM. 22. 28754 from India. Scale = 1 mm.

4.2.3 Principal Component Analysis (PCA) of Seventeen Characters

Fifty-seven individuals of *R. rouxii* from India (n=15) and Sri Lanka (n=42) were group together. Normally, *R. rouxii* from India and Sri Lanka could be divided into two big groups, but there is still overlap in both external and internal characters. However, the cranial and dental characters seem to be less overlapping than external characters. In fact, *R. rouxii* from India can be possibly divided into three subgroups and *R. rouxii* from Sri Lanka could be divided into two subgroups (Fig. 35).

4.2.4 Analysis of variance (ANOVA) Test

One-way ANOVA was applied to examine the variation between these two countries (India and Sri Lanka) of single species. Tukey HSD post hoc test was applied to test the both twenty-one external and cranial and dental characters that used in the PCA with maximum eighty-one individuals. There were twelve characters,

which were significantly (P<0.05) /different, there are considerably different in cranial and dental characters, but similarity of external characters (Table 5).

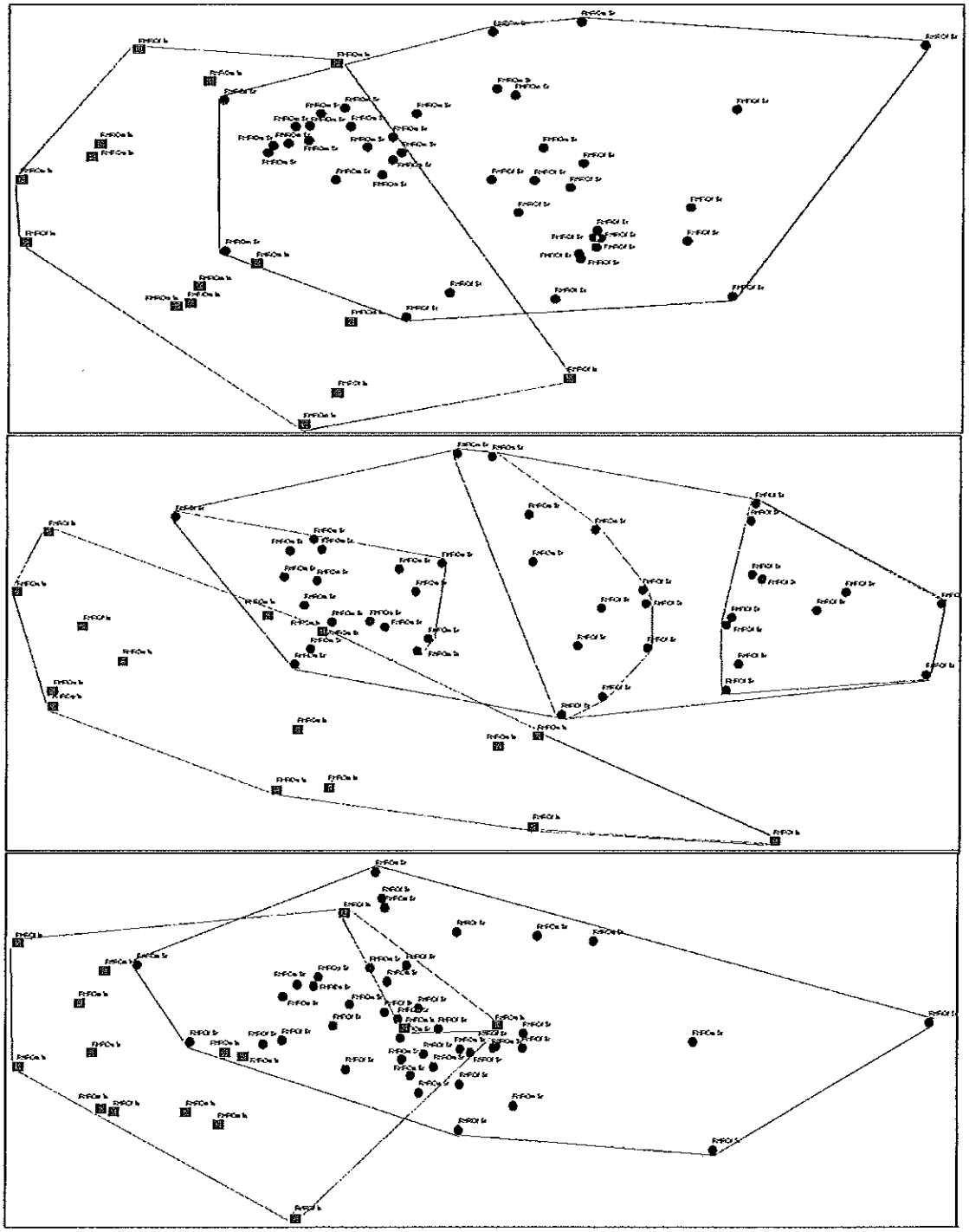


Figure 35: The Principal Component Analysis of external characters (Top), cranial and dental characters (middle) and external, cranial and dental characters (bottom) of *R. rouxii* from India (red square) and Sri Lanka (blues speckle).

4.2.5 Echolocation

In this study, there is no echolocation call recorded for *R. rouxii.* Because most voucher specimens were loan from the collections of the Harrison Institute (HZM) and the British Natural History Museum (London).

Table 5: Geographical variation in size of external, skull and cranial characters between *R. rouxii* from India and Sri Lanka

One-way ANOVA for Equality of means 95% confidence interval of the difference					
Characters	N	Minimum	Maximum	Mean ± SD	Sig (P)
HBL	70	48.17	66.00	53.87±3.69	.119
E	70	14.50	19.93	17.87±1.15	.813
TAIL	70	12.80	29.00	23.08±2.89	.071
HF	70	7.20	12.80	9.75±0.95	.111
TIB	69	19.05	24.10	21.69±1.03	.001*
FA	75	45.09	52.30	48.70±1.55	.007*
5Met	74	35.55	41.18	38.84±1.09	.059
4Met	74	34.69	41.59	38.56±1.12	.284
3Met	74	33.48	39.74	37.27±1.22	.150
3Met1ph	74	12.93	16.45	14.81±0.69	.705
3Met2ph	74	18.67	26.18	22.63±1.38	.219
GTL	59	20.93	23.61	22.28±0.67	.001*
CCL	69	17.08	20.33	19.02±0.72	.001*
SL	67	19.85	22.81	21.47±0.72	.001*
ZB	71	10.14	11.76	10.95±0.40	.001*
BB	70	9.60	10.68	10.17±0.24	.001*
CM3UP	73	7.99	9.35	8.66±0.33	.001*
M3M3	73	7.45	8.79	8.03±0.25	.010*
PL	73	2.07	2.90	2.42±0.15	.012*
CM3LOW	73	8.48	10.06	9.29±0.31	.001*
ML	73	13.65	16.35	15.11±0.59	.001*

* The mean difference is significant at the 0.05 level

4.3 *Rhinolophus affinis* from India and *Rhinolophus affinis* from Southeast-Asia versus *R. affinis* from Java

4.3.1 External Morphology Comparison

4.3.1.1 Sella Morphology and Variation

R. affinis showed some variation in sella shapes, but the highest percentage is typically sides' concave (61%), its side at the base arched and broader at the base of its vertical angle 18% of parallel sides (Fig. 36). Other component, there is undefined in sella shape (21%) because in some specimens showed an indication of a concavity, without being as convex as a (C) or as straight as a (A), it is not included in the figure below.

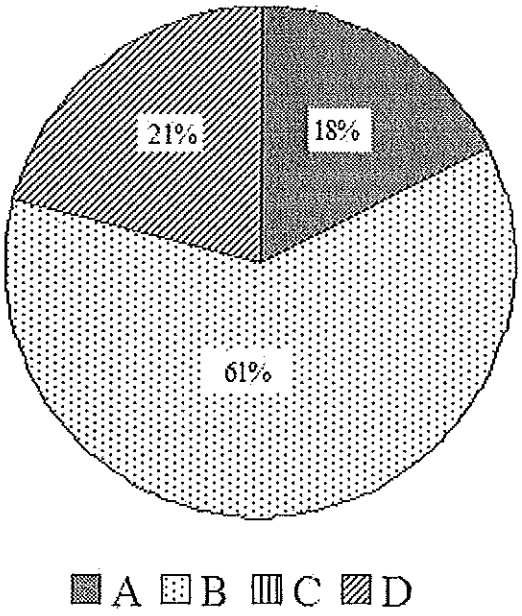


Figure 36: The charge is showing the sella variation in *R. affinis* (n=114) in southeast-Asia. The sella shape with parallel margined (A), concave sides (B) and convex sides (C) see in Fig 17, p 62.

4.3.1.2 Lancet Morphology and Variation

In *R. affinis,* lancet is almost wedge-shaped (Cuneate), the leaves with broad abruptly pointed apex and tapering to the base. Lancet is always straight sides (98%) and only small numbers (2%) of the concave sides that presented. Although, most of *R. affinis* lancet' shapes were not different in lancet shapes between

specimens from southeast-Asia, India and Java (Type specimen), but three specimens of *R. affinis* from Nala Pania cave, south and 7 km to Mussoorie, north India showed the difference in lancet shapes, it is the concave sides (2%), see in Fig. 37.

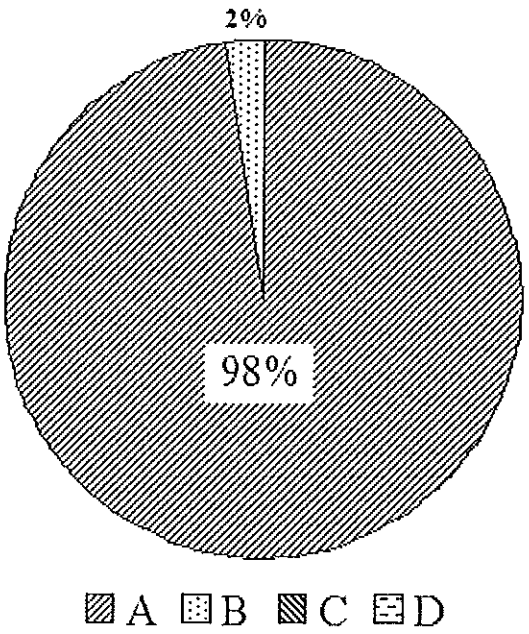


Figure 37: Variation in lancet shapes from *R. affinis* from India, southeast-Asia and Java, Indonesia (Type specimen). Straight sides with triangular shaped (A), the triangular shaped with concave sides (B), the dome shaped with straight sides tip (C) and the dome shaped with elongate sides tip (D), see in Fig. 18, p 63.

4.3.2 Morphometrics

Ten external and eleven cranial and dental character measurements of *Rhinolophus affinis* from India, Southeast-Asia and Java, Indonesia were taken and compared. Even thought, *R. affinis* from India and Southeast-Asia are mostly not significantly different in external, cranial and dental character measurements with *R. affinis* from Java, Indonesia, but some external, cranial and dental characters are statistically significantly different (Table 6).

Table 6: Mean ± SD, minimum-maximum (mm) and P-value (Mann-Whitney samples test) of external, cranial and dental characters of *R. affinis* from India, Southeast-Asia and Indonesia (Java)

Characters	India				Southeast-Asia and Java				Sig (P)
	N	Min	Max	Mean ± SD	N	Min	Max	Mean ± SD	
HBL	14	49.00	66.00	55.75±4.89	96	49.00	72.15	56.80±4.77	0.60
TAIL	14	20.00	29.00	24.10±2.87	97	16.53	32.00	23.81±2.94	0.38
HF	14	10.00	12.50	11.00±0.63	96	8.00	12.00	10.26±0.73	0.13
TIB	14	21.08	25.21	23.53±1.08	96	21.84	26.27	24.29±1.01	0.22
FA	14	51.00	55.00	53.58±1.06	97	46.72	54.55	50.88±1.50	0.15
5Met	14	41.44	44.32	43.00±0.99	96	37.71	44.26	41.15±1.43	0.27
4Met	14	40.05	43.20	41.67±1.01	96	36.92	44.14	40.04±1.46	0.56
3Met	14	38.50	42.02	39.90±1.01	96	35.84	42.67	38.64±1.38	0.82
3Met1ph	14	14.82	16.45	15.73±0.51	96	14.10	16.70	15.37± 0.62	0.48
3Met2ph	14	27.45	31.87	29.76±1.20	96	24.00	31.37	27.16±1.38	0.09
GTL	15	22.17	24.28	23.37±0.48	101	21.61	24.70	23.28±0.65	**0.01***
CCL	15	19.53	20.53	19.86±0.24	111	17.55	21.05	19.72±0.57	0.17
SL	15	22.25	23.23	22.49±0.26	111	20.10	23.64	22.39±0.59	0.30
ZB	15	11.06	11.76	11.39±0.18	114	1.74	11.97	11.07±0.94	0.10
BB	14	9.12	9.61	9.37±0.15	102	8.59	11.14	9.58±0.49	**0.01***
CM3UP	15	8.68	9.61	9.05±0.20	115	7.58	9.60	8.91±0.32	0.21
M3M3	15	8.29	9.15	8.85±0.20	115	7.30	9.25	8.48±0.28	0.18
PL	15	1.96	2.31	2.17±0.11	114	1.81	2.57	2.22±0.16	0.97
CM3LOW	15	9.17	10.05	9.65±0.22	115	7.85	10.10	9.42±0.34	0.54
ML	15	15.18	16.72	15.85±0.33	115	13.67	16.45	15.54±0.53	**0.01***
PC	15	1.74	2.35	2.08±0.16	115	1.80	2.76	2.19±0.20	0.61

* The mean difference is significant at the 0.05 level

4.3.2.1 Sexual Dimorphism

Statistically, males and females of *Rhinolophus affiis* from India and Southeast-Asia are different in size of cranial and dental characters ($P<0.05$). Even though, Mann-Whitney test of *R. affinis* from Java, Indonesia, there are no different

(significance) in both characters (P>0.05). A summary of the measurements and statistics test are included in Table 7 and Table 8.

Table 7: Independent samples test (mean ± SD, minimum-maximum and P-value) of external, cranial and dental characters of *R. affinis* from India and Southeast-Asia.

	India				Southeast-Asia				
Characters	N	Min	Max	Mean ± SD	N	Min	Max	Mean ± SD	Sig (P)
HBL	14	49.00	66.00	55.75±4.89	95	49.00	72.15	56.83±4.79	0.44
E	14	14.00	23.00	17.12±2.21	96	15.27	24.10	19.87±1.57	0.67
TAIL	14	20.00	29.00	24.10±2.87	96	16.53	32.00	23.83±2.95	0.35
HF	14	10.00	12.50	11.00±0.63	95	8.00	12.00	10.27±0.72	0.32
TIB	14	21.08	25.21	23.53±1.08	95	21.84	26.27	24.30±1.01	0.07
FA	14	51.00	55.00	53.58±1.06	96	46.72	54.55	50.91±1.49	0.19
5Met	14	41.44	44.32	43.00±0.99	95	37.71	44.26	41.16±1.44	0.24
4Met	14	40.05	43.20	41.67±1.01	95	36.92	44.14	40.05±1.46	0.28
3Met	14	38.50	42.02	39.90±1.01	95	35.84	42.67	38.64±1.38	0.46
3Met1ph	14	14.82	16.45	15.73±0.51	95	14.10	16.70	15.37±0.62	0.78
3Met2ph	14	27.45	31.87	29.76±1.20	95	24.00	31.37	27.19±1.37	0.62
GTL	15	22.17	24.28	23.37±0.48	93	21.61	24.70	23.23±0.62	**0.01***
CCL	15	19.53	20.53	19.86±0.24	97	17.55	20.87	19.69±0.56	**0.01***
SL	15	22.25	23.23	22.49±0.26	97	20.10	23.64	22.37±0.58	**0.01***
ZB	15	11.06	11.76	11.39±0.18	98	10.01	11.74	11.12±0.32	**0.01***
BB	14	9.12	9.61	9.37±0.15	87	8.59	9.98	9.42±0.27	0.57
CM3UP	15	8.68	9.61	9.05±0.20	98	7.58	9.54	8.89±0.31	**0.03***
M3M3	15	8.29	9.15	8.85±0.20	98	7.72	9.09	8.46±0.25	0.12
PL	15	1.96	2.31	2.17±0.11	98	1.81	2.55	2.22±0.16	**0.01***
CM3LOW	15	9.17	10.05	9.65±0.22	98	7.85	10.04	9.42±0.34	**0.01***
ML	15	15.18	16.72	15.85±0.33	98	13.67	16.45	15.58±0.51	**0.01***
PC	15	1.74	2.35	2.08±0.16	98	1.80	2.76	2.19±0.21	0.71

* The mean difference is significant at the 0.05 level

Table 8: Mann-Whitney samples test and descriptive statistics (Mean ± SD, minimum-maximum, and P-value) of cranial and dental characters of *R. affinis* from Java, Indonesia.

Characters	Mann-Whitney test for Equality of Means 95% Confidence Interval of the Difference				
	N	Mean ± SD	Minimum	Maximum	Sig (P)
GTL	8	23.89±0.69	22.35	24.68	.513
CCL	14	19.91±0.67	18.74	21.05	.983
SL	14	22.54±0.66	21.13	23.50	.273
ZB	16	10.75±2.43	1.74	11.97	.037*
BB	15	10.55±0.33	9.85	11.14	.470
C-M^3	17	9.01±0.34	8.38	9.60	.958
M3-M3	17	8.57±0.43	7.30	9.25	.429
PL	16	2.23±0.17	1.92	2.57	.543
C-M_3	17	9.41±0.37	8.78	10.10	.492
ML	17	15.25±0.58	13.77	16.11	.833
PC	17	2.14±0.90	1.90	2.31	.334

* The mean difference is significant at the 0.05 level

4.3.2.2 Wing Structure Measurements

The second phalanx of the third metacarpal is long about three fourth the length of the third metacarpal (71%, 64% – 79%, n= 112), see in the (Fig. 38). First phalanx of third digit is less than half length of metacarpal (about 40% length of the third metacarpal (37% - 43%, n= 112).

The second phalanx of third digit is always more than 1.59 mm lengths the first phalanx of the third digit (1.78, 1.59 mm – 2.07 mm, n= 112) see in Fig. 39.

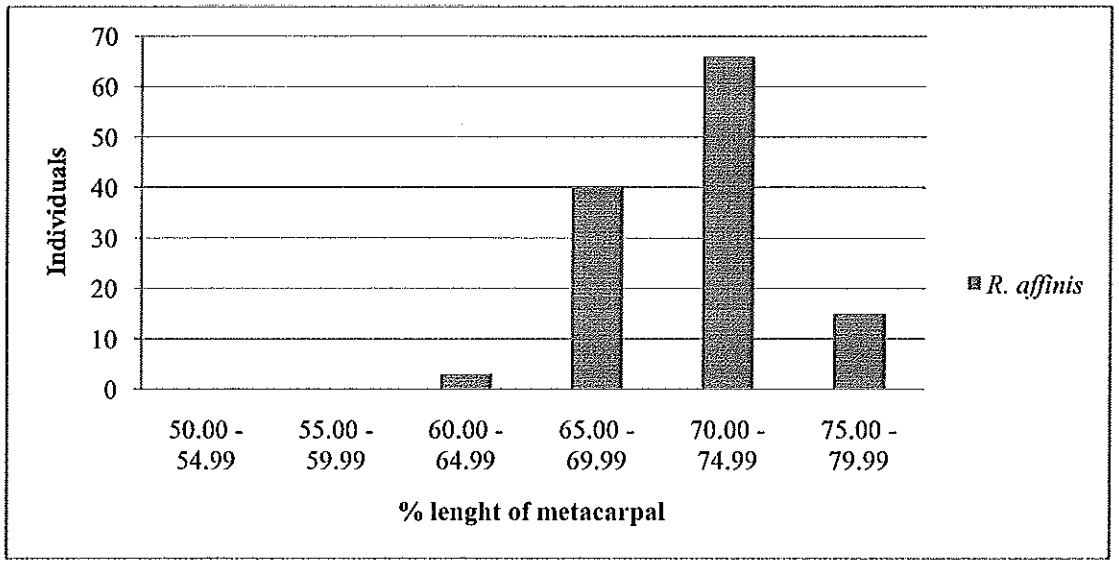


Figure 38: The comparison of the length of wing structures of *R. affinis* between the second phalanx of the third digit and the third metacarpal (n= 122).

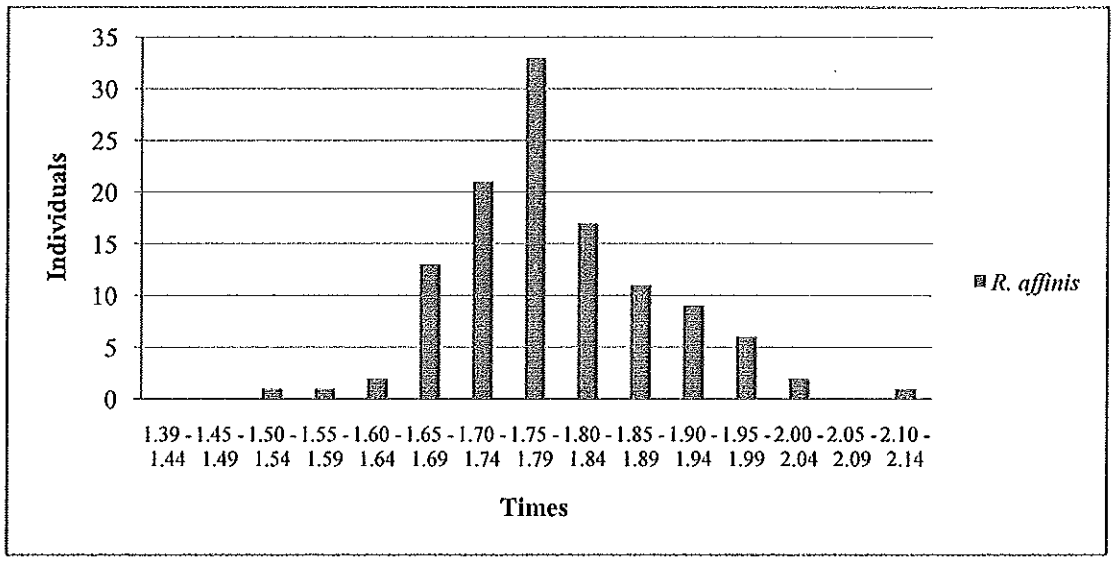


Figure 39: Variation of wing structure measurements within *R. affinis* (n= 112) from India, Southeast-Asia and Indonesia.

4.3.2.3 Palatal Bridge

Palatal bridge is relatively short, about one fourth (24.84%, 21.43% - 28.19%, n= 109) of maxillary upper toothrow length or even less. Palate bridge is especially short; it is emarginated anteriorly to the level of the parastyle of the first upper molar (M^1) and middle to the mesostyle of second upper molar (M^2).

4.3.2.4 First Upper Premolars (P^2)

The position and size of the first upper premolars are mostly medium sized and situated in the toothrow. First upper premolar is medium sized and partly extruded from the toothrow, in 16.47% of specimens, while 1.67% of specimens there are small and situated in the toothrow. However, in 2.35% of specimens, there are large size and situated in toothrow of the first upper premolars. The upper canine (C^1) is typically enormous and not in contact with the second upper premolar (P^4). See in the (Fig. 19: A, B, C; p 65).

4.3.2.5 Second Lower Premolars (P_3)

Second lower premolar is exterior and extremely small, rarely within or partly within toothrow 12.35% of specimens. P_3 is usually minute and extruded from the toothrow (83.54% of specimens). P_3 is small or very tiny, usually fully situated in toothrow (4.11%). P_2 and P_4 are generally in contact or more distinctly separated. But some specimens, P_2 and P_4 are not in contact, but nearly so (Fig. 20: A, B; p 65).

4.3.2.6 Bacular Morphology

The average length of baculum is 2.71 mm (2.45 – 3.00 mm, n= 5). The greatest width of baculum is 0.87 - 0.90 mm, and average of the greatest is width 0.894 mm in lateral profile. In addition, all of five baculum seem to be similar in size and length, the dorsal view, the shaft of baculum is long and parallel-margined, the basal cone is expanded and deeply emarginated. The basal cone is expanded with angled ventrally in lateral view and bended the tip (Fig. 40).

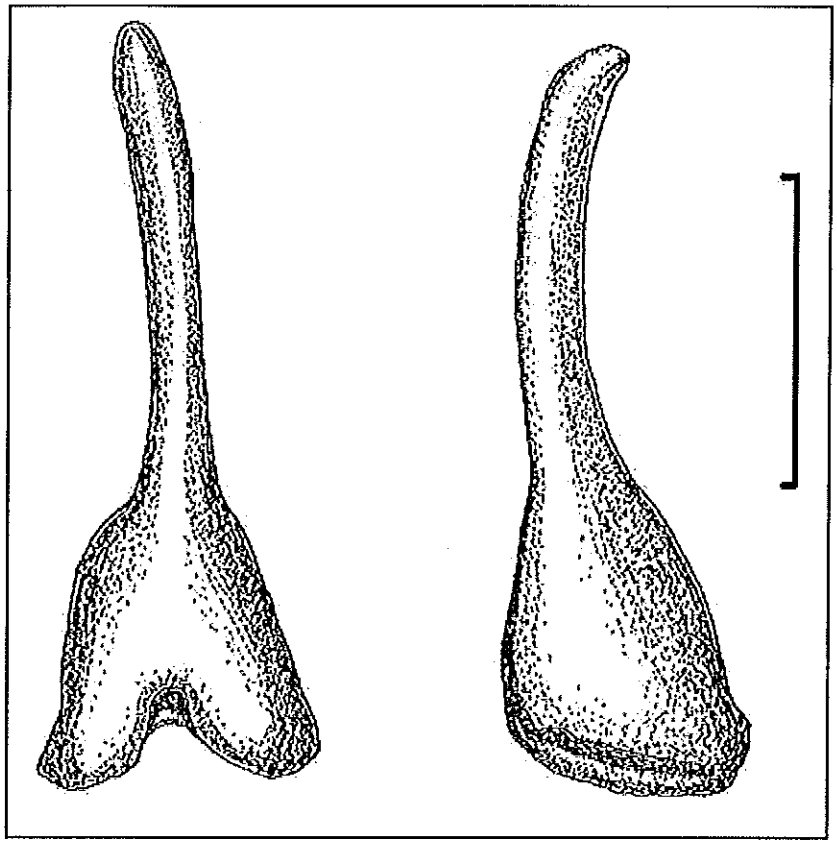


Figure 40: Dorsal (left) and lateral (right) views of baculum shapes of *R. affinis* CP2, Hala Bala Wildlife Sanctuary, Narathiwat, Thailand. Scale = 1 mm.

4.3.3 Principal Component Analysis (PCA) of Ten Cranial and dental Characters

Only ten cranial and dental character measurements of *R. affinis* were examined by principal component analysis (PCA). Because the specimens from Java that deposited in the British Natural History Museum, London is damage.

Ten cranial and dental characters were CCL, SL, ZB, BB, PC, C-M3, M3-M3, PL, C-M3 and ML (see abbreviation in pp.59 - 60). From PCA, *R. affinis* from India, Southeast-Asia and Java (Indonesia) could be separated from each other (Fig. 41).

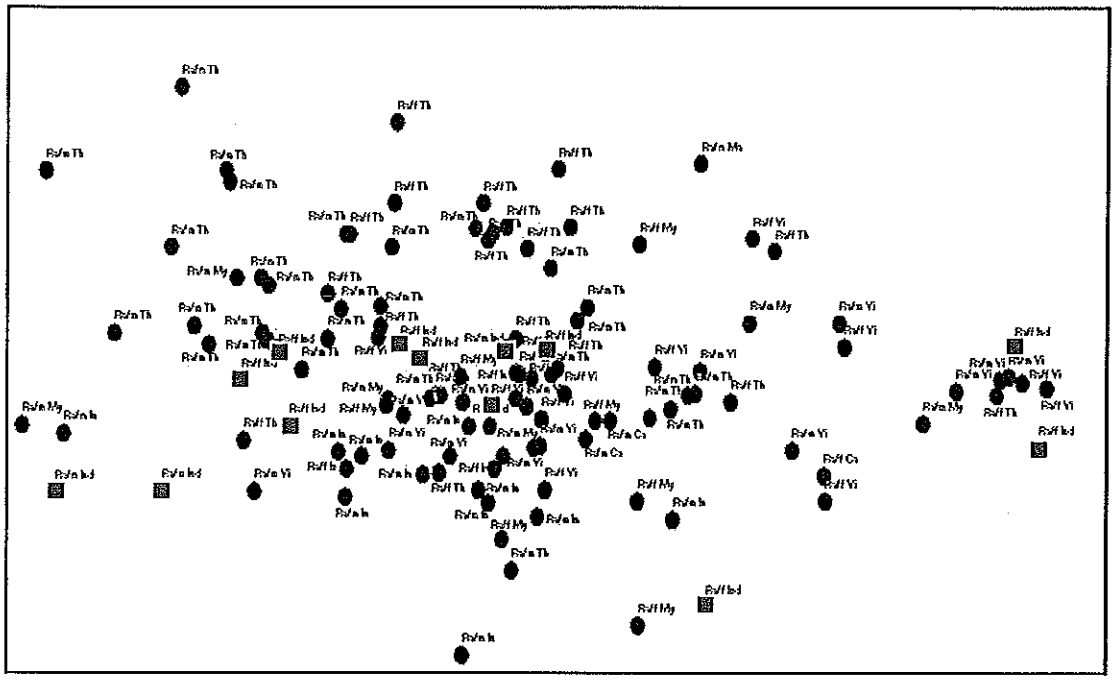


Figure 41: The principal component analysis (PCA), *R. affinis* from India and Southeast-Asia (blues speckle) versus *R. affinis* from Java, Indonesia (red square).

4.3.4 Analysis of variance (ANOVA) Test

Geographical variations between the *R. affinis* from these two regions (Indian, Southeast-Asia land versus Indonesian land) were examined. Only three characters were significantly different between two regions (Table 9).

4.3.5 Echolocation

Recent studies, six parameters (D, SF, EF, FINT and MF, see the abbreviation in pp 67 - 68) of echolocation calls of 66 *R. affinis* were examined. Based on the recent collection, only echolocation calls from Thailand were analyzed. There is variation in call frequency; it ranges from 65.5 kHz – 77.3 kHz. Noteworthy the call from Tarutao Island is rather low 65. 5 kHz – 67 kHz. In contrast, the echolocation calls of *R. affinis* from Chiang Mai province is ranging from 75.8 kHz - 77.3 kHz, in the southern of Thailand 66.5 – 71.5 kHz (Fig. 42).

Table 9: One-way ANOVA test of the ten metric characters used in the Principal Component Analysis (PCA) between *R. affinis* from India, Southeast-Asia and Indonesia (Java)

	India				Southeast-Asia and Java				
Characters	N	Min	Max	Mean ± SD	N	Min	Max	Mean ± SD	Sig (P)
HBL	14	49.00	66.00	55.75±4.89	96	49.00	72.15	56.80±4.77	0.57
TAIL	14	20.00	29.00	24.10±2.87	97	16.53	32.00	23.81±2.94	0.50
HF	14	10.00	12.50	11.00±0.63	96	8.00	12.00	10.26±0.73	0.10
TIB	14	21.08	25.21	23.53±1.08	96	21.84	26.27	24.29±1.01	0.35
FA	14	51.00	55.00	53.58±1.06	97	46.72	54.55	50.88±1.50	0.12
5Met	14	41.44	44.32	43.00±0.99	96	37.71	44.26	41.15±1.43	0.37
4Met	14	40.05	43.20	41.67±1.01	96	36.92	44.14	40.04±1.46	0.57
3Met	14	38.50	42.02	39.90±1.01	96	35.84	42.67	38.64±1.38	0.84
3Met1ph	14	14.82	16.45	15.73±0.51	96	14.10	16.70	15.37± 0.62	0.54
3Met2ph	14	27.45	31.87	29.76±1.20	96	24.00	31.37	27.16±1.38	0.09
GTL	15	22.17	24.28	23.37±0.48	101	21.61	24.70	23.28±0.65	**0.01***
CCL	15	19.53	20.53	19.86±0.24	111	17.55	21.05	19.72±0.57	0.20
SL	15	22.25	23.23	22.49±0.26	111	20.10	23.64	22.39±0.59	0.35
ZB	15	11.06	11.76	11.39±0.18	114	1.74	11.97	11.07±0.94	0.09
BB	14	9.12	9.61	9.37±0.15	102	8.59	11.14	9.58±0.49	**0.01***
CM3UP	15	8.68	9.61	9.05±0.20	115	7.58	9.60	8.91±0.32	0.23
M3M3	15	8.29	9.15	8.85±0.20	115	7.30	9.25	8.48±0.28	0.48
PL	15	1.96	2.31	2.17±0.11	114	1.81	2.57	2.22±0.16	0.83
CM3LOW	15	9.17	10.05	9.65±0.22	115	7.85	10.10	9.42±0.34	0.59
ML	15	15.18	16.72	15.85±0.33	115	13.67	16.45	15.54±0.53	**0.01***
PC	15	1.74	2.35	2.08±0.16	115	1.80	2.76	2.19±0.20	0.39

* The mean difference is significant at the 0.05 level

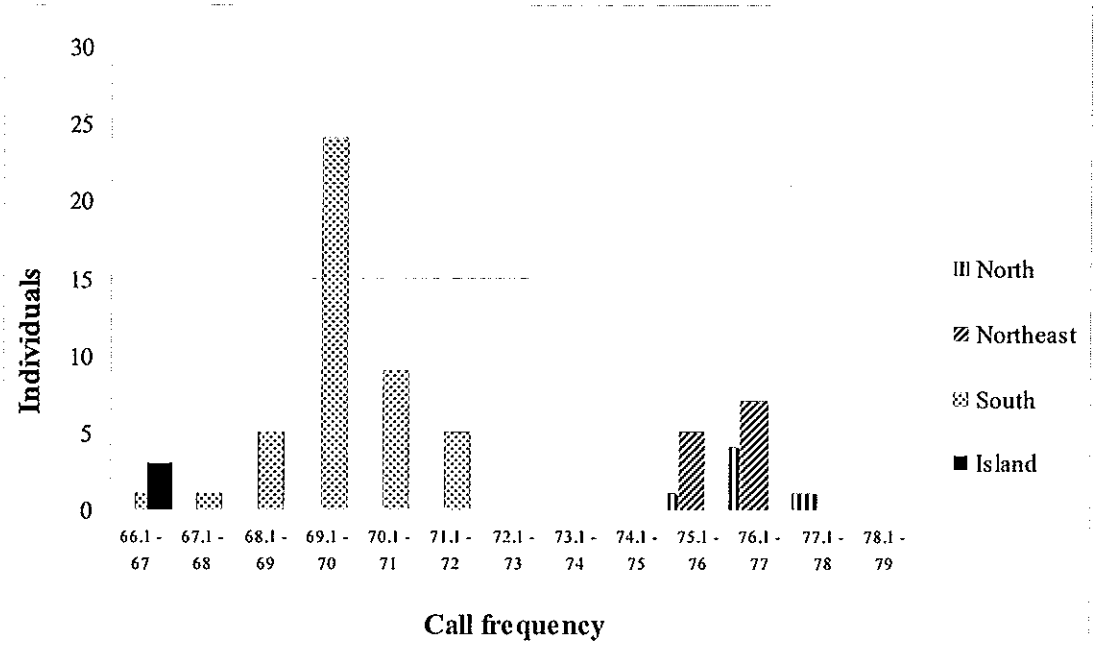


Figure 42: The relationship of call frequency of two groups between the southern-Tarutao Island and northern-northeast of *R. affinis* in Thailand.

4.4 *Rhinolophus affinis* from India versus *Rhinolophus rouxii* from India

4.4.1 External Morphology Comparison

4.4.1.1 Sella Morphology and Variation

The sella shapes of *R. affinis* from India are normally concave sides (77%), but some other shapes were exist as well, there are parallel/straight sides (8%) and undefined/or without convex and straight sides (15%), see in the Fig 43 (left). Whether, a similarity of *R. affinis* from India and *R. rouxii* were determined. It was found that the straight or/parallel sides of sella was mainly presented in *R. rouxii* (62%) and 38% were concave sides, see in Fig. 43 (right).

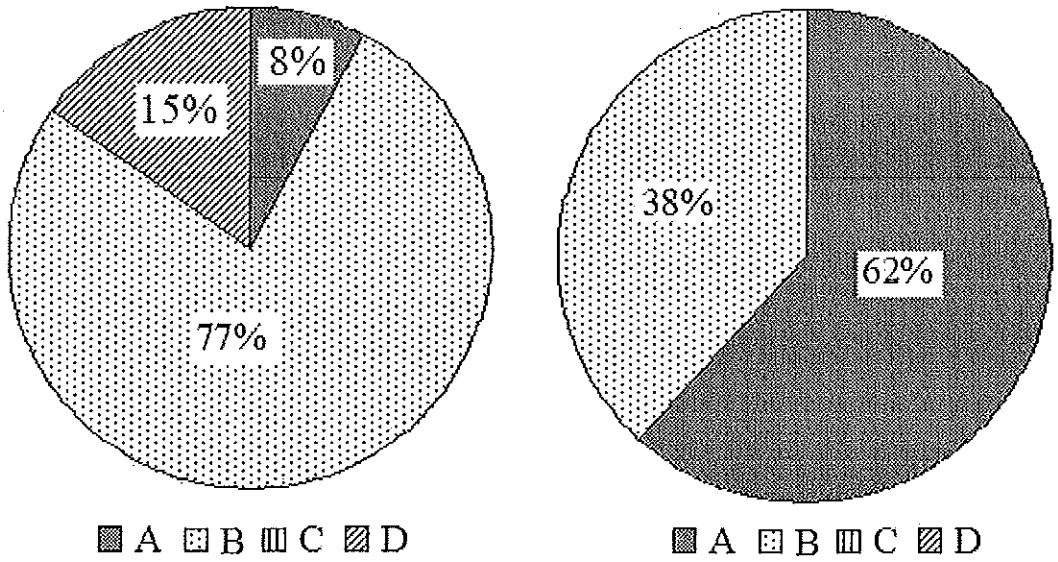


Figure 43: Sella variations in *R. affinis* (n=13) from India (left) and *R. rouxii* (n=13) from India (right). The sella shape with parallel margined (A), concave sides (B), convex sides (C), and undefined (D) see in Fig 17, p 62.

4.4.1.2 Lancet Morphology

Fourteen individuals of *R. affinis* were determined of the lancet characters. Most lancet is characteristically triangular shape with concave sides (79%) and 21% of the triangular shape with straight sides was also presented (Fig. 44, left). However, in *R. rouxii,* lancet shape highly varied, but the majority was the dome shape with straight sides tip (58%). Another, three types of lancet shapes were exist in *R. rouxii,* there was triangular shape with concave sides (21%), triangular shaped with concave sides (7%) and dome shape with elongate sides 14% (Fig. 44, right).

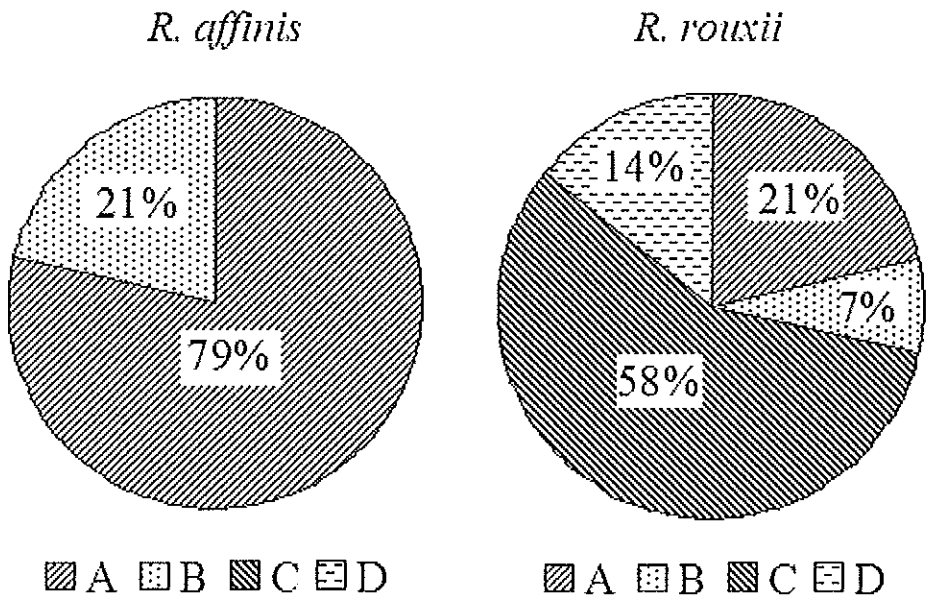


Figure 44: The comparison of lancet shape of *R. affinis* and *R. rouxii* from India. Triangular shape with straight sides (A), the triangular shape with concave sides (B), the dome shape with straight sides tip (C) and the dome shape with elongate sides tip (D), see in Fig. 18, p 63.

4.4.2 Skull Morphology

In *R. affinis,* the skull is modurate to large size, with CCL: 19.79 mm in average. The rostal profile is always deepped as U shape (Fig 49 a). The zycoma is modurate and a well developed, with a high jugal projection and curvature (Fig 50 a). However, the skull of *R. rouxii* is smaller than the skull of the *R. affinis.* The rostal profile is shallow and slopping forward (Fig. 49 b). The zygoma is slightly low rearfard of jugal with strongly built (Fig. 50 b).

4.4.3 Morphometrics

Fifteen *Rhinolophus affinis* and twenty-five *R. rouxii* from India were examined of eleven external, eleven cranial and dental characters. Statistically, many characters were significantly ($P<0.05$) different between both species. Including, TIB, FA, 5Met, 4Met, 3Met, 3Met2ph, GTL, CCL, SL, PC, M^3-M^3, PL and C-M_3 (see the abbreviation in pp. 57- 60). This result was summarized in the Table 10 below.

4.4.3.1 Sexual Dimorphism

Generally, sexual dimorphism was not found in both species, but only one cranial character, the mandible length ($P<0.05$). The statistic tests of both species were concluded in the Table 11.

Table 10: Comparison of external, cranial and dental characters of *Rhinolophus affinis* and *R. rouxii* from India

	R. rouxii				*R. affinis*				Sig
Characters	Min	Max	Mean ± Std	n	Min	Max	Mean ± Std	n	(P)
HBL	49.78	66.00	56.83±5.27	16	49.00	66.00	55.75±4.89	14	0.61
E	14.50	19.80	17.52±1.33	16	14.00	23.00	17.12±2.21	14	0.16
TAIL	22.00	28.50	25.00±1.94	16	20.00	29.00	24.10±2.87	14	0.26
HF	7.20	12.80	10.29±1.53	16	10.00	12.50	11.00±0.63	14	0.27
TIB	20.91	24.10	22.79±0.91	16	21.08	25.21	23.53±1.08	14	0.02*
FA	48.03	52.30	50.43±1.24	19	51.00	55.00	53.58±1.06	14	0.01*
5MET	37.80	41.18	39.76±0.92	19	41.44	44.32	43.00±0.99	14	0.01*
4MET	37.22	41.59	39.04±1.12	19	40.05	43.20	41.67±1.01	14	0.01*
3MET	35.58	39.74	37.94±1.05	19	38.50	42.02	39.90±1.01	14	0.01*
3MET1ph	14.21	16.45	15.40±0.58	19	14.82	16.45	15.73±0.51	14	0.11
3MET2ph	21.10	26.18	24.19±1.39	19	27.45	31.87	29.76±1.20	14	0.01*
GTL	21.54	23.61	22.65±0.69	15	22.17	24.28	23.37±0.48	15	0.01*
CCL	17.08	20.33	19.33±0.82	21	19.53	20.53	19.86±0.24	15	0.04*
SL	20.59	22.81	21.86±0.71	21	22.25	23.23	22.49±0.26	15	0.01*
ZB	10.34	11.76	11.29±0.39	21	11.06	11.76	11.39±0.18	15	0.70
BB	8.59	9.70	9.35±0.26	15	9.12	9.61	9.37±0.15	14	0.74
PC	1.98	2.88	2.43±0.24	21	1.74	2.35	2.08±0.16	15	0.01*
C-M^3	8.12	9.35	8.83±0.36	21	8.68	9.61	9.05±0.20	15	0.16
M^3-M^3	7.83	8.79	8.32±0.22	21	8.29	9.15	8.85±0.20	15	0.01*
PL	2.18	2.76	2.49±0.15	21	1.96	2.31	2.17±0.11	15	0.01*
C-M^3	8.62	10.06	9.40±0.41	21	9.17	10.05	9.65±0.22	15	0.04*
ML	13.88	16.35	15.41±0.65	21	15.18	16.72	15.85±0.33	15	0.08

* The mean difference is significant at the 0.05 level

4.4.3.2 Wing Structure Measurements

The percentage of second phalanx of the third metacarpal compared to the third metacarpal of *R. affinis* and *R. rouxii* are considerably different. The second phalanx of the third metacarpal is usually more than 75% (70% -79%, n= 14) of the third metacarpal in *R. affinis,* in *R. rouxii* it is shorter than 64% (56% - 71%, n=16) of the third metacarpal (Fig. 45). In terms of ration between the second phalanxes of the third metacarpal to the record on *R. affinis* is more than 1.71 mm in average (1.71 – 2.01). In other hand, this ration of *R. rouxii* is shorter than 1.72 (1.44 – 1.72 mm, n=16), see in the Fig. 46.

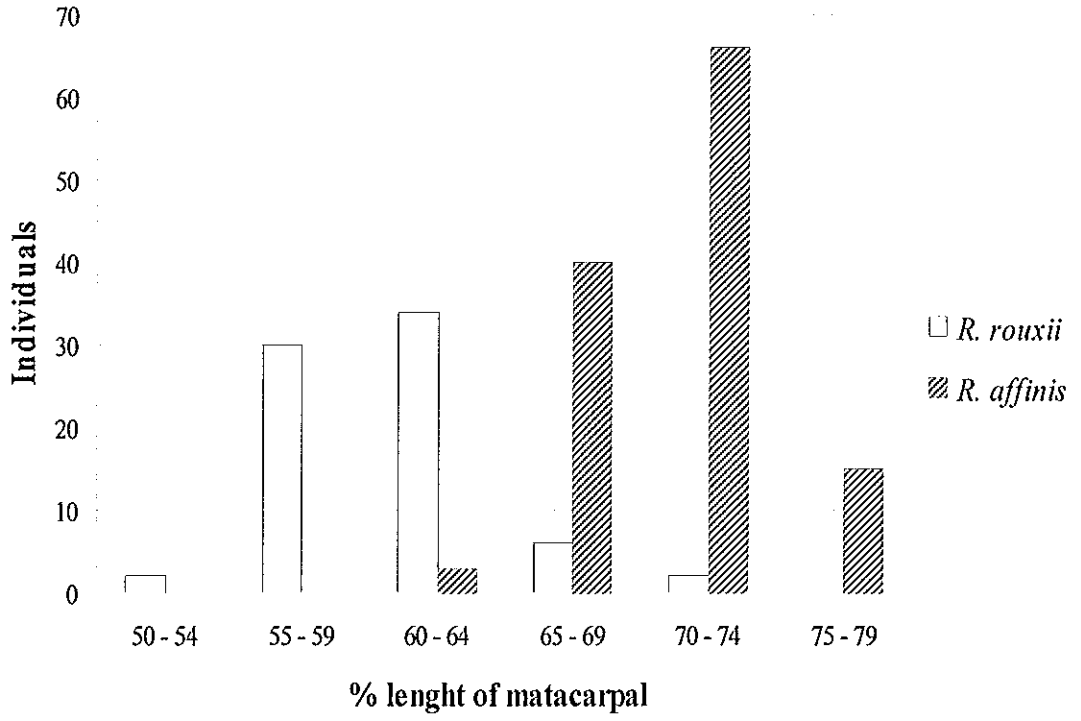


Figure 45: Percentage of the length of the second phalanx of the third metacarpal to the third metacarpal of *R. affinis* and *R. rouxii* from India.

Table 11: Mean ± SD, minimum-maximum (mm) and P-value for external, cranial and dental measurements of *Rhinolophus affinis* and *R. rouxii* from India.

Characters	*Rhinolophus affinis*									*Rhinolophus rouxii*								
	Males				Females					Males				Females				
	Mean ± SD	Max	Min	N	Mean ± SD	Max	Min	N	Sig (P)	Mean ± SD	Max	Min	N	Mean ± SD	Max	Min	N	Sig(P)
HBL	55.40±4.23	62.00	49.00	**11**	57.00 ± 7.94	66.00	66.00	**3**	0.93	56.63 ± 4.62	63.63	49.78	**12**	57.42 ± 7.77	66.00	50.68	**4**	1.00
E	17.57±2.24	23.00	15.00	**11**	15.50 ± 1.32	16.50	16.50	**3**	0.11	17.30 ± 1.44	19.80	14.50	**12**	18.20 ± 0.72	18.90	17.20	**4**	0.14
TL	23.68±2.21	27.00	20.50	**11**	25.67 ± 4.93	29.00	29.00	**3**	0.38	24.50 ± 1.91	28.50	22.00	**12**	26.50 ± 1.29	28.00	25.00	**4**	0.06
HF	11.10±0.64	12.50	10.20	**11**	10.67 ± 0.58	11.00	11.00	**3**	0.30	10.40 ± 1.56	12.80	7.20	**12**	9.98 ± 1.61	11.50	8.30	**4**	0.90
TIB	23.53±1.18	25.21	21.08	**11**	23.53 ± 0.83	24.08	24.08	**3**	0.93	22.65 ± 0.95	23.66	20.91	**12**	23.24 ± 0.72	24.10	22.60	**4**	0.33
FA	53.47±1.09	55.00	51.00	**11**	54.00 ± 1.00	55.00	55.00	**3**	0.52	50.39 ± 1.32	52.30	48.03	**14**	50.56 ± 1.15	51.80	49.00	**5**	0.78
5Met	42.91±0.97	44.32	41.44	**11**	43.35 ± 1.22	44.13	44.13	**3**	0.39	39.79 ± 0.96	41.18	37.80	**14**	39.70 ± 0.91	40.94	38.42	**5**	0.85
4Met	41.58±0.92	43.20	40.05	**11**	42.01 ± 1.47	43.07	43.07	**3**	0.48	39.01 ± 0.98	40.64	37.22	**14**	39.11 ± 1.62	41.59	37.37	**5**	0.92
3Met	39.78±1.03	42.02	38.50	**11**	40.36 ± 0.97	41.27	41.27	**3**	0.39	37.96 ± 1.05	39.49	35.58	**14**	37.92 ± 1.18	39.74	36.86	**5**	0.78
3Met1ph	15.65±0.53	16.45	14.82	**11**	16.06 ± 0.29	16.25	16.25	**3**	0.31	15.35 ± 0.54	16.18	14.21	**14**	15.56 ± 0.76	16.45	14.44	**5**	0.35
3Met2ph	29.57±1.08	30.90	27.45	**11**	30.45 ± 1.65	31.87	31.87	**3**	0.31	24.23 ± 1.26	25.87	21.90	**14**	24.09 ± 1.91	26.18	21.10	**5**	1.00
GTL	23.39±0.50	24.28	22.17	**12**	23.32 ± 0.52	23.73	23.37	**3**	1.00	22.76 ± 0.60	23.61	21.60	**11**	22.36 ± 0.94	23.21	21.54	**4**	0.43
CCL	19.88±0.24	20.53	19.53	**12**	19.77 ± 0.27	20.07	20.07	**3**	0.47	19.45 ± 0.81	20.33	17.08	**14**	19.10 ± 0.88	20.25	17.92	**7**	0.26
SL	22.50±0.28	23.23	22.25	**12**	22.48 ± 0.20	22.61	22.61	**3**	0.94	22.06 ± 0.56	22.81	20.73	**14**	21.49 ± 0.87	22.75	20.59	**7**	0.20
ZB	11.38±0.19	11.76	11.06	**12**	11.44 ± 0.16	11.62	11.34	**3**	0.94	11.40 ± 0.25	11.68	10.96	**14**	11.08 ± 0.54	11.76	10.34	**7**	0.20
BB	9.37±0.17	9.61	9.12	**11**	10.45 ± 0.08	9.39	9.31	**3**	0.58	10.38 ± 0.16	10.68	10.15	**14**	10.15 ± 0.30	10.61	9.74	**7**	0.69
C-M^3	9.06±0.21	9.61	8.68	**12**	9.01 ± 0.13	9.16	8.93	**3**	0.51	8.89 ± 0.28	9.35	8.42	**14**	8.71 ± 0.49	9.33	8.12	**7**	0.37
M^3-M^3	8.85±0.22	9.15	8.29	**12**	8.87 ± 0.14	8.99	8.72	**3**	1.00	8.37 ± 0.20	8.79	8.04	**14**	8.22 ± 0.24	8.55	7.83	**7**	0.17
PL	2.19±0.11	2.31	1.96	**12**	2.07 ± 0.06	2.13	2.01	**3**	0.11	2.52 ± 0.14	2.76	2.21	**14**	2.43 ± 0.17	2.65	2.18	**7**	0.19
C-M_3	9.67±0.24	10.05	9.17	**12**	9.60 ± 0.21	9.84	9.47	**3**	0.56	9.47 ± 0.35	10.06	9.08	**14**	9.28 ± 0.53	10.00	8.62	**7**	0.23
ML	15.85±0.36	16.72	15.18	**12**	15.86 ± 0.19	16.06	15.68	**3**	088	15.66 ± 0.44	16.35	14.89	**14**	14.91 ± 0.76	15.93	13.88	**7**	**0.02***

* The mean difference is significant at the 0.05 level

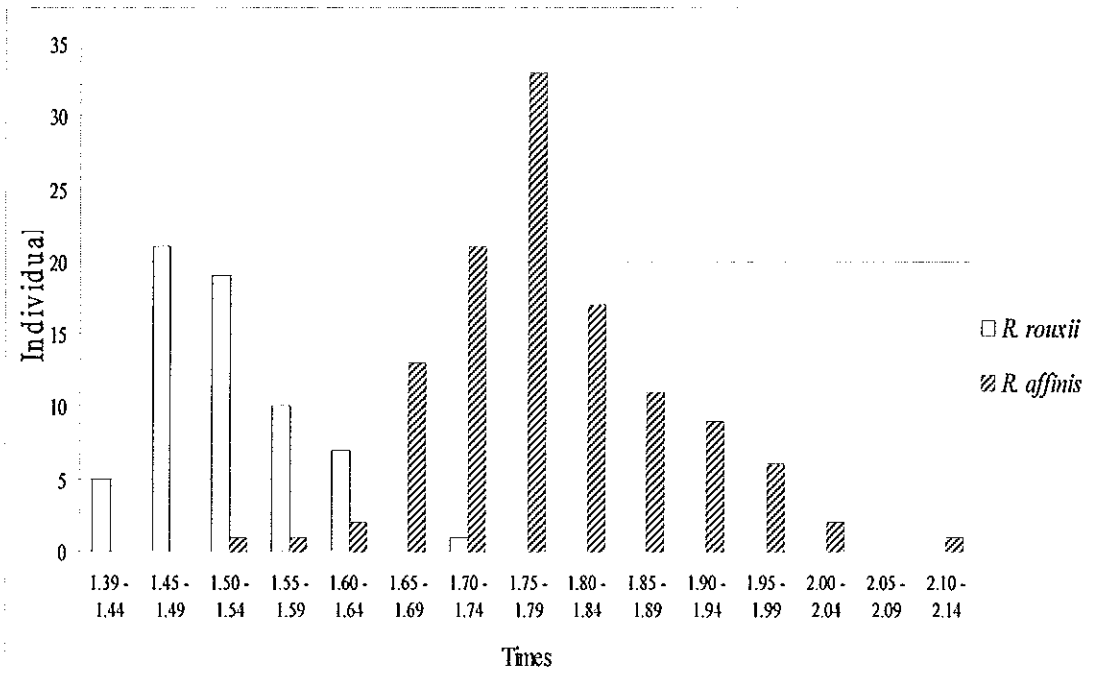


Figure 46: The ration of *R. affinis* (cross column) and *R. rouxii* (open column) from India.

4.4.2.3 Palatal Bridge

In *R. affinis,* the palatal bone is less than one fourth of maxillary upper toothrow length (average 22.48%); it is about 1.96 – 2.31 mm (2.17 mm). Conversely, the palatal bridge *R. rouxii* is more than one fourth of the maxillary upper toothrow length (average 26.52%) and range 2.18 – 2.76 mm (2.49 mm), see in above of the Table 10.

4.4.2.4 First Upper Premolar (P^2)

Both species are very similar in size and position of the first upper premolars. In *R. affinis,* most 64% of the first upper premolars are medium size and lying in the troothrow and the rest 36% partly extruded of the toothrow (n=14) (Fig. 19, p 68). In *R. rouxii,* it is medium size and 73% situated in the toothrow, and 27% partly extruded from the toothrow (n=21) (Fig. 19, p 66). The upper canine (C^1) and second upper premolar (P^4) of these species are not in contact.

4.4.2.5 Second Lower Premolars (P_3)

In *R. affinis,* the second lower premolars are mostly medium sizes (79%), but in some cases, the minute size (21%) was present. The positions of the second lower premolars are typically extruded from the toothrow (93%), but there is small number of partly extruded from the toothrow (7%). Conversely, in *R. rouxii* the second lower premolars are mostly minute sizes (87%) and medium sizes (13%), but its position is similar to *R. affinis* 69% extruded from the toothrow, 19% situated in toothrow and 12% partly extruded from the toothrow. For the size and position with explanation see Fig. 20, p 66.

4.4.2.6 Bacular Morphology

The baculum of *R. rouxii* from India was determined, there are three bacular of *R. rouxii* were extracted. There is nearly the same length, but one of them was morphologically different (Fig. 47: A-C). In this study, there is no baculum of *R. affinis* from India, but only the baculum of *R. affinis* from Thailand was extracted and compared, it is a similarity in length of *R. rouxii* and different in morphology/shapes (Fig.47: D - E). The baculum of *R. affinis* from Thailand is expanded in basal cone with angled ventrally in lateral view and bended the tip, and in overall it is fatter than those India baculum.

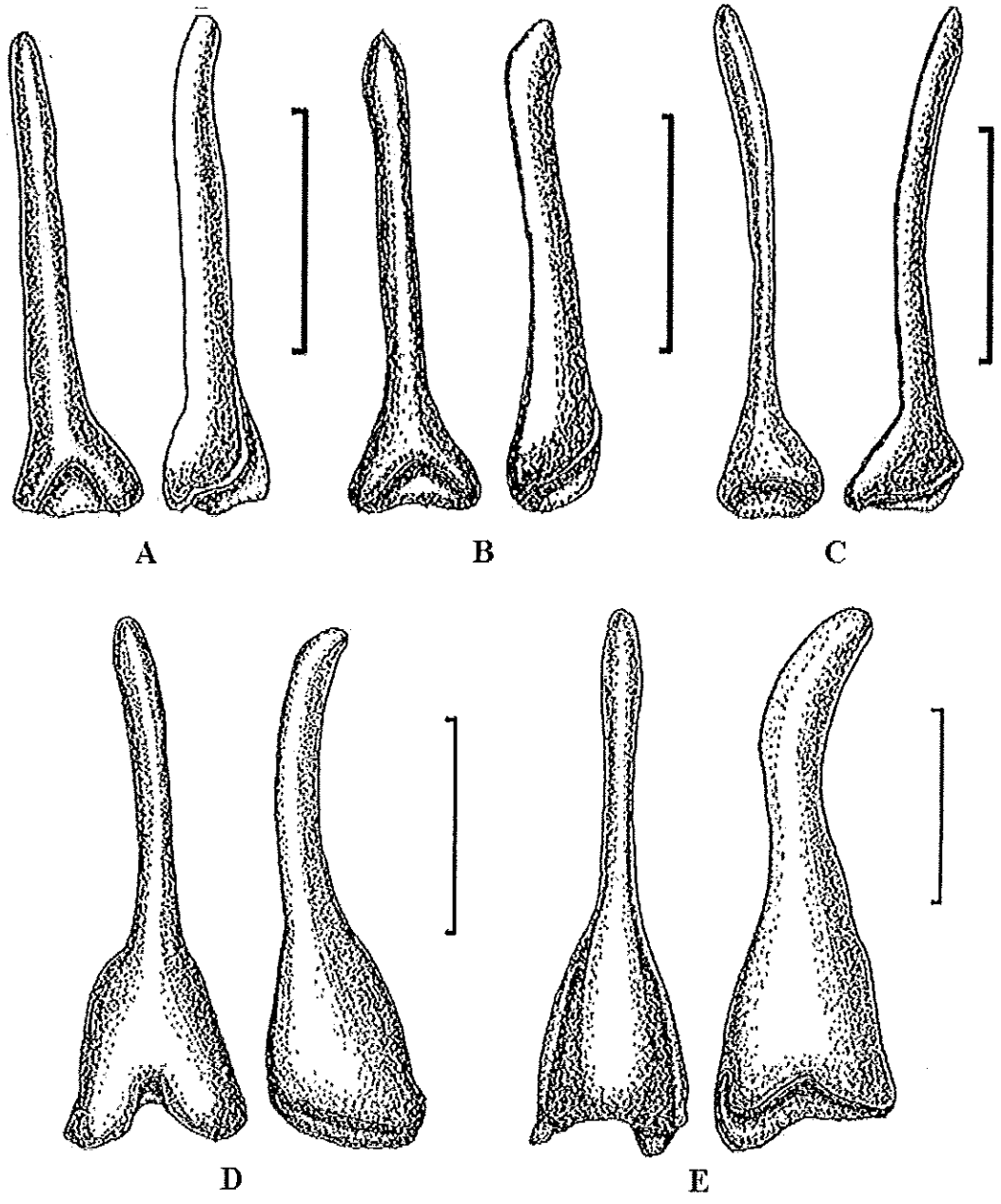


Figure 47: The comparison of the baculaum series of *R*hinolophus species, dorsal view (left) and lateral view (right) of each pair. Three specimens from India, (A) *R. rouxii* HZM. No. 26.28158, (B) *R. rouxii* HZM. No. 25.28157, (C) *R. rouxii* HZM. No. 22.28574 and two specimens of *R. affinis* from Thailand, (D) *R. affinis* SB.07038.5 and (E) *R. affinis* CP. 2 from Hala Bala wildlife sanctuary, Thailand. Scale: 1 mm.

4.4.4 Principal Component Analysis (PCA) of Sixteen Characters

Sixteen metric characters included external, cranial and dental characters of *R. affinis* (n= 14) and *R. rouxii* (n=15) were included in PCA. The result showed that they are completely separated from each other. In *R. rouxii* group, it seems to has three small populations inside, it is only one populations are from the same location (green square), see in the Fig. 48.

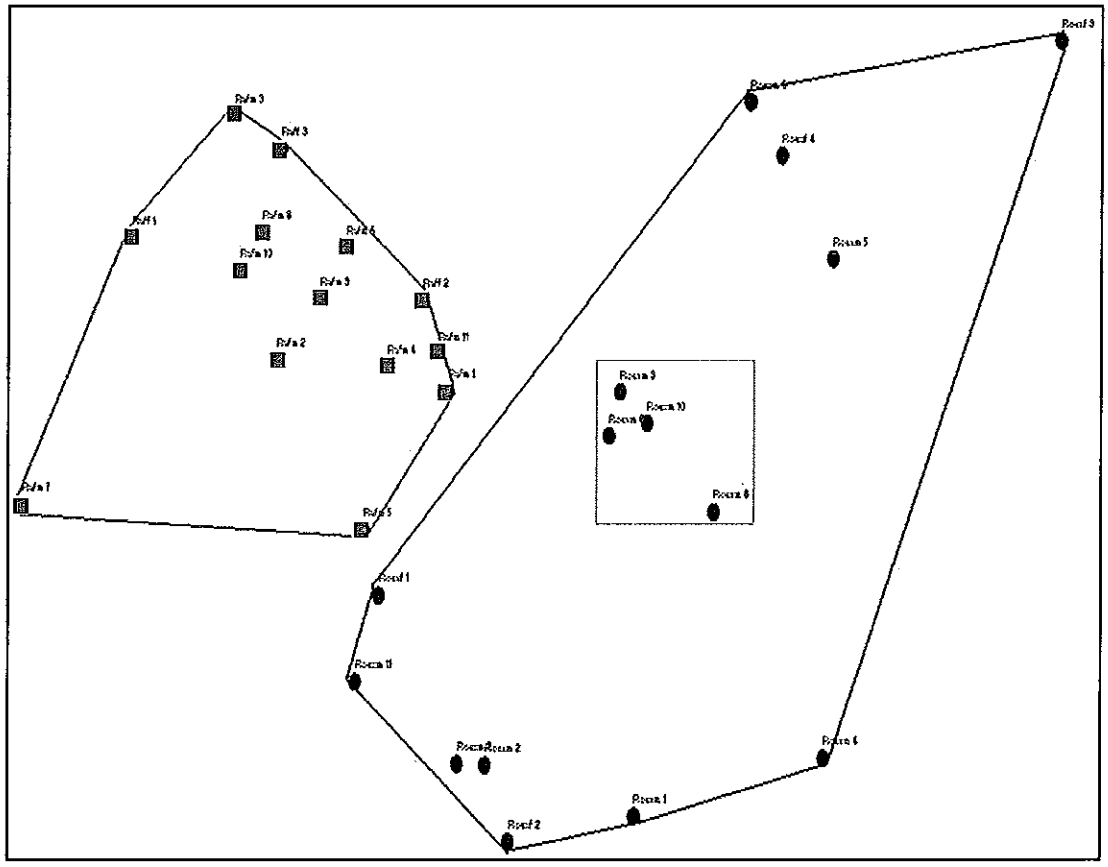


Figure 48: Principle component analysis of sixteen characters of *R. affinis* (red square) and *R. rouxii* (blue speckle). There are three sub groups inside the *R. rouxii*, and only one population are present the same locality (green square).

4.4.5 Analysis of Statistics (Mann-Whitney Samples Test)

Twenty-two external, cranial and dental character measurements were examined for interspecific variation between *R. affinis* and *R. rouxii*. The wing structures were the highly different between two these cryptic species, and also seven cranial and dental characters ($P<0.05$). The results of the PCA and Mann-Whitney test were similar separated from each (Table 11).

4.4.6 Echolocation

The echolocation calls of *R. affinis* and *R. rouxii* from India are unavailable in this study. However, most voucher specimens from India were loan from the Harrison Institute, England and some from the Natural History Museum, London.

4.5 The Comparison and Principal Component Analysis (PCA) of *R. affnis, R. rouxii, R. sinicus* and *R. thomasi* from India and Southeast-Asia.

4.5.1 Cranial and Dental Characters Coparison

In *R. affinis,* the skull is modurate to large size, with CCL: 19.79 mm in average. The rostal profile is always deep U-shape (Fig 49 a). The zygoma is modurate and well developed, with a high jugal projection and curvature (Fig 50 a). However, the skull of *R. rouxii* is smaller than the skull of the *R. affinis.* The rostal profile is shallow and slopping forward (Fig. 49 b). The zygoma is slightly low rearward and jugal bone is robust (Fig. 50 b). The skull of *R. sinicus* is bigger than *R. thomasi*, with CCL: 17.81 mm in average. The rostral depression profile is narrowly convex (Fig. 49 c). The zygoma is well developed and concave in shape, with high jugal projection (curve) (Fig 50 c). The palatal bridge is 1.62 – 2.46 mm or 27.04% in average of the maxillary toothrow length ($C\text{-}M^3$). In *R. thomasi*, the skull is a small and robust, with CCL: 16.20 mm. The nasal swelling is high and rostal depression profile is deeper and slopping rearward than *R. sinicus* (Fig. 50 d). The zygoma is slightly strong with low jugal projection (convex) (Fig 50 d). The palatal bridge is 1.45 – 2.50 mm/27.84 % in average of the maxillary toothrow length ($C\text{-}M^3$).

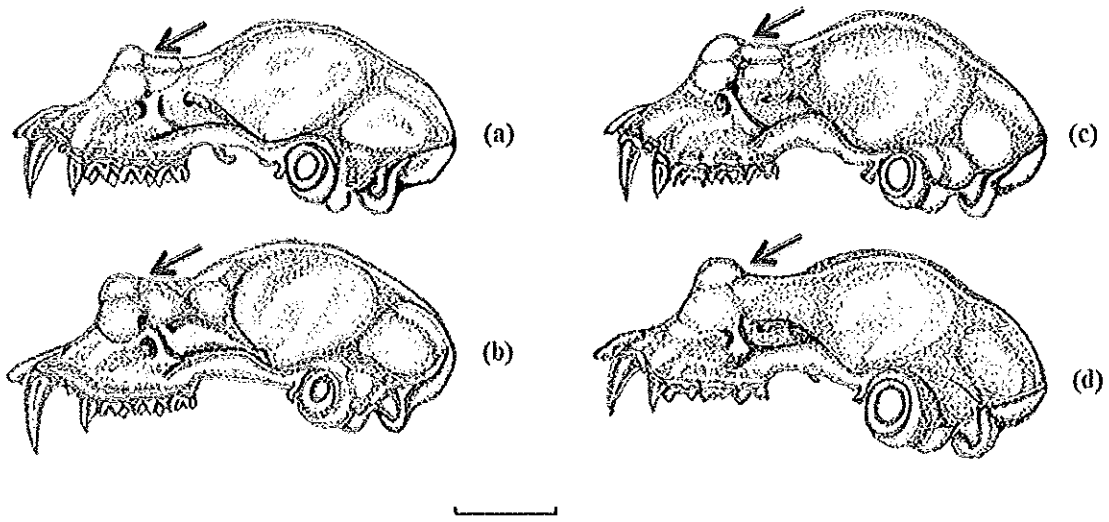


Figure 49: The differentiation of the rostal depression shapes of *R. affinis* (a), *R.rouxii* (b), *R. sinicus* (c) and *R. thomasi* (d).

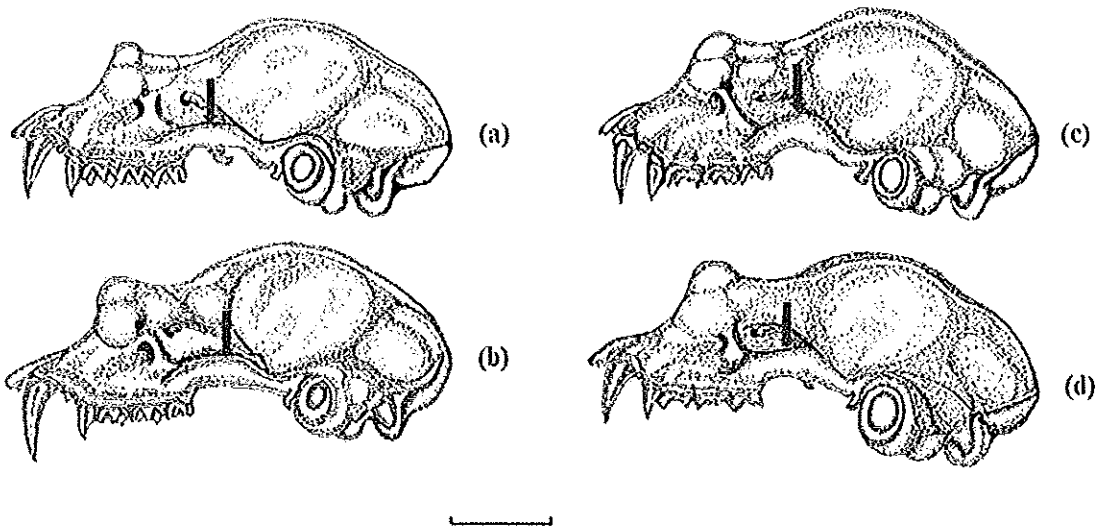


Figure 50: The differentiation of the zygomata shapes of *R. affinis* (a), *R.rouxii* (b), *R. sinicus* (c) and *R. thomasi* (d).

4.5.2 Principal component analysis of *R.affinis, R. rouxii, R. sinicus* and *R. thomasi* from India and southeast-Asia

121 specimens of four species, *R. affnis* (63), *R. rouxii* (33), *R. sinicus* (13) and *R. thomasi* (12) from India and southeast-Asia, with fifteen external, cranial

and dental characters were examined. The principal component analysis showed an overlap between *R. affinis* and *R. rouxii,* but *R. sinicus* and *R. thomasi* were clearly distinguished from the others (Fig 51).

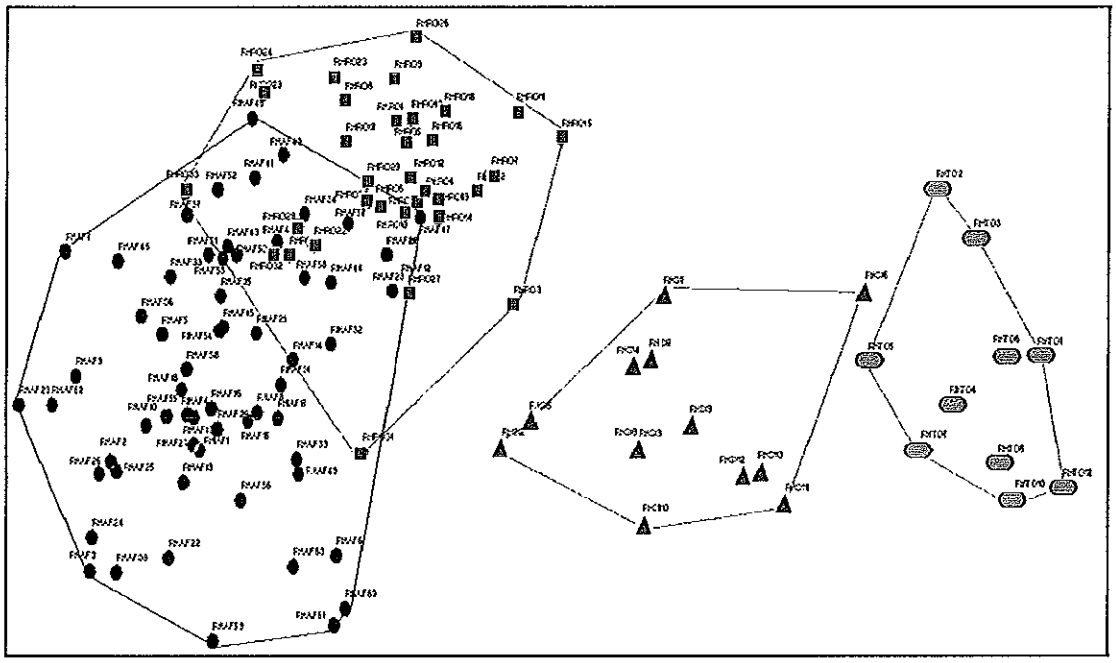


Figure 51: The principal component analysis (PCA) of fifteen external, cranial and dental characters of *R. affinis* (blue speckles), *R. rouxii* (red squares), *R. sinicus* (green trangle) and *R. thomasi* (pink elongates).

4.6 Systematics Summary

The information of four species in this study and all character measurements were described and stated below, base on species list that collected in hand with some photograph available.

4.6.1 *Rhinolophus affinis* Horsfield, 1823

Intermediate horseshoe bat

External characters

R. affinis is generally large sized horseshoe bat than *R. rouxii,* with having an average forearm length of 51.43 mm (46.72 – 55.00 mm). The ears are also longer than, in average 19.53 mm. The lower lip has three grooves. The horseshoe morphology is rounded in anterior section and roughly horseshoe shaped, the horseshoe being relatively broad, but does not cover the muzzle. The lancet is mostly cuneate shape with straight margins (98%) and well developed and only small number is concave margins (see in the variations). Sella is variable in margins ranging from concave (61%) to parallel (18%) and undefined (21%) because in some specimens showed an indication of a concavity, without being as convex or parallel margins. In general, the forearm length is 51.22 mm (46.72 – 55.00 mm) average longer than those three species from this study. In the wing structure is differs significantly to *R. rouxii, R. sinicus* and *R. thomasi.* The second phalanx of the third metacarpal is long about three fourth the length of the third metacarpal (71%, 64% – 79%). First phalanx of third digit is less than half length of metacarpal (about 40% length of the third metacarpal (37% - 43%). The second phalanx of third digit is always more than 1.59 mm lengths the first phalanx of the third digit (1.78, 1.59 mm – 2.07 mm). The pelage colour is typically grayish brown and occasionally bright orange.

Cranial and dental characters

The skull is medium and slightly bigger than *R. rouxii* with an average condylo-cranine length (CCL) of 19.73 mm (17.55-21.05 mm). The zygomata are usually greater than mastoid width. Palatal bridge is relatively short, about one fourth (24.84%, 21.43% - 28.19%) of maxillary upper toothrow length or even less. Palate

bridge is especially short; it is emarginated anteriorly to the level of the parastyle of the first upper molar (M^1) and middle to the mesostyle of second upper molar (M^2).

Dentition

The Upper toothrow length (C-M^3) average 8.93 mm (7.58 - 9.61 mm) in length. The position and size of the first upper premolars are mostly medium sized and situated in the toothrow. The upper canine (C^1) is typically enormous and not in contact with the second upper premolar (P^4). Second lower premolar is exterior and extremely small, rarely within or partly within toothrow 12.35% of specimens. P_3 is usually minute and extruded from the toothrow (83.54% of specimens). P_3 is small or very tiny, usually fully situated in toothrow (4.11%). P_2 and P_4 are generally in contact or more distinctly separated.

Baclular morphology

The average length of baculum is 2.71 mm (2.45 – 3.00 mm). The greatest width of baculum is 0.87 - 0.90 mm, and average of the greatest is width 0.894 mm in lateral profile. In addition, all of five baculum seem to be similar in size and length, the dorsal view, the shape of baculum is long and parallel-margined, the basal cone is expanded and deeply emarginated. The basal cone is expanded with angled ventrally in lateral view and bended the tip.

Echolocation

Based on this study, six parameters (D, SF, EF, FINT and MF, see the abbreviation in pp 68 - 69) of echolocation calls of 66 *R. affinis* were examined. Based on the recent collection, only echolocation calls from Thailand were analyzed. There is variation in call frequency; it ranges from 65.5 kHz – 77.3 kHz. Noteworthy the call from Tarutao Island is rather low 65. 5 kHz – 67 kHz. In contrast, the echolocation calls of *R. affinis* from Chiang Mai province is ranging from 75.8 kHz - 77.3 kHz, in the southern of Thailand 66.5 – 71.5 kHz. Other studies had reported that 80 kHz in Malaysia (Csorbar *et al.* 2003). There has no significant in sexual variation in echolocation calls.

Geographical distribution and conservation status

In the recent study, *R. affinis* has wide distribution; it ranges from India to south China, Vietnam, Cambodia, Myanmar to Thailand, Malaysia and Java, Indonesia (Bates and Harrison, 1997). This (Fig. 52) below shows the additional record from literatures and recent specimens collected of specimen examined for this study and the geographical distribution of *R. affinis*. Status of *R. affinis* in the IUCN 2003 and IUCN/SSC Action Plan (2001) is Lower Risk and least concern (Simmons, 2005).

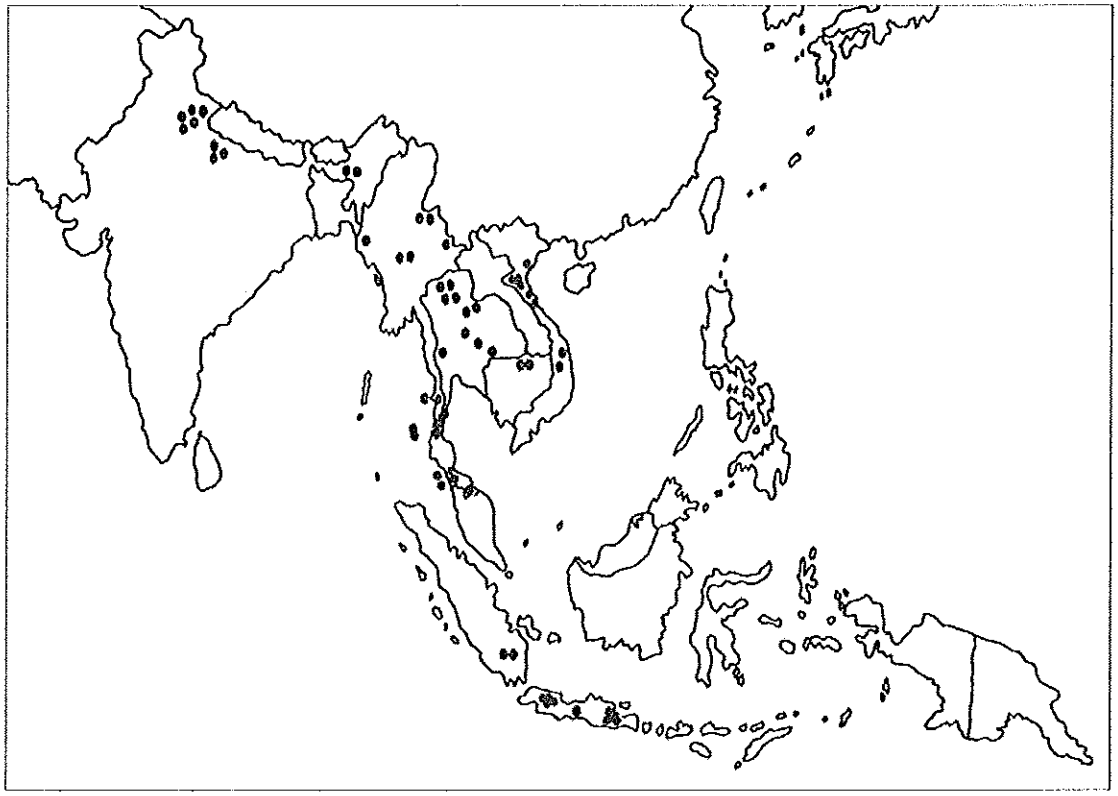


Figure 52: The additional records from literature and new localities record of geographical distribution of *Rhinolophus affinis* in this study.

4.6.2 *Rhinolophus rouxii* Temminck, 1835

Rufous horseshoe bat

External characters

Rhinolophus rouxii is variable in body size, but considerably a medium size and having an average forearm length 48.70 mm (45.09 – 52.30 mm). The noseleaf is broader and the emargination of horseshoe is slightly dark or darken. The ears are also longer than, in average 17.87 mm (14.50 – 19.93 mm). In general, *R. rouxii* have had a high variation of lancet morphology. In this study, *R. rouxii* showed a discrepancy in lancet shapes (lancet-margin). There are two types of lancet shapes, concave sides and straight sides. Sella of *R. rouxii* is varying in shapes and side, Sella is particularly straight-sided or parallel-margined (59%) from the base to the apex; the apex is broadly rounded off. The pelage colour is normally dark orange to buffy brown or rufous. The morphology of wings are vary and notably to the *R. affinis.* The second phalanx of the third digit is more than 1.50 length of the first phalanx of third digit (in average length 1.53). On the other hand, there are some taxa shows less numbers 1.50 length of the first phalanx (about 40%). The first phalanx of the third metacarpal is always less than half length of the third metacarpal, it is about 40%, and the second phalanx of the third metacarpal is typically less than 61% in average length of the third metacarpal.

Cranial and dental characters

The skull is medium and considerably with the condylo-cranine length (CCL) ranging from 17.08 – 20.33 mm (an average 19.02 mm). The zygomatic width is slightly greater or subequal to mastoid width. Palatal length is rather long and tends to be up to 1/3 of maxillary toothrow length or even more. Palate is especially longer than *R. affinis* and *R. sinicus* in average length 2.30 mm, it is emarginated anteriorly to the level of the parastyle of the first upper molar (M^1) and posteriorly to the metacone of second upper molar (M^2). Palatal bridge is relatively long 26.94% - 28.11% of the upper toothrow.

Dentition

Upper toothrow length (C-M^3) average 8.66 mm (7.99 – 9.35 mm). First Upper premolars (P^2): is usually medium sized and completely in the toothrow (81.49%) but occasionally partly of extruded (18.51%). The size of P^2 and the gap between the canine (C^1) and the second upper premolar (P^4) are unstable. P^2 is usually medium sized and situated in the toothrow, although it slightly partly extruded from the toothrow in specimens from Sri Lanka and four specimens from Tamil Nadu, south India, comprise in a medium sized and partly extruded from the toothrow. Second Lower premolars (P_3): second lower premolar is most regularly rather external or fully external (66.68%), sometimes partially in the toothrow (25.92%), and occasionally is absent (1.23%). Additionally, a few specimens showed 6.17% of position of the second lower premolar within toothrow. The cingular of the first lower premolar (P_2) and third lower premolars (P_4) are mostly in contacted, but sometime of specimens are nearly so or distinctly separated.

Baclular morphology

The shape of the baculum is parallel-sided of the baculum shape; the basal is expanded and near the tip is bent. The lengths of bacular are 2.07 – 2.70 mm, and average length is 2.25 mm. The greatest widths are 0.42 – 0.85 mm, and the mean of greatest width 0.58 mm. However, the baculum of *R. rouxii* (HZM. No.13.27453) from Sri Lanka is special longer than each specimens of *R. rouxii*, but the shape character is different (very thin) and it is very similarity length to bacular of *R. affinis* when it is examined, which bigger and fatter than specimens from India. Although, these measurements and shape characters, it supports for new evidence to propose of *R. affinis* that might be existed in Sri Lanka.

Geographical distribution and conservation status

From additional record of literature studies, *R. rouxii* ranges India, Sri Lanka to southern China and Vietnam (Bates and Harrison, 1997). However, in recent study, *R. rouxii* are ranging from western India to Sri Lanka and jump across to Myanmar (Fig. 53). Status of *R. rouxii* in the IUCN 2003 and IUCN/SSC Action Plan (2001) is Lower Risk and least concern (Simmons, 2005).

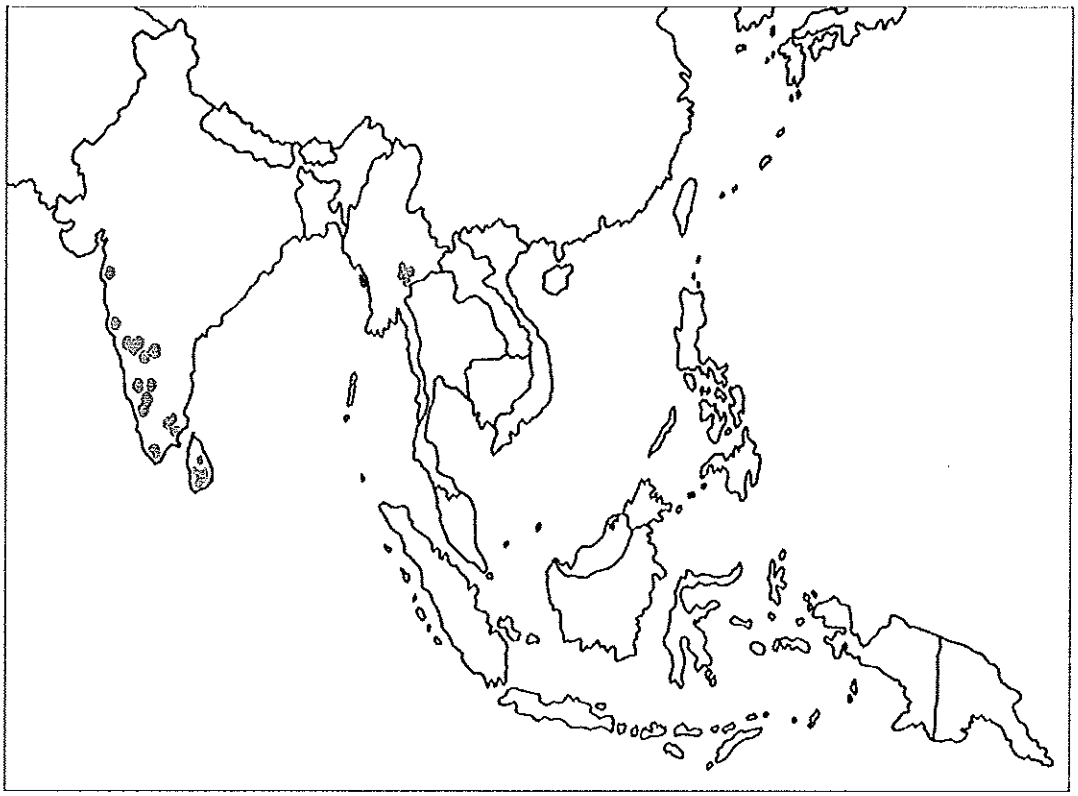


Figure 53: Recent species examined record of geographical distribution of *Rhinolophus rouxii.*

4.6.3 *Rhinolophus sinicus* Andersen, 1905

Chinese Rufous Horseshoe bat

External characters

The horseshoe morphology of *R. sinicus* is moderately wide, but it does not cover the snout or muzzle and the whole horseshoe is slightly dark. Usually, well developed secondary leaflet is clearly present on noseleaf. *R. sinicus* is generally bigger than *R. thomasi* by size with an average forearm length is 47.53 mm (46.20 – 48.80 mm). The ear is ranging from 14.38 – 20.00 mm (an average 17.63 mm). In *R. sinicus,* the lancet is typically elongate-margined and with a blunt tip or sometimes straight foreword-tip. It is always very short/or short in *R. sinicus,* there are the dome shape with elongated sides (94%) and the straight sides (6%). However, the sella shape of *R. sinicus* is typically parallel-margined with a widely obtuse apex (100%).

The first phalanx of the third metacarpal of *R. sinicus* is less than half (43%) the average length of the third digit. The second phalanx of the third digit is relatively short compared to *R. affinis*; it averages 68.9% of the length of the third metacarpal. In addition, the average length of the second phalanx of the third digit is mostly less than 1.60 mm the length of the first phalanx of the third metacarpal. The pelage colour is typically greyish brown to dark brown and occasionally bright foxy orange.

Cranial and dental characters

The skull is modulated size considerably with the condylo-cranine length; it is ranging from 17.29 – 18.87 mm (an average 17. 80 mm). The zygomatic width is slightly greater than mastoid width. In *R. sinicus,* the average length of the bony palate is about 27.40% of maxillary upper toothrow length (25.72 - 31.30%) or even more and sometime up to one third of maxillary toothrow length. Palate is emarginated posterioly to the level of the mesostyle of the first upper molar (M^1) and anterioly to the level metacone of the second upper molar (M^2).

Dentition

In *Rhinolophus sinicus,* the upper toothrow length (C-M^3) average is 7.83 mm (7.52 – 8.31 mm). The first upper premolar is small to fairly sized, but even though extremely tiny in some specimens and usually lying in the toothrow (88.24%), but with a minority (11.76%) slightly extruded from the toothrow. The upper canine (C^1) and the second upper premolar (P^4) are greatly separated, nevertheless the cingular are both relatively close together, but not in contact. In *R. sinicus,* the second lower premolar (P_3) is small or very tiny. It is partially or fully extruded from the toothrow. The first lower premolar (P_2) and third lower premolar (P_4) are fully in contacted. Although, the average length of the bony palate is about 27.40% of maxillary upper toothrow length (25.72 - 31.30%) or even more and sometime up to one third of maxillary toothrow length.

Geographical distribution and conservation status

From the current study, *R. sinicus* is known range from northern India to central Nepal through eastern Myanmar, Vietnam and southern China (Fig. 54).

Status of *R. sinicus* in the IUCN 2003 and IUCN/SSC Action Plan (2001) is Lower Risk /least concern (Simmons, 2005).

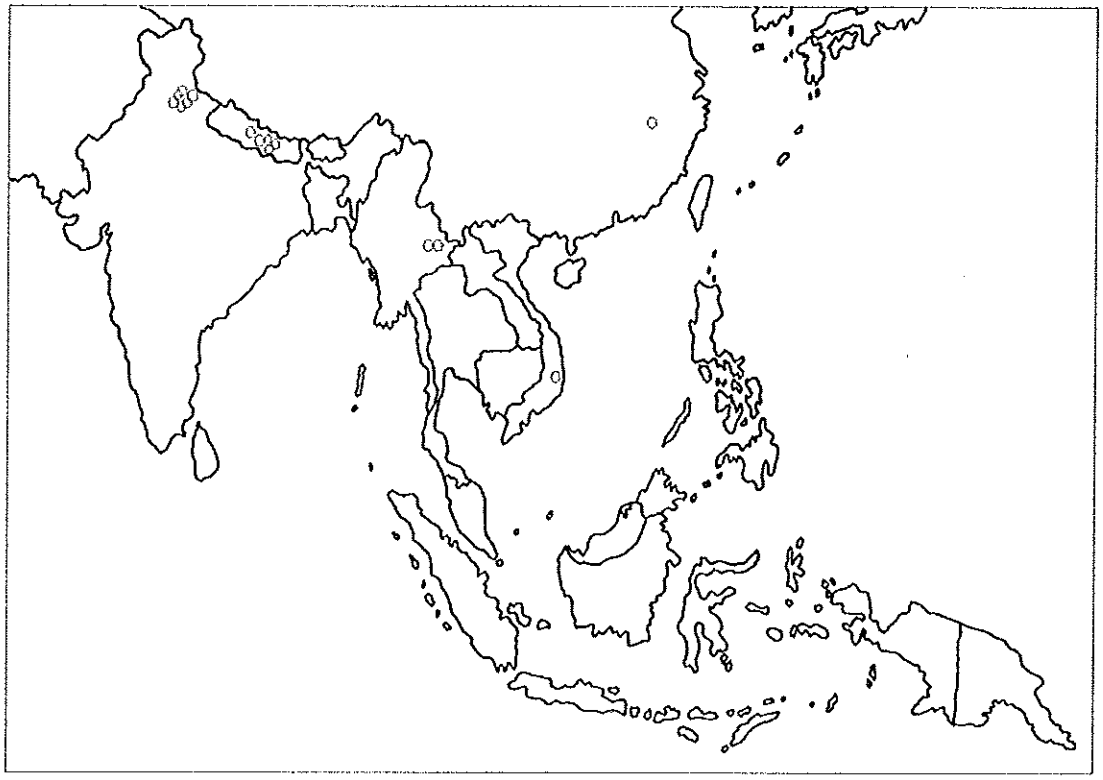


Figure 54: Geographical distribution of *Rhinolophus sinicus* from recent record.

4.6.4 *Rhinolophus thomasi* Andersen, 1905

Thomas's horseshoe bat

External characters

In *Rhinolophus thomasi* is small size and smallest size than *R. affinis, R. rouxii* and *R. sinicus.* The forearm length ranges from 41.50 – 45.10 mm (an average 43.81 mm). Tthe horseshoe shape is narrower than *R. rouxii* and *R. sinicus,* it is abroad, but does not cover the complete muzzle and the second leaflet is well developed and clearly presented on the noseleaf. The ears are 19.53 mm in average (14.45 -19.30 mm). In *R. thomasi,* the lancet is short, the tip is almost simple or straightforward with elongated sides, is completely shown the dome shape with elongated sides of lancet (100%). *R. thomasi* is very variable in sella morphologies, the majorities (55%) of the sella shapes of *R. thomasi* are straight or parallel margined with a blunt tip. However, a sizeable minority have convex sides (18%) or an

indication of a concavity (27%). The pelage colour is typically dark brown to buffy and occasionally bright orange.

Cranial and dental characters

The skull is small, the zygomatic width is slightly greater or usually subequal to mastoid width. The condylo-canine length is averages 16.25 mm (15.59 - 16.83 mm). Palatal is fairly lengthening about 29.75% of maxillary toothrow length or even less. Palate is emarginated posterioly to the level of the mesostyle of the first upper molar (M1) and posteriorly to the middle level commissures of the second upper molar (M2). Palatal bridges are rather long 21.94 – 34.44% of the upper toothrow.

Dentition

The upper toothrow length (C-M^3) average 7.06 mm (6.73 – 7.44 mm). Generally, the first upper premolar of *R. thomasi* is medium sized, and is situated in the toothrow. The upper canine (C^1) and second upper premolar (P^4) are not in contacted. The second lower premolar of *R. thomasi* is very small and usually partly or completely extruded from the toothrow. The first lower premolar (P_2) and the third lower premolars (P_4) are in contact.

Baclular morphology

In lateral view, the baculum has a simple shaft and is usually slightly curved towards the bluntly pointed tip. The basal cone is expanded and flattened. Average baculum length is 1.81 mm (1.67 – 1.95 mm). The baculum of specimen from Vietnam had a slightly more robust tip.

Echolocation

Based on this study, there is no echolocation data are available for *Rhinolophus sinicus* and *R. thomasi* since the study was based on existing voucher specimens held in the collections of the Harrison Institute and the Natural History Museum (London).

Geographical distribution and conservation status

From additional literature and current study, *R. thomasi* is ranging from Myanmar to Thailand and Vietnam (Fig. 55). There are very poor of information and specimen record in Southeast Asia. Status of *R. thomasi* in the IUCN 2003 and IUCN/SSC Action Plan (2001) is Lower Risk and near threatened (Simmons, 2005).

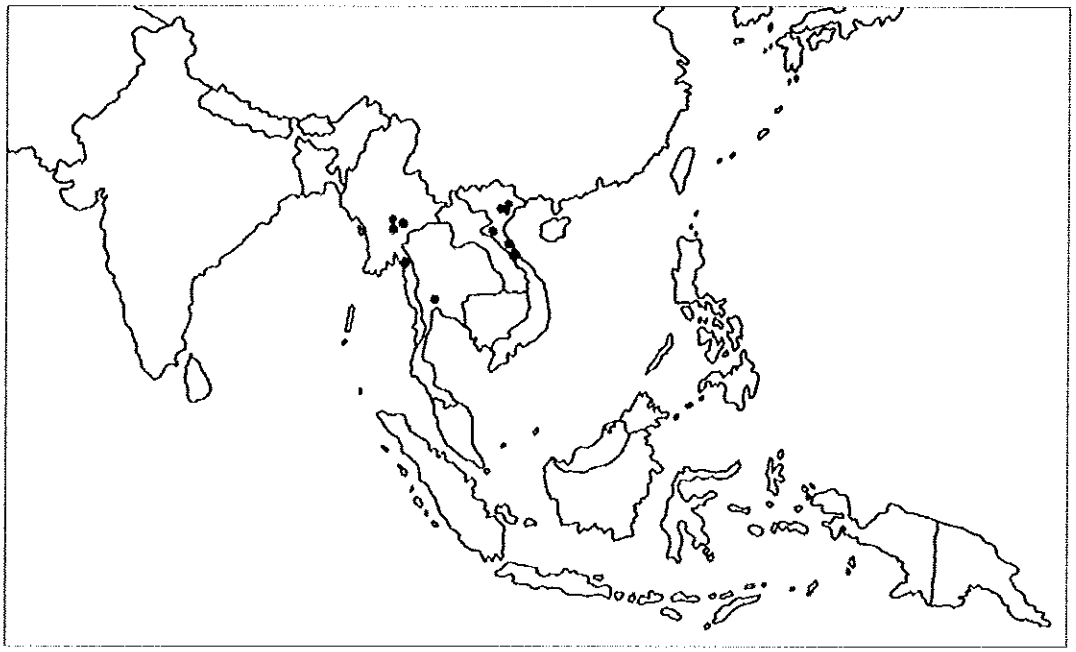


Figure 55: Geographical distribution of *Rhinolophus thomasi* from additional literature and recent record.

CHAPTER 5
DISCUSSION

5.1 General Comparisons

In this study, four cryptic species had been evaluated of their taxonomic characters and interspecific comparison was undertaken. *R. affinis, R. sinicus* and *R. thomasi* can be distinguished from each other by external morphology, cranial and dentition characters. However, *R. rouxii* is very complicated and it is similar in body size and morphology to *R. affinis* which made their species identification very difficult. Previously, these two rhinolophid bats were reported to be differentiated by their forearm length, which is ranging from 41 - 55 mm, and wing bone measurements (the second phalanx of the third metacarpal) (Bates and Harrison, 1997, Csorba *et al.,* 2003). Lancet shapes is very variable (Bates and Harrison, 1997), but palatal length (Csorba *et al.,* 2003) and skull morphologies including dentition characters can be used to distinguish between species (Bates and Harrison, 1997). Therefore, the results from this study confirmed and provide additional supported previous authors and strengthen of using some characters to separate *R. affinis* from *R. rouxii.*

Regularly, the external morphology is not useful character for identification of the cryptic species because there can be high intraspecific variation of some taxonomic characters such as sella shapes and lancet morphology. Some studies apply echolocation call to identify cryptic species (Soisook *et al.,* 2008). Unfortunately, only the echolocation call of *R. affinis* was available and lack of information in echolocation calls of *R. rouxii*, *R. sinicus* and *R. thomasi* in the present study. Geographical variation was found from this study, including a gradual integration of characters and body sizes from south to north such as in *R. affinis.* In these taxa, individuals from southern Thailand and Java, Indonesia are smaller than those from northern Thailand and Myanmar, and India. Generally, the forearm length is very useful character to identify species such as *R. affinis* from India which has the

longest forearm length (average forearm length 53.59 mm) than other species, while *R. thomasi* has the shortest one from this study. Furthermore, the specimens of *R. affinis* from Vietnam are slightly smaller than Indian one, but bigger (51.07 mm) than specimens from Myanmar. Overall, a specimen of *R. affinis* from Cambodia 47.44 mm is smallest of all *R. affinis* from this study. In this study, morphological and morphometric measurements approach is quite useful in defining taxa. Although, *R. affinis* and *R. rouxii* are very analogous in external morphology, skull morphology and dentition characters, they can be differentiated since *R. affinis* has longer average length of the second phalanx of the third metacarpal and bigger skull than *R. rouxii*, but the palatal bridge of *R. rouxii* is longer than *R. affinis*. Furthermore, the tiny bone of bacular (Bates and Harrison, 1997; Csorba *et al.*, 2003) of both species is completely different in length (Table 11) and morphology. It is very interesting to note that, the bacular of *R. rouxii* from Sri Lanka is similar in length to bacular of *R. affinis*, but it is thinner than those *R. affinis* and different in shape from baculum of *R. affinis*.

R. rouxii and *R. sinicus* are also analogous in morphology and size, but considerably different in length of the first and the second of the third metacarpal. *R. rouxii* is shorter than *R. sinicus* and the lancet shape in *R. rouxii* is triangular shape with concave sides, but dome shape with elongate margined in *R. sinicus*. The cranial and dental characters of *R. rouxii* are completely bigger than *R. sinicus*, and palatal length is longer. Evidentially, the baculum of *R. rouxii* was clearly described in this study, this organ was used to differentiate between this species and others, and its shape is identical within species. Unfortunately, the baculum *R. sinicus* was missed and it is not cleared to separate between *R. rouxii* and *R. sinicus*. *R. thomasi* was completely distinguished from *R. sinicus* by its smaller size, and they are different in some characters, such as sella shape, lancet morphology and wing measurements. The relationships and specific boundaries between *R. sinicus* and *R. rouxii* and *R. thomasi* are clearly distinguished by size. However, *Rhinolophus sinicus* is very similar to the smaller *R. thomasi* of Myanmar, Vietnam, Lao PDR and Thailand, to which it is closely related, but they are sapalately by character measurements. Note that, these two species showed sexual dimorphism (male is general larger than female). The result from this study is strongly supported the view of Csorba *et al.*, (2003) who

proposed that the type specimen of *R. sinicus* is larger than the type specimen of *R. thomasi.*

5.2 Taxonomic Notes

In this study, the populations of *R. rouxii* from India are absolutely differed from those Sri Lanka populations by sizes. This result was supported Thomas (1997) who suggested that *R. rouxii* from southern India is relatively larger than *R. rouxii* from Sri Lanka. This species has widely distribution and ranging from India to Sri Lanka and spread to northern Myanmar (Bates and Harrison 1997). From the result, it is not different between *R. rouxii* from Myanmar and Sri Lanka. This species is not found in Thailand yet; it is not clear why its distribution is limited there. Similarity, *Hipposideros ater* was also limited only in Myanmar (Douangboubpha *et al.* submitted).

R. affinis has wide distribution; it ranges from India to south China, Laos, Vietnam, Cambodia, Myanmar to Thailand, Malaysia and Indonesia (Bates and Harrison, 1997). In current study, the call frequency of *R. affinis* from the north and northeast of Thailand are similar in range, it is about 70 - 77 kHz. However, the lower call frequency of *R. affinis* was found in mainland southern Thailand (69 - 73 kHz) and the lowest is from Tarutao Islands (65 - 69 kHz). However, *R. affinis* from Tarutao Islands are similar to *R. affinis* from mainland southern Thailand by size and morphology. In case of this relationship, it may suggest that disjunction pattern was occurred after its separation, these two populations are geographically isolated and showed evidence of echolocation call variation between them. The molecular study for *R. affinis* in Thailand is recommended, as this country covers relatively high latitudinal variation and shows considerably geographical variation of echolocation call from the north to the south. It is interesting to make a note that *R. affinis* was not found in central part of Thailand during this study. Thus, survey should be conducted for *R. affinis* in central part of Thailand in both rainy and dry seasons.

Further DNA study of *R.rouxii* and *R. sinicus* are recommended, especially specimens from Myanmar, Thailand and Laos. Because their geographical distritbutions are slightly spreadout from Sri Lanka, to southern India through northern Myanmar, it may exist in northern Thailand since it is similar in

environmental conditions. The echolocation call of *R. sinicus* is a long constant frequency signal, with a brief frequency-modulated start and tail. Frequencies with most energy recorded from hand-held bats ranged between 80 and 88.2 kHz (n= 13). Some evidence of males calling at lower frequencies (80-84.2 kHz) than females (84-88.2 kHz), as found in the closely related *R. rouxi* in Sri Lanka which calls at 73.5 - 79 kHz (Neuweiler *et al.* 1987). Call frequencies is not overlap with those used by *R. sinicus*, so it is a diagnostic feature for separating these species. An echolocation call frequency of R. sinicus is not overlap with those emitted by *R. affinis*. *R. affinis* is also typically a larger species, though overlap occurs with *R. sinicus* at forearm lengths between 50-51 mm. Call frequency for *R. thomasi* in Lao PDR is reported to be 76 kHz (Francis & Habersetzer 1998), and so the two taxa may use different call frequencies. Echolocation calls of these species can thus be used to separate them since it is species specific.

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APPENDIXES

APPENDIC 1: Goegraphical and coordinations of *Rhinolophus affinis*

Species	Collection No	Locality	Coordination
R.affinis	HZM. 4. 28148	Nala Panai cave, 3 km south of Mussoorie. Up India	30. 26 N 78.04 E
R.affinis	HZM. 5. 28149	Nala Panai cave, 3 km south of Mussoorie. Up India	30. 26 N 78.04 E
R.affinis	HZM. 6. 28150	Nala Panai cave, 3 km south of Mussoorie. Up India	30. 26 N 78.04 E
R.affinis	HZM. 2. 28146	Nala Panai cave, 3 km south of Mussoorie. Up India	30. 26 N 78.04 E
R.affinis	HZM. 8. 28152	Kaladungi, 7 km west of Haldwani, Uttar Pradesh, India	26.55 N 80.34 E
R.affinis	HZM. 3. 36075	Holin village, Cave, Keng Taung, Myanmar	21° 27'483" N 99°32'648"E
R.affinis	HZM. 31. 35976	Head Chey Khang Gu cave, Kyauk Taung, Thanintaryi Division, Myanmar	12° 19' N 99°01'E
R.affinis	HZM.28.34945	Mant Hai village, Muse Township, Shan State, Myanmar	23.54'962"N 97.49'442"E
R.affinis	HZM.29.35223	Taung Pauk village, Inle lake, Shun state, Myanmar	20.21'175"N 96.53'189"E
R.affinis		Mant Hai village, Muse Township, Shan State, Myanmar	23.55'962"N 97.49'442"E
R.affinis	HZM. 25. 34147	M'Lou Prey, Preah Vihear prov. Cambodia	Not located
R.affinis	HZM.27.34176	M'lou Prey, Preah Vihear, Combodia	Not located
R.affinis	HZM. 11.32182	Kon Ka Kinh Natural Reserve. Vietnam	14.19'N 108.24'E
R.affinis	HZM.14.32185	Kon Ka Kinh Natural Reserve. Vietnam	14.28'N 108.36'E
R.affinis	HZM.13.32184	Kon Ka Kinh Natural Reserve. Vietnam	14.19'N 108.24'E
R.affinis	HZM.17.32188	Kon Ka Kinh Natural Reserve. Vietnam	14.17'N 108.23'E
R.affinis	HZM.16.32187	Kon Ka Kinh Natural Reserve. Vietnam	14.19'N 108.25'E
R.affinis	HZM.19. 32198	Bong, Cuc Phoung National Park, Vietnam	20° 18' N 105°38'E
R.affinis	HZM. 24. 32193	Kon Cha Rang, Nature reserve, Gai-lai prov, Vietnam	14° 29' N 108°36'E
R.affinis	HZM. 18. 32199	Mon Son-Nam Son cave, Pu Mat nature reserve, Vietnam	18° 58' N 104°46'E
R.affinis	HZM. 10.32181	Kon Cha Rang, Nature reserve, Gai-lai prov, Vietnam	14° 28' N 108°36'E
R.affinis	HZM. 21.32196	Stream nr. Moa Son village, Ke Bang, Phong Nha Nature reserve, Vietnam	17° 47' N 105°52'E
R.affinis	BNHM	Nala Panai Cave, 3 km. Sook, 7 km Mussoorie, Up India	30. 26 N 78.04 E

APPENDIC 1: Goegraphical and coordinations of *Rhinolophus affinis* (Continued)

Species	Collection No	Locality	Coordination
R.affinis	BNHM	Nala Panai Cave, 3 km. Sook, 7 km Mussoorie, Up India	30. 26 N 78.04 E
R.affinis	BNHM	Nala Panai Cave, 3 km. Sook, 7 km Mussoorie, Up India	30. 26 N 78.04 E
R.affinis	BNHM	Nala Panai Cave, 3 km. Sook, 7 km Mussoorie, Up India	30. 26 N 78.04 E
R.affinis	BNHM	Nala Panai Cave, 3 km. Sook, 7 km Mussoorie, Up India	30. 26 N 78.04 E
R.affinis	BNHM	Not located	Not located
R.affinis	HZM. 25. 34147	M'Lou Prey, Preah Vihear prov. Cambodia	Not located
R.affinis	HZM.27.34176	M'lou Prey, Preah Vihear, Combodia	Not located
R.affinis	PSU-M05.108	Phu soun sai National Park, Loei, Thailand	17° 30'323" N100°56'295"E
R.affinis	PSU-M05.109	Phu soun sai National Park, Loei, Thailand	17° 30'323" N100°56'295"E
R.affinis	PSU-M05.110	Phu soun sai National Park, Loei, Thailand	17° 28'429" N 101°58'539"E
R.affinis	PSU-M05.111	Mae Ja cave, Chieng dao National Park, Chieng dao district, Chieng Mai, Thailand	19° 31'915" N 98°50'440"E
R.affinis	PSU-M05.113	Khao Kram cave, Patiew district, Chumporn prov, Thailand	10° 55'131" N 99°22'440"E
R.affinis	PSU-M05.114	Khao Kram cave, Patiew district, Chumporn prov, Thailand	10° 55'131" N 99°22'440"E
R.affinis	PSU-M05.102	River bank, Boripatr Waterfall, Ton Nga-chang Wildlife Sanctuary, Satun, Thailand	7°00'049" N 100°08'534"E
R.affinis	PSU-M05.103	River bank, Boripatr Waterfall, Ton Nga-chang Wildlife Sanctuary, Satun, Thailand	7°00'049" N 100°08'534"E
R.affinis	PSU-M05.104	River bank, Boripatr Waterfall, Ton Nga-chang Wildlife Sanctuary, Satun, Thailand	7°00'049" N 100°08'534"E
R.affinis	PSU-M05.117	Khao Kram cave, Patiew district, Chumporn prov, Thailand	10° 55'131" N 99°22'440"E
R.affinis	PSU-M05.106	Boripatr Waterfall, Ton Nga-chang Wildlife Sanctuary, Songkhla Province	06° 59' N 100 100°08'E
R.affinis	PSU-M05.89	North Surin, Thailand	Not located
R.affinis	PSU-M05.90	North Surin, Thailand	Not located
R.affinis	PSU-M05.92	North Surin, Thailand	Not located
R.affinis	PSU-M05.94	Tham Khao Tieb, Ton Nga-chang Wildlife Sanctuary, Songkla province, Thailand	6°59'975" N 100°17'872"E
R.affinis	PSU-M05.95	Tham Khao Tieb, Ton Nga-chang Wildlife Sanctuary, Songkla province, Thailand	6°59'975" N 100°17'872"E
R.affinis	PSU-M05.96	Tham Khao Tieb, Ton Nga-chang Wildlife Sanctuary, Songkla province, Thailand	6°59'975" N 100°17'872"E
R.affinis	PSU-M05.82	Tamean Thom, Huai Tubtun, WS. Surin province, Thailand	14.21'08"N 103. 15'54"E
R.affinis	PSU-M05.83	Ao Son -Ao Chak road, Tarutao island, Thailand	6.39'38"N 99.38'2"E
R.affinis	PSU-M05.85	Kavackee, HQ of East Thung Yai, WS, Tak province, Thailand	15.42'26"N 98.59'28"E

APPENDIC 1: Goegraphical and coordinations of *Rhinolophus affinis* (Continued)

Species	Collection No	Locality	Coordination
R.affinis	PSU-M06.	Klao Rak Kiet, Rattaphum district, Songkhla, Thailand	07° 04'264" N 100°15 '098"E
R.affinis	PSU-M07.12	km 7, road to Talowao, Tarutao National Park, Thailand	06° 39'292" N 9939 '455"E
R.affinis	PSU-M07.128	Ao - Son road, Tarutao National Park, Thailand	06° 39'541" N 9937 '960"E
R.affinis	PSU-M07.	Huay wang cave, T. Khao Talu, Sawi district, Chumporn, Thailand	10° 10' 996" N 98°55'183"E
R.affinis	PSU-M07.	Klao Plu cave, Lamae district, Chumporn, Thailand	09° 43' 601" N 99°06'495"E
R.affinis	PSU-M05.119	Mae Ja cave, Chieng dao National Park, Chieng dao district, Chieng Mai, Thailand	19° 31'915" N 98°50'440"E
R.affinis	PSU-M05.120	Mae Ja cave, Chieng dao National Park, Chieng dao district, Chieng Mai, Thailand	19° 31'915" N 98°50'440"E
R.affinis	PSU-M07	Hala - Bala wildlife sanctuary, Nara Thiwat, Thailand	05° 47'54" N 101°49 '495"E
R.affinis	PSU-M07	Hala - Bala wildlife sanctuary, Nara Thiwat, Thailand	05° 47'54" N 101°49 '495"E
R.affinis	VN	Ban Khon cave, Pu Hoat natural Reserve, Que Phong district, Nghe An Prov, Vietnam	Not located
R.affinis	VN	Ban Khon cave, Pu Hoat natural Reserve, Que Phong district, Nghe An Prov, Vietnam	Not located
R.affinis	VN	Ban Khon cave, Pu Hoat natural Reserve, Que Phong district, Nghe An Prov, Vietnam	Not located
R.affinis	24290	Vietnam	Not located
R.affinis	65	Vietnam	Not located
R.affinis	1997.35	NA HANG NATURE REV, TUYEN QUANG PROV, VIETNAM	Not located
R.affinis	PSU-M05.91	Ao Son -Ao Chak road, Tarutao island, Thailand	6°38'76" N 99°37'4"E
R.affinis	PSU-M05.112	km.6, Tarutao island	6° 39'48" N 99°39'4"E
R.affinis	B.M. 8. 2. 25. 8	Batu Caves, Kuala Lumpor	Not located
R.affinis	B.M. 9. 1. 5. 149	Kalipoctjang, West, Java	Not located
R.affinis	B.M. 9. 1. 5. 148	Tjelatjah, Java	Not located
R.affinis	B.M. 9. 1. 5. 150	Kalipoctjang, Java	Not located
R.affinis	B.M. 9. 1. 5. 151	Kalipoctjang, West, Java	Not located
R.affinis	9.1.5.141	Sockabocmi, Java, Indonesia	Not located
R.affinis	B.M. 20.11. 1. 21	Khasi Hills	Not located
R.affinis	B.M. 21. 1. 17. 1	Up Chinduvin	Not located

APPENDIC 1: Goegraphical and coordinations of *Rhinolophus affinis* (Continued)

Species	Collection No	Locality	Coordination
R.affinis	T 20	Bach Ba National Park, Thua Thien-Thue prov, Central Vietnam	Not located
R.affinis	HZM. 3. 28147	Nala Panai cave, 3 km south of Mussoorie. Up India	30. 26 N 78.04 E
R.affinis	HZM. 7. 28151	Nala Panai cave, 3 km south of Mussoorie. Up India	30. 26 N 78.04 E
R.affinis	BNHM	Nala Panai Cave, 3 km. Sook, 7 km Mussoorie, Up India	30. 26 N 78.04 E
R.affinis	HZM. 26.34175	M'lou Prey, Preah Vihear, Combodia	Not located
R.affinis	HZM. 22. 32195	Cave at Khe Mat ride to Pu Mat, Nature reserve, Vietnam	18° 58' N 104°46'E
R.affinis	HZM. 20. 32197	Phu Nong mountain, Pu Mat nature reserve, Vietnam	18° 58' N 104°46'E
R.affinis	HZM. 23. 32194	Khe Dap Trostream, Phong Nha Nature reserve, Vietnam	17° 35' N 106°19'E
R.affinis	HZM.15.32186	Koncha Rang Nature Reserve. Vietnam	14. 28' N 108. 36' E
R.affinis	HZM.12.32183	Kon Ka Kinh Natural Reserve. Vietnam	14.19'N 108.24'E
R.affinis	HZM.9.30654	Cuc-Phoung National Park, Vietnam	Not located
R.affinis	HZM.71.35978	Cave nr. Katalu village nt. Nawayat lake, Kadan LD. Tanintharyi. Myanmar	12.28'436"N 98.24'191"E
R.affinis	HZM.74.35981	Cave nr. Katalu village nr, Kadan LD. Tanintharyi.Div. Myanmar	12.28'436"N 98.24'191"E
R.affinis	HZM.70.35977	Stream.nr. Leik- Kyi village. Kadan ID, Tanin - Tharyi, Myanmar	12.30'113"N 98.24'333"E
R.affinis	HZM.30.35224	Lake Shan State, Taung Pauk Village, Myanmar	20.21' 175"N 96.53'189"E
R.affinis	HZM. 73. 35980	Cave nr. Katalu village, Kadanr ID, Thanintaryi Division, Myanmar	120° 28'436" N 98°24'191"E
R.affinis	HZM. 72. 35979	Cave nr. Katalu village, Kadanr ID, Thanintaryi Division, Myanmar	120° 28' N 4306'E
R.affinis	B 114	Vietnam	Not located
R.affinis	B. 12	Pu Hoat Nature reserve, Nghe Anh prov, central Vietnam	Not located
R.affinis	BM. 06	Bach Ba National Park, Thua Thien-Thue prov, Central Vietnam	Not located
R.affinis	T 91	Vietnam	Not located
R.affinis	79.11.21.70	Java, Indonesia	Not located
R.affinis	PSU-M05.84	Wang Saithong waterfall, Ma-Nong district, Satun province, Thailand	47N 0600469, UTM0783824
R.affinis	PSU-M05.86	Thung Kamong, Phukiew, WS. Chaiyapum province, Thailand	Not located
R.affinis	PSU-M05.87	Thailand	Not located

APPENDIC 1: Goegraphical and coordinations of *Rhinolophus affinis* (Continued)

Species	Collection No	Locality	Coordination
R.affinis	PSU-M05.88	Thailand	Not located
R.affinis	PSU-M05.201	Mae Ja cave, Chieng dao National Park, Chieng dao district, Chieng Mai Prov.	19° 31'915" N 98°50'440"E
R.affinis	PSU-M05.122	Mae Ja cave, Chieng dao National Park, Chieng dao district, Chieng Mai Prov.	19° 31'915" N 98°50'440"E
R.affinis	PSU-M07.	Klao Plu cave, Lamae district, Chumporn province	09° 43' 601" N 99°06'495"E
R.affinis	PSU-M07.	Knad Dai cave, A. La-Aun, Ranong province	10° 01'910" N 98°55'183"E
R.affinis	PSU-M07.	Pra Kayang cave, T. Lum lieng, A. Kraburi, Chumporn province	10° 19'569" N 98°45 '923"E
R.affinis	PSU-M05.97	Tham Khao Tieb, Ton Nga-chang Wildlife Sanctuary, Songkla province, Thailand	6°59'975" N 100°17'872"E
R.affinis	PSU-M05.98	Tham Khao Tieb, Ton Nga-chang Wildlife Sanctuary, Songkla province, Thailand	6°59'975" N 100°17'872"E
R.affinis	PSU-M05.99	Tham Khao Tieb, Ton Nga-chang Wildlife Sanctuary, Songkla province, Thailand	6°59'975" N 100°17'872"E
R.affinis	PSU-M05.100	Rever bank, Boripatr Waterfall, Ton Nga-chang Wildlife Sanctuary, Satun, Thailand	7°00'049" N 100°08'534"E
R.affinis	PSU-M05.101	River bank, Boripatr Waterfall, Ton Nga-chang Wildlife Sanctuary, Satun, Thailand	7°00'049" N 100°08'534"E
R.affinis	PSU-M05.115	Khao Kram cave, Patiew district, Chumporn prov, Thailand	10° 55'131" N 99°22'440"E
R.affinis	PSU-M05.116	Khao Kram cave, Patiew district, Chumporn prov, Thailand	10° 55'131" N 99°22'440"E
R.affinis	PSU-M05.118	Khao Kram cave, Patiew district, Chumporn prov, Thailand	10° 55'131" N 99°22'440"E
R.affinis	PSU-M07.129	Ao - Son road, Tarutao National Park	06° 39'541" N 9937 '960"E
R.affinis	PSU-M07.130	Taluwao - Tarow, Ou - Rang raod, Tarutao National Park	06° 36'265" N 99°40 '518"E
R.affinis	PSU-M05.105	Mae Ja cave, Chieng dao National Park, Chieng dao district, Chieng Mai Prov.	19° 31'915" N 98°50'440"E
R.affinis	PSU-M05.107	Thung sa lang luang National Park, Nhong mae na, Loei	16° 34'283" N 100°52'583"E
R.affinis	PSU-M07	Hala - Bala wildlife sanctuary, Nara Thiwat, Thailand	05° 47'54" N 101°49 '495"E
R.affinis	VN	Ban Khon cave, Pu Hoat natural Reserve, Que Phong district, Nghe An Prov, Vietnam	Not located
R.affinis	VN	Ban Khon cave, Pu Hoat natural Reserve, Que Phong district, Nghe An Prov, Vietnam	Not located
R.affinis	VN	Ban Khon cave, Pu Hoat natural Reserve, Que Phong district, Nghe An Prov, Vietnam	Not located
R.affinis	B.M. 21.1. 17. 2	Holin village, Cave, Keng Taung, Myanmar	Not located
R.affinis	B.M. 9. 1. 5. 142	Kattamnah cave, Sockabolmi, Java	Not located
R.affinis	B.M. 10. 4. 6.21	Kangcan, East of Java	Not located

APPENDIC 1: Goegraphical and coordinations of *Rhinolophus affinis* (Continued)

Species	Collection No	Locality	Coordination
R.affinis	9.1.5.146	Sockabocmi, Java, Indonesia	Not located
R.affinis	9.1.5.145	Sockabocmi, Java, Indonesia	Not located
R.affinis	B.M. 9. 1. 5. 154	Kalpoctjang,West of Java	Not located
R.affinis	B.M. 9. 1. 5. 147	Sockabcimi, Java	Not located
R.affinis	B.M. 9. 1. 5. 143	Sockabcimi, Java	Not located
R.affinis	B.M.9. 1. 5. 155	Kalipoctjang West, Java	Not located
R.affinis	B.M. 9. 1. 5. 144	Kattamnah cave, Sockabolmi, Java	Not located
R.affinis	B.M. 9. 1. 5. 152	Kalipoctjang West, Java	Not located
R.affinis	B.M. 9. 1. 5. 153	Kalipoctjang, West. Java	Not located
R.affinis	B.M. 10. 4. 6. 20	Kaunggan, East of Java	Not located
R.affinis	B.M. 8. 2. 25. 9	Batu caves, Kaula Lumpor	Not located
R.affinis	VN. 9601-B027	Na Hang Nature reserve, Tuyen Quang prove Vietnam	Not located

APPENDIC 2: Goegraphical and coordinations of *Rhinolophus rouxii*

Species	Collection No	Locality	Coordination
R. rouxii	B.M. 94. 455	BomBay, National History Society, Viagenoa, India	Not located
R. rouxii	85.3. 20.1	Coonoor Nilgiri hills, India	Not located
R. rouxii	B.M. 12. 6. 29.17	Dharwar Dist, BomBay N. H. S, Indai	Not located
R. rouxii	B.M. 12. 6. 29. 16	Dharwar Dist, BomBay N. H. S, Indai	Not located
R. rouxii	B.M. 12. 6. 29. 18	Dharwar Dist, BomBay N. H. S, Indai	Not located
R. rouxii	B.M. 12. 6. 29. 19	Dharwar Dist, BomBay N. H. S, Indai	Not located
R..rouxii	BNHM	Eevengallar Dam, Venniar state, High Vavu mountain, Tamil Nadu, India	10.59 N 78.25 E
R. rouxii	BM 2003. 386	India	Not located
R. rouxii	BM 79.11.12.146=150	India	Not located
R. rouxii	No. Reg. No	India	Not located
R. rouxii	BM 79.11.12.146=149	Mussoorie, India	Not located
R. rouxii	HZM. 27. 28159	Rachel Seminary, Salcete, approx 15 km Nam Colva, Goa, India	15.50 N 73.57 E
R. rouxii	HZM. 70. 36210	Sengaltheri cave, Kalakad hills, Mundantherai Tiger reserve, Tamil Nadu, South, India	08.22"N 77.20"E
R. rouxii	HZM. 71. 36211	Shenkaltheri Kalakad hills, near Pachayer Prov, Tamil Nadu, south, India	10.59 N 78.25 E
R..rouxii	BNHM	Sriranga Patnam 16 km Trum Mysore, Karnataka, India	Not located
R..rouxii	BNHM	Sriranga Patnam 16 km Trum Mysore, Karnataka, India	Not located
R..rouxii	BNHM	Sriranga Patnam 16 km Trum Mysore, Karnataka, India	Not located
R. rouxii	HZM. 10. 25680	Talewadi, 50 km south-west Belgaum, Karnataka, India	15.31"N 75.31'E
R. rouxii	HZM. 11. 25681	Talewadi, 50 km south-west Belgaum, Karnataka, India	15.31"N 75.31'E
R. rouxii	HZM. 12. 25682	Talewadi, 50 km south-west Belgaum, Karnataka, India	15.31"N 75.31'E
R. rouxii	HZM. 72. 36548	Tunnel 1, Kodaiar Beat, Kodaiar, KMTR, Tirunelveli Tamil Nadu, South, India	08.41'283"N 77.31'098"E
R. rouxii	HZM. 26. 28158	Venniar estate, High wany, mountains. Tamil Nadu, India	20.21'175"N 96.53'189"E
R. rouxii	HZM. 25. 28157	Venniar Estate, High Wany mountain, Tamil Nadu, India	20.21'175"N 96.53'189"E
R. rouxii	HZM. 24. 28156	Venniar Estate, High Wany mountain, Tamil Nadu, India	20.21'175"N 96.53'189"E
R. rouxii	BNHM	Venniar estate, High wany, mountains. Tamil Nadu, India	20.21'175"N 96.53'189"E
R. rouxii	BM. 59.5.31.68		Not located

APPENDIC 2: Goegraphical and coordinations of *Rhinolophus rouxii* (Continued)

Species	Collection No	Locality	Coordination
R. rouxii			Not located
R. rouxii	66. 2. 13. 8		Not located
R. rouxii	9.11.18.3		Not located
R. rouxii	9. 11. 18. 1		Not located
R. rouxii	9. 11. 18. 2		Not located
R. rouxii	9. 11. 18. 4		Not located
R. rouxii	HZM. 53. 29292	Althwella cave, Pallama, Matale Dist, Sri Lanka	07.31'55"N 80.39'24"E
R. rouxii	HZM. 48. 29287	Aluthwella cave, Pallama, Matale Dist Sri Lanka	07.31'55"N 80.39'24"E
R. rouxii	HZM. 54. 29330	Aluthwella cave, Pallama, Matale Dist Srilanka	07.31'55"N 80.39'24"E
R. rouxii	HZM. 50. 29289	Aluthwella cave, Pallama, Matale Dist Srilanka	07.31'55"N 80.39'24"E
R. rouxii	HZM. 69. 31608	Aluthwella cave, Pallama, Matale Dist Srilanka	7.32' N 80.39' E
R. rouxii	HZM. 68. 31607	Aluthwella cave, Pallama, Matale Dist Srilanka	7.32' N 80.15' E
R. rouxii	HZM. 51. 29190	Aluthwella cave, Pallama, Matale Dist Srilanka	7.32' N 80.39' E
R. rouxii	HZM. 57. 31076	Bogala Watta, near Grapite works, Ruwanwella, Sabaragamuwa,Srilanka	7.02' N 80.15' E
R. rouxii	HZM. 66. 31291	Bogala Watta, near Grapite works, Ruwanwella, Sabaragamuwa,Srilanka	7.02' N 80.15' E
R. rouxii	HZM. 65. 31290	Bogala Watta, near Grapite works, Ruwanwella, Sabaragamuwa,Srilanka	7.02' N 80.15' E
R. rouxii	HZM. 15. 27455	Bogala Watta, near Ruwanwella, Sabaragamuwa,Srilanka	7.02' N 80.15' E
R. rouxii	HZM. 58. 31077	Bogala Watta, near Grapite works, Ruwanwella, Sabaragamuwa,Srilanka	7.02' N 80.15' E
R. rouxii	HZM. 47. 29286	Gampaha, near Hewelkandura, Srilanka	06.41'55"N 81.03'59"E
R. rouxii	HZM. 46. 29285	Gampaha, near Hewelkandura, Srilanka	06.41'55"N 81.03'59"E
R. rouxii	HZM. 44. 29283	Gampaha, near Hewelkandura, Srilanka	06.41'55"N 81.03'59"E
R. rouxii	HZM. 43. 29282	Gampaha, near Hewelkandura, Srilanka	06.41'55"N 81.03'59"E
R. rouxii	HZM. 49. 29288	Gampaha, near Hewelkandura, Srilanka	06.41'55"N 81.03'59"E
R. rouxii	HZM. 42. 29281	Gampaha, near Hewelkandura, Srilanka	06.41'55"N 81.03'59"E
R. rouxii	HZM. 45. 29284	Gampaha, near Hewelkandura, Srilanka	06.41'55"N 81.03'59"E
R. rouxii	HZM. 52. 29291	Gampaha, near Hewelkandura, Srilanka	06.41'55"N 81.03'59"E
R. rouxii	HZM. 60. 31079	Giritale Wildlife Training Centre. Giritale. north. Central Provnce. srilanka	7.59' N 80.54' E

APPENDIC 2: Goegraphical and coordinations of *Rhinolophus rouxii* (Continued)

Species	Collection No	Locality	Coordination
R. rouxii	HZM. 67. 31292	Hagala Estate, supervisors bungalow, Hagala, Sabaragamuwa, central province, Sri Lanka	Not located
R. rouxii	HZM. 39. 28566	Ingiriya, western Prov, Sri Lanka	06.45' N 80.05' E
R. rouxii	HZM. 36. 28563	Ingiriya, western Prov, Sri Lanka	06.45' N 80.05' E
R. rouxii	HZM. 28. 28555	Ingiriya, western Prov, Sri Lanka	06.45' N 80.05' E
R. rouxii	HZM. 35. 28562	Ingiriya, western Prov, Sri Lanka	06.45' N 80.05' E
R. rouxii	HZM. 30. 28557	Ingiriya, western Prov, Sri Lanka	06.45' N 80.05' E
R. rouxii	HZM. 37. 2864	Ingiriya, western Prov, Sri Lanka	06.45' N 80.05' E
R. rouxii	HZM. 40. 28567	Monaragala, southern prov, Srilanka	06.52' N 80.22' E
R. rouxii	HZM. 32. 28559	Monaragala, southern prov, Srilanka	06.52' N 80.22' E
R. rouxii	HZM. 31. 28558	Monaragala, southern prov, Srilanka	06.52' N 80.22' E
R. rouxii	HZM. 29. 28556	Monaragala, southern prov, Srilanka	06.52' N 80.22' E
R. rouxii	HZM. 41. 28568	Monaragala, southern prov, Srilanka	06.52' N 80.22' E
R. rouxii	HZM. 33. 28560	Monaragala, southern prov, Srilanka	06.32' N 81.22' E
R. rouxii	HZM. 19. 27476	Pussahena Tunnel near Ruwanwella, Sabaragamuwa, Srilanka.	7.02' N 80.15' E
R. rouxii	HZM. 56. 31075	Pussahena Tunnel near Ruwanwella, Sabaragamuwa, Srilanka.	7.02' N 80.15' E
R. rouxii	HZM. 20. 27477	Pussahena Tunnel near Ruwanwella, Sabaragamuwa, Srilanka.	7.02' N 80.15' E
R. rouxii	HZM. 16. 27473	Pussahena Tunnel near Ruwanwella, Sabaragamuwa, Srilanka.	7.02' N 80.15' E
R. rouxii	HZM. 62. 31287	Pussahena Tunnel near Ruwanwella, Sabaragamuwa, Srilanka.	7.02' N 80.15' E
R. rouxii	HZM. 63. 31288	Pussahena Tunnel near Ruwanwella, Sabaragamuwa, Srilanka.	7.02' N 80.15' E
R. rouxii	HZM. 18. 27475	Pussahena Tunnel near Ruwanwella, Sabaragamuwa, Srilanka.	7.02' N 80.15' E
R. rouxii	HZM. 59. 31078	Pussahena Tunnel near Ruwanwella, Sabaragamuwa, Srilanka.	7.02' N 80.15' E
R. rouxii	HZM. 17. 27474	Pussahena Tunnel near Ruwanwella, Sabaragamuwa, Sri Lanka.	7.02' N 80.15' E
R. rouxii	HZM. 55. 30958	River Valley, near Hagala factory,Hagala, Sabaragamuwa, central province, Srilanka	Not located
R. rouxii	HZM. 64. 31289	River Valley, near Hagala factory,Hagala, Sabaragamuwa, central province, Srilanka	Not located
R. rouxii	HZM. 14. 27454	Wavulpane,7 miles south of Pallebedda, Sabaracumuwa, Srilanka	06.25' N 80.40' E
R. rouxii	HZM. 13. 27453	Wavulpane,7 miles south of Pallebedda, Sabaracumuwa, Srilanka	06.25' N 80.40' E
R. rouxii	HZM. 34. 28561	Wellawaya, southern prov, Srilanka	06.44' N 81.07' E

APPENDIC 3: Geographical and coordinations of *Rhinolophus sinicus*

Species	Collection No	Locality	Coordination
R. sinicus	99.3.1.6	Chintah, Anhwei. Lower Yangtse, China	
R. sinicus	HZM. 22. 28154	Nala Panai cave, 3 km south of Mussoorie, Up India	30. 26 N 78.04 E
R. sinicus	HZM. (BNHS)	Nala Panai cave, 3 km south of Mussoorie, Up India	30. 26 N 78.04 E
R. sinicus	HZM. (BNHS)	Nala Panai cave, 3 km south of Mussoorie, Up India	30. 26 N 78.04 E
R. sinicus	HZM. 21. 28153	Nala Panai cave, 3 km south of Mussoorie, Up India	30. 26 N 78.04 E
R. sinicus	HZM. 23. 28155	Nala Panai cave, 3 km south of Mussoorie, Up India	30. 26 N 78.04 E
R. sinicus	HZM. 4. 16294	Godavari, Nepal	
R. sinicus	HZM. 7. 16297	Godavari bridge, on Pulchowki Track, Nepal	
R. sinicus	HZM. 2. 16292	Mahendra Limbu, Nepal	
R. sinicus	HZM. 8. 16298	Godavari, Nepal	
R. sinicus	HZM. 1. 16291	Godavari, Nepal	
R. sinicus	HZM. 6. 16296	Pulchowki, Nepal	
R. sinicus	HZM. 5. 16295	Godavari, Nepal	
R. sinicus	HZM. 3. 16293	Pulchowki, Nepal	
R. sinicus	HZM. 9. 16299	Godavari, Nepal	
R. sinicus	HZM. 2. 36074	Holin village, Cave, Keng Taung, Myanmar	21° 27'483" N 99°32'648"E
R. sinicus	HZM. 1. 32326	Kon Ka Kinh Nature reserve, Gai Lai Prov. Vietnam	14.18"N 108.24"E

APPENDIC 4: Geographical and coordinations of *Rhinolophus thomasi*

Species	Collection No	Locality	Coordination
R. thomasi	HZM.10.35115	Naga cave, Taunggyi Township, Shane State, Myanmar	20.45'277"N 97.01'067"E
R. thomasi	HZM.11.35116	Naga cave, Taunggyi Township, Shane State, Myanmar	20.45'277"N 97.01'067"E
R. thomasi	HZM.9.35103	Myin-Ma-Hti cave, Kalaw Township, Shan State, Myanmar	20.34'312"N 96.36'177"E
R. thomasi	*4*	Myin-Ma-Hti cave, Kalaw Township, Shan State, Myanmar	20.34'312"N 96.36'177"E
R. thomasi	*5*	Indian Single Rock cave, Mon State, Myanmar	16.19'N 97.43'E
R. thomasi	HZM. 8. 32320	Khe Dap Tro, Phong Nha Nature reserve, Vietnam	17.35"N 106.19"E
R. thomasi	HZM. 4. 32324	Kanh Trail stream, Cuc Phoung National Park, Vietnam	20.18"N 105.38"E
R. thomasi	HZM. 6. 32322	Cave in Toong Ching karst area, Pu Mat Nature reserve, Vietnam	18.58"N 104.46'E
R. thomasi	HZM. 3. 32325	Khe Da Nui stream, Phong Nha-Ke Bang Prop. Park. Vietnam	17.45"N 106.51'E
R. thomasi	HZM. 1. 30623	Cuc Phoung National Park, Vietnam	
R. thomasi	HZM. 7. 32321	Khe Dap Tro, Phong Nha Nature reserve, Vietnam	17.35"N 106.19"E
R. thomasi	HZM. 5. 32323	Khe Dap Tro, Phong Nha Nature reserve, Vietnam	17.35"N 106.19"E
R. thomasi	HZM. 2. 30651	Cuc Phoung National Park, Vietnam	
R. thomasi	90.4.7.10	Ta Ho	
R. thomasi	90.4.7.9	Karin Hills, Myanmar	
R. thomasi	*6*	Ta Ho	
R. thomasi	*7*	Karin Hills, Myanmar	

VITAE

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List of Publication and Proceeding

Kingsada, P., Bumrungsri, S. and P. J. J. Bates. 2009. A taxonomic evaluation of four cryptic species of Rhinolophus (Chiroptera: Rhinolophidae) in Southern India and Southeast-Asia. Proceeding of the First International South-East Asian Bat Conference. Club Andaman Resort Beach Hotel, Patong, Phuket, Thailand, 7-10 May 2007.