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SYSTEMATICS AND BIOGEOGRAPHY OF SHREWS (SORICOMORPHA: SORICIDAE) IN PENINSULAR MALAYSIA

Field of Study : **WILDLIFE CONSERVATION**

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ABSTRACT

A total of 122 samples were examined in this study were trapped by using pitfall traps, except for *Suncus murinus* species, which was caught with a rat trap. The 14 skull characters of seven species measured from the past and present studies were subjected to principal component analysis. Although *Crocidura negligens* was never trapped in this study, earlier studies indicated that this species was distinct from other *Crocidura* species. Currently, *Crocidura monticola* are commonly found in Peninsular Malaysia and widely distributed in Sundaland. Previous studies indicated that *Crocidura fuliginosa* was dominant species in this region. Meanwhile, the smallest shrew *Suncus malayanus* formerly known as *Suncus etruscus* proved distinct morphologically from *C. monticola*. Finally, in this study I provide identification keys for shrew species, morphological measurements and skull drawings that may help in identification of Malay peninsula shrews for future studies.

Small crocidurinae shrews (weight < 8g) from Southeast Asia have been poorly studied to date, mainly because of the difficulty to catch them and the concomitant paucity of reference specimens available in museums. Hence their systematics is still debated, and most small *Crocidura* shrews from Sundaland are assigned to the *monticola* species complex. Morphometric analyses based on 14 skull measurements showed that these shrews tend to be larger with increasing altitude, but showed otherwise no consistent variation. When compared to museum specimens of the *monticola* species complex sampled in the Sundaland (total: 77 specimens), the Malay shrews tend also to be larger than those living on Kalimantan and Sumatra. All are, however, morphologically distinct from the other species, *C. maxi*, found in eastern Java and on the Lesser Sundas. Molecular analyses of a subset of these small shrews based

on a mitochondrial (cytochrome *b*) and a nuclear gene (Apolipoprotein B) suggest that samples from the central region of Peninsular Malaysia (Bukit Rengit and Ulu Gombak) differ genetically from other Malaysian populations (by about 7% K2P distance at the cyt *b* gene) and are more closely related to some samples from Sumatra and Borneo. These differences did not correlate with the altitudinal variation evidenced from the morphological analysis. Reference sequences from the terra typica of *monticola* and *maxi* (both species were originally described from Java) are however needed to determine if these unexpected genetic differences warrant additional taxonomic subdivision within the Sundaland.

A portion of the mitochondrial cytochrome *b* gene was used to investigate the phylogenetic relationships of *Suncus* pygmy shrews and to help clarify the taxonomic status of the Malayan Pygmy Shrew (*S. malayanus*) and the Malagasy Pygmy Shrew (*S. madagascariensis*). Phylogenetic reconstructions were performed using neighbour-joining and Bayesian analysis methods and revealed that *S. malayanus* is related to, but distinct from *S. etruscus*. The K2P-corrected genetic distance among the Malaysian taxon and other pygmy shrews for the cytochrome *b* gene was between 6.1 and 8.5%, supporting recognition that *S. malayanus* represents a distinct species from the geographically widespread *S. etruscus* species complex. A close (1.5% K2P distance) sister-group relationship was revealed between *S. etruscus* from Sri Lanka and *S. madagascariensis* from Madagascar, which has been considered an island endemic, and suggests that these animals are not specifically distinct. The Malagasy population of this shrew most probably was translocated to the island by human intervention, with the lineage originating from Southeast Asia or the Indian subcontinent.

ABSTRAK

Sejumlah 122 sampel yang diperiksa dalam kajian ini telah ditangkap menggunakan kaedah perangkap kecuali bagi spesis *Suncus murinus* ditangkap dengan perangkap tikus. 14 karakter tengkorak bagi tujuh spesis diukur daripada kajian dahulu dan terkini telah diserahkan kepada analisis komponen utama. Walaupun *Crocidura negligens* tidak pernah terperangkap dalam kajian ini, ianya disokong daripada data kajian terdahulu bahawa spesis ini berbeza daripada spesis *Crocidura* yang lain. Sekarang, spesis *C. monticola* biasa ditemui di Semenanjung Malaysia dan tersebar meluas di Sundaland. Kajian dahulu menunjukkan bahawa *Crocidura fuliginosa* adalah merupakan spesis dominan di rantau ini. Sementara itu, tikus terkecil *S. malayanus* yang dahulunya dikenali sebagai *S. etruscus* terbukti secara morfologi berbeza daripada *C. monticola.* Akhir sekali, kajian ini telah menyediakan kunci pengenalan spesis, ukuran morfologi dan lukisan-lukisan tengkorak yang boleh membantu mengenalpasti cencurut semenanjung Malaya untuk kajian di masa hadapan.

Cencurut-cencurut crocidurinae kecil (berat < 8g) dari Asia Tenggara tidak dikaji secara sempurna sehingga kini, terutamanya disebabkan oleh kesukaran untuk menangkap mereka dan kekurangan spesimen-spesimen rujukan yang terdapat di muzium. Oleh kerana sistematik mereka masih diperdebatkan, kebanyakan cencurut *Crocidura* kecil dari Sundaland adalah dikategorikan sebagai *monticola* spesis komplek. Analisis morfologi berdasarkan 14 pengukuran tengkorak menunjukkan bahawa cencurut ini cenderung untuk menjadi lebih besar kepada ketinggian altitud yang semakin meningkat, tetapi sebaliknya menunjukkan tiada perubahan yang konsisten. Apabila dibandingkan dengan spesimen *monticola* spesis komplek dari Sundaland (berjumlah: 77 spesimen), cencurut Malaya juga cenderung lebih besar daripada cencurut yang terdapat di Borneo dan Sumatra. Semuanya, walau bagaimanapun, secara

morfologi adalah berbeza daripada spesis lain, *C. maxi*, yang dijumpai di pulau Jawa Timur dan di Lesser Sundas. Cencurut kecil ini subset kepada analisis molecular berdasarkan kepada gen mitokondria (cytochrome *b*) dan gen nuklear (Apolipoprotein B) telah mencadangkan bahawa sampel dari kawasan tengah Semenanjung Malaysia (Bukit Rengit dan Ulu Gombak) genetiknya berbeza daripada populasi-populasi Malaysia yang lain (lebih kurang 7% jarak K2P pada gen cyt *b*) dan lebih rapat kepada beberapa sampel dari Sumatra dan Borneo. Jujukan urutan-urutan dari "terra typica" *monticola* dan *maxi* (kedua-dua specis pada asalnya adalah dari pulau Jawa), bagaimanapun perlu ditentukan jika perbezaan genetik yang tidak diduga ini adalah merupakan sebahagian taksonomi di antara Sundaland.

Separuh daripada gen mitokondria cytochrome *b* telah digunakan untuk mengkaji hubungan filogeni cencurut kecil *Suncus* dan untuk membantu menjelaskan status taksonomi Cencurut Kecil Malaya (*S. malayanus*) dan Cencurut Kecil Malagas (*S. madagascariensis*). Pembinaan semula filogenetik telah dilakukan menggunakan kaedah "neighbor-joining" dan kaedah analisis Bayesian, dan telah mendedahkan *S. malayanus* adalah berbeza daripada *S. etruscus*. Jarak genetic K2P antara takson Malaysia dan cencurut-cenrutut kecil lain untuk gen cytochrome *b* adalah antara 6.1 dan 8.5% yang mana menyokong bahawa *S. malayanus* mewakili spesis yang berbeza daripada *S. etruscus* spesis komplek yang boleh didapati secara meluas. Pendedahan hubungan "sister-group" yang rapat (1.5% jarak K2P) antara *S. etruscus* dari Sri Lanka dan *S. madagascariensis* dari Madagascar, yang dianggap pulau endemik, telah membuktikan bahawa secara khususnya, haiwan-haiwan ini tidak berbeza antara satu sama lain. Populasi cencurut Malagas kemungkinan telah dipindahkan ke pulau ini disebabkan oleh campur tangan manusia yang barasal dari Asia Tenggara atau benua kecil India.

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CHAPTER 1

INTRODUCTION

1.1 GENERAL INTRODUCTION

Shrews are small, mouse-like mammals, having long and pointed snouts, skin covered with short and dense fur such as brown or black colour, small eyes and ears, short legs and certain shrew have long and hairy tails. This animal is unique, shows some of the earliest mammalian features and retains a number of primitive characters such as the skulls are long and narrow, small brain, lacking the zygomatic arches on the lateral sides of skull, foot possesses five toes with a simple claw and the mandible possesses a double surface (Churchfield, 1990). The shrews characters remained unchanged since in the late Eocene/early Oligocene some 38 million years ago (Repenning, 1967; Yates, 1984), although it have undergone a slightly reduction in size. According to Repenning (1967), this animal is probably evolved soon after dinosaurs disappeared.

The Soricidae is one of the most diversified mammalian families, comprises 376 species in 26 genera and can be found on several major lands such as Africa, Europe, Asia, North and Central America (Churchfield, 1990; Hutterer, 2005). This family divided into three subfamilies. The Soricinae (red-toothed shrews) are distributed in Holarctic region. It contained 146 species within 13 genera. The Crocidurinae (white-toothed shrews) shrews are well-distributed throughout Africa and Eurasia is represented by 210 species within 9 genera. The Myosoricinae which are restricted in Africa contain only 18 species within 3 genera. The genus *Crocidura* and *Suncus* of the subfamily Crocidurinae contributed the highest number of species in Asia, Europe and

Africa, with a sum of 172 species in genus *Crocidura* and 18 species in genus *Suncus* (Hutterer, 2005).

The Southeast Asia region is one of the world biodiversity hotspot, has sparked curiosity among biogeographers and taxonomists (Wallace, 1860), as it covers 20% of the world's mammalian species (Corbet & Hill, 1992). The shrews have one of the most diversified species in Southeast Asia. It can be regarded as good models for terrestrial animal for biogeography and evolutionary study (Turner *et al*., 2001; Ohdachi *et al*., 2004; Motokawa *et al*., 2005). However, the high species diversity has arisen much complication and confusion in the taxonomy, particularly for genus *Crocidura* (Jenkins, 1982; Davison, 1984; Corbet & Hill, 1992; Kitchener *et al.*, 1994).

There are 43 species with more than 40 junior synonyms in the genus *Crocidura* from East and Southeast Asia (Hutterer, 2005; Motokawa *et al*., 2005). This increasing number of *Crocidura* species was the result of taxonomic re-evaluation that was based on morphological studies conducted in Sunda shelf and Sulawesi (Corbet & Hill, 1992; Ruedi, 1995), Philippine (Heaney & Ruedi, 1994), Japan (Motokawa *et al*., 1996 & 2003; Motokawa, 1998, 1999 & 2003; Takada *et al*., 2004), Taiwan (Fang *et al*., 1997; Motokawa *et al*., 1997; Fang & Lee, 2002; Motokawa *et al*., 2003 & 2004), China (Hoffmann, 1996; Lazell, 1998; Jiang & Hoffmann, 2001; Motokawa *et al*., 2001), Korea (Iwasa *et al*., 2001; Han *et al*., 2002; Motokawa *et al*., 2003), continental areas (Medway, 1978; Davison *et al*., 1982; Jenkins, 1982; Heaney & Timm, 1983; Davison, 1984; Ruedi *et al*., 1990; Jenkins & Smith, 1995; Motokawa & Harada, 1998; Smith *et al*., 1998; Robinson, 1999; Feiler & Zeigler, 1999; Lunde *et al.*, 2003 & 2004) and Southeast Asian Islands (Kitchener *et al*., 1994; Heaney *et al*., 1998; Meek, 2000).

Besides classical morphology, the number of species increased recently because of genetic markers was used as important tool to reconstruct phylogenetic relationships. The taxonomic confused of specific species in many species had been clarified using several genetic markers such as karyological analyses (Ruedi *et al*., 1990; Maddalena & Ruedi, 1994; Ruedi & Vogel, 1995; Motokawa *et al*., 1997, 2001 & 2004; Fang *et al*., 1997; Motokawa & Harada, 1998; Zima *et al*., 1998; Biltueva *et al*., 1999 & 2001; Fang & Lee, 2002) and allozyme or biochemical analyses (Maddalena, 1990); Ruedi *et al*., 1990 & 1993; Heaney & Ruedi, 1994; Ruedi, 1996), where allowed much progress on species level assignment.

More recently, the information in mitochondrial DNA and nuclear genes markers are useful to understand the higher taxonomic level within the Soricidae. Genetic markers of mitochondrial DNA sequences and band analyses were used in phylogeographical studies, as it is maternally inherited (Avise, 1989) which allowed a much finer resolution at lower taxonomic level such as intrageneric relationships. The mitochondrial cytochrome *b* gene as an effective marker for phylogeographical studies and has been used to examine many studies in mammalian patterns of evolution at intra- and interspecific levels. Besides that, the lower mutation rate of nuclear genes is expected to help obtaining a good resolution at a higher taxonomic level such as subfamilies, tribes and genera (Bannikova *et al*., 1996; Ohdachi *et al*., 1997, 2004 & 2006; George, 1998; Hausser *et al*., 1998; Ruedi *et al*., 1998, 2012; Fumagalli *et al*., 1999; Motokawa *et al*., 2000 & 2001; Iwasa *et al*., 2001; Quérouil *et al*., 2001; Han *et al*., 2002; Vogel *et al*., 2003; Dubey *et al*., 2006, 2007a, 2007b, 2007c & 2008; Esselstyn *et al*., 2009; Esselstyn & Oliveros, 2010; Omar *et al*., 2011 & 2013; Weyeneth *et al*., 2011; Meegaskumbura *et al*., 2012a & 2012b; Heaney *et al*., 2012).

1.2 PROBLEM S STATEMENT

In Peninsular Malaysia, seven species of shrews were listed in Hutterer (2005). The list was a compilation of finding by Chasen (1940), Harrison (1958) and Medway (1978) on new records, taxonomic studies at species level (Davison, 1979; Davison *et al*., 1982; Jenkins, 1982; Davison, 1984; Ruedi, 1995) and more recently in karyological relationships and molecular phylogeny (Ruedi *et al*., 1990, 1996 & 1998; Ruedi & Vogel, 1995; Ruedi, 1996; Omar *et al*., 2011; Omar *et al*., 2013).

At the beginning, Chasen (1940) and Harrison (1958) recorded all species of shrews in Southeast Asia based on finding from new localities but with little or no consideration towards examining further details on morphological characters. Therefore, the tendency for individual species to be misidentified was high. In 1982, Davison *et al*., examined specimens of one species that was classified as *S. etruscus*. The classification of these species was based from morphological of external and skull features. A re-examination of unicuspid teeth at upper tooth row enough provide valuable significant of findings to conclude that the specimens were made up of possibly different species. At the same time, the medium-sized shrews that are very similar in morphological features were still inconclusive and debated. This is especially on *C. fuliginosa* which is believed to be widely distributed in Southeast Asia (Corbet & Hill, 1992; Hutterer, 2003). However, karyological and genetic evidence (Ruedi *et al*., 1990) and extensively morphological analyses (Ruedi, 1995), pointed the taxonomic status to a three medium-sized.

The *Crocidura* species in Peninsular Malaysia is re-examined. Ruedi (1995) carried out extensive work on shrew's survey in Peninsular Malaysia, Indonesia and

Borneo. His work contributed to a major advancement in shrew's systematic though there are more studies that could be carried out. Shrews are very shy animals and difficult to trap. The long-worth traps and shrew drive techniques had not yielded good number of captures. A long trapping session had not guarantee of good captures. Despite of the interest to the recent systematics studies, there was no attempt to extend the research work.

An attempt to capture forest frogs using large pitfall traps, occasionally trapped shrews. A trial using 100 large pitfalls in one hectare in Gombak Forest had yielded a considerable numbers of shrews caught. With this success, the research on shrews was reinitiated. Manuel Ruedi and a well known internationally and locally recognized mammal scientist, Professor Zubaid Akbar was consulted. An objective to elucidate phylogenetic relationships of *C. monticola* and *S. malayanus* to compare with Southeast Asian, Eurasian and African shrews was attempted. As a result, the taxonomy of these two species which is critically and unresolved within subfamily Crocidurinae has yielded new insight. The status of current distribution the Crocidurinae in P. Malaysia was updated to also include of two large shrews, *S. murinus* and *Chimarrogale hantu*.

1.3 SIGNIFICANT OF THIS STUDY

The gap of information available for the distribution of shrews in Peninsular Malaysia after more than 30 years (from 1978 to 2013) was studied. This study is important to clarify the systematics and biogeography of Soricidae in Peninsular Malaysia. Since Ruedi's work from 1990 to 1998, no study on small shrew's systematics in Peninsular Malaysia had been conducted, thus, this extended study for *C. monticola* and *S. malayanus* provides further baseline information for similar studies in the future.

Among Peninsular Malaysia shrews, *C. monticola* and *S. malayanus* have been among the least known species and this study contributed valuable result in solving their taxonomic issues. This study not only contributed to the further clarification of the shrew diversity in Peninsular Malaysia as well as in wide species ranged localities including Madagascar, but also opens the future study about animal dispersal with human activities as interdisciplinary science. Therefore, this study contributed adequate scientific significance findings in zoology and other related fields.

1.4 RESEARCH OBJECTIVES

The objectives of this study were as follows.

I) To examine and update distribution pattern of shrews in Peninsular Malaysia based on past and present collections and to establish identification keys for shrews in Peninsular Malaysia based on various skull characteristics (Chapter 2).

II) To assess taxonomic status of *Crocidura monticola* (based on skull characteristics and DNA data) by comparing specimens obtained from Peninsular Malaysia with reference collections from the Sundaland and Indochina (Chapter 3).

III) To assess the taxonomic relationships between *Suncus malayanus* of Peninsular Malaysia and *Suncus madagascariensis* of Madagascar based on DNA data (Chapter 4).

1.5 THESIS OVERVIEW

Generally, this thesis consists of six chapters. Following the introduction, Chapter 2 presents the taxonomic revisions of shrews in Peninsular Malaysia and adjacent neighboring country, i.e. Thailand and Singapore. This chapter is useful to provide a preliminary data to the perspective on the occurrence of shrews in Peninsular Malaysia, particularly on *C. monticola* and *S. malayanus* that will be discussed in details on their taxonomic status in Chapter 3 and Chapter 4, respectively.

Chapter 3 explained and clarified morphological confusion of *C. monticola* using molecular phylogenetic. Although morphological identification of *C. monticola* in Peninsular Malaysia has been done by Davison *et al.* (1982), Jenkins (1982) and Davison (1984), however, shrews relationship based on molecular evidence are still unavailable. From above studies, I raised several questions as follow. 1) Does genetic divergence and phylogenetic relationships support morphological differences between *C. monticola* and *C. maxi* as stated by Kitchener *et al.*, (1994) and Ruedi (1995)? 2) Is *C. monticola* species widely distributed in Asia according to presumption by Ruedi (1995)? With respect to this, two additional questions are raised. 3) Is there any morphological variations among *C. monticola* in Peninsular Malaysia and does it associate with altitudinal variation? 4) Do the phylogenetic relationships of *C. monticola* distinct from any other small *Crocidura* species in Sundaland and Indochina regions?

The relationship among pygmy shrews (*Suncus* spp) is evaluated in Chapter 4. The genetic divergent between *S. malayanus* from Peninsular Malaysia and *S. madagascariensis* from Madagascar (and other Eurasian species) is revealed based on

mitochondrial DNA sequence data. Four synonyms of *S. etruscus* have sparked taxonomic issue due to geographical restriction (Hutterer, 2005). Hence, this study question whether Malayan pygmy shrew (*S. malayanus*) distinct from Malagasy pygmy shrew (*S. madagascariensis*) as mentioned in Hutterer (2005)?

Chapter 5 presents general discussion related to the question of geographical evolutionary process that affect morphological variation and genetic differences of *C. monticola* and *S. malayanus*. Finally, Chapter 6 concludes the study based on the results presented in the previous three main chapters (Chapter 2, 3 and 4). Related papers resulted from this study that has been published in international journals as listed below are as attached in Appendices D and E.

1. Omar, H., Adamson, E.A.S., Bhassu, S., Goodman, S.M., Soarimalala, V., Hashim, R. & Ruedi, M. (2011) Phylogenetic relationships of Malayan and Malagasy Pygmy Shrews of the genus *Suncus* (Soricomorpha: Soricidae) inferred from mitochondrial cytochrome *b* gene sequences. *The Raffles Bulletin of Zoology*, **59** (2), 237-243 (see Appendix D).

2. Omar, H., Hashim, R., Bhassu, S. & Ruedi, M. (2013). Morphological and genetic relationships of the *Crocidura monticola* species complex (Soricidae: Crocidurinae) in the Sundaland. *Mammalian Biology.* doi.org/10.1016/j.mambio.2013.04.004 (see Appendix E).

3. Omar, H., Hashim, R., Bhassu, S. & Ruedi, M. (in progress). Taxonomic revision of shrews (Soricidae) from Malay peninsula.

CHAPTER 2

TAXONOMIC REVISION OF SHREWS (SORICIDAE) FROM THE MALAY PENINSULA

2.1 INTRODUCTION

Historical review on shrews in Malay peninsula started from the work of H. C. Robinson and C. B. Kloss that named many species (see in Corbet & Hill, 1992) based on size and color variations which subsequently were all declared to be synonyms under the name of *Crocidura fuliginosa*. Chasen (1940) listed 31 *Crocidura* species from the Sunda regions with seven of them are from the mainland and islands of Malay peninsula. However, the list was commented by many authors (Laurie & Hill, 1954; Hill, 1960; Davis, 1962; Medway, 1965; Harrison, 1966; Medway, 1969; 1977; 1978), because Chasen did not include a taxonomic revision for the proliferation of nominal species and subspecies. In addition to the study on Crocidurinae shrews, Harrison (1958) listed a new water shrew *Chimarrogale hantu* from the subfamily Soricinae as the first recorded red-toothed shrew existing in Peninsular Malaysia. Furthermore, Medway (1978) recognized four species from Peninsular Malaysia known as *C. fuliginosa* (Southeast Asian White-toothed Shrews), *S. etruscus* (Savi's Pygmy Shrew), *S. murinus* (House Shrew) and *Chimarrogale himalayica* (Himalayan Water Shrew).

From the series, Davison (1979) worked on a partial review on several individuals of *S. etruscus* from Selangor, which Davison *et al.* (1982) and Jenkins (1982) realized later were actually *C. monticola* and not *S. etruscus*. Davison *et al.* (1982) separated the shrews into different genera based on the presence of the last unicuspid teeth in the upper toothrow, i.e. *S. etruscus* has four and *C. monticola* has

three. This categorization has been accepted by many researchers and considered as a valuable contribution. Next, Ruedi (1995) took responsibility for the taxonomic revision of Malay shrews in significant additional works using multivariate analyses and recognized three species of *Crocidura* shrews from Peninsular Malaysia. Subsequently, Hutterer (2005) put an effort in compiling earlier work on shrews and listed seven species from Peninsular Malaysia: *C. malayana*, *C. fuliginosa*, *C. negligens*, *C. monticola*, *S. malayanus* (formerly *S. etruscus*), *S. murinus* and *Chimarrogale hantu*.

Earlier taxonomists identified shrews based on classical measurements of external morphology (head and body length, hind foot length, tail length, ear length, weight, color of body) and skull (condylobasal length, upper toothrow, mandible width M2 - M2, and braincase breadth). Many of these works were uncertain because species are difficult to distinguish and museum specimens are limited. This had resulted in critical descriptions and increased number of synonyms from one individual in most of published literature. Ruedi and colleagues have applied various methods such as chromosomal study, genetic evidence and multivariate analyses to resolve the taxonomic issue concerning *Crocidura* shrews (Ruedi, 1995 & 1996; Ruedi & Vogel, 1995; Ruedi *et al*., 1996 & 1998). However, as of recently, there is no new data on *Suncus* and *Chimarrogale* species, and details distributional of shrews distribution in Peninsular Malaysia is still lacking.

During field surveys conducted between January 2008 and December 2010, many specimens of shrews in Peninsular Malaysia were trapped, thus assist in updating distribution data of shrews, allow review on their morphology and provide historic and current distributions as well as identification keys for shrews of Malay peninsula.

2.2 MATERIALS AND METHODS

2.2.1 Sampling method

Shrews were captured from 12 localities in Peninsular Malaysia with pitfall traps set up within a 45 m by 45 m grid per site at Lata Belatan (Terengganu), Maxwell Hill (Perak), Ulu Gombak (Selangor), Fraser Hill (Pahang), Pangkor Island (Perak), Ulu Kenas (Perak), Endau-Kluang (Johor), Kenaboi (Negeri Sembilan) and Cameron Highlands (Pahang), and with pitfall traps set up within a 20 m by 45 m grid per site at Wang Kelian (Kedah), Ulu Gombak (Selangor), Belum Royal Park (Perak) and Aur Island (Johor). Plastic buckets (50 to 100 buckets) with 25 cm height and 21 cm diameter were buried at 5 to 10 m intervals. Every bucket has 10 to 12 small holes (5 mm) at the bottom to allow water to drain out. Pitfalls were opened for few days (usually 3 to 5 days) without bait and were checked in the early morning and afternoon for the presence of shrews. The types of forests and shrews captured at each locality were recorded. A total of 122 specimens were measured and prepared for morphological study and euthanized using chloroform. Sex and external measurements were recorded immediately and specimens were then either prepared as flat skin or fixed in 5% formalin and preserved in 70% ethanol with cleaned skulls preserved separately. The preserved specimens are kept at the Zoological Department (Institute of Biological Sciences), University of Malaya.

In addition, 15 museum specimens (Raffles Museum of Singapore - ZRC) of *C. malayana* from Cheras Road (Kuala Lumpur), Port Dickson (Negeri Sembilan), Dusun Tua (Selangor), Bukit Timah and Nee Soon Swamp Forest (Singapore) and 2 specimens of *S. malayanus* from Triang (Negeri Sembilan) and Sungai Sedim (Kedah) were also

included in this study. Information regarding the reference number, locality, finding notes and external measurements of all specimens used from museum were also recorded.

2.2.2 Morphological analysis

For morphological comparison, five external and 14 skull characters were measured with a Vernier caliper to the nearest 0.01 mm and weight of freshly dead shrews (in gram) was taken using an electronic scale. External morphology of newly caught specimens such as head and body length (HB), hind foot length without claw (HF), tail length (TL), ear length (EL), and extent of bristle hairs along the tail (BH; measured from the tail base, in mm) were measured. Measurements of skull involving cranial and mandibular bones (see Figure 2.1) were conducted following Heaney & Timm (1983) and Ruedi (1995). These are greatest length of skull (GLS), upper tooth row length (IM3S), post palatal length (PPL), length of upper molariform teeth (PM3), rostral breadth (ROB), palatal width at the third molars (M3B), greatest width at the second molars (M2B), breadth of braincase (BB), interorbital breadth (IOB), rostral length (ROL), post palatal depth (PPD), condyle to glenoid length (CTG), lower tooth row length excluding first incisor (IM3I), and mandibular length (MAL).

The existence of sexual dimorphism was examined by ANOVA. As no significant sexual dimorphism was detected, further morphological analyses based on external body and skull measurements were done regardless of gender. The univariate analysis including sample size, mean and standard deviation, minimum and maximum range values were conducted. The principal component analysis (PCA) using correlated matrices without log-transformed data were done based on complete skull

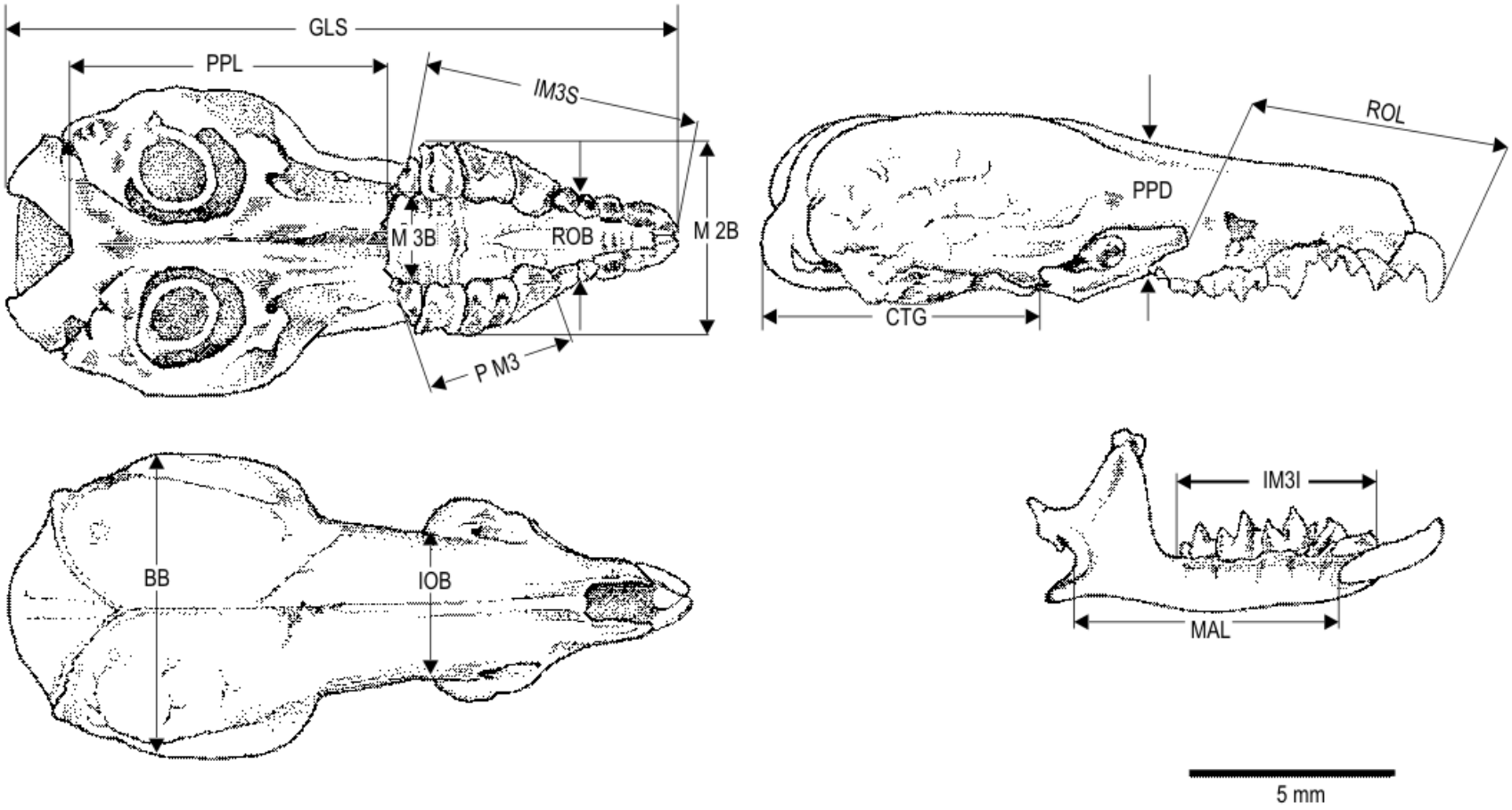


Figure 2.1 Skull of shrews illustrating 14 characters that were measured. See text (in materials and methods) for meaning of abbreviations. The scale bar represents 5 mm.

measurements of trapped shrews and museum specimens. All statistical analyses were performed using STATISTICA version 8.0 (StatSoft Inc., USA).

Besides Peninsular Malaysia shrews, holotypes and paratypes specimens that were preserved in British Museum of Natural History in London (BMNH), Rijksmuseum van Natuurlijke Historie in Leiden (RMNH), Institut de Zoologie et d'Ecologie Animale in Lausanne (IZEA), Museum Zoologicum Bogoriense in Bogor (MZB) and Raffles Museum in Singapore also were analysed. The skull measurements data of *C. malayana*, *C. fuliginosa* and *C. negligens* from Peninsular Malaysia that were examined by Ruedi (1995) in Peninsular Malaysia were also combined with this study. The combination data of skull measurements is useful in this study to see the occurrence of shrews in Peninsular Malaysia since Ruedi's work in 1990 until currently.

2.3 RESULTS AND DISCUSSION

2.3.1 Sample collections

Six species of shrews were trapped in 50 to 100 pitfall arrays. A total of 122 samples comprising of *Chimarrogale hantu* (3 samples), *S. murinus* (2 samples), *S. malayanus* (15 samples), *C. monticola* (53 samples), *C. malayana* (48 samples) and *C. fuliginosa* (1 sample) were obtained. *Chimarrogale hantu* can be easily identified based on external morphology (e.g. lack of visible ears, enlarged snout, dense and light fur), while *S. murinus* can be easily distinguished by its characteristic of large skull. The other two small shrews, *S. malayanus* and *C. monticola*, are very similar in morphological features and have been recognized by Davison *et al*. (1982) based on the number of the last unicuspid teeth in the upper tooth row. Meanwhile, the medium-sized shrews of genus

Crocidura (*C. malayana*, *C. negligens* and *C. fuliginosa*) are readily have been studied by Ruedi (1995 & 1996) which differentiated them using morphological, biochemical and molecular evidences.

2.3.2 Principal component analysis

A total of 111 of *Chimarrogale hantu*, *S. murinus*, *S. malayanus*, *C. monticola*, *C. fuliginosa*, *C. malayana* and *C. negligens* (see Figure 2.2) with complete skull characters were carried out using PCA. A total variance in first two axes of PCA accumulated 97% variation. The first principal component axis showed highly negative loadings with 95.8% of the total variance correlating to the general size of samples. Variations of skull width (ROB, M3B and IOB) are best explained by second axis demonstrating 1.2% of the total variance (see Table 2.1). Smaller shrews (groups 4 and 5) show complete separation from larger shrews (groups 6 and 7), while the intermediate size represented by *C. malayana* and C. *fuliginosa* (labelled as group 1 and group 2, respectively). Although *C. negligens* (group 3) was never trapped in this study, however analysis based on museum's specimens grouped it into medium-sized species of *C. malayana* and *C. fuliginosa*.

2.3.3 Taxonomic work

Two subfamily of Soricidae were discussed in taxonomic order consisting of Crocidurinae (*C. malayana*, *C. fuliginosa*, *C. negligens*, *C. monticola*, *S. malayanus*, and *S. murinus*) and Soricinae (*Chimarrogale hantu*). The 14 skull measurements of shrews were conducted based on univariate analysis using STATISTICA version 8.0 (StatSoft Inc., USA) with the results of sample size, mean and standard deviation,

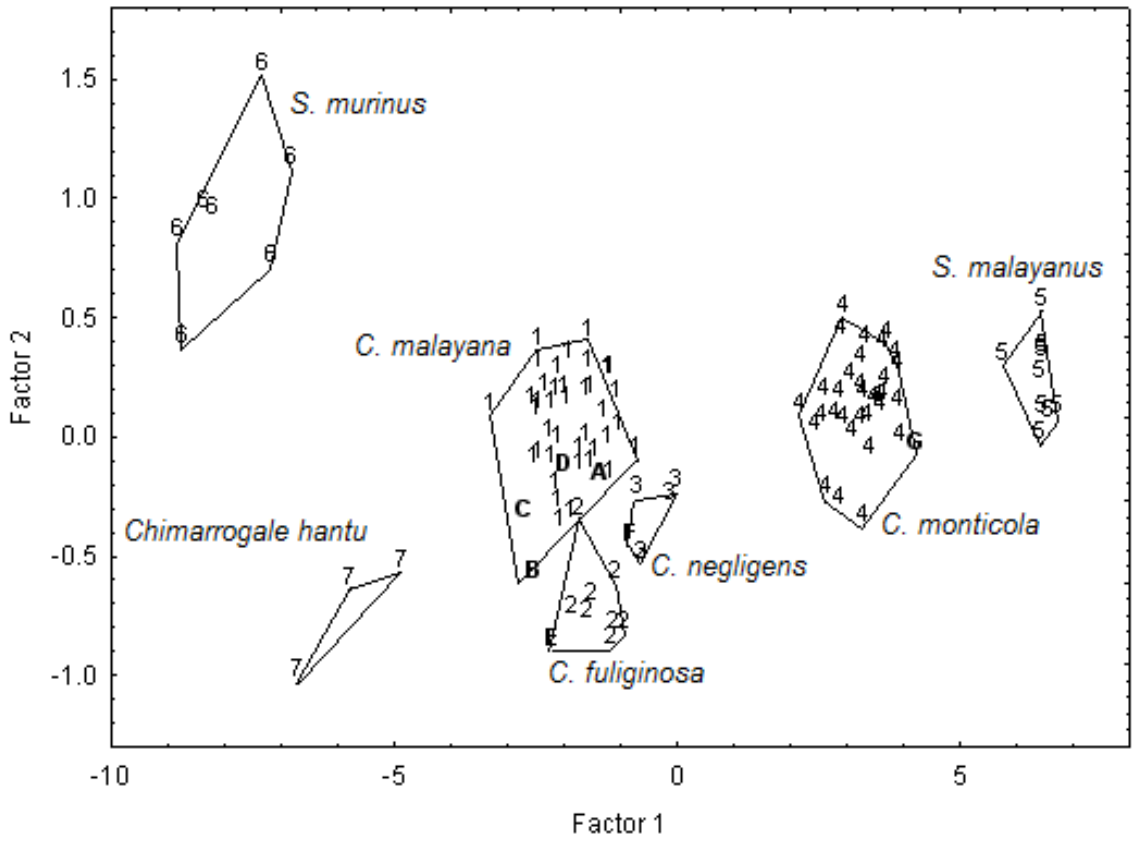


Figure 2.2 Plot of first and second principal component based on variations of 111 skull shrews from Peninsular Malaysia. Group 1 represents *C. malayana* and the holotypes of *malayana* (**A**), *klossi* (**B**), *aoris* (**C**) and *aagaardi* (**D**). Group 2 represents *C. fuliginosa* and the holotype of *gravida* (**E**). Group 3 represents *C. negligens* includes the holotype of *maporiensis* (**F**). All specimens in Group 1, 2 and 3 were combined from Ruedi's measurements (Ruedi, 1995) with current study. The small-sized *Crocidura* (*C. monticola*) represented in group 4 with the holotype of *monticola* (**G**) from Thailand, while the small-sized *Suncus* (*S. malayanus*) represented in group 5. Two large shrews of *S. murinus* and *Chimarrogale hantu* represents in group 6 and 7, respectively.

Table 2.1 Factor loadings of the variables used in the principal component analysis.

Variable	Factor 1	Factor 2
GLS	-0.996	0.015
IM3S	-0.994	-0.004
PPL	-0.979	0.041
PM3	-0.985	-0.060
ROB	-0.937	0.319
M3B	-0.961	-0.140
M2B	-0.989	0.074
BB	-0.980	-0.081
IOB	-0.974	-0.133
ROL	-0.991	-0.034
PPD	-0.969	0.079
CTG	-0.974	-0.088
IM3I	-0.989	0.005
MAL	-0.986	0.018
Eigenvalue	13.42	0.17
% Total variance	95.8	1.2

minimum and maximum values as given in Table 2.2. The taxonomic conclusions for each species took into account their scientific name and its synonyms, locality, specimens examined from present study and previous records, external measurements, morphological observation, distributions and comments. Finally, sketches of the skull, distribution maps, and identification keys of each species were given as following.

Table 2.2 Skull measurements on seven species of shrews from Peninsular Malaysia with sample size (n). Values of mean ± standard deviation (and range) are given for each taxon. All measurements are in millimeters.

Variable	*Crocidura malayana* (n = 38)	*Crocidura fuliginosa* (n = 10)	*Crocidura negligens* (n = 5)	*Crocidura monticola* (n = 34)	*Suncus malayanus* (n = 9)	*Suncus murinus* (n = 7)	*Chimarrogale hantu* (n = 3)
Greatest length of skull (GLS)	23.1 ± 0.6 (22.0 – 24.2)	22.7 ± 0.8 (21.5 – 24.0)	21.4 ± 0.4 (20.9 – 22.0)	17.0 ± 0.5 (16.1 – 18.1)	14. 0 ± 0.3 (13.5 – 14.7)	30.6 ± 1.4 (28.6 – 32.3)	26.8 ± 0.9 (26.0 – 27.7)
Length of upper toothrow (IM3S)	10.4 ± 0.3 (9.8 – 11.0)	10.0 ± 0.5 (9.3 – 10.9)	9.5 ± 0.2 (9.3 – 9.7)	7.3 ± 0.2 (6.8 – 7.8)	5.9 ± 0.1 (5.7 – 6.1)	13.5 ± 0.5 (12.7 – 13.9)	12.2 ± 0.2 (12.0 – 12.5)
Post-palatal length (PPL)	10.0 ± 0.3 (9.5 – 10.9)	10.1 ± 0.3 (9.5 – 10.5)	9.4 ± 0.2 (9.1 – 9.7)	7.8 ± 0.4 (6.9 – 8.7)	6.7 ± 0.3 (6.4 – 7.2)	13.3 ± 0.8 (12.1 – 14.2)	11.3 ± 0.4 (11.0 – 11.7)
Length of upper molariform (PM3)	5.9 ± 0.2 (5.5 – 6.2)	5.6 ± 0.2 (5.2 – 6.0)	5.4 ± 0.1 (5.2 – 5.5)	4.1 ± 0.1 (3.9 – 4.4)	3.3 ± 0.1 (3.1 – 3.6)	7.1 ± 0.2 (6.8 – 7.4)	7.1 ± 0.2 (6.9 – 7.3)
Rostral breadth (ROB)	3.2 ± 0.2 (2.9 – 3.6)	2.6 ± 0.1 (2.4 – 2.9)	2.7 ± 0.1 (2.6 – 2.8)	2.4 ± 0.1 (2.1 – 2.7)	1.9 ± 0.1 (1.8 – 2.1)	4.5 ± 0.1 (4.3 – 4.6)	3.6 ± 0.2 (3.5 – 3.8)
Breadth at third molars (M3B)	2.7 ± 0.2 (2.3 – 3.2)	2.7 ± 0.1 (2.5 – 2.8)	2.5 ± 0.2 (2.3 – 2.7)	2.0 ± 0.1 (1.8 – 2.3)	1.4 ± 0.1 (1.3 – 1.5)	3.3 ± 0.2 (3.0 – 3.6)	3.5 ± 0.3 (3.2 – 3.7)
Breadth at second molars (M2B)	6.9 ± 0.2 (6.6 – 7.4)	6.6 ± 0.1 (6.4 – 6.7)	6.4 ± 0.2 (6.2 – 6.7)	5.2 ± 0.2 (4.8 – 5.5)	4.0 ± 0.1 (3.8 – 4.1)	9.3 ± 0.3 (8.7 – 9.7)	8.0 ± 0.4 (7.6 – 8.3)
Braincase breadth (BB)	10.1 ± 0.3 (9.4 – 10.7)	9.8 ± 0.2 (9.6 – 10.0)	9.7 ± 0.2 (9.4 – 10.0)	7.8 ± 0.3 (7.1 – 8.3)	6.3 ± 0.2 (6.0 – 6.5)	12.6 ± 0.6 (11.9 – 13.4)	13.2 ± 1.0 (12.3 – 14.3)

Table 2.2 (Continued)

Variable	*Crocidura. malayana* (n = 38)	*Crocidura fuliginosa* (n = 10)	*Crocidura negligens* (n = 5)	*Crocidura monticola* (n = 34)	*Suncus malayanus* (n = 9)	*Suncus murinus* (n = 7)	*Chimarrogale hantu* (n = 3)
Interorbital breadth (IOB)	4.8 ± 0.2 (4.4 – 5.1)	4.7 ± 0.2 (4.5 – 4.9)	4.5 ± 0.1 (4.3 – 4.7)	3.8 ± 0.2 (3.5 – 4.2)	3.1 ± 0.1 (3.0 – 3.2)	5.5 ± 0.2 (5.3 – 5.8)	5.6 ± 0.1 (5.5 – 5.7)
Rostral length (ROL)	9.5 ± 0.3 (8.7 – 10.2)	9.3 ± 0.4 (8.4 – 10.1)	8.6 ± 0.2 (8.3 – 8.9)	6.6 ± 0.3 (6.0 – 7.2)	5.4 ± 0.1 (5.3 – 5.6)	12.1 ± 0.6 (11.4 – 12.8)	11.3 ± 0.1 (11.2 – 11.4)
Post-palatal depth (PPD)	4.2 ± 0.1 (3.9 – 4.5)	4.1 ± 0.1 (3.9 – 4.2)	4.0 ± 0.1 (3.8 – 4.1)	3.3 ± 0.2 (3.0 – 3.6)	2.4 ± 0.1 (2.2 – 2.5)	5.8 ± 0.3 (5.4 – 6.3)	5.2 ± 0.2 (5.1 – 5.4)
Condyle to glenoid length (CTG)	8.7 ± 0.3 (8.1 – 9.3)	8.8 ± 0.3 (8.4 – 9.1)	8.3 ± 0.1 (8.2 – 8.5)	6.9 ± 0.3 (6.1 – 7.4)	5.9 ± 0.2 (5.6 – 6.2)	10.8 ± 0.9 (9.4 – 11.9)	10.2 ± 1.2 (9.5 – 11.6)
Lower toothrow length excluding first incisor (IM3I)	7.1 ± 0.2 (6.7 – 7.4)	6.9 ± 0.3 (6.5 – 7.4)	6.5 ± 0.1 (6.3 – 6.5)	5.0 ± 0.2 (4.6 – 5.4)	4.0 ± 0.1 (3.8 – 4.2)	9.1 ± 0.3 (8.7 – 9.6)	7.7 ± 0.5 (7.2 – 8.2)
Mandibular length (MAL)	9.3 ± 0.4 (8.4 – 10.3)	9.3 ± 0.4 (8.8 – 10.1)	8.6 ± 0.2 (8.3 – 8.9)	6.9 ± 0.4 (6.2 – 7.7)	5.5 ± 0.2 (5.3 – 5.9)	12.3 ± 0.4 (11.8 – 12.9)	10.4 ± 0.5 (10.0 – 11.0)

Crocidura malayana Robinson & Kloss, 1911 (Figure 2.3)

(Malayan Shrew)

Synonyms and type localities

Crocidura malayana Robinson & Kloss, 1911 – Journal Fed. Malay States Mus., 4: 243 (Maxwell Hill, Peninsular Malaysia).

Crocidura major Kloss, 1911 – Annals Mag. Nat. Hist., 7: 117 (Great Redang Island, Peninsular Malaysia)

Crocidura aoris Robinson, 1912 – Annals Mag. Nat. Hist., 10: 589 (Aur Island, Peninsular Malaysia).

Crocidura aagardi Kloss, 1917 – Journal Nat. Hist. Soc. Siam, 2: 283 (Bang Nara Patani, Peninsular Thailand).

Specimens examined

In this study: Lata Belatan, Terengganu - M1555 (1 male), M1554, 1562, LB0852 (3 female); Ulu Gombak, Selangor - M1547-49, PP0811, 0860-61 (6 male), M1550, PP0805-06, 0812-13, 0947-50, 0957, 0859, 0869 (12 female), PP0714-18, 0820-27, 0829, 0808, 0827, 0862, 0871 , 0858 (19 unknown); Aur Island, Johor - Ao0804, 0807 (2 male); Pangkor Island, Perak - Pa0933, 0935 (2 male), Pa0930, 0951 (2 female). Bukit Rengit, Pahang - BR10104 (1 male). Cheras Road, Kuala Lumpur - ZRC.4.5071 (1 male), ZRC.4.5070, 5073-74 (3 female), ZRC.4.5076-77 (2 unknown); Port Dickson, Negeri Sembilan - ZRC.4.5081 (1 female), ZRC.4.5079 (2 unknown); Dusun Tua, Selangor - ZRC.4.5078 (1 male); Bukit Timah Nature Reserve & Nee Soon Swamp Forest, Singapore - ZRC.4.7910-12, ZRC.4.7914-15 (5 unknown).

Previous study: Holotype of *malayana* from Maxwell Hill, Perak - BMNH 49.427 (1 female). Holotype of *klossi* from Great Redang Island, Terengganu - BMNH 47.1422

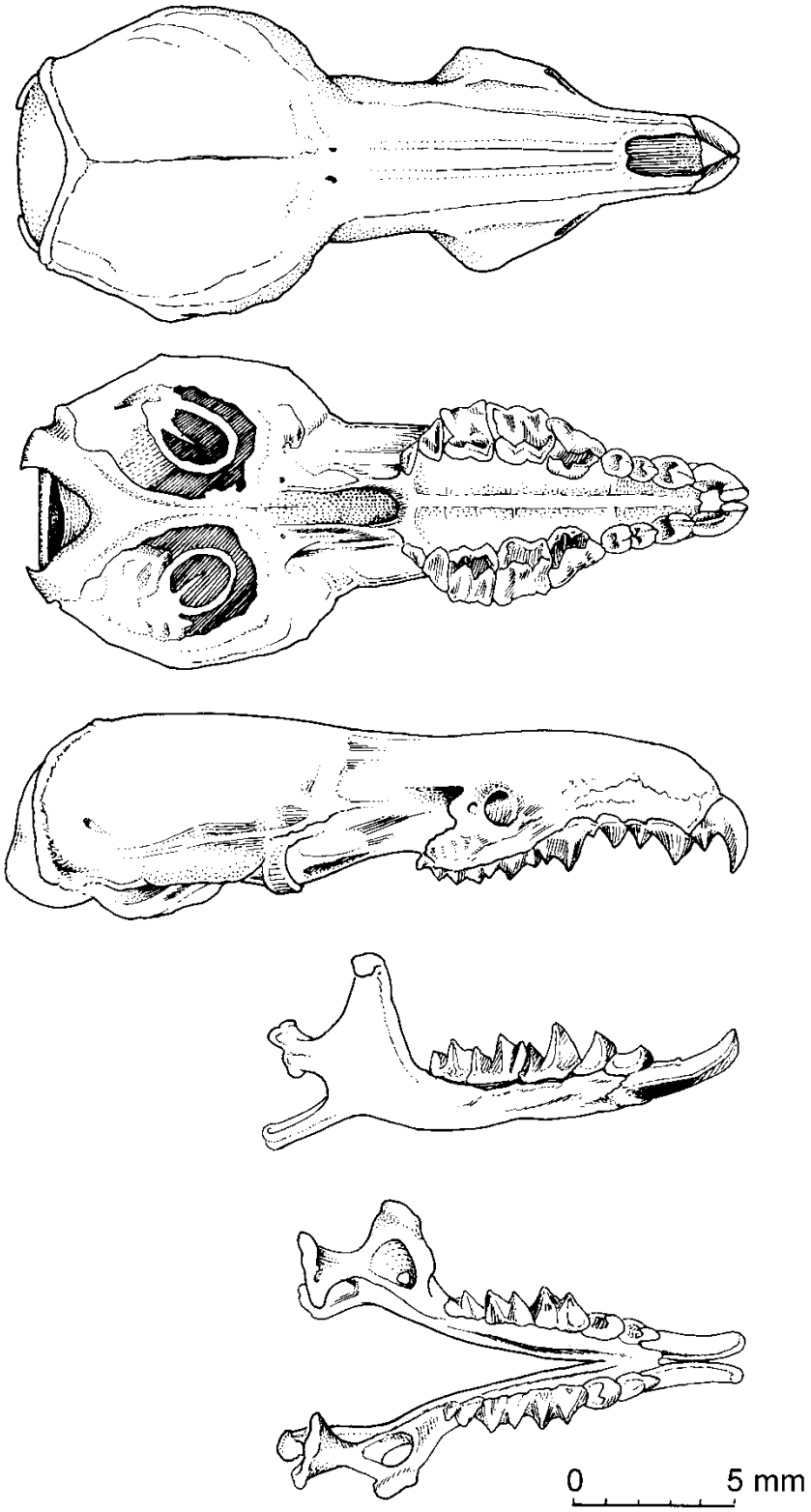


Figure 2.3 Illustration of dorsal, ventral and lateral view of cranium and mandible of female *Crocidura malayana* from Pangkor Island, Perak (Pa0951).

(1 male). Paratype of *klossii* from Tioman Island, Pahang - ZRC.4.2408 (1 female); Great Redang Island, Terengganu - ZRC.4.2083 (1 male), ZRC.4.2084 (1 female). Holotype of *aoris* from Aur Island, Johor - BMNH 47.1423 (1 female); Fraser Hill, Pahang - IZEA 3611 (1 male); Ulu Gombak, Selangor - IZEA 3611, 3977, 3992 (3 male), IZEA 3620, 3991 (2 female). Holotype of *aagaardi* from Patani, Peninsular Thailand - BMNH 47.1420 (1 female) (Figure 2.4).

External measurements of this study

HB: 70-99, T: 50-66, HF: 13.2-15.0, EL: 7.0-11.8, BH: 10-37, Weight: 9.7-14.3g.

Morphological observation

Crocidura malayana is very similar in external dimensions to *C. fuliginosa*, except the hind foot size of *C. malayana* is generally smaller. However, on the basis of skull dimensions, *C. malayana* has a larger broader rostrum (ROB) and palate (M2B) than *C. fuliginosa* with sizes more than 2.9 mm and 6.6 mm, respectively (see Table 2.2). Based on morphological features, both species have dark brown to black fur, which usually leads to misidentification at sampling sites.

Distribution

Crocidura malayana is a common species found in a variety of forest habitats at approximately 50 to 1500 m above sea level (a.s.l) and several islands (Pangkor, Aur, etc.) of Peninsular Malaysia. It is sympatric with *C. monticola*, *S. malayanus* and *Chimarrogale hantu* at Ulu Gombak, Selangor, and also lives sympatric with *S. malayanus* at Ulu Kenas, Perak and Lata Belatan, Terengganu.

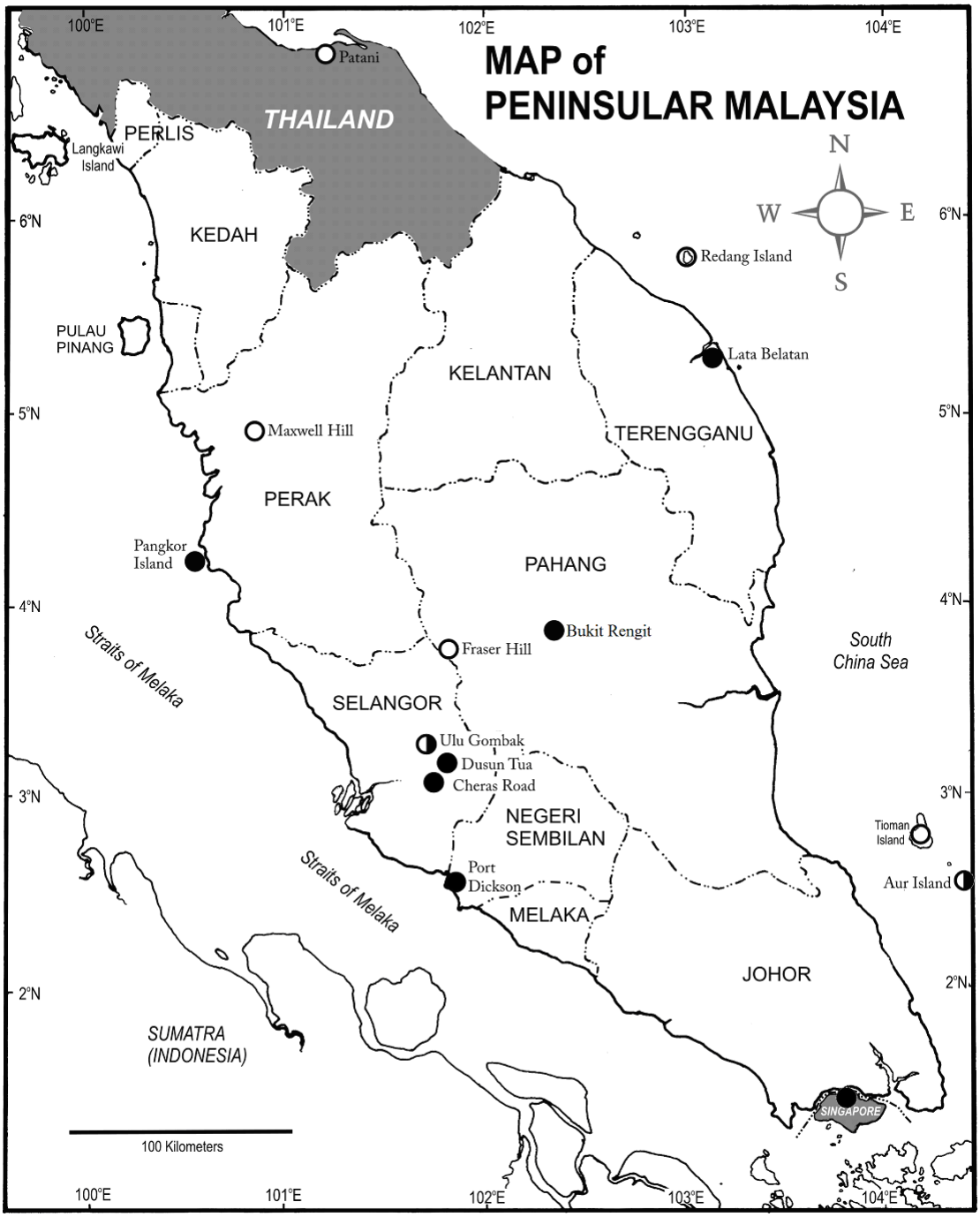


Figure 2.4 Map of *Crocidura malayana* survey in thirteen localities indicated with ● current record, ○ historical record and ◑ current and historical record.

Comments

This species was included under the name *C. fuliginosa* by previous authors (such as Jenkins, 1982; Corbet & Hill, 1992) as synonyms or subspecies. Ruedi *et al.* (1990) study on Peninsular Malaysia shrews and have been separated *C. fuliginosa* species complex into two subspecies using karyotypes evidence, which represent *C.* cf. *malayana* (2n = 38, 39 or 40) and *C.* cf. *fuliginosa* (2n = 40). In addition, Ruedi (1995) have proved that *C. malayana* has rounded palatine between pterygoid and rectangular processes in *C. fuliginosa*. This wild shrew is endemic in Peninsular Malaysia and southern Thailand (Corbet & Hill, 1992) and is listed as a concern species (Lunde, 2008a).

Crocidura fuliginosa (Blyth, 1856) (Figure 2.5)

(Southeast Asian Shrew)

Synonyms and type localities

Sorex fuliginosa Blyth, 1856 – Journal Asiat. Soc. Bengal, 24:362 (Schwegyin, Burma).

Crocidura dracula Thomas, 1912 – Annals Mag. Nat. Hist., 9: 686 (Mongtze/Mengtsz, South Yunnan, China).

Crocidura gravida Kloss, 1917 – Journal Fed. Malay State Mus., 7: 127 (Dayang Bunting Island, Peninsular Malaysia).

Specimen examined

In this study: Cameron Highlands, Pahang - CH0973 (1 female).

Previous study: Holotype of *gravida* from Dayang Bunting Island, Langkawi, Kedah - BMNH 47.1419 (1 male). Tanah Rata, Cameron Highlands, Pahang - IZEA 3553-55, 3753, 3542 (5 male), IZEA 3610, 3747, 3752 (3 female) (Figure 2.6).

External measurements of this study

HB: 80, T: 70, HF: 15.1, EL: 9.5, BH: 35, Weight: 10g.

Morphological observation

Although confused with *C. f. dracula* from Indochina regions, *C. fuliginosa* from Peninsular Malaysia has smaller external and skull dimensions as given by Heaney & Timm (1983) (also see Table 1 in Ruedi, 1995). In this study, one sample of *C. fuliginosa* was caught from Cameron Highlands. It is obviously more blackish in pelage color compared to the wild shrew of *C. malayana*.

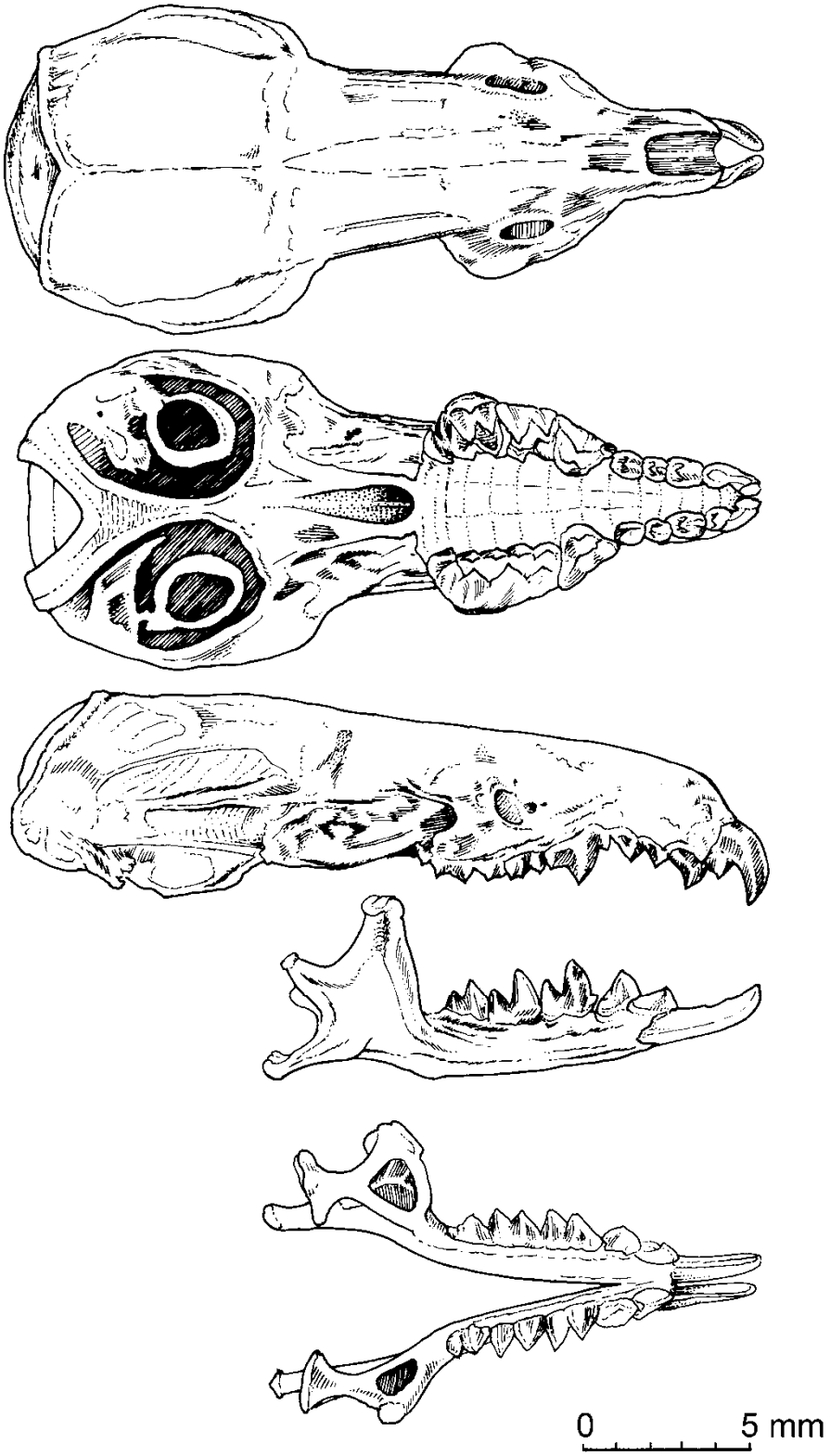


Figure 2.5 Illustration of dorsal, ventral and lateral view of cranium and mandible of female *Crocidura fuliginosa* from Cameron Highlands, Pahang (CH0973).

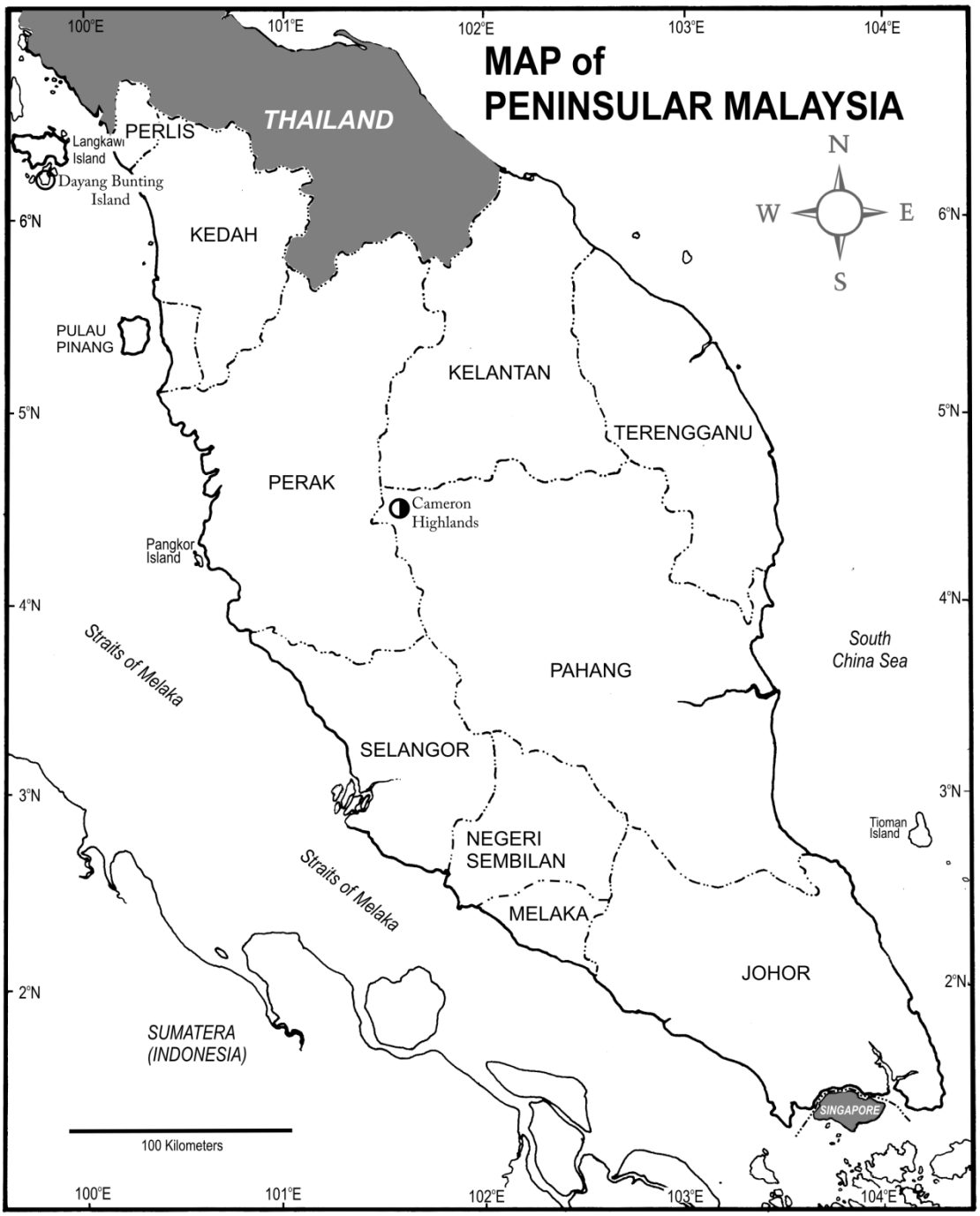


Figure 2.6 Map of *Crocidura fuliginosa* survey in two localities indicated with ○ historical record and ◑ current and historical record.

Distribution

This species is commonly found at Cameron Highlands (Pahang). This place is located at the backbone of Peninsular Malaysia surrounded by montane forest which is approximately 1500 m. Ruedi *et al.* (1990) had captured several individuals of *C. fuliginosa* in the area. Hence, it seems that *C. fuliginosa* is restricted to the highland forest and does not exist in other locality, including adjacent islands.

Comments

Originally, *C. fuliginosa* consists of 31 recognized taxa (see Chasen, 1940) and it is widely distributed in Southeast Asia, including Burma, China, Vietnam, Thailand, Peninsular Malaysia, Borneo, Sumatra and Java. However, Jenkins (1976) and Medway (1977) have successfully narrowed down a large number of the species complex into a single species which was named *C. fuliginosa* (based on separation of external and skull characters). According to Jenkins (1982), a complex taxonomic history of the *C. fuliginosa* is caused by direct comparison within geographical range that is relevant for only one region, but irrelevant for others. They were endemic to specific regions and slightly differ in several characteristics which were affected by environment pressure. Jenkins (1982) had discussed various geographical restricted species of *fuliginosa* group, i.e. from China and Vietnam - *C. f. dracula* (Thomas, 1912); from Peninsular Malaysia - *C. f. malayana* (Robinson & Kloss, 1911); from Borneo - *C. f. foetida* (Peter, 1870), *C. f. baluensis* (Thomas, 1898), *C. f. kelabit* (Medway, 1965); from Sumatra - *C. f. villosa* (Robinson & Kloss, 1918) and lastly, six forms were described from Java - *C. f. brunnea*, *orientalis*, *brevicauda*, *melanorhyncha*, *lawuana*, *pudjonica*. Subsequently, *C. fuliginosa* has also been confused externally with other medium-large size *C. attenuata* from Thailand. Heaney & Timm (1983) reviewed *C. attenuata* and discovered that they are differs from *C. fuliginosa* by shorter tail length and head-body length. Moreover, *C.*

attenuata were formerly known to exist only on Gunung Tahan in Peninsular Malaysia, but it was revised as incorrect labels by Davison (1984). As discussed in Ruedi & Vogel (1995), *C. fuliginosa* are distinct from any other shrews with chromosome variation 2n = 40 and FN = 54, 56, 58. *Crocidura fuliginosa* is listed as of least concern species because of limited information on their populations (Molur, 2008).

Crocidura negligens Robinson & Kloss, 1914 (Figure 2.7)

(Peninsula Shrew)

Synonyms and type localities

Crocidura negligens Robinson & Kloss, 1914 – Annals Mag. Nat. Hist. 13: 223 (Samui Island, Peninsular Thailand).

Crocidura maporiensis Robinson & Kloss *in* Robinson, 1916 – Journal Fed. Malay States Mus. 7: 73 (Mapor Island, Singapore).

Crocidura tionis Kloss, 1917 – Journal Fed. Malay States Mus., 7: 127 (Tioman Island, Peninsular Malaysia).

Specimen examined

Previous study: Holotype of *C. negligens* from Koh Samui, Thailand - BMNH 47.1425 (1 male). Holotype of *C. maporiensis* from Mapor Island, Riau, Singapore - BMNH 47.1421 (1 unknown). Tioman Island, Pahang - IZEA 3557 (1 male), 3560, 3563, 3747 (3 female); Gunung Tahan, Pahang - BMNH 6.10.4.14 (1 female) (Figure 2.8).

External measurements of historical study

Four specimens of the Peninsular Malaysia and one holotype of *C. negligens* from Thailand were taken from Ruedi (1995) (in mm): HB: 75-82, T: 55-69, HF: 14.0-15.8, EL: 9.5, BH: 20-43, Weight: 9.5-12.5g.

Morphological observation

According to Ruedi (1995), *C. negligens* is more similar to *C. attenuata* from Indochina as measured by Heaney & Timm (1983). However, *C. negligens* has shorter tail, upper toothrow and rostrum.

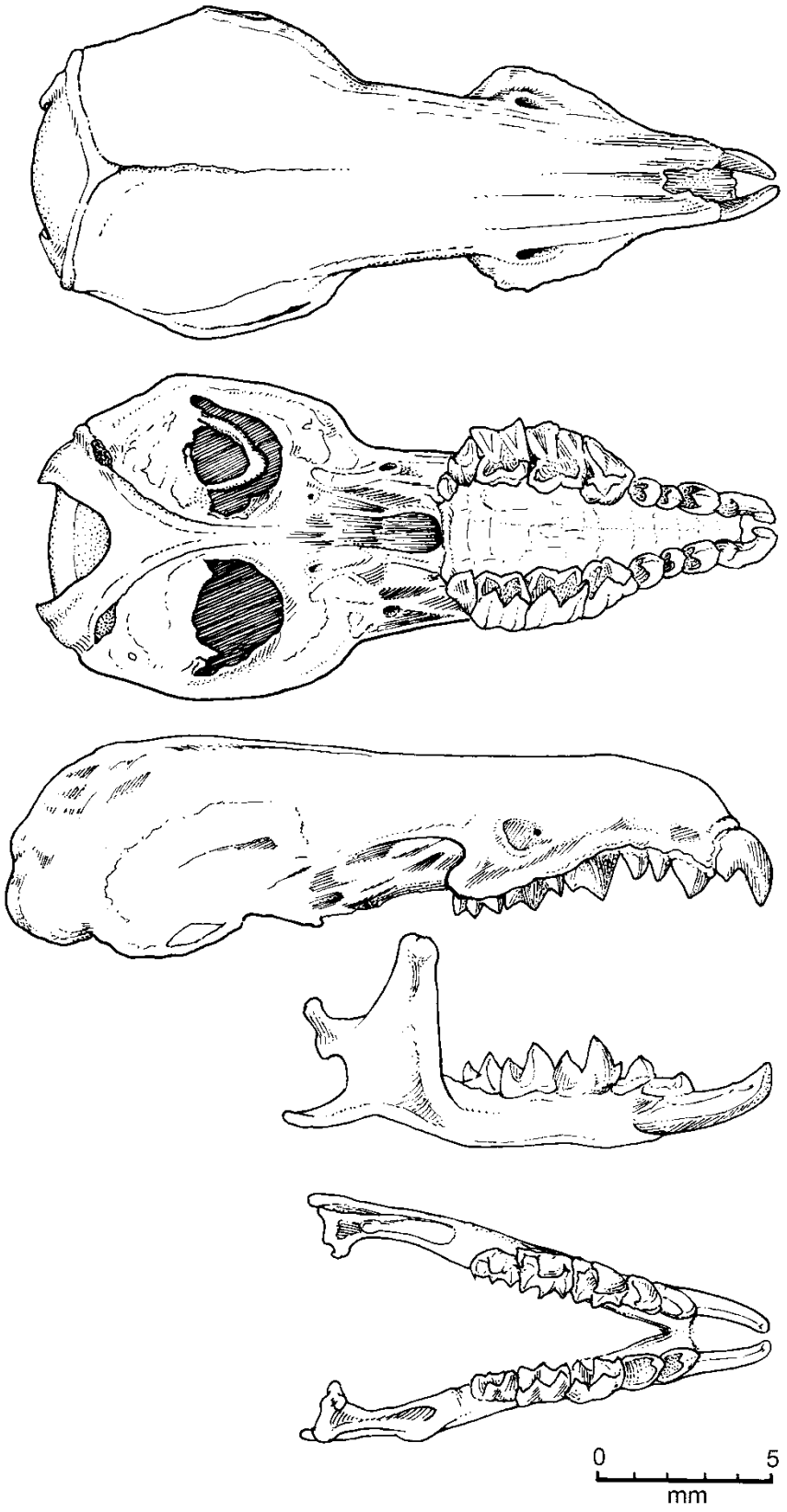


Figure 2.7 Illustration of dorsal, ventral and lateral view of cranium and mandible of female *Crocidura negligens* from Tioman Island, Pahang. The skull loan from the Institut de Zoologie et d'Ecologie Animale in Lausanne, Switzerland (IZEA 3565).

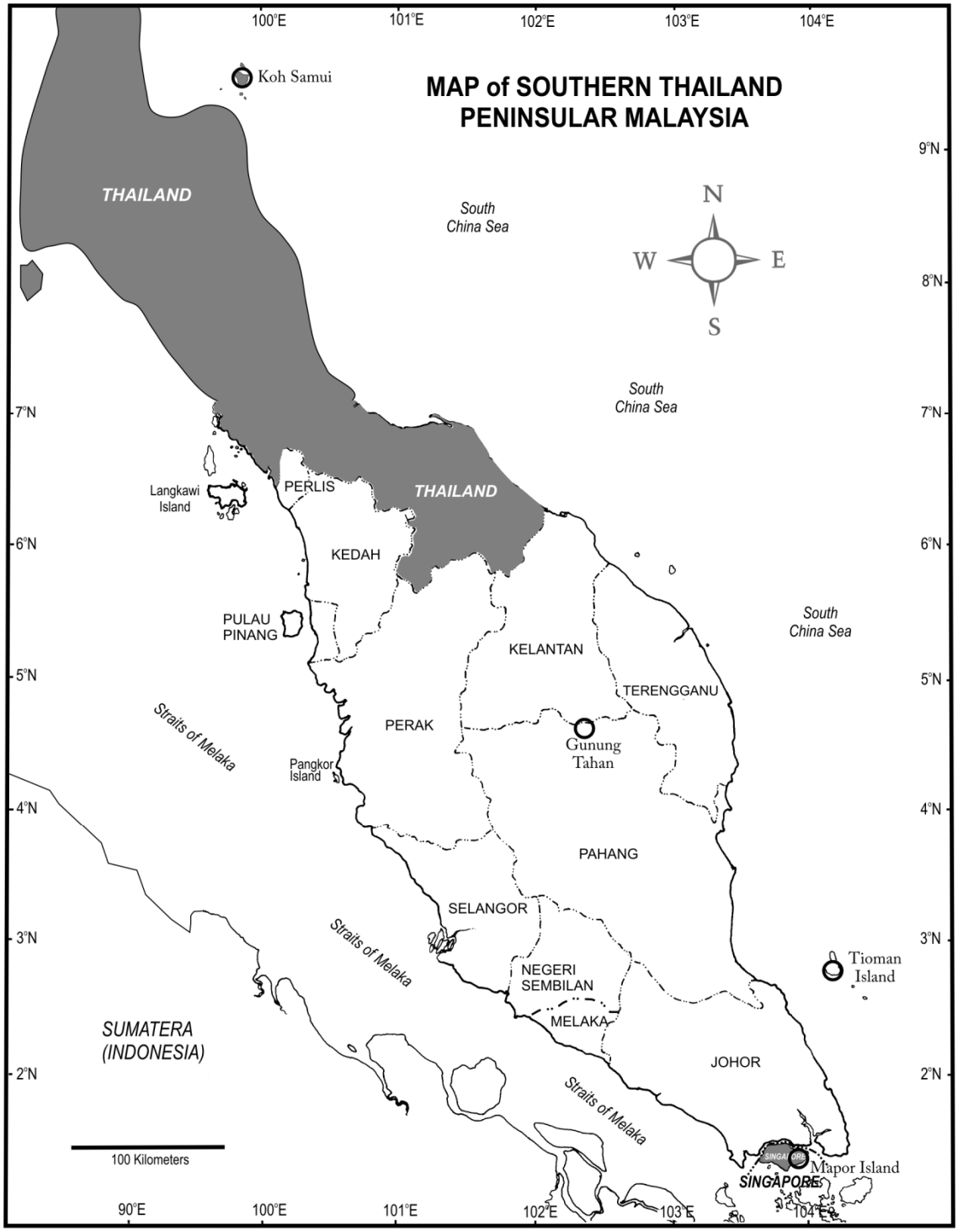


Figure 2.8 Map of *Crocidura negligens* survey in three localities from Peninsular Malaysia, Koh Samui (Thailand) and Mapor Island (Singapore) with O indicate historical record.

Distribution

Documented exist in the Malay Peninsula and adjacent islands - Koh Samui, Tioman and Mapor (Ruedi, 1995; Hutterer, 2005; Francis, 2008). Ruedi (1995) also believed that *C. negligens* and *C. malayana* are possibly sympatric in Maxwell Hill (Perak) and Genting Highland (Pahang).

Comments

Medway (1978) included this species in *C. fuliginosa*, but Ruedi & Vogel (1995) treated them as two cryptic species based on karyotype formula of *C. negligens* 2n = 38 and FN = 62. Meanwhile, Ruedi (1995) analysed three medium-sized shrews from Peninsular Malaysia (*C. malayana*, *C. fuliginosa* and *C. negligens*) using multivariate analyses and proved that *C. negligens* are morphologically distinct and does not overlap with *C. malayana*, but overlapping happens more extensively between *C. malayana* and *C. fuliginosa*.

Crocidura monticola Peter, 1870 (Figure 2.9)

(Sunda Shrew)

Synonym and type locality

Crocidura monticola Peter, 1870 – Monatsberichte K. Preuss. Acad. Wiss., 1870: 584 (Mount Lawu, near Surakarta, Java).

Specimen examined

In this study: Wang Kelian, Perlis - M1530-31, WK1075-77 (5 male), M1537, WK1074 (2 female); Ulu Gombak, Selangor - M1546, PP1080, PP0963-64, 0870, 1079 (6 female), PP0815-16, 0824, 0829, 0940 (5 unknown); Maxwell Hill, Perak - MH1096, 10101 (2 male), MH1097-100, 10102 (5 female), MH0801, 0802, 0810 (3 unknown); Fraser Hill, Pahang - FH1082-83 (2 male), FH0941, 0809, 0938-39 (4 unknown); Cameron Highlands - CH1088, 1091-93, 1094-95 (6 male), CH0972, 1089 (2 female); Bukit Rengit, Pahang - BR10105, 10107-108, 10110-111 (5 male), BR10103, 10109 (2 female); Endau-Kluang, Johor - EnK0834, 0837, 0842 (3 unknown).

Previous study: Ulu Gombak, Selangor - (1 female) and (1 unknown); Khow Ram, Peninsular Thailand - ZRC.4.5060, (1 male), 5059 (1 female) (Figure 2.10).

External measurements of this study

HB: 41-69, T: 30-48, HF: 9.8-12.7, EL: 5.4-8.9, BH: 10-20, Weight: 3.4-6.1g.

Morphological observation

Upper part fur body greyish to brown colour, while the under part is paler grey. Ear, hind foot and tail almost paler than body. Tail fur usually sparse, scattered and have

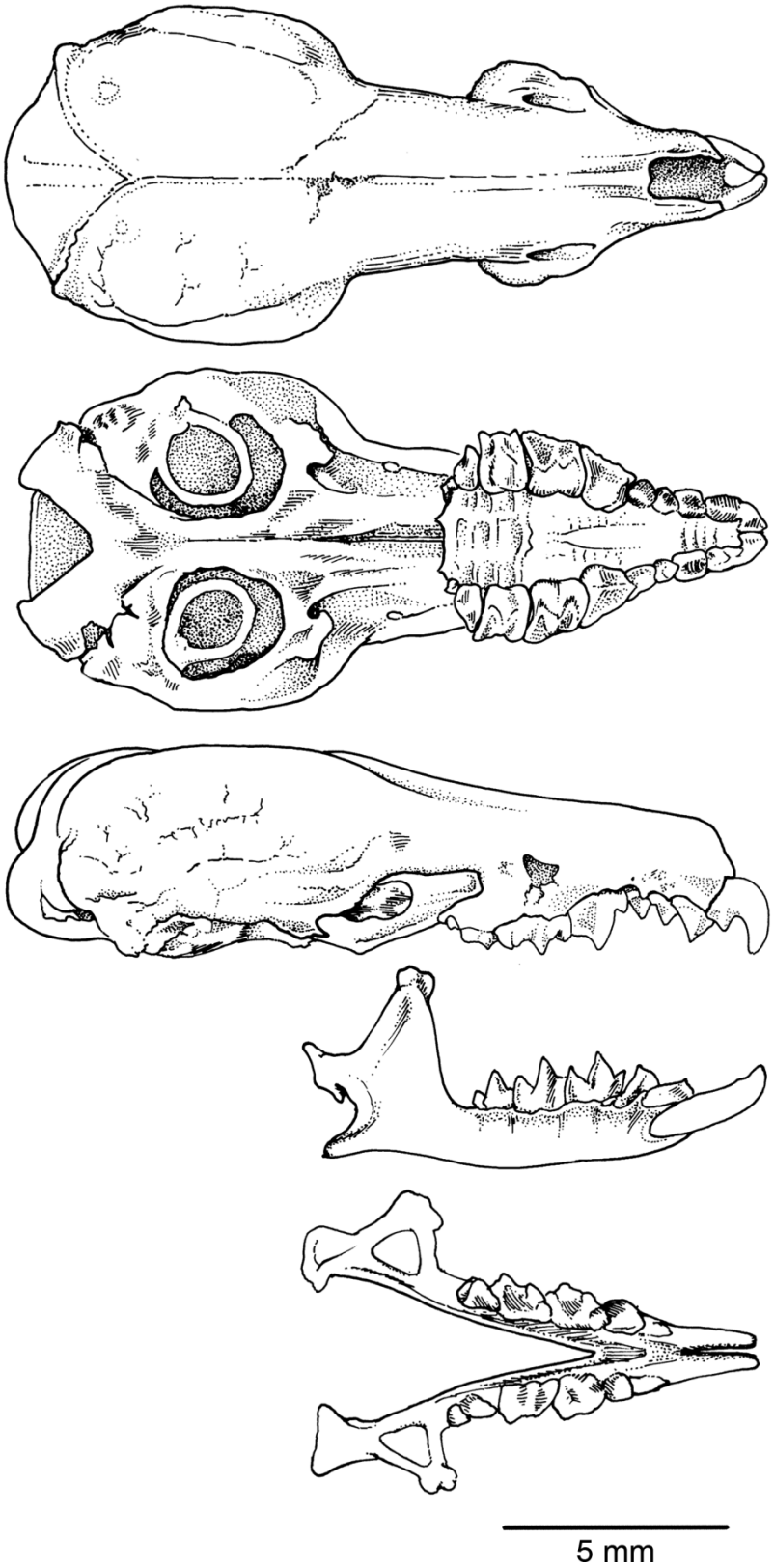


Figure 2.9 Illustration of dorsal, ventral and lateral view of cranium and mandible of male *Crocidura monticola* from Wang Kelian, Perlis (WK1075).

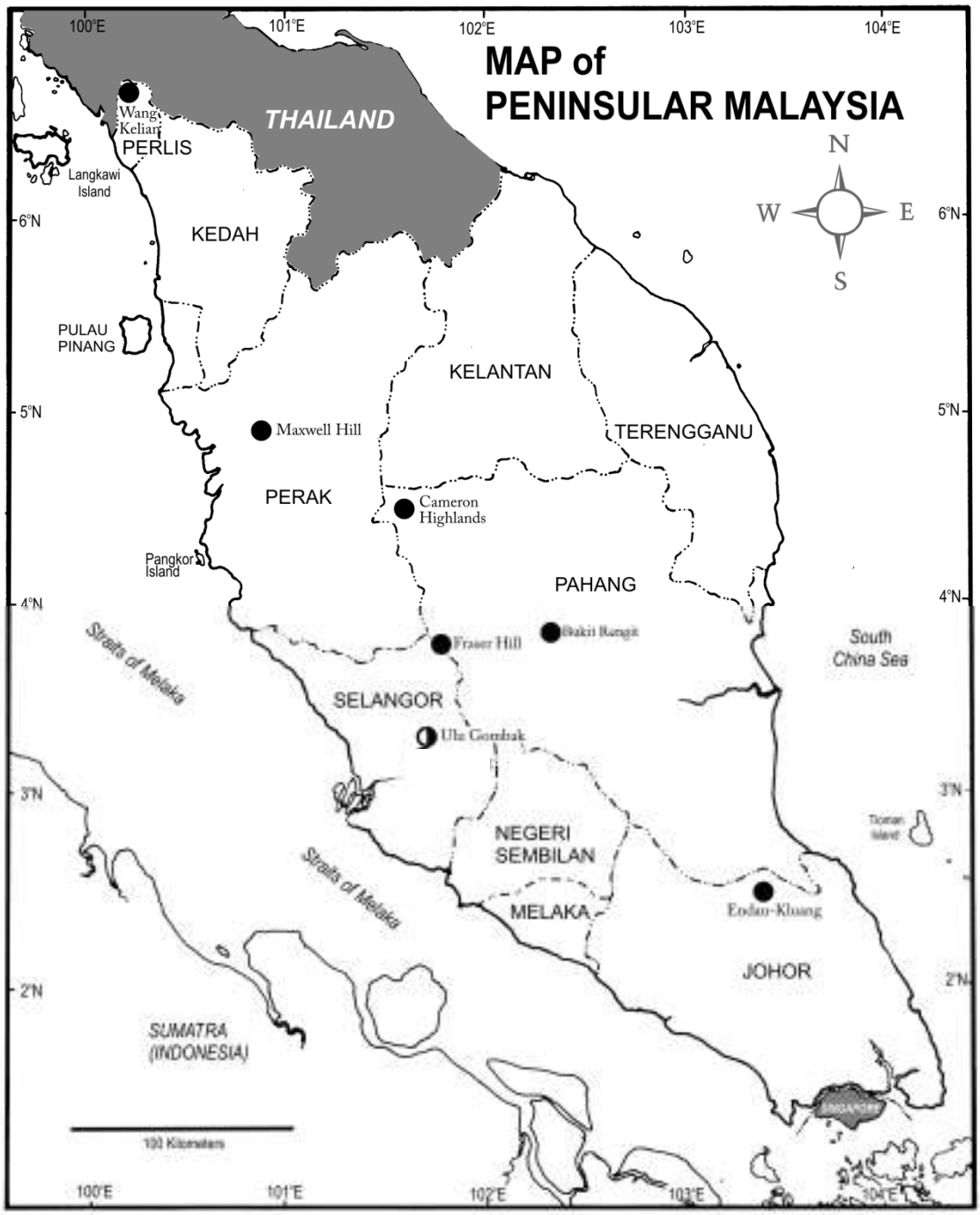


Figure 2.10 Map of *Crocidura monticola* surveys in eight localities with ● indicate current record, O indicate historical record and ◑ indicate current and historical record.

long whitish hair. Previously, it is very similar in external morphological features to *S. malayanus* and thought as one species (Davison, 1979).

Distribution

Widespread from north to south of Peninsular Malaysia, but during this study they were not found on islands (e.g. Pangkor and Aur). This tiny shrew can live in lowlands and highlands (see Table 3.1). Based on previous studies, *C. monticola* can only be found at Ampang and Ulu Gombak forests in Selangor by Davison *et al.* (1982) and Jenkins (1982). *Crocidura monticola* is widely distributed in Sunda regions (Ruedi, 1995), possibly also widespread in Indochina regions.

Comments

The Sunda Shrew originated from Java and was distributed extensively in Peninsular Malaysia, Sumatra and Borneo. Previously, *C. monticola* has been associated with other species from Sunda regions such as *C. minuta*, *C. neglecta and* C. *bartelsii*. The taxonomic condition was in confusion because of inadequate materials for description which led to their status remain unsolved. Subsequently, Ruedi (1995) referred all *C. maxi* from eastwards Java to Lesser Sunda as synonymous to *C. monticola*. This is contrary to Kitchener *et al.* (1994) that believed *C. monticola* is present in west to central Java, while *C. maxi* can be found in east to Lesser Sunda Islands. No specimens of *C. monticola* or *C. maxi* for comparison purposes. In Chapter 3, further study on *C. monticola* has been conducted to clarify their taxonomic status in Sundaland and other countries in Southeast Asia. According to Chiozza (2008a), this species is categorized as lower risk.

Suncus malayanus Kloss, 1917 (Figure 2.11)

(Malayan Pygmy Shrew)

Synonym and type locality

Pachyura malayana Kloss, 1917 – Journal Nat. Hist. Soc. Siam, 2: 282 (Bang Nara, Patani, Thailand).

Specimen examined

In this study: Ulu Gombak, Selangor - PP1081, 1084 (2 male), PP10117 (1 female), PP0346, 0943, 0956, 10129 (4 unknown); Cameron Highlands, Pahang - CH1090 (1 male); Ulu Kenas, Perak - UK0855 (1 male), UK0831, 0844 (2 female); Lata Belatan, Terengganu - LB0836 (1 female); Triang, Negeri Sembilan - Tr0954 (1 male); Sungai Sedim, Kedah - Se1085 (1 female); Bukit Rengit, Pahang - BR10106 (1 male).

Previous study: Krau Wildlife Reserve, Kuala Gandah, Pahang - ZRC.4.8116 (1 unknown) (Figure 2.12).

External measurements of this study

HB: 37-51, TL: 22-34, HF: 6.7-8.2, EL: 4.4-6.5, BH: 12-24, Weight: 1.1-2.3 g.

Morphological observation

The body size is too small and eyes are invisible to the naked eye. Upper part of body is blackish but and has whitish grizzled fur, while the under part is more greyish. Ear and foot are black in colour. Under part of long tail is thickened and concealed with dense short hair compared to upper part, which has sparse fur.

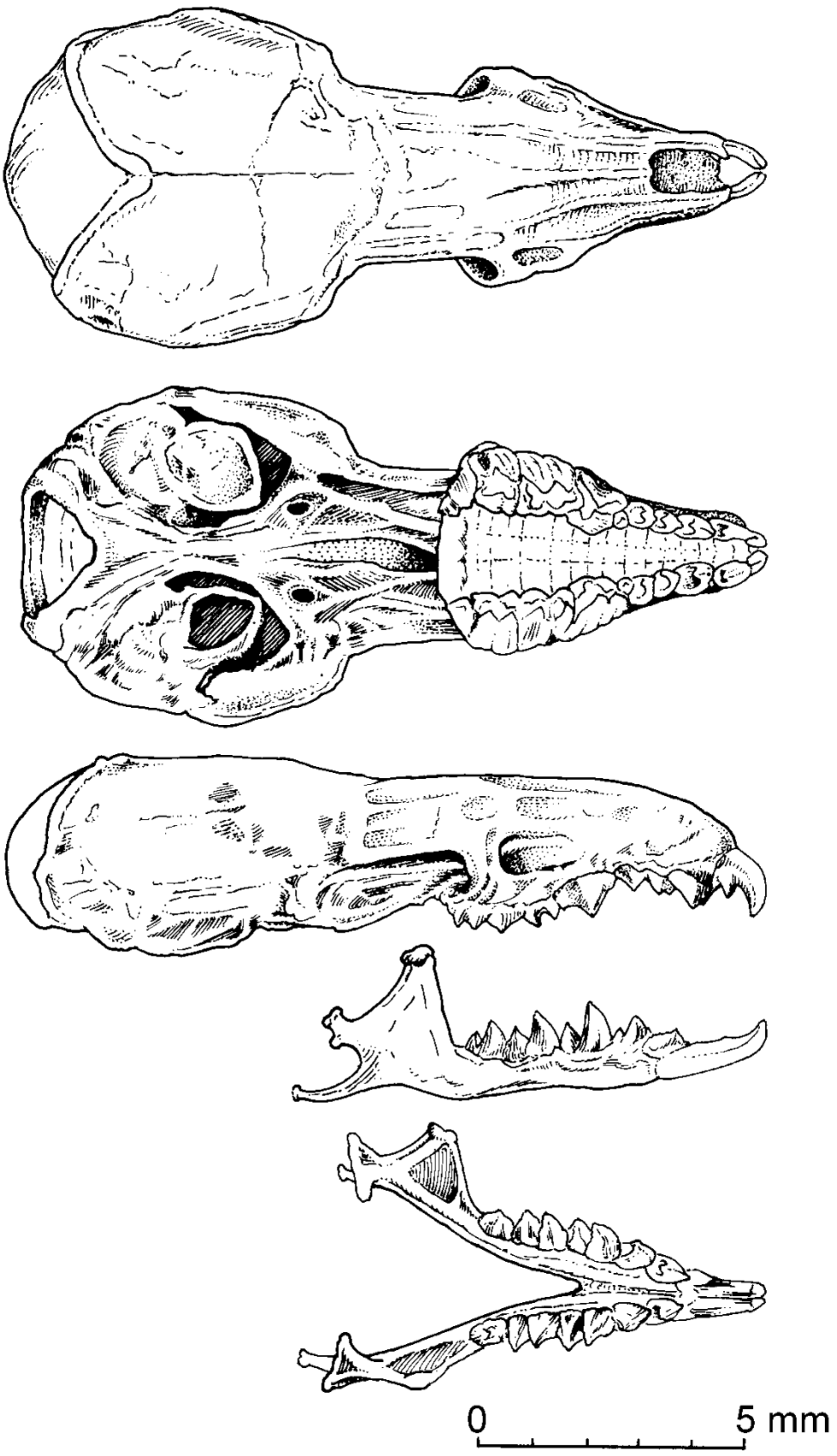


Figure 2.11 Illustration of dorsal, ventral and lateral view of cranium and mandible of male *Suncus malayanus* from Cameron Highlands, Pahang (CH1090).

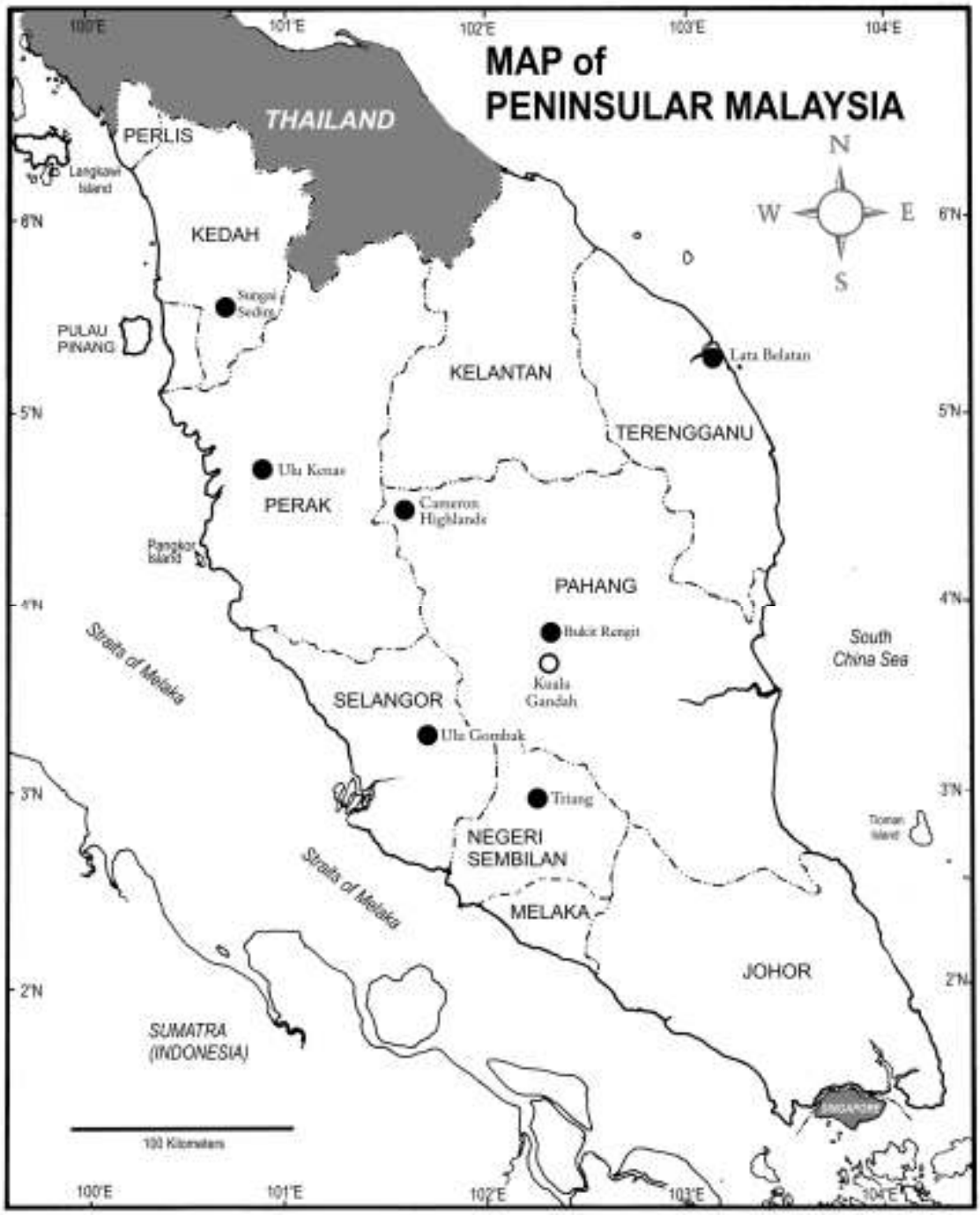


Figure 2.12 Map of *Suncus malayanus* survey in eight localities with ● indicate current record and O indicate historical record.

Distribution

This species is very small and seldomly trapped. It is widely distributed in Peninsular Malaysia. Mostly found at lower altitude forests. For high altitude, *S. malayanus* can only be found at Cameron Highlands, Pahang with one sample was captured. *Suncus malayanus* is sympatric species to *C. monticola* in Peninsular Malaysia (e.g. Ulu Gombak, Cameron Highlands and Bukit Rengit), which lead to confusion in identifying live animals. In this study, *S. malayanus* was never trapped in Peninsular Malaysia islands (e.g. Pangkor Island and Aur Island).

Comments

Formerly, many authors include *Suncus* pygmy shrews in *etruscus* group. However, four valid species were isolated from the *etruscus* group due to its morphologies does not fit to their geographical range. They were endemic to Madagascar (*S. madagascariensis*), Borneo (*S. hosei*), Malay-Thai peninsula (*S. malayanus*) and Sri Lanka (*S. fellowesgordoni*) (see Hutterer, 2005). In recent publications, Meegaskumbura *et al.* (2012a & 2012b) have proved that *S. fellowesgordoni* differed morphologically and genetically when compared to *S. etruscus* from Sri Lanka and India. For this study, *S. malayanus*, *S. fellowesgordoni*, and European *S. etruscus* will be discussed in details in Chapter 4 based on their taxonomic status using DNA sequences. Info on *S. malayanus* and need more data such as on distribution, taxonomic status and ecological studies (Lunde, 2008b).

Suncus murinus Linnaeus, 1766 (Figure 2.13)

(House Shrew)

Synonym and type locality

Sorex murinus Linnaeus, 1766 – Amoenitates Academicae, 6: 74 (Java)

Specimen examined

In this study: Ulu Gombak, Selangor - PP0868, (1 female), MZUM 060, 095, 097, 217, 264, M65.118, R1805, (7 unknown); Tapah, Perak - Ta1078 (1 female) (Figure 2.14).

External measurements of this study

HB: 100-117, T: 67-72, HF: 18.0-20.3, EL: 11.3-12.5, BH: 26.4-58.0, Weight: 18.1g.

Morphology observation

Categorized as large-sized shrew. The whole body and tail are grey in colour. Large skull size but tail and hind foot are shorter than *Chimarrogale hantu*.

Distribution

Because of it large-sized and commonly frequents human abodes, gardens and open habitats, use of pitfall traps is inappropriate to capture this species. A single individual was found coincidentally at Rest and Relax Tapah Highway, Perak and instantly captured by hand, while the individuals from Ulu Gombak were trapped using rat trap.

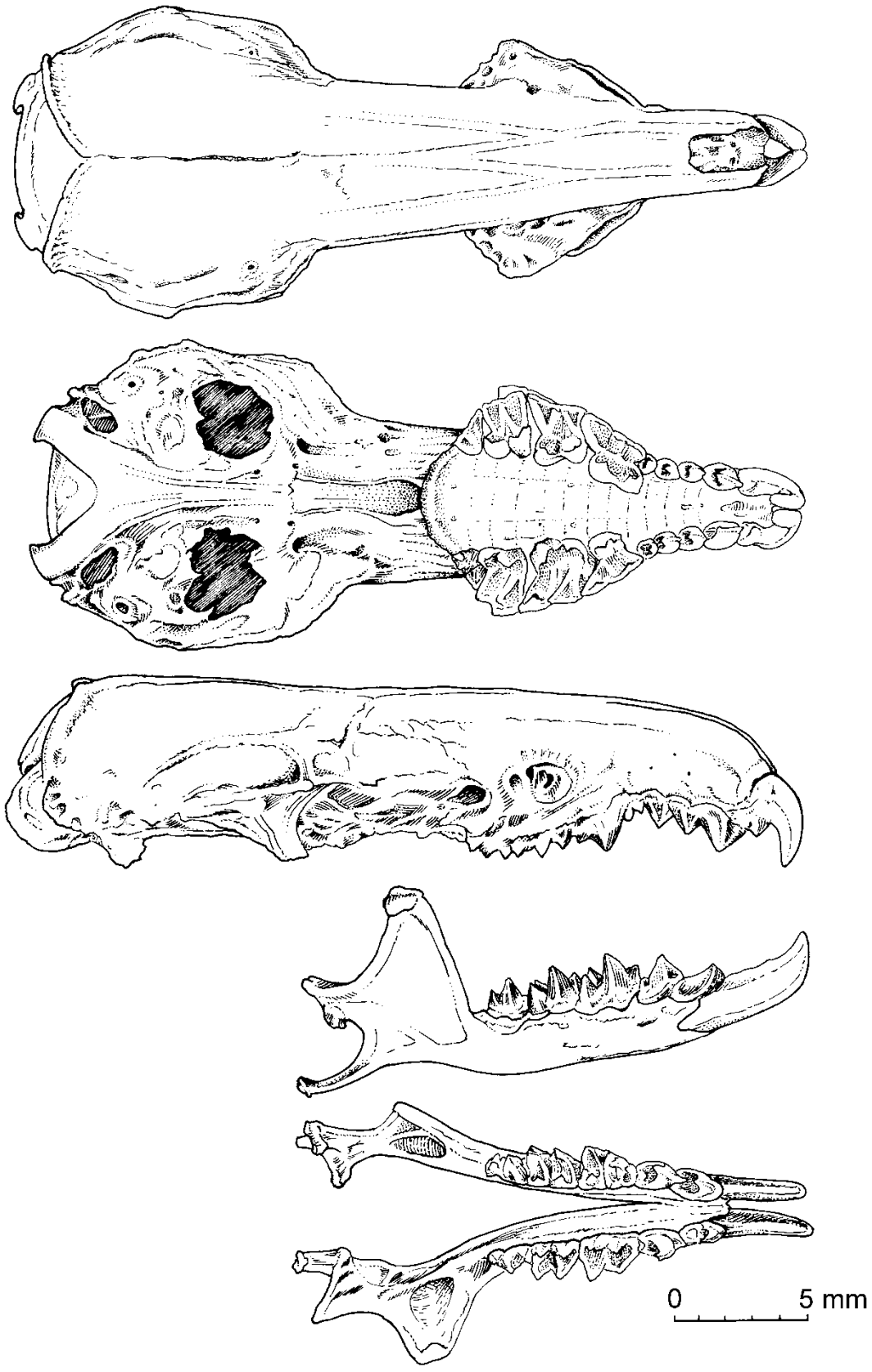


Figure 2.13 Illustration of dorsal, ventral and lateral view of cranium and mandible of female *Suncus murinus* from Tapah, Perak (Ta1078).

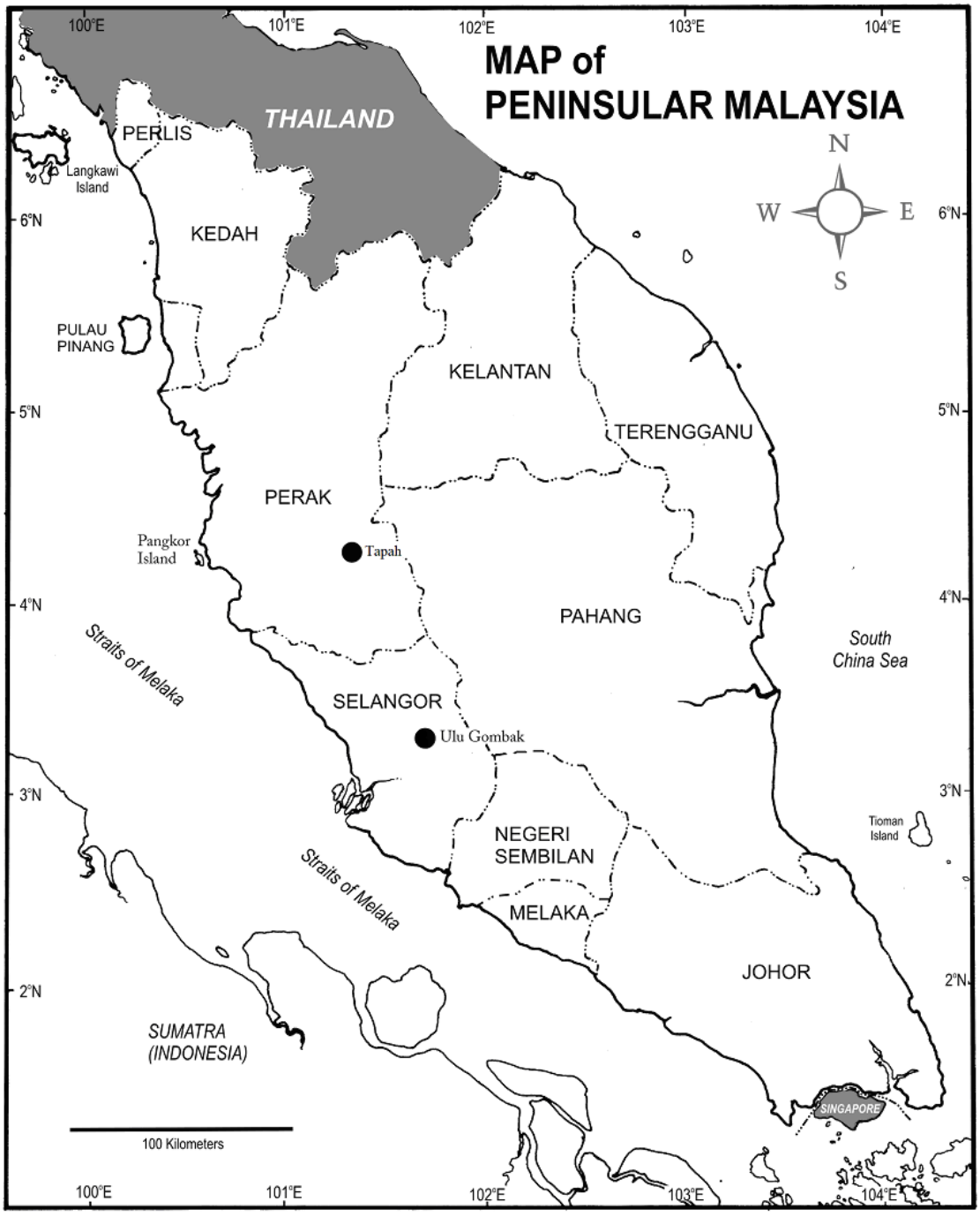


Figure 2.14 Map of *Suncus murinus* survey in two localities with ● indicate current record.

Comments

Initially, *S. murinus* was first found and recognized in Java by Linnaeus (1766), but subsequently several authors (Ellerman, 1961; Hutterer & Tranier, 1990; Jenkins *et al.*, 1998; Burney *et al.*, 2004) believed it was originated from central India and migrated to Asia and Africa by ship. Earlier taxonomists have classified House Shrew as subspecies based on its body size and tail length. However, majority of these subspecies have been overlooked in mammalian taxonomy until recently (Corbet & Hill, 1992; Hutterer, 2005). Thus, this shrew became a species complex. For example, in Sri Lanka, four species were recognized as *Sorex*, which have very similar morphological characteristics with *S. murinus*, but later were classified as synonyms: *S. zeylanicus* (Phillips, 1928), *S. montanus* (Kelaart, 1850), *S. kandianus* (Kelaart, 1852), and *S. caerulescens* (Shaw, 1800). However, all synonymized shrews were later reclassified as subspecies of *S. murinus* by Phillips (1980): *S. m. murinus*, *S. m. montanus*, *S. m. kandianus* and *S. m. caerulescens*. From these, the three former species were described from Sri Lanka and the latter species from India. Meegaskumbura & Schneider (2008) and Meegaskumbura *et al.* (2010) further studied mitochondrial DNA sequence data and morphometric analyses to reveal systematic relationships and taxonomic status of *S. murinus* complex in Sri Lanka and India.

Chimarrogale hantu Harrison, 1958 (Figure 2.15)

(Malayan Water Shrew)

Synonym and type locality

Chimarrogale hantu Harrison, 1958 – Annals Mag. Nat. Hist., 1: 282 (Ulu Langat Forest Reserve, Selangor).

Specimen records

In this study: Belum Royal Park, Perak - Be0803 (1 unknown), Ulu Gombak, Selangor - PP1086 (1 male), PP0965-66 (2 unknown) (Figure 2.16).

External measurements of current record

HB: 90-112, T: 74-90, HF: 19.9-21.0, EL: 6.9-8.1, Weight: 20.5g.

Morphology identification

Silky fur with dark grey to brown colour, fit for waterproof purposes with silvery protector hairs most obvious on hindquarters, slightly short hair edging the surface of the feet, tail slender with thick hair.

Distribution

Chimarrogale hantu is native to Peninsular Malaysia. It was found in Ulu Langat Forest Reserve, Selangor (Corbet & Hill, 1992; Hutterer, 2005) and Sungai Nenggiri, Kelantan (Medway, 1978). It also possibly exists in southern Thailand. This is a very unique mammal because it can survive in water and lives in forests adjacent to waterfalls or rivers. The Water Shrew feeds on freshwater aquatic invertebrates and adapts well to an almost aquatic life.

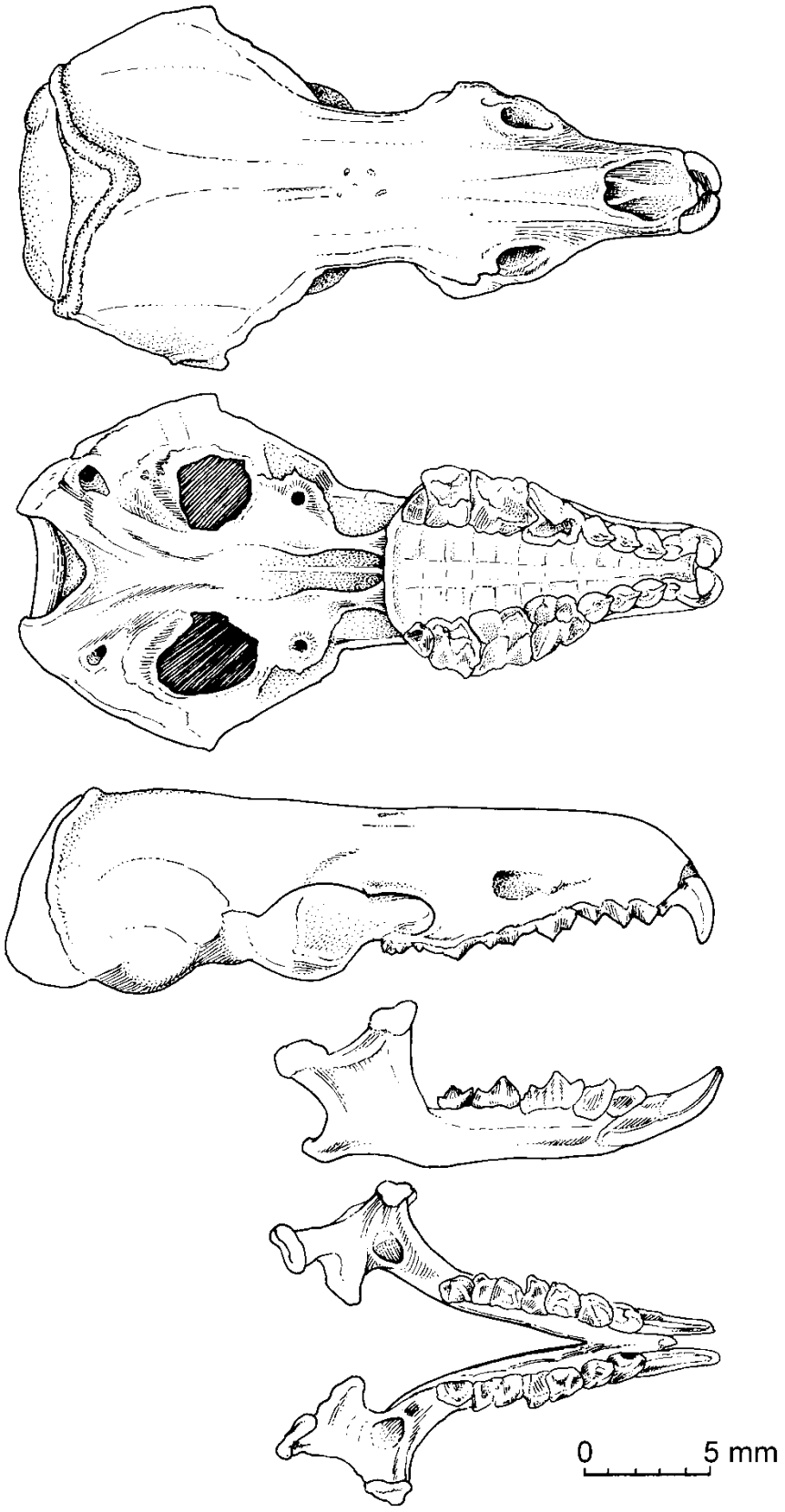


Figure 2.15 Illustration of dorsal, ventral and lateral view of cranium and mandible of *Chimarrogale hantu* from Belum Royal Park, Perak (Be0803).

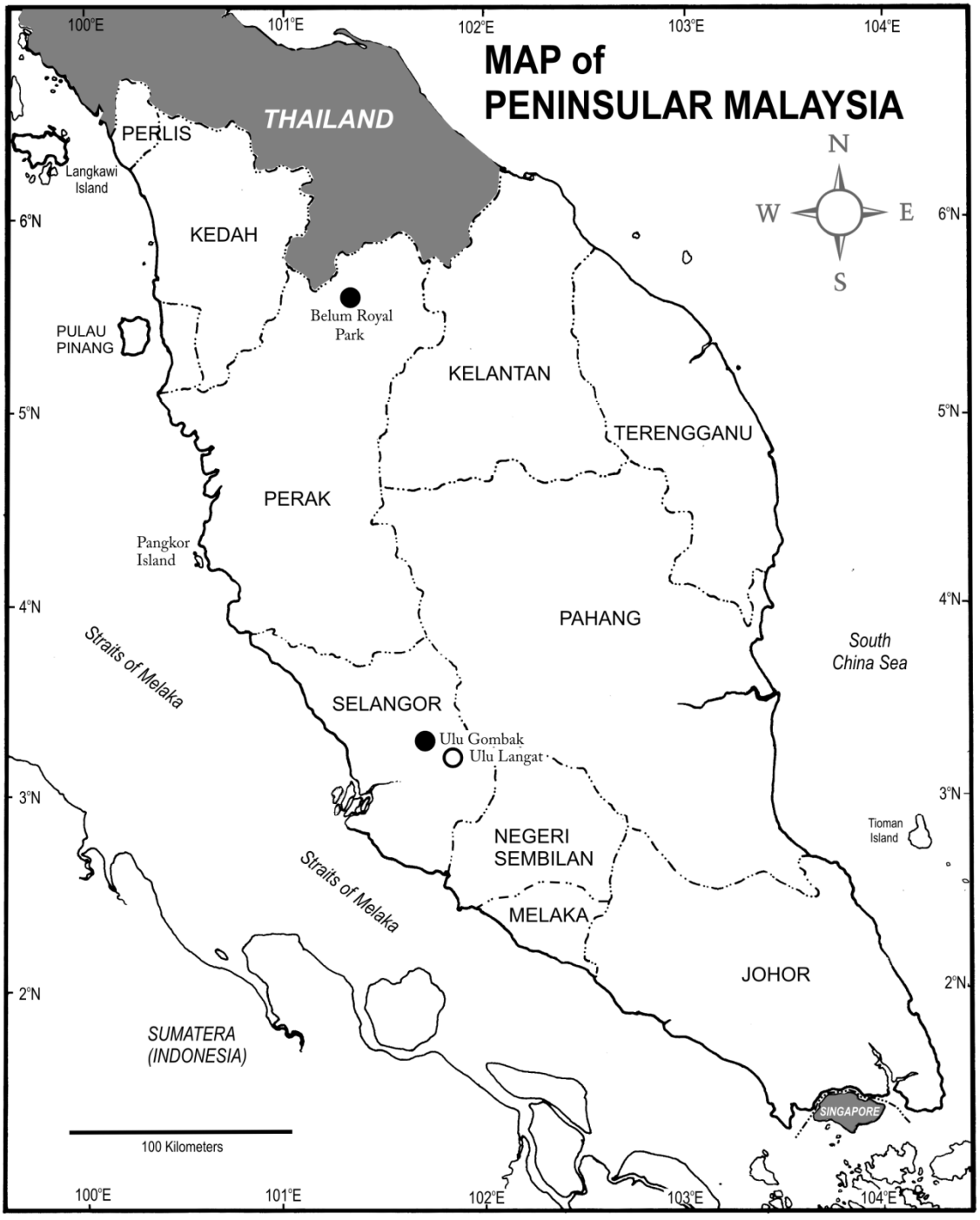


Figure 2.16 Map of *Chimarrogale hantu* survey in three localities with ● indicates current record and ○ indicate historical record.

Comments

This shrew is rarely discovered and hard to trap. Previously, *Chimarrogale hantu* were included in *Chimarrogale himalayica* from India by Medway (1978), but because their morphology and habitat more different from *Chimarrogale himalayica*, it was then assigned as a valid species (see Hutterer, 2005). *Chimarrogale hantu* have cusps on upper incisors, compared to *Chimarrogale himalayica* which lacks the character (Francis, 2008). According to Corbet & Hill (1992), *Chimarrogale hantu* and *Chimarrogale phaeura* (from Borneo) are slightly different on the basis of deeper braincase (7.7 - 8.0 mm and 7.3 - 7.5 mm respectively). *Chimarrogale hantu* is listed as near threatened by IUCN because their abundance and population size are not known (Chiozza, 2008b). The Water Shrew may not be at risk, but could be threatened by degradation of water quality from pollution or habitat destruction.

2.4 SUMMARY

In this study, *C. monticola* is distributed throughout Peninsular Malaysia, in contrast to previous studies which recorded them in Selangor only (Davison *et al.*, 1982; Jenkins, 1982). Although this tiny shrew has minimal number of museum specimens it is possibly widespread in East and Southeast Asia and could be treated as a single species (Ruedi, 1995). Further studies are needed to extend more trapping sites to capture *C. negligens* and *C. fuliginosa*, as well as two large shrews (i.e. *Chimarrogale hantu* and *S. murinus*) which more info on distributions and ecology.

2.5 IDENTIFICATIONS KEYS FOR MALAY PENINSULA SHREWS (CROCIDURINAE AND SORICINAE)

A. Large shrews; weight > 18 g; HF ≥ 18 mm..... B

A'. Small shrews; weight < 17 g; HF < 17 mm..... C

B. Larger (HB: 100 – 117 mm; HF about 18 – 20 mm; T < 73 mm; GLS about 32 mm; IM3S about 14 mm; ROB about 4.4 mm; mandible part of MAL about 13 mm and IM3I about 9.1 mm); snout triangular; ear visible through fur; fur greyish; tail thick; often found around homes such as pipe channel and drain ..... *Suncus murinus*

B'. Smaller (HB: 90 – 112 mm; HF about 20.0 – 21.0 mm; T ≥ 74 mm; GLS < 28 mm; IM3S < 13 mm; ROB ≤ 3.8 mm; mandible part of MAL ≤ 11 mm and IM3I ≤ 11.6 mm); snout enlarged; no visible ears; fur dark grey-brown, thick and dense; tail slender; lives in water or near forest stream .....*Chimarrogale hantu*

C. Size very small (HB < 70 mm; HF < 13 mm; T < 49 mm)..... D

C' Size larger (HB: 75 – 99 mm; HF > 13 mm; T > 50 mm) ..... E

D. Head flattened; fur very short and dark brown; ears prominent; foot dark in colour; weight ≤ 2.3 g (GLS < 15 mm; IM3S ≤ 6.1 mm; M2B < 4.1 mm; MAL ≤ 5.9 mm; IM3I < 4.2) ..... *Suncus malayanus*

D' Head rounded; grey brown fur and dense; weight about 3-6 g (GLS > 16.0 mm; IM3S ≥ 6.8 mm; M2B ≥ 4.8 mm; MAL > 6.0 mm; IM3I ≥ 4.6 mm) ..... *Crocidura monticola*

E Larger; weight about 9.7 – 14.3 g; (HB: 70 – 99 mm; HF: 13.2 – 15 mm; T: 50 – 66 mm; GLS ≥ 22.0 mm; IM3S: 9.8 – 11.0 mm; ROB: 2.9 – 3.6 mm; M2B: 6.6 – 7.4 mm; MAL: 8.4 – 10.3 mm)..... *Crocidura malayana*

E' Similar size or smaller; T: 14 – 15.8 mm..... F

F Larger; weight about 10 g; (GLS: 21.5 – 24.0 mm; IM3S: 9.3 – 10.9 mm; ROL: 8.4 – 10.1 mm; CTG: 8.4 – 9.1 mm; MAL: 8.8 – 10.1 mm; IM3I: 6.5 – 7.4 mm)..……..……. *Crocidura fuliginosa*

F' Smaller; (GLS about 21.0 – 22.0 mm; IM3S: 9.3 – 9.7 mm; ROL: 8.3 – 8.9 mm; CTG: 8.2 – 8.5 mm; MAL: 8.3 – 8.9 mm; IM3I: 6.3 – 6.5 mm) …….. *Crocidura negligens*

CHAPTER 3

MORPHOLOGICAL AND GENETIC RELATIONSHIPS OF THE *CROCIDURA MONTICOLA* SPECIES COMPLEX (SORICIDAE: CROCIDURINAE) IN SUNDALAND

3.1 INTRODUCTION

Crocidura monticola (Peters, 1870) is a small shrew (weight less than 8 grams) that was originally described from central Java. This species is apparently widely distributed in the Sundaland, unlike other wild crocidurine shrews (Ruedi, 1995). Based on few available records (Davison *et al*., 1982; Davison, 1984; Corbet & Hill, 1992), *C. monticola* is apparently restricted to the forested area and can be found over a broad altitudinal range (100 to 1144 m above sea level; see Figure 3.1), up to 2400 m in western Java (Ruedi,1995). Specimens from eastern Java and Lesser Sunda islands (east to Aru) are slightly larger. It was recorded live in more open habitats and was ascribed by Kitchener *et al*. (1994) to *C. maxi* (Sody, 1936). *Crocidura maxi* is currently considered either as vicariant species (Jenkins, 1982; Kitchener *et al*., 1994; Hutterer, 2005), or subspecies to *C. monticola* (Ruedi, 1995). Other small forms of Indonesian *Crocidura* (e.g. *C. bartelsii* or *C. neglecta*) were synonymized with *C. monticola* (Jenkins, 1982; Ruedi, 1995; Hutterer, 2005). Precise taxonomic assessment of C. monticola was hindered by confusion with original dimensions (Sody, 1936), inaccurate type descriptions (e.g. for *C. minuta*) or lack of direct comparisons with adequate material from extensive distribution range. Furthermore, no comparisons with similar-sized shrews found in the mainland north to the Isthmus of Kra were made, and possible conspecificity with closely related taxa such as *C. indochinensis* (Robinson and Kloss, 1922) or *C. wuchihensis* (Shaw *et al*., 1966) was never carried out (see

Figure 3.1 Picture of a live adult male *Crocidura monticola* collected in a lowland forest near Wang Kelian, Peninsular Malaysia (location 1). This small Sunda shrew weighted 4.4 g and had 97 mm in total length (including tail).

Jenkins *et al*., 2009). Therefore, taxonomic status of *C. monticola* and its exact geographical distribution is therefore still debated.

Davison *et al*. (1982) revised a series of presumed pygmy shrews (*Suncus malayanus*) caught in Selangor, Peninsular Malaysia which included three specimens of *C. monticola*. Although all these shrews were of similar overall size, *Crocidura* specimens can be easily distinguished from true pygmy shrews by dentition, *Crocidura* has only three unicuspid teeth in the upper tooth row, instead of four, as in *Suncus*. These specimens represented first record of *C. monticola* from the Thai-Malay peninsula region.

During the survey on distribution and taxonomic status of wild shrews in Peninsular Malaysia, a significant numbers of small-sized shrews of the genus *Suncus* (*S. malayanus*, see Omar *et al*., 2011), and *Crocidura* were trapped. In this report, I describe morphological and genetic variations of Malay *C. monticola* and compare them with reference specimens of the Sundaland and mainland Indochina to clarify their taxonomic status.

3.2 MATERIALS AND METHODS

3.2.1 Sample collection and field methods

A total of 52 individuals of *C. monticola* were collected during several field surveys conducted between 2008 and 2010 in various parts of Peninsular Malaysia (Figure 3.2). These locations include: (1) Wang Kelian, dominated by secondary lowland forest; (2) Maxwell Hill, an upper dipterocarp forest; (3) Cameron Highlands, in remnants of

Figure 3.2 Seven sampling sites of *C. monticola* in Peninsular Malaysia. Numbers refer to the following locations: 1 Wang Kelian, Perlis; 2 Maxwell Hill, Perak; 3 Cameron Highlands, Pahang; 4 Fraser Hill, Pahang; 5 Bukit Rengit, Pahang; 6 Ulu Gombak, Selangor; 7 Endau-Kluang, Johor. For location of samples outside Peninsular Malaysia, see Fig. 1 in Ruedi (1995).

montane forest bordering tea plantations; (4) Fraser Hill, covered with secondary montane forest; (5) Bukit Rengit, a lowland peat-swamp forest; (6) Ulu Gombak at the University of Malaya Field Studies Centre, covered with secondary lowland evergreen forest; and (7) Endau-Kluang, dominated by lowland dipterocarp forests (see Table 3.1 and Appendix A).

Shrews were trapped using pitfalls consisting of large buckets (height = 25 cm, diameter = 21 cm) with 10 to 12 small holes (5 mm) drilled at the bottom to drained water. 50 to 100 buckets were buried every 5 to 10 m interval within a 45 m by 45 m grid per site. Trapped shrews were euthanized using chloroform; sex and external measurements were taken immediately. Specimens were then either prepared as a flat skin or fixed in 5% formalin and preserved in 70% ethanol, with cleaned skulls preserved separately. Tissue samples were taken from representative exemplars and preserved in 95% ethanol or stored at -80^{o}C for genetic study. All specimens are currently deposited at the zoological collections of the University of Malaya.

Additionally, a total of 50 museum specimens assigned as *C. monticola* species complex were morphologically examined. These speciemens were stored in the following collections: the Museum Zoologicum Bogoriense, Indonesia (MZB; 31 specimens), the Raffles Museum, Singapore (ZRC; 2 specimens), the Museum of Vertebrate Zoology of the University of California at Berkeley, USA (MVZ; 2 specimens), Rijksmuseum van Natuurlijke Historie in Leiden, Netherlands (RMNH; 12 specimens), the British Museum of Natural History in London (BMNH; 1 specimen) and the Institut de Zoologie et d'Ecologie Animale in Lausanne, Switzerland (IZEA; 2 specimens) (see details in Appendix A). External measurements for these museum specimens were recorded from the original labels. Measurements of two *C. maxi* from

Table 3.1 Geographic location of the sampling sites where *Crocidura monticola* was trapped in Peninsular Malaysia. Number of females (F), males (M) or unsexed (U) individuals captured at each site is also given.

Sampling (State)	GPS	Altitude a.s.l.	Number and Sex
1. Wang Kelian (Perlis)	N06^{o}41.977' E100^{o}11.925'	152 m	2 F, 5 M
2. Maxwell Hill (Perak)	N06^{o}41.977' E100^{o}11.925'	1160 m	5 F, 2 M, 3 U
3. Cameron Highlands (Pahang)	N04^{o}28.460' E101^{o}23.05'	1460 m	2 F, 6 M
4. Fraser Hill (Pahang)	N03^{o}43.041' E101^{o}44.256'	1260 m	2 M, 4 U.
5. Bukit Rengit (Pahang)	N03^{o}35.987' E102^{o}10.758'	132 m	2 F, 5M
6. Ulu Gombak (Selangor)	N03^{o}60.170' E101^{o}60.46'	300 m	5 F, 1 M, 5 U
7. Endau-Kluang (Johor)	N02^{o}29.284' E103^{o}26.597'	55 m	3 U

Komodo as reported by Kitchener *et al*. (1994) were also included for morphometric study.

3.2.2 Morphological analyses

Measurements of external morphology of all caught specimens and 14 skull characters (including cranial and mandibular, see Figure 2.1) were conducted as presented in Chapter 2. Possibly of sexual dimorphism in external and skull measurements were tested using ANOVA. Since the analysis indicated no dimorphism between sex, further morphological analyses were done regardless of gender. The descriptive statistics (i.e. sample size, mean and standard deviation, minimum and maximum values) were calculated for all external body and skull measurements (Tables 3.2 and 3.3).

To provide broader overview on the variation of shrews for entire Sundaland, two principal component analyses (PCA) based on correlation matrices of raw data was performed on 14 skull measurements. Initially, PCA only comprised specimens from Peninsular Malaysia, but later analysis include all specimens from Sundaland as listed in Appendix B. Since many skulls were extracted from unsexed specimens, the PCAs ignore possible sexual dimorphism. Scores of the Malay shrews on the PC1 of the first analysis were also used to test the effect of altitude of sampling locations on their cranial morphology. The significance of the correlation was tested with the Spearman's rank correlation coefficient. All statistical analyses were performed using STATISTICA version 8.0 (StatSoft Inc., USA).

Table 3.2 External measurements of *Crocidura monticola* and *C. maxi* from Sundaland and Komodo. Sample size (n) is followed by mean ± standard deviation and range (in parenthesis). Values for two Komodo shrews are taken from Kitchener *et al.* (1994). All measurements are in millimeters and weight are in grams. Unavailable measurements are indicated as NA.

Character	*C. monticola* (Peninsular Malaysia)	*C. monticola* (Thailand)	*C. monticola* (Borneo)	*C. monticola* (west and central Java)	*C.* cf. *maxi* (Sumatra)	*C. maxi* (east Java)	*C. maxi* (Komodo)
Head and body length (HB)	n = 50 57.8 ± 6.7 (41 – 69)	n = 2 59.0 ± 8.5 (53 – 65)	n = 10 58.1± 6.5 (47 – 69)	n = 8 60.9 ± 8.1 (52 – 78)	n = 2 54.5 ± 0.7 (54 – 55)	n = 19 62.4 ± 5.9 (53 – 72)	n = 2 63.0 ± 4.2 (60.0 – 66.0)
Tail length (T)	n = 52 39.3 ± 4.6 (30 – 48)	n = 2 33.5 ± 4.9 (30 – 37)	n = 11 34.2 ± 3.3 (30 – 39)	n = 8 46.0 ± 10.6 (36 – 68)	n = 2 37.5 ± 2.1 (34– 41)	n = 19 46.7 ± 5.6 (35 – 55)	n = 2 45.0 ± 1.4 (44.0 – 46.0)
Hind foot length without claw (HF)	n = 52 11.1 ± 0.8 (9.8 – 12.7)	n = 1 11.0	n = 10 10.4 ± 0.5 (10 – 11)	n = 8 10.8 ± 0.8 (10 – 12)	n = 2 10.0 ± 0.3 (9.8 – 10.2)	n = 19 12.0 ± 0.6 (11.0 – 13.5)	n = 2 11.6 ± 0.2 (11.4 – 11.7)
Ear length (E)	n = 39 7.5 ± 1.0 (5.4 – 8.9)	NA	n = 10 7.9 ± 0.6 (7.0 – 9.0)	NA	n = 2 7.5 ± 0.7 (7 – 8)	NA	n = 2 7.8 ± 0.4 (7.5 – 8.1)
Bristle hairs (along the tail)	n = 38 14.2 ± 2.5 (10 – 20)	NA	NA	n = 9 18.2 ± 6.3 (10 – 27)	n = 2 12 ± 1.5 (11 – 13)	n = 19 23.5 ± 4.5 (17 - 32)	NA
Weight (W)	n = 30 4.6 ± 0.7 (3.4 – 6.1)	NA	n = 10 4.4 ± 0.5 (3.8 – 5.1)	NA	n = 1 5	NA	NA

Table 3.3 Skull measurements of *Crocidura monticola* and *C. maxi* from Sundaland and Komodo. Sample size (n), mean (± standard deviation) and range (in parenthesis) are given for each taxon. All measurements are in millimeters.

Character	*C. monticola* (Peninsular Malaysia) n = 42	*C. monticola* (Thailand) n = 1	*C. monticola* (Borneo) n = 11	*C. monticola* (west and central Java) n = 8	*C.* cf. *maxi* (Sumatra) n = 2	*C. maxi* (east Java) n = 9	*C. maxi* (Komodo) n = 2
Greatest length of skull (GLS)	17.0 ± 0.5 (16.2 – 18.1)	16.1	16.3 ± 0.5 (15.2 – 16.8)	16.7 ± 0.7 (15.8 – 17.7)	15.9 ± 0.1 (15.8 – 15.9)	18.1 ± 0.6 (17.1 – 18.9)	18.1 ± 0.1 (18.0 – 18.1)
Length of upper toothrow (IM3S)	7.3 ± 0.2 (6.7 – 7.7)	6.9	6.9 ± 0.2 (6.5 – 7.1)	7.0 ± 0.3 (6.7 – 7.5)	6.7 ± 0.2 (6.5 – 6.8)	7.7 ± 0.3 (7.2 – 8.2)	7.9 ± 0.1 (7.8 – 7.9)
Post-palatal length (PPL)	7.8 ± 0.4 (6.9 – 8.6)	7.2	7.4 ± 0.5 (6.3 – 8.0)	7.6 ± 0.4 (7.1 – 8.3)	7.6 ± 0.5 (7.2 – 7.9)	7.8 ± 0.4 (6.9 – 8.6)	8.1 ± 0.1 (8.0 – 8.1)
Length of upper molariform (PM3)	4.1 ± 0.1 (3.9 – 4.4)	3.9	3.9 ± 0.1 (3.7 – 4.1)	4.1 ± 0.2 (3.8 – 4.4)	3.8	4.3 ± 0.1 (4.2 – 4.5)	4.4 ± 0.1 (4.3 – 4.4)
Rostral breadth (ROB)	2.4 ± 0.1 (2.1 – 2.7)	2.1	2.2 ± 0.1 (2.0 – 2.4)	2.1 ± 0.2 (2.0 – 2.5)	2.2	2.2 ± 0.1 (2.0 – 2.5)	2.0
Breadth at third molars (M3B)	2.0 ± 0.1 (1.8 – 2.3)	1.9	1.8 ± 0.1 (1.6 – 1.9)	1.8 ± 0.2 (1.6 – 2.1)	1.9 ± 0.1 (1.8 – 2.0)	1.9 ± 0.1 (1.7 – 2.0)	1.9 ± 0.1 (1.9 – 2.0)
Breadth at second molars (M2B)	5.2 ± 0.2 (4.8 – 5.5)	4.9	4.8 ± 0.1 (4.5 – 5.0)	4.8 ± 0.2 (4.6 – 5.1)	4.9 ± 0.1 (4.8 – 4.9)	5.3 ± 0.2 (5.1 – 5.5)	5.3 ± 0.1 (5.2 – 5.4)
Braincase breadth (BB)	7.8 ± 0.3 (7.1 – 8.3)	7.7	7.5 ± 0.3 (6.7 – 7.9)	7.7 ± 0.5 (7.2 – 8.5)	7.5 ± 0.1 (7.4 – 7.6)	8.1 ± 0.4 (7.6 – 8.9)	7.9 ± 0.1 (7.8 – 7.9)
Interorbital breadth (IOB)	3.7 ± 0.1 (3.5 – 4.2)	3.6	3.5 ± 0.2 (3.4 – 3.7)	3.7 ± 0.3 (3.4 – 4.4)	3.7 ± 0.1 (3.6 – 3.7)	3.7 ± 0.3 (3.0 – 4.1)	3.7 ± 0.1 (3.6 – 3.7)

Table 3.3 (Continued)

Character	*C. monticola* (Peninsular Malaysia) n = 42	*C. monticola* (Thailand) n = 1	*C. monticola* (Borneo) n = 11	*C. monticola* (west and central Java) n = 8	*C.* cf. *maxi* (Sumatra) n = 2	*C. maxi* (east Java) n = 9	*C. maxi* (Komodo) n = 2
Rostral length (ROL)	6.6 ± 0.3 (6.0 – 7.2)	6.2	6.3 ± 0.2 (6.0 – 6.5)	6.4 ± 0.3 (6.1 – 7.0)	5.9 ± 0.3 (5.7 – 6.1)	6.9 ± 0.3 (6.4 – 7.3)	6.9 ± 0.1 (6.8 – 7.0)
Post-palatal depth (PPD)	3.4 ± 0.2 (3.0 – 3.6)	3.1	3.1 ± 0.1 (2.9 – 3.3)	3.2 ± 0.2 (2.8 – 3.5)	3.1 ± 0.1 (3.0 – 3.1)	3.2 ± 0.1 (3.1 – 3.4)	NA
Condyle to glenoid length (CTG)	6.9 ± 0.2 (6.4 – 7.2)	6.1	6.7 ± 0.3 (6.0 – 6.9)	6.9 ± 0.3 (6.5 – 7.5)	6.7 ± 0.2 (6.5 – 6.8)	7.4 ± 0.2 (7.0 – 7.7)	NA
Lower tooth row length excluding first incisor (IM3I)	5.0 ± 0.2 (4.6 – 5.4)	4.7	4.7 ± 0.1 (4.6 – 4.9)	4.8 ± 0.3 (4.2 – 5.2)	4.7 ± 0.1 (4.6 – 4.8)	5.2 ± 0.2 (4.9 – 5.5)	5.3 ± 0.1 (5.2 – 5.4)
Mandibular length (MAL)	6.8 ± 0.3 (6.2 – 7.6)	6.6	6.5 ± 0.3 (5.8 – 6.8)	6.5 ± 0.3 (6.2 – 7.1)	6.7 ± 0.1 (6.6 – 6.7)	6.9 ± 0.2 (6.5 – 7.2)	NA

3.2.3 DNA sequencing

Deoxyribonucleic acid (DNA) was extracted from ethanol-preserved kidney samples using Promega Wizard extraction kits following the manufacturer's protocol (Promega Co.). The mitochondrial cytochrome *b* gene (cyt *b*) was amplified using primer pairs L14724 and H15915 (Irwin *et al.*, 1991), while part of the Apolipoprotein B nuclear gene (ApoB) was amplified using primer pairs of ApoBf and ApoBr (Dubey *et al.*, 2007a). DNA was amplified by polymerase chain reaction (PCR) in 25 µl reactions containing 5 µl of DNA template (~20 ng/µl), 2.5 µl of each primer (10mM), 0.5 µl of dNTP (10 mM), 2.0 µl of $MgCl_2$ (25 mM), 2.5 µl of 5x colorless GoTag ® Flexi buffer and 0.2 µl of GoTaq DNA Polymerase (5 µ/µl). Amplification condition for the cyt *b* gene consisted of 40 thermal cycles of denaturation at 93^{o}C for 45 s, annealing at 47.6^{o}C for 45 s, extension at 72^{o}C for 1 min, with a final extension at 72^{o}C for 5 min. Amplification conditions for the ApoB gene were the same, except for annealing temperature at 54.1^{o}C. PCR products were electrophoresed on a 1% agarose gel and visualized with ethidium bromide (ETBR) staining to check the quality of amplified DNA. Amplicons were purified with Promega Wizard SV Gel and PCR Clean up System (Promega Co.). Cleaned amplicons were sequenced in both direction using the same primers (as in PCA amplification) by a commercial laboratory (First Base Co., Selangor, Malaysia) using BigDye ® Terminator version 3.0 Cycle Sequencing Kit with products run on an ABI 3730 automatic sequencer.

3.2.4 Phylogenetic analyses

All obtained sequences were edited with Chromas version 1.45 (MacCarthy, 1996) and aligned with Clustal X 1.81 (Thompson *et al.*, 1997). As no stop codon or alignment

gaps were detected on the alignments, it is assumed that the sequences represent orthologs, not pseudogenes. To infer phylogenetic relationships in a broader perspective, the tissue samples of two Bornean shrews identified as *C. monticola* were also sequenced. These specimens were obtained from two distinct locations in West Kalimantan and were stored at the University of Michigan Museum of Zoology under voucher numbers UMMZ 174668 (field number AJG 134) and UMMZ 174683 (field number AJG 198), respectively. 18 additional sequences of Oriental *Crocidura* species and two outgroups (*Suncus etruscus* and *S. murinus*) for the cyt *b* data set and 13 Oriental *Crocidura* and one outgroup (*S. murinus*) for the ApoB data set were downloaded from GenBank, as detailed in Appendix C. All partial sequences with missing nucleotides were completed by adding Ns in the final alignment. Pairwise distances of cyt *b* gene for the different species of *Crocidura* were estimated using Kimura's two-parameter (K2P) model (Kimura, 1980) implemented in MEGA 5.0 software (Tamura *et al.*, 2011).

Phylogenetic analyses were inferred with Bayesian analysis (BA) and the maximum likelihood (ML) method to illustrate relationships for all sampled taxa. BA trees were reconstructed in MrBayes version 3.0 (Huelsenbeck & Ronquist, 2001). Akaike Information Criterion (Akaike, 1974) was used to determine the best-fit-model of DNA sequence evolution for each gene using Modeltest 3.7 (Posada & Crandall, 1998). GTR + I + G and HKY + G were selected for the cyt *b* and ApoB data sets, respectively. Analysis for the combined data set (cyt *b* + ApoB) was done on a partitioned model, where each gene was allowed to have specific model parameters. These combined analyses were performed on a slightly reduced set of specimens, as not all were sequenced for both genes (see Appendix C). Bayesian analyses were performed with one million generations implementing Metropolis-Coupled Markov Chain Monte

Carlo (MCMCMC) with trees sampled every 1000 generation. Tree parameters reached stationarity after a burn-in period of about 200,000 generations. Posterior probabilities (PP) were calculated on the remaining sampled trees. Best ML trees were obtained with RAXML v. 7.0.3 (Stamatakis, 2006) using the GTR GAMMA model in all analyses and genes were analyzed separately or in combination with a partitioned model. One thousand bootstrap replicates were performed to estimate confidence support to the ML tree nodes.

3.3 RESULTS

3.3.1 Morphological variation analysis

Principal component analysis (PCA) was carried out on 42 individuals collected from various parts of Peninsular Malaysia such as Wang Kelian, Maxwell Hill, Cameron Highlands, Fraser Hill, Ulu Gombak, Bukit Rengit and Endau-Kluang (Figure 3.3). These samples are presented in Figure 4 as two distinct altitudinal groups corresponding to the highland (1160-1460 m, filled circles) and lowland (55-300 m, hollow circles) locations, respectively. The first two principal components explained a total of 61% of the total variance. In the first axis, (factor 1: 50% of the total variance), most variables (e.g. GLS, ROL, CTG and IM3S) showed similar negative loadings (see Appendix B) and indicate that this factor is certainly correlated with general size of the samples. The second axis (factor 2: 10%) is most correlated with M3B, IOB and ROB, i.e. expressing broadness of the skull. In a projection of skull measurements onto the first two components (Figure 3.3), specimens collected in highland areas (i.e. above 1100 m) tend to be larger than those from the lowlands, but both altitudinal groups overlap broadly. This trend is confirmed by a modest ($r = -0.57$) but highly significant

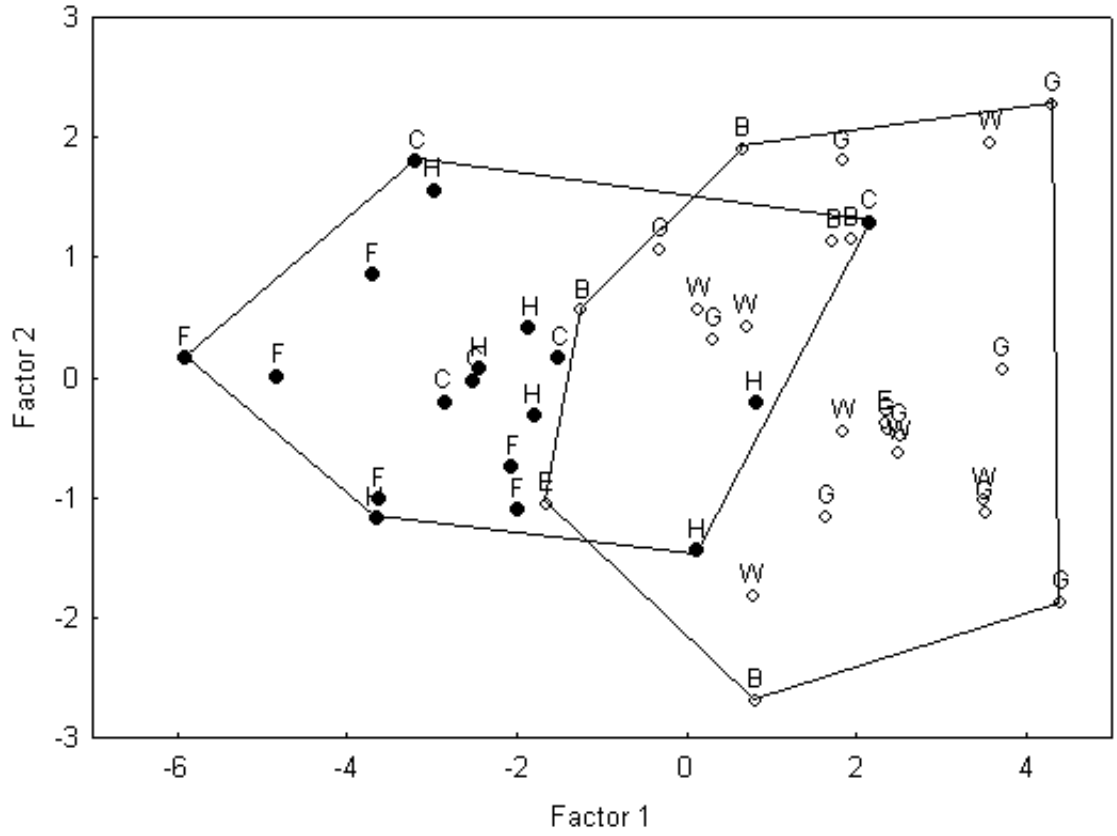


Figure 3.3 Plot of the first and second principal components of the analysis based on 42 skulls of *C. monticola* from Peninsular Malaysia. Samples from highland areas (above 1100 m a.s.l.) are marked with filled circle (●), while those from the lowlands (below 300 m a.s.l.) are represented by hollow circle (○). Letters correspond to samples from the following localities: Cameron Highlands (C), Maxwell Hill (H), Fraser Hill (F), Bukit Rengit (B), Endau-Kluang (E), Ulu Gombak (G) and Wang Kelian (W). See Table 1 for details of altitudinal range.

($p > 0.0001$) negative correlation between PC1 scores of skulls and altitude of the sampling sites. No other subdivision or trend is apparent on this multivariate morphospace.

The second PCA was conducted on the whole sample of 77 skulls of *monticola* shrews from the Sundaland and Komodo. The first two axes of this second PCA explained 71% of the total variance. Factor 1 alone explained 60% of the variance with 14 skull measurements loading heavily and negatively on that factor 1, suggesting again that it represents primarily a size axis. Factor 2 (11% of the total variance) is negatively correlated with ROB, M3B and PPD (see Appendix B), thus expressing broadness of the rostrum and medial parts of the cranium. On a projection of these two components (Figure 3.4), *C. maxi* from eastern Java and Komodo are clearly distinct on the second axis, indicating that this species has a relatively narrower skull when compared to *C. monticola*. Two individuals from Sumatra and assigned to "*C. maxi*" by Esselstyn *et al.* (2009) without comments did not coincide morphologically with other *C. maxi* sampled in Java and Komodo, but grouped together with *C. monticola*, notably with specimens from Kalimantan, Borneo (Figure 3.4). Within *C. monticola*, shrews from Sumatra (S) and Kalimantan (K) tend to be smaller (i.e. have positive loadings on factor 1; Figure 3.4) than those collected in Peninsular Malaysia (P). This trend is also evidence by their smaller mean dimensions (both external and skull) in univariate comparisons with populations from elsewhere in the Sundaland (Table 3.2 and 3.3).

3.3.2 Phylogenetic relationships

A total of 18 sequences of the mitochondrial gene (cyt *b*) and 13 sequences of the nuclear gene (ApoB) were obtained and deposited in GenBank under accession numbers

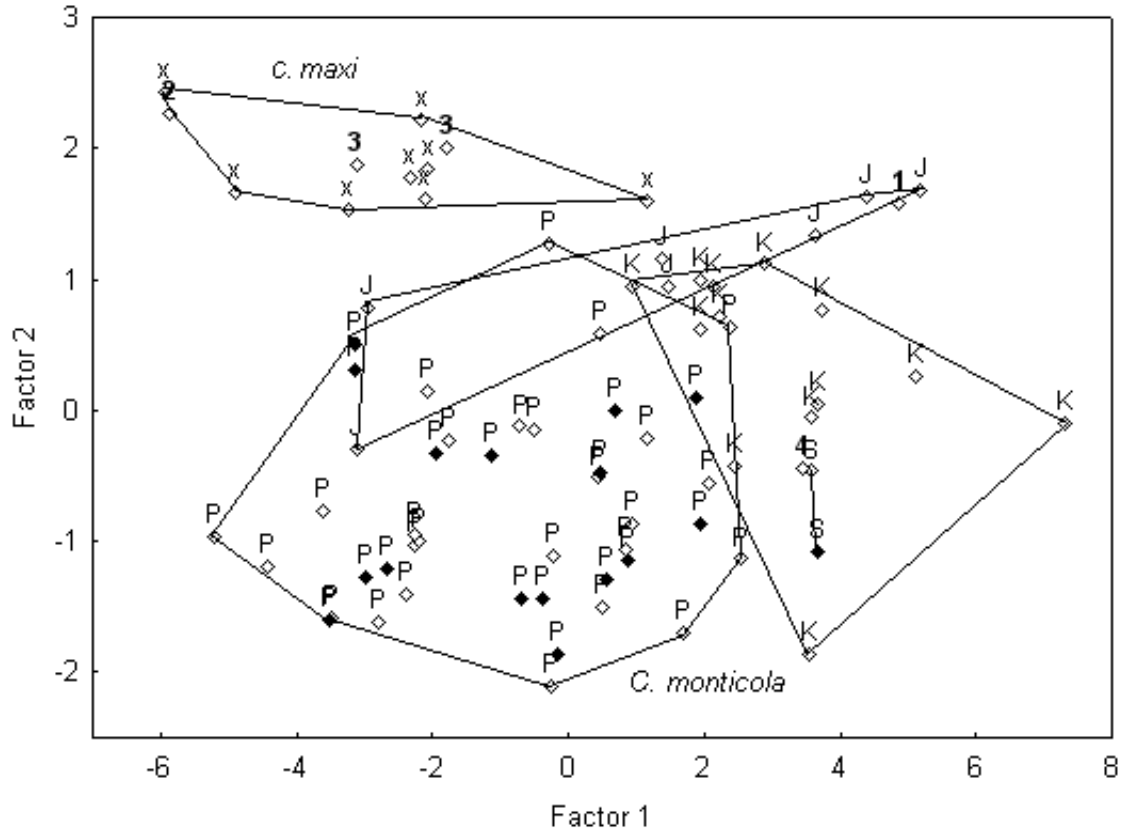


Figure 3.4 Projection of the first two principal components of an analysis based on 14 skull measurements of 77 shrews from the *C. monticola* species complex. These shrews include the holotype of *C. bartelsii* from Java (**1**), the holotype of *C. maxi* from Java (**2**), 2 specimens of *C. maxi* from the Lesser Sundas (Kitchener *et al.*, 1994) (**3**) and one *C. monticola* from Thailand (**4**). Other samples of *C. maxi* from Java are represented by X. Specimens of *C. monticola* from Java are represented by J, from Kalimantan by K, from Peninsular Malaysia by P and from Sumatra by S. Filled diamonds indicate samples also used in genetic analyses, while hollow diamonds those used only in morphological comparisons.

JX162650 - JX162667 and JX162668 - JX162680, respectively (Appendix C). The alignment (1140 bp) for the cyt *b* data set of these sequences and those downloaded from GenBank (Appendix C) represented 707 conserved and 433 variable sites, of which 346 were parsimony-informative. The alignment (524 bp) of the ApoB data set consisted in 469 conserved and 55 variable sites, of which 18 were parsimony-informative.

Phylogenetic reconstructions based on the cyt *b* gene (Figure 3.5) or on a combination of cyt *b* and ApoB genes (Figure 3.6) are similar and both strongly support (100% posterior probability or bootstraps) a monophyletic origin of Sundaland *C. monticola*. Other small shrews from continental Southeast Asia, such as *C. indochinensis* or *C. wuchihensis* from Vietnam and China (see Bannikova *et al.*, 2011), or those from Sulawesi [represented here by *C. musseri* or *C. nigripes*; see Ruedi *et al.* (1998) or Esselstyn *et al.* (2009) for a more complete picture of Sulawesian shrews belong to other, unrelated groups in the *Crocidura* radiation. The sister-group relationship of *C. monticola* is not determined, as phylogenetic reconstructions of deeper nodes lack resolution (Figures 3.5 and 3.6). Within this clearly monophyletic clade, the various samples of *C. monticola* from Peninsular Malaysia are not each group as closely related. Two specimens from the central regions (Ulu Gombak (6) and Bukit Rengit (5); Figure 3.2) appear more closely related to shrews from Sumatra and Borneo, than shrews from the northern regions of the Thai-Malay peninsula (Figures 3.5 and 3.6). Likewise, the two shrews collected in different parts of the Kalimantan province in Borneo are not each other's closest relatives on the cyt *b* reconstruction (Figure 3.5), although their exact position within the *C. monticola* radiation is not firmly established (low posterior probability or bootstrap support).

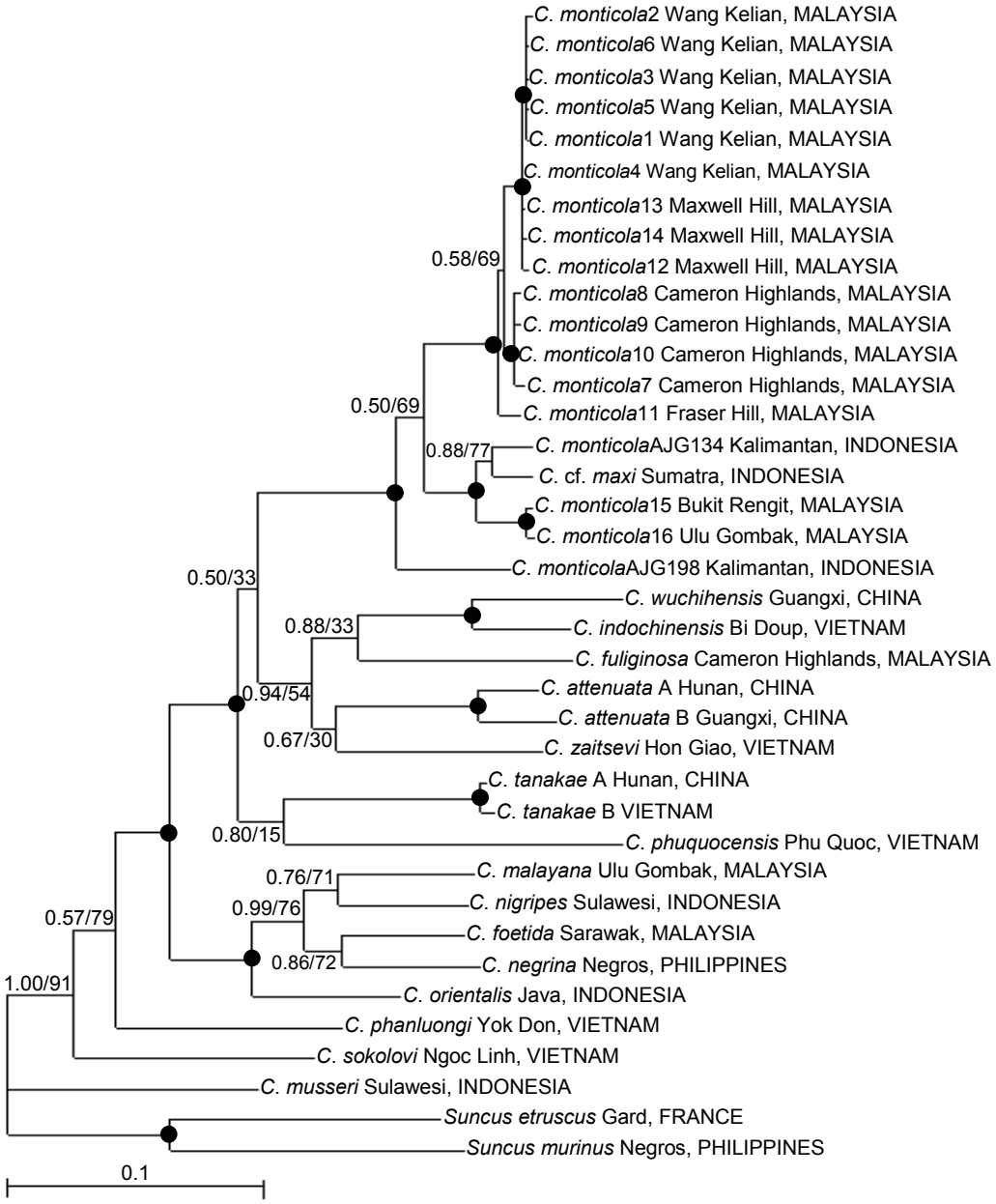


Figure 3.5 Bayesian consensus tree illustrating phylogenetic relationships of Oriental *Crocidura* based on cytochrome *b* data set (1140 bp) and using *Suncus etruscus* and *S. murinus* as outgroups. Nodal support is represented as posterior probabilities and percent ML bootstrap values; a black dot indicates support values higher than 95% for both methods.

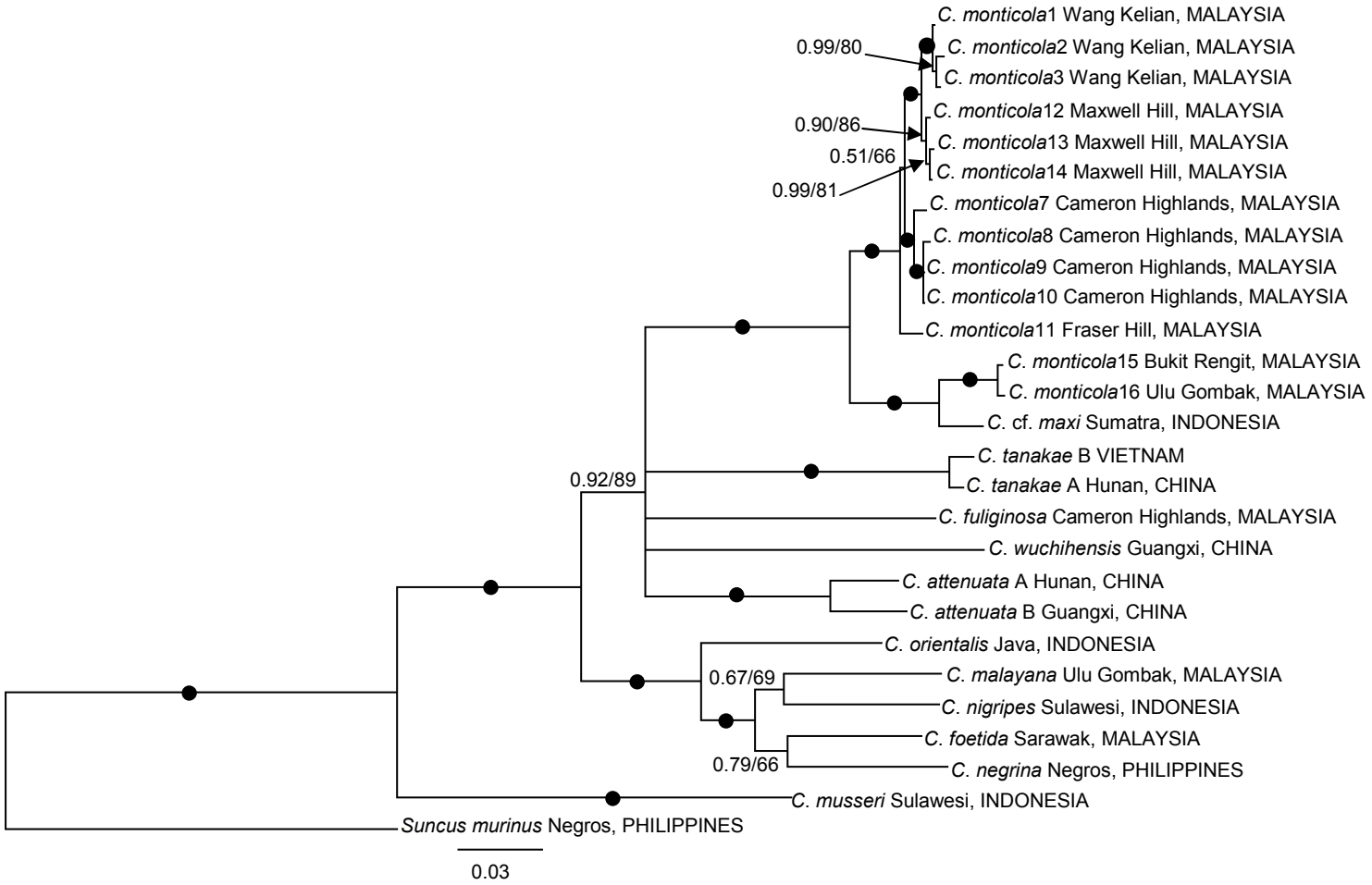


Figure 3.6 Bayesian consensus tree illustrating phylogenetic relationships of Oriental *Crocidura* species based on an alignment combining 1140 base-pairs (bp) of cytochrome *b* and 524 bp of Apolipoprotein B genes. *Suncus murinus* were used as outgroups. Nodal support is represented as posterior probabilities and percent ML bootstrap values; a black dot indicates support values higher than 95% for both methods.

These general relationships among samples of *C. monticola* are also reflected in the comparisons of pairwise K2P genetic distances measured among representatives of each location (Table 3.4). Three classes of genetic distances can be distinguished. First, various individuals trapped in the same locality have virtually identical cyt *b* sequences with maximum divergence of 0.7%. However, in the next distance class, shrews differ by up to 3.4% can be found in various locations within northern Peninsular Malaysia (i.e. among those trapped in Wang Kelian, Maxwell Hill, Cameron Highlands or Fraser Hill), or in a group of shrews from central Peninsular Malaysia, Sumatra and Kalimantan. The third level of genetic differentiation, at about 7% K2P distance, separates the later two groups from each other, or distinguishes the two lineages of *C. monticola* found on Kalimantan (Borneo). Other small, unrelated shrews such as *C. wuchihensis* from Guangxi, China diverged to 13% and over K2P distance.

3.4 DISCUSSION

Recent studies on the biogeography of *Crocidura* shrews from Southeast Asia (Heaney & Ruedi, 1994; Ruedi, 1996; Ruedi *et al.*, 1998; Esselstyn *et al.*, 2009; Jenkins et al., 2009; Esselstyn & Oliveros, 2010; Bannikova *et al.*, 2011) showed that all species have restricted distributions in the Malay Archipelago or in the Indochina peninsula, with very few species found on multiple islands or on both sides of the Isthmus of Kra. Apparently, a singular exception is for *C. monticola* that is widely distributed across islands of the Sundaland (Jenkins, 1982), and perhaps also for *C. maxi* (Kitchener *et al.*, 1994). It is however unclear if such extensive, inter-island distributions are due to inadequate taxonomic considerations, or if a single taxon would really have reached several islands.

Table 3.4 Kimura-2 parameter distances (%) at the cytochrome *b* gene among individuals of the *Crocidura monticola* species complex, with *C. wuchihensis* serving as unrelated outgroup.

	Sample	1	2	3	4	5	6	7	8	9	10
1	*C. monticola* (Wang Kelian)	-									
2	*C. monticola* (Maxwell Hill)	0.6	-								
3	*C. monticola* (Cameron Highlands)	1.7	1.4	-							
4	*C. monticola* (Fraser Hill)	1.9	1.6	1.4	-						
5	*C. monticola* (Bukit Rengit)	6.7	6.5	6.6	6.4	-					
6	*C. monticola* (Ulu Gombak)	6.8	6.6	6.7	6.5	0.3	-				
7	*C.* cf. *maxi* (Sumatra)	6.1	5.8	6.1	6.1	3.3	3.4	-			
8	*C. monticola* A (Borneo)	6.8	6.6	6.8	6.5	3.2	3.1	2.7	-		
9	*C. monticola* B *(*Borneo*)*	7.7	7.6	7.7	7.4	6.9	6.8	6.6	6.6	-	
10	*C. wuchihensis* (Guangxi)	14	14	14	14	14	14	14	13.9	13.9	-

On taxonomic perspective, this study provides further support that the skull morphology of *C. maxi* from east Java and Komodo and *C. monticola* from Sundaland are distinct (Figure 3.4), hence, both taxa are certainly represent valid and separate species. Both species differ on external morphology and certain skull characters. *Crocidura maxi* has larger mean body size, hind foot and tail lengths, as well as in mean skull dimensions such as skull length, tooth row length, or breadth of braincase (Tables 3.2 and 3.3). The current geographic sampling in Java is inadequate to infer precise relationship between *C. monticola* and *C. maxi*. It is unclear whether these species have have overlapping or parapatric distributions on the island, or if these differences are due to altitudinal variation. Multivariate analyses showed that *C. monticola* from Peninsular Malaysia tend to have larger skulls with increasing altitude (Figure 3.3) with no significant sexual dimorphism. On contrary, populations of *C. maxi* from Flores showed no size variation due to altitude, but males were significantly larger than females (Kitchener *et al*., 1994). It is therefore difficult to extrapolate general trends in morphological variation of *C. monticola* and *C. maxi* throughout their distributional range without dedicated altitudinal or latitudinal transects.

According to the multivariate morphological assignment of the examined skulls (Figure 3.4), it is confirm that specimens identified as *C. monticola* have a widespread distribution covering most of the Sunda Shelf, from central Java and Borneo, west to Sumatra and the Thai-Malay peninsula (see details of locations in Appendix A). Kitchener *et al.* (1994) showed that *C. maxi* is also widely distributed from east Java, eastward to the Lesser Sunda Islands and Aru (Moluccas). Both species of *Crocidura* are therefore indeed distributed on several islands, in line with previous morphological studies (Jenkins, 1982), but *C. monticola* is apparently restricted to continental islands of the Sundaland, while *C. maxi* reached many oceanic islands. This may underlie

unequal dispersal abilities of these shrews, regardless whether inter-island colonization have been natural, or facilitated by humans. Significant morphological and genetic variations (at allozyme loci) among insular populations of *C. maxi* were reported by Kitchener *et al.* (1994). It is suggested that these populations were evolved in isolation for extended periods, a situation similar to other wild shrews (*Crocidura* spp.) studied in the Philippines archipelago (Esselstyn & Brown, 2009). This pattern of relatively high genetic diversity is therefore very distinct from that of typically anthropophilic shrews such as *Suncus murinus* or *S. etruscus*, which have been widely translocated passively by humans (Hutterer & Tranier, 1990; Omar *et al.*, 2011). These commensal species show minimal allozyme or molecular variation over large geographic areas, including on oceanic islands (Ruedi *et al.*, 1996; Omar *et al.*, 2011), unlike in *C. monticola* or *C. maxi* (Kitchener *et al.*, 1994).

Phylogenetic reconstructions based on a mitochondrial DNA (Figure 3.5) or a combination of a mitochondrial DNA and nuclear gene (Figure 3.6) clearly supports a monophyletic origin of all *C. monticola* sampled in the Sundaland, it also suggests that other similar-sized shrews from continental Southeast Asia or Sulawesi are unrelated. Unfortunately, genetic material from Java, the terra typica of *maxi* and *monticola*, or from the Lesser Sunda Islands was unavailable to place *C. maxi* in this phylogenetic radiation and confirm its species status from a genetic perspective. The cyt *b* and ApoB sequences deposited in GenBank by Esselstyn *et al.* (2009) and labeled "*C. maxi*" (specimen from Bukit Lawang, Sumatra, MVZ 192178; see Appendix A and Appendix C), appear within the *C. monticola* clade in all reconstructions (Figures 3.5 to 3.6), which is in line with the multivariate analyses of the skull morphology of this specimen (Figure 3.4). Thus current morphological and molecular evidences suggest that this record of a putative *"maxi"* from Sumatra should best be assigned to *C. monticola*, or to

C. neglecta, if this poorly known, endemic taxon from Sumatra (Ruedi, 1995) proves to be different from Javan *monticola*. Again, stronger taxonomic conclusions await direct comparisons with reference molecular sequences from the type localities of these taxa.

The phylogeography of *C. monticola* in the Sunda shelf, as inferred from mitochondrial lineages, is also more complex than anticipated. Two samples from Kalimantan (Borneo), and Peninsular Malaysia showed unexpectedly large genetic differences at the cyt *b* gene (up to 6.8% K2P distance; Table 3.4), which is unusual for intra-specific comparisons in mammals (Bradley & Baker, 2001), or in shrews (Ruedi *et al.*, 1998; ; Dubey *et al.*, 2006; Bannikova *et al.*, 2011).

Phylogenetic reconstructions show for instance a closer relationship between shrews from central Peninsular Malaysia (localities 5 and 6 in Figure 3.2) and Sumatra (plus Borneo) than with specimens collected less than 100 km to the north (localities 1 to 4) Malay peninsula (Figures 3.5 and 3.6). This pattern of relationship is not supported by variations of the ApoB gene analyzed alone (results not shown), or by the PCA analyses on skull morphology (Figures 3.3 and 3.4). It is unclear if mitochondrial differences underlie cryptic taxonomic diversity in *C. monticola*, or reflect historical movements of ancient lineages. Range-shifts of populations during sea-level changes of the Pleistocene affect a number of species in the Sunda shelf (see e.g. Hewitt, 2000; Esselstyn & Brown, 2009; Esselstyn *et al.*, 2009). It is therefore possible that populations of *C. monticola* that evolved in isolation during high sea-levels of interglacial periods were temporarily connected when lowered sea-levels exposed dry land between islands of the Sunda shelf (Bird *et al.*, 2005) which favored secondary exchanges of mitochondrial lineages. These past connections may explain both the low divergence of lineages among samples from Sumatra, central Peninsular Malaysia and

Borneo, and the coexistence of divergent lineages within the Thai-Malay peninsula or within Borneo. On the other hand, large rivers and corridors of dry savannah dissected the exposed lands of the Sunda shelf during cooler periods of Pleistocene (Bird *et al.*, 2005). The impact of these potential ecological barriers on inter-island colonization by shrews is unknown, as small *Crocidura* species are associated with to a variety of habitats ranging from forests to dry savannah (Kitchener *et al.*, 1994). Clearly, extensive sampling of shrews from southern parts of Peninsular Malaysia, Borneo, Sumatra and Java are needed to understand full phylogeographical picture of the evolution of *C. monticola* in the Sundaland.

CHAPTER 4

PHYLOGENETIC RELATIONSHIPS OF MALAYAN AND MALAGASY PYGMY SHREWS OF THE GENUS *SUNCUS* (SORICOMORPHA: SORICIDAE) INFERRED FROM MITOCHONDRIAL CYTOCHROME *B* GENE SEQUENCES

4.1 INTRODUCTION

Due to their extreme small size (about two grams), pygmy shrews of the genus *Suncus* (Family Soricidae) are difficult to trap and are therefore poorly represented in reference collections, which in turn has added to ongoing taxonomic confusion regarding species limits within the genus. For instance, *S. malayanus* (Figure 4.1), the principal focus of this study, was initially named *Pachyura malayana* by Kloss (1917) and subsequently considered a subspecies of the Etruscan shrew *S. etruscus* (Savi, 1822) under the name *S. etruscus malayanus* (see Medway, 1978). Ellerman & Morrison-Scott (1966) and Corbet & Hill (1992), with some reservation, considered all pygmy shrews that occur in the Indomalayan region as a part of a widespread species, *S. etruscus*, originally described from specimens collected in Italy. Chasen (1940) and Corbert & Hill (1992) including recent review on Southeast Asian mammals (Francis, 2008), have adopted this view. However, Hutterer (2005) recognized four additional, geographically restricted species in the *etruscus* group: *S. fellowesgordoni* (Phillips, 1932) from the highlands of Sri Lanka, *S. malayanus* from the Thai-Malay peninsula, *S. hosei* (Thomas, 1893) from Borneo and *S. madagascariensis* (Coquerel, 1848) from Madagascar. These forms are slightly differ in morphology from European *S. etruscus* but live in very different habitats, therefore were considered endemic to their specific regions (Hutterer, 2005).

Figure 4.1 Male Malayan Pygmy Shrew (*Suncus malayanus*) captured in the Cameron Highlands, Pahang, Malay Peninsula, in a pitfall trap set on the forest floor. Notice the characteristic of large ears and dark fine pelage.

Molecular phylogenetic techniques have been employed to clarify aspects of the systematic relationships among other groups within the family Soricidae and subfamily Crocidurinae (e.g. Jenkins *et al*., 1998; Ruedi *et al*., 1998; Motokawa *et al*., 2000; Quérouil *et al*., 2001; Ohdachi *et al*., 2004; Dubey *et al*., 2007a; Meegaskumbura & Schneider, 2008; Meegaskumbura *et al*., 2010). These studies have shown that within the Crocidurinae the genus *Suncus* is paraphyletic to genera *Sylvisorex* and *Crocidura* (Quérouil *et al*., 2001; Dubey *et al*., 2007a), with *Suncus etruscus* basal to other Asian *Suncus* and the remaining members of the Crocidurini tribe (Dubey *et al*., 2008). Large divergence among populations of this genus formally classified as a single taxon (e.g. *S. murinus*; Meegaskumbura & Schneider, 2008), suggests that additional cryptic taxonomic diversity exists. Although adding to systematic knowledge of the group, these studies have generally not addressed the relationships among members of Asian pygmy shrews including *S. malayanus*.

This study presents an analysis on mitochondrial DNA sequence data to determine phylogenetic relationships and genetic distances between *S. malayanus*, *S. madagascariensis* and *S. etruscus*. The results have clarified taxonomic status of pygmy shrews and documented new occurrences of *S. malayanus* at three sites across the Thai-Malay peninsula, including one from a montane location.

4.2 MATERIALS AND METHODS

4.2.1 Sample collection

Samples of *Suncus malayanus* (Figure 4.1) were collected from three locations in Peninsular Malaysia: Ulu Gombak (N03^{o}60.170' E101^{o}60.46', altitude 200 m), Bukit Rengit (N03^{o}35.987' E102^{o}10.758', altitude 132 m) and Cameron Highlands (N04^{o}28.460' E101^{o}23.05', altitude 1465 m). The former two locations are covered by extensive secondary lowland evergreen forests, while the latter location is covered by montane forests surrounding tea plantations. Shrews were captured by 50 - 100 unbaited pitfall traps, set within a 45 x 45 m grid. All individuals were immediately euthanized after capture using chloroform. Sex and external body measurements were recorded, and the specimens were either prepared as flat skins or fixed in 5% formalin and subsequently transferred to 70% ethanol. Kidney samples were removed soon after death and stored at -80^{o}C or preserved in 95% ethanol for genetic analysis. The voucher specimens are housed at the Zoological Department (Institute of Biological Sciences), University of Malaya. The specimens of *S. madagascariensis* were collected in the dry deciduous forest of Beanka, near Maintirano (S18°03.75' E44°31.5'E, altitude 320 m) with the use of pitfall traps. Muscle tissue samples from these animals were preserved in EDTA. In phylogenetic analysis, other *Suncus* samples were also employed in this study and the genus *Myosorex* was used as the outgroup (see below and Table 4.1).

4.2.2 DNA extraction, PCR amplification and sequencing

DNA was extracted from preserved tissue samples using Promega Wizard extraction kits following the manufacturer's protocol (Promega Co.). Polymerase chain reaction

Table 4.1 List of all *Suncus* and *Myosorex* (outgroup) specimens that were used in this study with information on collection site, country and GenBank accession number. The numbers given after the species names are associated with the sample numbers of the different taxa and are used in Table 2.

Species	Collection site (Locality)	Country	GenBank accession number (cytochrome *b*)	Reference
*Suncus malayanus*1	Ulu Gombak (Selangor)	Malaysia	JF817391	This study
*S. malayanus*2	Cameron Highlands (Pahang)	Malaysia	JF817392	This study
*S. malayanus*3	Bukit Rengit (Pahang)	Malaysia	JF817393	This study
*S. madagascariensis*1	Beanka Forest	Madagascar	JF817394	This study
*S. madagascariensis*2	Beanka Forest	Madagascar	JF817395	This study
*S. etruscus*1	Anuradhapura	Sri Lanka	FJ716836	Meegaskumbura & Schneider (2008)
*S. etruscus*2	Fivizzano	Italy	DQ630397	Dubey *et al*. (2007)
*S. etruscus*3	Camargue	France	DQ630396	Dubey *et al*. (2007)
S. etruscus4	Gard	France	JF817396	This study
*S. montanus*1	Agarapathana	Sri Lanka	GQ290378	Meegaskumbura & Schneider (2008)
*S. montanus*2	Morningside	Sri Lanka	FJ716835	Meegaskumbura & Schneider (2008)
S. niger (previously analysed as *montanus*)	Nilgiri Hills	India	DQ630388	Dubey et al. (2007)
*S. murinus*1	Negros Island	Philippine	FJ813963	Esselstyn *et al*. (2009)
*S. murinus*2	-	India	EU122224	Meegaskumbura & Schneider (2008)
S. remyi	Moueva	Gabon	DQ630399	Dubey *et al*. (2007)
S. dayi	Nilgiri Hills	India	DQ630432	Dubey *et al*. (2007)
*S. stoliczkanus*1	-	Nepal	AB175077	Ohdachi *et al*. (2004)
*S. stoliczkanus*2	-	Nepal	AB175076	Ohdachi *et al*. (2004)
S. varilla	-	South Africa	DQ630434	Dubey *et al*. (2007)
Myosorex cafer	Serola	South Africa	FJ814024	Dubey *et al*. (2007)
M. sclateri	Natal	South Africa	FJ814043	Dubey *et al*. (2007)

(PCR) amplification of the target fragment of the mitochondrial cyt *b* gene was performed using primers L14724 and H15915 (Irwin *et al*., 1991). 25 µl PCR reactions contained 5 µl of DNA template (~20 ng/µl), 2.5 µl of each primer (10 mM), 0.5 µl of dNTP (10 mM), 2.0 µl of $MgCl_2$ of (25 mM), 2.5 µl of 5x colorless GoTaq ® Flexi buffer and 0.2 µl of GoTaq DNA Polymerase (5 u/µl). PCR conditions were as follows: 40 cycles of denaturation at 93^{o} C for 45 s, annealing at 45.9^{o} C for 45 s, extension at 72^{o}C for 1 min, with a final extension at 72^{o} C for 5 min. PCR products were electrophoresed on a 1% agarose gel then visualized with ethidium bromide staining to check success of PCR amplification. PCR products were gel purified using Promega Wizard SV Gel and PCR Clean up System with microcentrifuge (Promega Co.). Cleaned amplicons were sequenced in both directions using the same primers (as in PCR amplification) by a commercial laboratory (First Base Co., Selangor, Malaysia) using a BigDye ® Terminator v3.0 Cycle Sequencing Kit with products run on an ABI 3730 automatic sequencer.

4.2.3 Sequence alignment and phylogenetic analyses

New sequence data from the cyt *b* gene of three *S. malayanus* from Malaysia, two *S. madagascariensis* from Madagascar and one *S. etruscus* from France were obtained in the current study. The sequences were edited using Chromas version 1.45 (MacCarthy, 1996). Another 13 *Suncus* cyt *b* sequences, representing additional samples of *S. etruscus* from France and Italy and a further six *Suncus* species were compiled from GenBank records, along with cyt *b* sequences for two outgroup taxa, *Myosorex cafer* and *M. sclateri* (Table 4.1). The initial 270 base-pairs of the sequences of *S. etruscus* from Italy deposited by Dubey *et al*. (2007a) under GenBank number DQ630397 were omitted because of unreliable readings. Multiple alignments of all sequences were

performed with ClustalX 1.81 (Thompson *et al*., 1997). Missing nucleotides in partial sequences were placed by N in the final alignment. Estimates of pairwise genetic distance among cyt *b* gene fragments from the different forms of pygmy shrew were calculated using the Kimura two-parameter (K2P) model (Kimura, 1980) implemented in MEGA 4 software (Tamura *et al*., 2007).

Phylogenetic analyses were conducted to illustrate the level of divergence and relationships among all sampled taxa. Trees were constructed by using neighbour-joining (NJ) and Bayesian analysis (BA) methods. All trees were rooted with *Myosorex* spp. as the outgroup. The NJ analysis was conducted using MEGA 4 (Tamura *et al*., 2007) with the K2P distance matrix and uniform rates across sites. Bootstrap analysis used 1000 replications to compute 50% majority rule consensus trees for node confidence limits (Felstenstein, 1985). For BA phylogenetic analysis, trees were constructed using the TrN + I + G model of DNA evolution that best fits data according to the protocol of Posada & Crandall (1998) implemented in Modeltest version 3.7. BA was performed in MrBayes version 3.0 (Huelsenbeck & Ronquist, 2001) with one million generations implementing Metropolis-Coupled Markov Chain Monte Carlo (MCMCMC) with trees sampled every 1000 generation. Tree parameters reached stationary distributions after a burn-in period of 100,000 generations. Posterior probabilities (PP) were calculated for each node as the percentage of sampled trees containing the node in question.

4.3 RESULTS

A total length of mitochondrial sequences (between 1013 from the three Malayan samples) and (1140 base-pairs from the single French and two Malagasy samples) was obtained from cyt *b* gene, which are deposited in GenBank under numbers JF817391 - JF817396. All 21 sequences used in the phylogenetic reconstructions and distance calculations represented 10 putative *Suncus* and two *Myosorex* species (Table 4.1). The final alignment represented 704 conserved sites, with 366 of the 436 variable sites proved to be parsimony-informative. Corrected (K2P) pairwise genetic distances among the pygmy *Suncus* are shown in Table 4.2, with the African Pygmy Shrew, *S. remyi*, also included for comparison as a distant relative (Dubey *et al*., 2008). They reveal relatively high inter-specific genetic distances (6.1 - 8.5%) between *S. malayanus* and *S. madagascariensis* or *S. etruscus*, but much greater distances when compared to *S. remyi* (23.6 – 24.0%). Among the three *S. malayanus* sequenced, the two lowland individuals from Bukit Rengit and Ulu Gombak were more closely related to each other (0.3% K2P divergence), than they were to the individual from the Cameron Highlands (1.1 and 1.4% divergence). Intra-specific divergences among *S. etruscus* individuals collected in Europe were minimal (0.1-0.4% which corresponds to one to three point mutations), but higher (up to 3.6% or 39 point mutations) when the sample from Sri Lanka was included. This distance is comparable to the divergences between European samples of *S. etruscus* and *S. madagascariensis* (3.0-3.3%). The lowest inter-specific divergence (1.4-1.5%) was observed between the Sri Lankan *S. etruscus* and Malagasy *S. madagascariensis*, identifying a close genetic distance between these individuals.

Table 4.2 Percent pairwise corrected (K2P) genetic distance among the pygmy shrews *Suncus malayanus*, *S. madagascariensis*, *S. etruscus* and *S. remyi* cytochrome *b* sequences.

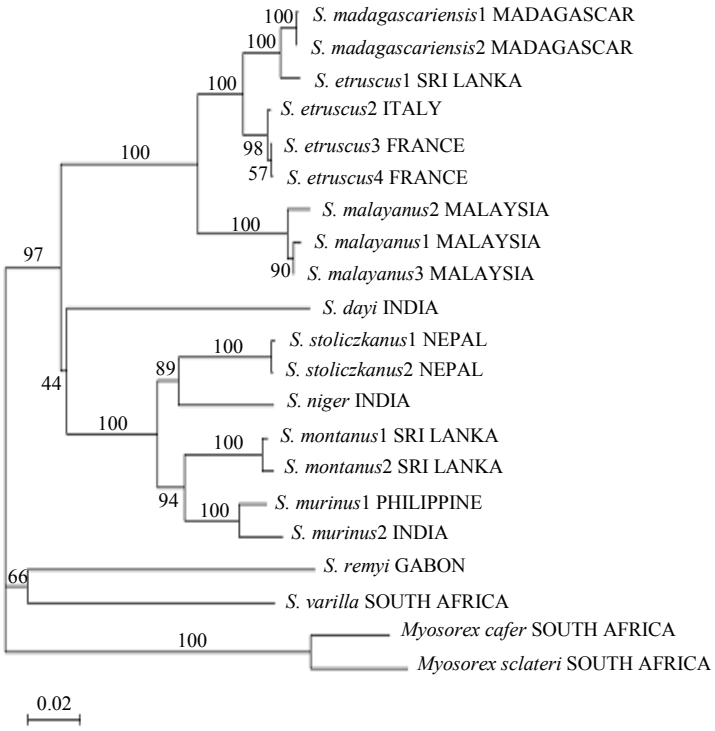
	Sample	1	2	3	4	5	6	7	8	9
1	*S. malayanus*1 Malaysia	-								
2	*S. malayanus*2 Malaysia	1.4	-							
3	*S. malayanus*3 Malaysia	0.3	1.1	-						
4	*S. madagascariensis*1 Madagascar	8.0	8.5	7.7	-					
5	*S. madagascariensis*2 Madagascar	8.0	8.5	7.7	0.1	-				
6	*S. etruscus*1 Sri Lanka	7.6	8.0	7.3	1.5	1.4	-			
7	*S. etruscus*2 Italy	6.6	6.9	6.1	3.3	3.3	3.4	-		
8	*S. etruscus*3 France	6.9	7.3	6.4	3.1	3.1	3.3	0.4	-	
9	*S. etruscus*4 France	7.1	7.6	6.8	3.1	3.0	3.6	0.3	0.1	-
10	*S. remyi* Gabon	24.0	23.9	23.6	23.5	23.5	23.3	21.7	21.1	21.8

The phylogenetic trees constructed using the two methods (NJ and BA) were largely congruent (Figure 4.2). Both analyses identified a major clade among *Suncus* corresponding to all members of this genus sampled with Eurasian/Madagascar distributions, and excluding the African species *S. remyi* and *S. varilla*. Within the Asian clade, two groups were resolved, corresponding to the pygmy and the larger *Suncus* species. The monophyletic pygmy shrew clade contained *S. madagascariensis*, *S. etruscus* and *S. malayanus* and was well supported, with a BA posterior probability of 1.0 and 100% bootstrap support in the NJ analysis. Within this clade, the monophyly of *S. malayanus* was well supported. Both analyses indicated that *S. etruscus* is paraphyletic, with the Sri Lankan *S. etruscus* consistently sister to *S. madagascariensis*, reflecting the low divergence observed between these populations in pairwise estimates.

4.4 DISCUSSION

The phylogenetic reconstruction presented here demonstrates that pygmy shrews from Italy, France, Sri Lanka, Madagascar and Malaysia form a monophyletic clade to the exclusion of other Asian and African shrews of the genus *Suncus*. Although the taxon sampling in southeastern Asia is still incomplete, it is confirmed that the morphological resemblance among Eurasian taxa included in the *S. etruscus* species complex (sensu Corbet & Hill, 1992) is also evidence at the molecular level. This results show that Eurasian *S. etruscus* are genetically closely related to *S. madagascariensis*, but not to *S. malayanus*. Aside from the pygmy shrews, the other relationships identified herein among *Suncus* species are in agreement with earlier findings, such as close relationships between *S. murinus* and *S. montanus* (Meegaskumbura & Schneider, 2008), and the distinct position of *S. niger* relative to other large *Suncus* (Meegaskumbura *et al*., 2010). In addition, the separation of the African and Eurasian

A)



B)

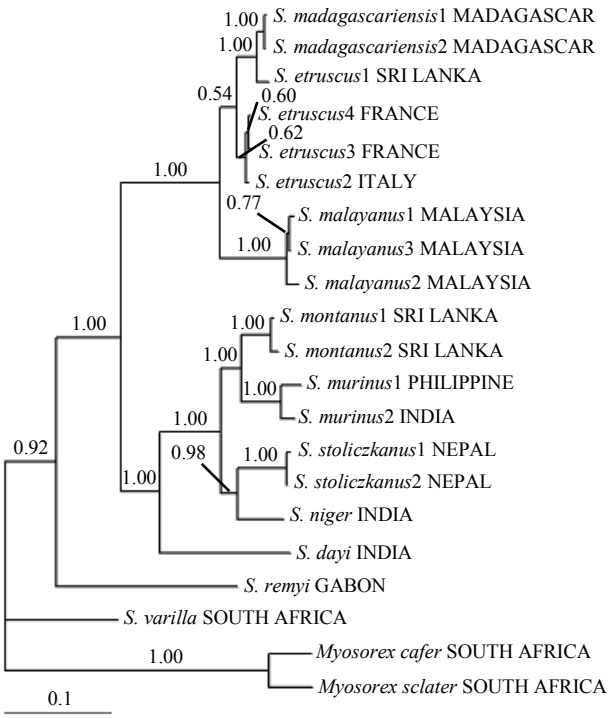


Figure 4.2 The neighbour-joining (A) and Bayesian (B) trees for *Suncus* inferred from 1140 base-pairs of cytochrome *b* gene sequence. Bootstrap and posterior probability values are given above branches.

taxa into two clades (Figure 4.2A) mirrors the relationships observed by Dubey *et al*. (2008) based on a more comprehensive taxonomic and larger character sampling of Crocidurinae shrews.

Previous studies of divergence in the cyt *b* gene of mammals in general (Bradley & Baker, 2001) or Crocidurinae in particular (Ohdachi *et al*., 2004; Dubey *et al*., 2006) have found that divergences of between 1.5 and 2.4% indicate intra-specific variation, while divergences of 7.5% or greater are present at the inter-specific level (see also Ruedi *et al*., 1998; Meegaskumbura & Schneider, 2008). Although relying on a genetic species concept based solely on mitochondrial DNA to classify samples of uncertain taxonomic rank can be problematic (Ferguson, 2002), this approach is still useful when the morphology of the taxa being compared is quite conserved, as is the case in pygmy shrews.

The notably low "inter-specific" divergence of 1.4% between *S. madagascariensis* and Sri Lankan *S. etruscus* was significantly less than the intra-specific divergence present among the most distinct *S. etruscus* individuals (up to 3.6% K2P distance; Table 4.2). This indicates that *S. madagascariensis*, that is treated as endemic to Madagascar (Hutterer, 2005), and *S. etruscus* should not be considered as separate species and that the former is a junior synonym of the latter. Furthermore, shrews originally identified as *S. madagascariensis* are more closely related to Asian *S. etruscus* than those from Europe.

It is hypothesize that the Madagascar population was recently colonized the island with founding animals originating from the Indian Subcontinent or Southeast Asia, presumably transported by maritime travellers. The earliest evidence of humans

on Madagascar is slightly more than 2,000 years ago and during the 11th-14th centuries there was extensive maritime movement between portions of Asia and Madagascar (Burney *et al*., 2004). This situation is similar to that of the large, commensal musk shrew (*S. murinus*), where all African and Malagasy populations are considered as recent introductions due to human trade (Hutterer & Tranier, 1990). Unlike the relatively robust musk shrew, however, *S. etruscus* is a notably small species, but given its broad geographical range, it appears to be adapted to a wide range of environmental conditions (see below), and might have survived translocation in, for example, soil associated with the shipment of live commercial plants (tea or coffee) or in boat holds containing invertebrate populations. Elsewhere, in the Mediterranean region, *S. etruscus* is also believed to have reached remote islands though passive human transport (Dobson, 1998). Another potential species of pygmy shrew, *S. coquereli*, is named from Mayotte in the Comores Archipelago in close proximity to Madagascar. Although the tissue samples of this population are lacking, Hutterer (2005) indicated that close genetic similarity exists between this taxa and Malagasy individuals. It is strongly suspect that its status is similar to *S.* "*madagascariensis*" and therefore, *S. coquereli* actually represents another introduced form of *S. etruscus*.

In contrast, the level of genetic divergence present between *S. malayanus* and the other Eurasian pygmy shrews (6.1-8.5%) suggests that Malayan populations have been evolving independently for a relatively long period and are best considered as a distinct species. In addition, *S. malayanus* is ecologically different from its European relative, as it lives in tropical humid forests, including montane habitats (see Figure 4.1 and trapping sites in Materials & Method section), whereas Eurasian *S. etruscus* is adapted to much more xeric and lowland habitats (Spitzenberger, 1990). The Malagasy population of *S. etruscus* occurs in different biomes on Madagascar, ranging from

notably dry habitats to tropical humid forests across an elevational gradient from near sea level to 1200 m (Soarimalala & Goodman, 2011). Hutterer (2005) mentioned possible morphological differences between *S. malayanus* and *S. etruscus*, but besides darker pelage coloration in the former (Figure 4.1), I did not investigate this aspect further.

The three *S. malayanus* individuals used in this study were collected from three different localities within Peninsular Malaysia. The Cameron Highlands site is situated at an elevation of about 1460 m, while the other two locations (Bukit Rengit and Ulu Gombak) are at lower elevations (< 500 m) and within about 65 km distance from each other. Greater genetic similarity was observed between the lowland individuals than among lowland and highland individuals (0.3% vs 1.1 - 1.4% K2P genetic distance). As the highland location is also geographically more distant than the two lowland locations (> 150 km), the greater genetic distance may be associated with isolation by distance (Wright, 1943). Alternatively, the divergence between the Cameron Highlands sample and lowland individuals may indicate that historically, elevation has been an important factor in limiting gene flow among *S. malayanus* populations in this region. In conclusion, more geographically extensive sampling across the Thai-Malay peninsula is needed to ascertain whether either of these hypotheses is true for *S. malayanus* and to unravel its northern and western range limits. Further, samples from northern and central Thailand also need to be included in subsequent studies to investigate the level of divergence between *S. malayanus* and the endemic Borneo pygmy shrew *S. hosei.*

CHAPTER 5

GENERAL DISCUSSION

ARE GENETIC DIFFERENCES AND MORPHOLOGICAL VARIATIONS OF *CROCIDURA MONTICOLA* AND *SUNCUS MALAYANUS* IN PENINSULAR MALAYSIA INFLUENCED BY GEOGRAPHICAL EVOLUTIONARY PROCESS?

Fragmentation of Earth's plates shaped the distribution patterns of biota through the process of splitting and collision of land masses (Cox & Moore, 2005). Southeast Asia archipelagos consist of numerous oceanic islands which had undergone a long and complex geological process. Historical geographic of Southeast Asia was formed by active tectonic occurrence which correlated with two main plate collides; first, between India and Eurasia approximately 50 - 65 mya and second between Southeast Asia and Australia about 15 mya (Hutchison, 1989; Hall, 1998). Southeast Asia regions have been subjected for the study of biological diversification by biogeographers, ecologists and evolutionists where they raised up some questions related to biogeography and evolution (e.g. Darwin, 1859; Wallace, 1860; MacArthur & Wilson, 1967).

The correlation between biogeography and evolution most probably can be explained by the occurrence of land bridge connecting between islands and mainland during ancient period. Drastic climate changes during Pleistocene Epoch with rising and lowering of sea water level have greatly affected the geographical structure of the Sunda shelf (Biswas, 1973). Formation of ice during glaciations causes exposure of land bridge which allowed the inter-connection between mainland and islands that had caused migration and hence mixing of the biota (Heaney, 1986; Hall, 1993; Ruedi & Fumagalli, 1996; Karns *et al*., 2000; Inger & Voris, 2001; Riginos, 2005). This

Pleistocene Epoch (with the fluctuation of climate changes) had major impact on the habitat fragmentation and would be one of the possible forces drove the evolution of new species (Whitmore, 1987; Comes & Kadereit, 1998; Pearson & Dawson, 2003). When sea water level increase after the melting of ice sheets during late Pleistocene, these inter-connected islands were disconnected due to the formation of sea as a physical barrier for the previously inter-connected islands (Heaney, 1985; Voris, 2000; Turner *et al*., 2001). Hence, these four territories of Sumatra, Java, Borneo and Peninsular Malaysia in the present-day have been known as "Sundaland" (Mollengraaff, 1921; Tweedie, 1978).

Phylogeographic studies generally explain the historical events for current distribution patterns of biota across Pleistocene land bridge and evolutionary history of Sunda shelf rain forest taxa. The important of these periodic connections to the evolution of the Sunda shelf fauna has been studied in detail for some groups, such as non-volant mammals, gymnures, Asian water snake, frogs and snakes (Heaney, 1986; Ruedi & Fumagalli, 1996; Karns *et al*., 2000; Inger & Voris, 2001). Nowadays, researchers are increasingly studying the phylogeography that happened on a Pleistocene epoch to test the existence, location and duration of hypothesized refugia (Brunhoff *et al*., 2003; Dubey *et al*., 2006; Riddle & Hafler, 2007). Mitochondrial DNA (mtDNA) is maternally inherited (Avise, 1989) and a very useful tool for phylogeographic studies to understand the evolutionary relationships among individual, populations and species (Avise, 1989; Gorog *et al*., 2004; Esselstyn & Brown, 2009; Esselstyn & Oliveros, 2010). However, such results may be due to misleading information in mitochondrial genes at higher taxonomic level, (e.g. for other mammals and for fishes; see Janke *et al*., 1997 and Rasmussen & Arnason, 1999), therefore,

nuclear gene markers are good resolution for higher taxonomic level of subfamilies, tribes and genera (Dubey *et al*., 2007a; Esselstyn *et al*., 2009).

In this study, I have shown that all *C. monticola* from Sundaland formed a monophyletic clade differing from any other small *Crocidura* species in Asia. The results revealed that the two samples from central Peninsular Malaysia (Ulu Gombak and Bukit Rengit) form a monophyletic clade to Sumatra and Kalimantan samples with high bootstrap value and posterior probability in phylogenetic relationships (see Figures 3.5 and 3.6) supported with low genetic distance (2.7-3.4% K2P). The high similarity in these sequences may reflect the formations of Sunda landscape, which had permitted free migration across mainland and islands by Pleistocene land bridge, presumably they were essentially similar haplotype at one time before the geographical isolation.

The geographical separation by rising sea water level during the Pleistocene may cause restriction of gene flow between Peninsular Malaysia and Kalimantan or Sumatra. These have caused genetic drift that allows an evolution of a gene adapted to local environment. In addition, high intra-specific divergence of the two samples obtained from Ulu Gombak and Bukit Rengit plus two samples from Kalimantan to their provinces (> 6.8% K2P distance), usually did not happened in shrews' studies (e.g. Dubey *et al*., 2006; Ruedi *et al*., 1998; Bannikova *et al*., 2005; Bannikova *et al*., 2011). In this case, the Titiwangsa range is considered as the geographical barrier dividing Peninsular Malaysia from southern Thailand, into East and West Coast regions towards southeastwards into Johor. A physical barrier of the Titiwangsa range had prevented gene flow between Ulu Gombak and Bukit Rengit shrews from other populations in Peninsular Malaysia such as Cameron Highlands, Fraser Hill, Maxwell

Hill and Endau-Kluang. Kalimantan also is a geographically mountainous area and could be resulted high intra-specific level to the other closest relatives. More samples from Ulu Gombak, Bukit Rengit and Kalimantan need to be sampled for more understanding of the relationships of *C. monticola* between mainland and islands of Sundaland.

Although the samples of *C. maxi* is lacking for phylogenetic analysis, the morphological analysis had demonstrated *C. monticola* and *C. maxi* are clearly a distinct species. Morphological variation within both taxa may be reflected by Wallace's Line (Wallace, 1902), the supposed boundary between the Oriental and Australian regions. The extensive inter-island distributions may possibly cause change to occur in allele frequencies within the gene pool in parapatric populations between *C. monticola* and *C. maxi*. Thus, a parapatric population across the environmental gradient ultimately results in genetically distinct sister species. Therefore, more detail studies are required to clarify such population structure. The two samples from Sumatra that were assigned as "*C. maxi*" by Esselstyn *et al*. (2009) were assigned as *C. monticola* in this study due to its in *C. monticola* group. However, future studies especially from genetic point of view are needed to ascertain its taxonomic status.

Morphological variations within *C. monticola* from Sundaland revealed that the skull characters correspond to the larger size of mainland and continental islands population. Differences in morphological characters between the Peninsular Malaysia, Sumatra and Kalimantan shrew's population only happened in certain characters of skull and not consistent (see Table 3.3). This may be caused by adaptation to compete for food, water supplies and territory. *Crocidura monticola* from higher altitude (1160 - 1140 m) in Peninsular Malaysia is larger than lowlands altitude (55 - 300 m) but

showed overlap in both altitudes. Those highlands *monticola* expressed larger skull characters such as palatal width at the third molars (M3B), interorbital breadth (IOB) and rostral breadth (ROB) compared to lowlands *monticola*. This skull characters are appropriate for daily activities, indicated that shrews in high elevation needs to climb and using the snout for burrowing and obtaining food such as catching small insect or mealworm.

This study also addressed the relationships among pigmy shrew (*Suncus* spp). Previously, other researchers (e.g. Corbet & Yalden, 1972; Medway, 1978; Davison, 1979; Corbet & Hill, 1992; Hutterer, 2005; Francis, 2008) were classified *S. malayanus* and other *Suncus* pygmy shrews as *S. etruscus*. However, this study had confirmed that the taxon from Peninsular Malaysia represents a distinct species that is genetically divergent from widespread Eurasian taxa. This study also reveals that the Malagasy taxon does not warrant a different species from Eurasian forms. The results show high inter-specific genetic distances (6.1-8.5%) between *S. malayanus* from Peninsular Malaysia and *S. madagascariensis* from Madagascar or *S. etruscus* from Sri Lanka, Italy and France. This study also corroborates with Meegaskumbura *et al*. (2012a), which revealed that *S. fellowesgordoni* of Sri Lanka forms a distinct species from *S. malayanus* with representing genetic distance of 9.1-9.7%. Although *S. hosei* from Sarawak is still not yet been studied in taxonomic and phylogenetic position, it is assumed that it also will differs genetically from *S. fellowesgordoni*, *S. malayanus*, *S. madagascariensis*, and agreed with Hutterer (2005).

The result on genetic divergence patterns of *S. malayanus* revealed geographical distance by elevation. This may be caused restriction to gene flow between highland (Cameron Highlands) and lowlands (Ulu Gombak and Bukit Rengit) population and

this may possibly had a significant impact on the evolution of certain morphological trait. High inter-specific divergence due to geographical isolation presumably have caused evolution of shrews' phenotype. This character had adapted to local environmental stresses onward causing genetic drift from species main population. Thus, this study on phylogenetic relationships of *S. malayanus*, *S. madagascariensis* and European *S. etruscus* had provided valuable information for further evaluation on divergence among various populations of different regions.

CHAPTER 6

CONCLUSIONS

This study examined five species of shrews belong to subfamily Crocidurinae and one species of shrew belong to subfamily Soricinae. Surprisingly, *C. fuliginosa* that was commonly found in earlier studies by many authors are currently absent in pitfall traps but luckily one individual was captured from Cameron Highlands, Pahang. Peninsular Malaysia shrews were listed as least concern and data still deficient in the IUCN, except Malayan Water Shrew, listed as near threatened species. Further studies are needed to obtain more information on distribution, abundance, threats and conservation of all shrews' species in Malaysia.

In Chapter 3, *C. monticola* from Ulu Gombak and Bukit Rengit of Peninsular Malaysia showed very close lineages to the major Sunda Islands shrews (Kalimantan and Sumatra), represents a unique species from those found elsewhere in Peninsular Malaysia. This study also provides enough information for Peninsular Malaysia based on morphological and phylogenetic data. All *C. monticola* in Peninsular Malaysia are morphologically similar and the data also supports that *C. maxi* does not exist in this region. Additional field sampling and other analyses of molecular work are necessary to determine the degree of relationships for unclear species occur in western part of Peninsular Malaysia, Sumatra and Kalimantan. It would be important to sampling shrews from southern and eastern parts of Peninsular Malaysia, Borneo and Sumatra, to determine the full range of morphological variation and the exact distribution of these mitochondrial lineages.

Chapter 4 demonstrates that *S. malayanus* which is endemic to the Thai-Malay peninsula, represents a unique lineage among *Suncus* pygmy shrews distinct information on the extent of genetic variation present among *S. etruscus* individuals, and shows that shrews previously considered as *S. madagascariensis* are taxonomically synonymous to *S. etruscus*. The Malagasy shrews were most likely introduced to Madagascar via human intervention from an Indian Subcontinent or Southeast Asia. For a better understanding of the evolutionary history of different forms in the *etruscus* group, notably *S. hosei* also need to be included in future studies to investigate their level of genetic divergence.

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APPENDIX A

The list of comparative specimens examined for morphometric analysis. Acronyms of institutions are: MZB, the Museum Zoologicum Bogoriense; ZRC, the Raffles Museum, Singapore; MVZ, the Museum of Vertebrate Zoology of the University of California at Berkley, USA; RMNH, Rijksmuseum van Natuurlijke Historie in Leiden, Netherlands; BMNH, the British Museum of Natural History in London; IZEA, the Institut de Zoologie et d'Ecologie Animale in Lausanne, Switzerland.

West Java: Cibuni plantation – *C. monticola* RMNH Tjib 50. Mount Gede-Pangerango – holotype of *C. bartelsii* RMNH 12723, *C. monticola* RMNH 24032, 15456, 11141, 23988, 23990, IZEA 4579-80, MZB 13693, 124. Western Java – RMNH 15456. Mount Salak – MZB 109.27, 107.25. Mount Wayang – MZB 27057-8.
East Java: Besuki – holotype of *C. maxi* RMNH 23992 and paratypes RMNH Z15, 23993. Pudjon – RMNH Poedjon 45, 23985. Punten Batu – RMNH 23986, MZB 8691, 8692, 2168, 2169. Eastern Java – MZB 871, 8705-6, 8693, 8695-6, 8698.
Lesser Sunda: Komodo – MZB 32984, 32991.
Kalimantan, Borneo: Mount Palung – MZB 20612-14, 20616-19. Raja Hill – MZB 20620-23.
Sumatra: Bukit Lawang, Gunung Leuser N.P. **–** *C.* cf. *maxi* MVZ192177-8 (Esselstyn *et al*., 2009).
Thailand: Khao Ram – *C. monticola* ZRC 5059. Khao Luang – ZRC 5060.
Peninsular Malaysia: Wang Kelian – M1530-1, M1537, WK1074-7. Fraser Hill – FH0809, FH0938-9, FH0941, FH1082-3. Ulu Gombak – M1546, PP0815-6, PP0824, PP0829, PP0870, PP0940, PP0963-4, PP1079-80. Maxwell Hill – MH0801-2, MH0810, MH1096-9, MH10100-2. Cameron Highlands – CH0972, CH1088-9, CH1091-5. Bukit Rengit – BR10103, BR10105, BR10107-11. Endau-Kluang – EK0834, EK0837, EK0842.

APPENDIX B

Two first axes of factor-loadings from the principal component analyses based on 14 skull measurements of specimens from the *C. monticola* species complex. The first two components were calculated only on specimens from the Thai-Malay peninsula, while the next two axes were based on the complete sampling from the Sundaland (i.e. Borneo, Java, Sumatra, and the Thai-Malay peninsula).

Variable	Peninsular Malaysia *C. monticola*		Sundaland *C. monticola* + *C. maxi*	
	PC1	PC2	PC1	PC2
GLS	-0.94	0.149	-0.933	0.278
IM3S	-0.822	0.272	-0.895	0.258
PPL	-0.762	-0.18	-0.814	0.069
PM3	-0.646	0.417	-0.809	0.251
ROB	-0.425	-0.44	-0.387	-0.693
M3B	-0.537	-0.661	-0.602	-0.580
M2B	-0.746	-0.308	-0.854	-0.227
BB	-0.747	0.132	-0.821	0.068
IOB	-0.416	-0.518	-0.633	-0.198
ROL	-0.845	0.188	-0.871	0.232
PPD	-0.576	-0.031	-0.665	-0.448
CTG	-0.835	0.003	-0.807	0.273
IM3I	-0.771	0.276	-0.873	0.093
MAL	-0.634	0.056	-0.714	-0.105
Eigenvalue	7.05	1.43	8.43	1.48
% Total variance	50.4	10.2	60.2	10.5

APPENDIX C Origin (place and country) of the different *Crocidura* species used in the genetic comparisons. GenBank accession number corresponding to the mitochondrial (cyt *b*) and nuclear (ApoB) genes used for reconstructions, and references are also given.

Collection sites	Country	Species	Accession Numbers		Reference
			Cyt *b*	ApoB	
Wang Kelian	Malaysia	*C. monticola*1	JX162650	JX162668	This study
		*C. monticola*2	JX162651	JX162669	This study
		*C. monticola*3	JX162652	JX162670	This study
		*C. monticola*4	JX162653	-	This study
		*C. monticola*5	JX162654	-	This study
		*C. monticola*6	JX162655	-	This study
Cameron Highlands	Malaysia	*C. monticola*7	JX162656	JX162671	This study
		*C. monticola*8	JX162657	JX162672	This study
		*C. monticola*9	JX162658	JX162673	This study
		*C. monticola*10	JX162659	JX162674	This study
Fraser Hill	Malaysia	*C. monticola*11	JX162660	JX162675	This study
Maxwell Hill	Malaysia	*C. monticola*12	JX162661	JX162676	This study
		*C. monticola*13	JX162662	JX162677	This study
		*C. monticola*14	JX162663	JX162678	This study
Bukit Rengit	Malaysia	*C. monticola*15	JX162664	JX162679	This study
Ulu Gombak	Malaysia	*C. monticola*16	JX162665	JX162680	This study
Kalimantan, Borneo	Indonesia	*C. monticola* AJG134	JX162666	-	This study
		C. monticola AJG198	JX162667	-	This study
B. Lawang, Sumatra	Indonesia	*C.* cf. *maxi*	FJ814024	FJ813798	Esselstyn *et al*. (2009)
Bi Doup	Vietnam	*C. indochinensis*	HM587023	-	Bannikova *et al*. (2011)
Phu Quoc	Vietnam	*C. phuquocensis*	HM587011	-	Bannikova *et al*. (2011)
Yok Don	Vietnam	*C. phanluongi*	HM587020	-	Bannikova *et al*. (2011)
Ngoc Linh	Vietnam	*C. sokolovi*	HM586999	-	Bannikova *et al*. (2011)
Hon Giao	Vietnam	*C. zaitsevi*	HM587021	-	Bannikova *et al*. (2011)
Ulu Gombak	Malaysia	*C. malayana*	DQ630381	EF524959	Dubey *et al*. (2007a)

APPENDIX C (Continued)

Collection sites	Country	Species	Accession Numbers		Reference
			Cyt *b*	ApoB	
Cameron Highlands	Malaysia	*C. fuliginosa*	FJ813925	EF524931	Esselstyn *et al*. (2009)
Sarawak, Borneo	Malaysia	*C. foetida*	FJ814053	FJ813827	Esselstyn *et al*. (2009)
Java	Indonesia	*C. orientalis*	FJ814029	FJ813803	Esselstyn *et al*. (2009)
Sulawesi	Indonesia	*C. nigripes*	FJ813928	EF524935	Esselstyn *et al*. (2009)
		C. musseri	FJ813927	FJ813701	Esselstyn *et al*. (2009)
Hunan	China	*C. tanakae* A	FJ814037	FJ813811	Esselstyn *et al*. (2009)
		C. attenuata A	FJ814034	FJ813808	Esselstyn *et al*. (2009)
Guangxi	China	*C. attenuata* B	FJ814039	FJ813813	Esselstyn *et al*. (2009)
		C. wuchihensis	FJ814043	FJ813817	Esselstyn *et al*. (2009)
	Vietnam	*C. tanakae* B	FJ814033	FJ813807	Esselstyn *et al*. (2009)
Negros	Philippines	*C. negrina*	FJ813962	FJ813737	Esselstyn *et al*. (2009)
		Suncus murinus	FJ813963	FJ813738	Esselstyn *et al*. (2009)
Gard	France	*S. etruscus*	JF817396	-	Omar *et al*. (2011)

APPENDIX D PUBLISHED MANUSCRIPT OF *SUNCUS MALAYANUS*

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PHYLOGENETIC RELATIONSHIPS OF MALAYAN AND MALAGASY PYGMY SHREWS OF THE GENUS *SUNCUS* (SORICOMORPHA: SORICIDAE) INFERRED FROM MITOCHONDRIAL CYTOCHROME *B* GENE SEQUENCES

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ABSTRACT. – This study used a portion of the mitochondrial cytochrome *b* gene to investigate the phylogenetic relationships of *Suncus* pygmy shrews and to help clarify the taxonomic status of the Malayan pygmy shrew (*S. malayanus*) and the Malagasy pygmy shrew (*S. madagascariensis*). Phylogenetic reconstructions were performed using neighbour-joining and Bayesian analysis methods and revealed that *S. malayanus* is related to, but distinct from *S. etruscus*. The K2P-corrected genetic distance among the Malaysian taxon and other pygmy shrews for the cytochrome *b* gene was between 6.1 and 8.5%, supporting recognition that *S. malayanus* represents a distinct species from the geographically widespread *S. etruscus* species complex. A close (1.5% K2P distance) sister-group relationship was revealed between *S. etruscus* from Sri Lanka and *S. madagascariensis* from Madagascar, which has been considered an island endemic, and suggests that these animals are not specifically distinct. The Malagasy population of this shrew most probably was translocated to the island by human intervention, with the lineage originating from Southeast Asia or the Indian subcontinent.

KEY WORDS. – Crocidurinae, cytochrome *b*, genetic distance, mitochondrial DNA, phylogenetic relationships, *Suncus*.

INTRODUCTION

Due to their extremely small size (about 2 g), pygmy shrews of the genus *Suncus* (Family Soricidae) are difficult to trap and are therefore poorly represented in reference collections, which in turn has added to ongoing taxonomic confusion regarding species limits within the genus. For instance, *S. malayanus* (Fig. 1), the principal focus of this study, was initially named *Pachyura malayana* by Kloss (1917) and subsequently considered a subspecies of the Etruscan shrew *S. etruscus* (Savi, 1822) under the name *S. etruscus malayanus* (see e.g. Medway, 1978). More generally, Ellerman &

APPENDIX E PUBLISHED MANUSCRIPT OF *CROCIDURA MONTICOLA*

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Original Investigation

Morphological and genetic relationships of the *Crocidura monticola* species complex (Soricidae: Crocidurinae) in Sundaland

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Abstract

Small Crocidurinae shrews (weight <8 g) from Southeast Asia have been poorly studied to date, mainly because of the difficulty to catch them and the concomitant paucity of reference specimens available in museums. Hence their systematics is still debated, and most small *Crocidura* shrews from Sundaland are assigned to the *monticola* species complex. Here, we report a study based on a survey of shrews caught with large pitfalls set on forest floors in Peninsular Malaysia. Morphometric analyses based on 14 skull measurements showed that these shrews tend to be larger with increasing altitude, but showed otherwise no consistent variation. When compared to museum specimens of the *monticola* species complex sampled in the Sundaland (total: 77 specimens), the Malay shrews tend also to be larger than those living on Kalimantan (Borneo) and Sumatra. All are, however, morphologically distinct from the other species, *C. maxi*, found in eastern Java and on the Lesser Sundas. Molecular analyses of a subset of these small shrews and based on a mitochondrial (cytochrome *b*) and a nuclear gene (Apolipoprotein B) suggest that samples from the central region of Peninsular Malaysia (Bukit Rengit and Ulu Gombak) genetically differ from other Malaysian populations (by about 7% K2P distance at the cyt *b* gene) and are more closely related to some samples from Sumatra and Borneo. These differences did not correlate with the altitudinal variation evidenced from the morphological analysis. Reference sequences from the terra typica of *monticola* and *maxi* (both species were originally described from Java) are however needed to determine if these unexpected genetic differences warrant additional taxonomic subdivision within the Sundaland.

Keywords

Shrew; Cryptic species; Principal component analysis; Phylogeny; Genetic distance