CHAPTER II

MATERIALS AND METHODS

Materials

Specimens

Chiropterans' hairs in Thailand from 10 families 38 genera 97 species were studied. The mid dorsal guard hairs from the scapular region were used in this study (Benedict, 1957 ; Pornpimol Singnoi, 1987). Hair in contact with the skin of 1-3 amimals from each species were carefully cut and preserved in 70% ethyl alcohol. The specimens were obtained from Thailand Institute of Scientific and Technological Research, Bangkok and Senckenberg Research Institute, Frankfert, Federal Republic of Germany. Specimens from the following species were involved in this study.

Order Chiroptera

Suborder 1 Megachiroptera

Family 1 Pteropodidae

Subfamily 1 Pteropodinae

Genus Cynopterus

Cynopterus brachyotis

C. sphinx

C. horsfieldi

Genus Chironax

Chironax melanocephalus

Genus Balionycteris

Balionycteris maculata

Genus Sphaerias

Sphaerias blanfordi

Genus Megaerops

Megaerops niphanae

M. ecaudatus

Genus Rousettus

Rousettus amplexicaudatus

R. leschenaulti

Genus Pteropus

Pteropus lylei

P. hypomelanus

P. vampyrus

Subfamily 2 Macroglossinae

Genus Macroglossus

Macroglossus sobrinus

M. minimus

Genus Eonycteris

Eonycteris speleae

Suborder 2 Microchiroptera

Family 2 Rhinopomatidae

Genus Rhinopoma

Rhinopoma microphyllum

Family 3 Emballonuridae

Genus Emballonura

Emballonura monticola

Genus Saccolaimus

Saccolaimus saccolaimus

Genus Taphozous

Taphozous longimanus

T. melanopogon

T. theobaldi

Family 4 Craseonycteridae

Genus Craseonycteris

Craseonycteris thonglongyai

Family 5 Nycteridae

Genus Nycteris

Nycteris tragata

Family 6 Megadermatidae

Genus Megaderma

Megaderma lyra

M. spasma

Family 7 Rhinolophidae

Genus Rhinolophus

Rhinolophus malayanus

R. stheno

R. thomasi

R. affinis

R. robinsoni

R. lepidus

R. acuminatus

R. pusillus

R. macrotis

R. pearsoni

R. yunanensis

R. coelophyllus

R. marshalli

R. shameli

R. paradoxolophus

R. trifoliatus

R. luctus

Family 8 Hipposideridae

Genus Hipposideros

Hipposideros bicolor

H. ater

H. cineraceus

H. halophyllus

H. galeritus

H. diadema

H. lekaguli

H. lylei

H. armiger

H. turpis

H. larvatus

Genus Coelops

Coelops frithi

C. robinsoni

Genus Aselliscus

Aselliscus stoliczkanus

Family 9 Vespertilionidae

Genus Myotis

Myotis muricola

M. siligorensis

M. annectans

M. rosseti

M. horsfieldi

M. hasseltii

M. chinensis

Genus Pipistrellus

Pipistrellus coromandra

P. tenuis

P. mimus

P. cadornae

P. javanicus

P. pulveratus

P. circumdatus

Genus Glischropus

Glischropus tylophus

Genus Eptesicus

Eptesicus serotinus

E. dermissus

E. pachyotis

Genus Ia

Ia io

Genus Hesperoptenus

Hesperoptenus blanfordi

H. tickelli

Genus Nyctalus

Nyctalus noctula

Genus Tylonycteris

Tylonycteris pachyotis

T. robustus

Genus Scotomanes

Scotomanes ornatus

Genus Scotophilus

Scotophilus kuhli

Genus Miniopterus

Miniopterus medius

M. macrodens

M. schreiber

Genus Murina

Murina tubinaris

M. cyclotis

M. huttoni

Genus Harpiocephalus

Harpiocephalus harpia

Genus Kerivoula

Kerivoula papillosa

K. picta

K. minuta

K. hardvickei

Genus Phoniscus

Phoniscus atrox

Family 10 Molossidae

Genus Chaerephon

Chaerephon plicata

Genus Cheiromeles

Cheiromeles torquatus

Genus Tadarida

Tadarida teniotis

Chemical Substance

Ethyl alcohol

n-Butyl alcohol

Hydrogen peroxide

Diethyl ether

Ammomium hydroxide

Xylene

Permount

Paraffin

Methods

Hairs with hair follicles were carefully plucked off by using fine tipped forceps. At least thirty hairs from each species were used for three different methods of studies. The middle one-third portions of filament were used for the studies of morphological structures. The preparations of specimen were made as follow :

Preparation of specimen for light microscpe

a. Preparation of the whole mount hair

Dark color hairs were bleached off prior to the dehydration with the mixture of equal part of hydrogen peroxide and ammonium hydroxide for 5-10 minutes, if necessary. Specimens were dehydrated through the series of 70%, 90%, 95% ethyl alcohol and n-butyl alcohol 5 minutes for each step. Hairs were then cleared in xylene for 10 minutes and mounted on slides with permount. Shape and length of hair were studied under stereo microscope. Form of medulla and distribution of pigment were observed under bright field microscope. The width of hair was measured from the widest part of the middle portion of the shaft (Benedict, 1957).

b. Preparation of the cross-section of hair

Hairs were wrapped in lens paper, and dehydrated through the series of 70%,90%,95% and absolute ethyl alcohol. The specimens were infiltrated with the mixture of xylene and melted paraffin in the ratio 2:1, 1:2 and then in pure paraffin at 60 C for 30 minutes in each step and embeded in paraffin. Cross-sections were cut at 8-10 um. (micron). Sections were mounted on slides with permount. Shape of cross-section of hair and the distribution of pigment were observed under bright field microscope. Thickness of medulla was measured from the center to the outer circumferential of medulla, while the thickness of cortex was measured from the junction of medulla-cortex to the outer circumferential of cortex of the same radius (Brunner & Coman, 1974).

Preparation of specimens for SEM

Hairs were dehydrated through the series of concentration of ethyl alcohol from 70%, 90%, 95% and in the mixture of equal part of alcohol and diethyl ether for 10 minutes and air dried. Specimens were mounted on SEM stubs by using double sides sticky tapes. All specimens were coated with gold in the ion sputtering apparatus for 8 minutes. Coated hairs were examined in scanning electron microscope at 15 kiloelectron volts (KV). The patterns and arrangements of scale were studied from these specimens under SEM. Selected scale for measurement was the largest scale on the widest part of the middle portion of the shaft.