สัณฐานวิทยา ชีววิทยาการสืบพันธุ์ และนิเวศวิทยา
ของงูผ้าขี้ริ้ว *Acrochordus granulatus* ในอ่าวพังงา ประเทศไทย

นางสาว ศันสรียา วังกุลางกูร

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MORPHOLOGY, REPRODUCTIVE BIOLOGY AND ECOLOGY OF THE LITTLE FILE SNAKE, *Acrochordus granulatus* AT PHANGNGA BAY, THAILAND

Miss Sansareeya Wangkulangkul

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By	Miss Sansareeya Wangkulangkul
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Thesis Advisor	Assistant Professor Kumthorn Thirakhupt, Ph. D.
Thesis Co-advisor	Harold K. Voris, Ph. D.

Accepted by the Faculty of Science, Chulalongkorn University in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

....Dean of Faculty of Science

(Professor Piamsak Menasveta, Ph.D.)

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....Thesis Advisor

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.... Thesis Co-advisor

(Harold K. Voris, Ph.D.)

.... Member

(Art-ong Pradatsundarasar, Ph.D.)

.... Member

(Assistant Professor Pipat Patanaponpaiboon, Ph.D.)

.... Member

(Lawan Chanhome, DVM.)

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จากการศึกษางูผ้าขี้ริ้ว (*Acrochordus granulatus*) จำนวน 119 ตัว จากอ่าวพังงา ในระหว่างเดือนมกราคมถึงเดือนธันวาคม 2545 พบว่าความหลากหลายของรูปร่างและสีของงูผ้าขี้ริ้วมีน้อย ลักษณะกะโหลกของงูชนิดนี้เป็นรูปแบบของงูที่พัฒนาแล้ว งูผ้าขี้ริ้วจะมีความสมบูรณ์เพศเมื่องูมีความยาวจากปลายจมูกถึงโคนหางอย่างน้อย 580 มิลลิเมตร และสามารถแบ่งกลุ่มตัวอย่างโดยใช้เพศและความสมบูรณ์เพศออกได้เป็น 4 กลุ่มดังนี้ งูผ้าขี้ริ้วเพศผู้ที่ยังไม่สมบูรณ์เพศ งูผ้าขี้ริ้วเพศผู้ที่สมบูรณ์เพศแล้ว งูผ้าขี้ริ้วเพศเมียที่ยังไม่สมบูรณ์เพศ และงูผ้าขี้ริ้วเพศเมียที่สมบูรณ์เพศแล้ว ซึ่งจากการทดสอบทางสถิติด้วย t-test พบว่างูผ้าขี้ริ้วที่สมบูรณ์เพศแล้วจะมีลักษณะทางสัณฐานวิทยาที่มีความแตกต่างกันระหว่างเพศอย่างมีนัยสำคัญทางสถิติ ($p<0.05$) จำนวน 11 ลักษณะจากลักษณะที่ใช้เปรียบเทียบทั้งสิ้น 14 ลักษณะ หว่าในงูผ้าขี้ริ้วที่ยังไม่สมบูรณ์เพศมีเพียง 2 ลักษณะเท่านั้นที่มีความแตกต่างกันระหว่างเพศอย่างมีนัยสำคัญทางสถิติ ($p<0.05$) และผลการคำนวณทางสถิติด้วยวิธีการ Discriminant function analysis พบว่าค่าความถูกต้องของสมการเพื่อการทำนายเพศของงูผ้าขี้ริ้วที่สมบูรณ์เพศแล้วมีค่าความถูกต้องที่ 98.3%

ผลการศึกษาช่วงเวลาการสืบพันธุ์พบว่างูผ้าขี้ริ้วเริ่มผสมพันธุ์ในเดือนกรกฎาคม โดยในระหว่างเดือนกรกฎาคมถึงเดือนธันวาคมนี้ งูผ้าขี้ริ้วเพศผู้จะมีขนาดของอัณฑะใหญ่ขึ้น ซึ่งมีขนาดใหญ่กว่าที่พบในช่วงเดือนมกราคมถึงเดือนมิถุนายน ส่วนเพศเมียจะเริ่มมีการสะสมไข่แดงมากขึ้นจนเห็นได้ชัดเจนและไข่เคลื่อนที่มายังมดลูกในช่วงเดือนพฤศจิกายน สังเกตเห็นตัวอ่อนที่เจริญบนไข่แดงในช่วงเดือนมกราคม พบตัวอ่อนที่เจริญสมบูรณ์แล้วในเดือนพฤษภาคม และพบลูกงูผ้าขี้ริ้วที่มีขนาดความยาวจากปลายจมูกถึงโคนหาง 360-400 มิลลิเมตรในเดือนมิถุนายน

งูผ้าขี้ริ้วมีการแพร่กระจายตามแนวชายฝั่งทะเลของประเทศไทย โดยมากมักพบอยู่ในบริเวณปากแม่น้ำ หรือบริเวณใกล้เคียงที่มีพื้นโคลนและป่าชายเลน และได้ศึกษาเปรียบเทียบความชุกชุมของงูผ้าขี้ริ้วจาก 3 พื้นที่ในอ่าวพังงา งูผ้าขี้ริ้วมีรูปร่างที่เหมาะสมกับการดำรงชีพในเขตน้ำทะเลที่ตื้น โดยอาหารหลักของงูผ้าขี้ริ้วคือปลา ซึ่งงูผ้าขี้ริ้วเพศเมียสามารถกินอาหารที่มีขนาดใหญ่กว่าเพศผู้

ลายมือชื่อนิสิต ....

สาขาวิชา วิทยาศาสตร์ชีวภาพ ลายมือชื่ออาจารย์ที่ปรึกษา ....

ปีการศึกษา 2547 ลายมือชื่ออาจารย์ที่ปรึกษาร่วม ....

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SANSAREEYA WANGKULANGKUL: MORPHOLOGY, REPRODUCTIVE BIOLOGY AND ECOLOGY OF THE LITTLE FILE SNAKE, *Acrochordus granulatus* AT PHANGNGA BAY, THAILAND. THESIS ADVISOR: ASST. PROF. KUMTHORN THIRAKHUPT, Ph.D., THESIS CO-ADVISOR: HAROLD K. VORIS, Ph.D., 96 pp. ISBN 974-53-1528-1.

A total of 119 little file snakes (*Acrochordus granulatus*), collected at Phangnga Bay from January - December 2002, were studied. The variation in body shape and coloration are low in this species. Their skull elements show the neomorphic form of advanced snakes. The size at maturation in both sexes is 580 mm SVL or more. The snakes were divided into four groups according to their sex and reproductive stage; juvenile males, adult males, juvenile females and adult females. The t-test was used to analyze the data on sexual size dimorphism. Significant differences (t-test, $p \leq 0.05$) in 11 of 14 morphological characters were found between the sexes of adult snakes. In juvenile snakes, only two morphological characters were significantly different between the sexes. Results from discriminant function analysis gave the equation for predicting the sexes of adult little file snakes with the original grouped case correctly classified at 98.3%.

Reproductive data indicates that the breeding season begins in July. From July to December, the testicular volume increased, surpassing that observed from January to June. Following an increase in size of follicles to vitellogenesis, ovulation was observed in November. The embryos were first observed in January and full term embryos were observed in May. The young snakes of about 360 - 400 mm SVL were first caught in June. Maternal size had a positive influence on clutch size.

A. granulatus is distributed widely in the coastal areas of Thailand. They are usually found in the estuary or nearby the river mouth where mudflats and mangrove forests are present. The relative abundance of snakes at the three locations in Phnangnga bay were compared. Their bodies are adapted for living in shallow sea water. Their main diet is fish and females tend to feed on bigger prey than do males.

Student's signature Sansareeya Wangkulangkul

Field of study Biological Science Advisor's signature K. Thirakhupt

Academic year 2004 Co-advisor's signature Harold Voris

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CHAPTER I

INTRODUCTION

1.1 Rationale

Beginning with fossorial ancestors from the early Cretaceous, the diversity of snakes has shown an impressive adaptive radiation in vertebrate evolutionary history. Snakes have the largest member of species of living reptile groups with over 2,900 species placed in 19 families. Because of their adaptability of form and function with specialized body plan, they can occur on all continents except the in the deep ocean and polar region. They have had a successful marine radiation but less success in dispersing onto oceanic islands (Lillywhite and Henderson, 1993; Ernst and Zug, 1996; Greene, 1997; Lee and Caldwell, 1998; Zug et al., 2001; Evans, 2003).

Of the 19 living snake families (Pough, et. al. 2004), four families contain marine species. They are in the Family Colubridae (Subfamily Homalopsinae), Family Acrochordidae, Family Laticaudidae and Family Hydrophiidae. The members in the Family Colubridae and the Family Acrochordidae are usually found in mangrove forest and estuaries while members in the Family Laticaudidae and the Family Hydrophiidae are entirely in marine (Heatwole, 1999; Zug et al., 2001; Pough, et. al. 2004).

In Thailand, the first report on marine snakes was published by Smith in 1914. Until the present time, a total of 32 species of marine snakes have been reported in Thai waters. There are 4 species in the Subfamily Homalopsinae, 1 species in Family Acrochordidae, 2 species in Family Laticaudidae and 25 species in the Family Hydrophiidae (Smith, 1915; Smith, 1926; Suvatti, 1967; Tu, 1974; Frith, 1977; Bussarawit, et. al., 1989; Cox, 1991; Murphy, et. al., 1999). However, the knowledge on the morphology, reproductive biology, taxonomy and ecology of each of them is still not complete (Gadow, 1968; Heatwole, 1999). In this study, the little file snake, *Acrochordus granulatus*, was chosen to initiate a study to clarify some of these topics. Although this species is widespread in estuarine habitats and locally abundant, its biology and ecology is still little known.

Phangnga Bay is located in Southern Thailand and has been for a long time abundant with natural resources. The bay has been classified as one of the most biologically productive and ecologically important bays in the world. (Chong, et. al., 1998) The bay is rich in marine fauna and flora; it also provides shelter and habitats for a wide variety of the Bay's life. Throughout the year, in the bay and along the shoreline, local fishermen have occasionally captured marine snakes in their fishing equipment. This event provided a good opportunity to survey *A. granulatus* in the bay.

This research emphasizes the morphology, reproduction and ecology of *Acrochordus granulatus* found in Phangnga Bay. The data on this species will provide a clear picture of the marine snake biology and ecology. This information and its present status will allow us to be able to assess its environmental impact and can arrange proper conservation management in the future.

1.2 Objectives

To study *Acrochordus granulatus*:

1.2.1 Morphology; external character, skull and skeletons, reproductive organs, and sexual dimorphism.

1.2.2 Reproductive biology; size at sexual maturity, reproductive cycle, embryonic development and clutch size.

1.2.3 Ecology; distribution, habitat, relative abundance and diet.

CHAPTER II

LITERATURE REVIEW

2.1 Classification and general description

Snakes are limbless reptiles. They capture, manipulate and consume their prey using only the body and mouth. This necessity has led to major modifications of snake anatomy. They have many anatomical structures that differ from those of other vertebrates in size, shape and location. The internal organs are modified greatly to fit in the long-shaped body. Major organs usually are staggered linearly and more elongated than in other squamates. In some snakes, a member of a pair of organs may be reduced or absent. For example, in marine snakes, the left lung is reduced or absent while the right lung is well developed and extends through much of a snake's body cavity and ends in a sack-like air storage area (Oldham et al., 1970; Greene, 1997; Zug et al., 2001).

Marine snakes are classified into four families. They are in Family Colubridae (Subfamily Homalopsinae), Acrochordidae, Laticaudidae and Family Elapidae (Subfamily Hydrophiinae). Seven genera of Subfamily Homalopsinae in Family Colubridae are marine and estuarine snakes. Family Acrochordidae contains only one genus with 3 species. Only the little file snake, *Acrochordus granulatus* can be found in a variety of aquatic habitats. Venomous sea snakes are included in the Families Laticaudidae and Elapidae (Hydrophiinae). Taxonomists, however, do not completely agree on the status of some taxa (Gadow, 1968; Heatwole, 1999; Zug, et al., 2001; Pough, et al. 2004).

The three acrochordid species are the Arafura file snake (*Acrochordus arafurea*), the Elephant's trunk snake (*A. javanicus*) and the little file snake (*A. granulatus*). They are strictly aquatic inhabitants of the Indo-Australian region. The fossil record of acrochordids shows that their relatives once were widespread in the Old World. At present these snakes are distributed along the seashores of South Asia, Southeast Asia and Australia. Acrochordids display a puzzling mixture of primitive and highly specialized traits. Among the latter are their exceptionally flabby and tubercular skins with marked vertical flattening of the body when in water. *A. arafurea* and *A. javanicus* are frequently referred to as freshwater snakes, whereas the little file snake; *A. granulatus* has a parallel with the true sea snakes in families Elapidae(Hydrophiinae) and Laticaudidae. Its extreme morphological and behavioral specializations are adapted for marine life. It can occupy a variety of marine habitats ranging from the brackish coastal habitat, the estuary and the sea (Greene, 1997; Shine and Houston, 1993; Heatwole, 1999, Zug, et al., 2001).

At present time, a total of 31 species of marine snakes were reported in Thai waters (Smith and Kloss, 1915; Smith, 1926; Smith, 1930; Taylor, 1965; Suvatti, 1967; Tu, 1974; Frith, 1977; Frith and Boswall, 1978; Frith and MacIver, 1978; Bussarawit , et al., 1989; Cox, 1991; Murphy, et al. 1999). There are three species of Subfamily Homalopsinae, one species of Family Acrochordidae, two species of Family Laticaudidae and 25 species of Subfamily Hydrophiidae. The taxonomy of coastal marine snakes however, is still not clear (Gadow, 1968; Heatwole, 1999; Zug, et al., 2001; Pough, et al. 2004). In historical accounts, *A. granulatus* has been reported by many authors (Smith, 1914; Smith and Kloss, 1915; Smith, 1930; Taylor and Elbel, 1958; Taylor, 1965; Suvatti, 1967; Tu, 1974; Frith, 1977; Cox, 1991; Murphy, et al. 1999; Pauwels et al. 2000).

A. granulatus (Figure 2.1) is described as a snake in which the head is not distinct from neck, scales are small juxtaposed, nostrils on the top of the snout and close together, very small eyes with pupil vertically elliptic, and teeth almost equal with 12-15 pieces in each maxillary. Its body is stout; compressed; covered with small, rhomboidal and juxtaposed scales with tubercle-like keel; spinose beneath; no ventral shields but a fold of the skin along the middle of the belly. The tail is short, compressed and prehensile. No rostal, a series of larger shields on the lips, separated from the mouth by a row of very small scales and no chin-shields. The scale row at the widest part of the body is approximately row 130-140 and the dorsal scales are largest. The black and cream bands are nearly equal in width, extending completely around the body (Rooij, 1917; Bourret, 1936; Taylor and Elbel, 1958; Gadow, 1968; Murphy, et al. 1999).

The biology and ecology of *A. granulatus* are not well known. In general, *A. granulatus* is nocturnal, feeding mainly on gobies and occasionally on marine crustaceans (Voris and Glodek, 1980; Voris and Voris, 1983; Shine and Houston, 1993; David and Vogel, 1996; Greene, 1997). Their pulmonary blood flow during voluntary diving was studied by Lillywhite and Donald (1989). The measurements of pressure and blood flow in pulmonary and systemic vessels indicate that blood flow completely bypasses the lung for significant periods during prolong and quiescent submergence. These reciprocating patterns of preferential blood flow reflect inverse relations between flow and vascular resistance. The result of systemic and pulmonary arterial pressures remains virtually constant throughout repetitive dive cycles.

Figure 2.1 *Acrochordus granulatus* from Phangnga Bay

The study of serological differences among sea snakes by Minton (1975) showed a weak reaction with serum of *A. granulatus*. The observation on antiserum showed a shared antigen of *Lapemis* and *Acrochordus*. However, the lack of close serological relationship between *Acrochordus* and the hydrophids is of interest, since *Acrochordus* has several of the anatomical and physiological adaptations of other sea snakes. In a study of haematology and blood chemistry of *A. granulatus*, Das and Padgaonkar (1998) reported that the erythrocytes are oval shape with a centrally located oval nucleus. During pregnancy erythrocyte size does not change but the numbers increases and the total leucocyte count is low, the neutrophils increase and lymphocytes decrease in number. The hemoglobin is constant but haematocrit values and ESR are higher. Levels of urea and creatinine in serum are found to be higher.

Furthermore, the sexes of *A. granulatus* are not easily distinguished on the basis of external features and the reproductive biology of *A. granulatus* is not well understood (Voris and Glodek, 1980; Shine, 1994; Heatwole, 1999). Although, in many species of animals, males and females differ from each other in body size, shape and color, either because of sexual selection or ecological divergence between the sexes, most snakes do not show extreme dimorphism (Shine, 1993; Heatwole, 1999; Gregory, 2004). Therefore, the study on the sexual dimorphism and reproductive biology of *A. granulatus* is of prime interest.

2.2 Sexual dimorphism

Males and females of all vertebrate species obviously differ in their reproductive organs. But in many species, there are also marked differences in the secondary sexual characteristics that are not directly associated with reproduction. The distinction in appearance is called sexual dimorphism. Sexual dimorphism is often visible in a body size difference in which one sex is larger than the opposite sex. Charles Darwin originally formulated his theory of sexual selection to account for the evolution of sexual differences in body size and other morphological characters since 1874. He recognized that in most cases males are usually larger, stronger and more colorful than females of the same species.

To understand the pattern of sexual dimorphism, the selective forces acting on the body size of both males and females needs to be explained. Many theoretical models suggest several ways in which significant sex differences may arise. Most explanations are focused on the relationship between differences in the body size and reproductive success of males and females. In this case, differences in the cost of reproduction between sexes can be the determination of sexual size dimorphism. Another explanation for the evolution of sexual dimorphism has been proposed as intra-specific niche divergence. Sexual differences in body size or morphology may evolve by ecological functions since it is an advantage for each sex to have a different ecological niche which reduces niche overlap and competition between the sexes. The details of sexual selection, cost of reproduction and intra-specific niche divergence for the evolution of sexual dimorphism in snakes has been discussed by many authors (Shine, 1988; Shine, 1989; Shine, 1993; Madsen and Shine, 1994; Pough, et al., 2001).

For the cost of reproduction, which is different between males and females as a consequence of their differences in reproductive biology, Shine (1980) suggested that, it can take several forms in reptiles. Since the survival can be reduced by reproductive activities, the survival cost is a trade-off between fecundity and survival of males and females. For example, gravid females of the montane skink, *Leiolopisma entrecasteauxii*, are more vulnerable to predators than non-gravids because the weight of the clutch can slow females considerably, reducing running speed and requiring basking for prolonged periods. In many species of reptiles, female fecundity is strongly correlated with body size and energy allocation to reproduction rather than to growth can depress fecundity at later reproductions. Therefore, trade-offs between fecundity and survival are likely to be the main evolutionary determinants of the optimal level of reproductive effort.

When considering the fecundity advantage model, larger females have more space inside their bodies to carry eggs or embryos. Then, larger female will leave more copies of their own genes in succeeding generations than will smaller females, resulting in gene for large body size in females becoming prevalent in the population. However, this hypothesis may not be true for all species, because the energy availability and survival rate of females may be an important determinant

(Shine, 1988). For example, in the pigmy rattlesnake, *Sistrurus miliarius*, there is no sexual dimorphism in color pattern and growth rate. The lack of sexual size dimorphism in this species may be the result of selection for large body size in both sexes (Bishop, et al. 1996)

Body size differences between sexes reflect different selection on males than on females. The study of male snakes of some species conducted by Shine (1978a) suggested that intra-sexual competition between males is the selective agent producing large body size in male snakes. His study coincides with the suggestion of Gibbons (1972) in that it might be the result of a positive selection for larger body size in male snakes which is linked to the aggressive male interaction. However, there are two exceptions. First, species with male combat but males are smaller than females. In this case, male combat may select for large male size, but other factors select for even larger female size. Second, species without male combat but males are larger than females. In this case, the large male size should also result from other selective forces rather than male combat (Shine, 1978a).

In many species of snakes, males and females differ in important ecological characteristics. Differences in diet and habitat use seem to be important in many cases of sex-based ecological divergence in snakes. Many of these differences are related to the differences in body sizes between the sexes. For example, adult male Arafura File snakes, *Acrochordus arafurae* are small (mean = 105 cm SVL, 660 g) and forage in relatively shallow water for small fish, whereas adult females are much larger (mean = 135 cm, 1.4 kg) and tend to feed on larger fish in deeper water (Shine, 1986; Houston and Shine, 1993). Differences in the body size may be selected because some important resources in the habitat are limited. Development of sexual size dimorphism will create the different use of resources between the sexes which will reduce their niche overlap and competition. The details of ecological factors that influence the evolution of sex-specific body size in snakes were reported by Shine (1986b).

Many of the sexually dimorphic attributes of snakes are likely to reflect a combination of selective forces. Each major factor (sex-specific reproductive biology, cost of reproduction and ecological divergence) may amplify the degree of sexual size dimorphism favored by the action of other factors (Shine, 1989; Shine, 1991). Although most snakes do not show extreme dimorphism but sex differences in size, shape, and color are often noticeable. Sexual selection should not be the sole agent for the evolution of sexual dimorphism in snakes. It appears to result from a complex interplay of intra-sexual selection, fecundity selection, and natural selection on ecological relevant attributes of morphology, color, and behavior (Shine, 1993).

There are some studies to explain sexual dimorphism in snakes, for example; sexual dimorphism is evident in adult *Acrochordus arafurae* and neonate for body size, weight/length ratio and head and tail lengths. Two hypotheses for the evolution of such dimorphisms are 1) sexual selection and 2) adaptation of the sexes to different ecological niches of which Shine (1986a) reported that both are selective forces for the evolution of sexual dimorphism in *Acrochordus arafurae*. Pearson et al. (2002) reported that in five isolated populations of the Australian carpet python; *Morelia spilota*, females grew larger than males and had larger head relative to body length. The adult males and females also diverged strongly in dietary composition; males consumed small prey, while females took larger mammals. It was also found that some morphologies such as head size between males and females of different populations are distinct. Thus, the high degree of geographical variation among python populations in sexually dimorphic aspects of body size and shape plausibly result from geographical variation in prey availability. Madsen (1987) reported that the sexual size dimorphism of grass snakes (*Natrix natrix*) follows a typical ontogenetic trajectory in which males and females do not differ in size at hatching but at adult stage, females are usually larger than males and have larger head and shorter tails relative to body length. Males of this species become mature before females. To get a maximum reproductive success female grass snakes have delayed maturation until the age of 5 years. Gregory (2004) suggesting that factors influencing female's grass snake biased sexual size dimorphism are faster growth and later maturation.

Shine (1993) reported that sexually dimorphic characters in snakes show a great degree of variation among related species and even among conspecific populations, providing an ideal opportunity for comparisons and tests of explanatory hypotheses. However, this potential has rarely been exploited. Ecological differences between the sexes such as dietary differences have also attracted little attention.

Furthermore, most studies have focused on adult snakes and much more remains to be learned about juveniles.

2.3 Reproductive biology

Two modes of reproduction are recognized in reptiles, oviparous (egg laying) and viviparous (live bearing) reproduction. Oviparous reproduction includes a variety of situations, ranging from eggs retained in the uterus after ovulation to embryonic development and hatching occurring outside the body. In contrast, viviparous reproduction retains eggs in the uterus for the entire period of embryonic development, so fully developed young are produced at birth (Shine, 1983; Tyagi and Prasad, 1991). Viviparity is a very interesting phenomenon found in snakes which is basically an adaptation to the environment and also shows a surprising similarity to the most advanced group i.e. Mammalia.

Some authors have explained that the origins of live bearing in reptiles are 1) an adaptation to cold environments and 2) an adaptation to provide maternal care of the eggs inside the body (Shine and Bull, 1979). Some ideas suggested that it is generally an adaptation to the environmental condition of the regions climate with shorter and cooler summers (Tyagi and Prasad, 1991). Alternately, Shine and Berry (1978) reported that, except in very cold environments in North America, environmental temperatures seem to play little role in the relative success of live bearing versus egg laying reptiles. Live bearing may be due to entirely different selective forces. The ideas are based on two possibilities: 1) females can regulate their body temperature and thus the temperature for the young, the total incubation time should decrease, thus decreasing the probability of mortality, 2) because temperature conditions are clinal, species invading colder areas might gradually increase retention time, and if retention does increase egg survivorship, the intermediate stages of viviparity can be seen as adaptive (Seigel and Ford, 1987).

The reproductive biology of snakes is considerably less well understood than any other reptilian groups. Especially, the reproductive biology of tropical snakes is still unknown. (Seigel and Ford, 1987).

2.3.1 Reproductive organs

All reptiles have internal fertilization. Snakes have evolved unique intromittent organs, the hemipenes, which develop as paired evaginations from the rear wall of the cloaca (Pough, et al. 2004). The snake's reproductive organs are located between small intestine and urinogenital system. Male snakes have testes in which the left side lies posterior of the right. Each testis has a long slender convoluted tube; the ductus deferens, that connects to the cloaca. At the vent opening, male snakes have two copulatory organs namely hemipenes, each one conducts sperm from the gonads and ductus deferens. Each side is functionally complete and either may be used at a time, but not both. When not in use, each hemipenes is inverted, thus the outside surface is inside. When the male everts one of its hemipenes into a female's cloaca, it unfolds, emerges at the base and enters deeply in the female's cloaca. The surface of a hemispenis is covered with spines which is suitable for insertion, holding and withdrawal (Oldham et al. 1970; Ernst and Zug, 1996).

In females, the kidney and urinary duct are similar to those of males. White multi lobed ovaries are much larger than the testes. The right ovary is situated anterior to the left one. The mesovarium supports each ovary. Swellings present in the ovaries are eggs. The oviduct on each side is supported by mesotubarium and it receives eggs through an anterior opening called the ostium of infundibulum. The anterior fallopian tube follows the infundibulum where the eggs pass to the uterus, where they remain to maturity (Figure 2.2) (Oldham et al. 1970).

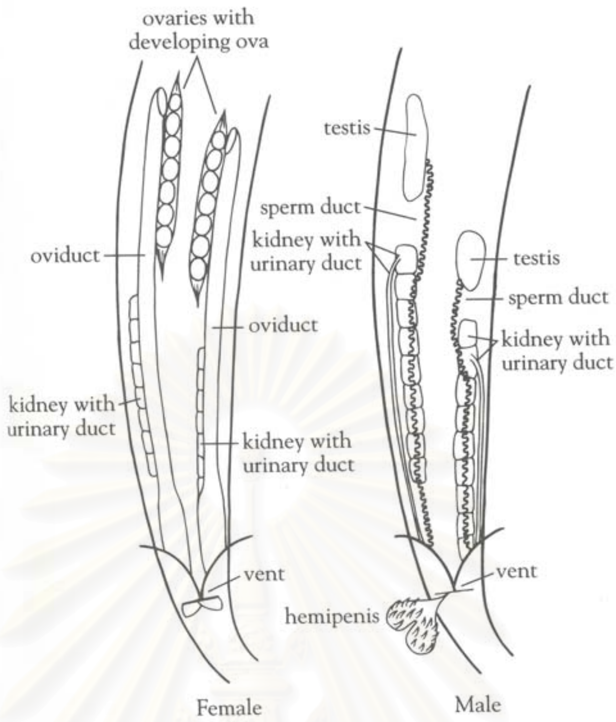


Figure 2.2 Reproductive organs of female and male cottonmouth snakes (*Agkistrodon piscivorus*) (From Ernst and Zug, 1996)

2.3.2 Reproductive cycle

Early studies on reproductive cycles in snakes concentrated mainly on female snakes and the researchers have recently begun to examine the physiological and behavioral changes in relation to reproductive cycles (Seigel and Ford, 1987). The male reproductive cycle has been considered in some ways, such as spermatogenetic cycle (Aldridge and Metter, 1973; Krohmer and Aldridge, 1985; Yokoyama and Yoshida, 1993) and hormonal cycle (Seigel and Ford, 1987). For example, Yokoyama and Yoshida, (1993) reported that the reproductive cycle of the male Habu, *Trimeresurus flavoridis,* is annual. Before mating season, testosterone increases and the size of testes enlarge. The testicular size shows a close relationship to spermatogenesis. During mating season, testicular size may be small because sperm have been transferred into the female’s cloaca. The study of testicular cycle and mating season of eight elapid snakes; *Austrelaps superbus*, *Hemiaspis signata*, *Notechis scutatus*, *Unechis gouldii*, *Hemiaspis daemelii*, *Pseudechis porphyriacus,* *Pseudonaja nuchalis* and *P. textilis*, showed a relationship between production times of spermatozoa and retention of spermatozoa in the vas deferens which is adapted to the timing of the mating season (Shine, 1977a). In a study of female reproductive cycles in seven species of elapid snakes, five were viviparous; *Austrelaps superbus, Hemiaspis signata, Notechis scutatus, Pseudechis porphyriacus, Unechis gouldii*, and two oviparous; *Pseudonaja nuchalis* and *P. textiles*. Although live bearing species have similar seasonal timing of ovulation and parturition. The egg laying species ovulate at about the same time as live bearing but oviposit earlier and may have a second clutch. The clutch size is correlated with maternal size in all the species (Shine, 1977b)

Considering successful reproduction; it requires that males and females must be physiologically and behaviorally ready for mating at the same time. Environmental conditions must also be suitable for the successful development of embryos and neonates. The seasonal change, temperature and photoperiod play important roles in the timing of the reproductive cycle. The temperature is clearly the primary dominant environmental cue controlling gonad and sexual cycle of lizards, but little is known for other orders of reptiles. Food supply appears to be an important factor affecting reproduction, indeed the moisture effect becomes manifested through effects on food production (Duvall et al., 1982; Pough, et al., 2004).

Generally, snakes exhibit two main types of reproductive cycles: associated and continuous. Associated cycles are the most common and characterize species living in the temperate zone and seasonal tropical environments where the period suitable for reproduction is moderately long (Pough, et al., 2004). Pizzatto and Marques (2002) studied the reproductive cycle of false coral snakes *Oxyrhophus guibei*, and the result showed a continuous cycle with vitellogenesis and spermatogenesis occurring throughout the year. Vitt (1983) suggested that the reproductive cycles of tropical snakes may depend on foraging ecology and seasonal variation in resource availability. The Acrochordids have been reported as viviparous, with seasonal reproduction (Shine and Houston, 1993). The study of Shine (1986b) showed that *A. arafurae* is seasonal, litter sizes range from 11-25. *A. granulatus* in the Philippines has a well defined seasonal breeding cycle (Gorman et al., 1981). However seasonal reproduction for *A. granulatus* in the Straits of Malacca is still not clear, it has either aseasonal or loosely seasonal reproduction (Voris and Glodek, 1979; Lemen and Voris, 1981).

2.3.3 Size and sexual maturity

Weight, spermatogenesis and testes size have been used as indicators of maturity in male snakes (Bacolod, 1983; Gibbons, 1972; Shine, 1986a; Aldridge, 2002). The female body size also has an important influence on the reproductive traits of snakes. It is significantly correlated with clutch size, and the number of offspring produced (Lemen and Voris, 1981; Seigel and Ford, 1987). The criterion of maturity in the female is the presence of embryos or yolked follicles (Gibbons, 1972). Brown and Shine (2002) suggested that due to the lost survival rate of keelbacks, *Tropidonophis mairii*, their growth rate in juveniles is rapid and followed by early sexual maturity. Energy is allocated to reproduction but also to continued growth, so the snakes rely upon a high rate of energy intake and high reproductive out put.

2.3.4 Clutch size

Clutch size varies within snake species and between populations. The studies of *Acrochordus granulatus* by Lemen and Voris (1981) and of *Acrochordus arafurae* by Shine (1986a) showed the correlation between relative clutch mass and female body size. In snakes, clutch sizes can be from one to more than 100 eggs per female and the litter size ranges from one to more than 150 newborns. Maternal body size has less effect on offspring size than on clutch size, because the clutch size depends on mother’s health. If food is low or if a female is injured, fat store will be insufficient to produce eggs and the female will skip the reproductive season (Lemen and Voris, 1981; Seigel and Ford, 1987; Ernst and Zug, 1996 Shine 2003).

CHAPTER III

METHODOLOGY

3.1 Study sites at Phangnga bay

Phangnga bay is located on the west coast of Southern Thailand. Its area covers parts of three provinces; Phuket, Phangnga and Krabi. The bay has the total area of about 2,800 km^2. The average depth of the water is about 14 m with a maximum depth of 40 m at the mouth of the bay (Sojisuporn et al., 1994). The bay has been proposed as one of the most biologically productive and ecologically important bays in the world (Chong et al., 1998). Since March 15, 1996 the bay is protected from illegal fishing gear by the Agricultural Ministry's Declaration (Figure 3.1).

There have been many studies concerning environmental factors and natural resources in the bay such as sedimentation, tidal current and velocity, economic fish, phytoplankton, and zooplankton. (Boonruang, 1985; Khokiattiwong et al., 1991; Carr et al., 1991; Sojisuporn et al., 1994). The study of Khokiattiwong et al. (1991) showed that the environment of Phangnga Bay varies by interaction of runoff, tidal current and topography of the bay, especially in the northern or the upper part of the bay where high variability of salinity gradients and mixing are found during the southwest monsoon. The study of the sediment by Carr et al. (1991) also showed that the main soil type in the northern part of the bay is mud or clay, and the mean grain size and sediment sorting increases to the south. Many macrobenthic fauna in Phangnga bay are economically important species such as *Phaphia undulata*, *Metapenaeus ensis*, *M. moyebi* and *M. lysianassa*. The maximum abundance of macrobenthic fauna was in the innermost part of the west bank of Phangnga bay where the mangrove forest was abundant (Sawangerreruke and Boonruang, 1986).

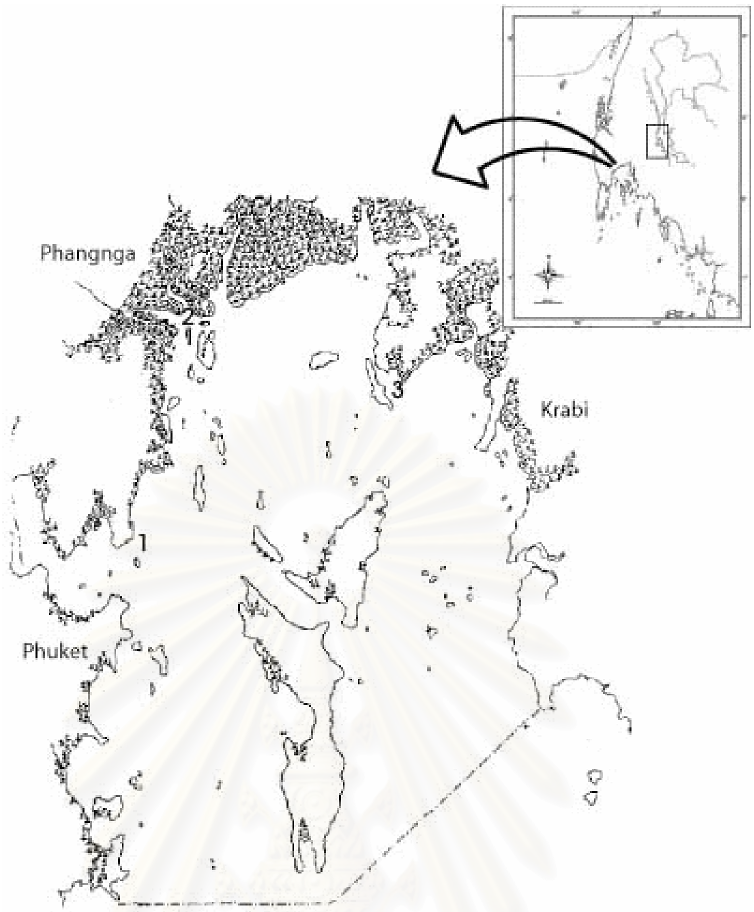


Figure 3.1 Phangnga Bay (1:250000): Mangrove forests occur mainly in the northern part where the water is shallow. *A. granulatus* specimens were collected at three locations; (1) Ban Klong Khien, (2) Ban Sam Chong and (3) Ban Leam Sak. The line at the mouth of the bay shows the area that is protected from illegal fishing gear.

In general, the depth of water in the northern part of the bay is between 2 and 6 meters. Many small and medium size estuaries and patches of mangrove forest occur along the shoreline. Mudflats are also present when the sea water is at low tide. In this study, 3 locations were chosen for *A. granulatus* sampling, they are at Ban Klong Khien, Ban Sam Chong and Ban Leam Sak. In all three locations, local fishermen have placed some fishing equipment such as the stake net and the floating or gill net regularly near the shore areas. The fish trapped in the nets are collected everyday during the low tide.

(1) Ban Klong Khien (N8.14042 E98.42471, Figure 3.2): This area was in the past a vast area of mangrove forest and mudflat. The fresh water from the land runs off through the mangrove forest by a small canal. At present, a large area of the mangrove forest has been changed to shrimp farms. Human activities have been increasing rapidly in this area and there are many permanent fishing traps along the shore.

(2) Ban Sam Chong (N8.29094 E98.50727, Figure 3.3): The mangrove forest in this area is still abundant and intact. There is a large canal runs through the mangrove forest. Bottom type in the canal and along the shore line is mud. Disturbance from human activities is relatively low compared to the other two sites.

(3) Ban Leam Sak (N8.28185 E98.60725, Figure 3.4): The mangrove forest in this area is relatively small compared to the other two sites. A small canal runs through the mangrove forest where the bottom type is mud. Some areas of the mangrove were changed to shrimp farms but not as much as at Ban Klong Khien. Human activities are high in some spots.

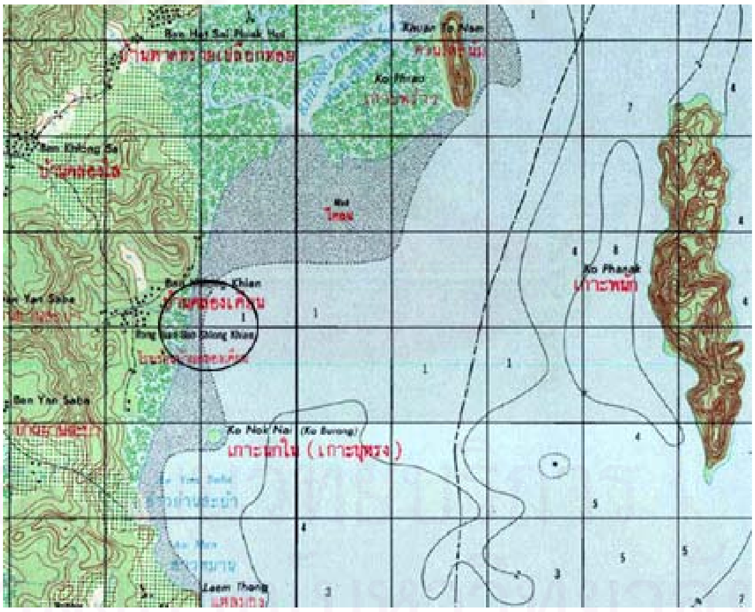


Figure 3.2 Map of Ban Klong Khien, showing the area of mangrove and mudflat where specimens were collected. (Map is 1:50,000, Hydrographic Department, 1984)

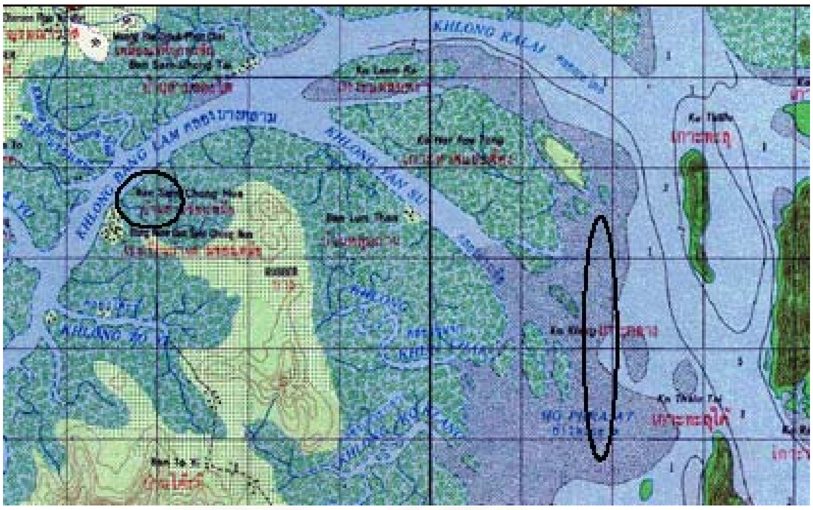


Figure 3.3 Map of Ban Sam Chong: black circles show areas where specimens were collected. (Map is 1:50,000, Hydrographic Department 1984)

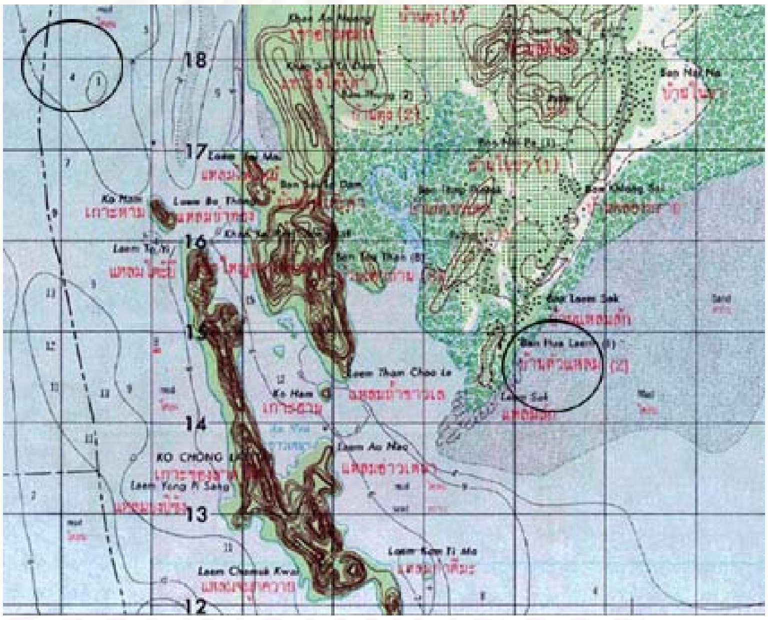


Figure 3.4 Map of Ban Leam Sak; black circles show areas where specimens were collected. (Map is 1:50,000, Hydrographic Department 1984)

3.2 Methods:

3.2.1 Collection of samples

Specimens of *Acrochordus granulatus* in Phangnga bay were trapped using 2 types of local fishing equipment, stake net (Figure 3.5) and gill nets (Figure 3.6). The stake net is a permanent trap since it was placed day and night in the sampling area throughout the study period (January – December 2002) while the gill net was placed overnight in the sampling area and was taken back by the fisherman in the morning. These traps were checked for snakes regularly in the morning throughout the study period. Snakes caught were taken from the trap and kept individually in each plastic bag. If snakes died they were placed in ice immediately to prevent decomposition and were moved to freezer at the laboratory of The Biodiversity Unit, Phuket Marine Biological Center (PMBC). After finishing the research sixteen snake specimens were deposited at the Museum of marine animals and plants of Phuket Marine Biological Center (PMBC 20271-20286). The other specimens were deposited at Museum of Natural History of Chulalongkorn University.

Figure 3.5 The illustration of a stake net.

(adapted from Department of Fisheries, 1969)

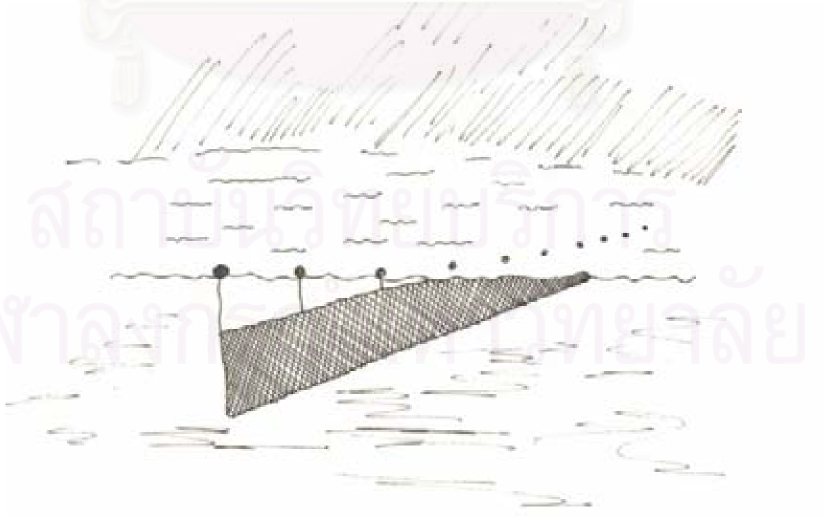


Figure 3.6 The illustration of a gill net.

(adapted from Department of Fisheries, 1969)

3.2.2 Morphological and sex difference

Specimens were euthenized by placing them in a freezer over night. Then the following data was recorded for each specimen:

1) Sex: the sex of snakes was determined by injecting water at the base of the tail. In males the hemipenes would emerge from the vent opening (Figure 3.7A), while for the females hemipenes would not emerge (Figure 3.7B).

2) Weight: the snakes were placed in a plastic bag of known weight. Then, they were weighed by digital balance. The weight was recorded in grams.

3) Head length (HL): the length of the little file snake's head was measured by Vernier calliper. It was the distance from the tip of the snout to the end of the lower jaw which is close to neck (Figure 3.8 -3.9A).

4) Head width (HW): the maximum width of the head was measured by Vernier calliper. The measurement was recorded at the lower jaws are highest distance from each other (Figure 3.9A).

5) Distance between the eyes (EYES): the measurement of distance between the right eye and the left eye was done by Vernier calliper (Figure 3.9A).

6) Snout to the corner of the mouth length (SCL): the measurement was recorded from the tip of snout to the corner of the mouth (Figure 3.8-3.9A) by Vernier calliper.

7) Snout to eye length (SEL): the measurement of the distance from the tip of the snout to the right eye by Vernier calliper (Figure 3.8-3.9A).

8) Mouth width (MW): the maximum size of the mouth opening was measured by plastic rod of known diameter (Figure 3.9C).

9) Snout vent length (SLV): the measurement was done with a measuring tape. It measured from the tip of snout along the body to the vent opening (Figure 3.8).

10) Tail length (TL): the measuring tape was used to measure from the vent opening to the tip of the tail (Figure 3.8-3.9).

11) Neck girth (NG): the neck is at the posterior end of lower jaws. The length around the neck was done with a measuring tape (Figure 3.8-3.9A).

12) Body girth (BG): the girth of the body was measured at the middle of the body length (Figure 3.8). A measuring tape was used to measure the girth around the body.

13) Tail girth (TG): the measurement was done at base of the tail at the vent opening. The length around was measured with a measuring tape (Figure 3.8).

14) Vent width (VW): the measurement of vent width was taken at the maximum width of the vent opening by Vernier calliper (Figure 3.9B).

15) Vent length: the measurement of vent length was taken at the maximum length of the vent opening by Vernier calliper (Figure 3.9B).

A

B

Figure 3.7 The hemipenes emerge from beside the vent opening of males after injection of water at base of tail (A). The female does not show emergence after injection (B).

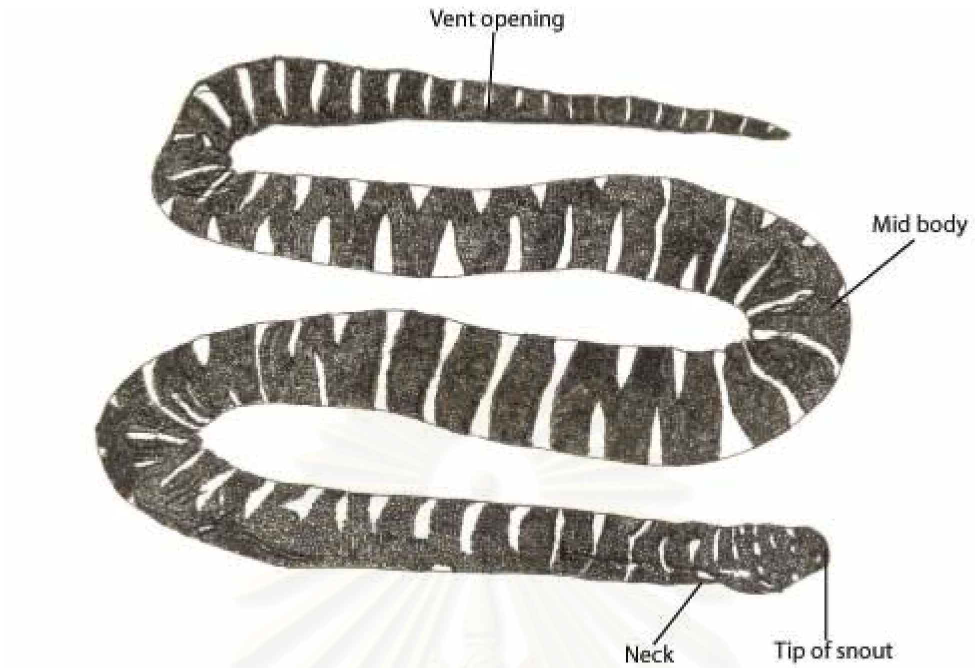
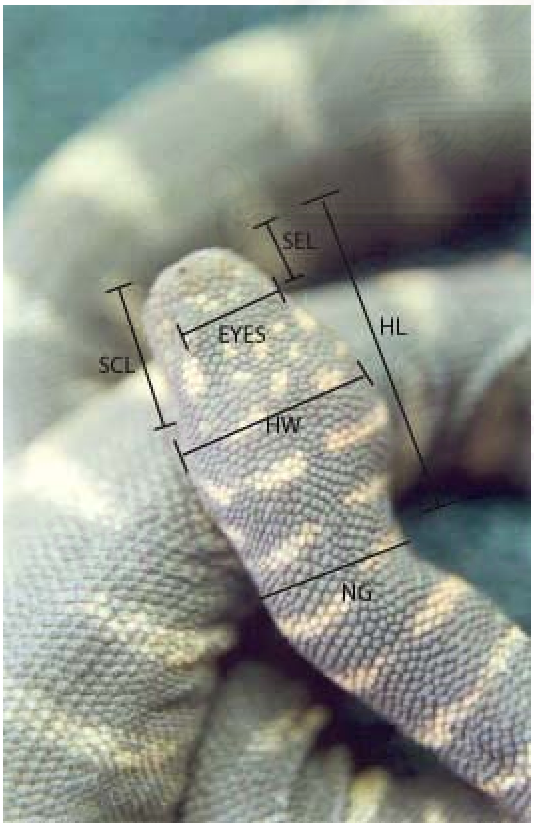


Figure 3.8 The position of major external features of *Acrochordus granulatus*.

A



B

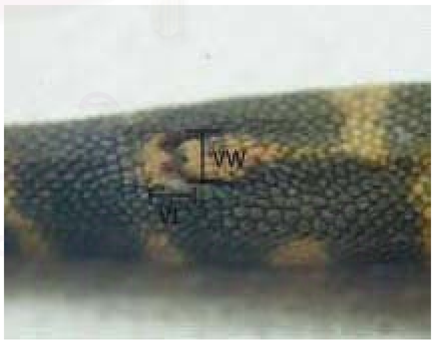


Figure 3.9 The position of measurements on the body of *Acrochordus granulatus* at the head (A) and vent (B)

C

Figure 3.9C The measurement of mouth width on *Acrochordus granulatus* by plastic rod of known diameter.

The morphological differences between the sexes were compared by independent sample T-tests ($p<0.05$). Equations for predicting the sex of *Acrochordus granulatus* were done using the Discriminant Function Analysis ($p<0.05$). However to avoid bias, the data were transform by $\log_3$ and divided by head width. The one factor ANCOVA ($p<0.05$) were used with sex as factor, SVL as covariate and the head length, head width and mouth width as the dependent variable. The statistical analysis was performed on computer by SPSS 12.00 for windows.

3.2.3 Reproductive biology

The sex of specimens was determined by observing internal sex organs. Tissue samples of heart, liver and muscle, including scales of each specimen, were immediately preserved in 95% alcohol for DNA analysis in a future study.

The stage of reproduction, size and number of follicle, vitellogenesis and embryo of each female were recorded during the study period. The follicular volume was estimated by mathematical calculation. The relationship between female's SVL and follicular size was examined. The clutch size was compared with females SVL by the bivariate correlation and the statistical analysis was performed on computer of SPSS 12.00 for windows.

The reproductive state of male snakes was studied by measuring the length and width of testes during the study period. The testis volume was estimated by a mathematic calculation. The relationship between male’s SVL and testis size was examined. The statistical analysis was performed on a computer by SPSS 12.00 for windows.

3.2.4 Diet

The stomach contents were removed from snakes captured in Phangnga bay. Prey items found were immediately preserved in 10% formaldehyde and identified. The maximum size of prey swallowed by a snake was estimated by measuring the width of the snake’s mouth, using a rod of known diameter. The comparison of prey size taken by different sexes was investigated. In addition, the width of the brain case and the length of lower jaws of *Acrochordus granulatus* were measured on skulls from Malaysia, deposited at The Field Museum of Natural History, Chicago (Male: FMNH 230049, 231721-2, 231727, 231729, 232779, 232781, 232786, 232790-1, 232796, 233197, 233203, 234140-1, 234143, 235457- 62, Females: FMNH 231719, 231723-6, 231728, 232778, 232782-5, 232792, 233198-9, 233201, 233204, 235446-8, 235456). The sample were compared by independent sample T-test ($p<0.05$). The statistical analysis was performed on computer by SPSS 12.00 for windows.

CHAPTER IV

RESULT AND DISCUSSTION

4.1 Morphology of *Acrochordus granulatus* in Phangnga bay

4.1.1 External characters

4.1.1.1 Body shape and coloration

The body of *Acrochordus granulatus* in Phangnga bay is shown in figure 4.1. Its body is stout and laterally compressed when supported in water, but slightly flattened when lying down on the sea floor. The body coloration and pattern consists of white or cream and dark brownish to black bands, which are wide on the back and narrow on the ventral side. The juvenile has brighter color than the adult.

Figure 4.1. Female *Acrochordus granulatus* from Phangnga bay. This large snake is an adult female and the smaller one is a juvenile female.

4.1.1.2 Head, neck and tail

Its head is medium to large and not easily distinguished from the neck (Figure 4.2A). The eyes are very small. The nostrils are on the top of the snout. The tip of the mouth at the lower jaw is concave. The tail is short and slightly laterally compressed (Figure 4.2B). The ratio of tail length to snout vent length is about 1:9 in both juvenile and in adult of both sexes.

A

B

Figure 4.2. The head of a female *Acrochordus granulatus* showing the small eyes, nostrils on top of the snout and a concave mouth (A). The slightly laterally flat tail is shown in B.

4.1.1.3 Body scales

The scales are diamond shaped and very small. The body scales are course to the touch. The shape of the dorsal and ventral scales are similar, but scale sizes are slightly smaller ventrally. Supralabial and sublabial scales are larger than adjacent scales (Figure 4.3A). Head shields are broken into small scales like the body scales and no chin-shield is present. The size of the ventral scales is reduced, forming a prominent fold of skin along the center of the belly (Figure 4.3).

A

B

Figure 4.3. Lateral side of *Acrochordus granulatus*, showing body, supralabial and sublabial scales (A). The ventral side of *Acrochordus granulatus*, showing small ventral scales and a fold line (B).

External morphology of *Acrochordus granulatus* in Phangnga bay was similar to other conspecifics reported by several authors (Greene, 1997; Shine and Houston, 1993; Heatwole, 1999, Zug, et al., 2001). It is possible that this species has little variation in body shape and coloration.

4.1.2 Skull and skeleton

4.1.2.1 Skull

The skull of *Acrochordus granulatus* is similar to that of *A. arafurae*, which is wider than those of other snakes and shows the advanced form (Romer, 1956; Camilleri and Shine, 1990, Cundall and Greene, 2000) (Figure 4.4A). The actual CT scan of *A. granulatus* from Malaysia (Figure 4.7A) shows the potential for flexibility of skull elements. The premaxilla of *A. granulatus* is a small bone, has dorsal contact with the septomaxilla, but has lost contact with the maxilla. The nasal is not in contact with frontals and prefrontal. The prefrontal is reduced and contacts at the anterior of the frontal where the prefrontal folds down and contacts with the maxilla laterally (Figure 4.4B). In the Python, an example of a primitive snake form, there is contact between the prefrontal and the shortened nasal (Romer, 1956).

In *A. granulatus*, a single bone forms the post orbital, expanding above the orbit. Anterior of the post orbital to arches the frontal and the ventral extends to the ectopterygoid. The absence of the supraorbital is a character of neomorphic snakes (Romer, 1956). The parietal is a single bone. The dorsal part of the quadrate connects with supratemporal. This bone is similar to those of their sister group, the Colubroidea (comprised of Viperidae, Elapidae and Colubridae) in that it is longer than in other snakes (Cundall and Greene, 2000).

On the ventral side, the vomer is contacted below the septomaxilla. The Palatine is contacted with maxilla laterally and contacted with the pterygoid at the posterior end. The maxilla is fixed to the ectopterygoid, of which the end of ectopterygoid bone bends to touch with the pterygoid laterally. The parasphenoid and basisphenoid perform as a palate and support the brain case (Figure 4.5).

4.1.2.2 Mandible

Mandibles (lower jaws) contact the quadrate and touch the pterygoid. They are connected by ligament (Figure 4.7B). Their ability to distend makes the mouth large enough to swallow bulky prey. The anterior part of the mandible is the dentary and bears teeth. The bone which is posterior ventral to the dentary is the splenial. It forms part of floor and inner wall of the Meckelian canal. Posterior to the splenial is a similar shaped bone named the angular. The posterior half of the mandible is a complex compound bone; articular, prearticular and surangular bones (Figure 4.6).

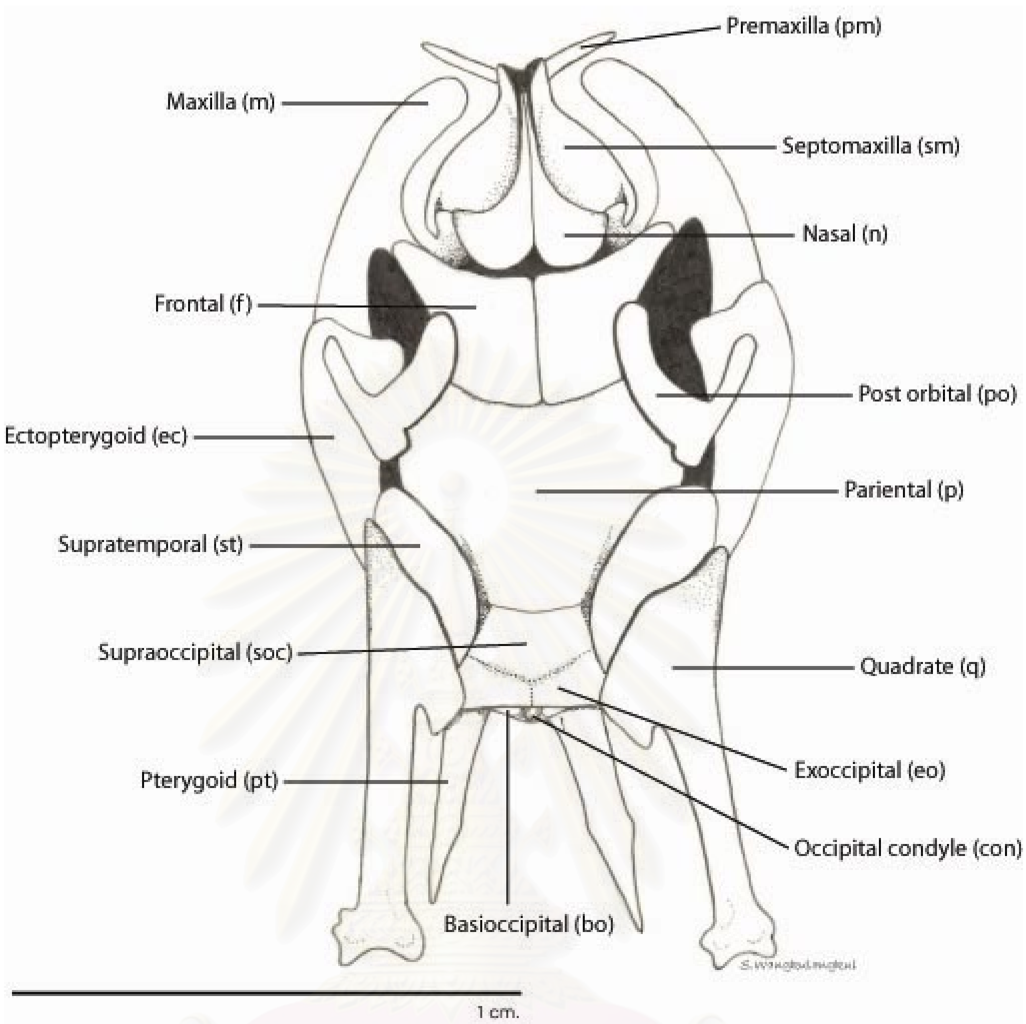
4.1.2.3 Teeth

The hook like teeth of *Acrochordus granulatus* occur on the maxilla, palatine and pterygoid. There are no fangs. The teeth are pleurodont, that are typical of snakes (Romer, 1956). There are no suitable sockets, and the teeth lie in grooves on the bones. Specimens in this study (CUBMZR 2005.10) have 12-14 teeth on jaws (Figure 4.6), 14-16 teeth on the maxillas (Figure 4.8), 7-8 teeth on palatines, and 5 teeth on pterygoids. The specimens from Malasia deposited at the Field Museum of Natural History, Chicago (FMNH 230049, 231721-2, 232790-1, 232796, 232781, and 235446) have approximately 11-14 teeth on the jaws, 11-14 teeth on maxilla, 5-9 teeth on palatine and 5-7 teeth on pterygoid. The CT scan of specimen number FMNH 201350 has 16 teeth on the left jaw and 16 teeth on maxilla at left side.

4.1.2.4 Vertebra

In *Acrochordus granulatus*, the atlas bone (Figure 4.8) is the first vertebra. Atlas and axis bones are modified (Figure 4.9) to allow head movement. The atlas is small and concave on both anterior and posterior. It has small hypapophysis but lacks a neural spine. The neural canal is formed by the neural arch at mid-dorsal and intercentrum at mid-ventral with occipital cotyles to receive the occipital condyle of skull. The axis has a long neural spine and two hypophyses. The odontiod process is locked with the atlas. The atlas and axis are free from contact with ribs. All other vertebrae are concave at the anterior end and convex at the posterior end (Figure 4.10). The size of anterior trunk vertebrae is smaller than posterior, and can not be distinguished from thoracics and lumbars. On each vertebra the neural spine is dorsal and neural arch embraces the neural canal. Long hypapophysis occur on the ventral side. The zygantrum and zygosphere act as connectors between vertebrae. Oldham et al. (1970) and Kardong (1998) reported that to prevent wringing of the long vertebra column of snakes a specialized bone element is needed on the truck vertebrae. This special element locks the vertebrae to each other. Then, the set of anterior zygosphene and posterior zygantrum is interlocking. The rib bears a coastal process attaching to the rib articulation and serves for muscle attachment (Figure 4.11).

A



B

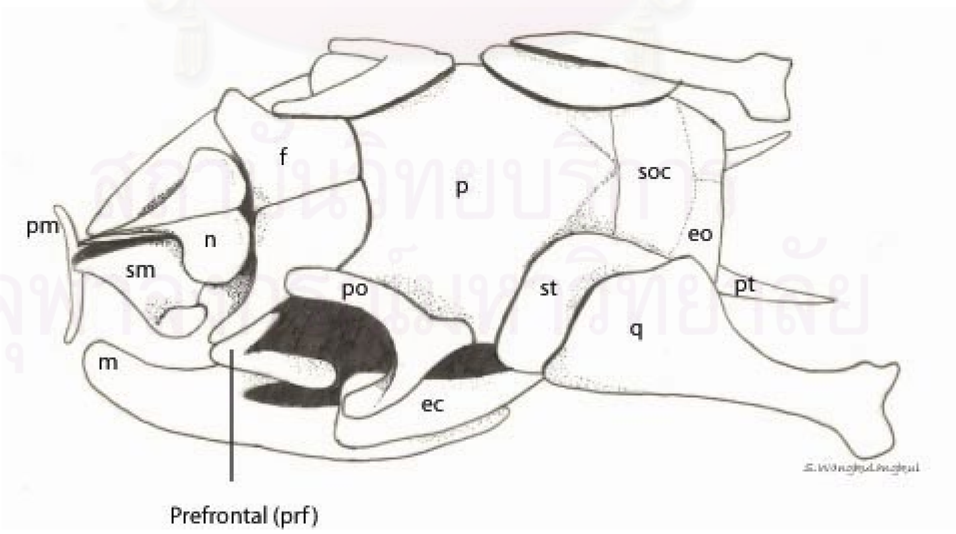


Figure 4.4. The skull elements of an adult female *Acrochordus granulatus* from Phangnga bay (CUBMZR 2005.10), dorsal view (A) and lateral view (B).

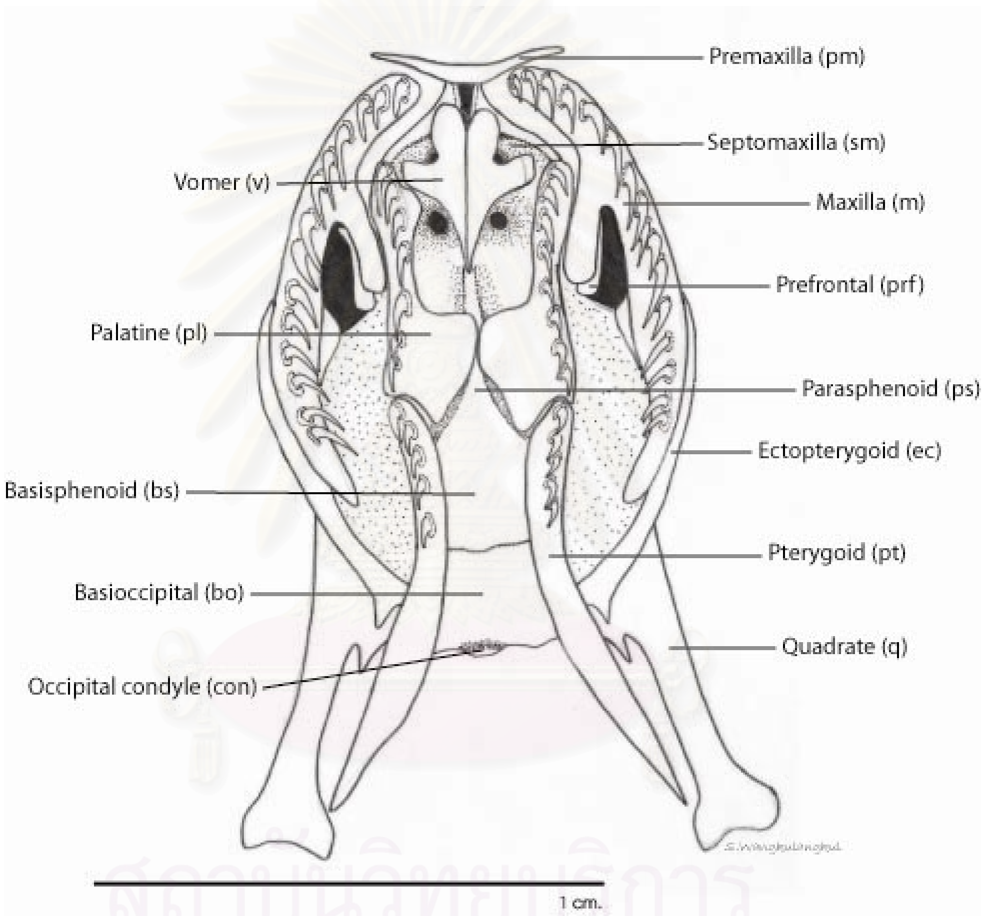


Figure 4.5. The skull elements of an adult female *Acrochordus granulatus* [CUBMZR 2005.10], ventral view.

A B

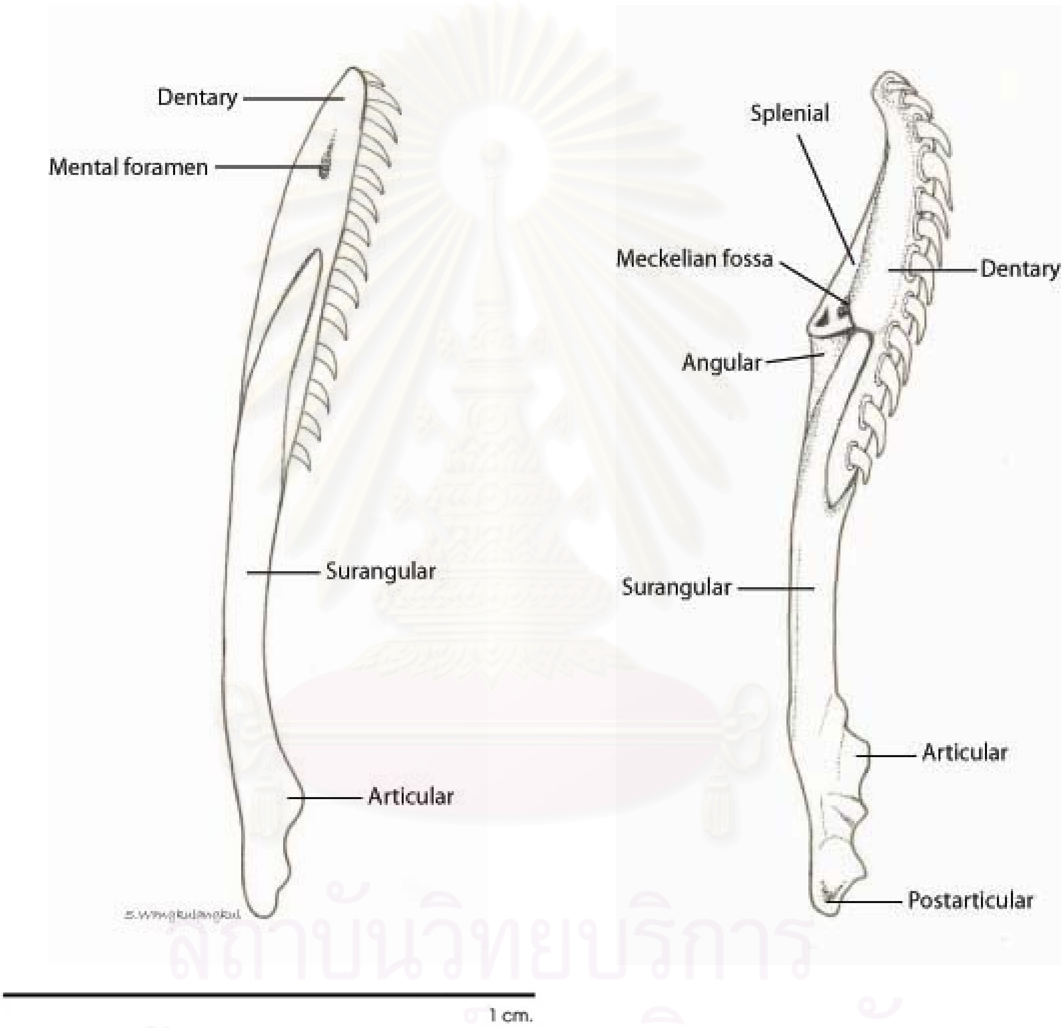
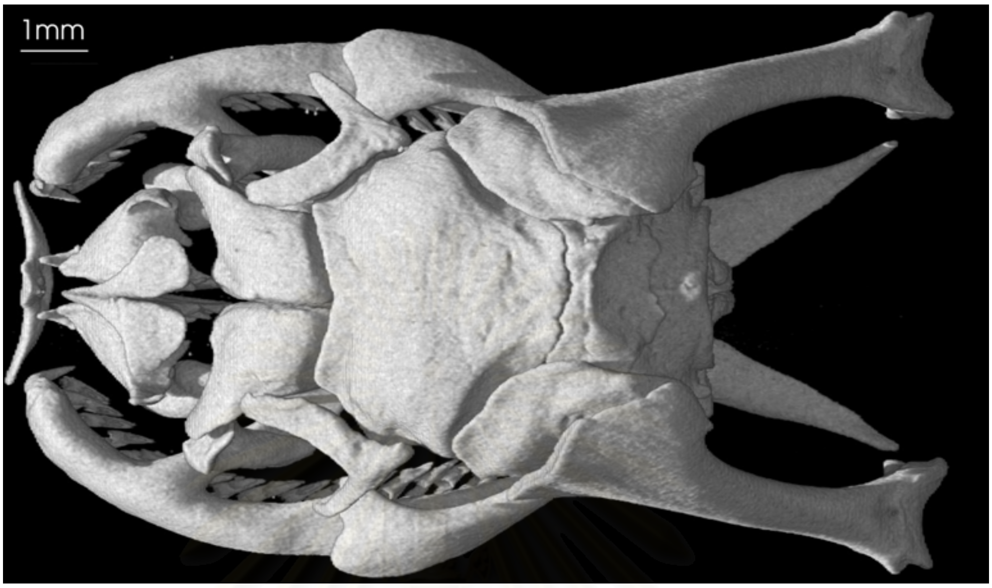


Figure 4.6 Mandibles of *Acrochordus granulatus*, [CUBMZR 2005.10] showing the lateral view of the outer side of the left jaw (A) and the inner side of the right jaw (B).

A



B

Figure 4.7 The dorsal (A) and lateral (B) views of CT scans of *Acrochordus granulatus* [FMNH 201350] from Malaysia.

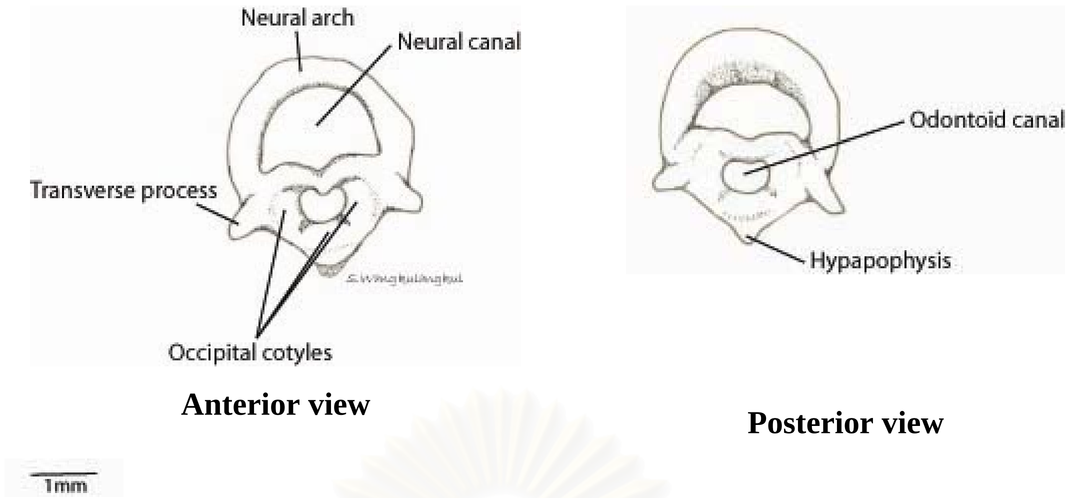


Figure 4.8 The atlas bone of *Acrochordus granulatus*, showing the anterior and posterior views.

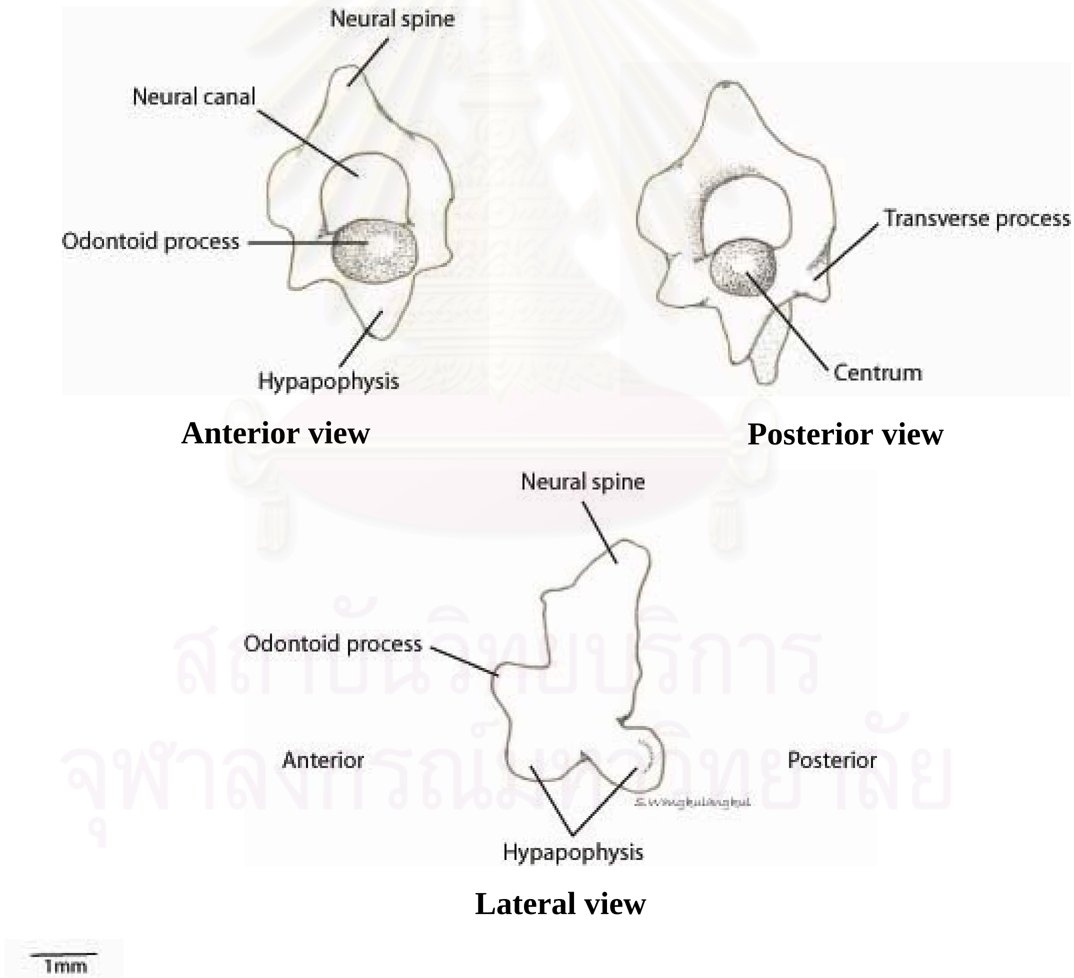
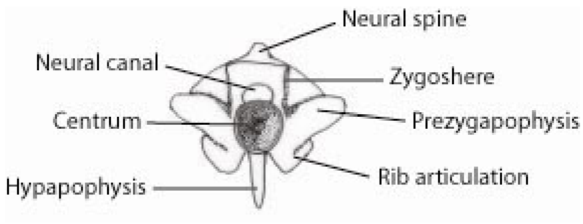
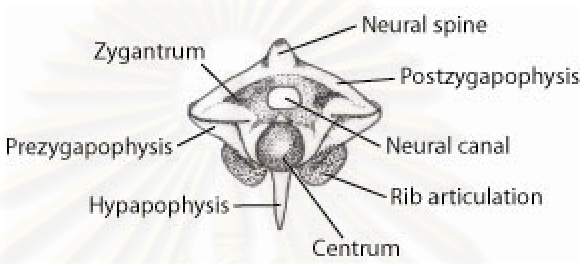


Figure 4.9 The axis of *Acrochordus granulatus*, showing the anterior, posterior and lateral views.



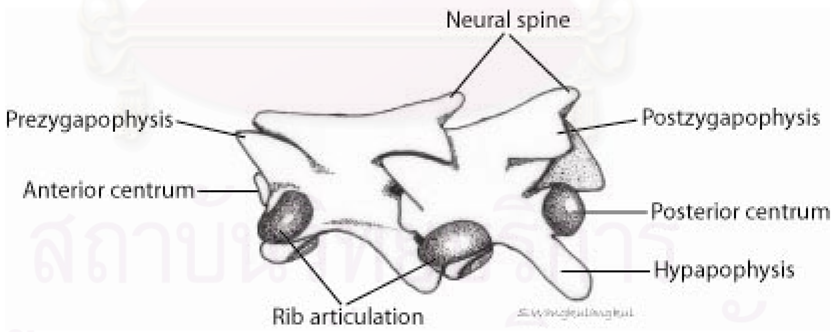
Anterior view



Posterior view

0.5 cm.

Figure 4.10 The vertebrae of *Acrochordus granulatus*, showing the anterior view and the posterior view.



Lateral view

0.5 cm.

Figure 4.11 Lateral view the vertebrae of *Acrochordus granulatus*, showing the locked position.

4.1.3 Reproductive organ

4.1.3.1 Testis

The testes and kidneys lie posterior to the stomach. The right testis and kidney are situated anterior to the left. Testes are pink, depressed and attached with white convoluted tubes, namely ductus deferens. Kidneys are a gray to brown color and elongated; they are close to the renal portal vein and ureters. All these tubes lead posterior to join with the cloaca and the vent opening (Figure 4.12).

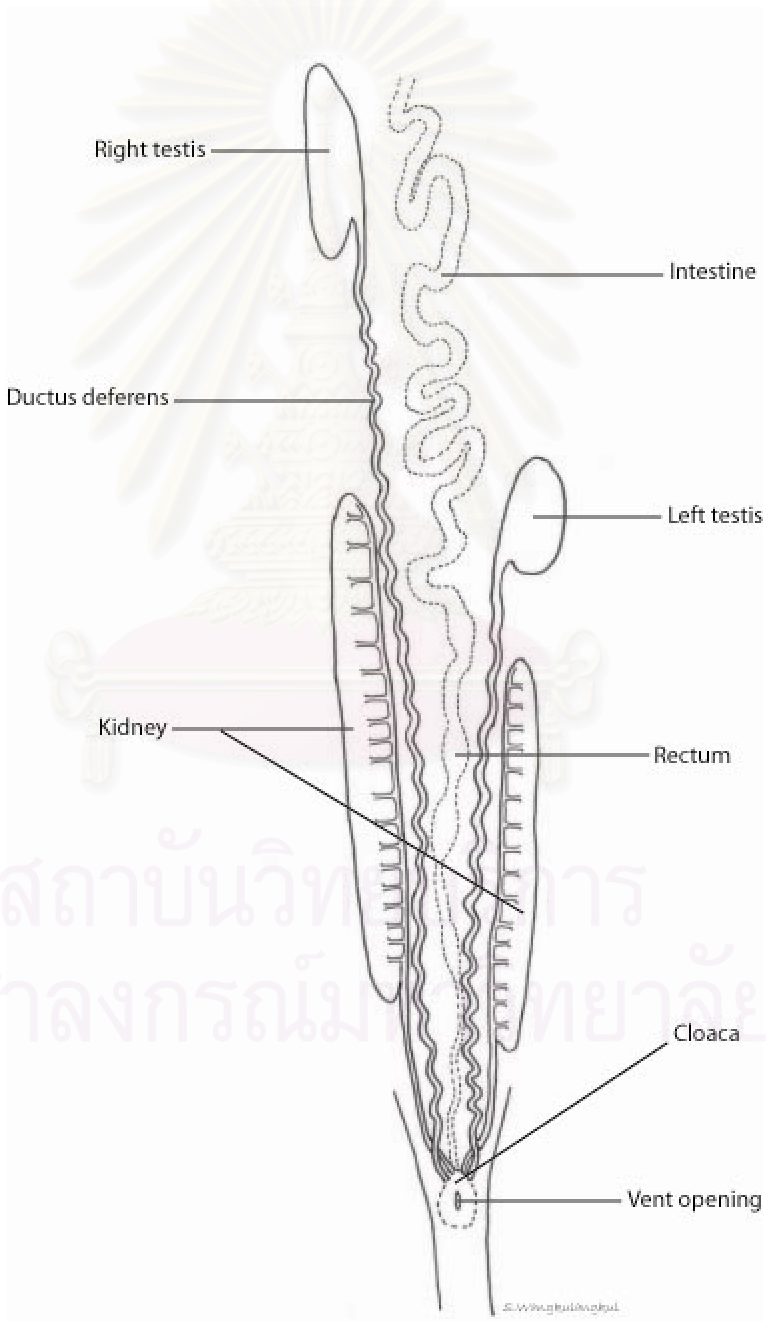


Figure 4.12 Male reproductive organs of *Acrochordus granulatus* [CUBMZR 2005.11]

4.1.3.2 Ovary

The kidney and ureters are similar to that of the male in figure 4.11. The right ovary is situated anterior to the left. The mesentery supports each ovary which lies close to the oviduct. The follicles with oocytes were found on the ovary (Figure 4.13). The oviduct is longer on the right side than the left side and the ostrium is at the anterior end.

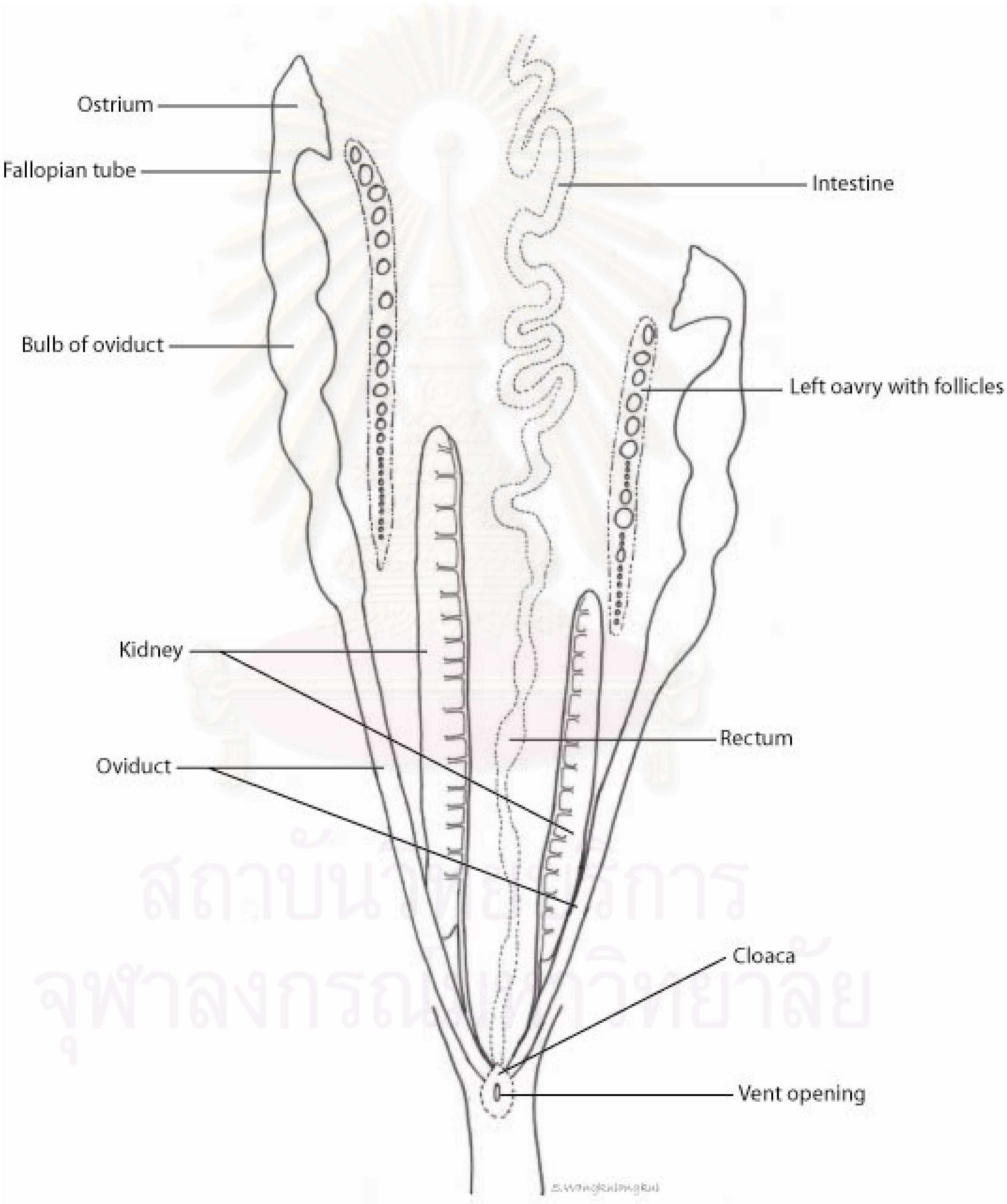


Figure 4.13 Female reproductive organs of *Acrochordus granulatus* [CUBMZR 2005.12]

4.2. Sexual dimorphism

4.2.1 Size distribution

The frequency distribution of 13 characters from the male little file snake's population from Phangnga bay show 7 normally distributed characters; distance between eyes, snout to corner of the mouth length, snout to right eye length, neck girth, body girth, vent width and vent length. The female population showed 4 normally distributed characters; snout to right eye length, neck girth, body girth and tail girth. Other characters measured; head length, head width, snout-vent length, tail length, and mouth width, do not demonstrate a normal distribution in either sex.

4.2.1.1 Head length

The frequency distribution of head length for 44 male little file snakes is shown in Figure 4.14A. Males have an average head length (±SE) of 16.61 ± 0.26 mm (median = 17.00 mm, variance = 3.09). For male snakes in the sample the smallest head length is 11.80 mm, and the largest is 19.80 mm. The skewness value of -0.99 indicates that these data are asymmetrical and the mean is less than the median. The kutosis value of 1.28 indicates that the data are concentrating around the mean. The normality test gave a significant difference (Shapiro-Wilk = 0.01, $p<0.05$).

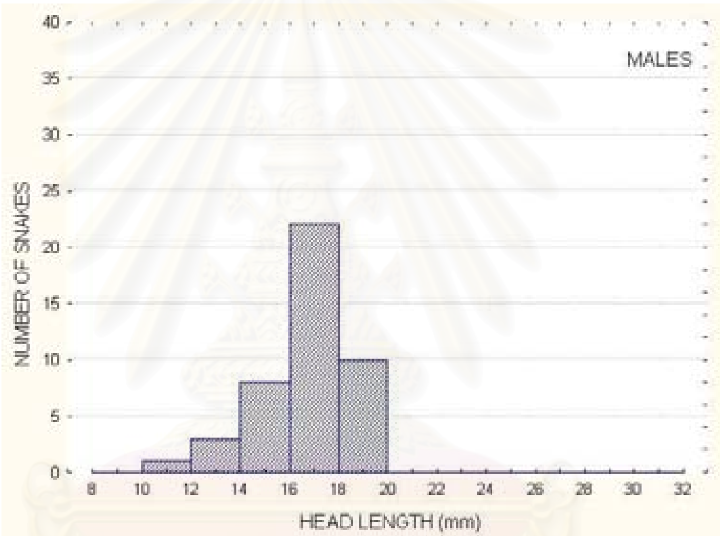
Seventy nine females have an average head length (±SE) of 18.30 ± 0.29 mm (Figure 4.14B). The median is 18.70 mm and the variance is 6.77. The smallest head length for female snakes captured is 10.50 mm, and the largest is 28.90 mm. The skewness value of -0.09 indicates that these data have tend toward asymmetrical and the mean is less than the median. The kutosis value of 3.75 indicates that the data are concentrating around the mean. The normality test gave a significant difference (Kolmogorov-Smirnov = 0.00, $p<0.05$).

4.2.1.2 Head width

Male little file snakes have an average head width (±SE) of 10.54 ± 0.20 mm, the median is 10.50 mm and the variance is 1.69 (Figure 4.15A). For male snakes in the sample the smallest width is 6.7 mm, and the largest is 13.10 mm. The skewness value of -0.74 indicates that these data are asymmetrical and the mean is less than the median. The kutosis value of 2.03 indicates that the data are concentrating around mean. The normality test gave significant difference (Shapiro-Wilk = 0.01 $p<0.05$).

The females little file snakes have an average head width ($\pm$SE) of 12.22 $\pm$ 0.24 mm (Figure 4.15B). The median is 12.10 mm and the variance is 4.64. The smallest head width of female snakes captured is 8.00 mm, and the largest is 18.60 mm. The skewness value of 0.80 indicates that these data are asymmetrical and the mean is more than the median. The kutosis value of 1.55 indicates that the data are concentrating around the mean. The normality test gave significant difference (Kolmogorov-Smirnov =0.00, $p<0.05$).

A



B

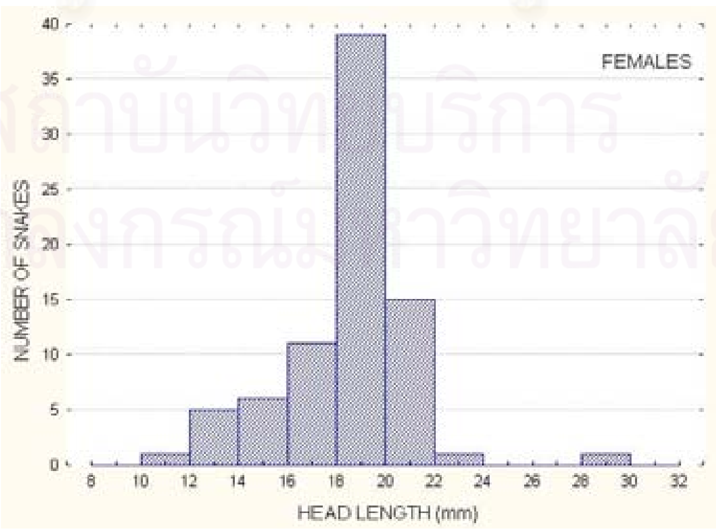
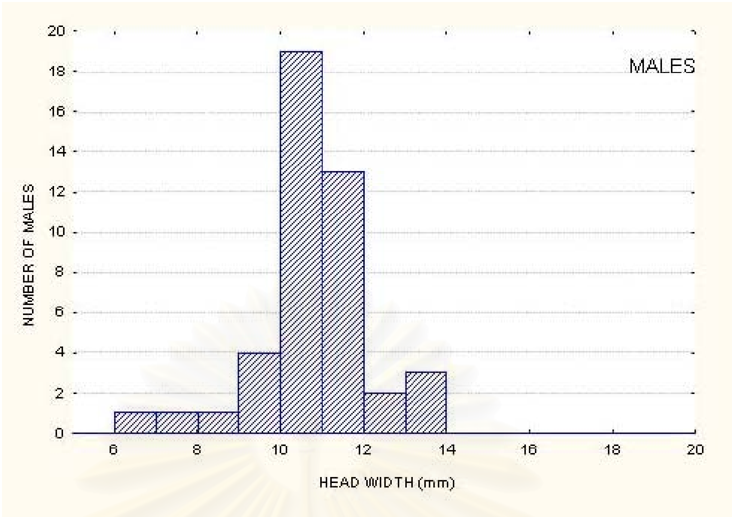


Figure 4.14 The frequency distribution of head lengths for males (A) and females (B) of *Acrochordus granulatus* from Phangnga bay.

A



B

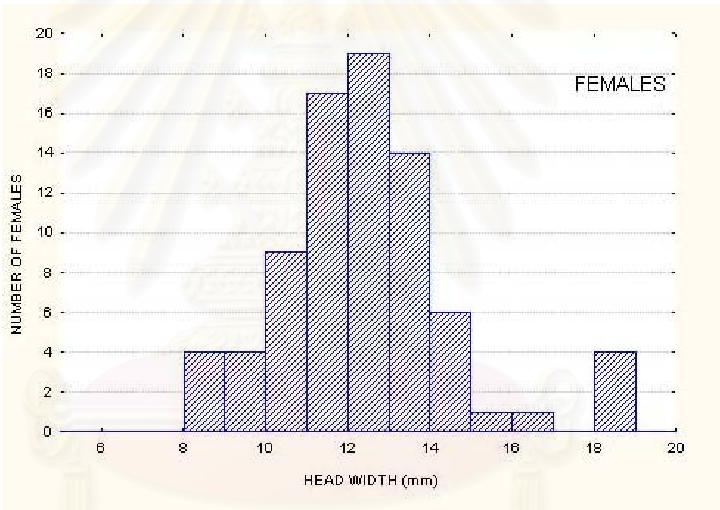


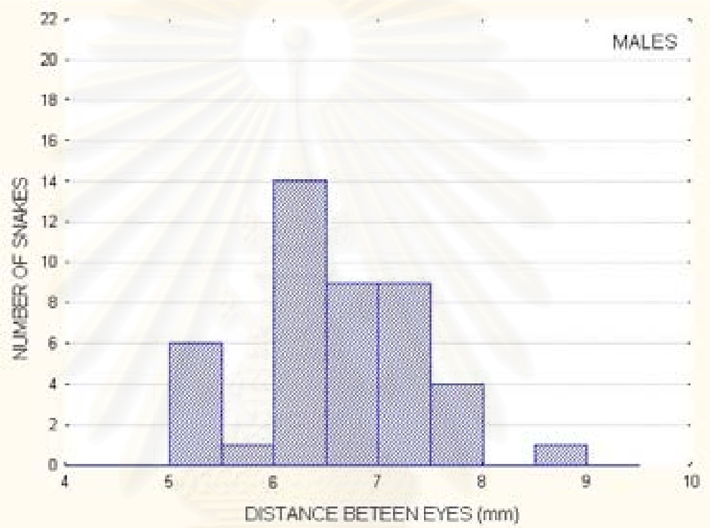
Figure 4.15. The frequency distribution of head width for males (A) and females (B) of *Acrochordus granulatus* from Phangnga bay.

4.2.1.3 Distance between eyes

The frequency distribution of distance between eyes for the male little file snakes have an average value (±SE) of 6.55 ± 0.11 mm, the median is 6.60 mm and the variance is 0.62. The distance between eyes of smallest male snake captured is 5.00 mm, and the largest is 8.60 mm. The skewness value of -0.12 indicates that these data tend toward asymmetrical and the mean is less than the median. The kutosis value of 0.09 indicates that the data are concentrating around the mean. The normality test gave a non significant difference (Shapiro-Wilk = 0.23 $p<0.05$) (Figure 4.16A).

The females had an average value of distance between eyes (±SE) of 6.96 ± 0.11 mm (Figure 4.16B). The median is 7.20 mm and the variance is 0.94. Minimum value of distance between eyes of female snakes captured is 4.00 mm, and the maximum value is 9.00 mm. The skewness value of -0.79 indicates that these data are asymmetrical and the mean is less than the median. The kutosis value of 0.94 indicates that the data are concentrating around mean. The normality test gave significant difference (Kolmogorov-Smirnov = 0.02, $p<0.05$).

A



B

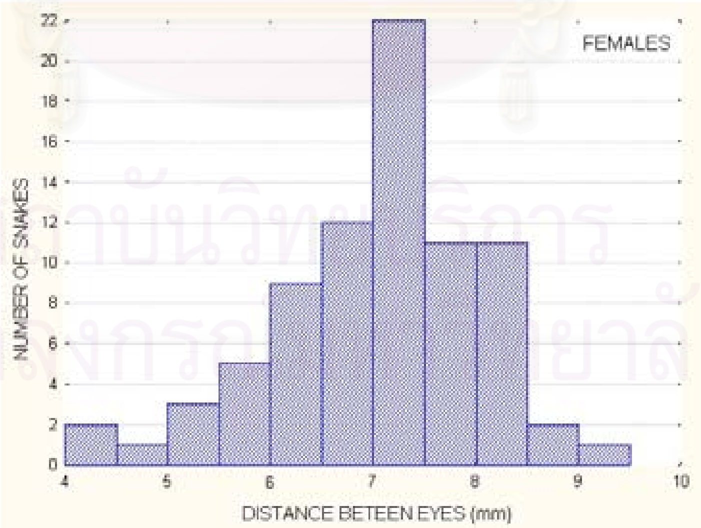


Figure 4.16 The frequency distribution of distance between eyes for males (A) and females (B) of *Acrochordus granulatus* from Phangnga bay.

4.2.1.4 Snout to corner of the mouth length

The frequency of snout to corner of the mouth length for 44 male little file snakes is shown in Figure 4.17A. Males have an average snout to corner of the mouth length (±SE) of 9.64 ± 0.17 mm (median = 9.40 mm, variance = 1.23). The minimum value of snout to corner of the mouth length of male snakes captured is 7.00 mm, and the largest is 11.50 mm. A skewness value of -0.44 indicates that these data tend toward asymmetrical and shows that the mean is less than the median. The kutosis value of -0.20 indicates that the data are more dispersion around the mean. The normality test gave non significant difference (Shapiro-Wilk = 0.10, $p<0.05$).

The females have an average snout to corner of the mouth length (±SE) of 10.52 ± 0.19 mm (Figure 4.17B). The median is 10.40 mm and the variance is 2.94. For female snakes in the sample the smallest snout to corner of the mouth length is 7.00 mm, and the largest is 18.00 mm. The skewness of 1.07 indicates that these data tend toward asymmetrical and show the mean is more than the median. The kutosis value of 3.97 indicates that the data are concentrating around the mean. The normality test gave a significant difference (Kolmogorov-Smirnov = 0.00, $p<0.05$).

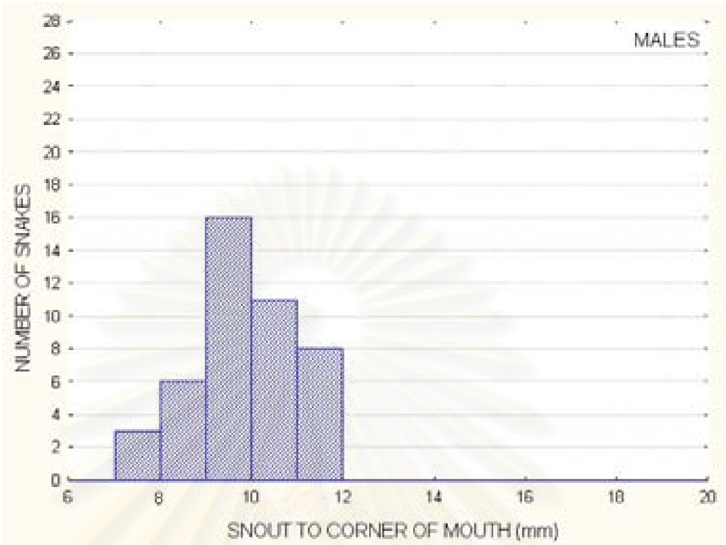
4.2.1.5 Snout to eye length

The frequency of snout to eye length (right side) of male little file snakes has an average value (±SE) of 4.66 ± 0.10 mm, the median is 4.65 mm and the variance is 0.45 (Figure 4.18A). The minimum value of the snout to eye length of male snakes captured is 3.00 mm, and the largest is 6.00 mm. The skewness value of -0.37 indicates that these data tend toward asymmetrical and the mean is less than median. The kutosis value of 0.44 indicates that the data are concentrating around mean. The normality test gave a non significant difference (Shapiro-Wilk = 0.10, $p<0.05$).

Females have an average value of snout to right eye length (±SE) of 4.81 ± 0.08 mm (Figure 4.18B). The median is 4.80 mm and the variance is 0.56. The smallest snout to eye length of female snakes captured is 3.40 mm, and the largest is 6.50 mm. The skewness value of 0.27 indicates that these data tend toward asymmetrical and the mean is more than the median. The kutosis value of -0.38

indicates that the data are more dispersed around the mean. The normality test gave a non significant difference (Kolmogorov-Smirnov = 0.06, $p<0.05$).

A



B

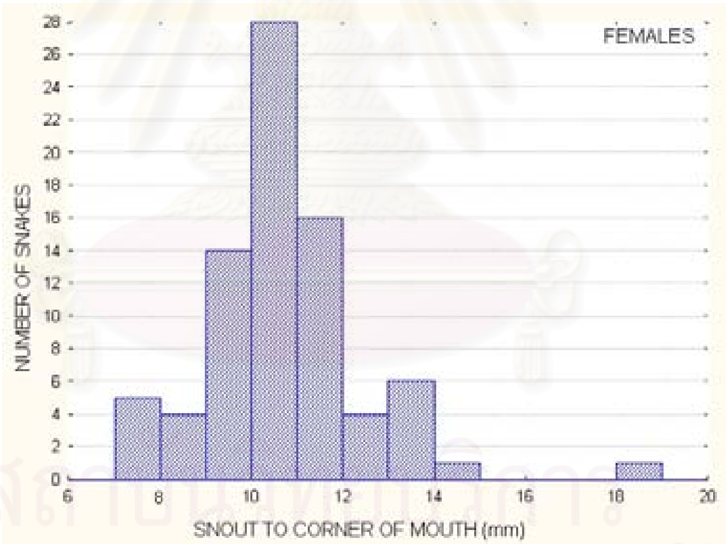
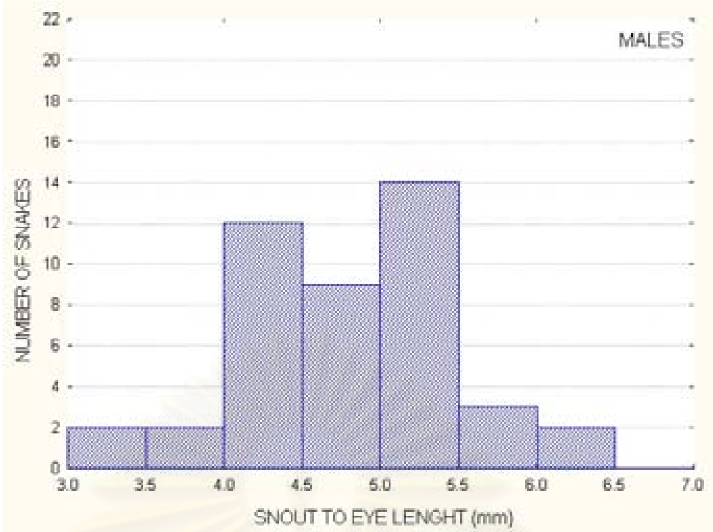


Figure 4.17 The frequency distribution of snout to corner for the mouth length of males (A) and females (B) of *Acrochordus granulatus* from Phangnga bay.

A



B

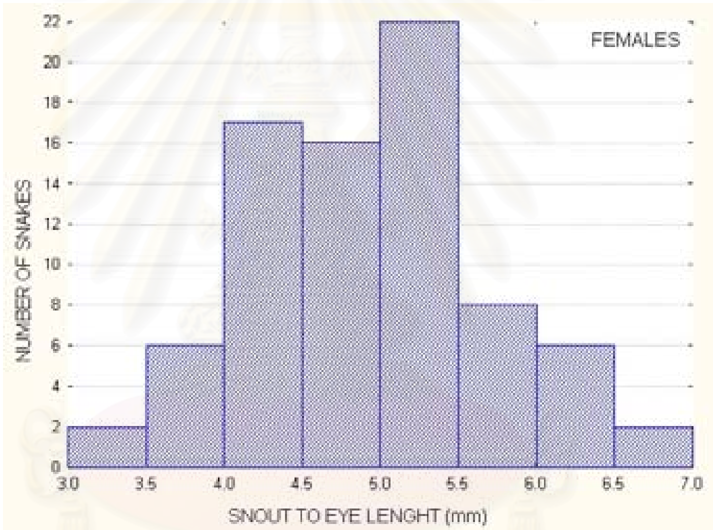


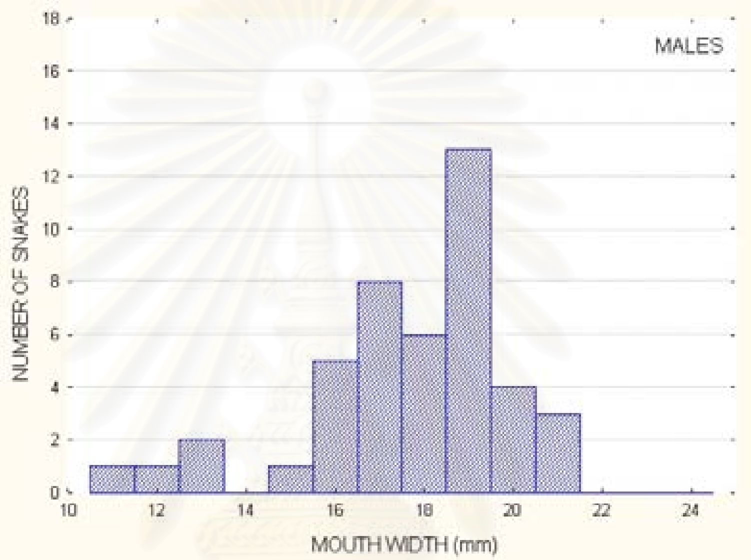
Figure 4.18. The frequency distribution of snout to eye length of males (A) and females (B) of *Acrochordus granulatus* from Phangnga bay.

4.2.1.6 Mouth width

The frequency distribution of mouth width for 44 male little file snakes is shown in Figure 4.19A. Males have an average mouth width (±SE) of 17.68 ± 0.34 mm, (median = 18.00 mm, variance = 5.19). For male snakes in the sample the minimum value of mouth width is 11.00 mm, and the largest is 21.00 mm. The skewness value of -1.12 indicates that these data are asymmetrical and the mean is less than the median. The kutosis value of 1.34 indicates that these data concentrate around mean. The normality test gave a significant difference (Shapiro-Wilk = 0.00, $p<0.05$).

Females have an average value of mouth width (+SE) of 20.17 + 0.30 mm (Figure 4.19B). Median is 20.00 mm and the variance is 7.19. The smallest mouth width of female snakes captured is 12.00 mm, and the largest is 24.00 mm. The skewness value of -1.09 indicates that these data are asymmetrical and the mean is less than the median. The kutosis value of 1.43 indicates that these data are concentrated around mean. The normality test gave a significant difference (Kolmogorov-Smirnov = 0.00, $p<0.05$).

A



B

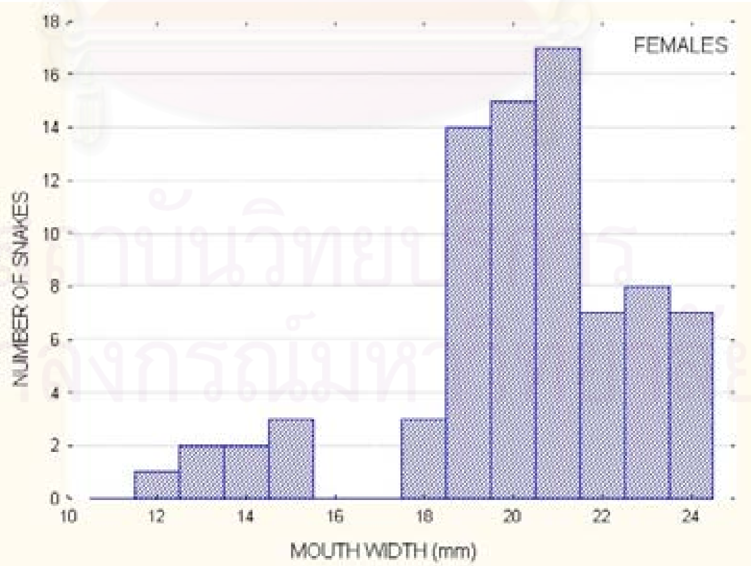


Figure 4.19 The frequency distribution of mouth width for males (A) and females (B) of *Acrochordus granulatus* from Phangnga bay.

4.2.1.7 Snout - vent length

The frequency distribution of snout-vent length for male little file snakes has an average value (±SE) of 596.18 ± 11.25 mm, the median is 600.00 mm and variance is 5568.10 (Figure 4.20A). The minimum value snout-vent length of male snakes captured is 364.00 mm, and the largest is 690.00 mm. The skewness value of -1.51 indicates that these data are asymmetrical and the mean is less than the median. The kutosis value of 2.51 indicates that these data concentrate around the mean. The normality test gave a significant difference (Shapiro-Wilk = 0.00, $p<0.05$).

Females have an average value of snout-vent length (±SE) of 629.23 ± 10.58 mm (Figure 4.20B). The median is 646.00 mm and the variance is 8854.30. The smallest snout-vent length of female snakes captured is 374.00 mm, and the largest is 850.00 mm. The skewness value of -0.82 indicates that these data are asymmetrical and the mean is less than the median. The kutosis value of 0.94 indicates that these data are concentrated around the mean. The normality test gave a significant difference (Kolmogorov-Smirnov =0.00, $p<0.05$).

A

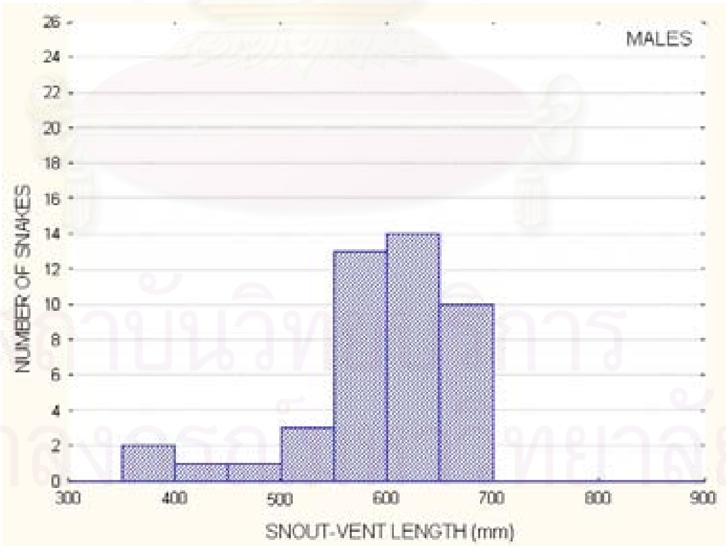


Figure 4.20A The frequency distribution of snout-vent length for males of *Acrochordus granulatus* from Phangnga bay.

B

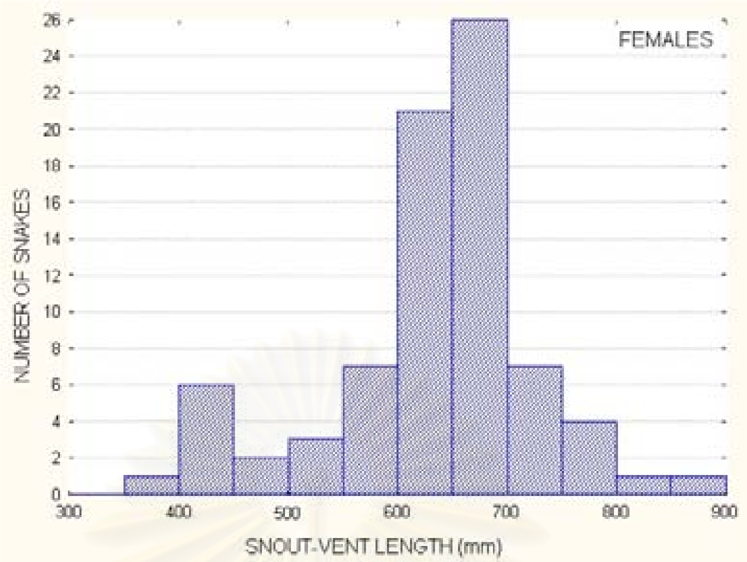


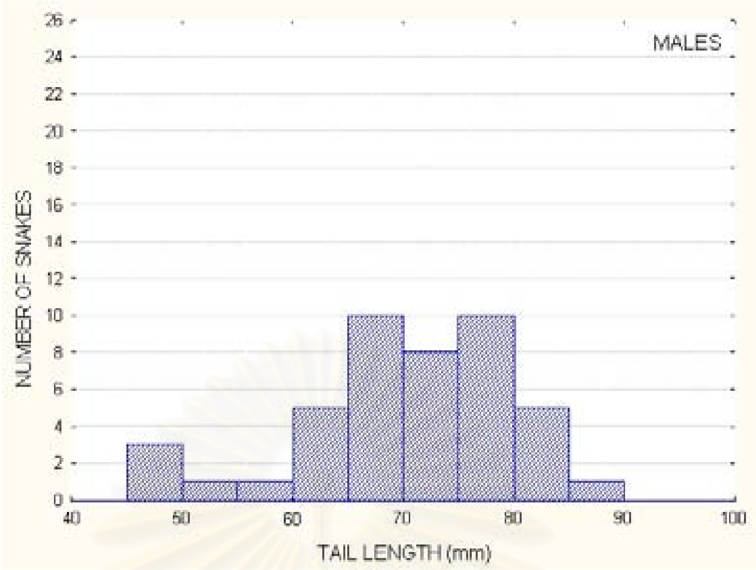
Figure 4.20B The frequency distribution of snout-vent length for females of *Acrochordus granulatus* from Phangnga bay.

4.2.1.8 Tail length

Males have an average value of tail length (±SE) of 69.07 ± 1.47 mm (Figure 4.21A). The median is 70.00 mm and the variance is 95.84. The smallest tail length of male snakes captured is 45.00 mm, and the largest is 87.00 mm. The skewness value of -0.88 indicates that these data are asymmetrical and the mean is less than the median. The kutosis value of 0.56 indicates that these data are concentrated around mean. The normality test gave a significant difference (Shapiro-Wilk = 0.01, $p<0.05$).

The frequency distribution of tail length for female little file snakes have an average value (±SE) of 68.92 ± 1.10 mm (median = 70.00 mm, variance = 96.04). The minimum value tail length of female snakes captured is 40.00 mm, and the largest is 96.00 mm. The skewness value of -0.50 indicates that these data are asymmetrical and the mean is less than the median. The kutosis value of 1.19 indicates that these data are concentrated around mean. The normality test gave a significant difference (Kolmogorov-Smirnov = 0.00, $p<0.05$) (Figure 4.21B).

A



B

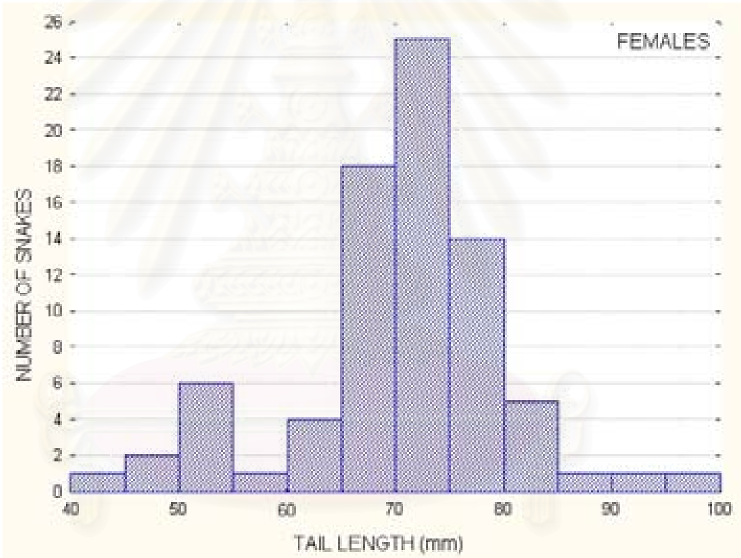


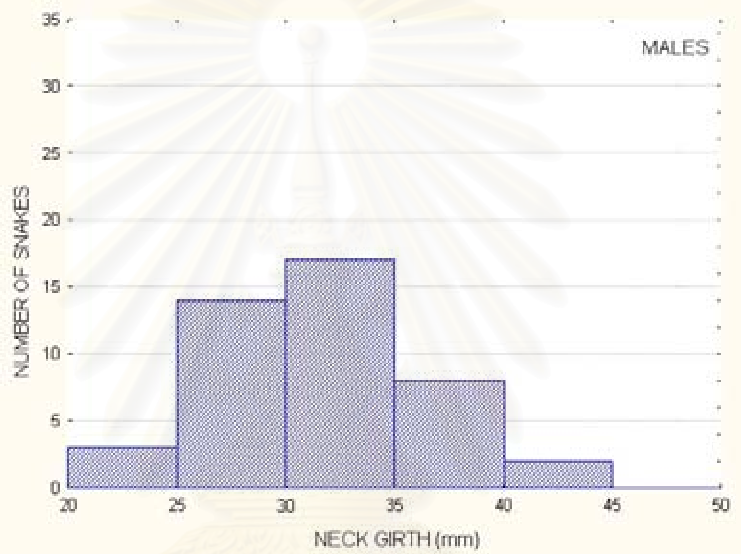
Figure 4.21 The frequency distribution of tail length for males (A) and females (B) of *Acrochordus granulatus* from Phangnga bay.

4.2.1.9 Neck girth

The frequency distribution of neck girth for male little file snakes have an average value (±SE) of 31.27 ± 0.68 mm, the median is 30.95 mm and the variance is 20.40 (Figure 4.22A). The minimum value of neck girth of male snakes captured is 20.00 mm, and the largest is 41.00 mm. The skewness value of -0.13 indicates that these data tend toward asymmetrical and mean is less than the median. The kutosis value of 0.39 indicates that these data concentrate around the mean. The normality test gave a non significant difference (Shapiro-Wilk = 0.47, $p<0.05$).

Females have an average neck girth (+SE) of 34.23 + 0.56 mm (Figure 4.22B). The median is 34.00 mm and the variance is 25.28. The smallest neck girth of female snakes captured is 22.00 mm, and the largest is 48.00 mm. The skewness value of 0.24 indicates that these data are asymmetrical and mean is more than the median. The kutosis value of 0.34 indicates that these data are concentrated around the mean. The normality test gave a non significant difference (Kolmogorov-Smirnov = 0.20, $p<0.05$).

A



B

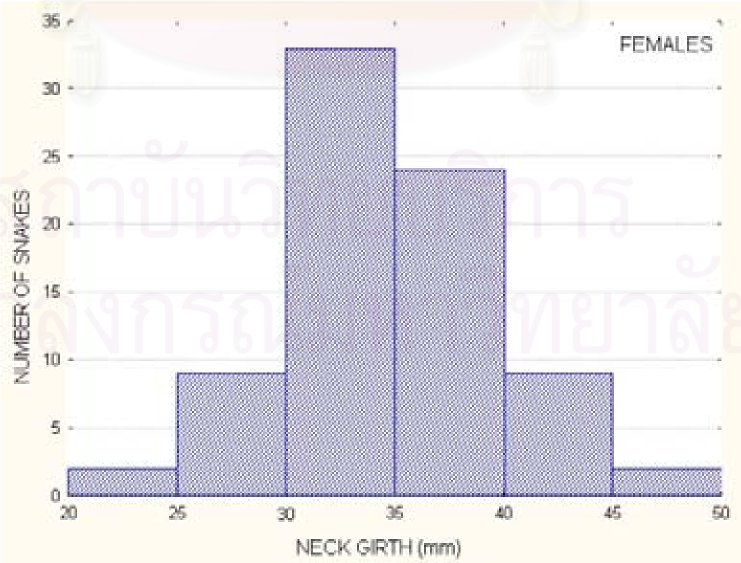


Figure 4.22 The frequency distribution of neck girth for males (A) and females (B) of *Acrochordus granulatus* from Phangnga bay.

4.2.1.10 Body girth

The Body girth of male little file snakes averages (+SE) 54.13 + 1.23 mm, the median is 54.00 mm and the variance is 66.93. The minimum value of body girth of male snakes captured is 32.00 mm, and the largest is 74.00 mm. The skewness value of -0.38 indicates that these data tend toward asymmetrical and mean is less than the median. The kutosis value of 0.92 indicates that these data concentrate around mean. The normality test gave a non significant difference (Shapiro-Wilk = 0.56, $p<0.05$) (Figure 4.23A).

Females have an average value of body girth (+SE) of 59.78 + 1.46 mm (Figure 4.23B). The median is 60.00 mm and the variance is 168.14. The smallest body girth of female snakes captured is 32.50 mm, and the largest is 98.00 mm. The skewness value of 0.93 indicates that these data tend toward asymmetrical and the mean is more than the median. The kutosis value of 0.34 indicates that these data are concentrated around mean. The normality test gave a non significant difference (Kolmogorov-Smirnov = 0.20, $p<0.05$).

A

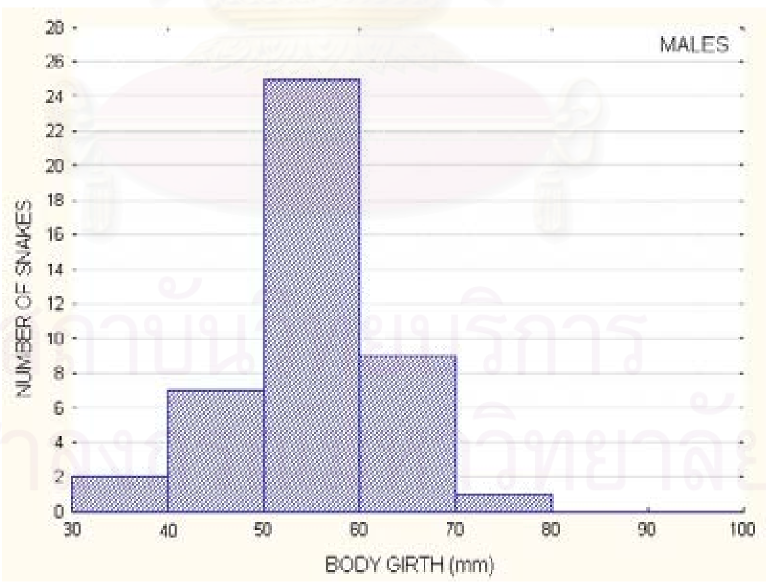


Figure 4.23A. The frequency distribution of body girth for males of *Acrochordus granulatus* from Phangnga bay.

B

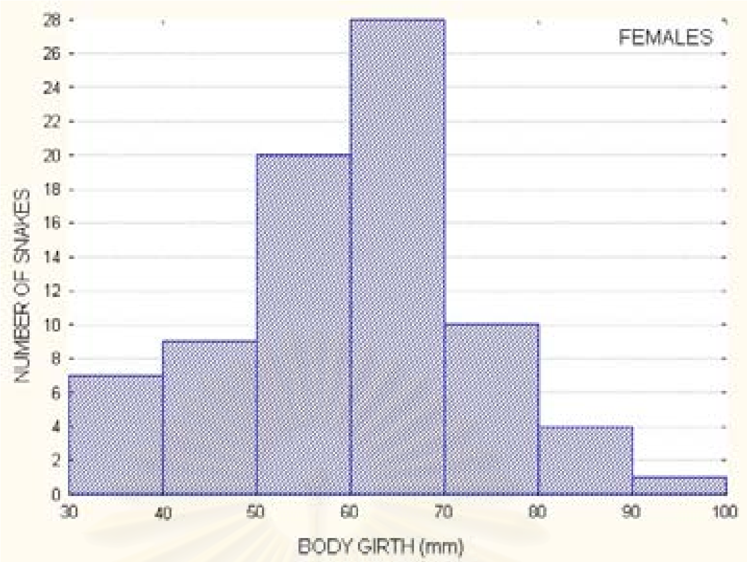


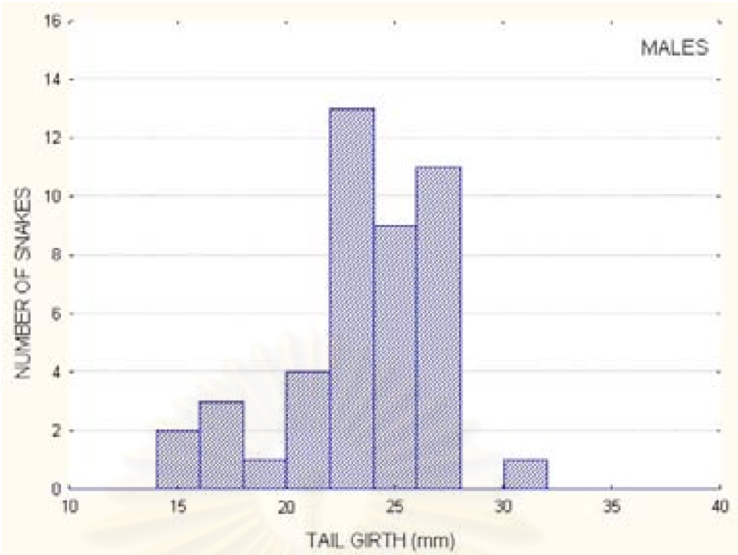
Figure 4.23B. The frequency distribution of body girth for females of *Acrochordus granulatus* from Phangnga bay.

4.2.1.11 Tail girth

The tail girth of male little file snakes averages (±SE) of 23.02 ± 0.52 mm, the median is 23.50 mm and the variance is 11.97. The minimum value of tail girth of male snakes captured is 14.00 mm, and the largest is 30.00 mm. The skewness value of -0.76 indicates that these data are asymmetrical and the mean is less than the median. The kutosis value of 0.36 indicates that these data concentrate around the mean. The normality test gave a significant difference (Shapiro-Wilk = 0.05, $p<0.05$) (Figure 4.24A).

Females have an average tail girth (±SE) of 24.56 ± 0.51 mm (Figure 4.24B). Median is 25.00 mm and variance is 20.37. Smallest tail girth of female snakes captured is 13.00 mm, and the largest is 36.00 mm. The skewness value of -0.12 indicates that these data are asymmetrical and mean is less than the median. The kutosis value of 0.49 indicates that these data are concentrated around the mean. The normality test gave a non significant difference (Kolmogorov-Smirnov = 0.20, $p<0.05$).

A



B

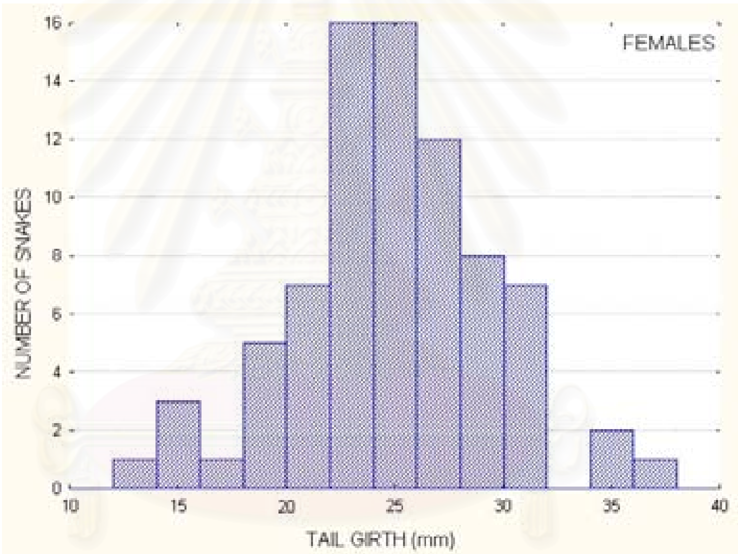


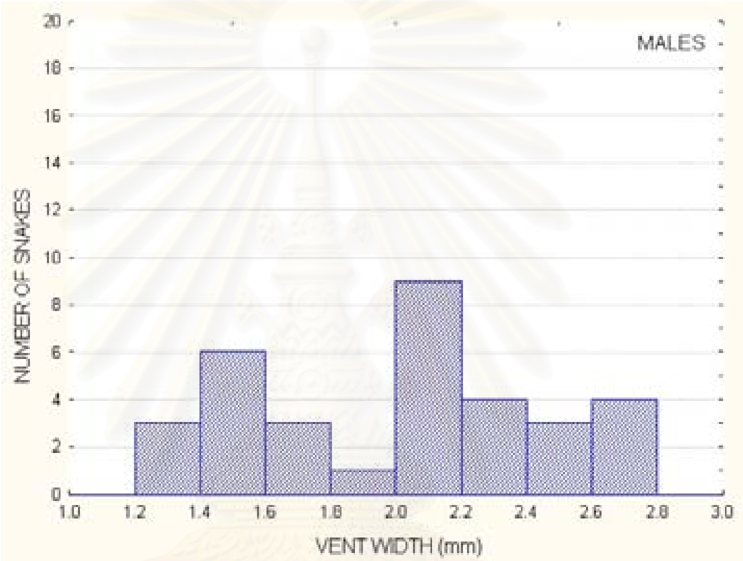
Figure 4.24 The frequency distribution of tail girth for males (A) and females (B) of *Acrochordus granulatus* from Phangnga bay.

4.2.1.12 Vent width

The vent width of male little file snakes shows an average value (±SE) of 1.96 ± 0.08 mm, the median is 2.00 mm and the variance is 0.22 (Figure 4.25A). The minimum value of the vent width of male snakes captured is 1.20 mm, and the largest is 3.00 mm. The skewness value of 0.62 indicates that these data are asymmetrical. The kutosis value of -0.79 indicates that these data are dispersed around the mean. The normality test gave a non significant difference (Shapiro-Wilk = 0.14, $p<0.05$).

Females have an average value of vent width (+SE) of 1.76 + 0.05 mm (Figure 4.25B). Median is 1.70 mm and variance is 0.17. The smallest vent width of female snakes captured is 1.00 mm, and the largest is 2.80 mm. The skewness value of 0.28 indicates that these data tend toward asymmetrical and the mean is more than the median. The kutosis value of -0.51 indicate that distribution of these data are more dispersed around the mean or the data might be a composite of two normal populations with the same variance but different means (Zar,1999) The normality test gave a significant difference (Kolmogorov-Smirnov = 0.00, $p<0.05$).

A



B

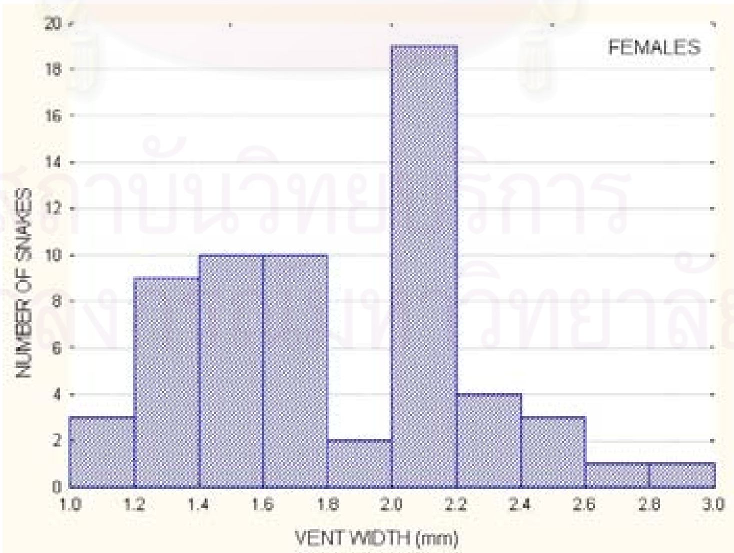


Figure 4.25 The frequency distribution of vent width for males (A) and females (B) of *Acrochordus granulatus* from Phangnga bay.

4.2.1.13 Vent length

The vent length of male little file snakes shows an average value (±SE) of 2.16 ± 0.09 mm, the median is 2.00 mm and the variance is 0.29 (Figure 4.26A). The minimum value of vent width of male snakes captured is 1.00 mm, and the largest is 3.60 mm. The skewness value of 0.50 indicates that these data are asymmetrical and mean is more than the median. The kutosis value of 0.62 indicates that these data are concentrated around the mean. The normality test gave a non significant difference (Shapiro-Wilk = 0.19, $p<0.05$).

Females have an average value of vent length (±SE) of 1.89 ± 0.08 mm (Figure 4.26B). The median is 1.90 mm and the variance is 0.36. The smallest vent length of female snakes captured is 0.50 mm, and the largest is 3.30 mm. The skewness value of 0.30 indicates that these data tend to asymmetrical. The kutosis value of -0.34 indicate that these data are more dispersed around the mean and it seem to be composed of two populations. The normality test gave a significant difference (Kolmogorov-Smirnov = 0.02, $p<0.05$).

A

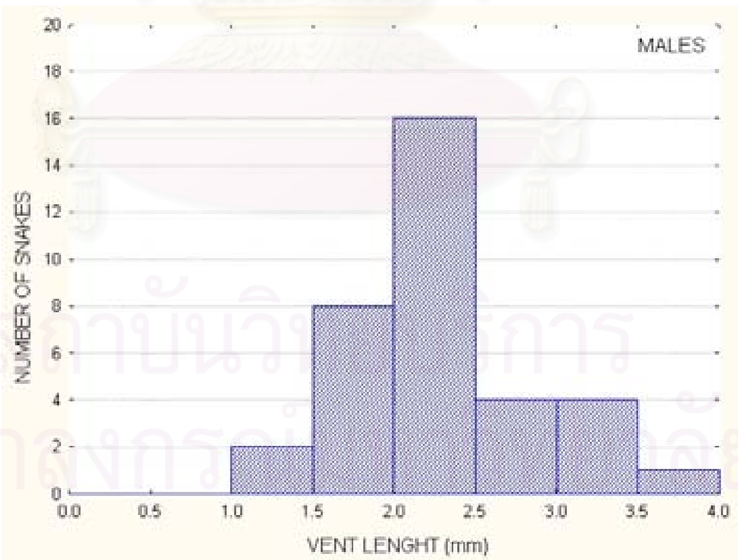


Figure 4.26A The frequency distribution of vent length for males of *Acrochordus granulatus* from Phangnga bay.

B

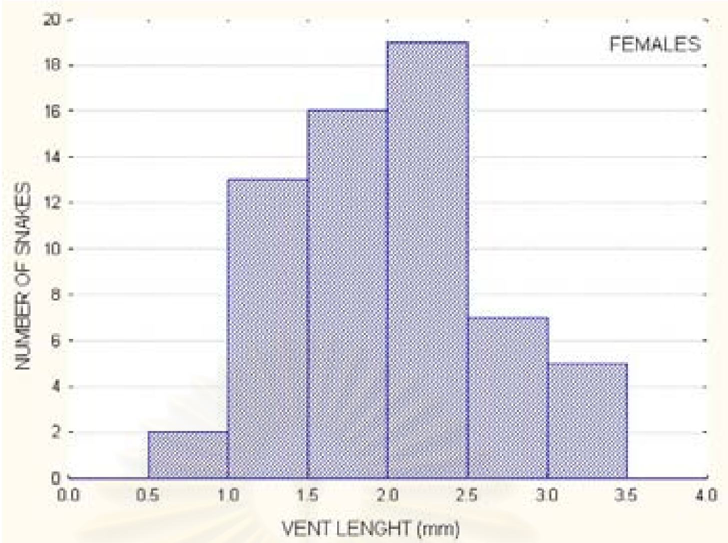


Figure 4.26B The frequency distribution of vent length for females of *Acrochordus granulatus* from Phangnga bay.

4.2.2 Sexual size dimorphism

According to the section on size at maturity in this document, the little file snake can be categorized into 4 groups based on SVL values. Juvenile males and females have SVLs less than 580 mm and adult males and females have SVLs equal to or greater than 580 mm. By this procedure 23 juvenile males, 32 juvenile females, 19 adult males, and 45 adult females were identified.

The results of the independent-sample *t* test on 14 morphological characters between juvenile males and females shown in table 4.1A indicates significant differences only in mouth width and vent length. Females have larger mouth width than males, but males show longer vent length than do females.

Table 4.1A. Sexual size dimorphism in juveniles of *Acrochordus granulatus* from Phangnga Bay, Thailand. Each table shows the mean values (± se) for 14 morphological characters and the statistical test results (independent-sample *t*-test).

Morphological character	Juvenile		p-value
	Males	Females	
Sample size	23	32	
Weight (g)	73.55 ± 6.56	83.60 ± 7.68	0.324
Head length (mm)	15.62 ± 0.36	16.46 ± 0.44	0.171
Head width (mm)	10.09 ± 0.30	10.84 ± 0.26	0.067
Distance between eyes (mm)	6.17 ± 0.15	6.30 ± 0.18	0.601
Snout to corner of the mouth length (mm)	9.35 ± 0.25	9.50 ± 0.21	0.640
Snout to eye length (mm)	4.43 ± 0.14	4.29 ± 0.09	0.441
Mouth width (mm)	16.65* ± 0.51	18.37* ± 0.51	0.020
Snout to vent length (mm)	548.39 ± 15.02	543.22 ± 14.59	0.880
Tail length (mm)	63.91 ± 2.06	62.66 ± 1.74	0.643
Neck girth (mm)	30.10 ± 1.03	30.96 ± 0.70	0.494
Body girth (mm)	52.44 ± 2.05	52.09 ± 2.11	0.908
Tail girth (mm)	21.74 ± 0.78	21.28 ± 0.65	0.651
Vent width (mm)	1.81 ± 0.13	1.57 ± 0.07	0.128
Vent length (mm)	2.21* ± 0.14	1.78* ± 0.11	0.026

* Significant difference at $p < 0.05$

Among adult males and females 11 out of 14 characters were significantly different; weight, head length, head width, distance between eyes, snout to corner of the mouth length, mouth width, snout to vent length, neck girth, body girth, tail girth and vent width (Table 4.1B). Adult females were larger than male counterparts in all measurements except vent width.

Table 4.1B. Sexual size dimorphism in adults of *Acrochordus granulatus* from Phangnga Bay, Thailand. Each table shows the mean values (± se) for 14 morphological characters and the statistical test results (independent-sample *t*-test).

Morphological characters	Adults		p-value
	Male	Female	
Sample size	19	45	
Weight (g)	104.72* ± 5.02	168.52* ± 8.28	0.000
Head length (mm)	17.78* ± 0.24	19.57* ± 0.27	0.000
Head width (mm)	11.11* ± 0.22	13.24* ± 0.30	0.000
Distance between eyes (mm)	6.95* ± 0.15	7.41* ± 0.09	0.014
Snout to corner of the mouth length (mm)	9.95* ± 0.21	11.18* ± 0.25	0.000
Snout to eye length (mm)	4.86 ± 0.13	5.15 ± 0.10	0.092
Mouth width (mm)	18.73* ± 0.34	21.46* ± 0.25	0.000
Snout to vent length (mm)	648.63* ± 6.33	686.26* ± 7.09	0.000
Tail length (mm)	74.95 ± 1.34	73.67 ± 1.00	0.462
Neck girth (mm)	32.92* ± 0.84	36.71* ± 0.64	0.001
Body girth (mm)	56.89* ± 1.07	65.83* ± 1.56	0.001
Tail girth (mm)	24.82* ± 0.51	27.03* ± 0.52	0.004
Vent width (mm)	2.21* ± 0.11	1.87* ± 0.07	0.016
Vent length (mm)	2.19 ± 0.13	1.98 ± 0.10	0.230

* Significant difference at $p < 0.05$

To calculate the sexual size dimorphism value between adult males and females, the sexual dimorphism index proposed by Gibbons and Lovinch (1990) was used. The index is positive if females were the larger sex and negative if males were larger.

$$\text{Sexual size dimorphism (SSD)} = \left(\frac{\text{Mean adult SVL larger sex}}{\text{Mean adult SVL smaller sex}} \right) - 1$$

Then, the SSD index of adult *A. granulatus* for this study was

$$\text{Sexual size dimorphism (SSD)} = \left(\frac{\text{Mean adult SVL of female}}{\text{Mean adult SVL male}} \right) -1$$

$$0.058 = \left(\frac{686.26}{648.63} \right) -1$$

The result of 0.058 is a positive value. Thus, female *A. granulatus* in Phangnga bay were larger than males.

Discriminant Function Analysis (DFA) of adult *A. granulatus* indicated highly significant discrimination (Wilks' Lambda = 0.297, $p < 0.05$, canonical correlation = 0.839). The eleven variables utilized in the discriminant function and discriminant scores showed sexes group functions as the following.

Y = 88.461(HL) – 50.317(HW) + 55.949(Eyes) + 55.116(SEL) –35.658 (SGL) – 3.198(SVL) + 153.555(TL) + 57.319 (NG) – 57.063(BG) + 70.885(TG) – 249.749(MW) – 16.021

Where the Y scores were less than 1.42, snakes were classified as male, and where scores were more than 1.42, snakes were classified as female. Twenty specimens of known sex were tested correctly by this function, the sexes showed a significant difference between centroids (males = 2380 and females = -0.963, units indicate discriminant scores).

The result of this study show that female *Acrochordus granulatus* in Phangnga bay have significantly larger head size, body size and longer maximum body length than the males. This is reflected in the positive value of the sexual size dimorphism index of 0.058. Thus, it is consistent with the hypotheses that many species of snakes are sexually dimorphic in adult body size with females larger than males (Shine, 1978; Bertona and Chiaraviglio, 2003). Larger female body size may predict that male–male combat for females is no likely in *A. granulatus*. To explain this sexual size dimorphism (SSD), Madsen and Shine (1994) suggested that adult males may be smaller than females for two reasons; firstly, they grow more slowly and secondly, they reach maturation sooner. However, *A. granulatus* from this study showed approximately the same maturation size at 580 mm SVL in both sexes. Another possibility is that after they become mature, females may continue grow to a size that will maximize their reproductive output. As with most vertebrates (Badyaev, 2002) *A. granulatus* male and female juveniles follow a similar growth curve. Hence, in snake species lacking male–male combat, males seem to allocate their available energy to mate searching behavior rather than to body growth (Shine, 1978).

To investigate the allometry of male and female *A. granulatus*, the relationship of head length, head width and mouth width is plotted against snout-vent length in figure 4.27-4.29. Head length, head width and mouth width increas slightly with snout-vent length. The females tend to have longer head length, head width and mouth width than males at the same snout-vent length. The head length has a r^2 value of 0.593 for males and 0.650 for females, the significant difference between sexes is shown after the effect of SVL was factored out of the analysis ($F_{1,104}$ = 11.840, $p<0.05$; slopes homogeneous test $F_{1,104}$ = 2.50, p = 0.117). Head width has a r^2 value of 0.365 for males and 0.474 for females, the significant difference between sexes is shown after the effect of SVL was factored out of the analysis ($F_{1,104}$ = 19.483, $p<0.05$; slopes homogeneous test $F_{1,104}$ = 1.699, p = 0.195). Mouth width has a r^2 value of 0.569 for males and 0.679 for females, the significant difference between sexes is shown after the effect of SVL was factored out of the analysis ($F_{1,104}$ = 32.225, $p<0.05$; slopes homogeneous test $F_{1,104}$ = 0.001, p = 0.975).

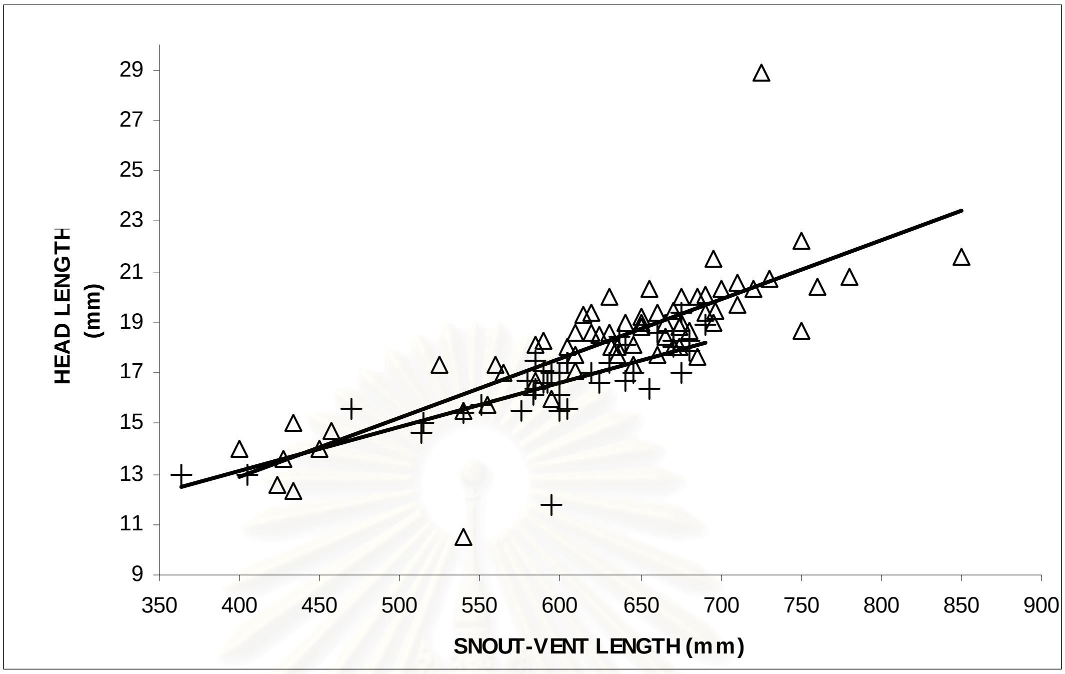


Figure 4.27 The relationship of head length to SVL of *Acrochordus granulatus* from Phangnga bay. The triangles (△) are females (r^2 = 0.650) and the pluses (+) are males (r^2 = 0.593).

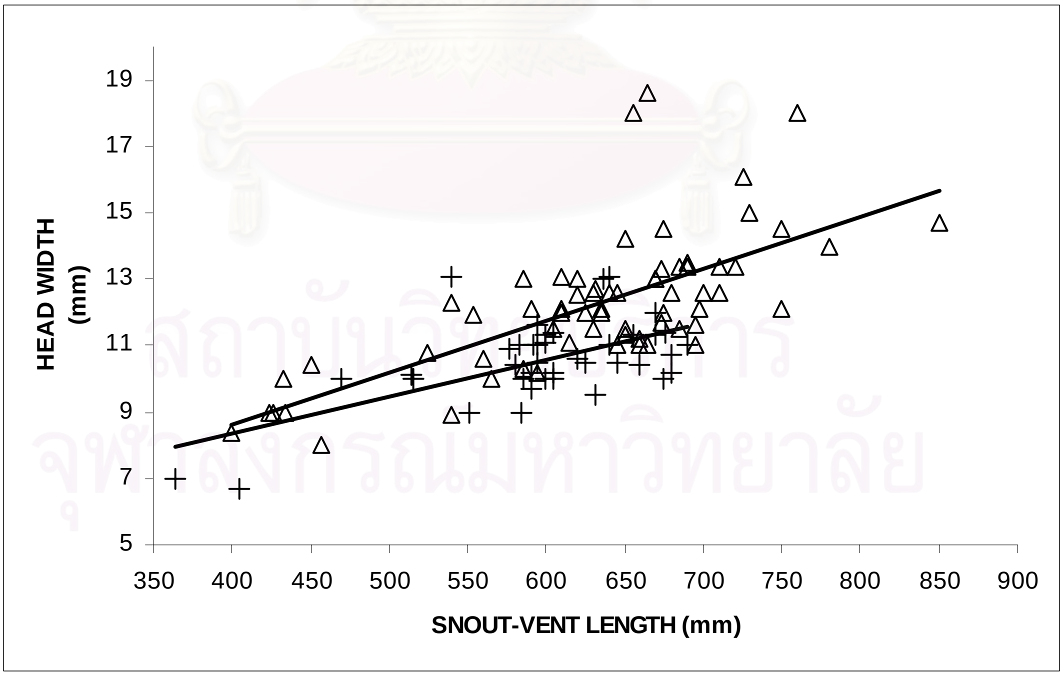


Figure 4.28 The relationship of head width to SVL of *Acrochordus granulatus* from Phangnga bay. The triangles (△) are females (r^2 = 0.474) and the pluses (+) are males (r^2 = 0.365).

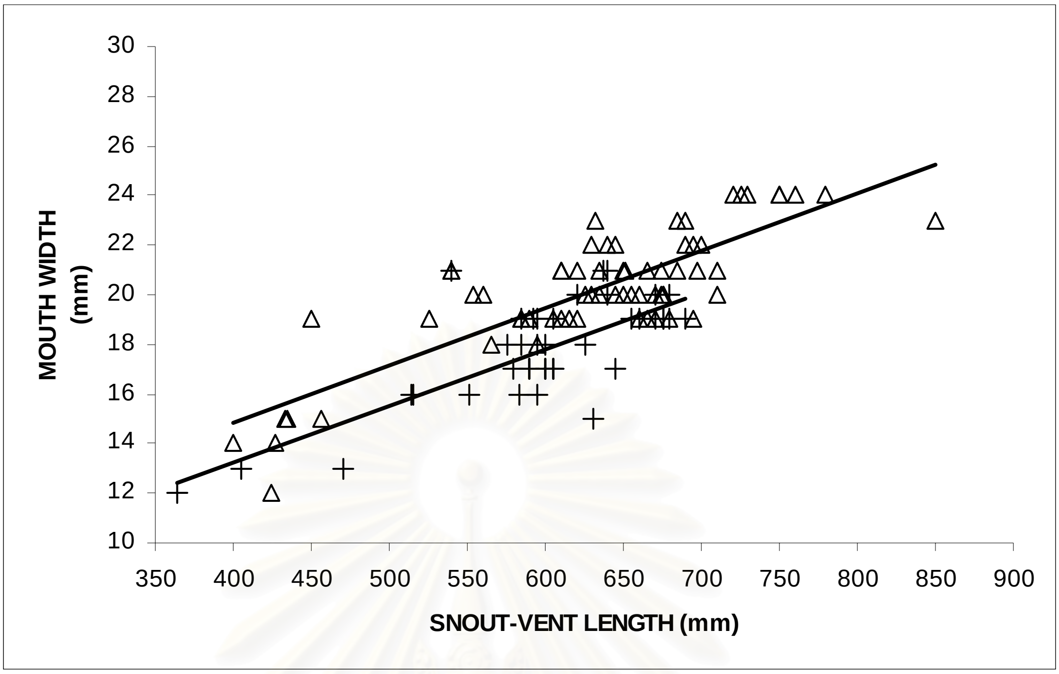


Figure 4.29 The relationship of mouth width to SVL of *Acrochordus granulatus* from Phangnga bay. The triangles (△) are females (r^2 = 0.679) and the pluses (+) are males (r^2 = 0.569).

Body size differences between sexes reflect selection acting on females and males (Shine, 1978). Dietary divergence has been an important selective force for the evolution of head size dimorphism in snakes. The larger head size in one sex has evolved to allow ingestion of larger prey (Shine, 1986; Camilleri and Shine, 1990; Shetty and Shine, 2002). It is likely that gape size also relates to prey diameter. Rotation at the quadrate supratemporal and dentary compound joint allow the skeleton to conform more closely to prey shape (Cundall and Greene, 2000). Sexual dimorphism in head size may occur as a result of habitat differences (Shine, 1989; Shine 1991). In adult *A. arafurae*, there are also ecological differences between the sexes with small males foraging in shallow water for small fishes while larger females tend to feed on larger fishes in deeper water (Houston and Shine, 1993).

4.3 Reproductive biology

4.3.1 Size at sexual maturity

To demonstrate the breeding season and size at sexual maturity of *Acrochordus granulatus*, the relationship of SVL to testicular and follicular volumes are presented in figure 4.30 and 4.31. During the study period, from July to December 16 male snakes with SVL over 580 mm were captured with enlarged testes (minimum = 403.19 mm^3, maximum = 3262.60 mm^3, mean = 1667.39 $\pm$ 218.89). They were larger than the testes of the same sized snakes collected in January to June (minimum = 23.11 mm^3, maximum = 1583.21 mm^3, mean = 352.64 $\pm$ 94.82). However, seven of 12 male snakes which have SVL over 580 mm captured in January to June show enlarged testes. Thus, male *A. granulatus* with SVL over 580 mm were mature. The beginning of initiation of spermatogenesis and testes size increase began in July.

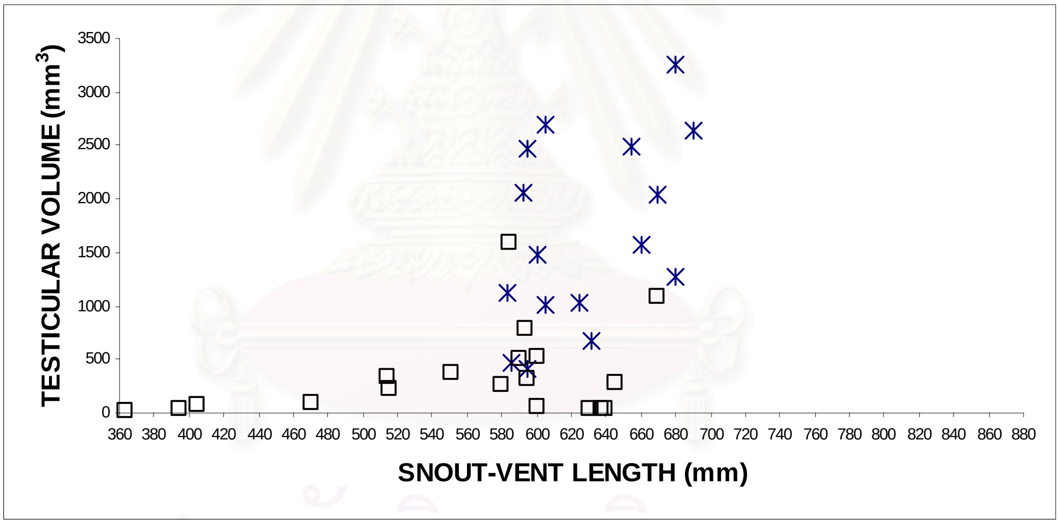


Figure 4.30 Plot of SVL versus testicular volume in *Acrochordus granulatus*. Squares (□) show specimens collected from January to June and asterisks (*) show specimens from July to December.

For the females, it was obvious that most snakes which have SVL over 580 mm were mature. During July to December, four females with SVL less than 580 mm and one female with SVL more than 580 mm did not have developing follicles, while the follicles of 30 females with SVL over 580 mm were developing (minimum = 4.17 mm^3, maximum = 27777.15 mm^3, mean = 8530.85 $\pm$ 962.26). During January to June, three of 34 female snakes which have SVL over 580 mm show enlarged follicles, while the other did not have developing follicles (minimum = 3.99 mm^3, maximum = 10399.98 mm^3, mean = 640.98 $\pm$ 384.39).

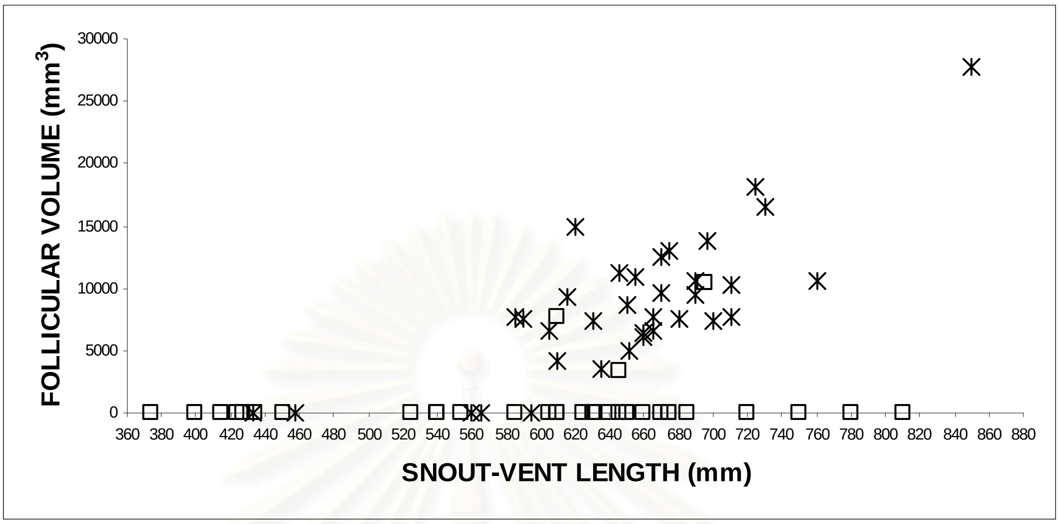


Figure 4.31 Plot of SVL versus folliclular volume in *Acrochordus granulatus*. Squares (□) show specimens collected from January to June and asterisks (*) show specimens from July to December.

These data suggest that the sexes can be divided on the basis of their size at maturity. Below 580 mm SVL were juveniles with sexually immature testes and ovaries whereas those above 580 mm were sexually mature adults. It is possible, however that those individuals measuring 580 – 600 mm SVL of males and females may be sub-adults, because of the mature and immature snakes were found in this narrow size range through out the breeding season.

4.3.2 Reproductive cycle

Reproductive data indicate that the breeding season of *Acrochordus granulatus* in Phangnga bay begins in July, the end of the monsoon season. Average values of the testicular volume per snout-vent length increased from July through October with the beginning of the dry season, and remained elevated in November to December. The volume of testes in September to October (3.776 $\pm$ 1.112 mm^3) was largest, being larger than testes in both July to August (2.765 $\pm$ 1.838 mm^3) and November to December (1.974 $\pm$ 0.908 mm^3) (Figure 4.32). However during January to June, testis sizes were smaller.

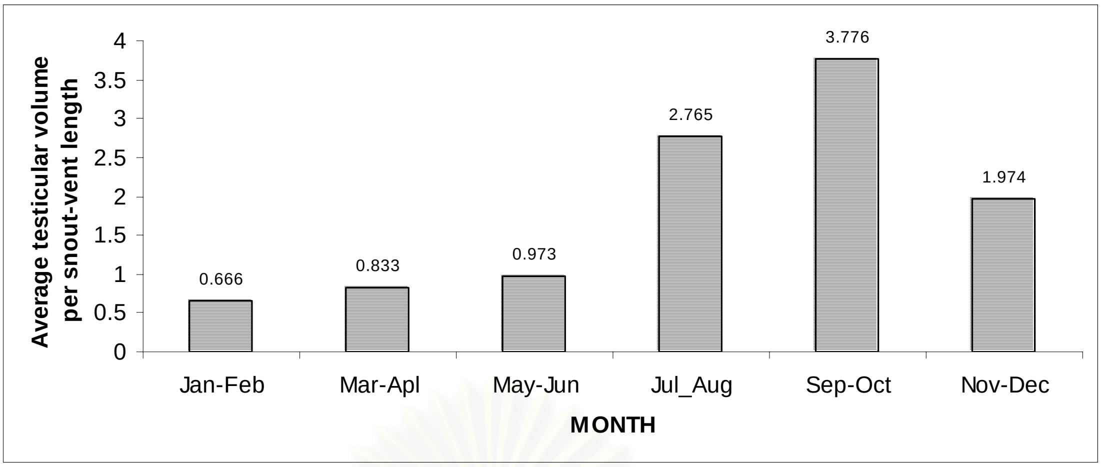


Figure 4.32 Monthly variations in the average testes volume/SVL of male *Acrochordus granulatus* in Phangnga bay.

The proportion of adult females reproductive (Table 4.2) shown by pregnancies was highest from September through January, which is a dry season in Phangnga bay. The reproductive cycle in females shows an ovulation, which is indicated by an increased size of follicles to yolk accumulated in September. This situation still remains until late February, the small follicles of one to two millimeters in diameter were present in the ovaries throughout the year (Figure 4.33). In January embryos were first observed. Fully developed embryos were present in May, however newborns (220 mm SVL; Ernst and Zug, 1996) were not evident in this study whereas juvenile snakes (360-400 mm SVL) were first caught in June, which is the beginning of the monsoon season in Phangnga bay.

Table 4.2 The proportion of reproductive female *Acrochordus granulatus* showing the gravid and non gravid periods.

	Number of female	Number of reproductive female	Proportion reproductive
January – February	12	8	0.66
March – April	11	1	0.09
May – June	20	0	0.00
July - August	4	0	0.00
September – October	6	6	1.00
November - December	24	22	0.90

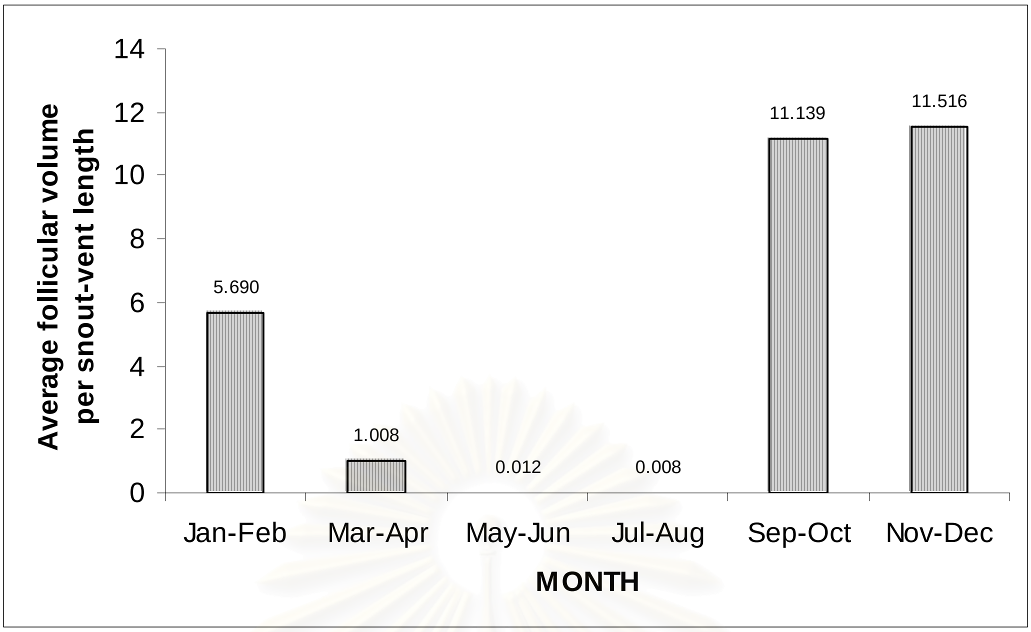


Figure 4.33 Monthly variations in the average follicular volume/SVL of female *Acrochordus granulatus* in Phnagnga bay.

This study agrees with the report that *Acrochordus granulatus* is viviparous, with seasonal reproduction (Shine and Houston, 1993). As in the study of Gorman et al. (1981) *A. granulatus* in the Philippines has a well defined seasonal breeding time from September to November. However *A. granulatus* seasonal reproduction in the Straits of Malacca is not clear, it has either aseasonal or loosely seasonal reproduction (Voris and Glodek, 1979). The breeding time of this species might vary between populations due to differences in sensitivity to climate (Gorman et al., 1981)

The male reproductive cycle was elucidated by testicular volume because the testis size has been reported to be positively related to the degree of expression of male sex hormone (Gorman et al., 1981; Yokoyama and Yoshida, 1993; Merilä and Sheldon, 1999). Examination of the testicular volume revealed that the reproductive activity in *A. granulatus* in Phangnga Bay was seasonal. The pattern is quite consistent with the findings of Gorman et al. (1981), a seasonal breeding of *A. granulatus* in the Philippines with snakes having the highest testosterone and testis weight in October and November.

4.3.3 Embryo development

Acrochordus granulatus is a viviparous snake; the embryos develop inside of maternal. The embryo is supported by yolk which they use for nourishment during their development. In snakes from Phangnga bay, female little file snakes have a small follicle approximate size was 1-2 mm diameter in ovaries throughout a year (Figure 4.34). These follicles were expanded from February (Figure 4.35) until in November (Figure 4.36) a vitellogenesis and ovulation occurred, yolks began to accumulate and move from the ovaries through the ostrium to the oviduct. The average diameter of eggs was 18.59 $\pm$ 0.35 mm. Eggs remained there until embryo first was observed on January (Figure 4.37), average diameter of embryo disk was 9.93 $\pm$ 0.55 mm and the average diameter of eggs was 19.45 $\pm$ 0.42 mm. In May (Figure 4.38) fully developed embryos with average SVL of 225.00 $\pm$ 5.33 mm and average tail length was 34.25 $\pm$ 0.75 mm were observed. The smallest little file snakes which have SVL range from 360 to 400 mm were first caught in June.

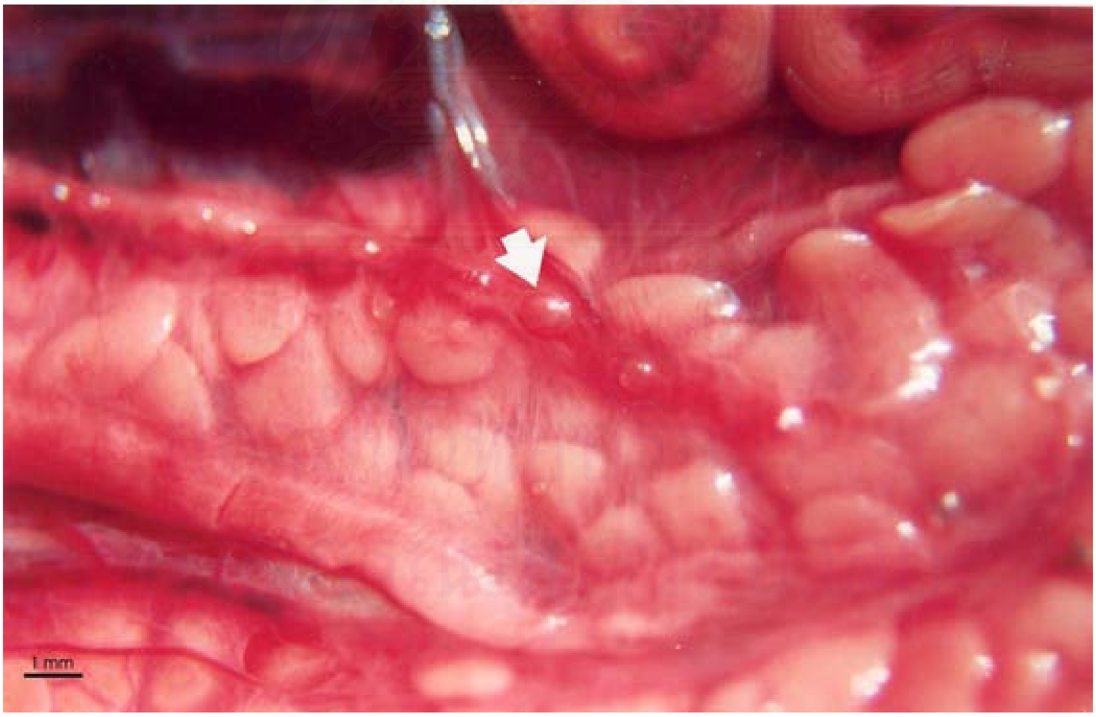


Figure 4.34 Small sized follicles of about 1 mm diameter found in February.

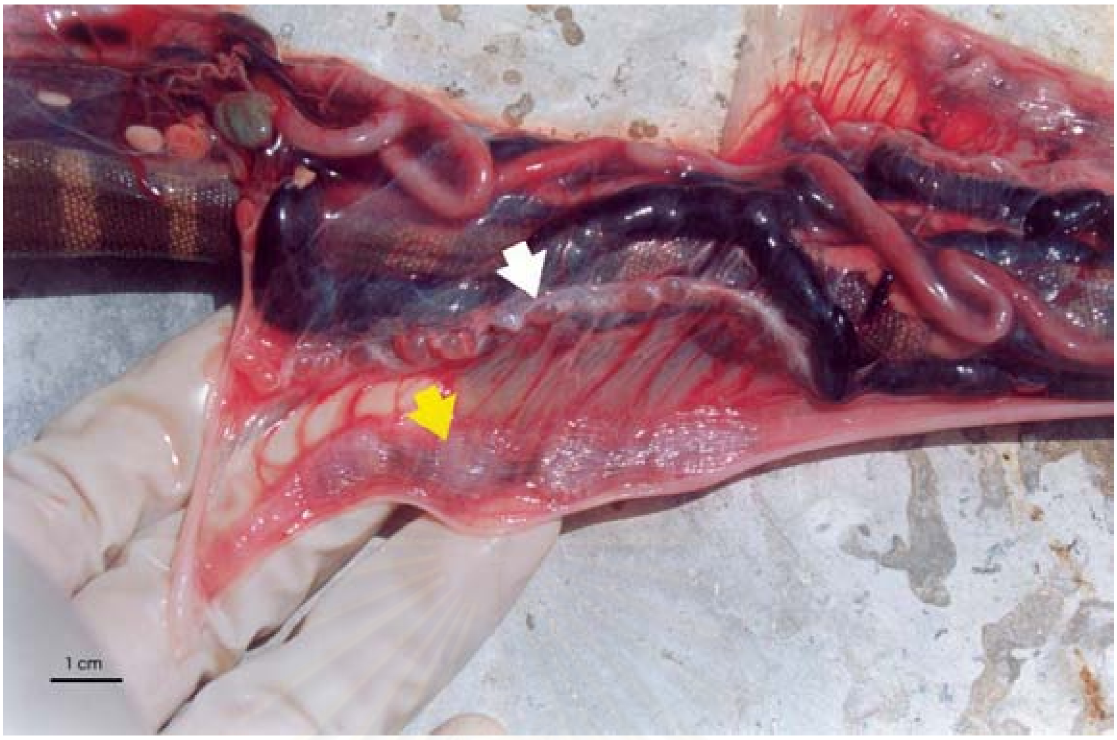


Figure 4.35 Follicles about 3-4 mm in diameter are from snakes collected in June (white arrow). The yellow arrow shows a bulge in the oviduct.

Figure 4.36 The accumulated yolk (white arrow) moved to the oviduct in November. The yellow arrow shows the oviduct.

Figure 4.37 The intermediate was found in January.

Figure 4.38 This near full term embryo was found in May.

4.3.4 Clutch size

The clutch size of *Acrochordus granulatus* in Phangnga bay averaged 5.18 ± 0.373 individuals (n = 37). Clutch size increases slightly with maternal body size (r^2 = 0.578, p < 0.05; Figure 4.39). The smallest female *A. granulatus* (590 mm SVL) was gravid with 4 eggs, while the biggest female (850 mm SVL) had 14 eggs developed.

However, the egg diameter in gravid females, averaged 18.93 ± 0.42 mm (n=37), and was not related to maternal size (r^2 = 0.000, p < 0.05). Egg size tends to be similar at all SVLs. The minimum diameter was 13.84 mm at 610 mm SVL and the maximum value was 23.40 mm at 620 mm SVL. The smallest SVL 590 mm snake had an average egg diameter of 18.68 mm and the biggest SVL 850 had an average egg diameter of 19.91 mm (Figure 4.40).

The reproductive success of female snakes in this study appears to be somewhat dependent on the body size with larger females producing more offspring. An important component of the reproductive ecology of marine snakes involves the relationships among female weight, clutch size, birth weight, and reproductive effort. (Lemen and Voris, 1981). Previous studies showed that larger female snakes of many species reproduce more frequently and contained more follicles than smaller females of the same species (Shine, 1977b; Shine, 1994; Bishop, et al., 1996; Bertona and Chiaraviglio, 2003). Thus, fecundity selection may favor the evolution of large body size in female snakes.

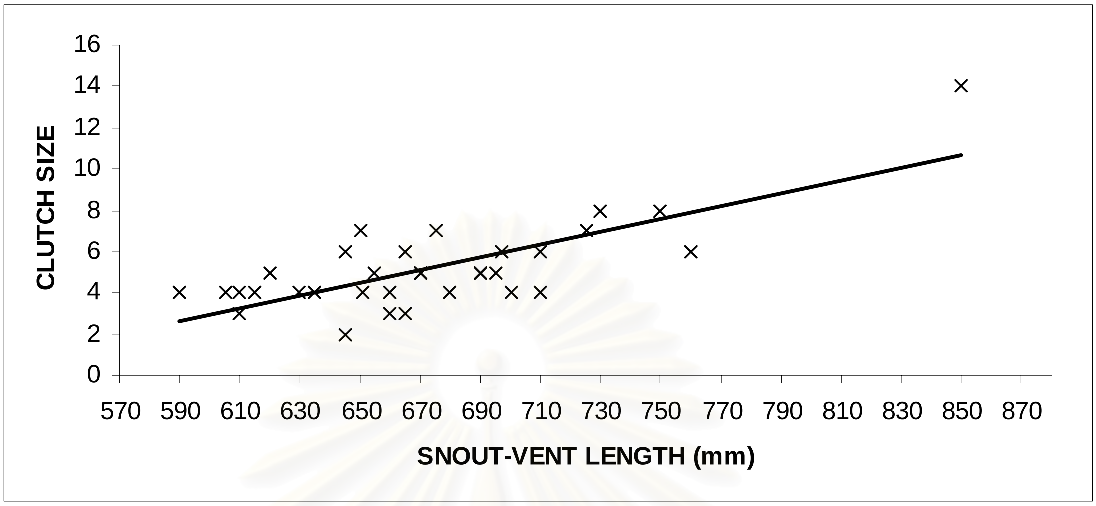


Figure 4.39 The relationship between snout-vent length (SVL) and clutch size in gravid female *Acrochordus granulatus*. (r^2 = 0.578; $p < 0.05$).

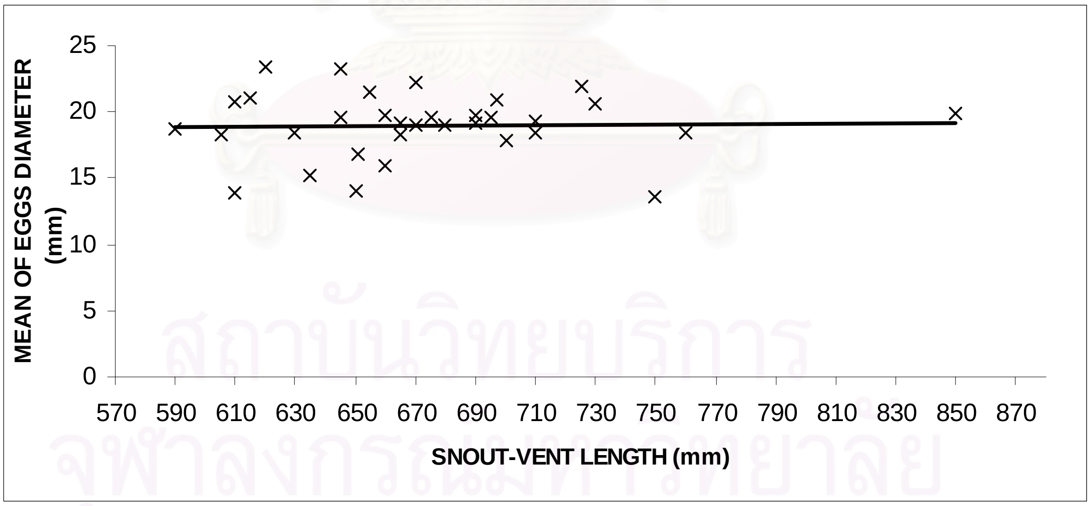


Figure 4.40 The relationship between snout-vent length (SVL) and the mean of eggs diameter in gravid female *Acrochordus granulatus*. (r^2 = 0.000; $p < 0.05$).

4.4 Ecology of *Acrochordus granulatus*

4.4.1 Distribution of *Acrochordus granulatus* in Thailand

Three sources of data; published documents (Table 4.3.1), museum specimens (Table 4.3.2) and this study (Table 4.3.3) were combined to show the recorded distribution of *A. granulatus* in Thailand (Figure 4.41). The plotted distribution indicates that *A. granulatus* may occur throughout coastal Thailand. In the previous studies, *A. granulatus* was reported to occur in the estuaries and mangrove forests along the Gulf of Thailand, and at Phangnga bay. The survey in the mangrove areas along the upper part of the Andaman sea coast in this study found two new localities for *A. granulatus,* one at Pratong Island, Phangnga province and one at Kraburi river, Ranong province.

Figure 4.41 The distribution of *Acrochordus granulatus* in Thailand. Locality records obtained from (●) references (■) museum specimens and (★) this study are shown.

Table 4.3 The detailed location data for *Acrochordus granulatus* in Thailand from 3 sources.

Table 4.31 The data from published documents

Species	Locality / Habitat	Reference
Chersydrus granulatus	Tacheen, Bangkok at the river mouth on high tide	Smith, 1914
	Koh Chang	Smith and Kloss,1915
	Andamans and Siam	Rooij, 1917
	Malay Peninsula: Northward to the Kra Isthmus through the south	Smith, 1930
	Cambodge et Siam	Bourret, 1936
	Tacheen, Chao Phya River; Bang pa kong River; Koh Chang; Outer Lake, Singora	Suvatti, 1967
Acrochordus granulatus	Phet Buri	Taylor and Elbel, 1958
	Gulf of Thailand: 10-20 miles off the coast of the Kra Isthmus	Tu, 1974
	Cave Panwa, Phuket: in sea off a fringing coral reef; Ko Panyi, Phangnga Bay	Frith, 1977
	Thailand: in brackish water, estuaries, coastal mangrove, and marine water	David and Vogel, 1996
	Thailand	Heatwole, 1999
	Gulf of Thailand	Murphy, et al. 1999
	Ko Mai Phai, Ko Boi Yai and Ka Lai in Phangnga Bay by fishing net close to mangrove	Pauwels et al. 2000

Table 4.3.2 Locality data from museum catalogs.

Species	Catalog Number	Province
Acrochordus granulatus	CAS-SU (Rep) 23687 - 23699	Chol Buri: fishing ground in Chol buri bay
	CAS-SU (Rep) 23700 - 23702	Bang Pakong river, Chachoengsao
	CUB-MZ (R) 1999.07.15.13	Ko Mai Phai, Phangnga
	CUB-MZ (R) 34189	Ang sila, Chol Buri: swamp in mangrove
	CUB-MZ (R) 556, 582, 585, 34617-8	Songkhla
	CUB-MZ (R) 1999.25	Pattani
	FMNH179308 - FMNH179310	Chon Buri
	FMNH179301 - FMNH179307	Nakhon Sitammarat
	FMNH250113	Phangnga
	FMNH252510	Phattalung
	FMNH242162, 2163, 242177, 242178, 242186, 242187	Phuket
	FMNH252509	Songkhla
	KU40064 - KU40066	Phet Buri
	MVZ Herp117544	Ang- Sila, Chon Buri
	QSMI1364	Samut Prakan

The abbreviations for the museum collections are:

CAS-SU (Rep) Department of Herpetology, California Academy of Sciences

CUB-MZ (R) Museum of Natural History, Chulalongkorn University

FMNH Field Museum of the Natural History, Chicago

KU University of Kansas Museum of Natural History

MVZ Museum of Vertebrate Zoology, University of California, Berkeley

QSMI Queen Saovabha Memorial Institute

Table 4.3.3 The capture location and number of *Acrochordus granulatus* from this study.

Species	Location	Habitat	Coordinate	Number of snakes
Acrochordus granulatus	Ranong	Mouth of Kra Buri river	N10.00701 E98.60751	5
	Pratong Island, Phangnga	Close to mangrove	N9.17146 E98.27281	8
	Ban Klong Khien, Phangnga Bay	In mangrove	N8.14042 E98.42471	10
	Ban Sam Chong, Phangnga Bay	Close to mangrove	N8.29094 E98.50727	63
	Ban Leam Sak, Phangnga Bay	Close to mangrove	N8.28185 E98.60725	50

Data from the literature review and this study document that, *Acrochordus granulatus* is usually found in tidal estuaries or nearby the river mouth where mudflats and mangrove forests occur. To date, there is no evidence that this species can inhabit other coastal habitats such as the area off the sandy beach or rocky shore, and the area of the coral reef and sea grass bed. There has been no report of *A. granulatus* in the Gulf of Thailand southward from Chumporn to Nakorn Sri Thammarach province and also from Krabi to Satun along the lower part of the Andaman sea coast. It might be possible that both gaps of locality records of *A. granulatus* are due to a lack of collection effort. Only a scatter of small estuaries and mangrove forests is present from Chumporn to Nakorn Sri Thammarach province. However, intensive surveys in these areas, especially where mangrove forests exist, should be conducted in the future before the distribution of *A. granulatus* in Thailand can be completely summarized.

4.4.2 Habitat of *Acrochordus granulatus*

Surveys of the little file snake, *A. granulatus* in its natural habitat were conducted at 3 locations in Phangnga Bay: (1) Ban Klong Khien, (2) Ban Sam Chong, and (3) Ban Lam Sak (Figure 4.42). Each survey was performed for a few days at each locality by walking through the mangrove forest area or on the mud flat during the low tide. Only at Ban Klong Khien (Figure 4.43) where five *A. granulatus* observed during a night survey. This could indicate that *A. granulatus* may be a nocturnal species. They were found in a small and shallow waterway inside the mangrove forest, lying at the bottom at water depths of about 10-30 cm. The bottom type is mud. In all three locations, no other snakes were found on the mudflat or in the area where mangrove trees were abundant.

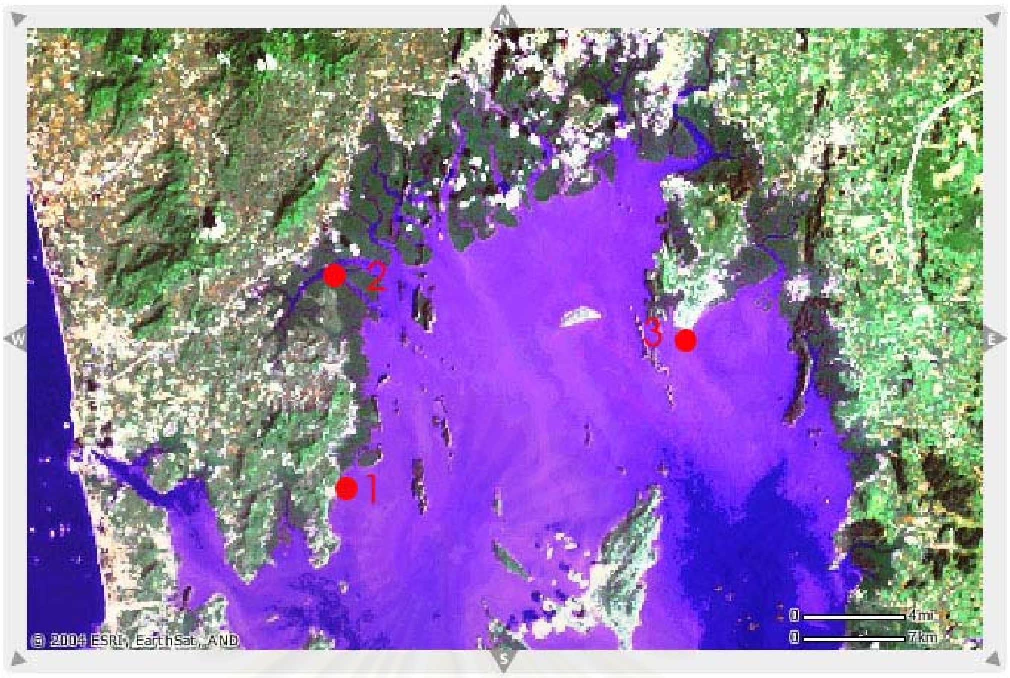


Figure 4.42 Phangnga Bay (08° 04´ - 08° 24´ N and 98° 04´ – 98° 39´ E, from ESRI Earth Satellite): showing patches of mangrove forests (dark green color) along the shoreline of the bay and three locations where *Acrochordus granulatus* specimens were collected, (1) Ban Klong Khien, (2) Ban Sam Chong, and (3) Ban Leam Sak.

Figure 4.43 The habitat of *Acrochordus granulatus* at Ban Klong Khien. A small inter tidal canal is behind the mangrove trees.

The results of this study are in accordance with previous reports indicating that *A. granulatus* usually lives at or nearby the river mouth where the mud flat and the mangrove forest are important for them (Rooij, 1917; Voris and Glodek, 1980; Lemen and Voris, 1981; David and Vogel, 1996; Heatwole, 1999; Murphy et al., 1999). Their morphology and behavior are closely related with their habitat. For example the reduced ventral scales and slightly flat tail allow them to swim properly in shallow water. Observation of captive snakes, kept in a 1 m^3 tank, indicates that the body of *A. granulatus* is very flexible and it becomes dorso-ventrally flattened when lying down at the bottom but laterally flattened when swimming (Figure 4.44). Captive snakes spent most of the time at the bottom of the tank. They rarely swam to the surface of the water for air.

In this study, *A. granulatus* habitat could be surveyed only in the area near to the shoreline when the water is shallow and where the land is exposed at low tide. The situation when the tide was high and the area further from the shore have not been investigated. Therefore, it cannot be assumed at this stage that *A. granulatus* inhabits only shallow water in the area of the mangrove forest or estuary. Voris and Glodek (1980) and Greene (1997) stated that *A. granulatus* commonly lives in mangrove swamps and other shallow saltwater habitats, but it is also found at depths of 4-20 m and as far as 10 km out to sea. In Phangnga bay, fish trawling at the water depth of about 6 to 10 m sometimes caught *A. granulatus*. Therefore, it may be possible that this species can inhabit a wider area further from the shore. They may move out to the deeper water for some periods of their life to find food or reproduce. Their movements and habitat use need to be studied more in the future.

A

B

Figure **4.44** The *Acrochordus granulatus* body becomes dorso-ventrally flattened when lying down at the bottom of the tank (A). Their body becomes laterally flat when swimming (B).

4.4.3 Relative abundance and status

During the period of one year from January to December 2002, one hundred and fourteen little file snakes, *Acrochordus granulatus,* were caught in Phangnga bay by two types of fishing equipment; the stake net and the floating gill net (Table 4.4). At Ban Klong Khien; 8 *A. granulatus* were caught, 7 by a gill net and 1 by stake net. At Ban Sam Chong; 56 snakes were caught, 52 by 5 stake nets and 4 by 1 gill net. Fifty snakes were caught at Ban Leam Sak, 26 by 3 stake nets and 24 by 2 gill nets.

The number of snakes caught per net for a period of one year at different localities is shown in table 4.4. Both types of fishing equipment were used throughout the study period and deployed in the area below the intertidal zone and at similar distances from the shore. The number caught in any one net at one time ranged from 1 to 12 individuals. The rates of capture by both types of fishing equipment seem similar, although the methods are distinct in their operation. The stake net reaches to the bottom and the water flows through the net bag from its open mouth to the end of the net. The floating gill net reaches from the surface vertically down but usually does not reach the bottom of the sea.

Table 4.4 The number of *Acrochordus granulatus* caught by net during January to December 2002 in Phangnga bay.

Locations	No. of snakes caught by stake net	No. of stake net	Proportion of snake caught/ stake net	No. of snakes caught by gill net	No. of gill net	Proportion of snake caught/ gill net
Ban Klong Khien	1	1	1	5	1	5
Ban Sam Chong	59	5	11.8	4	1	4
Ban Leam Sak	26	3	8.6	24	2	12
Total	86	9	9.5	33	4	8.3

The sex ratio of M : F observed from 3 localities was biased towards females at Ban Sam Chong. However, at Ban Klong Khien, the sex ration was 1 : 1 and at Ban Leam Sak the sex ratio was not significantly different from 1:1 (Table 4.5). This might indicate that Ban Sam Chong was a more suitable habitat for female

A. granulatus than males, although Ban Sam Chong was 16.3 km directly north of Ban Klong Khien and 25.6 km directly west of Ban Leam Sak.

Table 4.5 The sex ratio of *Acrochordus granulatus* caught from 3 localities in Phangnga bay.

Location	Number of males	Number of females	Sex ratio M : F	χ^2
Ban Klong Khien	5	5	1 : 1	0.00
Ban Sam Chong	18	45	1 : 2.5*	11.57
Ban Leam Sak	21	29	1 : 1.4	1.28

* Significant difference at $p<0.05$

Local fishermen provided information that the number of snakes captured by both types of fishing equipment was much higher in the past years, indicating that the population abundance of *A. granulatus* in the study areas is decreasing. In addition, a few other snake species that used to be occasionally captured or seen in the area have disappeared. *A. granulatus* specimens captured at Ban Klong Khien were a lot less than the numbers at Ban Sam Chong and Ban Leam Sak probably because a vast area of the mangrove forest at Ban Klong Khien has been changed to shrimp farms. Therefore, it may be assumed that one of the major factors affecting *Acrochordus granulatus* population is the increase of human activities in the area.

The number of *Acrochordus granulatus* caught during the study period fluctuated through the year (Figure 4.45). From July to September the number was lower than other months. In September, no snakes were captured. In November, the number of snakes caught was highest. One explanation of results is that most *A. granulatus* may move to other areas during the monsoon season which begins in June. Dunson (1975) reported that at the time of the monsoon season marine snakes sometimes move into the river. Thus, it is possible that during the time of strong currents and high waves *A. granulatus* may move into the rivers or canals.

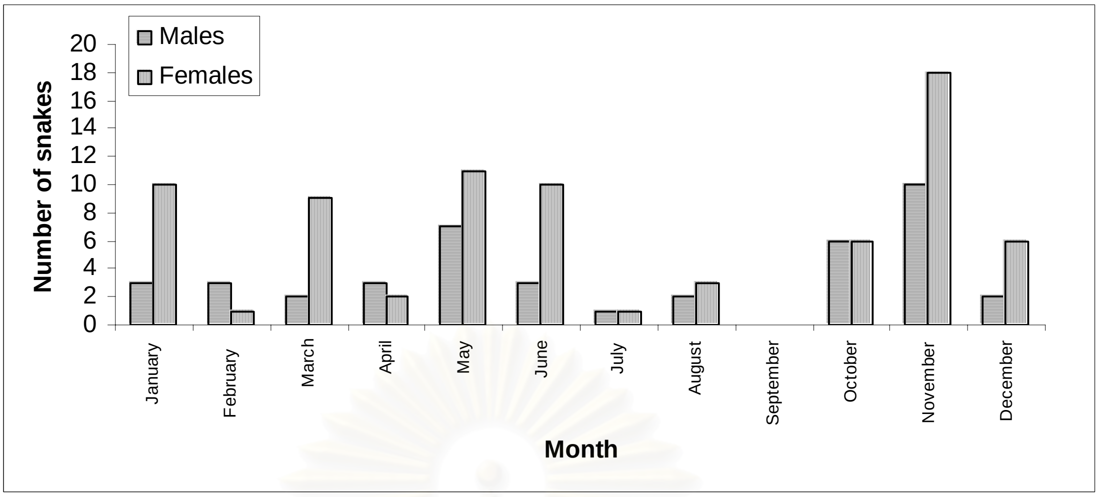


Figure 4.45 The number of male and female *Acrochordus granulatus* caught in Phangnga bay during January 2002 to December 2002.

The sex ratio of juvenile *A. granulatus* observed was not biased throughout the year (Table 4.6A). However the sex ratio of adults observed was biased towards females from January to August. In May to December the sex ratio was not significantly different from 1:1 (Table 4.6B).

Table 4.6 The sex ratio of *Acrochordus granulatus* caught during January to December 2002 from Phangnga bay.

A The male to female sex ratio of juvenile *Acrochordus granulatus*.

Month	Number of juvenile males	Number of juvenile females	Sex ratio M : F	χ^2
January – April	7	5	1 : 0.7	0.33
May – August	9	8	1 : 0.9	0.06
September - December	4	6	1 : 1.5	0.40

B The male to female sex ratio of adult *Acrochordus granulatus*.

Month	Number of adult males	Number of adult females	Sex ratio M : F	χ^2
January – April	4	18	1 : 4.5*	8.90
May – August	4	16	1 : 4*	7.20
September - December	16	26	1 : 1.6	2.38

* Significant difference at p<0.05

Juvenile males were caught throughout the year and the number exceeded adult males (Figure 4.46). Adult males were caught in high numbers only in October to November. Juvenile and adult females were caught in similar numbers throughout the year (Figure 4.47). Sex ratio results and number of snakes caught shows the possibility that females and juvenile males usually stay in the coastal area, while adult males move around and come to the coastal area only in the mating season, late in the year around October to November.

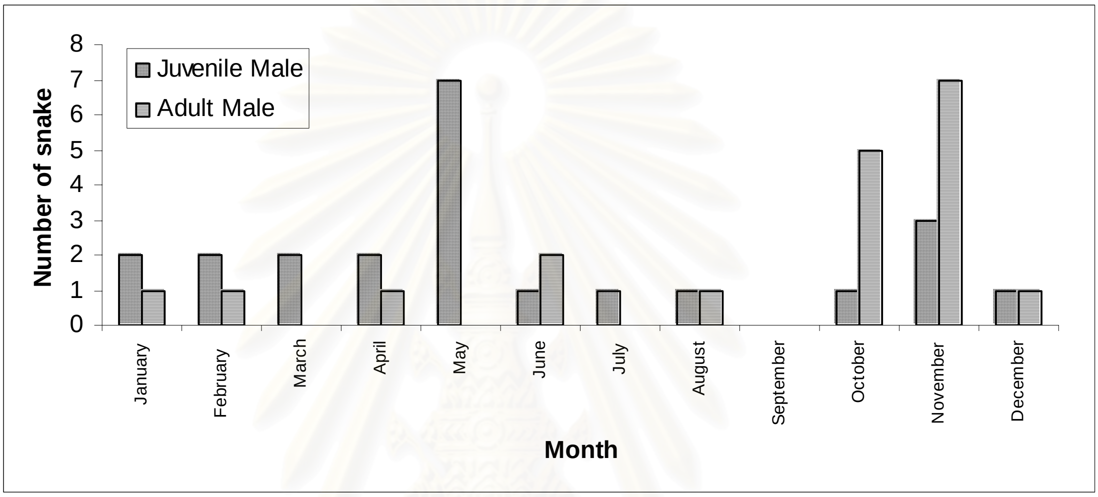


Figure 4.46 Juvenile and adult male *Acrochordus granulatus* caught in Phangnga bay during January 2002 to December 2002.

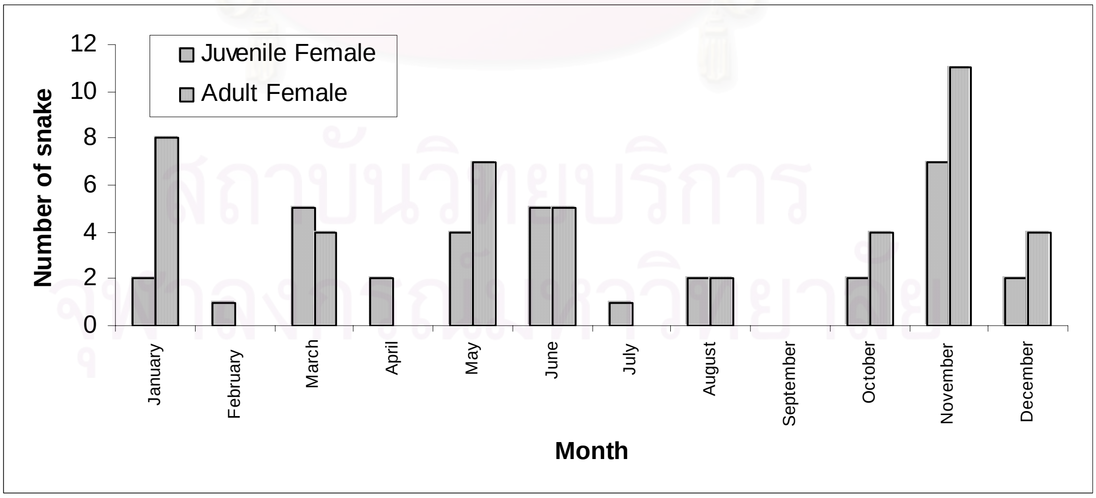


Figure 4.47 Juvenile and adult female *Acrochordus granulatus* caught in Phangnga bay during January 2002 to December 2002.

4.4.4 Diets

Most stomachs of *A. granulatus* specimen were empty. Some had many small fragments of food that were largely digested. There were only 9 *A. granulatus*, 2 males and 7 females, from 119 specimens that had incompletely digested food left in the stomach (Figure 4.48). All were identified as fish; consisting of 1 goby and 1 unidentified fish in males; 2 gobies, 2 Pony fish (*Leiognathus* sp.) and 3 unidentified in females. The total body length and body width of each fish was measured and its volume was estimated.

There are reports that the diet of *A. granulatus* consists mainly of crevice-inhabiting or sedentary fish such as gobies, blennies and mudskippers. Crustaceans and snails also have been reported as dietary items for this species (Voris and Glodek, 1980; Heatwole, 1999). In this study, only fish were found in the stomachs of *A. granulatus*, indicating that fish may be the main diet. Other prey items were not found.

The prey available in a habitat is certainly a major limiting factor on a predators diet (Zug, et. al., 2001). The fish in the family Leiognathidae and Gobiidae found in *A. granulatus* stomachs usually live in shallow coastal water and feed on benthic invertebrates (Nelson, 1994). This may explain why *A. granulatus* is usually found feeding at the bottom of coastal water. In addition, the flattened heads and eyes positioned dorsally in *A. granulatus* may allow them to see their prey above them.

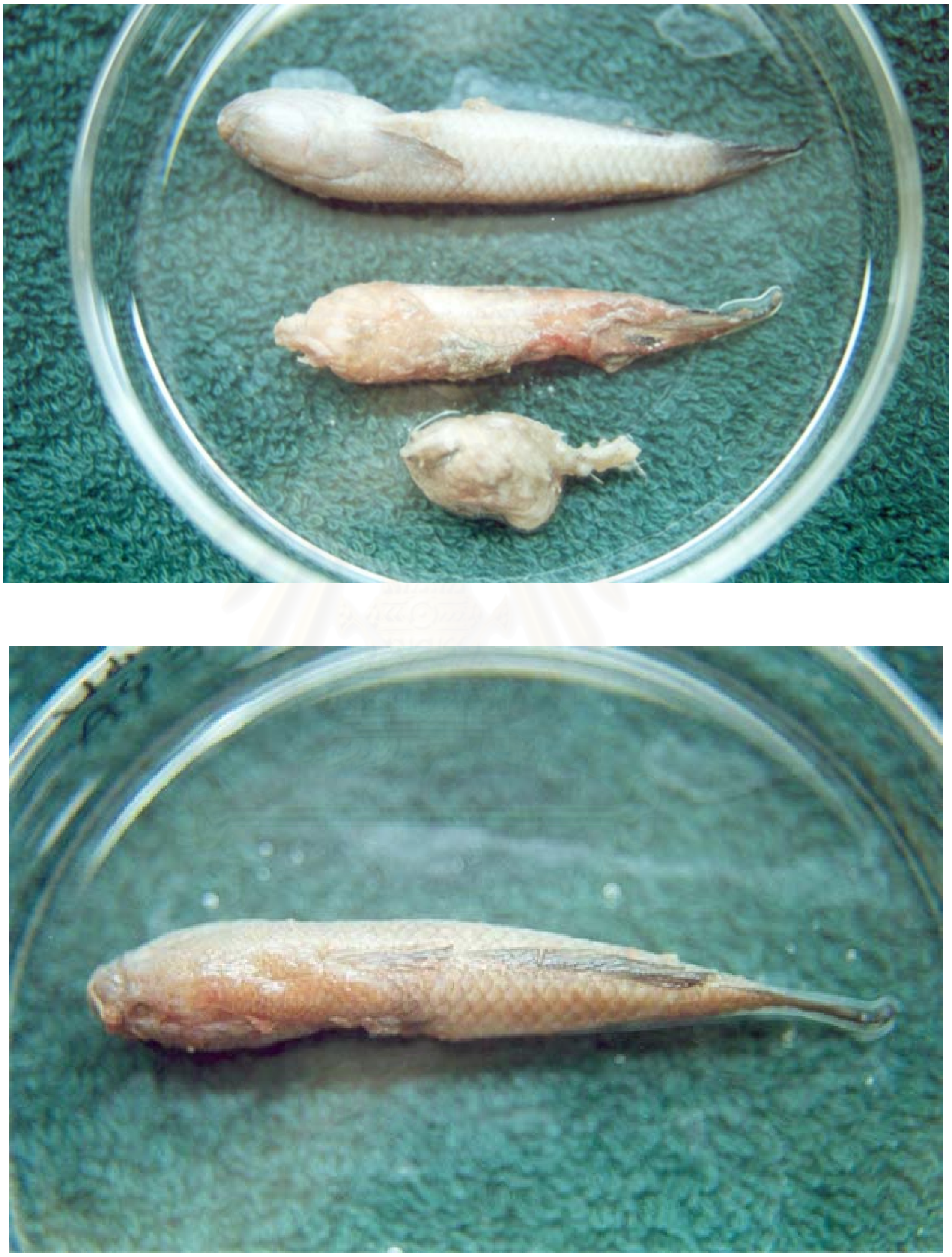


Figure 4.48 Gobies from in the stomachs of *Acrochordus granulatus*.

There is a positive relationship of gape size and prey size in snakes of the Family Hydrophiidae (Voris and Voris, 1983). The comparison of prey size and mouth width of male and female *A. granulatus* was considered from the available data. Although the sample size of each group is very small, the comparison may provide some information and a trend for future research. The result in table 4.7 and figure 4.49 shows that female snakes, on average, had larger mouth width than males and the fish found in the stomachs of females were also larger, indicating that there is a positive relationship between the prey size and mouth width in *A. granulatus*.

Table 4.7 Average food size (± SE) of stomach contents of male and female *Acrochordus granulatus*, mean to snake mouth widths.

	Fish length (mm.)	Fish width (mm.)	Fish volume (mm.)	Mouth width (mm.)
Male (n = 2)	35.25 ± 0.25	6.35 ± 2.35	224.42 ± 84.42	18.00 ± 2.00
Female (n = 7)	54.51 ± 7.11	15.00 ± 3.51	1,829.15 ± 1,099.37	19.57 ± 1.39

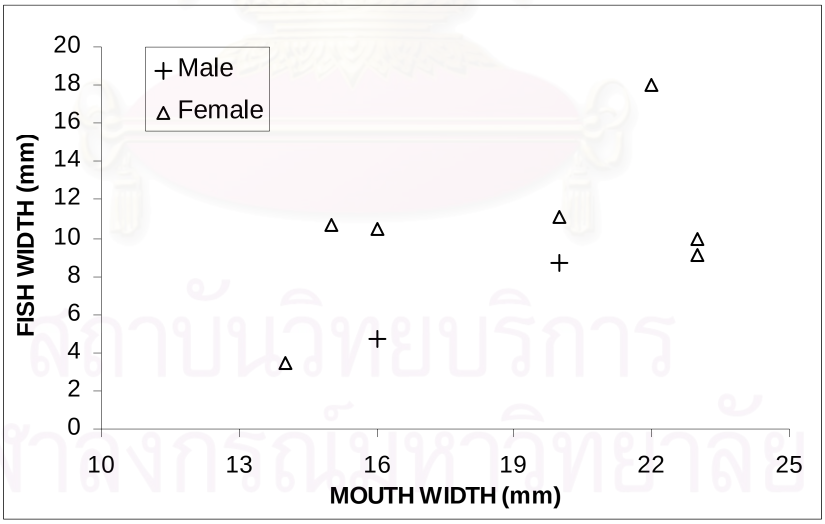


Figure 4.49 The positive relationship of fish width from stomachs of male and female *Acrochordus granulatus* to snake mouth widths.

Results of skull measurements on the brain case and the lower jaw of adult *A. granulatus* from Malaysia deposited in the collection of the Division of Reptiles and Amphibians, Field Museum of Natural History, Chicago support the above data in that there are significant size differences of lower jaws between sexes. The jaws of females were significantly larger than of males whereas the sizes of the brain cases between them were not significantly different (Table 4.8).

Table 4.8 Comparisons of the average width of brain cases and the average length of lower jaws between adult male and female *Acrochordus granulatus*.

	Brain case width (mm.)	Left jaw (mm.)	Right jaw (mm.)
Male (n = 21)	10.17 ± 0.67	18.71* ± 0.27	17.99* ± 0.84
Female (n = 20)	11.46 ± 0.31	20.94* ± 0.36	20.85* ± 0.37
P- value	0.094	0.000	0.004

* Significant difference at $p<0.05$

The size difference of mouth components such as jaw length may allow females to be able to open their mouths wider than the males. Cundall and Greene (2002) reported that the lower jaws are a primary determinant of gape size. Therefore, it is likely that females can feed on larger fish than do males as shown in table 4.4. The study in the sea krait, *Laticauda colobrina*, by Shetty and Shine, (2002) showed that head size is important for prey handling and ingestion and there is a positive relationship between prey size and snake size.

CHAPTER V

CONCLUSIONS AND RECOMEMDATIONS

5.1 Conclusions

The external morphology and coloration of the little file snake, *Acrochordus granulatus*, in Phangnga bay shows low level of variation. The little file snake has acquired more adaptations to a marine life style than its two congeners. Their bodies are adapted for living in shallow sea water. They demonstrate a neomorphic skull that is advanced compared with that found in Pythonidae.

The *A. granulatus* in this study could be divided into four groups according to their sex and reproductive stage: juvenile males, adult males, juvenile females and adult females. Independent sample T-test analysis shows significant differences between these groups ($p \leq 0.05$). The adult females have bigger body size than males reflected in head length, head width, distance between eyes, snout to corner of the mouth length, mouth width, snout to vent length, neck girth, body girth, tail girth and vent width. However, in male and female juvenile snakes, only two morphological characters, mouth width and vent length, were significantly different. The results from discriminant function analysis give the equation for predicting the sex of adult little file snakes with the original grouped case correctly classified at 98.3%.

The size at maturation of *A. granulatus* in Phangnga bay is 580 mm SVL in both sexes. However, female snakes reached larger sizes and the average size of adult females is bigger than males. This may be because the female little file snake becomes mature earlier than the male and after they become mature, females may continue grow to a size that will maximize their reproductive output. Reproductive data indicated that the breeding season of *A. granulatus* begins in July. From July to December, the testicular volume is largest surpassing that observed from January to June. Following an increase in size of follicles to vitellogenesis, ovulation was observed in September. The embryos were first observed in January and young snakes of about 360 - 400 mm SVL were first caught in June. The maternal size has a positive influence on clutch size, whereas it does not affect the egg size.

A. granulatus may distribute throughout coastal Thailand. In this study they were usually found in tidal estuaries or nearby the river mouth where mudflats and mangrove forests were present. However, there is no evidence that this species can inhabit sandy beach, rocky shore, coral reef or sea grass beds. The relative abundance of *A. granulatus* in three locations in Phnangnga bay is low. Their morphology and behavior are closely related to their habitat, for example the body is very flexible since it becomes dorso-ventrally flattened when lying down at the bottom but laterally flattened when swimming with the reduced ventral scales and slightly flat tail allowing them to swim properly in shallow water. *A. granulatus* were observed active during the night time, indicating that they may be a nocturnal species. The main diet of *A. granulatus* may be fish, females tend to feed on bigger prey than do males.

5.2 Recommendations

1. The little file snake, *Acrochordus granulatus* lives in shallow brackish water estuary and near mangrove forest which are complex areas of extreme physical factors. Their home range, micro habitat preference and feeding behavior are less known and need to be observed in the future.

2. The distribution and the status of *A. granulatus* in Thailand are not clear. Intensive surveys especially where mangrove forests exist, should be conducted in the future before the distribution of *A. granulatus* in Thailand can be completely summarized.

3. *A. granulatus* may inhabit a wider area further from the shore than this study demonstrated. For example, they may move out to deeper water during some periods. Their movement and habitat use need to be studied more in the future.

4. Habitat destruction and human activities are two major factors likely affecting the abundance of *A. granulatus*. Conservation efforts need to be promoted in order to protect important species and their natural habitats.

CHAPTER VI

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BIOGRAPHY

Miss Sansareeya Wangkulangkul was born on July 7, 1973 in Songkla Province. She graduated with a Bachelor degree of Science from the Faculty of Science, Prince of Songkhla University in 1995. She graduated with a Masters degree of Science from the Faculty of Science, Chulalongkorn University in 1998. She furthered her education in Biological Science in the Faculty of Science, Chulalongkorn University in 2000.