CHAPTER II
LITERATURE REVIEW

2.1 General Characters of Freshwater Leeches

Freshwater leeches share many characteristics with the oligochaetes, especially in their hermaphroditic characters. Nevertheless, they differ from other oligochaetes in many significant structures such as having no appendages or chaetae, having a fixed number of 34 of body segments, their external segmentation does not correspond with the arrangement of the internal organs, and possessing two ventral suckers, one at each posterior and anterior ends of the body (Moore, 1927; Sawyer, 1986). External and internal characters of freshwater leeches are presented in figures 2-1 to 2-3.

2.1.1 External Characters

External form and segmentation.—Freshwater leeches are segmented worms consisting of a constant number of 34 somites (Moore, 1927). Each somite has secondary subdivision transverse furrows, known as annuli. The number of annuli per somite is varying from single annulus in anterior somites to five annuli in mid-body somites, and each central annulus of the somite bears disc-shaped sensory receptors called "sensillae" (Figure 2-1). The somites are traditionally designated by the Roman numerals of I to XXXIV. The first six somites belong to the oral sucker; the somites VII to IX are the pre-clitellar region; somites X to XXIV are the middle body region, which are further subdivided into the clitellar or genital region (somites X to XIII) and the post-clitellar region (somites XIV to XXIV), the somites XXV to XXVII are the anal region, and the somites XXVIII to XXXIV are the caudal region or region of caudal sucker (Keegan, Toshioka, and Suzuki, 1968; Mann, 1962; Moore, 1927).

Oral sucker.—It is slightly smaller than body width, never widely explanate, but muscular and mobile, and capable of extension. It is a depression on the ventral surface of somites I-IV, passing posteriorly into an oral chamber, and end up by transverse muscular septum, called velum, perforated in the center by the triangular mouth (Figure 2-2) (Mann, 1962; Moore, 1927).

Eyes.—Eyes of freshwater leech are highly developed, five pairs, almost invariably, forming a regular arch on somites II to VI of the oral sucker (Figure 2-1). They are glorified sensillae, consist of a colorless transparent epithelial lens, a nerve-axis and optic ganglion, and surrounded by a tubular sheath of visual cells each containing a vitreous body and a dense pigment-cup (Moore, 1927).

Middle region.—It is the largest and most complex region, containing reproductive organs, the regularly paired and metameric nephridia, the ganglia of the central nerve cord, and the digestive region (the stomach and intestine) of the alimentary canal. The middle region may be further divided into the clitellum, the area where the reproductive organs present, and the post clitellum sub-region (Moore, 1927; Mann, 1962). The prominence number of specific segments involved of the clitellum varies among species. In hirudiformes, there are 15 annuli, usually from somite X to XIII (Moore, 1927; Sawyer, 1986).

Gonopores.—Gonopores are external opening of genital organs, located ventrally at the median of the body. The male pore appears anteriorly in advance of the female, at somites XI and XII, respectively (Figure 2-2). Most frequently, the gonopores open in the furrows, but in some species situated within the annuli. The male pore is rounded or represented as transverses slit (Keegan et al., 1968; Moore, 1927).

Nephropore.—It is an external opening of nephridia, located ventrally on the furrow of the second annulus (b2) of somites VIII to XXIV. In Hirudiformes, there are sixteen or seventeen pairs (Moore, 1927; Sawyer, 1986).

Sensillae.—Sensillae are tegumentary sense organ, comprising a group of ciliated bipolar cells, the specialized epithelial cells, whose somata lie in the peripheral tegument. They are scattered over various parts of the body. Sensillae in freshwater leeches are regularly arranged on the middle annulus of each segment, known as segmentally receptors (Figure 2-1). The function of senillae is probably mechanoreceptors or chemoreceptors (Derosa and Friesen, 1981; Mann, 1962; Sawyer, 1986).

Caudal sucker.—It is well developed as a simple but highly muscular disk, and distinctly larger than the oral sucker. Ventral surface of caudal sucker is provided with radiating ridges ending in marginal papillae, and dorsal surface may be roughly mosaic pattern. The caudal sucker is powerful organ of adhesion, serving for attachment and locomotion. It is provided with interlacing circular, radial, and oblique muscle fibers, in addition to the powerful erecter and depressor muscles that join it to the body (Moore, 1927).

Anal region.—It is a short transitional section of three incomplete somites (XXV to XXVII), contains the rectum and anus but no nephridia (Moore, 1927).

2.1.2 Internal Characters

Digestion system.—Digestive system in freshwater leeches is divided into an oral chamber, buccal sinus, pharynx, esophagus, stomach, intestine, rectum, and anus (Moore, 1927).

The oral chamber is the cavity of the oral sucker, ranged from the overarching lip to the buccal sinus (Figure 2-3). In the Hirudidae the oral chamber is separated from the buccal sinus by the velum.

Buccal sinus is an area extremely anterior end of the pharynx, where the jaw lies. Jaw of freshwater leeches is trignathous, consisting of one in dorsomedial and two in ventrolateral positions, respectively (Sawyer, 1986). The jaw is supplied with a number of ductules from the salivary glands or salivary papillae, which considerable are varied between species, in term of size and shape (Phillips, 2012). Each jaw bears a single row of sharp teeth made from specialized epithelial cells as a hard mineralized cuticle (Sawyer, 1986). Jaw morphology and teeth number are commonly used as diagnostic characters for hirudiniformes leeches (Moore, 1927; Phillips, 2012).

The pharynx in freshwater leeches is short and bulbous muscular organ, usually confined to somite VII and VIII. It is characterized by the great development of radial muscles connecting it with the body wall.

The esophagus is short and narrow, appeared anteriorly of the stomach preceding the caeca. The stomach reaches from the pharynx in somites IX or X to XIX. It is varied in development, offering valuable taxonomic characters in higher taxonomy (Moore, 1927).

The intestine is not always clearly distinct from the rectum. It is more or less sacculated or chambered, the first chamber being especially distinct both in from and structure (Moore, 1927; Sawyer, 1986).

Reproductive organs.—All leeches are hermaphroditic, and cross-fertilization is general rule (Moore, 1927). They have both male and female reproductive organs, which are the most important key characters for taxonomic purposes, especially for the separation of genera and species of leeches (Keegan et al., 1968; Moore, 1927; Phillips, 2012).

The male reproductive organs are most extensive, with terminal organs lying anteriorly and the testes in posteriorly. The terminal organ composes of two epididymis, two ejaculatory bulb, and single atrium. The atrium consists of two parts, a basal bulb and a penis sheath. There are several pairs of testes, with an equal number of vas deferens (Keegan et al., 1968).

The female reproductive organs are well developed, consists of paired ovaries and oviducts, common oviduct surrounded by albumin glands, single vagina caecum, and vagina that opens to the exterior through the female genital pore. The vagina caecum is usually very large, arises from the vagina and albumin gland (Keegan et al., 1968).

Nervous System.—The central nervous system of leeches is one of the most remarkably organized structures in the animal kingdom. Leeches have 34 segmental brains called ganglia, and each segmental ganglion arises two pairs of nerves. Each of these lateral segmental nerves connects with an inter-muscular nerve ring which circles the body between circular and longitudinal muscles (Figure 2-2) (Sawyer, 1986).

2.2 Biology of Freshwater Leeches

All leeches are hermaphrodites, which perform cross-fertilization as the general rule (Moser, Govedich, and Klemm, 2009; Sawyer, 1986) have a tube-like eversible male copulatory organ, which is inserted into the female gonopore of the partner (Elliott and Kutschera, 2011), but some Glossiphoniidae leeches may perform self-fertilization when mates are not available (Moser et al., 2009). Many leeches can breed multiple times and exhibit irregular growth after reaching maturity. In general, juveniles of freshwater leeches require a minimum of 3-5 times of blood meals to reach sexual maturity (Moser et al., 2009). The typical life span for leeches is 1-3 years, with life cycle in general consists of egg, juvenile, and mature hermaphroditic adults. Eggs are deposited inside a thickened or spongy cocoon secreted by the clitellum, which is filled with nutrient solution for the developing eggs and young leeches (Moser et al., 2009). In *Hirudo medicinalis* Linnaeus, 1758, each mature leech can lay 1 to 8 cocoons, with usually 12 to 16 eggs per cocoon (Elliott and Kutschera, 2011). The cocoons of freshwater leeches are either released into the sediment or attached to vegetation depends on leeches species, but typically abandoned by the parent (Elliott and Kutschera, 2011; Moser et al., 2009; Reish and Pernet, 2009). However, parental care behavior is commonly observed in the jawless worm leeches family Erpobdellidae, which represents the most ancient state in leech evolution (Kutschera and Wistz, 2001).

Freshwater leeches spend the day under rocks or leaves on the lakes, streams, canals, or paddy field bottoms, and use water vibrations to detect their hosts (Klemm, 1972). They feed on sucking blood from various kinds of animals, such as fish, frogs, turtles, and mammals (Moore, 1927). The most important environmental factors which determine the abundance of leeches are the availability of hosts; nature of substrate; depth of water; water current (lentic or lotic); physical factor such as hardness, pH, and temperature of the water; minimum concentration of dissolved oxygen; siltation and turbidity; and water salinity (Sawyer, 1974).

2.3 Freshwater Leeches and Human Relationship

Leeches have been familiarly connected to humans throughout nearly 2000 years of recognized history in western medicine. While in ancient time, the haematophagous medicinal leech, *Hirudo medicinalis* was considered as a panacea, nowadays for its bio-active anticoagulant and anti-inflammatory substances. These are solicitation medical and pharmaceutical attention (Sohn et al., 2001; Whitaker et al., 2004). Furthermore, freshwater leeches are also useful to restore blood circulation after reconstructive surgery or, recently, to treat osteoarthritis (Pilcher, 2004). There are more than 600 described species of leeches worldwide, but only 15 species have been used for medical purposes, namely *Haementaria costata*, *Haementaria officinalis*, *Haemopis sanguisuga*, *Hirudinaria javanica*, *Hirudinaria manillensis*, *Hirudo medicinalis*, *Hirudo michaelseni*, *Hirudo nippcnia*, *Hirudo orientalis*, *Hirudo quinquestriata*, *Hirudo trichina*, *Hirudo troctina*, *Hirudo verbena*, *Macrobdella decora*, *Placobdella ornate*, *Poecilobdella granulosa* (Eldor, Orevi, and Rigbi, 1996; Elliott and Kutschera, 2011; Michalsen, Roth, and Dobos, 2007; Oosthuizen, 1991; Trontelj and Utevsky, 2005; Utevsky et al., 2010; Utevsky and Trontelj, 2005; Whitlock et al., 1983; Zaidi et al., 2011). Although the leeches are now commercially bred in leech farms, the annual consumption still exceeds the supply. In nineteenth century, up to 100 million leeches were imported to France per year.

Recently, it has been found that the most commercially used leeches are not the species officially known, *Hirudo medicinalis*, but rather its related species, *Hirudo verbena* Carena, 1820 or *Hirudo orientalis* Utevsky and Trontelj, 2005 (see Sket and Trontelj, 2008). The main Southeast Asian genus *Hirudinaria* has also been used for medicinal purposes. Nowadays, freshwater leech culture is very popular in many countries, especially in Malaysia, China and Japan. In Thailand, there are some provinces being the hubs for exporting for example Nakorn Phanom province.

2.4 Taxonomy of Freshwater Leeches

Sawyer (1986) provided a classification of freshwater leeches as follows:

Phylum Annelida

Subphylum Clitellata

Class Hirudinea

Subclass Euhirudinea

Order Arynchobdellida

Family Hirudinidae

The first taxonomic study of freshwater leeches began in 1758 which the first genus *Hirudo* was named by Linnaeus. The classification was based on only external morphology such as body color pattern and number of eyes and its position. Fourteen species of the genus *Hirudo* were recorded in the Systema Naturae. At that time, taxonomists still have not paid much attention on the leeches. Moquin-Tandon (1846) studied and summarized the classification of this genus based on external morphology and internal morphological characters. The two species *Hirudo* *medicinalis* and *Hirudo troctina* were appeared as the first two nominated species. Whitman (1886) studied leech fauna of Japan, and described a genus *Hirudinaria*. Moore (1927) studied leeches in Burma and published a book name "*The Fauna of British India and Burma*" which included the new classification. He classified freshwater leeches into 4 families, namely Ichthyobdellidae, Glossiphonidae, Erpobdellidae, and Hirudidae, based mainly on external and internal morphological characters, for examples body color, locations of male and female pores and distance between them, vaginal stalk, and size of caudal.

Nevertheless, the most important leeches that strongly associated with human are freshwater leeches in the subfamily Hirudinae, called horse leech or buffalo leech. Moore (1967) suggested the classification of the subfamily into six genera. There were *Myxobdella* Oka, 1917, *Whitmania* Blanchard, 1887, *Dinobdella* Moore, 1927, *Hirudo* Linnaeus, 1758, *Limnatis* Moquin-Tendon, 1826, and *Hirudinaria* Whitman, 1886.

Sawyer (1986) reclassified the family Hirudinidae (= Hirudidae sensu Moore, 1927) into six subfamily, which the genera of freshwater leeches classified by Moore (1967) were separated into several different subfamilies. There were *Dinobdella* and *Myxobdella* in subfamily Praobdellinae, *Hirudinaria* in sumfamily Hirudinariinae, and *Hirudo* and *Limnatis* in subfamily Hirudininae. He moved the genus *Whitmania* in to Family Haemopidae.

Phillips and Siddall (2009) used molecular evidence to study phylogeny of family Hirudinidae. They found that the Hirudinidae were not monophyletic, but appearing into two distinct and unrelated clades. Therefore, they argued that the family Hirudinidae must be refined to include only the clade containing *Hirudo medicinalis* which related to blood feeding behavior taxa.

2.5 Thailand Freshwater Leeches

Thailand freshwater leeches are poorly investigated. The first scientific report of freshwater leeches from Thailand was Baird (1869). He reported one species, *H. manillensis*, from Siam (=Thailand). De Qual (1917) later reported the same species from Bangkok. Moore (1927) studied leeches in Myanmar and also included *H. manillensis* collected from Thailand into the list, but without the specific collected locality. Keegan (1968) studied leeches in Japan, and also examined living specimens of *H. manillensis* collected from Southeast Asian countries including Thailand.

The most recent record of freshwater leeches in Thailand was interpreted by Phillips (2012). The three south Thailand species of the genus *Hirudinaria* has been reported with proposed molecular phylogenic relationships. They are *H. javanica*, *H. manillensis*, and described *H. bpling*.

2.6 Karyotypes Study

Different organisms have very different sets of chromosomes (or karyotypes), and in general, the karyotypes of the closely related species are more similar to each other than that of distantly related species (O'Connor, 2008). Changes in karyotypes, the chromosome size and morphology, are therefore evidently characteristic of the evolutionary process, and it is possible to describe the numerous ways in which chromosomes and the whole genomes change through evolution (Sumner, 2003). Karyotypic analysis has been selected as an alternative approach to compare with the morphological identification (Utevsky et al., 2009). It is quite an acceptable technique for assisting reliable identification and cheap. Karyotypes can be prepared from variety of tissue types, which contain mitotic cells that have been arrested in the metaphase or prometaphase portion of the cell cycle, when chromosomes assume their most condensed conformations (O'Connor, 2008).

Up to now, chromosomes and karyotypes have been applied in the taxonomy works, such as in the study of land operculate snails genus *Cyclophorus* (Kongim et al., 2006) and in polychaets family Nereididae (Ipucha et al., 2007). Cichocka and Bielecki (2008) studied on karyotypes of many leeches families. They reported the diploid numbers of some families such as the Glossiphoniidae ranged from 14 to 32, from 20 to 32 in the Piscicolidae, from 16 to 22 in the Erpobdelliformes and from 24 to 28 in the Hirudiniformes. The karyological analyses have been also used to show phylogenetic relationship between the main groups of Hirudinida, which interpreted the diploid number of 16, and this number was suggested as a primitive stage (Cichocka and Bielecki, 2008; Utevsky et al., 2009) studied chromosome numbers of three freshwater leeches species: *Hirudo medicinalis*, *Hirudo verbana* and *Hirudo orientalis*, determined both from mitotic and meiotic stages of spermatogenesis using the propionic Haematoxylin staining technique. All three species were found to have different haploid chromosome numbers: 14, 13, and 12 for *Hirudo medicinalis*, *Hirudo verbana* and *Hirudo orientalis* respectively (Figure 2-4). Chromosomal studies can be used to confirm and clarify the taxonomic status that are previously classified by morphological characters. These make the evolutionary view of organisms more understandable. Moreover, Wenrowsky (1928) suggested that the evolutionary trend in size and number of the

Hirudinea chromosomes began from smaller size and larger numbers in the primitive forms.

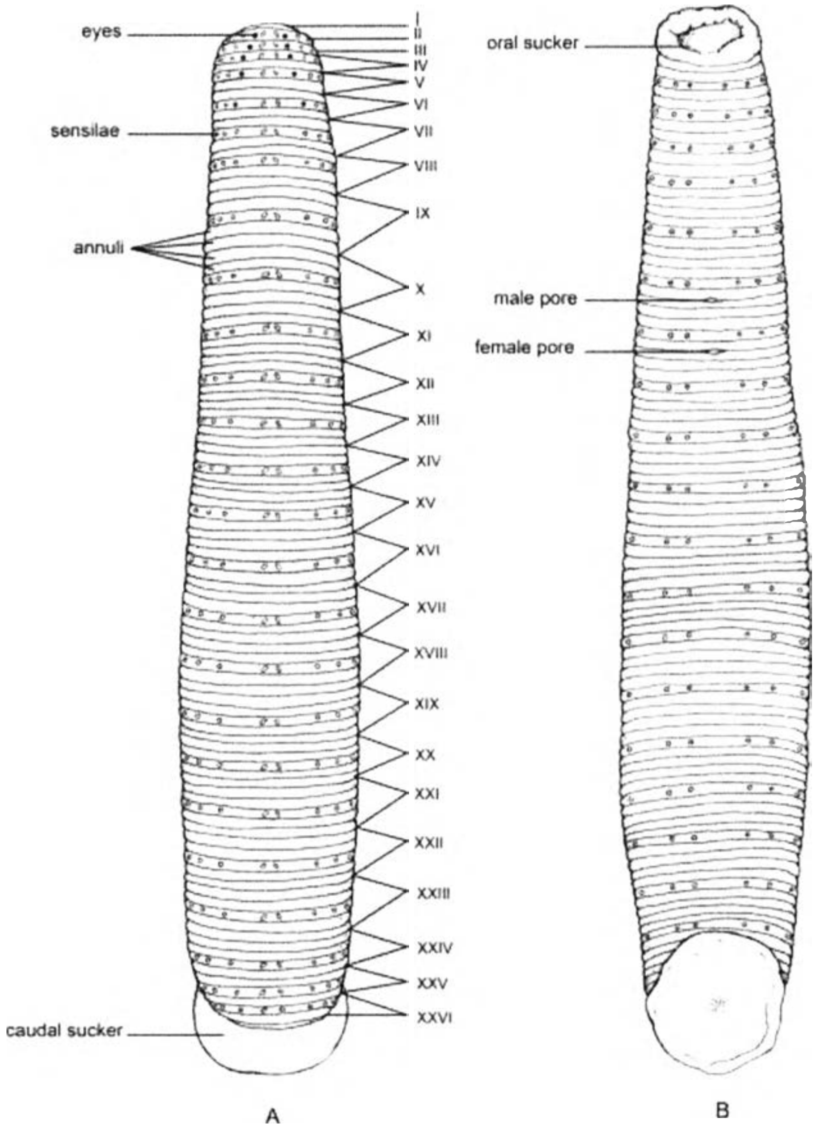

Figure 2-1. Dorsal (A) and ventral (B) view of *H. manillensis* (Lesson, 1842) showing general external characters. Specimen CUMZ 3403 collected from Nawa, Nakhon Phanom province.

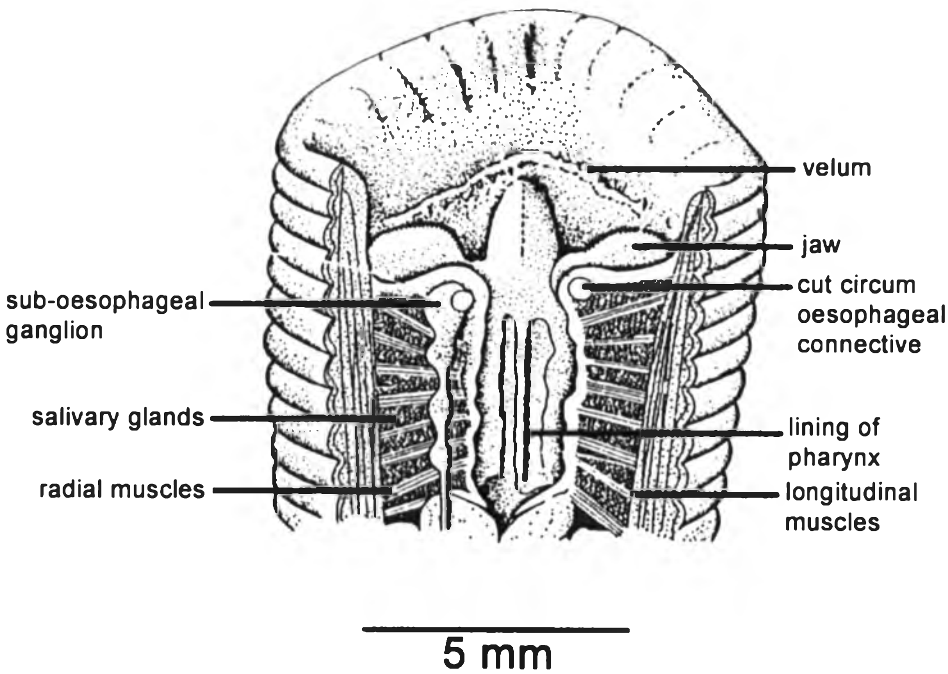

Figure 2-2. Ventral dissection of the head of *Hirudo medicinalis* showing jaws protruded in biting position (after Mann, 1962).

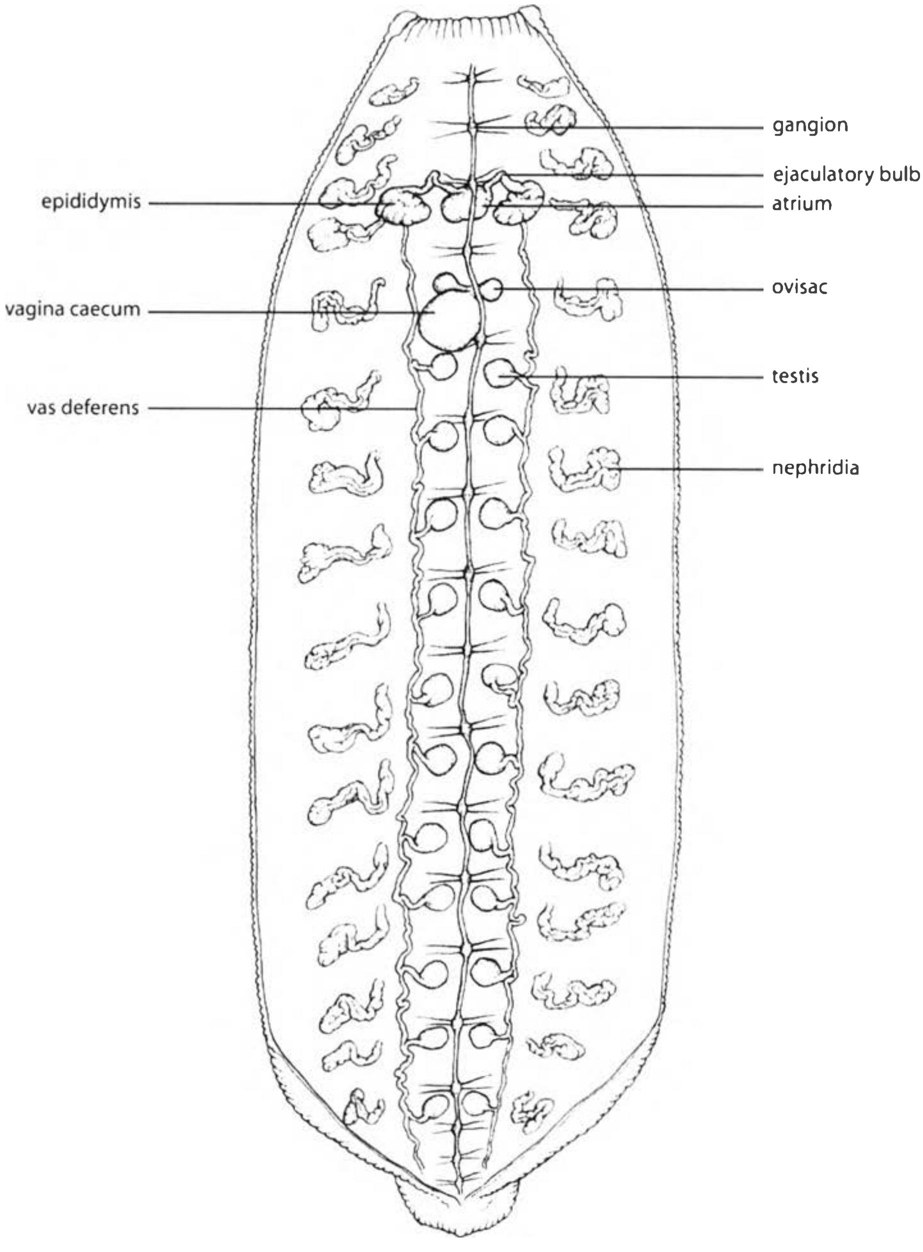

Figure 2-3. Internal characters of *H. javanica* (Wahlberg, 1856). Specimen CUMZ 3404 from Ban Donsala, Na Wa, Nakhon Phanom province.

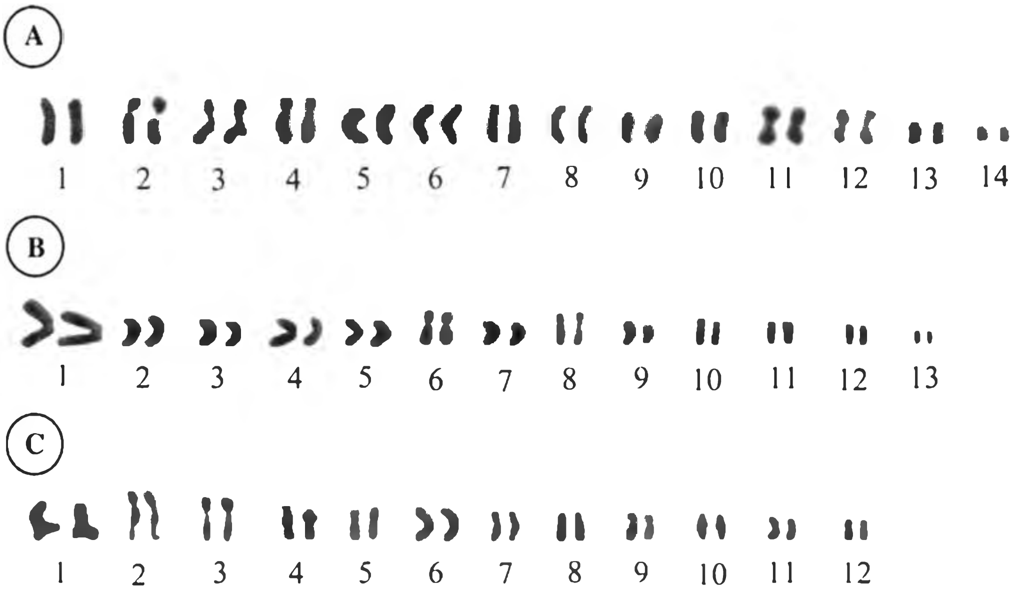

Figure 2-4. Karyotypes of three medicinal leeches species: *Hirudo medicinalis* (A) *Hirudo verbena*; (B) *Hirudo orientalis*; (C). (after Utevsky et al., 2009).