

## CHAPTER IV

### RESULTS

#### 4.1. Freshwater Leeches Subfamily Hirudinae in northeastern Part of Thailand

In total, 435 specimens of freshwater leeches in the subfamily Hirudinae were examined. The identification was based on the following literatures: Lesson (1842), Wahlberg (1856), Whitman (1886), Moore (1927), Richardson (1969), Klemm (1972), Lai and Chen (2010), and Phillip (2012). All examined specimens were classified into the genus *Hirudinaria* Whitman, 1886. Three species were identified, namely *H. javanica*, *H. manillensis*, and *Hirudinaria* sp., an unidentified morphotype (Table 4-1).

#### Taxonomy

Family Hirudidae Pinto, 1823

Subfamily Hirudinae Pinto, 1823

Genus *Hirudinaria* Whitman, 1886

*Hirudinaria* Whitman, 1886: 373. Moore, 1927: 207.

**Type species:** *Sanguisuga javanica* Wahlberg, 1856, by original designation.

**Diagnosis:** Size medium to very large (27-248 mm of body length). Live specimens have green body or brown, or reddish-brown, with a very precise pattern of black



stripes and metameric spots. There are fifteen complete somites (IX to XXIII). Head boarded with large five pairs eyes well-developed. Gonopore separated by five or seven annuli, the male characters locate at XI and the female's at XII or XIII. Sensillae large. Jaw large, with numerous prominent salivary papillae and more than one hundred acute monostichodont teeth. Gastric caeca have two pairs per somite, unequal, spacious, and somite much lobulated. Atrium pyriform, ejaculatory bulb present. Vagina caecum large, opens by a separated duct, but along with the common oviduct, either into the female bursa or into a vaginal stalk. Strictly sanguivorous.

Key to species of the northeastern Thailand freshwater leeches in the genus

*Hirudinaria*

- 1a. Male and female gonopores separated by 5 annuli .....2
- 1b. Male and female gonopores separated by 7 annuli.....*H. javanica*
- 2a. Middle dorsal line distinct, black, dorsal sensillae on caudal sucker 4-5 sensillae per radius; jaw with approximately 148 teeth..... *H. manillensis*
- 2b. Middle dorsal line absent; dorsal sensillae on caudal sucker two sensillae per radius; jaw with approximately 167 teeth.....*Hirudinaria* sp.



*Hirudinaria javanica* (Wahlberg, 1856)

(Figures 4-1 to 4-5)

*Sanguisuga javanica* Wahlberg, 1856: 233. Type locality: Samarang, Java [Semarang, Central Java, Indonesia].

*Hirudinaria javanica* — Whitman, 1886: 373-376, pl. 20, fig. 56.

*Limnatis (Poecilobdella) javanica* — Blanchard, 1897: 349-351, text figure 7.

*Limnatis javanica* — Kaburaki, 1921: 711.

*Hirudinaria javanica* — Moore, 1927: 210-218, Figures 50-52.

**Material examined:** Ban Donsala, Na Wa, Nakhon Phanom: CUMZ 3402 (17 specimens), 3404 (18 specimens; Figures 4-1, 4-2, 4-3, 4-4, 4-5B), 3429 (9 specimens). Ban Nongwang, Tao Ngoi, Sakon Nakhon: CUMZ 3413 (9 specimens). Ban Janpen, Tao Ngoi, Sakon Nakhon: CUMZ 3415 (16 specimens). Ban Nonghai, Khamchae, Mukdahan: CUMZ 3422 (4 specimens), 3424 (9 specimens; Figures 4-5A, 4-15A, 4-15B). Chaturaphak Phiman, Roi Et: CUMZ 3419 (1specimen).

**Diagnosis:** Live specimens have greenish on the dorsal side of the body or dark green, or yellow brown, with black line on middle dorsal. Gonopore separated by seven annuli. Dorsal sensillae on caudal sucker 4-5 sensillae per radius. Atrium short



and small, with short penis sheath. Vas deferens straight. Vagina caecum short, ovate in shape, no vaginal stalk.

**Description:** The preserved adult specimens, body length ranges 41-184 mm, body width at 5-16 mm (Table 4-3). Live specimens have greenish on the dorsal side of the body or dark green or yellow brown. Middle dorsal line distinct, black, continuous, paralleled with two series of black spots on both side, two faint black stripes present on each side. Body margin yellow with one ordered series of black spots. Ventral side green without marker (Figure 4-1). Jaw trignathous, approximately 134 teeth. Number of salivary papillae, both small and large, is 43 glands (Figure 4-2; Table 4-2). Gonopores separated by seven annuli. Male reproductive system located in middle of the body between somites XI and XIII. Ejaculatory bulbs short and small. Ejaculatory ducts long, connect with atrium side in somite XI. Atrium short, small, pear-shape with unclear penis sheath. Vas deferens straight, runs along almost entire of the body, with 11 testisac pairs (12 pairs in some specimens). Nerve cord runs along body length on right side of atrium. Ovisacs stout, albumin gland not well developed, common oviduct short, open into the female bursa. Vagina caecum short, ovate in shape, no vaginal stalk (Figure 4-3). Dorsal sensillae on caudal sucker 4-5 sensillae per radius (Figure 4-4).

**Variation:** Two color forms were observed on the dorsal region of the body from 83 specimens of *H. javanica*.



Form A: This form is characterized by an absent of black stripe on each side of the middle dorsal. They were recorded from four localities: Ban Donsala, Na Wa, Nakhon Phanom (24 specimens); Ban Nongwang, Tao Ngoi, Sakon Nakhon (9 specimens); Ban Janpen, Tao Ngoi, Sakon Nakhon (7 specimens); and Ban Nonghai, Khamchae, Mukdahan (9 specimens; Figure 4-5A, locality 8).

Form B: This form is distinct from the Form A by the presenting of two additional black stripe lined on each side of the middle dorsal line. They were found at Ban Donsala, Na Wa, Nakhon Phanom (20 specimens; Figure 4-5B, locality 1); Ban Janpen, Tao Ngoi, Sakon Nakhon (9 specimens); Chaturaphak Phiman, Roi Et (1 specimen); and Ban Nonghai, Khamchae, Mukdahan (4 specimens).

**Distribution:** China, India, Myanmar, Thailand, Java, Borneo, and Sumatra (Moore, 1927; Keegan, 1968).

**Habitat:** This species was found in swamps, ponds, and especially padcy field.

*Hirudinaria manillensis*, (Lesson, 1842)

(Figure 4-6 to 4-10)

*Hirudo manillensis* Lesson, 1842: 8. Type locality: Philippine Islands.



*Hirudo sanguisorba* Tennent, 1859: 305. Type locality: Ceylon. Tennent, 1861: 483-484, with text figure. Type locality: Ceylon [Sri Lanka].

*Hirudo multistriata* Schmarda, 1861: 3, Taf. 16, fig. 141. Type locality: Ceylon [Sri Lanka].

*Hirudo luzoniae* Kinberg, 1866: 356. Type locality: Manila [Philippines].

*Hirudo maculosa* Grube, 1868: 39-40, Taf. 4, fig. 6. Type locality: Singapore.

*Hirudo maculata* Baird, 1869: 315. Type locality: Siam [Thailand].

*Limnatis (Poecilobdella) granulosa* Blanchard, 1893: 28. Type locality: Java, Indonesia  
Blanchard, 1897: 338-349, figs 3-6. Kaburaki, 1921: 673-675.

*Limnatis granulosa* — Robertson, 1909: 676-679, fig. 4.

*Hirudo boyntoni* Wharton, 1913: 369-371. Type locality: Philippines Islands.

*Limnatis maculosa* — Dequal, 1917: 9.

*Limnatis (Poecilobdella) manillensis* — Moore, 1924: 376.

*Hirudinaria manillensis* — Moore, 1927: 218-226, fig. 53.

**Material examined:** Ban Donsala, Na Wa, Nakhon Phanom: CUMZ 3401 (21 specimens), CUMZ 3403 (4 specimens; Figures 4-6, 4-7, 4-8, 4-9, 4-10), 3430 (57 specimens). Ban Majang, Na Wa, Nakhon Phanom: CUMZ 3427 (51 specimens). Ban Nongwang, Tao Ngoi, Sakon Nakhon: CUMZ 3412 (4 specimens). Phang Khon, Sakon Nakhon: CUMZ 3428 (2 specimens). Mueang, Sakon Nakhon: CUMZ 3417 (2



specimens). Phu Phan, Sakon Nakhon: CUMZ 3416 (6 specimens). Ban Janpen, Tao Ngoi, Sakon Nakhon: CUMZ 3414 (1 specimen). Ban Nonghai, Khamchaee, Mukdahan: CUMZ 3423 (13 specimens). Khong Chai, Kalasin: CUMZ 3409 (28 specimens). Ban Thatoom, Mueang, Mahasarakham: CUMZ 3407 (4 specimens; Figures 4-15C, 4-15D). Huai E-pong, Phu Wiang, Khon Kaen: CUMZ 3425 (11 specimens). Bung, Mueang, Amnat Charoen: CUMZ 3410 (30 specimens). Pa Tio, Yasothon: CUMZ 3411 (3 specimens). Khemarat, Ubon Ratchathani: CUMZ 3408 (26 specimens). Chaturaphak Phiman, Roi Et: CUMZ 3418 (5 specimens).

**Diagnosis:** Live specimens have dark green on the dorsal side of the body or reddish-brown, yellow brown with black line on middle dorsal. Gonopore separated by five annuli. Dorsal sensillae on caudal sucker 4-5 sensillae per radius. Atrium large, relatively elongated, with penis sheath. Vagina caecum relatively long, ovate, no vaginal stalk.

**Description:** In preserved adult specimens, body length ranges 27-248 mm, body width at 3-30 mm (Table 4-3). Live specimens have dark green on the dorsal side of the body or reddish-brown, yellow brown. Middle dorsal line distinct, black, incontinuous, with two faint black stripes on each side. Body margin yellow, with disrupted black spots. Ventral side brown, without marker (Figure 4-7). Jaw trignathous, approximately 148 teeth. Number of salivary papillae, both small and large sizes, is 30 glands (Figure 4-8; Table 4-2). Gonopores separated by five annuli. Male reproductive system located in middle of body between somites XI and XII.



Ejaculatory bulbs long and large. Ejaculatory ducts short, connect with atrium side in somite XI. Atrium relatively long, large, elongated in shape, with penis sheath. Vas deferens curved, runs along almost entire body, with 11 testisac pairs. Nerve cord runs along body length on left side of atrium. Ovisacs stout, albumin gland well developed, common oviduct short, open into the female bursa. Vagina caecum relatively long, ovate in shape, no vaginal stalk (Figure 4-9). Dorsal sensillae on caudal sucker same with *H. javanica* but smaller (Figure 4-10).

**Variation:** Three color forms were observed on the dorsal region of the body from 271 specimens of *H. manillensis* which can be differentiated into three color forms.

Form A: It can be characterized by the middle dorsal line distinct, black, continuous, and with two black stripes lined on each side of the body; ventral side of body with green color and without black stripes and marker. This form recorded in Khemarat, Ubon Ratchathani (26 specimens); Bung, Mueang, Amnat Charoen (30 specimens); Pa Tio, Yasothon (3 specimens); Ban Nongwang, Tao Ngoi, Sakon Nakhon (4 specimens); Phu Phan, Sakon Nakhon (6 specimens); Ban Nonghai, Khamchaee, Mukdahan (13 specimens); and Huai E-pong, Phu Wiang, Khon Kaen (11 specimens; locality 11; Figure 4-10A).

Form B: This has middle dorsal line distinct, black, continuous, and with two faint black stripes on each side; ventral side brown or red-brown, with two black stripes. This form known from Khong Chai, Kalasin (2 specimens); Kaset Wisai, Roi Et (12 specimens); Ban Majang, Na Wa, Nakhon Phanom (9 specimens; locality 2; Figure 4-10B); and Ban Donsala, Na Wa, Nakhon Phanom (2 specimens).





Form C: This form is distinct from others by the middle dorsal line distinct, with blackish color, discontinuous, and with two brown stripes on each side. The ventral side of body with brown or reddish-brown, with two black stripes. Characteristic of the dorsal side of Form C is similar to that of Form B, but different on dorsal side. This form found from Ban Donsala, Na Wa, Nakhon Phanom (22 specimens); Ban Majang, Na Wa, Nakhon Phanom (42 specimens; locality 2; Figure 4-10C); Ban Thatoom, Mueang, Mahasarakham (4 specimens); Khong Chai, Kalasin (26 specimens); Kaset Wisai, Roi Et (52 specimens); Phang Khon Sakon Nakhon (2 specimens); Huai Saneng Reservoir, Surin (5 specimens).

**Distribution:** China, India, Bangladesh, Sri Lanka, Taiwan, Indochina, Peninsula Malaysia, Philippines, and Indonesia (Blanchard, 1897; Lai and Chen, 2010).

**Habitat:** This species was found in swamps and ponds, where water buffaloes often use for their normal habits.

*Hirudinaria* sp.

(Figure 4-11 to 4-14)

**Material examined:** Ban Donsala, Na Wa, Nakhon Phanom: CUMZ 3405 (1 specimen; Figs 2C, 4C, 5G-I), CUMZ 3431 (4 specimens). Ban Majang, Na Wa, Nakhon Phanom: CUMZ 3406 (1 specimen; Figs 3C, 6E-F).



**Diagnosis:** Live specimens have dark green on the dorsal side of the body or dark brown. Middle dorsal line not present. Gonopores separated by five annuli. Atrium moderate, somewhat elongated, with penis sheath. Vas deferens relatively smooth, runs along almost entire body, with 11 of testisac pairs. Vagina caecum long, elongated in shape, no vaginal stalk (Figure 4-16).

**Description:** The preserved adult specimens, body length ranges 107-140 mm, width at 11-16 mm (Table 4-3). Live specimens have dark green on the dorsal side of the body or dark brown. Middle dorsal line not present. Two brown stripes present on each side of the middle dorsal. Body margin yellow or orange with one ordered series of short black line. Ventral side brown or dark brown without marker (Figure 4-9). Jaw trignathous, approximately 167 teeth. Number of salivary papillae, both small and large sizes, is 25 glands (Figure 4-15; Table 4-2). Gonopores separated by five annuli. Male reproductive system located in middle of body between somites XI and XII. Ejaculatory ducts short, connect with atrium side in somite XI. Atrium moderate, penis sheath curved, opening ventral side. Vas deferens relatively smooth, runs along almost entire body, with 11 testisac pairs. Nerve cord runs along body length on right atrium side. Ovisacs somewhat long, albumin gland well developed, common oviduct long, opening into the female bursa. Vagina caecum long, elongated no vaginal stalk (Figure 4-16). Dorsal sensillae on caudal sucker two sensillae per radius (Figure 4-17).

**Variation:** No color pattern variation was observed across 6 examined specimens.



**Distribution:** This species were found in wetland area at Ban Donsala and Ban Majang Na Wa, Nakhon Phanom Province (localities 2, Figure 4-11).

**Habitat:** This species was found in swamps and several ponds with variety of aquatic plants.

#### 4.2 Karyotype Results

The metaphase chromosomes were typical indistinct because of their small size as reported in many papers (Cichocka and Bielecki, 2008; Sawyer, 1986; Utevsky et al., 2009). Nevertheless, all cleared metaphase arrangements could be observed and the spermatogonial meiotic and mitotic chromosome numbers could also be confirmed for all the examined species (Figure 4-15). Little variation in diploid number was observed among the metaphase screened in this study. The loss of one or two chromosomes was occasionally seen in a few metaphases that appeared too widely spread. However, analyses confirmed with the haploid number during meiotic diakinesis reported here for all three species. Ten to fifteen metaphases that showed no chromosome overlapping or signs of chromosome loss were selected for karyotype analysis.

Chromosomal data of the three investigated *Hirudinaria* species obtained in the present study are summarized in Table 4-1. Haploid and diploid numbers of three species of *Hirudinaria* were found differ, ranking from  $n = 13$ ,  $2n = 26$  in *H. javanica*,  $n = 12$ ,  $2n = 24$  in *H. manillensis*, and  $n = 14$ ,  $2n = 28$  *Hirudinaria* sp., as



shown in Figure 4-13 and 4-14, and did not differ within each species across their respective geographic populations. Karyotypes of all three species consisted of the chromosome pairs arranged in gradually decreasing size. They were asymmetric karyotype, and mostly are telocentric chromosomes (Figure 4-19). The distinct bi-armed chromosome appear very few and varied among the three species, being found on the first pair in *H. javanica*, on pairs 1, 2, 3, and 5 for *H. manillensis* and on pairs 3 and 5 for *Hirudinaria* sp. Moreover, the karyotype of *H. manillensis* shows distinctive chromosome markers of chromosome pair number 10, 11 and 12 exhibits telocentric with a wider angle  $100^\circ$  arrangement differ from the others.

Interestingly, the population of *H. manillensis* from Ban Thatoom, Mahasarakham (locality 10, Figure 4-17) exhibited the abnormality of chromosome condensation that cannot be determined the type or shape of chromosome and also a large number of chromosomes were found.



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Figure 4-2. SEM images of the jaws of *H. javanica* CUMZ 3404 from Nakhon Phanom; (A) overall jaw, (B) each jaw characteristic and, (C) salivary papillae.



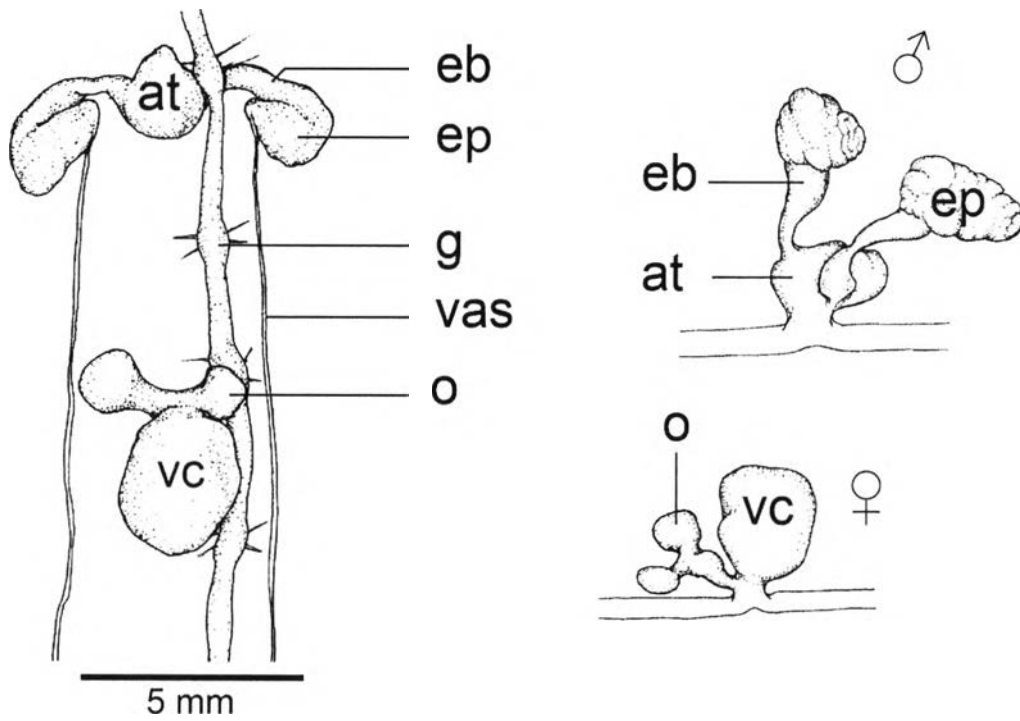


Figure 4-3. The reproductive system of *H. javanica* CUMZ 3404 from Ban Donsala, Na Wa, Nakhon Phanom. Abbreviations: ag = albumin gland, at = atrium, cod = common oviduct, eb = ejaculatory bulb, ep = epididymis, g = ganglion, o = ovary, ps = penis sheath, vas = vas deferens, vc = vagina sac, vd = vagina duct.

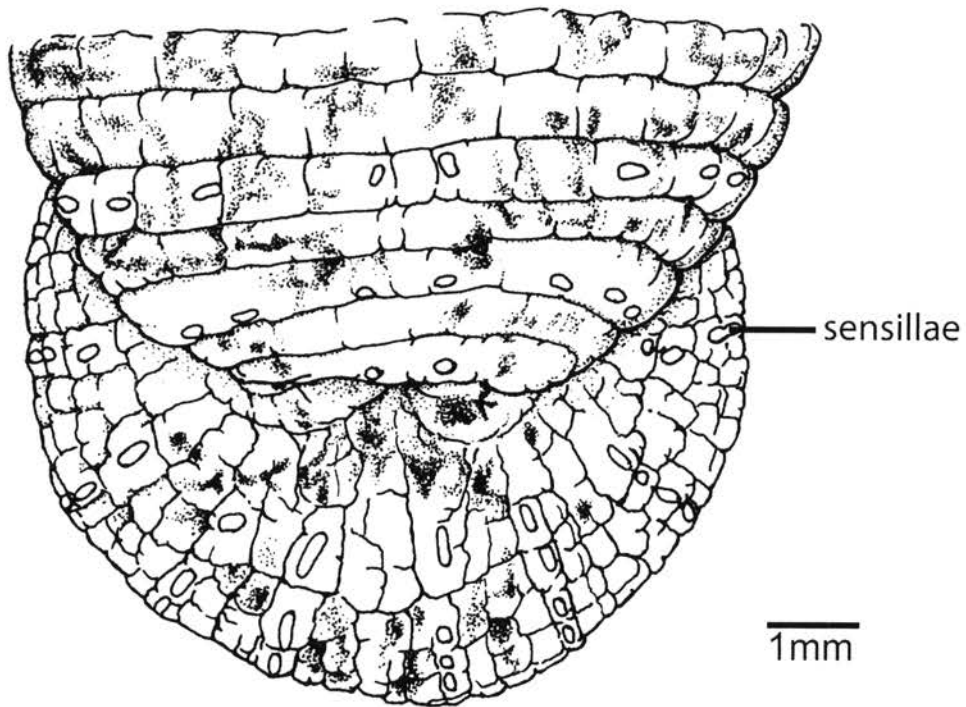


Figure 4-4. External morphology of *H. javanica* CUMZ 3404 from Nakhon Phanom showing caudal sucker and sensillae.



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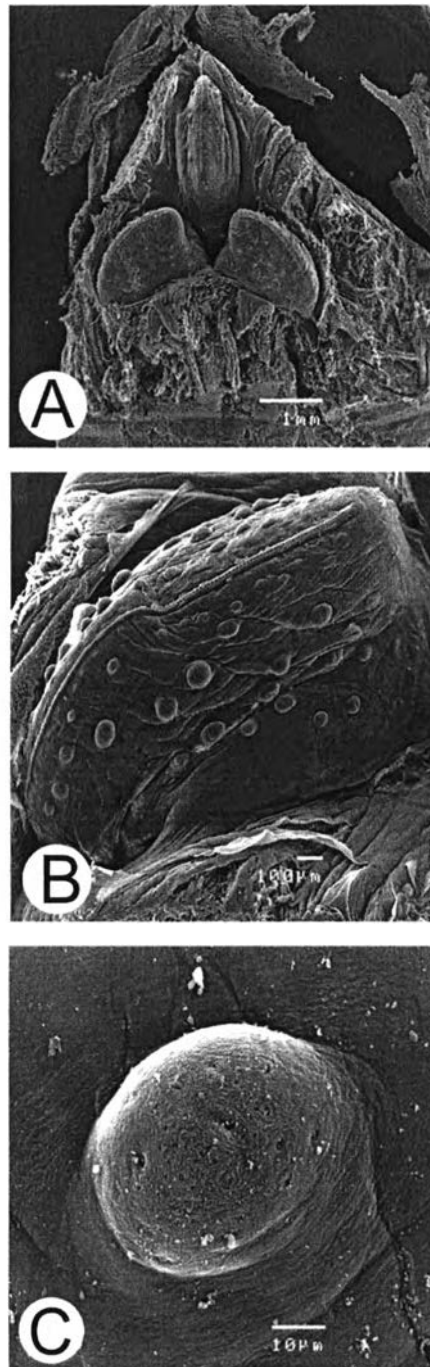


Figure 4-7. SEM images of the jaws of *H. manillensis* CUMZ 3403 from Nakhon Phanom; (A) overall jaw, (B) each jaw characteristic and, (C) salivary papillae.



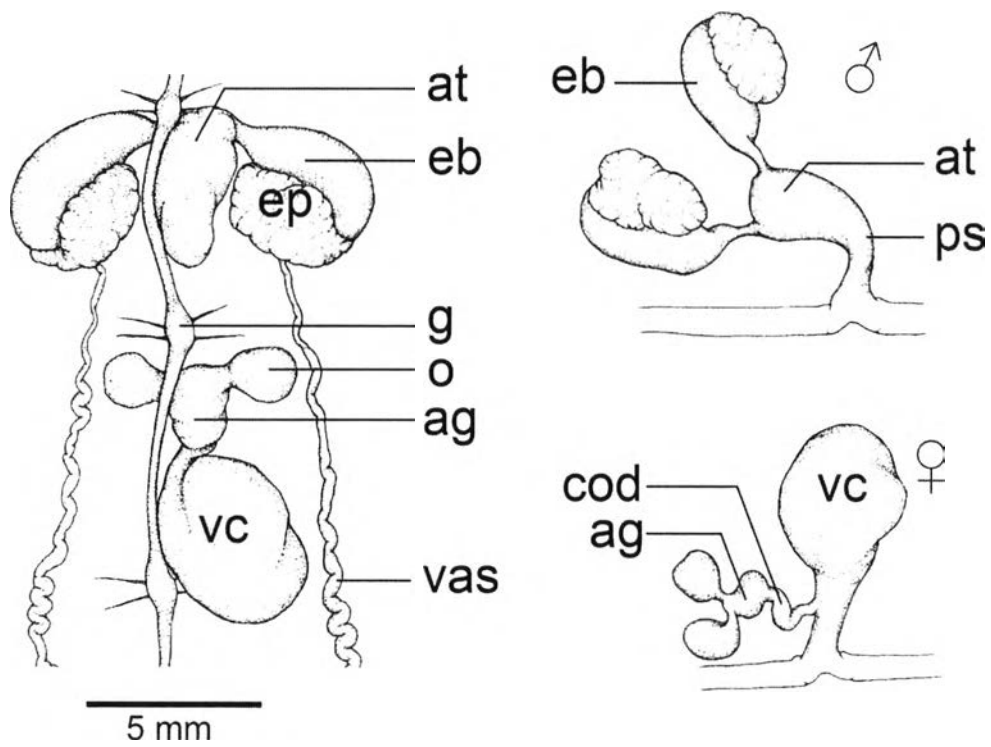


Figure 4-8. The reproductive system of *H. manillensis* CUMZ 3403 from Nakhon Phanom. Abbreviations: ag = albumin gland, at = atrium, cod = common oviduct, eb = ejaculatory bulb, ep = epididymis, g = ganglion, o = ovary, ps = penis sheath, vas = vas deferens, vc = vagina sac, vd = vagina duct.



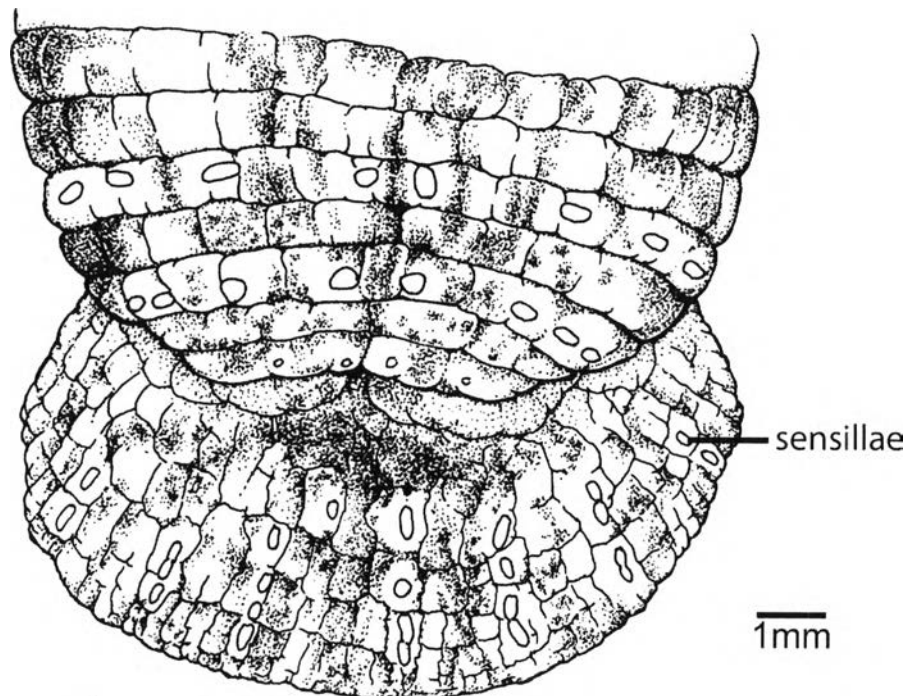


Figure 4 9. External morphology of *H. manillensis* CUMZ 3403 from Nakhon Phanom showing caudal sucker and sensillae.

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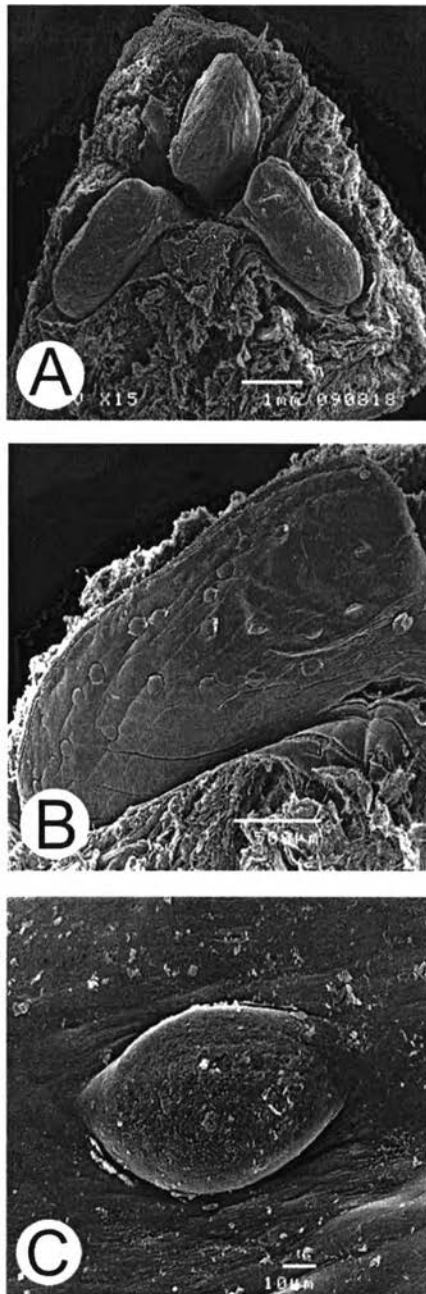


Figure 4-12. SEM images of the jaws of *Hirudinaria* sp. CUMZ 3405 from Nakhon Phanom; (A) overall jaw, (B) each jaw characteristic and, (C) salivary papillae.





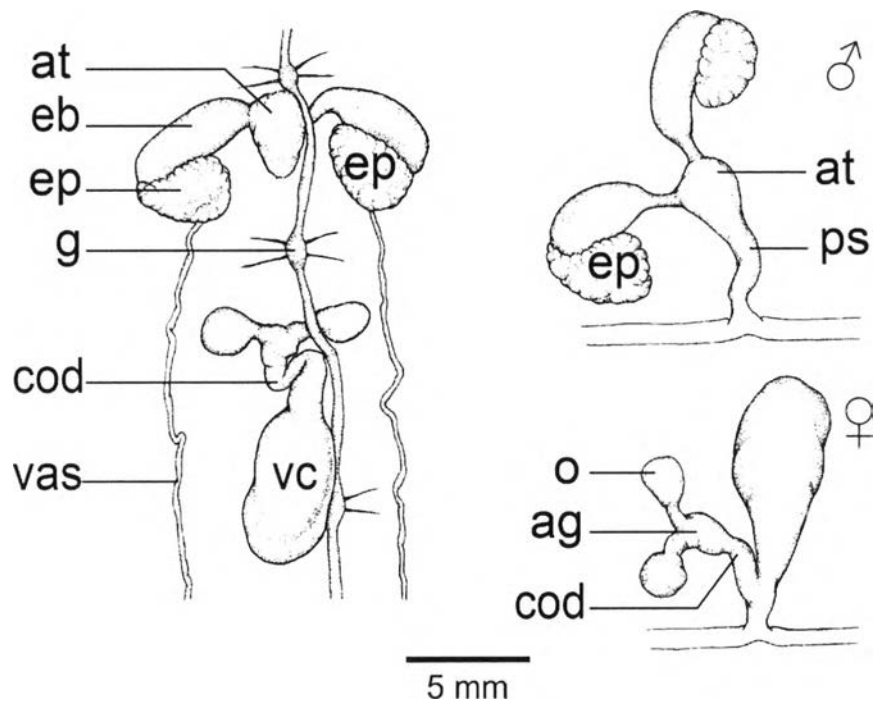


Figure 4 13. The reproductive system of *Hirudinaria* sp. CUMZ 3405 from Nakhon Phanom. Abbreviation: ag = albumin gland, at = atrium, cod = common oviduct, eb = ejaculatory bulb, ep = epididymis, g = ganglion, o = ovary, ps = penis sheath, vas = vas deferens, vc = vagina sac, vd = vagina duct.



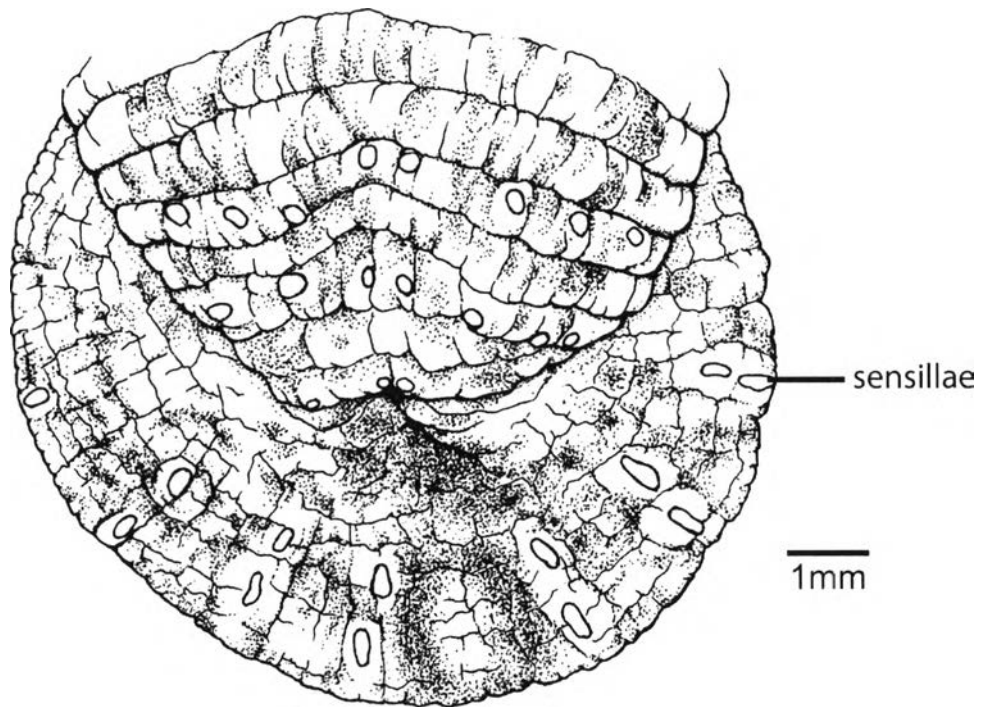


Figure 4-14. External morphology of *Hirudinaria* sp. CUMZ 3405 from Nakhon Phanom showing caudal sucker and sensillae.

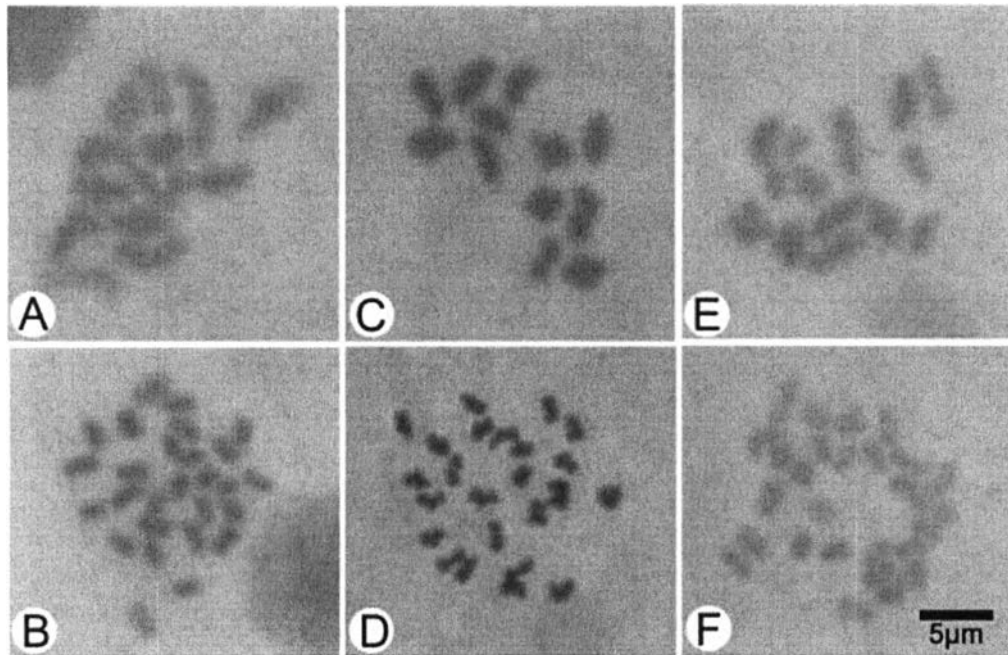


Figure 4-15. Meiotic and mitotic metaphase chromosome spreads of (A, B) *H. javanica* ( $n = 13$ ,  $2n = 26$ ), CUMZ 3424 from Ban Nonghai, Khamcha-i, Mukdahan; (C, D) *H. manillensis* ( $n = 12$ ,  $2n = 24$ ), CUMZ 3407 from Ban Thatoom, Mueang, Mahasarakham; and (E, F) *Hirudinaria* sp. ( $n = 14$ ,  $2n = 28$ ), CUMZ 3406 from Ban Majang, Na Wa, Nakhon Phanom.



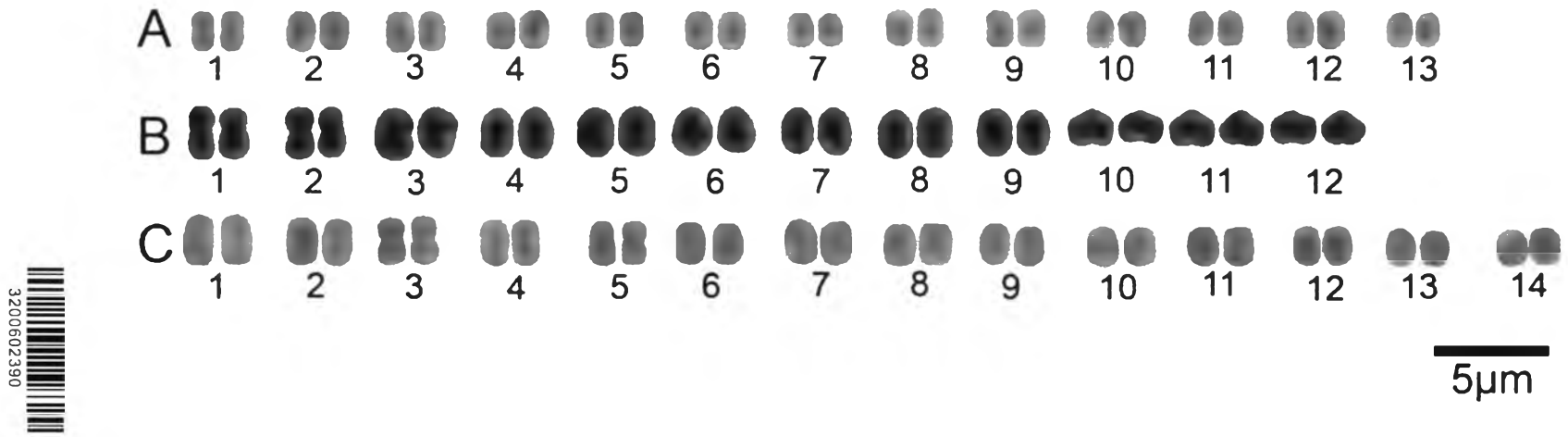


Figure 4-16. Karyotypes of three *Hirudinaria* species: (A) *H. javanica*, (B) *H. manillensis*, and (C) *Hirudinaria* sp.

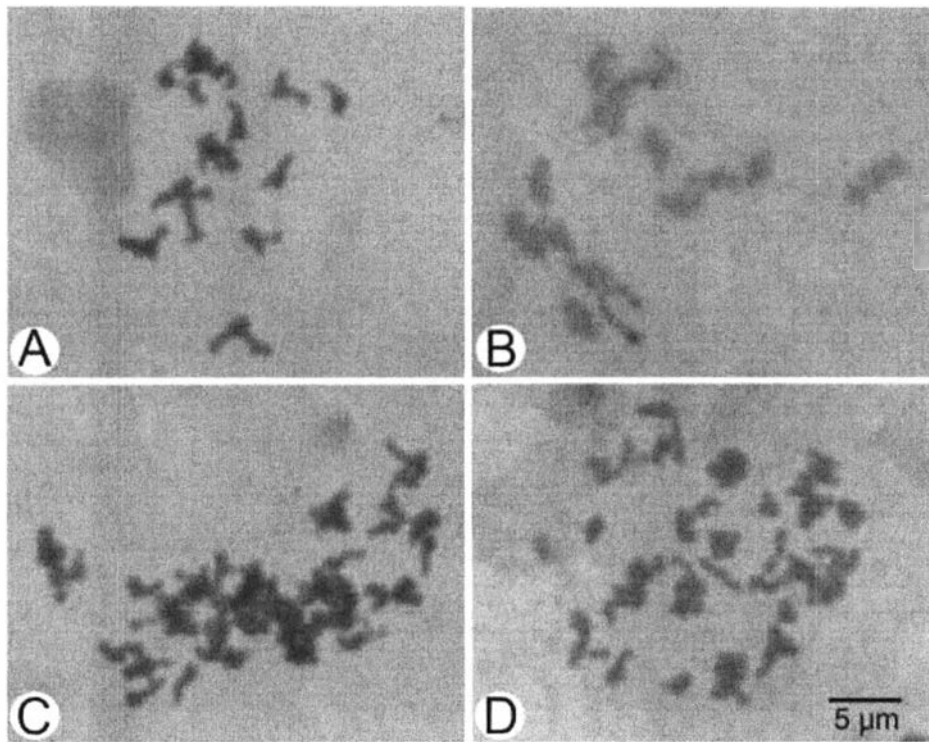


Figure 4-17. The chromosome aberration of *H. manillensis* from Ban Thatoom, Mahasarakham, CUMZ 3407 from Ban Thatoom, Mueang, Mahasarakham, showing (A-B) chromosome of undefined shape and (C-D) chromosomes in a large number.



Table 4-1. Chromosome numbers of freshwater leeches genus *Hirudinaria* collected from northeastern Thailand.

Species	Locality <sup>1</sup>	No. <sup>2</sup>	Haploid (n)	Diploid (2n)
<i>H. javanica</i>	1	4	13	26
	3	1	13	26
	7	2	13	26
	8	3	13	26
	15	1	13	26
<i>H. manillensis</i>	1	5	12	24
	2	3	12	24
	3	1	12	24
	4	1	12	24
	5	1	12	24
	6	2	12	24
	8	2	12	24
	9	2	12	24
	10	2	12	24
	11	2	12	24
	12	3	12	24
	13	2	12	24
	14	4	12	24
	16	4	12	24
17	4	12	24	
<i>Hirudinaria</i> sp.	1	5	14	28
	2	1	14	28

<sup>1</sup>Locality number corresponds to number in figure 3-1.

<sup>2</sup>No= Number of specimens examined



Table 4-2. Number of salivary papillae and number of teeth of genus *Hirudinaria*.

Species	No. <sup>1</sup>	Min-max (salivary papillae)	Salivary papillae $\bar{x} \pm SD$	Min-max (teeth)	Teeth $\bar{x} \pm SD$
<i>H. javanica</i>	20	42-45	43±0.9	130-138	134±1.7
<i>H. manillensis</i>	30	30-32	30±0.6	145-153	148±1.7
<i>H. sp.</i>	6	25-26	25±1	166-167	167±0

<sup>1</sup>No= Number of specimens examined



Table 4-3. The body length and body width of freshwater leeches genus *Hirudinaria*.

Species	Locality <sup>1</sup>	No. <sup>2</sup>	Length min-max (mm)	Width min-max (mm)
<i>H. javanica</i>	1	44	40.4-184.0	5.0-13.4
	3	9	62.8-83.8	8.9-13.3
	7	16	62.4-111.8	6.4-13.3
	8	13	62.9-122.9	6.4-16.4
	15	1	41.0	6.0
<i>H. manillensis</i>	1	82	34.2-198.9	6.4-13.8
	2	51	38.4-82.3	7.1-13.6
	3	4	31.2-45.8	6.2-6.9
	4	2	62.9-68.3	7.2-7.4
	5	2	32.8-103.8	5.2-14.7
	6	6	50.3-78.5	8.9-9.6
	7	1	248.0	30.5
	8	13	57.8-102.0	8.3-14.9
	9	28	31.7-58.5	5.2-11.2
	10	4	75.1-96.2	7.7-8.2
	11	11	52.7-98.9	8.3-14.5
	12	30	27.3-53.4	3.8-8.3
	13	3	101.0-112.5	14.0-15.3
	14	26	62.7-112.4	6.9-14.9
	15	5	126.7-133.7	11.2-11.9
	16	67	32.5-189.0	5.9-14.4
	17	11	105.4-184.7	11.1-15.1
<i>Hirudinaria</i> sp.	1	5	107.2-114.0	11.9-13.6
	2	1	140.0	16.6

<sup>1</sup>Locality number corresponds to number in figure 3-1.

<sup>2</sup>No= Number of specimens examined

