CHAPTER V
DISCUSSION & CONCLUSION

All 435 examined specimens were identified by morphological characteristics to belong to three distinct species within the genus *Hirudinaria*. They are *H. javanica*, *H. manillensis* and an unidentified morphospecies, *Hirudinaria* sp. The dichotomous key based on internal and external characters of northeastern freshwater leech species has been constructed. The external and internal morphological characters that are useful for classifying freshwater leeches species are as follows: color pattern of live specimens, distance (number of annuli) between male and female pore, position of male and female organs, size and shape of atrium, number of testisac pairs; shape of common oviduct, size and shape of vagina caecum, number of salivary papillae and teeth on jaw, and dorsal sensillae on caudal sucker. All these above details are summarized and provided in Table 5-1, which also included all species that reported by Phillips (2012), *H. bpling*.

The unidentified species (*Hirudinaria* sp.) was different from the other two (*H. javanica* and *H. manillensis*) in both its internal morphology and external morphology. Morphologically, *Hirudinaria* sp. have fewer salivary papillae (25) than the other two species (43 and 30 for *H.* and *H. manillensis*, respectively) and a higher estimated number of teeth per jaw (167 versus 134 and 148 for *H. javanica* and *H. manillensis*, respectively) (Figure 4-2). Although previous studies have reported a higher number of teeth for *H. javanica* and *H. manillensis* at 150 and 145, respectively (Moore 1927, Phillips 2012), than found in this study, these were still lower than that found for *Hirudinaria* sp. in this study. Comparisons of all the

taxonomic characters (Table 5-1) revealed that *Hirudinaria* sp. was quite similar to *H. manillensis* in terms of having the gonopores separated by five annuli, but it differs in color pattern (Figures 4-5 and 4-9). In addition, dorsal sensillae on caudal sucker of *Hirudinaria* sp. represents by two sensillae per radius, whereas 4-5 sensillae per radius in *H. javanica* and *H. manillensis*.

Surprisingly, the northeastern Thailand population of *H. manillensis* examined in this study showed a distinctly different internal morphology from that previously reported elsewhere. It contained a nerve cord running along on the left side of the atrium, instead of the right side as previously reported (Lai and Chen 2010), and also as found in *H. javanica* and *Hirudinaria* sp. in this study (Figure 4-7). The future molecular studies will be of great assistant for clarifying the mentioned problem.

Regarding the habitat characteristics and distribution, the two species and unidentified morphospecies occur in the same habitat throughout northeastern Thailand. They were found in various habitats including swamps, muddy ponds used by buffaloes, paddy fields, and ponds with variety of aquatic plants (Figure 3-2). The most frequently used habitat types by freshwater leeches observed in this study was the ponds, which more than hundred individuals found in the same ponds. There must be the founder groups occurred since colonization has been taken place and gradually increasing after ponds have been modified both naturally and artificially from time to time. *H. manillensis* was the most abundant and the most frequent collected species for this study (346/435 or 79.5% of the collected specimens and found in all 17 sampled localities), compared to 83 (19%) specimens from five locations for *H. javanica* and the seemingly rarer 6 samples (1.4%) from only two locations, Ban Donsala, Na Wa, Nakhon Phanom, for unidentified *Hirudinaria* sp.

The karyological analyses, in term of chromosome numbers, have been used to show phylogenetic relations between the main groups of Hirudinida, which the chromosome number tends to increase as the evolution progresses (Cichocka and Bielecki, 2008). In general, it is believed that the original or ancestors have low chromosome number (Sumner, 2003). Sawyer (1986) suggested that the primitive haploid chromosome number of various leeches in the subclass level is eight (Sawyer, 1986). This number is much smaller than this findings. The haploid and diploid numbers of the three specie ranged from n = 12, 2n = 24 for *H. manillensis*, n = 13, 2n = 26 for *H. javanica*, and n = 14, 2n = 28 for *Hirudinaria* sp. The results agree with Cichocka and Bielecki (2008), which reported diploid chromosome numbers of Hirudiniformes ranged from 24 to 28. Moreover, Utevsky et al. (2009) who studied in the close related freshwater leeches genus *Hirudo* also found the similar, which were diploid chromosome number of 28 in *Hirudo medicinalis*, 2n = 24 in *Hirudo orientalis* and 2n = 26 in *Hirudo verbena*. Follow the general trend of increasing chromosome number as the evolution progresses (Cichocka and Bielecki, 2008), it could be hypothesized that *Hirudinaria* sp. (n = 14, 2n = 28) is a more recent diverse species from *H. manillensis* (n = 12, 2n = 24) and *H. javanica* (n = 13, 2n = 26). In addition, *Hirudinaria* sp. was morphologically quite similar to *H. manillensis*, it thus allow for further suggestion that *Hirudinaria* sp. has been probably descended from or shared the common ancestor with *H. manillensis*, rather of *H. javanica.*

Moreover, karyotypes of *Hirudinaria* leeches in this study showed some variation in chromosome size across collected localities (figure B-1 to B-3 of appendix B). These chromosome size variations may be caused by environment and other

influences (Swanson, 1957). In addition, arm ratios and relative chromosome length sometimes are affected by differential contraction of chromosome arms or whole chromosomes (Rothfels and Siminivitch, 1958).

Furthermore, all examined species in the present study showed the typically indistinct small chromosomes. This is suggested the evolutionary trend in size and number of the Hirudinea chromosomes, which become smaller and larger in number from the primitive characteristics, as mentioned by Sawyer (1986).

Distinctive karyotypic chromosome markers were also presented, such as a distinct bi-arm chromosomes that whereas only found on the first pair in *H. javanica*, on pairs 1, 2, 3 and 5 in *H. manillensis*, and on pairs 3 and 5 in *Hirudinaria* sp. In addition, the karyotypes of *H. manillensis* have distinctive chromosome markers of chromosome pair number 10, 11, and 12 exhibits a wider angle 100 degree arrangement differ from the two species. The unidentified species (*Hirudinaria* sp.) in this study exhibits the same haploid and diploid chromosome numbers as *Hirudo medicinalis*, but their karyotypes were different (Utevsky et al. 2009) (Table 5-2). This finding of chromosome shape differentiation among species has been suggested as a common evolutionary trend within genera, which is generally found in the Hirudinida (Cichocka and Bielecki, 2008).

The current identification of these 435 samples to three morphospecies (two nominal species and one unidentified morphospecies) was quite clear because of the distinct appearance of their external and internal organs, and was supported by the distinct chromosome numbers and karyotypes of the analyzed samples of each species. However, given the apparent variation between that reported here, for

example, *H. manillensis* and that reported for the same nominal species elsewhere, indicates a need for further comparative studies utilizing type specimens and additional molecular analysis covering the reliable identified species and congeners will be great tools for future systematic revision of freshwater hirudine leeches of the region. Comprehensive taxonomic study of freshwater leeches will be crucial for biodiversity management in the near future.

Table 5-1. Comparative morphological characters among Thai *Hirudinaria* species.

Characters	*H. bpling*	*H. javanica*	*H. manillensis*	*Hirudinaria* sp.
Color	dark brown	dark green	dark brown/brown	dark green/brown
Distance (annuli) between male & female pores	5	7	5	5
Number of salivary papillae	N/A	43	30	25
Number of teeth	120	134	148	167
Position of male and female organs	XI-XII	XI-XIII	XI-XII	XI-XII
Atrium	bulbous	short	long	relative long
Pair of testisacs	N/A	12	11	11
Common oviduct	short	short	short	long
Vagina caecum	wide, long	small, ovate	small, ovate	large, elongate
Reference	Phillips (2012)	This study	This study	This study

Table 5-2. Comparison of chromosome numbers of the genera *Hirudo* and *Hirudinaria*.

Species	No.[1]	Haploid (n)	Diploid (2n)	Reference
Hirudo medicinalis	5	14	28	Utevsky et al. (2009)
Hirudo verbana	6	13	26	Utevsky et al. (2009)
Hirudo orientalis	7	12	24	Utevsky et al. (2009)
Hirudinaria javanica	11	13	26	This study
Hirudinaria manillensis	38	12	24	This study
Hirudinaria sp.	6	14	28	This study

[1]No= Number of specimens examined