

A STUDY ON THE ANATOMY FEATURES OF GREEN AND DARK BROWN BUFFALO LEECH (*HIRUDINEA MANILLENSIS*)

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Abstract: Leeches (Phylum: Annelida, Class: Hirudinea) are widely distributed all over the world in diverse habitats. In this study, the anatomy and features of *Hirudinae manillensis* or buffalo leech were observed and recorded. The external and internal anatomy of the leech was identified to know if there exist any differences between the green buffalo leech and the dark brown buffalo leech. The internal and external anatomy features both green and dark brown leeches were similar. The external anatomy features observed were; body pattern, colour of the body, size of the body, size of the sucker, body length and width, mouth, number of teeth and eyes, absence of papillae and number of annuli while the internal anatomy features observed were; organ such as crop, rectum, male and female reproductive organ and testes sack can be found. In contrast, the colour and pattern of the dorsal and the abdominal was different between the dark brown buffalo leeches and the green buffalo leeches. The Green leech had simple pattern with 5 stripes from a combination of 3 different colours namely green, brown and black, while the dark brown leech had a much more complex pattern with square shapes pattern on the dorsal.

KEYWORDS: Green leech, dark brown leech, external anatomy, internal anatomy.

Introduction

Morphology constitutes the study of the external and internal structures of living beings in their fully developed conditions. Approximately, there are 650 species of leeches worldwide. Although there is general agreement on the position of leeches in the classification of invertebrates, opinions differ on some of the finer points of their placement. Leeches are also placed in the Class Hirudinae (from Latin for leech, *Hirudo*) in the Sub- Phylum Clitella, together with the Class Oligochaeta (earthworms) distributed all over the world in a diversity of habitats, such as fresh water, seas, desert, and oases (Gouda, 2006). The term Hirudinae was used to describe the monophyletic group that includes the *Acanthobdellida*, the two known species of *Acanthobdella* and the *Euhirudinea* includes all 'true' leeches (Fredric *et al.*, 2003). Leeches are distributed in several countries in Southeast Asia such as Southern China, Philippines, Thailand,

Vietnam and Malaysia. These local leeches are known as Buffalo Leech or *Lintah Kerbau* (406th Medical Lab. Special Report, 1968).

The leech industry in this country was previously operated by the Department of Wildlife Protection and National Parks (PERHILITAN) and since 22 January 2009, the Department of Fisheries Malaysia has taken over the operation (Department of Fisheries Malaysia, 2009). The Fisheries Department have since identified the leech species used for commercial purposes. The taxonomic status of the local leech are as follows: Order: Hirudinea; Species: *Hirudinaria manillensis*; Local name: Buffalo Leech or "Lintah Kerbau". In Malaysia, there are two types of different local species namely "dark brown buffalo leech" and "green buffalo leech". However, it is not known or proven conclusively that the locally named Buffalo Leech is not of *H. manillensis* although this has been confirmed by Department of Fisheries

Malaysia. In addition, local taxonomists have not been able to identify those species used for medicinal purposes (Hj. Rosly Hassan, personal communication¹, April 2, 2010).

Several naturalists have studied many aspects of animal morphology throughout history but in Malaysia the study on anatomy of local leeches is lacking. Without a proper taxonomic identification system can lead to unnecessary confusion similar in species identification as shown by Siddal *et al.*, (2007) where it was found that the *Hirudo medicinalis* in some commercial farms in Europe were actually *Hirudo verbana*, whereby it was noted that both external species body showed similar pigmentation with different patterns (Trontelj *et al.*, 2004; Siddal *et al.*, 2007). The lack of detail integrated taxonomy study on the local Buffalo leech regardless of the body pigmentation is currently classified as *H. manillensis*. The objective of the current study was to revisit the external and internal anatomy features of dark and green Buffalo leech.

Material and Methods

The leeches used for the study was provided by Pt Dynamic Consultant Co., Kota Bharu, Kelantan. The leech was reared in an indoor glass aquarium measuring 30cm (L) x 19cm(W) x 27cm(H) filled with non-chlorinated freshwater. The water was regularly maintained by replacing 50% of the water every 3 days. The external anatomy of the leech observed parameters were body colour, body pattern, numbers and arrangement of the eyes, the number of annulations, the presence or absence of papillae, and the number of annulations between male and female gonophores. The dissected specimen was observed under stereo microscope. The internal organs of the leech such as the crop, caeca, penis sac, testes and ovary were identified and recorded. All measurements were recorded in centimetre (cm). The parameters that require imaging were recorded using a digital camera (Sony Cyber-shot™).

Results and Discussion

External Anatomy

From the study conducted on the anatomy of *Hirudinaria manillensis*, the descriptions on key features of the dark Buffalo leech and green Buffalo leech were collected. The local leech has a cylindrical, dorsoventrally flattened body divided into thirty-three or thirty-four segments. In comparison, the body of the dark brown leech was observed to have dark brownish body colour with black line and square pattern along the dorsal side, black stripe at lateral side and dark green colour on ventral side (Figure 1b). The lines of black spot pattern of the body were observed to spread from the middle line and becoming less prominent toward the lateral side. Toward the lateral side, a number of black square stripes were observed on alternating segment of the dorsal side. On the ventral side, a live specimen appears to have dark green colour.

As for green leech, the dorsal colour was dark greenish, dark olive, or dark brownish (Figure 1a). It also contain longitudinal, black or grey, mid-dorsal stripe. Next to the mid dorsal stripe, there are two longitudinal lines with orange yellowish colour at the right and left side of the mid dorsal stripes. Between the orange yellowish margin and the mid-dorsal stripe on each side, there are narrow and more or less broken stripes, of the dark brown or dark olive dorsum. For the ventral side, the colour pattern is fully dark brown colour except for two longitudinal stripes at the both side of body and the stripes colours are dark green (Figure 2a).

Both dark brown leech and green leech have at least 10 eyes which are arranged in pairs of 2. The eye can be observed under the microscope as multiple large black spot on the head arranged in parabolic arc. Five pairs of eyes are located on dorsal side toward the lateral side which is arranged closely for the first 3 pairs starting at 2nd annulus to 4th annulus. Meanwhile the 4th pair is located on the 6th annulus and the last pair is located on the 9th annulus as shown in Figure 4a and 4b.

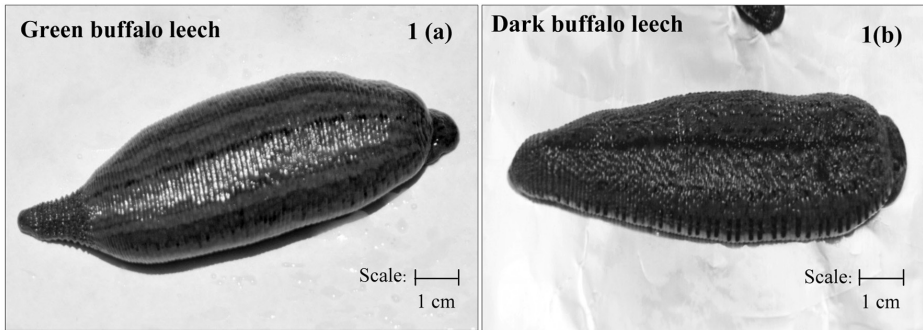


Figure 1(a) and 1(b): Comparison of Body Color and Pattern on Dorsal Side.

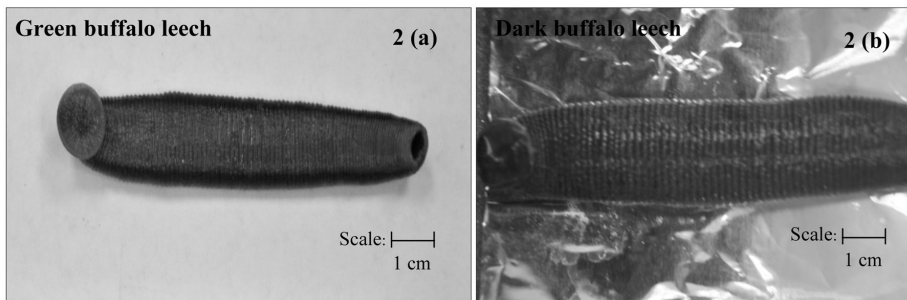


Figure 2(a) and 2(b): Comparison of Body Color and Pattern Onventral Side.

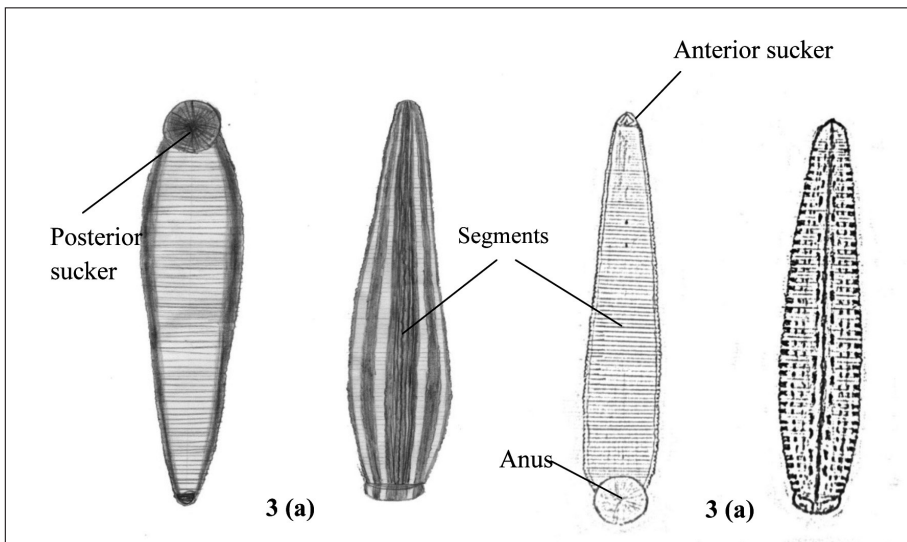


Figure 3(a) and 3(b): Illustration of Pattern on Dorsal Side of Green Leech (3a) and Dark Leech (3b).

Both dark and green buffalo leech had 102 annuli. Annulus found at the middle of the body was larger compared to the annulus at the end of the body. Both species also was observed to

have papillae on the body surface which appear as small protruding stub on the dorsal side of the body. The body surface of both species appear rough from the presence of papillae as

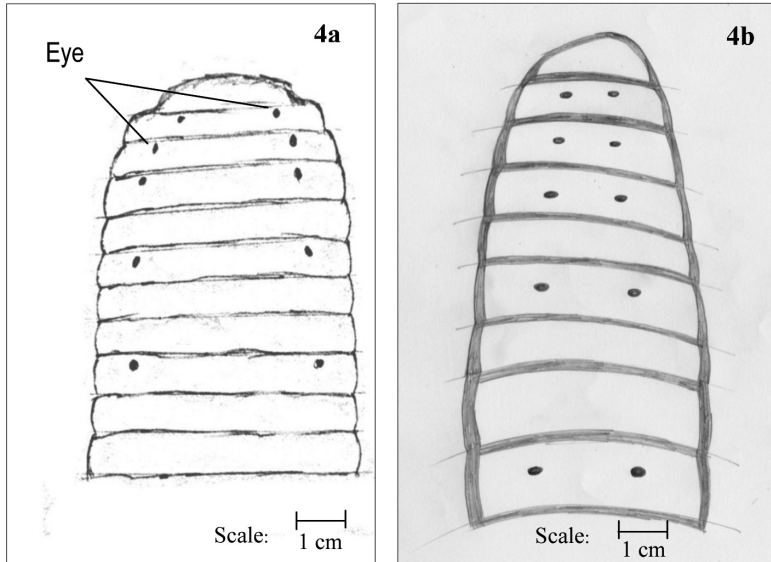


Figure 4a and 4b: Comparison of Eye Arrangement of Green Leech (4a) and Dark Leech (4b).

well. Other similar external feature found was the number of annulations between gonophores. Male and female gonophores of both dark and green leech were separated by five annuli as shown in Figure 5. Male gonopore was located at the 31st or 32nd annulus while the female gonopore was located at the 36th or 37th annulus.

Internal Anatomy

The present study shows that there was no difference between the internal anatomy for both green and dark brown buffalo leech. The internal organs observed were the male reproductive organ, female reproductive organ, testes sacs, crop, caeca and jaw. The structure of the male reproductive organ consists of 3 lobes which were positioned next to the female reproductive organ. The female reproductive organ of the leech was oval, orange in colour and bigger than the male reproductive organ (Figure 6).

Ten pairs of testes sacs were observed along the body (Figure 7). The testes sacs are small and are red swallow bean in structure which was connected to the crop. The crop is a protruding vein-like structure and located at the center of the body (Figure 8), which can only be seen

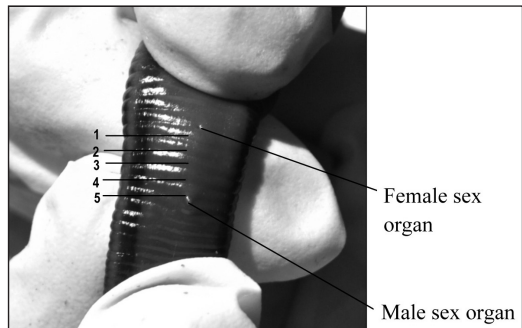


Figure 5: Annulations of Local Leeches.

clearly when the leech consumes blood. The vein like structure is colourless and becomes black in colour when filled with blood. The crop is connected to caeca, located at the end of the crop ending of the posterior sucker (Figure 9).

The jaws of *Hirudinea manillensis* are relatively small, rounded and soft, rough-surfaced teeth, and arranged in a Y-shape (Figure 10). The Y-shape arrangement is sometime referred to the three pointed star. Each jaw normally consists of approximately 100 teeth and papillae and was reported to secrete saliva.

The only anatomical differences between the green and dark buffalo leech was the colour

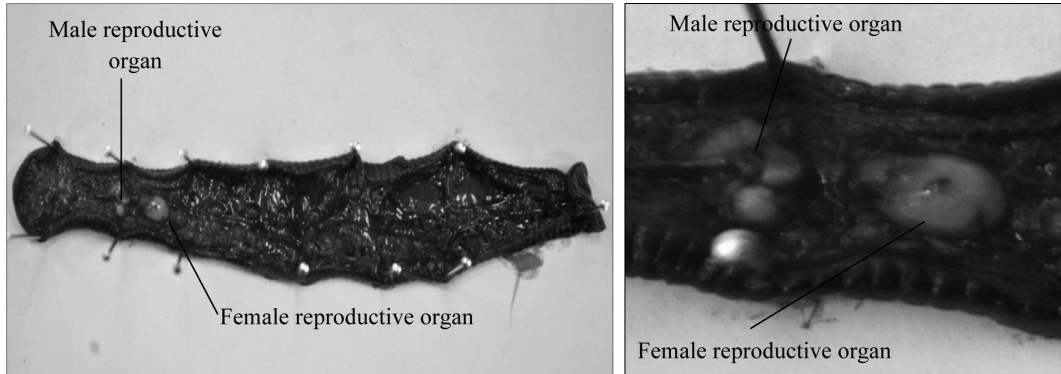


Figure 6: The Male and Female Reproduction Organ of Local Leeches.

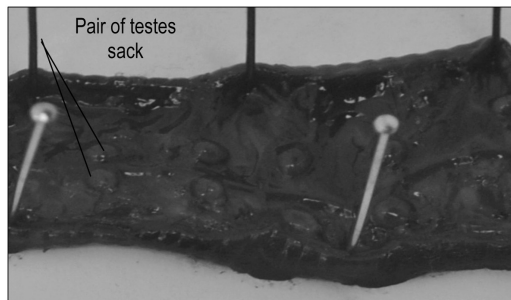


Figure 7: Pair of Testis Sack in Local Leech.



Figure 8: Crop of the Specimen.

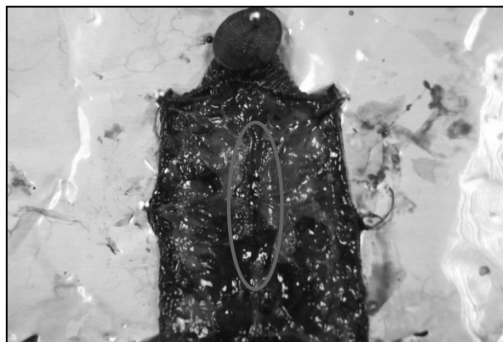


Figure 9: Caeca of the Specimen.

and pattern of the external body structure. The anatomy of the reproductive organs or annulations was not considered a reliable tool to characterise above the species level (Sket, 1968). The use of integrative taxonomy combining modern molecular and chemical data to supplement morphological observation are now becoming the current approach in species delineation (Glaw *et al.*, 2013; Riedel *et al.*,

2013), yet there still exist differences when using this approach for taxonomical study (DeSalle *et al.*, 2005).

Conclusion

The major differences between the green and dark buffalo leech in this study was its external body colour and pattern. Other external anatomy features such as the number of eyes, arrangement of eyes, number of annulations, presence of papillae and number of annulations between male and female gonophores were similar for both green and dark brown leeches. The internal anatomy also shows similarities between the dark and green leech. In the present study, only the external and internal anatomy features were used to differentiate the local leech species, thus a more detailed studies should include both molecular and traditional taxonomic approach to certify differences.

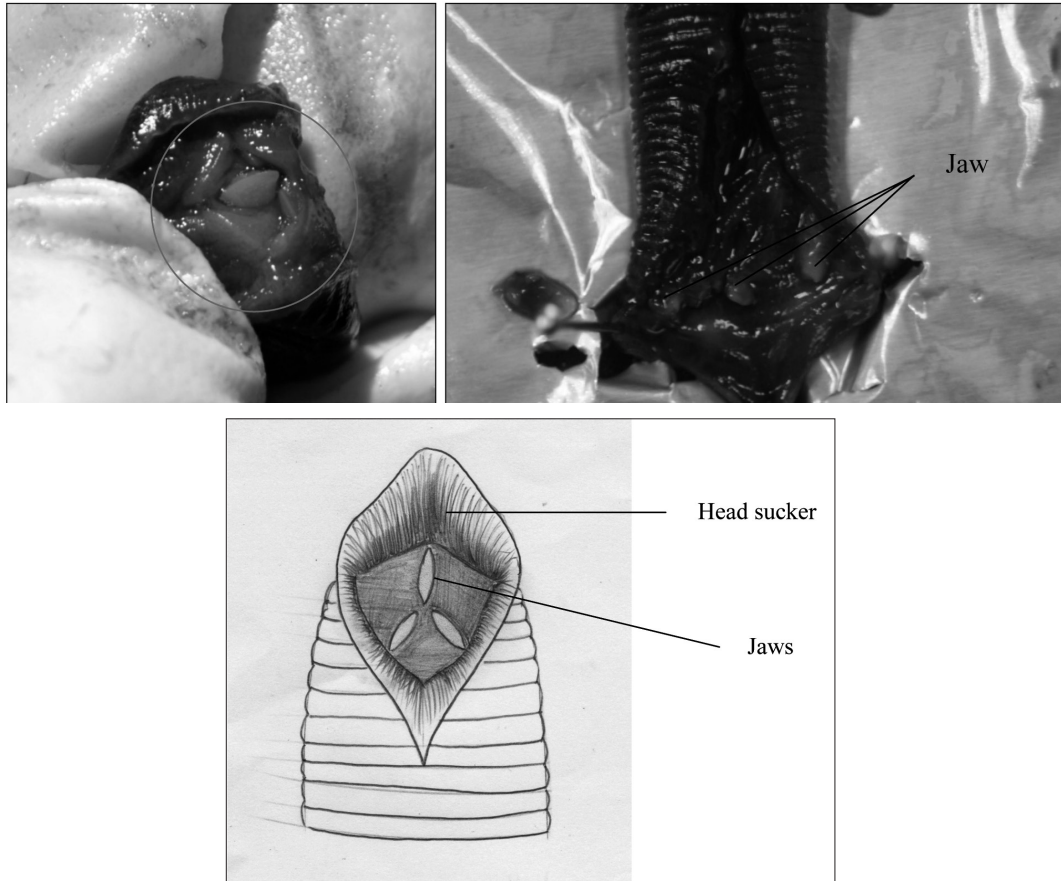


Figure 10: Mouth and Jaw.

Acknowledgement

The author is grateful to the Faculty of Agro Industry and Natural Resources for providing space and facilities to carry out the study. This study was funded by Universiti Malaysia Kelantan and supported by PTDynamic Consultant Co., Kota Bharu, Kelantan.

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