

**TREMATODE (DIGENEA) INFESTATION ON
FRESHWATER SNAILS (MOLLUSCA:
GASTROPODA) COLLECTED OFF RIVERS
OF CROCKER RANGE PARK, SABAH**

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SNAILS (MOLLUSCA: GASTROPODA) COLLECTED OFF RIVERS OF
CROCKER RANGE PARK, SABAH**

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DECLARATION

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29 February 2019



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CERTIFICATION

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GASTROPODA) COLLECTED OFF RIVERS
OF CROCKER RANGE PARK, SABAH**

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ABSTRACT

Freshwater snail is the first intermediate host for trematode parasite that can cause snail-borne diseases in human as well as animals. This study is the first kind that deals with the parasitology of freshwater snail population in rivers around the Crocker Range Park, Sabah. The objectives of this study are to determine the parasite prevalence of freshwater snail species in rivers in this study, to compare parasite prevalence between disturbed and undisturbed rivers, to establish a checklist of trematode parasite infesting freshwater snail and finally to compare freshwater snail diversity between disturbed and undisturbed rivers in this study. This study was conducted in 14 rivers consist of seven disturbed and seven undisturbed rivers and sampling was conducted for seven months from July 2017 to January 2018. Snails were collected from each river and observed for parasite infestation. Parasite prevalence and snail diversity were then compared between disturbed and undisturbed rivers by using Mann-Whitney U test and Sample based rarefaction curve respectively. A total of five morpho-types of trematode parasite, which consist of 11 different species were released from 1709 individuals of freshwater snail belong to 10 species and nine families collected from 14 rivers in this study. The overall parasite prevalence of the freshwater snail species was 4.45%. Unidentified snail species under the family of Hydrobiidae showed the highest parasite prevalence followed by *Paludomus* sp. (Paludomidae) and *Clea* sp. (Pachychilidae), whereas three species of snail which are *Sinotaia guangdungensis* (Viviparidae), *Pomacea* sp. (Ampillaridae) and *Physa acuta* (Physidae) recorded with zero prevalence. The comparison between undisturbed and disturbed rivers for parasite prevalence showed that there was a significant difference ($p < 0.05$) where undisturbed rivers (11.30%) are more prevalent compared to disturbed river (3.66%). All parasites reported in this study considered as new record to Sabah. Freshwater snail species richness and diversity comparison between the two types of rivers in this study showed significant difference where disturbed rivers contain higher diversity and more specious ($0=8$, $1=3.822$, $2=2.881$) compared to undisturbed rivers ($0=3$, $1=2.55$, $2=2.44$). This study has provided useful information regarding freshwater snail parasitology in Malaysia.

Key Words

freshwater snail, disturbed, undisturbed, rivers, parasite infestation, prevalence, diversity, species richness

ABSTRAK

Infestasi Trematode (Digenea) pada Siput Air Tawar (Mollusca: Gastropoda) Dikumpulkan dari Sungai-sungai Terletak di Kawasan Taman Banjaran Crocker, Sabah

*Siput air tawar merupakan perumah pertengahan pertama bagi parasit trematoda yang boleh menyebabkan penyakit jangkitan helmin bawaan siput pada manusia dan juga haiwan. Kajian ini merupakan yang pertama melibatkan jangkitan parasit pada populasi siput air tawar di Sabah, Malaysia. Objektif kajian ini adalah untuk menentukan kelaziman parasit pada siput air tawar di sungai-sungai dalam kajian ini, untuk membandingkan kelaziman parasit diantara sungai terganggu and sungai tidak terganggu, untuk membuat senarai semak parasit trematoda yang menjangkiti siput dan untuk membandingkan nilai kepelbagaian siput pada jenis sungai yang berbeza. Kajian ini dijalankan pada 14 buah sungai yang terdiri daripada tujuh sungai terganggu dan tujuh sungai tidak terganggu dan kajian ini dijalankan pada Julai 2017 sehingga Januari 2018. Siput dikumpulkan dari setiap sungai dan diperiksa untuk serangan parasit. Kelaziman parasit and kepelbagaian siput kemudiannya dibandingkan di antara sungai yang terganggu dan tidak terganggu dengan masing-masing menggunakan Mann-Whitney U test dan Sample-base Rarefaction curve. Sebanyak lima jenis morfologi serkaria, yang terdiri daripada 11 spesis yang berbeza telah didapati dari 1709 individu siput air tawar, dengan 10 spesis siput dari sembilan keluarga telah didapati dari 14 buah sungai yang berlainan dalam kajian ini. Kelaziman parasit secara menyeluruh dalam kajian ini ialah 4.45%. Spesis siput yang tidak dikenalpasti di dawah keluarga Hydrobiidae menunjukkan kelaziman parasit yang tertinggi diikuti dengan *Paludomus* sp. (*Paludomidae*) dan *Clea* sp. (*Pachychilidae*), sementara tiga spesis siput iaitu *Sinotaia guangdagensis* (*Viviparidae*), *Pomacea* sp. (*Ampillaridae*) dan *Physa acuta* (*Physidae*) direkodkan dengan sifar kelaziman. Perbandingan diantara sungai terganggu and tidak terganggu untuk kelaziman parasit oleh Mann-Whitney U test menunjukkan bahawa terdapat perbezaan ketara ($p < 0.05$) dimana kelaziman parasit lebih tinggi di sungai tidak terganggu (11.30%) berbanding di sungai terganggu (3.66). semua parasit yang dicatatkan dalam kajian ini adalah rekod baru di Sabah. Perbandingan kepelbagaian dan kekayaan spesis siput air tawar di antara dua jenis sungai dengan menggunakan Sample-base rarefaction curve dalam kajian ini menunjukkan perbezaan di sungai terganggu mempunyai kepelbagaian yang lebih tinggi dan mempunyai lebih banyak spesis ($0=8$, $1=3.822$, $2=2.881$) berbanding dengan sungai jenis tidak terganggu ($0=3$, $1=2.55$, $2=2.44$). Kajian ini telah memberikan maklumat berguna mengenai parasitologi siput air tawar di Malaysia.*

Kata kunci: siput air tawar, terganggu, tidak terganggu, sungai, infestasi parasit, kelaziman, kepelbagaian, kekayaan spesis

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LIST OF ABBREVIATIONS

BLAST	Basic Local Alignment Tool
BOLD	Barcoding of Life Database
CRP	Crocker Range Park
DNA	Deoxyribonucleic acid
E	East
GI	Geneinfo Identifier
GPS	Global Positioning System
ID	Identification
N	North
NCBI	National Center of Biotechnology Information
No.	Number
PCR	Polymerase Chain Reaction
US	United State
Sg.	<i>Sungai</i> (river)
Sp.	Species
a.s.l	above sea level
ln	Log
p	Proportion
km	Kilometer
m	Meter
mm	Millimeter

LIST OF SYMBOLS

°	Degree
%	Percent
&	and
H'	Index value
H ₀	Null hypothesis
H _A	Alternative hypothesis
μ	Micro
\$	Dollar



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CHAPTER 1

INTRODUCTION

1.1 Introduction

Parasitism is one of the most interesting and medically important relationships between living organisms both in the medical and conservation fields nowadays (Pakarinen, 2011). Parasitism can be defined as the association between two species, the parasite and the host (Dhaliwal & Juyal, 2013) in which, the parasite survive by getting everything it needs from the host while damaging the host (Drisdelle, 2010). Parasitic infestation are ubiquitous in wildlife, livestock and human populations, and healthy ecosystems are often parasite rich (Cable *et al.*, 2017). Yet, their negative impacts can be extreme and human disturbance has been claimed to influence parasite prevalence therefore this need immediate amelioration (Conlan *et al.*, 2011). Thus, this interaction cannot be neglected as parasites are highly pathogenic and economically important (Yizar *et al.*, 2006; Horwitz & Wilcox, 2005).

Trematode parasites are one of the most well-known class of parasite because they are significantly pathogen of high medical, veterinary and environmental importance (Rottu *et al.*, 2014). Trematode is grouped together with cestode under the phylum of Platyhelminthes (Parija *et al.*, 2003). Unlike cestode, trematode parasites are known to generally occur in aquatic habitat (Hasnath & Khan, 2016). The trematodes (or flukes) are leaf shaped with an outer cover called the tegument which may be smooth or spiny (Thomas, 1985). They are internal parasites of molluscs and vertebrates including human (King *et al.*, 2011). The subclass of digenea under the trematode class is the only group known to be zoonotic as they can pose risk to human health (Parija *et al.*, 2003).



Human and animals can be infected with this (digenea) parasite in two main ways. First is by direct penetration and secondly by oral ingestion (Magil, 2013; Belizario *et al.*, 2004). Some species of digenean trematode can directly penetrate the skin of the host and infect the host. Human can easily become infected with this parasite through a simple act just by spending a few minutes in contact with contaminated water that contains parasitic larvae (cercariae) released by infected freshwater snail (Lawton *et al.*, 2015). Human and animals can also be infected by the ingestion of contaminated or improper prepared food (Liat *et al.*, 1978). Although this parasite can infect a massive array of animal groups at each point of its development, they always infect molluscs particularly freshwater snail at the first point of their life cycles (Sandland *et al.*, 2007; Prenter *et al.*, 2004).

Freshwater snail act as an important intermediate hosts for digenetic trematode parasite, which can cause snail-borne helminth diseases in human as well as in animals (King *et al.*, 2011). Freshwater snail is categorized under the phylum of Mollusca and grouped under the class of Gastropod and under the subclass of Pulmonata, together with slug (Tompa *et al.*, 1984). The major difference between these two invertebrates that make up the subclass of Pulmonata is the presence of shell, possessed or present in snails and absent in slugs. The Mollusca is an extraordinarily varied phylum of organisms with estimates of 80,000–100,000 described species (Strong *et al.*, 2007). There are approximately 14 families of freshwater snail that represented in Southeast Asia so far (Medsen & Hung, 2014) and nine families of freshwater snail have been reported in Malaysia by Ng *et al.*, (2017). Most of the snail species under the families reported in the east Malaysia, which is in the state of Sabah are an important intermediate host for trematode parasite that generally dominated the freshwater habitat particularly in river system (Ng *et al.*, 2017).

The river systems in Sabah are important not just for the aquatic organisms such as snail to live in but also, crucial for used in agriculture and for economic resources especially in the rural areas. However, as agricultural activities intensifies and become a major source of income in Sabah (Marsh & Greer 1992), effects can be seen on hydrology and sediment yield. According to Chappell *et al.* (1999), agricultural, logging and ground clearance increases the river sediments by two to fifty times in some rivers in Sabah. Soil erosion can have impact on water quality,

of which can affect humans as users of drinking water (Seve, 1999). Crocker Range Park, Sabah the longest conservation area in Sabah contain the remaining undisturbed rivers that located considerably far from any human activities as it located in the protected area. The river inside the Crocker Range Park is strictly protected from any human activities (Chung *et al.*, 2016). According to Gurvich *et al.* (2005), disturbed habitat is any relative events that disrupt ecosystem, community or population structure and change resources, substrate availability or the physical environment, while undisturbed habitat can be defined as the natural forest without any implementation and exploration of human activities. Rivers in Crocker Range Park can be considered undisturbed since it has also been claimed to have an excellent water quality (Long *et al.*, 2002).

The prevalence of digenetic parasite in freshwater snail is highly influenced by the condition of the river system itself. Human activities and climate change have been claimed by many authors as the major factors in regulating the parasite prevalence among aquatic organisms including freshwater snail (Poulin, 2011). Poulin, (2011) commented parasite prevalence in rivers depends on the level of the pollutant that embedded in the system as well as types of parasite and host. Study conducted by Sri-aroon *et al.*, (2007) and Kulsantiwong *et al.* (2015) also claimed that altered or disturbed rivers which in close contact to the agricultural west and artificial irrigation provide a suitable habitat for snails and probably for parasite as well. Besides that, snail diversity was also proven to influence parasite prevalence in the freshwater snail population (Tomba, 2006; Mohammad, 2015). Yizhar *et al.* (2006) also stated that introduced gastropod highly influences the parasite prevalence in the freshwater ecosystem. Even though, habitat disturbance and snail population has been claimed to play an important role in parasite prevalence of freshwater snail, information regarding parasite prevalence between disturbed and undisturbed rivers as well as snail population particularly in Sabah, has not been investigated and still very limited.

Since the late 19th century, the study of molluscs in Malaysia was often related to the limestone hills which can be found in Peninsular Malaysia as well as in Sabah and Sarawak (Schilthuizen *et al.*, 2003). The studies of molluscs in Sabah are slowly growing, and quite a number of research articles regarding molluscs have been published lately, yet most of the researches conducted were focusing on

land snail (Schilthuisen *et al.*, 2003; Schilthuisen & Rutjes, 2001; Liew *et al.*, 2010). Whereas, two studies conducted on the freshwater snail diversity dynamic in Sabah which was conducted by Supian & Ikhwanuddin (2002) and the latest study was conducted by Ng *et al.* (2017).

However, very little is known about the parasitology of the freshwater snail population in Malaysia especially in Sabah. The only study conducted regarding the parasite infestation of freshwater snail in Sabah was published about 30 years ago and it is the only record of parasite prevalence of freshwater snail in Sabah ever since. The study was conducted by Liat *et al.* (1976), where they conducted a survey of parasite among wild rodent and molluscan host within the vicinity of Tuaran, Sabah. They found that 56 out of 382 molluscs which include land snail, slugs and freshwater snail were naturally infected with rat-lung worm (*Angiostrongylus malaysinensis*). Hospital records during that time which was in 1974 and 1975 were examined and clinical human eosinophilic meningoencephalitis was rarely reported in Sabah. A couple of years after that, in 1978, Liat *et al.* (1978), the same person published another study regarding the freshwater snail consumption and human angiostrongyliasis infection in Peninsular Malaysia. Where Liat *et al.* (1978), claimed that rat-lung worm is the causative agent of human eosinophilic meningoencephalitis and can be transmitted to human via ingestion of the freshwater snail.

Therefore, this study aimed to fill the gaps of knowledge regarding parasitology of molluscs in Malaysia especially in Sabah that is currently almost nothing is known.

This study embarks several objectives such as firstly;

1. To determine parasite prevalence of freshwater snail species in rivers located around the Crocker Range Park, Sabah.
2. To compare parasite prevalence of freshwater snail between disturbed and undisturbed rivers.
3. To establish a checklist of trematode parasite that infests freshwater snails in rivers located around the Crocker Range Park, Sabah.
4. To compare the freshwater snail diversity and abundance between disturbed and undisturbed rivers in this study.

Hence the knowledge of parasitology of molluscs in Sabah is still lagging. There has been a very little attempt to survey the parasite infestation among molluscs group which was conducted three decades ago and the study only focuses on one species of parasite and species of freshwater snail. Ultimately this study will serve as a basis for future research not just in conservation biology but in other related fields as well such as medicine and veterinary science. This may also improve our current understanding of parasitology in freshwater snail in Malaysia especially in Sabah.

1.2 Justification

This research aimed to determine the parasite prevalence status of freshwater snail within the vicinity of Crocker Range Park, Sabah Malaysia since there is paucity regarding parasitology research in relation to the freshwater snail in Malaysia, especially in Sabah. This knowledge is crucial in various fields such as conservation biology, medical and veterinary science. A parasite can be the stabilizer and destabilizer of population size in particular areas and one of the useful environmental health indicators in an ecosystem (Yizhar *et al.*, 2006). In other fields such as veterinary science and medical field, this data is highly important to provide a more realistic information regarding the parasite that infect freshwater snail (Hasnath & Khan, 2016). It is crucial to take note that most trematode parasite that naturally infests freshwater gastropod can infect humans and animals such as liver fluke and intestinal fluke are highly prevalent in Southeast Asia countries (Chai *et al.*, 2015; Hasnath & Khan, 2016). Therefore these organisms cannot be neglected as it is medically important and it also plays a decisive role sustaining the structure and biodiversity in ecosystem (Yizhar *et al.*, 2006).

Other than that, Malaysia also has an extensive river system which could support a high density of freshwater snail and probably for parasite as well. In the states of Sabah and Sarawak specifically, rural people are still highly dependent on resources derived from extensive river systems for their livelihoods (Marsh & Greer 1992). A significant number of rural populations in Malaysia, especially in Sabah and Sarawak, consume freshwater snails which can easily be found in almost every native market around Sabah (Hamli *et al.*, 2013). As well as supporting a great diversity of freshwater snails and the livelihoods of local people, extensive river

systems also serve as a suitable ecological environment for zoonotic parasites. Moreover, some snail-borne helminth diseases have been reported to infect humans in Malaysia, yet the information about the diseases and parasite is still scarce. Thus, the risk of the rural community being infected by snail-borne parasitic diseases transmitted by animals living near river systems cannot be neglected as there are already records of snail-transmitted disease in Sabah and Sarawak (Sagin *et al.*, 2001; Rohela *et al.*, 2005).

Despite the growing number of data regarding gastropod in the past decade, there is only a single study conducted regarding the parasite interaction between parasite and freshwater snail in Sabah which was conducted about three decades ago by Liat *et al.* (1976). Hence, this research will provide very useful information that can help contribute to a more effective method in ecosystem management in the conservation field as well as in diseases management in the veterinary and medical field. Ultimately this study will help fill the gap of knowledge regarding parasite and freshwater snail interaction in Sabah that is currently far from being complete.

CHAPTER 2

LITERATURE REVIEW

2.1 Parasitism

Among all the interesting interaction involving two species of organisms, parasitism receives the greatest attention globally for its medical value and for other variety of reasons. Parasitism is an intimate interaction between two species of either plants or animals in which one live internally or externally and at the expense of the other (Esch & Fernandes, 2013). The most classical explanation that defines this relationship is the implication of this type of lifestyle which implies harm to the host and benefit to the other (parasite). The parasite will usually get the full benefit from the host while the host is harmed (Esch & Fernandes, 2013; Dhaliwal & Juyal, 2013). The parasite is usually smaller than the host and depends fully on the host for shelter and nutrients to survive. They can cause significant diseases problems particularly in developing countries and may lead to death if left untreated (Poulin, 2011).

Parasitologists have been classifying these groups of organism into two major categories based on their interaction with their host. The two major categories of the parasite are endo-parasites and ectoparasites. Ecto-parasite is referred to the parasite that lives outside or on the surface of its host, for example, lice and mites. If the parasite infects the host internally which is inside its host body, then it is known as endo-parasite. Almost all parasites are an obligate parasite, in which they cannot survive without spending at least half of their lifetime fully depending on their host as in a parasitic relationship. While, a facultative parasite is a parasite that does not necessarily require a host to reproduce and survive but can become so by accidentally eaten or direct penetration through open wounds (Robert & Janovy,2005).



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