# ENDOPARASITES OF VARIOUS SPECIES OF ANURANS IN PENANG ISLAND, PENINSULAR MALAYSIA

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by

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## Table of Contents

## LIST OF TABLES

## LIST OF FIGURES

## LIST OF PLATES

## LIST OF ABBREVIATIONS

| | |
|---|---|
| BOD | Biochemical Oxygen Demand |
| cm | centimetre |
| g | gram |
| PALAPES | Pasukan Latihan Pegawai Simpanan |
| SVL | Snout-Vent Length |
| TL | Total Length |
| USM | Universiti Sains Malaysia |

## LIST OF SYMBOLS

| | |
|---|---|
| ♀ | Female |
| ♂ | Male |

**ENDOPARASIT DI DALAM PELBAGAI SPESIES KATAK DI PULAU PINANG, SEMENANJUNG MALAYSIA.**

## ABSTRAK

Haiwan liar termasuk katak anuran sering dijangkiti oleh beberapa spesies parasit. Populasi katak juga sering terancam disebabkan kematian yang secara tiba-tiba kerana perkembangan dan serangan oleh banyak parasit. Katak adalah sangat penting oleh beberapa sebab. Pertama, mereka mengawal populasi serangga dan kedua bertindak sebagai kayu ukur terhadap persekitaran. Katak boleh diinfestasi oleh banyak spesies parasit. Di serata dunia, banyak kajian dijalankan ke atas pelbagai spesies katak tetapi sangat sedikit kajian dilaporkan di Malaysia. Daripada kajian ini, sebanyak 300 ekor katak dari 14 spesies telah dikumpulkan dari lapan kawasan kajian: *Duttaphrynus melanostictus*, *Phrynoidis aspera, Hylarana erythraea, H. labialis, H. nigrovittata, Polypedates leucomystax, Fejervarya cancrivora, F. limnocharis, Limnonectes blythii, L. paramacrodon, L. ibanorum, L. ingeri, Microhyla butleri* dan *Kaloula pulchra*. Terdapat 11 species cacing parasit telah di rekod iaitu *Heterakis* sp., *H. vesicularis, Trichostrongylus* sp., *Pharyngodon* sp., *Ascaris* sp., *Oswaldocruzia* sp., *Rhabdias* sp., *Glypthelmins staffordi, Diplodiscus sacculosus, Manodistomum* sp., dan *Macracanthorynchus* sp.), lapan parasit darah (ricketsia*, Hepatozoon* sp., *Haemogregarina* sp., *Lankesterella* sp., *Trypanosoma loricatum, T. rotatorium, T. chattoni,* dan microfilaria*)* dan satu protozoa (*Nyctotherus* spp.). Terdapat perkaitan di antara parasit katak dengan kadar hujan, dan sama juga untuk parasit darah dengan Biochemical Oxygen Demand (BOD) di air.

Kata kunci: anuran, katak, parasit, trematoda, acanthocephala, nematoda, protozoa, ricketsia

**ENDOPARASITES OF VARIOUS SPECIES OF ANURANS IN PENANG ISLAND, PENINSULAR MALAYSIA.**

## ABSTRACT

Wild animals, including anuran are usually infected with several species of parasites. The population is often threatened by massive deaths among other things because of the dispersal and territorialism of most parasites species. Frogs are important for a variety of reasons. Firstly, they control populations of insects and secondly they can act as indicator species to the environment. Studies have shown that anurans can harbor many species of parasites. Worldwide, there are numerous researches carried out on anuran parasites but few represented from Malaysia. From this study, 300 anurans from 14 species were collected from eight study sites: *Duttaphrynus melanostictus*, *Phrynoidis aspera, Hylarana erythraea, H. labialis, H. nigrovittata, Polypedates leucomystax, Fejervarya cancrivora, F. limnocharis, Limnonectes blythii, L. paramacrodon, L. ibanorum, L. ingeri, Microhyla butleri,* and *Kaloula pulchra*. About 11 helminths (*Heterakis* sp., *H. vesicularis, Trichostrongylus* sp., *Pharyngodon* sp., *Ascaris* sp., *Oswaldocruzia* sp., *Rhabdias* sp., *Glypthelmins staffordi, Diplodiscus sacculosus, Manodistomum* sp., and *Macracanthorynchus* sp.), eight blood parasites (a rickettsia*, Hepatozoon* sp., *Haemogregarina* sp., *Lankesterella* sp., *Trypanosoma loricatum, T. rotatorium, T. chattoni,* and microfilaria*)* and one protozoa (*Nyctotherus* spp.) were recorded from frogs. There were correlations between the present helminth parasites and distribution of rainfall, and similarity between blood parasites and Biochemical Oxygen Demand (BOD) of water.

Key words: anuran, frog, parasites, trematodes, acanthocephala, nematodes, protozoa, rickettsia

# 1.0 INTRODUCTION

Over the last few decades, it was realized that numerous species of amphibians have suffered vast declines in many regions of the world. This is mainly due to exposure infectious diseases and pollution of the amphibian environment.

Frogs and toads belong to the class of Amphibian, which is known to be the most diverse group of vertebrates. Most of frog species are found in tropical rainforests, although their range of distribution are from tropic to sub-arctic regions. Taxonomists have described more than 5,000 species of frogs (Duellman and Trueb, 1994). Disease has been a factor in the decline of amphibian populations worldwide, although other factors including habitat loss and fragmentation, chemical pollution, climate changes, introduction of exotic species, increased ultraviolet radiation, and natural pollution have also been responsible for the decline (Hayes et al., 2010).

Malaysia is a country with an equatorial climate with high annual humidities ranging from 60% to 90% and rainfalls of 2000 to 3000 mm, resulting with a rich diverse biodiversity of wildlife (Department of Meteorology Malaysia, 2011). Malaysia harbours about 165 species from six families of anurans of which more than 150 species are found in Borneo (Inger and Stuebing, 2005), 107 species in Peninsula, including 26 species in Penang (Ibrahim et al., 2008).

Frogs are important for the environment and ecosystem. For example, frogs play an important role in a food web as reported by Ibrahim et al., (1997), where two species of frogs (*Fejervarya limnocharis* and *F. cancrivora*) were shown to control the population of green plant hopper (*Nephotettix* spp.) and leaf hoppers (*Nilaparvata lugens*), as well as insect pests in paddy lands. On the other hand,

tadpoles also play an important role, where in general, tadpoles are commonly found in environments like puddles, streams, ponds and roadside ditches, where they clean their food by scraping off the substrates or feeding themselves on particles suspended in the water (Duellman and Trueb, 1986). Tadpoles maintain their waterways clean by feeding on algae while adult frogs eat large quantities of insects, which include disease vectors that can transmit fatal illnesses to humans (Kerry, 2011).

Frogs are also known as bio-indicators to the environment. Most frogs need suitable habitats in both terrestrial and aquatic environments. Its permeable skin can easily absorb toxic chemicals from the environment. Frogs are indicators of environmental disturbances. For example, people consume various types of medicines, particularly those containing progestogen hormone and finally released it into the environment through sewage system. Progestogens are hormone preparations used in contraceptives, cancer treatments and hormone replacement therapy for menopausal discomfort. Frogs are known to be sensitive to progestogens. Female tadpoles that swim in water containing a specific progestogen, levonorgestrel, are subject to abnormal ovarian and oviduct development, resulting in adult sterility (Kvarnryd et al., 2011).

Frogs are important in research and medicines. Skins of frogs can produce a variety of secretions which are beneficial in pharmaceuticals. For example, scientists have discovered proteins in frog skins could be used to treat cancer, diabetes, stroke, and transplant patients by regulating the growth of blood vessels. In addition, a lot of Nobel Prizes in the field of physiology and medicine used frogs in their research. Frogs are also used as model specimens for students in the field of biology and anatomy.

As reported by Duellman and Trueb (1986), there are two main threats to amphibians resulting from habitat destruction and environmental pollution and concurred in Malaysia by Ibrahim et al., (1997). Firstly, rapid developments for the last fifteen years have led to changes of breeding habitats for survival of amphibians. Negative outcomes from forest logging, draining swamps, covering streams of land developments, damming of rivers and draining of irrigation, introduction of weeds and livestock are the main causes that witnessed the inevitable decrease of amphibian habitats such as swamps, natural waterways, wetlands, forests, rural areas and agricultural lands (Tyler et al., 2007). Secondly is parasitic infection, which is one of the uprising concerns in the history of frogs.

The first aimed of this study is to identify various species of anurans found in Penang Island, Peninsular Malaysia. The second, current knowledge on endoparasites of frog in Penang Island is lacking. Therefore, special attention is needed in survey on endoparasites of frog. Thirdly, environmental factors such as rainfall distribution, pH, Biochemical Oxygen Demand (BOD) and temperature of water were correlated to anuran population and parasite prevalence.

# 2.0 LITERATURE REVIEW

## 2.1 Biology of Frogs

Frogs and toads have distinctive characteristics that make them easily recognizable. They have no tail as it was gradually absorbed during metamorphosis. Besides, they have warty skin, short figure with stocky body shape, long hind legs with short front ones, large eyes and a very wide mouth. The hind legs of frog are adapted for swimming and jumping. It was created with a very special extra joint, for providing extra power for jumping leap. In addition, frogs and toads have short backbones, which can help them keep their eyes face straight during launching and landing. Adult frogs and toads are carnivorous. They eat mainly insects and small animals and have short digestive tracts. However, Inger and Stuebing (2005) reported that in the Indian subcontinent there is one species of frog known to feed on aquatic plants. Frogs and toads have a broad fleshy tongue attached at the front of the lower jaw. They catch their preys by the sticky tips of the long tongue and grip the food by using their small teeth (Nichikawa and Roth, 1991). Frogs and toads have teeth but do not use them to chew food.

Frogs and toads spend the early part of their lives in freshwater as they are freshwater animals. They lay their eggs in freshwater rather than saltwater (Davenport, 2011). However there is a species known as crab-eating frog, *Fejervarya cancrivora* which is found in Borneo that can tolerate saltwater and can be found swimming in the sea at Pulau Tiga, Sabah (Inger and Stuebing, 2005). As reported by Elliot and Karunakaran (1974), *F. cancrivora* can live in salinities <18 for long periods, while its tadpoles have been found in a ditches with water salinities

<35. Another study found that the African clawed frog, *Xenopus laevis* can tolerate salinities of 12-15 after it is introduced into estuarine habitats for purposes of medical trading (Lafertty and Page, 1997). In temperate zones, most frogs hibernate in mud or in tree holes whereas, in springs or breeding seasons, the females are known to lay thousands of eggs. Furthermore, during the summer season, frogs will undergo metamorphosis (Christiansen and Bailey, 1991).

Male fertilizes eggs of frogs immediately as they are being excreted by female. The eggs are covered with jelly-like substances that serve as a protective coating. Tadpoles hatch out after several days to several weeks depending on species, water temperature and amount of sunlight exposure (Banks and Beebee, 1988). Adult females lay as many as a thousand eggs at one time, so that there are always many tadpoles alive and fighting for survival in the possible presence of many predators.

A study by Bolek et al., (2009), reported that *Gorgoderina attenuata* and *Phylodistomum* sp. use tadpoles, arthropods, odonatas, molluscs and other frogs as second intermediate hosts. Another study reported that *Bufo melanostictus* (currently *Duttaphrynus melanostictus*) and *Rana limnocharis* (currently *Fejervarya limnocharis*) serve as intermediate hosts of a species of Pentastomida, *Kiricephalus pattoni*. The nymphs are found in the anuran hosts, while the adults occur in the lungs and respiratory passageways of snakes (Bursey and Goldberg, 2004). At these forms, frogs serve as paratenic hosts (Bursey et al., 2008a). For example, tadpoles and frogs act as paratenic host for life cycle of nematode, *Dracunculus insignis*. Typically the life cycle has two intermediate hosts, the first being a copepod where the larvae form is in third-stage larvae (L3) of *D. insignis.* The second is a frog,

where the *D. insignis* larvae continued through the metamorphosis of the tadpoles into adult frogs. This is the way where the paratenic host act in transporting infective larvae to the definitive hosts, mammalia carnivores (Eberhard and Brandt, 1995).

From the viewpoint of parasitology, tadpoles are noteworthy subject to study as they have abiotic associations between aquatic and terrestrial ecosystems. Besides, it also transmits the larvae of digenetic trematodes to terrestrial animals (Combes et al., 2002). Another study found that instead of terrestrial snakes, tadpoles and adult of *Rana nigromaculata* are also known as the second intermediate hosts for *Fibricola seoulensis* (Hong et al., 1985). Also, some metacercariae may cause limb deformities and deaths in tadpoles and adult amphibian populations as well (Johnson et al., 2004).

Sexing frogs can be difficult as it depends on species. Male frogs are usually smaller and smoother in appearance, unlike females, which are often larger and tougher. Males are noticeably smaller than females for most species of frog. A typical male frog is one and one half to two and one-quarter inches long from head to bottom (Oplinger, 1996). Females are two to three inches long and have the visible cloaca for *Xenopus laevis* (Anonymous, 2006). Besides, the other indicator that can distinguish males and female is the ear, which is located near the brain. Big ears belong to males and small ears to females. Oplinger, (1996) found that males of bullfrogs and green frogs in Minnesota have larger eardrums than females.

High infections of parasites regularly occur in mature male vertebrate hosts compared to females (Zuk and Mckean, 1996). This ratio is based on several factors, such as host establishment and breeding season of the vertebrate. Poulin (1996) reported that parasites may grow and establish well in male hosts rather than in

females. It is therefore used in meta-analysis to compare rates of growth of helminths in male and female hosts. During the period from 1956 to 1960, a large number of frogs (*Rana temporaria*) have been examined for helminths. Intensity of parasites found in male frogs is significantly higher when compared to females. The helminth parasites found were *Polystoma integerrimum, Gorgoderina vitelliloba*, *Rhabdias bufonis*, and *Acanthocephalus ranae* (Lees and Bass, 1960).

The skin of frogs is highly permeable. The irregular ventral surfaces provide a greater surface area in contact with the substrate, which ultimately result in greater rates of water absorption (Duellman and Trueb, 1986). Since the skin of frogs can easily absorb toxic chemicals and detect any environment pollution, these circumstances have made frogs to be environmental indicators. In addition, frogs also live in terrestrial and aquatic environments and can act as accurate indicators of environmental disturbance (Kerry, 2011). In order to conserve the species in the future, continuous research must be carried out. Besides, the permeable skin can harbour various species of parasites, which mean penetrations of free-living larvae to the skin of frogs as an example is *R. ranae,* a common lungworm of North American ranids (Baker, 1979a citation in Gendron et al., 2003).

Apart from its integral part of the food web and environmental indicator, frogs are also used in medical research especially in the pharmaceutical field. For example, the white tree frog (*Litoria caerulea*) secretes a chemical called a caerin that block Human Immunodeficiency Virus (HIV) transmission (Kerry, 2011). This finding is a good contrivance for future generations if we can discover more benefits of amphibians from now. A few studies on amphibians has been done in Malaysia including inventories and guides (Ibrahim et al., 1997), population ecology and

density and abundance of anurans, life history, feeding biology, reproduction biology and amphibian conservation (Inger et al., 2009). However these studies on amphibians are still superficial and insufficient. Also, studies on frog parasites are few.

Frogs have also economic importance in human society. Certain species of anurans are sold in the market. For example in India, hind legs of frogs are hunted extensively for protein source. Besides, frogs *Xenopus laevis* were also used in pregnancy tests during early 1950's, whereby urine from pregnant women can be used to stimulate spawning in frogs (Gurdon and Hopwood, 2000). Frogs are cultivated for leather. In Japan, France, and the United States, frog skins are tanned and made into fine soft leather (Thy and Eastoe, 2010). In many places in United States, France, Japan and Malaysia, there are large farms where frogs are raised for market. The species of *Rana catesbeiana* is the largest American species that is cultivated in the United State. In Malaysia, farmers use to cultivate frogs in many small pools while in the Philippines, frogs are cultivated in swamps, marshes or in rice paddies.

Amphibians harbour many kinds of parasites. This is because amphibians, particularly frogs and toads live in aquatic ecosystems, thus making them exposed to a large variety of parasites (Barta and Desser, 1984). Besides, the behaviour and food intake by frogs and toads also contributed to differences in helminth fauna they're harbouring (Holmes et al., 2008).

## 2.2 Species of Frogs

Some species of frogs existing in Penang Island are *Duttaphrynus melanostictus, Phrynoidis aspera*, *Hylarana erythraea, H. labialis*, *H. nigrovittata*, *Polypedates leucomystax*, *Fejervarya cancrivora, F. limnocharis, Limnonectes blythii, Limnonectes paramacrodon*, *L. ibanorum*, *L. ingeri, Microhyla butleri* and *Kaloula pulchra* (Inger and Stuebing, 2005).

*Duttaphrynus melanostictus* (Common Sunda Toad) occurs in many areas and increasing its numbers with time because it is a species easily adapted to its environmental habitat. In addition, this species is not commonly found in closed forests. The larvae are always found in the breeding sites. While the adults are found on terrestrial or under ground cover like rocks, logs and leaf-litter (Inger and Stuebing, 2005).

*Phrynoidis aspera* (River Toad) is easy to recognize as it is large of size with warty and rough skin. This species inhabit primary and old secondary forests but usually they remain on rocks along stream banks (Inger and Stuebing, 2005).

*Hylarana erythraea* (Green Paddy Frog) is bright to dark green and white colour under its belly. *Hylarana erythraea* lives in disturbed freshwater habitats like rice fields, irrigation ditches and lakes with eutrophication. They are commonly found perching on grasses and on pond banks (Inger and Stuebing, 2005).

*Hylarana labialis* (White-lipped Frog) is a small green frog with white upper lip, sometimes in pale yellow colour at the back. Earlier it was known as *Hylarana raniceps*, but redescribed as *H. labialis* by Inger et al. (2009). This species inhabit

disturbed forests and are usually found perching on small trees at the edges of ponds (Inger and Stuebing, 2005).

*Hylarana nigrovittata* (Black-striped Frog) has a smooth skin surface with brown colour back with dark broad stripes are on each side. The species inhabit small river banks and artificial pools (Inger and Stuebing, 2005).

*Polypedates leucomystax* (Four-lined Tree Frog) is well-known in disturbed habitats which are close to human activities. The colour of its skin is almost light to mid brown. Besides, the species has distinctive four narrow stripes at the back and some has scattered brown spots on their body (Inger and Stuebing, 2005).

*Fejervarya cancrivora* (Crab-eating Frog) has an oval body and it is easy to recognise from dark marking spots on its body in the form of the letter 'W'. The body colour of *F. cancrivora* is grey to brown, and whitish colour on its underside. The frog lives in disturbed habitats especially in swampy areas and semi brackish waters (Inger and Stuebing, 2005).

*Fejervarya limnocharis* (Grass Frog) occur in disturbed habitats which are near to human activities such as agriculture. The shape of the body is oval and has a streak down the middle of the back. The colour of streak varies from light brown to light orange and olive green. While the underside of females are totally white, whereas in male, it has a black band in the shape of the letter 'M' across its throat.

*Limnonectes blythii* (Blyth's River Frog) is common on the ground along the rivers and stream. The colour of its back is brownish, grey, but some are yellow. They are with or without a vertebral stripe on its back. Whereas the underside colour of the species is white to yellowish (Inger and Stuebing, 2005).

*Limnonectes paramacrodon* (Lesser Swamp Frog) inhabits swampy and muddy areas along river banks in primary or disturbed habitats. The basic colour of its dorsal is greyish to reddish brown. It also has a distinctive longer first finger than the second one, while the fourth is longer than the second (Inger and Stuebing, 2005).

*Limnonectes ibanorum* (Rough-backed River Frog) is of large size with a tough body with a long pointed snout. The colour of the back is greyish to brackish brown while, the ventral surface is white. This species lives in primary and secondary forests and always occur in the river backs of streams which preference to broad, clear stream with rocky bottom (Inger and Stuebing, 2005).

*Limnonectes ingeri* (Greater Swamp Frog) is similar to *L. leporinus* but the snout is rounded. The dorsal colour is reddish to dark brown while the ventral body is greyish white with misty mottling. Besides, the chin and throat are greyish brown colour and greatly mottled. The species inhabit lowland primary and secondary forests which can be found along muddy streams and swampy areas (Inger and Stuebing, 2005).

*Microhyla butleri* (Butler's Rice Frog) lives in disturbed areas and are always found on the ground or along a small stagnant ponds. The skin is smooth and the tympanum is unseen. The colour of the back is usually brownish and has a symmetrical wavy marking along its body (Inger and Stuebing, 2005).

*Kaloula pulchra* (Banded Bullfrog) is abundantly found in towns, cities, and villages area. They always go out during the nights after rain. The skin is tubular with no folds and the throats of males are roughly granular. The back colour of the

species is dark brown and there is a dark horizontal line from the rear of eye to the groin (Inger and Stuebing, 2005).

### 2.3 Endoparasites of frogs

#### 2.3.1 Blood protozoan.

Many hematological studies have been done on various species of *Rana*, especially on blood cell counts, measurements and hematological parameters examination such as the volume and the hematocrit value (Prosser and Weinstein, 1950 citation in Omomona and Ekpenko, 2011). Frogs are confronted with different kinds of threats especially disease and health problems, which are generally caused by parasites. Achariya et al., (2011) reported there are several researches in different geographical localities that reported that anurans harbour various kinds of blood pathogens comprising of viruses, rickettsiae, protozoans, and microfilariae. *Trypanosoma* and hemogregarine are common blood parasites of amphibians. *Trypanosoma* are transmitted by blood-sucking leeches while hemogregarines are transmitted by blood-sucking insects (Duellman and Trueb, 1986).

Zickus (2002) reported that most trypanosomes of amphibians have been found in species of *Rana* (Anura: Ranidae) and *Bufo* (Anura: Bufonidae) in Europe, the Americas, Africa, and Asia. Trypanosome is one of blood protozoans that parasitize anurans. Desser (2001) reported that, there are more than 70 species of trypanosomes harbored in anurans, even though the validity of several species is questioned. It is because some of trypanosomes may have different morphology depending on their mature phase. Thus, identification of species is difficult. Some papers reported that trypanosomes of a particular species of vertebrate host were

identified primarily on the basis of parasite restriction. Trypanosomes of anurans are polymorphic (Bardsley and Harman, 1973; Ray, 1983). A misperception of the taxonomy of anuran trypanosomes happen where *Trypanosoma rotatorium* is seen as a polymorphic species with a wide geographical distribution, while *Trypanosoma chattoni* is a monomorphic species discovered in Asia, Europe, and America (Martin et al., 2002; Achariya et al., 2011). Insufficient study of polymorphism stages of anuran trypanosomes may due to two possibilities, which is the variation in morphology in different host's species or the influence of different abiotic factors on parasite development in a host (Bardsley and Harman, 1973). Podlipaev (1990) described species of *Trypanosoma* were recorded in the amphibians that occur in Lithuania: *T. belli*, *T. inopinatum*, *T. elegans*, *T. rotatorium*, *T. hylae*, *T. costatum*, and *T. ranarum*.

Miyata and Yong (1990) made a finding in Malaysia discovering a *Trypanosoma hosei* in forest frog, *Rana Hosei*. However, malaria parasites (*Plasmodium*) were not described in amphibians, even though the vector, *Anopheles* mosquitoes were observed as biting anurans (Duellman and Trueb, 1986). Likewise, *Hepatozoon* spp. (Apicomplexa: Hepatozoidae) and *Opalina ranarum* are considered as the most common protozoa parasites in amphibians as well (Achariya et al., 2011).

Desser (1993), citation in Zickus, 2002 stated that species of *Haemogregarina*, *Hepatozoon*, *Lankesterella*, and *Schellackia* were found in blood of amphibians. Some studies have been done since the last few decades and found that *Rana boyli* was infected by *Karyolysus sonomae*, *Haemogregarina aurora*, and

*T. boyli* (Lehmann, 1959a, b, c). Besides that, *H. aurora* was also reported in *Rana aurora* (Lehmann, 1960). While in *Rana pretiosa*, the occurrence of species of *Lankesterella* sp. and *Trypanosoma* sp. were reported by Stenberg and Bowerman (2008). In addition, Zickus (2002) detected *T. rotatorium* in *Rana esculenta-lessonae*, *R. temporaria*, *Bufo bufo*, and *B. viridis.* There are two types of haemogregarines found in the blood of frogs, either intracorpuscular and extracellular.

*Hepatozoon* spp. (Apicomplexa: Hepatozoidae) is often found in amphibians, appearing as large banana-shaped organisms in the cytoplasm of host erythrocytes. *Hepatozoon* species possess heteroxenous life cycles, with sexual reproduction and sporogony occurring in an arthropod definitive host. Transmission occurs when such an arthropod, infected with mature oocysts, often containing thousands of sporozoites then migrate to the visceral organs, primarily to the liver, and undergo merogony. The meronts are released into the bloodstream where they form gamonts in erythrocytes (Achariya et al., 2011).

*Lankesterella* spp. (Apicomplexa: Lankesterellidae) is recognized as parasites of frogs and some other ectotherms. Merogony and sporogony occur in vascular endothelial cells in the visceral organs of the vertebrate hosts. Mature sporozoites are released into the bloodstream and invade erythrocytes. Intra-erythrocytic sporozoites are taken up by vector (leeches and mosquitoes) during feeding and it is believed that frogs become infected upon the digestion of sporozoite-bearing vectors (Desser, 1993 citatation in Achariya et al., 2011).

However, histological findings showed that frogs, which are infected with *T. rotatoriun* and *T. chattoni* do not show any inflammatory lesion like frogs infected with *Hepatozoon* sp. and *Lankesterella minima*, where the liver and the spleen of the frogs showed an accumulation of melanomacrophage centres (MMCs) around meronts and merozoites, also lessions occur in the visceral organs (Achariya et al., 2011). In addition, there are seventeen species of *Hepatozoon* described from the genus *Rana* (Smith et al., 2000).

Table 2.1: List of blood pathogens found in anurans and classification on type of host, organ, country and references.

| Species of blood pathogens | Amphibian species | Type of host | Organs | Country | References |
|---|---|---|---|---|---|
| *Trypanosoma hosei* | *Rana hosei* | Definitive host | Blood | Malaysia | Miyata and Yong, 1990 |
| *T. rotatorium* | *R. esculenta-lessonae, R. temporaria, Bufo bufo, B. viridis* | Definitive host | Blood | Lithuania | Zickus, 2002 |
| *Haemogregerina aurora* | *R. boyli, R. aurora* | Definitive host | Blood | North America, California | Lehmann, 1959a, b, c; Lehmann, 1960. |
| *Karyolysus sonomae* | *R. boyli* | Definitive host | Blood | Benton Counties, Oregon | |
| *T. boyli* | *R. boyli* | Definitive host | Blood | | |

### 2.3.2 Nematodes

Nematodes are important parasites in the gastrointestinal tract, lungs and blood vessels of anurans. Examples of anuran nematodes are *Rhabdias ranae*, *Cammalanus* sp. and *Oswaldocruzia* sp. Nematode has a direct life cycle compared to other helminths like digenetic trematodes and cestodes. Frogs become infected as they come into contact with infective larvae of the parasite living in the soil. At this stage, the free-living larvae penetrate the frog's skin, then move to the lungs, and reach their maturity (Baker, 1979b citation in Gendron et al., 2003).

Table 2.2: List of nematodes found in anurans and classification on type of host, organ, country and references.

| **Species of nematode** | **Amphibian species** | **Type of host** | **Organs** | **Country** | **References** |
|---|---|---|---|---|---|
| *Foleyella* sp. | *Rana catesbeiana* | Primary host | Blood | New Jersey | Crans, (1969) |
| *Cosmocercoides dukei* | *R. septentrionalis* | Primary host | Intestine | Minnesota, USA | Schotthoefer et al., (2009) |
| *Rhabdias sphaerocephala* *R. fulleborni* | *Bufo marinus* | Definitive host | Lung | Central America | Kuzmin et al., (2007) |
| *R. kuzmini* | *B. occidentalis* | Definitive host | Lung | Mexico | Salazer and Regagon, (2007) |
| *Africana telfordi* *Aplectana hylambatis* | *R. forreri* | Definitive host | Intestine | Costa Rica | Bursey and Goldberg, (2005) |

| | | | | | |
|---|---|---|---|---|---|
| *Aplectana inserta*<br>*Aplectana itzocarensis*<br>*Cosmocercoides podicipiny*<br>*Foleyellidei striatus*<br>*Subulascaris falcaustriformis*<br>*Oswaldocruzia costaricensis*<br>*Rhabdias savagei*<br>*Brevimulticaecum* sp. | *Rana forreri* | Definitive host | Intestine | Costa Rica | Bursey and Goldberg, (2005). |
| *Aplectana tarija*<br>*Cosmocercoides lilloi* | *Chaunus arenarum* | Definitive host | Intestine | Argentina | Ramello et al., (2007). |
| *Falcaustra lowei*<br>*F. inglusi*<br>*Foleyellidei striatus*<br>*O. pipieny*<br>*R. ranae*<br>*S. falcaustriformis* | *R. tarahumarae* | Definitive host | Intestine | Sonora, Mexico | Bursey and Goldberg, (2001). |
| *R. ranae*<br>*R. joaquinensis*<br>*R. bakeri* | Ranidae and Hylidae<br>*R. sylvatica* | Primary host<br>Definitive host | Lung | Neartic region.<br>North Dakota. | Tkach et al., (2006). |
| *Parapharyngodon garciae*<br>*Poekilostrongylus puertoricensis* | *Eleutherodactylus coqui* and *leptodactylid frogs*<br>*Eleutherodactylus coqui* | Primary host | Intestine | Puerto Rican | Dyer et al., (1995). |

| | | | | | |
|---|---|---|---|---|---|
| *Parathelandros allisoni* | *Nyctimystes trachydermis* | Primary host | Intestine | Papua New Guinea | Bursey et al., (2008b). |
| *Okinawandros goldbergi* *Ataronama sekii* | Rhacophord frogs | Primary host | Intestine | The Ryukyu Archipelago, Japan. | Hasegawa, (2005). |
| *Icosiella turgeocauda* | *Fejervarya cancrivora* | Primary host | Intestine | Luzon, Republic of Philippines. | Bursey et al., (2003). |

As reported by Crans, (1969), a preliminary observation of frog filariasis in New Jersey found that *Rana catesbeiana* was heavily infested with filarial worms of the genus *Foleyella* sp., Seurat 1917. The mosquitos, *Culex territans* known as a natural vector of the parasite, transmit the parasite through circulatory blood of the New Jersey bullfrogs.

In term of host-specificity, nematode is the least host specific among the helminth parasites. For example, cosmocercid nematode, *Cosmocerca brasiliensis* is recognised as a parasite to 15 species of South American anurans (Dyer and Altig, 1976), whereas *Cosmocercoides dukei* a species documented from *Rana septentrionalis* (Schotthoefer et al., 2009) is distinguished from 14 salamander hosts in North America (Dyer and Brandon, 1973). Species of *Rhabdias* nematodes are widely known parasites in lungs of amphibians and reptiles. This genus comprises of more than 40 nominal species, and about 15 species have been reported from Bufonidae. Out of this, only 11 of them seem to be host specific to the anuran family. *Rhabdias sphaerocephala* and *R. fulleborni* are recorded as parasites of *Bufo marinus* in Central America (Kuzmin et al., 2007).

Up to now, there are about 59 *Rhabdias* species, which have been described. Salazar and Regagnon (2007) reported that *Rhabdias kuzmini* is the 16th species that was discovered from an endemic anuran *Bufo occidentalis* in Mexico. The species differs from others in the genus by the possession of four lips, two subdorsal and two subventral, not inflated corpus, a larger barrel-shaped buccal capsul, equatorial vulva, and the presence of a slightly swollen cuticle in the anterior and posterior ends of the body. In Mexico, about ten species of this genus were recorded occurring in the lung of amphibians and reptile: *Rhabdias americanus, R. elegans, R. fuelleborni, R. fuscovenosa, R. lamothei, R. leonae, R. ranae, R. savagei, R. sphaerocephala* and *R. tobagoensis* (Salazar and Regagnon, 2007).

The Costa Rica frog, *Rana forreri* harbors many species of nematodes: *Aplectana incerta*, *A. itzocanensis*, *Cosmocerca podicipinus*, *Foleyellides striatus*, *Subulascaris falcaustriformis*, *Oswaldocruzia costaricensis*, *Rhabdias savagei* and larva of the nematode *Brevimulticaecum*. From the list, *O. costaricensis* represents the 77th species and differ from the other species in the genus by possessing a Type II bursa and long cervical alae. While *R. savagei* represents the 47th species and differs from others species in the genus by possession of four lips and a postequatorial vulva (Bursey and Goldberg, 2005).

Another study was done in Argentina as reported by Ramallo et al., (2007) who stated that two new species of Cosmocercid helminths, which are *Aplectana tarija* and *Cosmocercoides lilloi* were described from the intestines of the toad *Chaunus arenarum*. Both species are intestinal parasites of reptiles and amphibians. Species of *A. tarija* represents the 22nd species out of 41 species and the second reported from Argentina frogs. While *C. lilloi* is represents the 15th species of the 14 species and the first reported from South America.

As reported by Bursey and Goldberg (2001), various species of helminths parasitize the Tarahumara frog, *Rana tarahumarae* from Sonora, Mexico. They consist of seven species of nematodes: *Falcaustra lowei*, *F. inglisi*, *Foleyellides striatus*, *Oswaldocruzia pipiens*, *Rhabdias ranae*, *Subulascaris falcaustriformis* and larvae of *Physaloptera* sp.; three species of digenea: *Glypthelmins quieta*, *Haematoloechus breviplexus* and *Langeronia macrocirra*; one species of eucestoda, *Ophiotaenia magna* and one species of unidentified oligacanthorhynchid cystacanth acanthocephala. From above list, not one of the helminths found in this study was unique to *Rana tarahumarae* except for *Falcaustra lowei* because of the present of a pseudosucker. However *R. tarahumarae* is a newly recognized host for each of the helminth species reported. Furthermore, this is the first report of *Haematoloechus breviplexus*, *O. magna*, *Falcaustra inglisi, Rhabdias ranae, Subulascaris falcaustriformis* and the second report of *Glypthelmins quieta* and *Langeronia macrocirra* was from Mexico.

Nematodes of the genus *Rhabdias* is known as comman lung parasites of amphibians. As reported in Neartic region, there are three *Rhabdias* species recorded from anurans in from the order Ranidae and Hylidae, which is, *Rhabdias ranae*, *R. joaquinensis* and *R. bakeri*. Moreover, *R. bakeri* is a new species reported from the region and also known as a very common parasite of *Rana sylvatica* in North Dakota (Tkach et al., 2006).

During May 1997 to June 2002, about 1052 anurans representing 41 species were collected in Costa Rica and was reported harbouring 20 species of nematodes: *Africana telfordi, Aplectana hylambatis, A. inserta, A. itzocanensis, Cosmocerca parva, C. podicipinus, Cosmocercella minor, Cosmocercoides variabilis, Cruzia empera, Falcaustra costaricae, Foleyellides striatus, Ochoterenella digiticauda,*

*Oswaldocruzia costaricensis, Parapharyngodon duniae, Rhabdias alabialis, R. pseudosphaerocephala, R. savage, Schrankiana inconspicata, Spiroxys figueiredoi, Subulascaris falcaustriformis*; and five species of larvae: *Ascaridae* sp., *Contracaecum* sp., *Eustrongylides* sp., *Physaloptera* sp. and *Physocephalus* sp.. It was found that about 113 new host records of parasites were reported (Bursey and Brooks, 2005).

As reported from Northwestern Mexico by Goldberg and Bursey (2002), 13 species of nematodes were recorded from seven species of anurans. They were *Aplectana inserta, A. itzocanensis, Cosmocerca podicipinus, C. haberi, Cosmocercoides variabilis, Foleyellides striatus, Oswaldocruzia pipiens, R. americanus, Rhabdias ranae, Subulascaris falcaustriformis, Physaloptera* sp. (larva), *Physocephalus* sp. (larva) and *Spiroxys* sp. (larva). It was concluded that *A. incerta* is a common parasite of bufonids, *A. itzocanensis* commonly occurs on bufonids and a pelobatid, *C. haberi* discovered from hylids and ranids frog, *C. variabilis* found from various hosts of frogs, toads, salamanders, lizards, turtles, and snakes, which is similar to *O. pipiens* but exception to snakes, *R. americanus* of bufonids and *R. ranae* is a common nematode of hylids and ranids frog. These seven species of nematodes have a direct life cycle, which means they may not require many hosts during their life cycle and once they enter the body of the host, they will live all their lives there and complete their reproducing process. In contrast to *Physaloptera* sp., where it undergoes indirect life cycle because the adult is only obtained from the stomach of mammals and reptiles while the larvae are commonly found in amphibians and some reptiles. Whereas, the species of *Physecephalus* sp. and *Spiroxys* sp. have intermediate hosts to undergo a complete life cycle. The adults of *Physecephalus* sp. are found in the stomachs of swines, horses, cattle and rabbits;

while the adult of *Spiroxys* sp. is found in the gastric mucosa of turtles in North America. Both infective larvae are recovered from dung of beetles and found encapsulated in the tissues of frogs, newts, birds, snails and fishes (Anderson, 2000; Baker, 1987 citation in Goldberg and Bursey, 2002).

Two new species of *Rhabdias* were reported in Central America from the host cane toad, *Bufo marinus* (Kuzmin et al., 2007). *Rhabdias alabialis* differs from the genus by its head end morphological characteristic with an absence of lips, the slit-like oral opening and presence of triangle shape of the buccal capsule. Another species is *Rhabdias pseudosphaerocephala*, which, previously was identified as *R. sphaerocephala* but differs based on head end morphology and rDNA sequences.

A report found that a species of pharyngodonid nematode, *Parapharyngodon garciae* was recorded from two species of frogs in Puerto Rican, Puerto Rican tree frog, *Eleutherodactylus coqui* and leptodactylid frogs. This was the first report of the nematode species for *E. coqoi* and the second from leptodactylid frog. From this study, it was found that *E. coqoi* was the new host record for *P. garciae*. A previous study reported that the only nematode record from *E. coqoi* was *Poekilostrongylus puertoricensis* (Dyer et al., 1995).

As reported by Bursey et al., (2008b), a new species of *Parathelandros*, *P. allisoni* was found in the intestines of the Hylidae frog, *Nyctimystes trachydermis* from Central Province, Papua New Guinea. From the previous records, this species is the 12$^{th}$ described of genus *Parathelandros* and the ninth records from genus *Parathelandros* in Australo-Papuan region.

Nematodes of the Pharyngodonidae are a common parasite in cold-blooded vertebrates, particularly amphibians. As reported by Hasegawa, (2005), two new

species of nematode Pharyngodonidae, *Okinawandros goldbergi* and *Ataronema sekii* were recorded from Rhacophord frogs of The Ryukyu Archipelago, Japan.

The nematode of genus *Icosiella* is a well-known parasite of anurans from many regions. This species have been found in anurans from Indonesia, Japan, Malaysia, Papua New Guinea, Vietnam and Western Europe (Baker, 1987 citation in Bursey et al., 2003). As reported by Bursey et al., (2003), *Icosiella turgeocauda* n. sp. was found from the intestinal mesenteries of *Rana cancrivora* (currently *Fejervarya cancrivora*) in Luzon, Republic of Philippines. This finding represents the ninth species of the genus *Icosiella*. In addition, this species is easy to differentiate from the others in the genus by the position of the vulva, which occurs anterior to the junction of the muscular and granular part of the esophagus, and the posterior end of the males with bilateral umbos.

### 2.3.3 Trematodes

Table 2.3: List of trematodes found in anurans and classification on type of host, organ, country and references.

| Species of trematode | Amphibian species | Type of host | Organs | Country | References |
|---|---|---|---|---|---|
| *Glypthelmins quieta*<br>*Haematoloechus breviplexus*<br>*Langeronia macrocirra* | *Rana tarahumaraea* | Definitive host | Intestine | Sonora, Mexico | Bursey and Goldberg, (2007) |
| *H. parcivitellarius*<br>*Megalodiscus temperatus* | *R. forreri* | Definitive host | Intestine | Costa Rica | Bursey and Goldberg, (2005) |
| *Styphlodora* sp. | *Odontophrynus americanus, Elachistocleis bicolor* | Definitive host | Intestine | Northeastern Argentina | Hamann and Gonzalez, (2009) |
| *Opisthogonimus* sp. | *O. americanus, Physalaenus santafecinus* | | | | |
| *Lophosicyadiplostomum nephrocystis* | *Scinax nasicus* | | | | |
| *Bursotrema tetracotyloides* | *P. santafecinus* | | | | |
| *H. longiplexus*<br>*H. parviplexus*<br>*H. breviplexus*<br>*Cephalogonimus americanus*<br>*Loxogenes Arcanum*<br>*Gorgodera amplicava*<br>*G. multilobata* | *R. septentrioralis* | Definitive host | Intestine | Minnesota, USA | Schottoefer et al., (2009) |

The life cycle of trematode is complex because it requires an intermediate host like snails and arthropods to complete its life cycle. The cycle *Ribeiroia* sp. starts with the infected snail host, where the parasites leave its snail host and enters a tadpole and grow in the tadpole. Some young amphibians become prey for the