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THE PREVALENCE OF HAEMOPARASITES OF ANURANS IN AN URBAN HABITAT OF KOTA SAMARAHAN, SARAWAK, MALAYSIAN BORNEO

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Abstract: The prevalence of haemoparasites was investigated in Anurans from the urban area of Kota Samarahan, Sarawak, Malaysia. A total of 120 urban Anura individuals belonging to six species (*Duttaphrynus melanostictus*, *Fejervarya limnocharis*, *Polypedates leucomystax*, *Chalcorana raniceps*, *Haplobatrachus rugulosus*, and *Kaloula pulchra*) were collected from 12 sites in Kota Samarahan, Sarawak. The selected sampling site was close to the housing and dumping area. Approximately 22.5% of individuals of anurans were infected with haemoparasites. Three species of haemoparasites were identified: *Trypanosoma* sp. (7.5%), *Lankesterella* sp. (5%), and *Hepatozoon* sp. (10%). *Duttaphrynus melanostictus* (44.5%) had the highest infection rate among the six anura species, with all three haemoparasites present, followed by *C. raniceps* (22.2%), *F. limnocharis* (18.5%), and *K. pulchra* (14.8%). No haemoparasites were recorded in *P. leucomystax* and *H. rugulosus*. This study indicates that multiple haemoparasites species can infect anurans and thus potentially become zoonotic pathogens that can be transmitted to humans. Therefore, measures should be taken to safeguard these anuran species against these haemoparasites, as their transmission to people may pose a risk to public health. Local communities should be careful and keep their settlement environment clean to reduce the risk of infectious diseases for the health and well-being of the population.

Keywords: Haemoparasites, urban area, zoonotic agents, transmitted, infection.

Introduction

Anura, also called Salientia is one of the orders of the class Amphibia which, comprises frogs and toads. The ways to differentiate frogs and toads are by their physical state. Frogs have long pairs of limbs and smooth mucus-like skin, whereas toads have slightly shorter limbs and rough skin. According to Henry (2000), "Amphibia" is derived from Greek words that mean "two lives" and refer to any animal with two complete cycles of life. Anurans are the only wildlife that experiences aquatic and terrestrial ecosystems. They are prone to contamination in both environments since they have sensitive, semi-permeable skin. As of now, there are roughly 5,400 species of anuran worldwide.

Baharudin *et al.* (2015) claimed that urbanisation has led to an increase in amphibian mortality as well as population disappearance in urban areas since amphibians have always been a good healthy environment indicator. Rapid urbanisation has a negative impact on the environment, particularly amphibian populations. Their dual environment lifestyle makes them particularly vulnerable to detrimental changes in the environment (Budzik *et al.*, 2013).

Animal parasites, which are intrinsically linked to their hosts are similarly affected by the repercussions of urbanisation. According to Anderson and Sukhdeo (2010), urbanisation can directly influence parasites by exposing them to an altered physicochemical environment, as well as have a significant impact on animal physiology, behaviour, and fitness. The urban environment promotes and influences the transmission and success of animal parasites and pathogens (Giraudeau *et al.*, 2014).

Reports on haemoparasites of anuran species still need to be more sparse in Malaysia, particularly in urban areas. Until today, only a few researches have been carried out on the presence or detection of haemoparasites in anurans in Malaysia (Rahman *et al.*, 2008; Agustar *et al*., 2021). However, there have yet to be reported studies or findings about haemoparasites of anuran in East Malaysia, especially in Kota Samarahan. Rahman *et al.* (2008) have described and conducted a study of the parasitic fauna of two wild anuran species collected from a river area in Penang and found that some environmental parameters have influenced some populations of anurans and their parasitic fauna. The other research was conducted by Mohammad *et al.* (2013) on protozoan parasites of anuran in Zoo Negara and the latest research by Agustar *et al.* (2021) was on protozoa in anurans from lowland and highland golf courses in Malaysia. The presence of parasites in human settlements may affect the health status of humans and animals since these animals may act as reservoir hosts for parasites, which can be spread to other living organisms. Therefore, the objectives of this study are to determine the species of haemoparasites that occurred in urban anuran as well as the prevalence of haemoparasites in urban anuran at Kota Samarahan, Sarawak.

Materials and Methods

Study Area

The research was conducted at 12 distinct locations in the urban areas of Kota Samarahan, Sarawak, Malaysian Borneo: Taman Hill View (1.4082° N, 110.3653° E), Taman Samarindah (1.4470° N, 110.4119° E), Uni Garden (1.4618° N, 110.4575° E), Uni Square (1.4637° N, 110.4179° E), Place 2 Stay Hotel (1.4887° N, 110.4050° E), Kompleks Sukan UNIMAS (1.4614° N, 110.4366° E), Kampus Timur UNIMAS (1.4705° N, 110.4355° E), Aiman Mall (1.4554° N, 110.4464° E), Desa Ilmu (1.4529° N, 110.4575° E), Muara Tuang (1.4595° N, 110.4906° E), Asajaya (1.5461° N, 110.5191° E), and Samarahan jetty (1.4729° N, 110.4298° E) (Figure 1). Kota Samarahan is known as a knowledge town because it is home to many

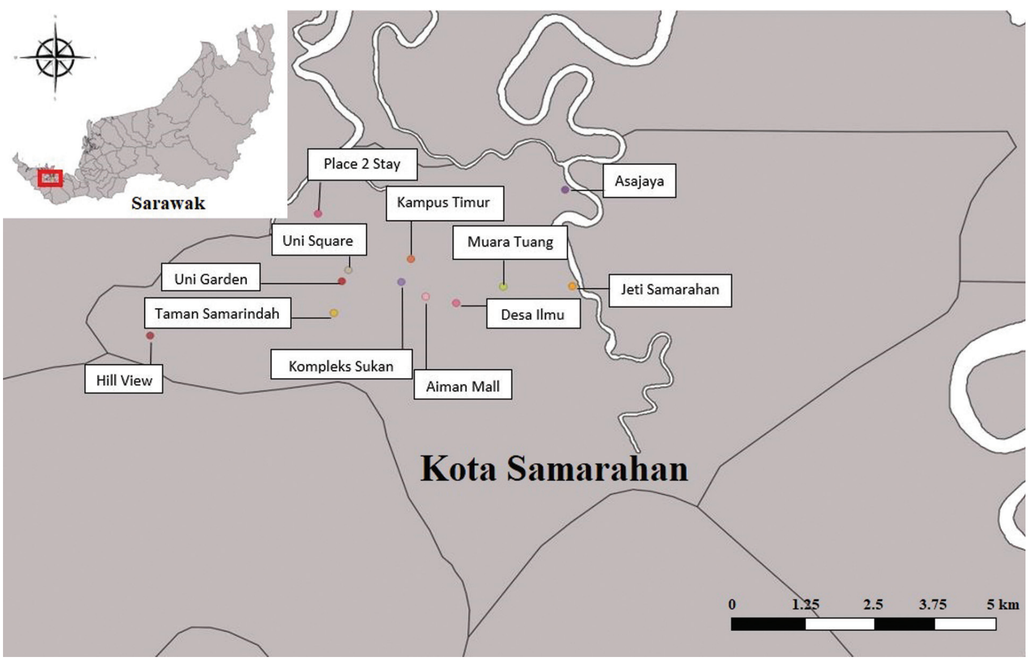


Figure 1: Location of study sites in Kota Samarahan, Sarawak
(Generated by: QGIS application)

educational and research facilities, including Universiti Malaysia Sarawak (UNIMAS), Universiti Teknologi MARA (UiTM), Tun Abdul Razak Teachers Training Institute, Sarawak General Hospital Heart Centre, National Institute of Public Administration (Intan), and Sarawak Tropical Peat Research Institute. Kota Samarahan was chosen due to its fast urbanisation. This neighbourhood is being developed with many housing estates, commercial centres, and offices, as well as a shopping mall. As development progresses, rubbish dumping activity grows.

Host Collection and Identification

A total of 120 anurans were collected between September 2019 and January 2020. All of the specimens were collected with bare hands. Different individual anurans were kept in separate zip lock bags since some individuals could secrete the toxic as a self-defend mechanism, resulting in fatality to another frog if they were mixed (Zainudin, 2006). The temperature and humidity were recorded at each sampling site and the location coordinates were determined using Global Positioning System Coordinate Finder (GPS). The weight of the anurans was taken and the specimens were identified following the description by Inger *et al.* (2017) and Das *et al.* (2017; 2018).

Collection of Blood for Haemoparasite Observation

The specimens were euthanised with excess diethyl ether and followed the animal ethic by Universiti Malaysia Sarawak (UNIMAS) Standard Operation Procedure under permit number UniMAS/AEC/R/F07/046. Once the animal had died, the blood was collected using a 1 cc/ml syringe. The blood was drawn straight from the heart wall. In most anuran species, blood can be collected from the ventral abdominal vein, lingual plexus, femoral vein, facial (maxillary) musculo-cutaneous vein, and the ventricle of the heart (Forzan *et al.*, 2012).

Preparation of Blood Films

In this study, both thick and thin blood films were prepared. Two droplets of blood were placed on a new slide to form a thick blood film. The blood was spread with the slider's corner edge in a circular motion. The smear should not be too thick, or it will fall off the slide, and it should be clear enough to read (Alias *et al*., 2014). After that, the thin blood smear was fixed on a glass slide by soaking it in 75% methanol for one minute. Then, the slide was soaked in a Quick Giemsa stain solution for one minute. Finally, it was drained with water and air-dried (Alias *et al*., 2014). A thick blood smear provides a better opportunity to detect the parasitic forms present (Alias *et al*., 2014). However, there is a limitation to an optimal review of parasite morphology. Therefore, a thin blood smear was made in order to identify haemoparasites based on their morphology.

To prepare thin blood films, one drop of blood was deposited at the end of the slide. A clean slider was held at a 45° angle and brought towards the blood droplets on the slide. Once the blood had spread evenly along the width of the slider, the slider was pushed rapidly along the slide. The slide was air-dried for a few minutes before being fixed with methanol. Once fixed with methanol, the slide was air-dried again and stained with quick Giemsa. The slide was air-dried for the last time before being identified under a microscope. The thin blood film is used to discover any parasites that infect the blood of the anurans. Thin smears consist of blood spread in a layer so that the thickness gradually reduces toward the mono-layer and it permits an ideal assessment of the morphology of any parasitic organisms that may be present (Alias *et al*., 2014).

Both thick and thin slides were observed under a compound microscope with 400x magnification for parasite screening and 1,000x magnification under oil immersion for parasite identification. Any observed parasites were identified based on Davies and Johnston (2000) and Rahman *et al*. (2008).

Statistical Analysis

All data were documented in a Microsoft Excel spreadsheet and the prevalence of haemoparasites was calculated in the form of percentages by using the formula described by Bush *et al.* (2001) as below:

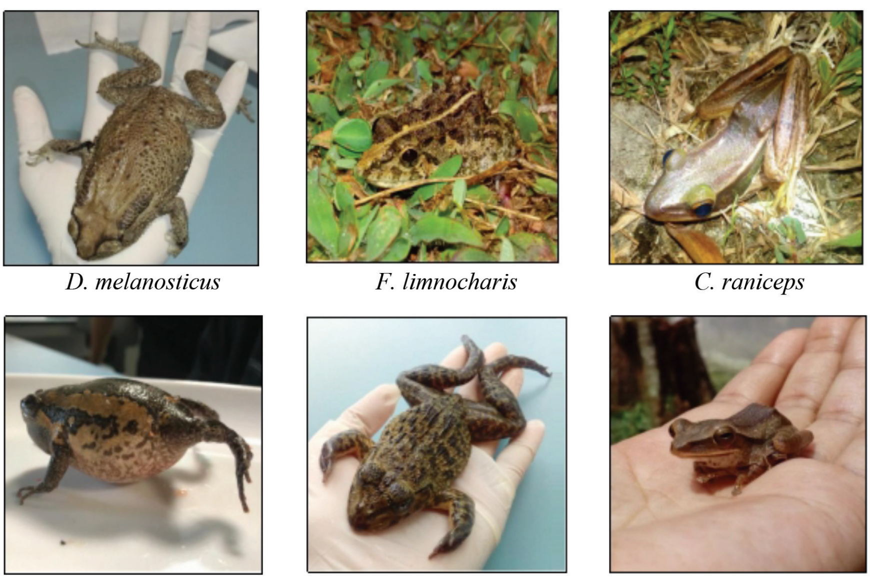
Prevalence (%) = (Number of host species infected by a particular parasite species/Total number of hosts examined) x 100

Results and Discussion

A total of 120 individuals of anurans were collected and they were identified and categorised into six species (Figure 2) namely *Chalcorana raniceps* (n = 20), *Duttaphrynus melanostictus* (n = 65), *Fejervarya limnocharis* (n = 24), *Haplobatrachus rugulosus* (n = 3), *Kaloula pulchra* (n = 7), and *Polypedates leucomystax* (n = 1) (Table 1). Of the six species collected, *P. lecuxomystax* is arboreal and *H. rugulosus* is aquatic meanwhile the rest are terrestrial anurans. The most dominant anuran species collected for this study was *D. melanostictus*, with the highest number of individuals collected with 65 individuals.

The number of anuran species varies in each site due to the fact that different species of anuran inhibit different habitat areas. Hence, that is why the distribution of anuran found was different for each sampling site. Some of the species found in this study had similarly been reported in other parasite studies worldwide (Davies & Johnston, 2000; Rahman *et al.*, 2008). From the study conducted by Rahman *et al.* (2008), they focused on one locality, which was located at Sungai Penang and collected two species of anurans, which were *Bufo melanostictus* and *Rana limnocharis*.

The common sunda toads (*D. melanostictus*) was the dominant species found and collected throughout this study. Compared to any other Anura, this species preferred to live in agricultural, rural, urban, suburban areas, and mostly open country. Since our study focuses on urban areas, this species can be found in most of our sampling sites, especially in the drain and watery areas since pools and puddles are known to be their reproduction site. This is congruent to a previous study done by Mohammad *et al.* (2013), as this species is obtained near water



D. melanosticus *F. limnocharis* *C. raniceps*

K. pulchra *H. rugulosus* *P. leucomystax*

Figure 2: Photo of caught anuran species in Kota Samarahan

Table 1: Prevelance of haemoparasites of urban anuran in Kota Samarahan, Sarawak

Anura Species	Total Number of Anuran Examined	Total Number of Anuran Infected	Haemoparasite Species		
			Hepatozoon sp.	*Trypanosoma* sp.	*Lankesterella* sp.
Chalcorana raniceps	20	6	5/6 (83%)	1/6 (17%)	-
Duttaphrynus melanostictus	65	12	5/12 (42%)	3/12 (25%)	4/12 (33%)
Fejervarya limnocharis	24	5	-	3/5 (60%)	2/5 (40%)
Haplobatrachus rugulosus	3	-	-	-	-
Kaloula pulchra	7	4	2/4 (50%)	2/4 (50%)	
Polypedates leucomystax	1	-	-	-	-
Total	**120**	**27**	**12**	**9**	**6**
Haemoparasite prevalence			27/120 (22.5%)		

sources such as artificial ponds, rivers, and lakes around their study area. The lowest number of Anura caught was *P. leucomystax*, with only one individual caught. Since the sampling site is emphasised around the housing area, it is rather difficult to find this species as this species is normally found in the pond areas. *Haplobatrachus rugulosus* was found in the watery drain. It is difficult to capture this species since it is a fast swimmer. *Kaloula pulchra* was mostly found under leaf litter. It is easier to find this species since it is a highly adaptable species and easily survives in disturbed habitats. Meanwhile, the other two species, *F. limnocharis* and *C. raniceps*, are found in grass and near lakes.

From the host collected, 22.5% of anurans were infected with haemoparasites. The highest infection of haemoparasite was found in *D. melanostictus* (10%, n = 12) and the lowest infection was *K. pulchra* (3.3%, n = 4) (Table 1). Both *P. leucomystax* and *H. rugulosus* were not infected with any haemoparasites during this study (Table 1, Figure 3). Three

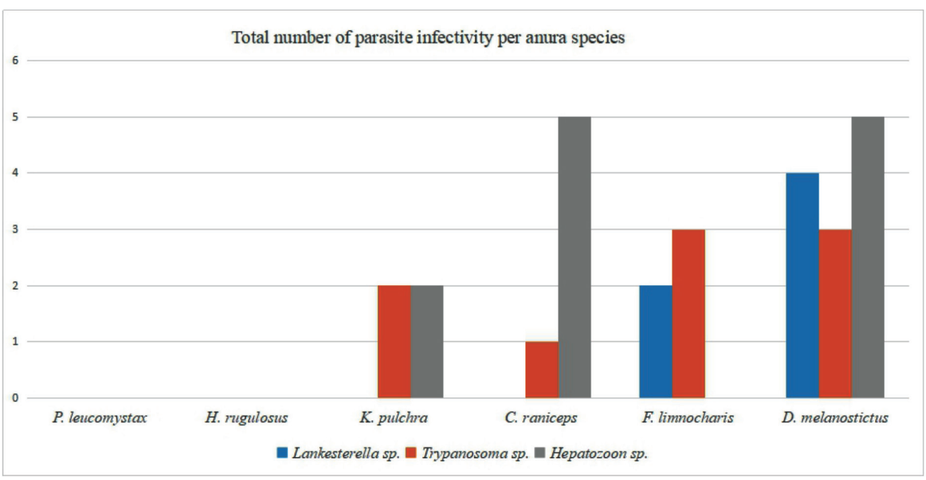


Figure 3: Total number of parasite infectivity per anura species

species of haemoparasites were detected from the Anura collected for this study (Figure 4). They comprised *Trypanosoma* sp. (Figures 4 a-c), *Lankesterella* sp. (Figures 4 d-f), and *Hepatozoon* sp. (Figures 4s g-i). *Hepatozoon* sp. (10%) recorded the highest prevalence rate of infection. Meanwhile, the lowest being seen was *Lankesterella* sp. (5%) (Table 1).

At least one species of Anura is infected with haemoparasite, except for *P. leucomystax* and *H. rugulosus*. Three species of haemoparasites were recovered from this study: *Hepatozoon* sp., *Trypanosoma* sp., and *Lankesterella* sp. In contrast to the haemoparasites study conducted in Malaysia, no haemoparasites were recovered on the comparable species studied by Mohammad *et al*. (2013), however, Rahman *et al*. (2008) were able to recover *Trypanosoma* sp. on *F. limnocharis*.

Hepatozoon sp. has accounted for most of the infection, followed by *Trypanosoma* sp. and *Lankesterella* sp. Out of six species of Anura, only *D. melanostictus* and *C. raniceps* were infected with *Hepatozoon* sp. Davies and Johnston (2000) stated that the only two haemogregarine species known to parasitise anuran hosts are *Hepatozoon* sp. *Hepatozoon* was later represented as the most common

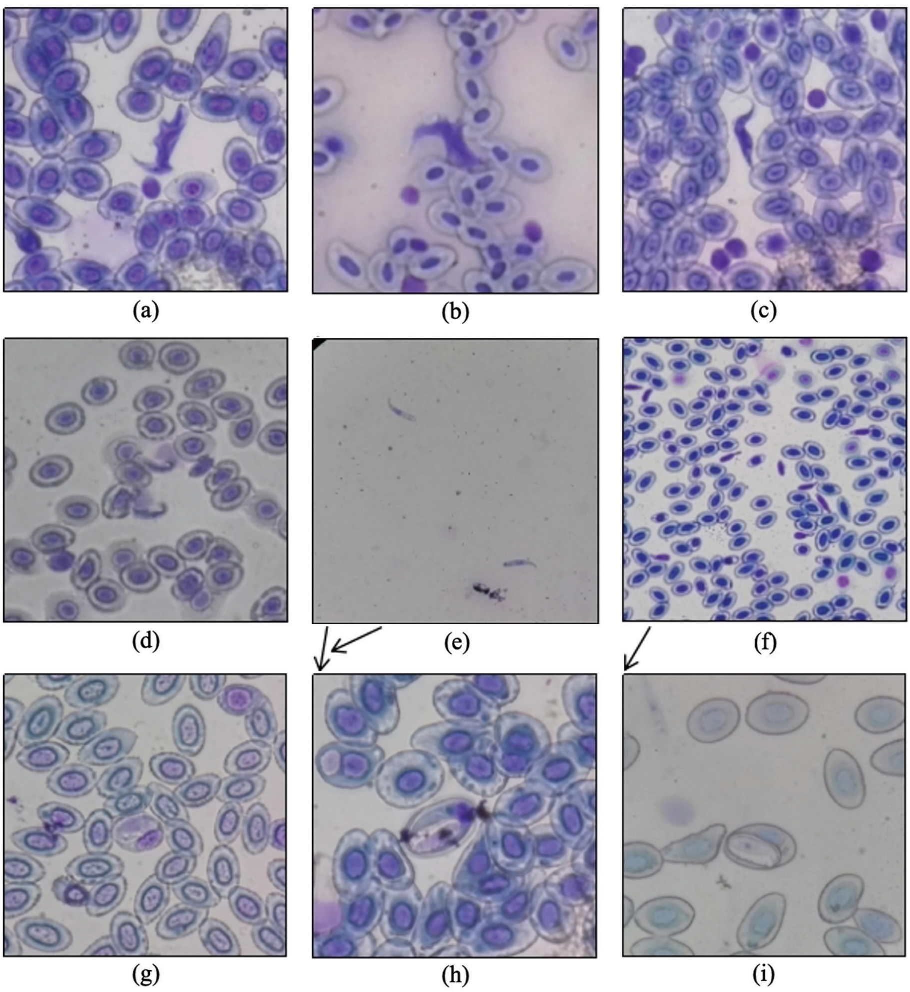


Figure 4: Photomicrographs of the haemoparasites of urban anuran in Kota Samarahan, Sarawak; *Trypanosoma* sp. (a-c), *Lankesterella* sp. (d-f), and *Hepatozoon* sp. (g-i) in red blood cell

intraerythrocytic protozoan parasite of anurans worldwide and it was suggested that the availability of insect vectors might have caused this (Smith, 1996). Mites, ticks, and various insects are known as the definitive hosts for *Hepatozoon* species (Uiterwijk *et al.*, 2023).

This study findings indicate that four Anura species are infected with *Trypanosoma* species. The discovery of *Trypanosoma* sp. in vertebrates such as anura is pertinent to earlier research that concluded midges biting frogs (Corethrellidae) in the order Diptera is the cause of *Trypanosoma* sp. transmission (Kvifte & Bernal, 2018). Besides that, it was also shown that insect vectors present in aquatic environments were also responsible for the spread of *Trypanosoma* sp. (Rahman *et al.*, 2008; Kaufer *et al.*, 2017; Desquesnes *et al.*, 2022). Since the anurans examined in this study were collected and located in drains, watery, and wet regions, as well as waste disposal locations, it is most likely that this haemoparasite species contracted infection possibly from an insect vector that lives in aquatic environments. Additionally, a *Trypanosoma* sp. transmission can also happen when parasite-containing bug excrement comes into contact with a bite wound or the mucous membrane of a host, like anura. This can lead to the parasites multiplying and spreading, which can cause a variety of symptoms of infection, including fever, fatigue, and serious neurological and cardiovascular issues.

There are roughly 60 species of Trypanosomes reported in anurans all over the world (Miyata *et al.*, 1978). *Trypanosoma* sp. was only found in *R. limnocharis* but was totally absent in *B. melanostictus* (Rahman *et al*., 2008). However, *Trypanosoma bilaspurensis* in *B. melanostictus* was discovered based on a study by Gupta *et al.* (2012). Anurans are edible for human consumption since most *Trypanosoma* species found in anurans were non-pathogenic compared to *Trypanosoma* species infecting humans (Smyth & Smyth, 1980).

Lankesterella sp. is known to be disseminated by a leech (*Placobdella marginata*) to anurans through blood-sucking activities (Kudo, 1971). *Lankesterella* sp. was recovered from two species of Anura, *D. melanostictus* and *F. limnocharis*. *Duttaphyrnus melanostictus* can be found inhabiting disturbed, stable, and watery habitats. As for *F. limnocharis*, most of these species are found in the drain, swimming, hence, providing a greater chance of being infected by leeches.

Many studies have been done on haemoparasites in mammals in Sarawak. However, no further studies have yet been done on Anura in this state. The importance of this study of Anura as reservoirs of potentially medically important zoonotic pathogens is undoubtedly necessary for a better understanding of the evolution of novel diseases. It is reported that *Trypanosoma* sp. can cause diseases such as onchocerciasis and lymphatic filariasis, which are transmitted through the bites of an insect vector, which are mosquitoes and black flies (Bakowski & McNamara, 2019). Therefore, this study proves that animals around us can be a causing agent of various type of diseases.

Conclusions

In conclusion, both haemoparasite prevalence and haemoparasites species infecting urban Anura in Kota Samarahan, Sarawak has been determined. Three haemoparasite species were recovered, namely, *Hepatozoon* sp., *Trypanosoma* sp., and *Lankesterella* sp. *Hepatozoon* sp. has the highest number of infections towards anurans since it is transmitted through ingestion of infected invertebrates. From the study, we can conclude that the species that inhibit most areas like *D. melanostictus* are prone to parasite infection due to their availability to adapt to various environments. Urbanisation increases waste dumping activity and causes the presence of parasitic vectors.

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Conflict of Interest Statement

The authors declare that they have no conflict of interest.

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