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Freshwater nematodes from Matang wild-life and Kubah national park rivers, Sarawak, Malaysia

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Abstract

Freshwater nematode surveys were carried out at Rayu River (Matang Wildlife Centre) and Cina River (Kubah National Park) to identify the species of freshwater nematodes that inhabited both rivers and to determine the relationship between nematode density and physico-chemical parameters of the rivers water. Sampling was carried out in six stations. Eight species of nematodes namely *Acanthopharynx* cf. *affinis, Afrodorylaimus bwana, Apodorylaimus bini, Cobbonchus palustris, Crocodorylaimus flavomaculatus, Ironus* cf. *ignavus, Laimydorus prolificus* and *Mononchulus* cf. *nodicaudatus* were recorded in both rivers. There is no significant correlation between dissolved oxygen and temperature with nematode density. The nematodes in both rivers were possibly attracted to food provided by decomposers on dead leaves that exist in the rivers.

INTRODUCTION

Most of Malaysia's freshwaters flowing in rivers, and most of the country's water requirements are supplied by the rivers [1]. Rivers arise in the mountain ranges where their flow is torrential, slowing down as the rivers reach the coastal plains and meander their way to the sea. As the rivers slow down in their lower reaches, their substrate changes from rocks to a more sandy and muddy base. Water quality such as temperature, oxygen levels, colour, and turbidity also change. In an upper catchments which respond very rapidly to rainfall, water is more clear, cold and oxygen levels increased compared to downstream which are more turbid, lower dissolved oxygen and temperature rise as the rivers become sluggish and less shaded. The rivers condition provides a different habitat and support different communities of invertebrates that have special adaptations to make them survive in such environments. Freshwater nematodes is one of the communities that has the ability to adapt in the river environment even though the accurate number of nematode species that live in the river is unknown. This is because most studies have been restricted to the macrofauna (organisms which are retained on a net with a mesh size of 500 μm), while the meiofauna (organisms which are retained on a net with a mesh size of about 40 μm) has received less attention; moreover, much less is known of the most abundant and species rich taxa - the nematodes [2].

In ecosystems, nematodes from phylum Nematoda play a role in the food chains as the consumer and decomposers. Recently, the nematode also has been known as potential live feed for fish, crabs and prawns [3]. Biedenbach *et al*. [4] has conducted study on the suitability of nematode *Panagrellus redivivus* as an *Artemia* replacement in a larval penaeid diet. In an experimental study conducted by Nelson & Coull [5], dead nematodes have been discovered in the fish guts other than dead copepods. Study on the response of bighead carp *Aristichthys nobilis* and Asian catfish *Clarias macrocephalus* larvae to free-living nematode *Panagrellus redivivus* as alternative feed has been conducted by Santiago *et al*. [6]. Other than that, it also possesses number of traits that make them useful especially for environmental studies. Although it also can provide a useful model system for fundamental ecological studies on the interactions between biodiversity and ecosystem functioning such as studies conducted by Bongers & Ferris [7], Kadadevaru *et al*. [8]; Mahmoudi *et al*. [9] and Wilson & Kakouli-Duarte [10], however studied on biology and life history of freshwater forms still received less attention [11]. Studies on the ecology of Malaysian free-living nematodes were also limited to the higher major taxa [12] with the exception for study carried out in Merbok mangrove estuary, Kedah [13]. These limitations are believed to be associated with difficulties in sampling, extraction, and identification of these small invertebrates [14]. Moreover, there is no study on freshwater nematodes in rivers from Matang Wild-life and Kubah National Parks to date.

Therefore the objectives of the present study are: (i) to identify the nematode species from Rayu River (Matang Wildlife Centre) and Cina River (Kubah National Park), (ii) to determine the relationship between nematode density and physico-chemical parameters of the water in Rayu and Cina Rivers.

METHODOLOGY

Sampling was carried out on July 2011 at Rayu River (Matang Wildlife Centre) and Cina River (Kubah National Park). Three stations were chosen from both rivers (station I to III - Rayu River; Station IV to VI - Cina River) and all stations were located at the upstream area. Temperature and dissolved oxygen of water parameters were measured using DO meter (Eutech Instrument). Coordinates, elevation and description of each station were recorded. Quantitative (2 replicates) and qualitative samplings were conducted in each station. Quantitative sampling was conducted using perspek corer with 3.5 cm diameter and 10 cm depth while sediment for qualitative sampling was collected using small scoop. Sediment obtained using perspek corer or scoop was transferred into a small pail and mixed properly with water. The mixture was then sieved with 500 µm (on top) and 45 µm (beneath) sieves. Sediment that retained on the 45 µm sieve was transferred into a labeled plastic bag and fixed with 5% formalin. The specimen was then brought back to laboratory for further analysis.

Nematode counting and identifying were following the methods proposed by Shabdin & Othman [15]. In the laboratory, nematode samples were poured into the 45 µm sieves and washed with tap water. The samples were made concentrated by washing it to the edge of the sieve and by using wash bottle; the samples were washed into a grid Petri dish. The nematode specimens were then scattered in a grid Petri dish. Then, the total number of nematodes in the grid Petri dish was counted under a stereo microscope. The numbers of organisms were finally converted to densities in units of individuals/10 cm square. Using a wire loop nematodes were then transferred to a glass cavity block containing 5% glycerine: 5% pure ethanol: 90% freshwater by volume and left in desiccators for few days [16]. This would allow the ethanol and water to evaporate slowly leaving the nematodes in pure glycerin. Finally, the nematodes were transferred to a fresh drop of anhydrous glycerin (phenol added) on a slide and a cover slip added, supported by several small glass blocks at both ends. Canada balsam was used as a sealant. The nematodes were identified to the species level under a compound microscope. Identification was performed using the key by Poinar [14] and Abebe *et al*. [11] and verified by various keys in the literature.

RESULTS

All sampling stations were located at the upper reaches of the rivers and covered by tree canopy (Table 1). The elevation of the stations ranged from 10.9 to 306 meter above the sea level. The water in both rivers (Rayu River and Cina River) was recorded as clear. The water current in the sampling area was classified as fast flowing water except at station 6 where the water current was very slow and almost stagnant. The sediments were recorded sandy and rubbles with big rocks located along the river bank. There was a little variation in water temperature for all stations in both rivers, this being not greater than about 2.94°C. Temperatures were generally within the range of 25.56ºC to 28.5°C (Table 2). The dissolved oxygen ranged between 56.46 to 98.01% (Table 2). There was a wide variation of dissolved oxygen values between stations 6 and other stations (station 1-5). Low dissolved oxygen level at station 6 possibly due to the slow water current and decomposition of dead leaves. In quantitative sampling, the maximum nematode's density was recorded at Station 6 (5.20

Table 1. Location and description of sampling stations in the study area.

Location	Station	Coordinates	Elevation (m)	Station description
Rayu River Matang Wildlife Centre	1	N01°35.958' E110°10.494'	93.00	Small stream, close to waterfall, fast flowing and clear water, covered by canopy, sandy sediment & rubble and surrounded by rock
Rayu River Matang Wildlife Centre	2	N01°36.669' E110°09.709'	46.63	Covered by canopy, fast flowing and water, sandy sediment and rubble.
Rayu River Matang Wildlife Centre	3	N01°6.601' E110°09.496'	10.97	Covered by canopy, fast flowing and clear water, sandy sediment and surrounded by rock.
Cina River Kubah National Park	4	N01º36.448' E110°11.516'	306.00	Small stream, fast flowing and clear water, covered by canopy, sandy sediment & rubble and surrounded by rock.
Cina River Kubah National Park	5	N01°36.545' E110°11.585'	265.00	Covered by canopy, small light penetration, fast flowing and clear water, sandy sediment and rubble and surrounded by rock.
Cina River Kubah National Park	6	N01º36.724' E110°11.838'	132.00	Covered by canopy, water clear, very slow current, sandy sediment & rubble covered by dead leaves and surrounded by rock

Table 2. Temperature, dissolved oxygen, nematode density (quantitative) and no.of individuals nematode (qualitative) at Rayu and Cina Rivers.

Station	Dissolved Oxygen (%)	Temperature (°C)	Density (No. Ind./10cm^2) (quantitative)	No. of ind. nematode (qualitative)
1	94.0	26.13	4.16	2
2	98.01	27.43	3.12	0
3	95.06	28.5	2.08	0
4	95.83	25.56	1.04	5
5	83.13	26.1	0.00	2
6	56.46	27.5	5.20	6

Table 3. No. of individuals nematode species found (quantitative and qualitative sampling) in Rayu River and Cina River (cf. confer)

Species	Station						Total
	1	2	3	4	5	6	
Acanthopharynx cf. *affinis*				2			2
Afrodorylaimus bwana	1						1
Apodorylaimus bini				1			1
Cobbonchus palustris						1	1
Crocodorylaimus flavomaculatus			1				1
Ironus cf. *ignavus*				3		4	7
Laimydorus prolificus					2	1	3
Mononchulus cf. *nodicaudatus*	2						2
Unidentified	2	3	1			5	11
Total	5	3	2	6	2	11	29

Table 4. Correlation between physico-chemical parameters of water and nematode density

Correlations				
		DO	Temperature	Individuals
DO	Pearson Correlation	1	-.282	-.598
	Sig. (2-tailed)		.588	.210
	N	6	6	6
Temperature	Pearson Correlation	-.282	1	-.102
	Sig. (2-tailed)	.588		.847
	N	6	6	6
Individuals	Pearson Correlation	-.598	-.102	1
	Sig. (2-tailed)	.210	.847	
	N	6	6	6

ind./m^2) and minimum at Station 4 (Table 2). Nematode was not found at station 5. In qualitative sampling the maximum individual of nematode was found at Station 6 and none in Station 2 and 3. High nematodes density at station 6 was dominated by *Ironus* cf. *ignavus* and a total of 11 unidentified nematode species (Table 3).

Eight species of nematodes (total of 18 individuals) were identified from the study area namely *Acanthopharynx* cf. *affinis, Afrodorylaimus bwana, Apodorylaimus bini, Cobbonchus palustris, Crocodorylaimus flavomaculatus, Ironus* cf. *ignavus, Laimydorus prolificus* and *Mononchulus* cf. *nodicaudatus* (Table 3). Another eleven individuals were unable to be identified to the species level due to damaged samples.

Pearson correlation coefficient shows that there is no significant correlation between the dissolved oxygen (DO), water temperature and the species density of the nematodes (Table 4). Negative correlation between the DO level and water temperature with the density of nematodes made it difficult to show clear relationship between these factors.

DISCUSSION

Historical studies of freshwater nematodes in Malaysia are related to parasitic species such as *Daubaylia malayanum* and *Hexamermis cathetospiculae* which parasitizes snail [17] and paddy [18] respectively. Poinar [14] stated that no general systematic studies on freshwater nematodes from Malaysia in his review of the Malaysian region. However, the freshwater nematodes studies in Sarawak has begun in 1995 when Abang *et al* [19] reported on the freshwater nematodes from lotic environment of Balui River, Bakun while in 1998 Shabdin and Abang [20] reported the freshwater nematodes from Pa' Dappur River, Bario. Studies on Balui and Pa' Dappur Rivers recorded the presence and absence of nematodes at higher taxonomic level (phylum) only while report on freshwater nematodes of east Lanjak-Entimau Wildlife Sanctuary rivers [21] recorded the density of freshwater nematodes in the study area. Four nematodes species are reported from various rivers in east Lanjak-Entimau Wildlife Sanctuary. In the current study, eight freshwater nematodes species are recorded from Rayu and Cina Rivers. This makes the total freshwater nematodes recorded in Sarawak to 11 species. It shows that the nematode species number present in Rayu and Cina Rivers is higher than Lanjak-Entimau Rivers. Both rivers are located in the National Park and their habitats are not disturbed by any agricultural activities resulting from herbicides or insecticides use in the farms which may reduce species number of aquatic animals including freshwater nematodes. However the species density in Rayu and Cina Rivers is lower than recorded in Lanjak-Entimau Rivers. This is common phenomenon where higher density usually dominated by one or two species whereas lower density occurs when more species exist in the habitat. Freshwater nematodes are said to regulate major ecosystem processes and play an important ecological role in detrital food webs [2]. However, the question on what mechanisms or factors that determined the community structure of the benthos community is still unresolved. There are researchers who shows that abiotic factors such as oxygen, sediment and resources are significant in determining the distribution of animals but the others suggested that the community structure of benthic invertebrate are influenced by the primary productivity and quality of organic matter. However, the effects of biotic factors such as competition and predation might also contribute to the community structuring [2]. In the present study, Pearson correlation coefficient shows that there is no significant correlation between the dissolved oxygen (DO), water temperature and the species density of the nematodes. The negative correlation however might describe that the nematodes can adapt in the environment with low dissolved oxygen. Slow water current (almost stagnant) in station 6 that consists of dead leaves might have a lot of food resources compared to the other station. The food abundance combined with very slow water current at station 6 attracts the colonization of nematodes. Nematodes feed on both primary decomposers such as bacteria and fungi as well as primary producers such as algae and higher plants [14,22]. Based on morphological characteristics of the head end of identified freshwater nematode in this study, the eight species of nematodes could be classified into three feeding type which are epistrate feeders (*Acanthopharynx*, *Ironus* and *Mononchulus*), chewers (*Cobbonchus*) and suction feeders (*Afrodorylaimus*, *Apodorylaimus*, *Crocodorylaimus* and *Laimydorus*). Epistrate feeders which feed on bacteria, unicellular eukaryotes, diatoms and other microalgae are dominant in both rivers. Deposit feeders are differentiated from epistrate feeders by the absence of teeth. Epistrate feeders have a small tooth. Chewers are recognised by the presence of voluminous, sclerotized buccal cavity with one or more teeth and denticles while suction feeders are characterised by the presence of a stylet.

The nematodes possibly did not possess special adaptations for holding on to the substrate so that they are not swept away by the flow thus preferred stagnant water [1]. Those possibilities are proved to influence the freshwater nematodes distribution. Traunspurger *et al*. [23] stated that free-living nematodes are present almost in all limnetic sediments including those subject to hot, acidic and anoxic conditions and more reports have demonstrated the survival and reproduction of nematodes in strict anoxic conditions [23].

CONCLUSIONS

The Rayu River in Matang Wildlife Centre and Cina River of Kubah National Park are found to provide habitat for eight species of freshwater nematodes namely *Acanthopharynx* cf. *affinis, Afrodorylaimus bwana, Apodorylaimus bini, Cobbonchus palustris, Crocodorylaimus flavomaculatus, Ironus* cf. *ignavus, Laimydorus prolificus* and *Mononchulus* cf. *nodicaudatus.* Both rivers are located in the National Park and the water is still clean and suitable for freshwater nematodes habitats.

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