Tropical Biomedicine 37(4): 1152–1157 (2020)

Short Communication

Morphological characteristics of microfilariae in blood smears of the common treeshrew *Tupaia glis* (Mammalia: Scandentia) in Gemas, Negeri Sembilan, Malaysia

Mat Udin, A.S.[1], Uni, S.[1,2*], Zainuri, N.A.[1], Abdullah Halim, M.R.[1], Belabut, D.A.[1], Mohamad Ikbal, N.H.[1], Saarani, M.A.F.[1], Chen, C.D.[1*], Uga, S.[2], Ramli, R.[1] and Sofian-Azirun, M.[1]
[1]Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia
[2]Department of Public Health, Faculty of Nursing, Kobe Women's University, Kobe 650-0046, Japan
*Corresponding author e-mails: unishigehiko@um.edu.my; chen_ctbr@um.edu.my
Received 22 July 2020; received in revised form 10 September 2020; accepted 10 September 2020

Abstract. Some filarial nematodes, such as *Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori*, cause lymphatic diseases in humans in the tropics, whereas other filarial parasites from wild animals cause zoonotic diseases in humans worldwide. To elucidate the prevalence and diversity of filarial parasites in Malaysia, we investigated the filarial parasites from wild animals in Gemas, Negeri Sembilan. To find adult filarial parasites, we dissected 26 animals, which included five frogs, one skink, one snake, two birds, six common treeshrews, and 11 rats. Then, we examined microfilariae in the blood smears and skin snips obtained from each animal. We found two types of microfilariae in the blood smears of common treeshrews: one was very similar to *Malayfilaria sofiani* and the other closely resembled *Brugia tupaiae*. These findings indicate an additional distribution of these filarial parasites in Gemas.

INTRODUCTION

Filarial nematodes, such as *Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori* in the superfamily Filarioidea, are well known to cause lymphatic diseases in humans in tropical regions of the world (World Health Organization, 2016). Recently, zoonotic infections with filarial nematodes from domestic or wild animals have been reported in humans worldwide (Otranto *et al*., 2011; Uni *et al*., 2015). In Malaysia, *Brugia pahangi*, a parasite of cats, dogs, and wild carnivores, has been found to cause zoonotic diseases in humans (Tan *et al*., 2011; Muslim *et al*., 2013).

To date, 34 filarioid species from 21 genera have previously been recorded in vertebrates in Malaysia (Yen, 1983; Gibbons, 2010). *Brugia tupaiae* and *Mansonella* (*Tupainema*) *dunni* are filarial parasites that have previously been recorded from the common treeshrew in Malaysia (Orihel, 1966; Mullin & Orihel, 1972). Recently, *Malayfilaria sofiani* has been described as a new species in a new filarial genus of common treeshrews captured in Jeram Pasu, Kelantan (located on the east coast of Peninsular Malaysia) (Uni *et al*., 2017). Multilocus sequence analyses by Lefoulon *et al*. (2015) indicated that members of the Onchocercidae could be divided into five clades (ONC1–ONC5). The first group of the ONC5 includes two genera, *Wuchereria* and *Brugia*. *Malayfilaria sofiani* was also included in the first group (Uni *et al*., 2017).

We previously examined 98 common treeshrews from 14 areas in nine states and the Federal Territory of Peninsular Malaysia, and found *M. sofiani* in only five common

treeshrews collected from a single rubber plantation in Jeram Pasu, Kelantan (Uni *et al.*, 2017). Furthermore, the specific location of *B. tupaiae* was not recorded in the original description from Malaysia (Orihel, 1966). In this study, we propose an additional distribution of these filarioids in Gemas.

MATERIALS AND METHODS

To elucidate the prevalence and diversity of filarial parasites in Malaysia, we participated in a scientific expedition in Gemas (02°33'18"N, 102°36'44"E; elevation 42 m), Negeri Sembilan, Malaysia, organized by the Institute of Biological Sciences, University of Malaya, between 8 and 12 August 2016 (Figure 1). From the secondary forest and oil palm plantation in the research area, we captured 26 wild animals: five frogs, one skink, one snake, two birds, six common treeshrews, and 11 rats (Table 1). We captured all animals with the permission given by the Department of Wildlife and National Park, Malaysia (Permit number W-00660-16-16), and used mesh wire cage traps baited with palm oil kernels or bananas for small mammals and mist nets for birds. We caught frogs by hand at night. We anesthetized and sacrificed animals in accordance with the policy and protocols approved by the Institutional Animal Care and Use Committee, University of Malaya.

Figure 1. Locality of the scientific expedition: Gemas, Negeri Sembilan in Peninsular Malaysia. Type locality of *Malayfilaria sofiani*: Jeram Pasu, Kelantan.

Table 1. List of wild animals captured from the secondary forest and oil palm plantation in Gemas, Negeri Sembilan, Malaysia

	Thick blood smears	Skin snips	Dissection
Amphibia, Anura			
1. Asian grass frog *Fejervarya limnocharis*, ID no. 9-1	–	ND	–
2. Common tree frog *Polypedates leucomystax*, ID no. 10-1	–	ND	–
3. Asian grass frog *F. limnocharis*, ID no. 10-2	–	ND	–
4. Common tree frog *P. leucomystax*, ID no. 10-12	–	ND	–
5. Asian giant stream frog *Limnonectes blythii*, ID no. 10-14	–	ND	–
Reptilia, Squamata			
6. Common sun skink *Eutropis multifasciata*, ID no. 9-2	–	ND	–
7. Mangrove snake *Boiga dendrophila*, ID no. 10-13	–	ND	–
Aves, Passeriformes			
8. White-chested babbler *Trichastoma rostratum*, ID no. 10-15	–	ND	1 nematode in thoracic cavity
9. White-chested babbler *T. rostratum*, ID no. 10-23	–	ND	–
Mammalia, Scandentia			
10. Common treeshrew *Tupaia glis*, ID no. 10-3	–	–	–
11. Common treeshrew *T. glis*, ID no. 10-10	–	–	–
12. Common treeshrew *T. glis*, ID no. 10-19	1 microfilaria with sheath: 245 × 5 µm (Fig. 2a)	–	–
13. Common treeshrew *T. glis*, ID no. 10-20	–	–	3 nematode larvae in mesentery
14. Common treeshrew *T. glis*, ID no. 10-21	–	–	–
15. Common treeshrew *T. glis*, ID no. 10-22	1 microfilaria with sheath: 272 × 5 µm (Fig. 2b)	1 microfilaria with sheath: 318 × 8 µm	1 oxyurid in intestine
Mammalia, Rodentia			
16. Malaysian field rat *Rattus tiomanicus*, ID no. 10-1	–	ND	–
17. Malaysian field rat *R. tiomanicus*, ID no. 10-5	–	ND	–
18. Malaysian field rat *R. tiomanicus*, ID no. 10-6	–	ND	–
19. Malaysian field rat *R. tiomanicus*, ID no. 10-7	–	ND	–
20. Malaysian field rat *R. tiomanicus*, ID no. 10-8	–	ND	–
21. Malaysian field rat *R. tiomanicus*, ID no. 10-9	–	ND	–
22. Malaysian field rat *R. tiomanicus*, ID no. 10-10	–	ND	–
23. Malaysian field rat *R. tiomanicus*, ID no. ID no. 10-16	–	ND	–
24. Malaysian field rat *R. tiomanicus*, ID no. 10-17	–	ND	–
25. Malaysian field rat *R. tiomanicus*, ID no. 10-18	–	ND	–
26. Rajah spiny rat *Maxomys rajah*, ID no. 10-4	–	ND	–

(–): No parasites.
ND: Not done.

To search for adult filarioids, we dissected these animals under a stereomicroscope in the field, and examined the lymphatic tissues, peritoneal cavity, and subcutaneous connective tissues of the mammals in detail. We made thick blood smears that were stained with 3% Giemsa solution (pH 7.4), and took skin snips from the back of the animals to examine microfilariae (Uni *et al.*, 2002). We investigated if there were microfilariae in the blood smears and skin snips under a compound microscope in the laboratory.

RESULTS AND DISCUSSION

We found two types of microfilariae in the blood smears of two of the six common treeshrews (2/6 or 33%; Table 1). The first microfilaria from a host animal (ID no. 10-19) had a body length of 245 µm, body width of 5 µm, and nerve ring that was 51.5 µm from the anterior end (21% of body length) (Figure 2a). The tail was 30 µm long (12% of body length) with a single nucleus at the tail end. The microfilaria had a sheath. No adult filarial parasites were found in the host animal.

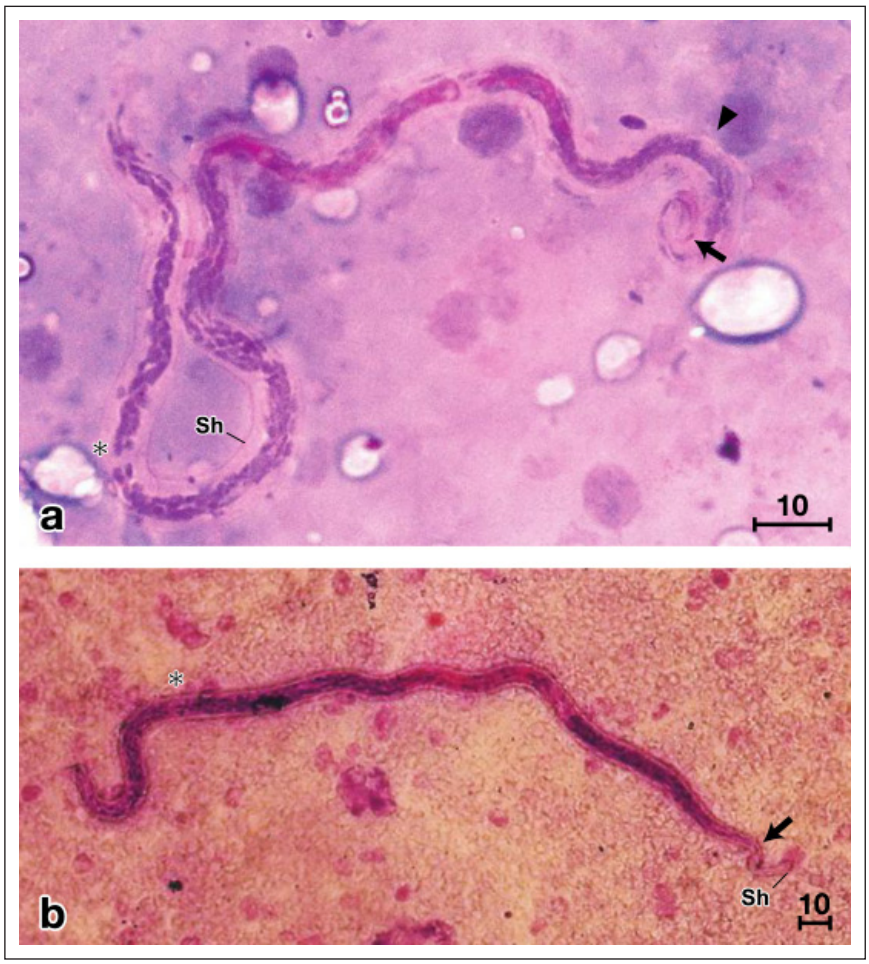

Figure 2. (a) A microfilaria in a thick blood smear of a common treeshrew (ID no. 10-19). *, nerve ring; arrowhead, anal pore; and arrow, terminal nucleus. Sh, sheath. Giemsa staining. Scale in µm. (b) A microfilaria in a thick blood smear of a common treeshrew (ID no. 10-22). *, nerve ring; arrow, terminal nucleus; and Sh, sheath. Giemsa staining. Scale in µm.

The second microfilaria that was obtained from a host animal (ID no. 10-22) had a body length of 272 µm, body width of 5 µm, and nerve ring that was 58 µm from the anterior end (21% of body length). The terminal nucleus and sheath were found in the smear (Figure 2b). In the skin snip of the same animal, only one microfilaria was detected and had a body length of 318 µm, body width of 8 µm, and was equipped with a sheath. No adult filarial parasites were found in this host animal.

One adult nematode, which was not a member of the superfamily Filarioidea, was found in the thoracic cavity of a white-chested babbler (ID no. 10-15). Three non-filarial nematode larvae were found in the mesentery of a common treeshrew (ID no. 10-20), and one oxyurid was found in the intestine of a common treeshrew (ID no. 10-22).

According to Mullin & Orihel (1972), the microfilaria of *M.* (*T.*) *dunni* detected in Giemsa-stained smears was 149 µm long and 4 µm wide. The microfilariae lack a sheath. Therefore, *M.* (*T.*) *dunni* was excluded from further consideration. The morphological characteristics of the first microfilaria (Figure 2a), including the body length, width, nerve ring position, tail length, and the presence of a terminal nucleus and sheath, were very similar to those characteristics of *M. sofiani* microfilariae recorded by Uni *et al.* (2017). Therefore, we propose the first microfilaria as *M. sofiani*.

In this study, we suggest an additional distribution of *M. sofiani* in Gemas, which is far from Kelantan. *Malayfilaria sofiani* displays more ancestral morphological and molecular characteristics than either *W. bancrofti* or *Brugia* species (Uni *et al.*, 2017). The *Wolbachia* endosymbionts that infect *M. sofiani* belong to supergroup D and are closely related to *Wolbachia* strains in *W. bancrofti* and *Brugia* species (Uni *et al.*, 2020). Therefore, we indicate coevolution between *Wolbachia* strains and their respective host filarioids in the *Wuchereria-Brugia* group of the ONC5.

The other microfilaria in the smear (Figure 2b) and the microfilaria in the skin snip of the common treeshrew (ID no. 10-22) closely resembled *B. tupaiae* in morphological characteristics such as body length, width, terminal nucleus, and sheath. In our previous studies performed in Malaysia, *B. tupaiae* was neither found in blood smears nor during dissection of common treeshrews (Uni *et al.*, 2017). The microfilaria obtained in the skin snip of the animal (ID no. 10-22) in this study was very similar to the formalin-fixed specimens of *B. tupaiae* (Orihel, 1966). The microfilaria in this skin snip appeared to have originated from the blood. Therefore, the present study reveals a new habitat for *B. tupaiae* in Gemas, Negeri Sembilan. Although adult filarioids were not detected in the host animals and sufficient microfilariae for molecular analysis were not obtained in this survey, the present morphological characteristics of microfilariae will assist in future attempts to identify filarial specimens in this area.

Acknowledgments. This study was supported by the Ministry of Higher Education, Malaysia (FRGS FP020-2012 to S. Uni). We thank Mallory Eckstut, PhD, from Edanz Group for editing a draft of this manuscript.

Conflict of Interests

The authors declare that they have no conflict of interests.

REFERENCES

Gibbons, L.M. (2010). Keys to the nematode parasites of vertebrates. Suppl Vol. Wallingford, UK: CABI, pp. 364-365.

Lefoulon, E., Bain, O., Bourret, J., Junker, K., Guerrero, R., Cañizales, I., Kuzmin, Y., Satoto, T.B.T., Cardenas-Callirgos, J.M., de Souza Lima, S., Raccurt, C., Mutafchiev, Y., Gavotte, L. & Martin, C. (2015). Shaking the tree: multi-locus sequence typing usurps current onchocercid (filarial nematode) phylogeny. *PloS Neglected Tropical Diseases* **9**: e0004233.

Mullin, S.W. & Orihel, T.C. (1972). *Tetrapetalonema dunni* sp. n. (Nematoda: Filarioidea) from Malaysian tree shrews. *Journal of Parasitology* **58**: 1047-1051.

Muslim, A., Fong, M.Y., Mahmud, R. & Sivanandam, S. (2013). Vector and reservoir host of a case of human *Brugia pahang* infection in Selangor, peninsular Malaysia. *Tropical Biomedicine* **30**: 727-730.

Orihel, T.C. (1966). *Brugia tupaiae* sp. n. (Nematoda: Filarioidea) in tree shrews (*Tupaia glis*) from Malaysia. *Journal of Parasitology* **52**: 162-165.

Otranto, D., Sakru, N., Testini, G., Gürlü, V.P., Yakar, K., Lia, R.P., Dantas-Torres, F. & Bain, O. (2011). Case report: first evidence of human zoonotic infection by *Onchocerca lupi* (Spirurida, Onchocercidae). *American Journal of Tropical Medicine and Hygiene* **84**: 55-58.

Tan, L.H., Fong, M.Y., Mahmud, R., Muslim, A., Lau, Y.L. & Kamarulzaman, A. (2011). Zoonotic *Brugia pahangi* filariasis in a suburbia of Kuala Lumpur City, Malaysia. *Parasitology International* **60**: 111-113.

Uni, S., Bain, O., Takaoka, H., Katsumi, A., Fujita, H. & Suzuki, Y. (2002). Diversification of *Cercopithifilaria* species (Nematoda: Filarioidea) in Japanese wild ruminants with description of two new species. *Parasite* **9**: 293-304.

Uni, S., Fukuda, M., Otsuka, Y., Hiramatsu, N., Yokobayashi, K., Takahashi, H., Murata, S., Kusatake, K., Morita, E., Maruyama, H., Hasegawa, H., Shiwaku, K., Ramli, R., Azirun, M.S. & Takaoka, H. (2015). New zoonotic cases of *Onchocerca dewittei japonica* (Nematoda: Onchocercidae) in Honshu, Japan. *Parasites & Vectors* **8**: 59.

Uni, S., Mat Udin, A.S., Agatsuma, T., Junker, K., Saijuntha, W., Bunchom, N., Fukuda, M., Martin, C., Lefoulon, E., Labat, A., Khan, F.A.A., Low, V.L., Cheah, P.L., Lim, Y.A.-L., Ramli, R., Belabut, D.M., Zainuri, N.A., Matsubayashi, M., Omar, H., Bhassu, S., Uga, S., Hashim, R., Takaoka, H. & Azirun, M.S. (2020). Description, molecular characteristics and *Wolbachia* endosymbionts of *Onchocerca borneensis* Uni, Mat Udin & Takaoka n. sp. (Nematoda: Filarioidea) from the Bornean bearded pig *Sus barbatus* Müller (Cetartiodactyla: Suidae) of Sarawak, Malaysia. *Parasites & Vectors* **13**: 50.

Uni, S., Mat Udin, A.S., Agatsuma, T., Saijuntha, W., Junker, K., Ramli, R., Omar, H., Lim, Y.A.-L., Sivanandam, S., Lefoulon, E., Martin, C., Belabut, D.M., Kashim, S., Abdullah Halim, M.R., Zainuri, N.A., Bhassu, S., Fukuda, M., Matsubayashi, M., Harada, M., Low, V.L., Chen, C.D., Suganuma, N., Hashim, R., Takaoka, H. & Azirun, M.S. (2017). Morphological and molecular characteristics of *Malayfilaria sofiani* Uni, Mat Udin & Takaoka n. g., n. sp. (Nematoda: Filarioidea) from the common treeshrew *Tupaia glis* Diard & Dubaucel (Mammalia: Scandentia) in Peninsular Malaysia. *Parasites & Vectors* **10**: 194.

WHO (2016). Lymphatic filariasis. http://www.who.int/lymphatic_filariasis /en/.

Yen, P.K.F. (1983). Taxonomy of Malaysian filarial parasites. In: Filariasis, Mak, J.W. (editor). Bulletin. No. 19. Institute for Medical Research Malaysia, pp. 17-35.