**Armigeres subalbatus** incriminated as a vector of zoonotic *Brugia pahangi* filariasis in suburban Kuala Lumpur, Peninsular Malaysia

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**Abstract**

**Background:** In 2011, we reported occurrence of natural human infections with *Brugia pahangi*, a filarial worm of dogs and cats, in a suburb of Kuala Lumpur, the capital city of Malaysia. Our preliminary entomological survey at that time suggested the mosquito species *Armigeres subalbatus* as the vector of the zoonotic infections. In this present report, we provide biological evidence to confirm our preliminary finding.

**Findings:** A total of 1798 adult female *Ar. subalbatus* mosquitoes was caught in the vicinity of the suburb, and 1599 were dissected for the presence of filarial larvae. Sixty-two mosquitoes were positive, and 27 of these were infected with L3 larvae. The L3 were inoculated into male gerbils. Microfilariae could be detected in the gerbils 92 days post-infection. Post-mortem on the gerbils recovered adult worms in the peritoneal cavity, heart, lungs, tail and testis. Male adult worms were confirmed to be *B. pahangi* by the ratio length of their spicules (left spicule: right spicule). Female adult worms were confirmed by the absence of minute cuticular bosses in the tail region. The worms were further confirmed to be *B. pahangi* by PCR.

**Conclusions:** Our results showed that *Ar. subalbatus* was the vector for the zoonotic *Brugia pahangi* infections. This mosquito species should now be categorised as a medically important mosquito species in Malaysia. Its role in the transmission of zoonotic *B. pahangi* must therefore be considered in future studies on filarial infections.

**Keywords:** *Armigeres subalbatus*, Vector, Zoonotic, *Brugia pahangi*, Filariasis
Malaysia [9]. In the early 1960s, it was found that *Ar. subalbatus* could serve as an efficient vector in experimental infections involving *B. pahangi*. Since then, numerous *B. pahangi*-*Ar. subalbatus* research work has been conducted. However, despite being widely used as a laboratory vector for several decades, hitherto there is no detailed description of *Ar. subalbatus* as a vector for *B. pahangi* in nature. In this report, we present biological findings to confirm *Ar. subalbatus* as a natural vector, particularly for the transmission of zoonotic *B. pahangi* filariasis.

**Methods**

**Location of entomological survey**

Entomological survey was carried out in the vicinity of Bukit Gasing–Kampung Kerinchi (3°5′25″N, 101°39′25″E – 3°6′51″N, 101°39′47″E), where zoonotic *B. pahangi* infections were previously reported [4]. This suburb consisted of houses, apartments, several construction projects and slum villages. A recreational secondary forest straddles the center of the suburb. Originally, the secondary forest was a plantation site and had been left idle for 50 years.

**Mosquito collection method**

Mosquito collection was carried out from 0600–0900 hours and from 1800–2030 hours using the human landing catches method. Approval for the collection was obtained from the University of Malaya Ethical and Research Review Committee [Ref. No. PAR/19/02/2013/AA (R)]. The predominant mosquitoes caught were *Ar. subalbatus* research work has been conducted. However, despite being widely used as a laboratory vector for several decades, hitherto there is no detailed description of *Ar. subalbatus* as a vector for *B. pahangi* in nature. In this report, we present biological findings to confirm *Ar. subalbatus* as a natural vector, particularly for the transmission of zoonotic *B. pahangi* filariasis.

**Identification of adult worm species by morphometric method**

The adult worm was mounted in glycerin in a permanent hanging drop preparation on a slide. It was then examined under the microscope which was attached with an Image Analyzer. The length of the left spicule (LS) and right spicule (RS) of male worms was measured and the LS:RS ratio was calculated. The ratio range for *B. pahangi* is 1.80-2.50:1, while for *B. malayi* is 2.90-3.80:1 [12]. The tail region of female worms was also examined to determine the presence of minute cuticular bosses, a characteristic of adult female worm of *B. malayi*, but absent in *B. pahangi*.

**Identification of adult worm species by PCR**

DNA was extracted from adult worms using DNeasy Blood and Tissue Kit (Spin-Column protocol, QIAGEN, Germany). The extracted DNA was amplified using primer pairs specific for the cytochrome oxidase I (COXI) gene of *B. pahangi* (forward primer 5′ TATTGCGCTGTATATGC 3′, reverse primer 5′ TGTATATGTGATGAC 3′). PCR was carried out in a 25 ml reaction mixture containing 10 mM Tris–HCl (pH 8·3), 2 mM MgCl2, 50 mM KCl, 0·01% gelatin, 200 mM of each deoxynucleoside triphosphate, 20 pmol of each primer, 1 U of Taq polymerase (Fermentas Life Sciences, Canada). The PCR mixture was pre-heated at 95°C for 10 min for initial denaturation before 30 cycles of amplification, which consisted of denaturation at 94°C for 1 min, annealing at 54°C for 1 min, and elongation at 72°C for 2 min. Final extension of the reaction was carried out at 72°C for 10 min. PCR product was analysed by agarose gel electrophoresis. The expected size of the PCR product was 633 bp.

**Results**

**Mosquito collection**

In the entomological survey, a total of 1878 mosquitoes was collected (Table 1). Four species of mosquitoes from two genera were found during the collection period. The predominant mosquitoes caught were *Ar. subalbatus*.
Table 1 Mosquito species and number collected in the entomological survey

| Mosquito species              | Number of mosquitoes collected (%) |
|------------------------------|------------------------------------|
| Armigeres subalbatus         | 1798 (95.7)                        |
| Armigeres kesseli            | 16 (0.9)                           |
| Aedes albopictus             | 55 (2.9)                           |
| Aedes aegypti                | 9 (0.5)                            |
| **Total**                    | **1878**                           |

mosquitoes were within the ratio range for *B. pahangi* (1.80-2.50:1) which is much smaller than the ratio of *B. malayi* (2.90-3.80:1) [12]. The 9 adult female worms were confirmed to be *B. pahangi* by examining their body cuticle in the tail region. The body cuticle of *B. pahangi* adult female worm is devoid of the minute cuticular bosses (Figure 1B), which are only seen in female *B. malayi* adult worm [12].

**Discussion**

*Armigeres subalbatus* is widely distributed in Malaysia. Previously, this species has never been considered a medically important mosquito in Malaysia as compared to other mosquitoes such as *Culex, Aedes, Mansonia* and *Anopheles* spp. Our present study highlights the role of *Ar. subalbatus* as a vector of zoonotic filariasis in Peninsular Malaysia.

Pioneering work on the vectors of *B. pahangi* in Malaysia was carried out mainly in the 1960s. Field and laboratory studies established *Ma. annulata* and *Ma. dives* as vectors of *B. pahangi* in nature [5]. The typical breeding place for *Ma. annulata* is the forest verge where open swamp and forest meet. Swamp forest having rootlets of trees, rattans and palms is the usual breeding ground for *Ma. dives*. Our study site, on the other hand, was a suburb with residential houses and flats, villages and a recreation hill park. This therefore explains the absence of *Mansonia* species in the site. The surroundings of the site were favourable for *Ar. subalbatus*, which breeds well around human dwellings with poor sanitation that contain polluted water [6]. This explains the abundance and predominance of *Ar. subalbatus* in the site as compared to other mosquito species, such as *Ar. kesseli, Ae. albopictus* and *Ae. aegypti*. The human landing catch method was used to collect the mosquitoes, and the high number of *Ar. subalbatus* obtained was surprising since it was previously reported that this species did not feed readily on humans [13]. Infection and infective rates obtained in our study were 3.9% and 1.7%, respectively. These rates are higher than those

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**Human identification and infective rates, and number of larvae recovered**

| Infection/infective rate | Number of infected mosquitoes (%) |
|--------------------------|----------------------------------|
| Total positive (infection rate) | 62 (3.9) |
| with L3ystem (infective rate) | 27 (1.7)  |
| with L2system | 32 (2.0)  |
| with L1system | 3 (0.2)  |
| **Larva stage** | **Total larvae recovered in mosquitoes** |
| L3system | 346 |
| L2system | 259 |
| L1system | 16 |

Post mortem on the gerbils was carried out 95 days post-infection. Eighteen adult worms were recovered, in which 16 were recovered in the peritoneal cavity, heart, lungs and tail of the first gerbil. Two were recovered from the testis of the second gerbil. Five of the 7 adult male worms were cleared and mounted for identification. They were confirmed to be *B. pahangi* by determining the ratio length of their spicules (LS:RS). The ratios of the adult male worms obtained were 2.47:1, 2.36:1, 2.38:1, 2.06:1 and 2.00:1 (Figure 1A), respectively. These ratios were within the ratio range for *B. pahangi* (1.80-2.50:1) which is much smaller than the ratio of *B. malayi* (2.90-3.80:1) [12]. The 9 adult female worms were confirmed to be *B. pahangi* by examining their body cuticle in the tail region. The body cuticle of *B. pahangi* adult female worm is devoid of the minute cuticular bosses (Figure 1B), which are only seen in female *B. malayi* adult worm [12].

**Species identification of adult worm by PCR**

The adult worms were further confirmed to be *B. pahangi* by PCR using primers specific for the COXI gene. The 633 bp region of the *B. pahangi* COXI gene was successfully amplified (Figure 2).
observed in previous entomological studies in endemic areas in Malaysia, which ranged from 0.1% to 3% [14-17].

A unique biological feature of *Ar. subalbatus* is its high susceptibility to *B. pahangi* but refractoriness to *B. malayi* mf infection. The mf of these filarial species are morphologically similar, although genetically they can be differentiated by various molecular methods. Whereas *B. pahangi* mf can easily develop to the infective L3 stage, *B. malayi* mf are rapidly destroyed in the haemocoel by melanotic encapsulation [18,19]. Before the advent of biochemical and molecular methods, distinguishing of *B. malayi* and *B. pahangi* mf could only be done by letting *Ar. subalbatus* feed on the infected animal or human, and detect the development of larvae in the mosquito [20]. Hence, the developing and infective larvae recovered in the *Ar. subalbatus* in our study were undoubtedly *B. pahangi*.

In laboratory experimental settings, *Ar. subalbatus* has been demonstrated to be an extremely efficient host, producing high number of L3 larvae [5]. In an experiment in which *Ar. subalbatus* was allowed to feed on a *B. pahangi* animal carrier with more than 1 mf/μl, all become infected and the larvae reached maturity as early as the 7th day after feeding. Almost all (99%) larvae were L3 by the 11th day. When fed on an animal carrier with 6.2 mf/μl, an average of 38.7 larvae per mosquito was obtained. Wharton [13] reported experimental infection index of 7.1 ± 1.1, for mf counts of 1 mf/μl. In other words, an average of 7 L3 per mosquito could be produced. In our study, a relatively high number of L3 larvae was seen in the naturally-infected mosquitoes, ranging between 7 and 25, with one reaching up to 45. This high infective rate is likely due to the size of the female *Ar. subalbatus*, which is comparatively larger than any other mosquito species. In a single blood meal, a female *Ar. subalbatus* can take an average of 4.5 μl of blood [13].

Upon ingestion of an infective blood meal by the female *Ar. subalbatus*, the mf penetrate the midgut epithelium and migrate to the thoracic musculature of the mosquito. Here, the mf moult several times to develop into infective L3. The L3 migrate to the head region and finally to the proboscis of the mosquito. The L3 are then transmitted to a vertebrate host when the infected mosquito takes a blood meal. In our study, most of the larvae were seen in

**Figure 1** Identification of species based on: (A) the ratio length of spicules (left spicule:right spicule, LS:RS) in adult male, the worm was confirmed to be *B. pahangi* with LS:RS = 2.00:1; and (B) tail region of adult female of *B. pahangi*, with body cuticle devoid of minute cuticular bosses (x40).
the usual sites in the mosquitoes: $L_1$ and $L_2$ in the thorax, and $L_3$ in the proboscis. Oddly, in some highly infected mosquitoes, $L_3$ could be found in the entire body. Zahedi et al. [21], who found developing larvae $L_2$ in the head, commented that erratic migration of larvae to unusual sites might be the consequence of heavy infection.

In our survey, *Aedes kesseli* was found but in smaller number. It shares similar morphology with *Aedes subalbatus*. The latter, however, can be identified by the presence of white scaling in the hind femur which tapers and terminates before the knee, and the broad black band on sternite 3–6. The white scaling in the hind femur of *Aedes kesseli* is broad, and the black band on its sternite is narrower [22]. Despite having morphological similarities and occupying similar habitats, none of the *Aedes kesseli* in our study was found to be infected with larvae.

**Conclusions**

Our study was conducted in a suburb where several cases of zoonotic *B. pahangi* infection were recently reported [4]. The findings of our study show that *Aedes subalbatus* was indeed the vector for the zoonotic infections. With a capacity to naturally harbour a high number of *B. pahangi* larvae and being a human biter which thrives in areas of human habitations, *Aedes subalbatus* should be considered a medically important mosquito species in Malaysia along with other mosquitoes such as *Aedes, Anopheles, Culex* and *Mansonia* spp. Its role in the transmission of zoonotic *B. pahangi* must be considered in future studies on filarial infections.

**Abbreviations**

L: Larva; LS: Left spicule; RS: Right spicule.

**Competing interests**

The authors declare that they have no competing interests.

**Authors’ contributions**

AM, MYF, and RM designed the study. AZ and SS performed field collection, mosquito identification and gerbil infection. AZ and YLL performed $L_1$ and $L_2$ in the thorax, and $L_3$ in the proboscis. Oddly, in some highly infected mosquitoes, $L_3$ could be found in the entire body. Zahedi et al. [21], who found developing larvae $L_2$ in the head, commented that erratic migration of larvae to unusual sites might be the consequence of heavy infection.

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AM, MYF, and RM designed the study. AZ and SS performed field collection, mosquito identification and gerbil infection. AZ and YLL performed molecular identification of the adult worms. AZ and MYF drafted the manuscript. All authors read and approved the final version of the manuscript.

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**References**

1. Denham DA, McGreevy PB: Brugian filariasis: epidemiological and experimental studies. Adv Parasitol 1977, 15:243–309.
2. Palmieri JR, Retwaysanto S, Masbar S, Titokusumo S, Rusch J, Manwoto HA: Evidence of possible natural infections of man with *Brugia pahangi* in South Kalimantan (Borneo), Indonesia. Trop Geogr Med 1985, 37:239–244.
3. Edeson JF, Wilson T, Wharton RH, Laing AR: Experimental transmission of *Brugia malayi* and *B. pahangi* to man. Trans R Soc Traph Med Hyg 1960, 54:229–234.
4. Tan LH, Fong MY, Mahmud R, Muslim A, Lau YL, Kamarulzaman A: Zoonotic *Brugia pahangi* filariasis in a suburbia of Kuala Lumpur City, Malaysia. Parasitol Int 2011, 60:111–113.
5. Edeson JF, Wharton RH, Laing AR: A preliminary account of the transmission, maintenance and laboratory vectors of *Brugia pahangi*. Trans R Soc Traph Med Hyg 1960, 54:439–449.
6. Rajavel AR: Larval habitat of *Armigera subalbatus* (COQ) and its characteristics in Pondicherry. Southeast Asian J Trop Med Public Health 1992, 23:470–475.
7. Liu H, Lu HJ, Liu ZJ, Jing J, Ren JQ, Liu YY, Lu F, Jin NY: Japanese encephalitis virus in mosquitoes and swine in Yunnan province, China 2009–2010. Vector Borne Zoonotic Dis 2013, 13:41–49.
8. Das P, Bhattacharya S, Palt CA, Das S, Ghosh KK, Hati AK: Diurnal man-biting activity of *Armigera subalbatus* (Coquillett, 1898) in a village in West Bengal. Indian J Med Res 1983, 78:794–797.
9. Cheong WH, Mak JW, Naidu S, Mahadevan S: *Armigera subalbatus* incriminated as an important vector of the dog heartworm *Dirofilaria immitis* and the bird Cardiofilaria in urban Kuala Lumpur. Southeast Asian J Trop Med Public Health 1981, 12:611–612.
10. Sivanandam S, Fredericks HJ: The "Innenkorper" in differentiation between the microfilariae of *Brugia pahangi* and *B. malayi* (sub-periodic form). Med J Malaysia 1966, 20:337–339.
11. Lim PKC, Sim BKL: Laboratory techniques in filariasis. Bull Inst Med Res Malaysia 1983, 19:95–104.
12. Buckley JJC, Edeson JFB: On the adult morphology of *Wuchereria sp.* (Malayi) from a monkey (*Macaca irus*) and from cats in Malaya, and on *Wuchereria pahangi* sp. from a dog and a cat. J Helminthol 1956, 301–30.
13. Wharton RH: The biology of *Manosia* mosquitoes in relation to the transmission of filariasis in Malaya. Bull Inst Med Res Malaysia 1978, 11:85–89.
14. Ramachandran CP, Cheong WH, Sivanandam S, Omar AH, Mahadevan S: Filariasis in Ulu Trengganu, West Malaysia: parasitological and entomological observations. Southeast Asian J Trop Med Public Health 1970, 1:505–515.
15. Cheah WC, Cheong WH, Mahadevan S, Lai KPF, Sivanandam S: Filariasis in Negri Sembilan - a follow-up study. Med J Malaysia 1977, 32:103–110.
16. Mak JW, Cheong WH, Omar AH, Sivanandam S, Mahadevan S: Filariasis in Perlis, Peninsular Malaysia. Med J Malaysia 1977, 31:198–203.
17. Mak JW, Lye MS, Sim BKL, Cheong WH, Lee CP: Studies on malaria and filariasis in Hilir Perak, Peninsular Malaysia. Trop Biomed 1985, 2:40–46.
18. Yamamoto H, Kobayashi M, Ogiwa N, Tsuoka H, Chiupsa Y: Studies on filariasis, VI. The encapsulation of *Brugia malayi* and *B. pahangi* larva in the mosquito, *Armigera subalbatus*. Japanese J Sanitary Zool 1985, 36:1–6.
19. Beerntsen BT, Luckhart S, Christiansen BM: *Brugia malayi* and *Brugia pahangi*: Inherent difference in immune activation in the mosquitoes *Armigera subalbatus* and *Aedes aegypti*. J Parasitol 1989, 75:76–81.
20. Cheong WH, Omar AH, Sivanandam S: An attempt to separate a mixed *Brugia* infection by biological means. Singapore Med J 1965, 5:43.
21. Zahedi M, Denham DA, White GB: An unusual site of development of *Brugia pahangi* in the mosquito, *Armigera subalbatus*. Trop Biomed 1990, 7:203–205.
22. Ramalingam S: On the restriction of *Armigera durhami* Edwards and the description of *Armigera kesseli* species (Diptera: Culicidae). Trop Biomed 1987, 4:55–65.

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