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Geographic variations in the size and behavior of common earthworms *Metaphire peguana* (Rosa, 1890) in Penang and neighboring states, Malaysia

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ABSTRACT

Metaphire peguana (Megascolecidae) is a terrestrial earthworm that was found to be widely distributed throughout the whole of Penang state during our field collecting. The differences in the average length and diameter of *M. peguana* between the island and mainland Penang population were correlated with their behavior and the environmental parameters of the biotope and soil type, pH and moisture content. Principle component analysis of the correlation matrix and dendrogram for *M. peguana* revealed significant differences in how the types of biotope and environmental factors may affect the size of *M. peguana*. Morphometric variations in *M. peguana* did not reflect any genetic difference. Concisely morphometric variation across different types of biotopes, combined with relatively low levels of gene flow, is expected to favor local adaptation of *M. peguana*.

1. Introduction

Metaphire peguana (Megascolecidae) is a cosmopolitan species of terrestrial earthworm that is widely distributed in Asia. The species was first described from Rangoon in Myanmar [1] but lately it has been reported from other localities in Myanmar, Thailand, Malaysia, Singapore, Cambodia, Java, St Paul's Cathedral Calcutta, India and Vietnam [2–4]. *Metaphire peguana* is claimed to be anthropochorous, where populations have been transported as aliens from one geographical area to another by direct or indirect human activities, typically inadvertently [2]. Accordingly, from prediction, it could be found in almost all of Southeast Asia. In an investigation throughout Thailand [3], *M. peguana* was found in a diverse variety of microhabitats, namely dipterocarp forest, deciduous forest, anthropogenic areas and even in wastewater saturated soil from households.

Based on our preliminary survey across Penang and its neighboring state in Malaysia, the collected *M. peguana* was found to exhibit a multifarious morphometric characteristic among the sampling sites, especially for the body length and diameter. However, phylogenetic analysis based upon cytochrome oxidase subunit 1 gene sequences showed no significant differences between the *M. peguana* populations at all sampling sites from Penang, Perak and Kedah state, Malaysia (data not shown), or from the reported sequences of samples from Thailand [5]. In order to fully answer the morphometric variation in *M. peguana*, further study on the impact of geographic variation or types of biotope, towards the morphological differences is necessary, since some of the specimens were collected from distinct and different habitats.

We addressed the above research question by selecting Penang state as a unique place with its small land area and availability of abundant forest types. Samples of *M. peguana* were collected from different habitats, including forests (coastal hill, low land, high land, recreational and reserved forest), parks (recreational and metropolitan) or garden (tropical spice garden) and housing area, across Penang and its neighboring states (Perak and Kedah).

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2. Materials and methods

2.1. Study site

Penang state is divided into the two parts, the island and the mainland. Altogether 26 sampling sites were examined for the presence of *M. peguana*, and at least one specimen was found in 15 of these sites (Table 1), located in the north of Peninsular Malaysia, and situated between latitude 4° 49′ to 5° 31′ N and longitude 100° 10′ to 100° 47′ E. The sampling sites were selected based on different types of biotopes around the Penang state and some sampling sites were from the neighboring states (Perak and Kedah).

2.2. Sample collection, identification and preservation

Searches for *M. peguana* were made under the leaf litter, and then the topsoil was carefully excavated manually and sorted by hand to sample earthworms of a similar morphology to *M. peguana*. The collected worms were washed with clean water to remove the unwanted debris, killed in 30% (v/v) ethanol, photographed, and transferred to 95% (v/v) ethanol for transit and storage prior to molecular and morphometric analysis when back at the laboratory. Samples were confirmed as *M. peguana* by examination of their key external and internal morphological characters, as reported [1–3,6–13], under a Stemi DV 4 ZEISS stereoscopic light microscope.

2.3. Morphometric analysis

The body length, diameter and number of segments of the collected samples were measured and counted. Note that only the body lengths of adult *M. peguana* were measured and used to plot the frequency length distribution, with juveniles from all sampling sites being excluded.

2.4. Geographic variation and environmental parameters

In an attempt to understand the geographic variation of *M. peguana,* the types of biotope where *M. peguana* was found were observed, photographed and compared among each sampling locality. Besides the biotope observation, any sampling site from which contained more than five individuals of *M. peguana* was selected for the subsequent soil analysis. Soil samples (600 g) of the 10 selected sites (with ≥ five individuals of *M. peguana*; but note site AHD has been excluded due to an insufficient soil sample) were collected and brought back to the laboratory for analysis of the soil type, pH, moisture level.

In order to examine the association of *M. peguana* from the mainland and island with the morphometric and environmental parameters, the sampling sites (from which ≥ 5 samples were taken) were clustered (Euclidean distance, complete linkage algorithm) into groups based on the relationship and similarity of 9 different variables between sampling sites using cluster observations analysis. Meaning of site codes used within the text is as given in Table 1. The variables which used in the clustering were site (mainland and island), disturbance (disturbed and undisturbed), type of biotopes (highland forest, lowland forest, recreational park, coastal hill forest and housing area), soil type (sandy loam, loamy sand, silt, sandy clay loam and silt loam), pH, moisture content, length, width and segment number of *M. peguana*. Then, the Principal Component Analysis of the correlation matrix (PCA-CM) was performed to identify any morphological differences that correlated to the type of biotope or soil parameters.

2.5. Genetic haplotypes of M. peguana at the different sampled sites

A complete genetic analysis of *M. peguana* based upon the mtDNA COI sequences. The muscular tissue (N = 12, one individual from each geographical region) of *M. peguana* (approximately 0.5 cm^2) near the anterior end was detached from the worm and used for the analyses. The detached muscular tissue was homogenized with a cutter and the total genomic DNA was extracted according to the standard protocol of the NucleoSpin® Tissue kit (Macherey-Nagel, Germany).

LCO 1490 and HCO 2198 were the universal primers used in amplifying a 650–700 bp fragment of the highly conserved regions of the mtDNA COI gene. The PCR amplifications were carried out in a reaction volume of 50 μL in a thermal cycle. The initialization step was performed for one cycle at a temperature of 94 °C for 2 min 35 cycles of denaturation step at 94 °C for 60 s, annealing step at 42 °C for 60 s and extension or elongation step at 72 °C for 90 s, followed by a final elongation which performed at a temperature of 72 °C for 5 min to ensure that the remaining single stranded DNA is fully extended. The procedure was ended with the final hold at a temperature of 20 °C. The extracted strands were then sent for sequencing. The sequence for *M. peguana* was submitted to GenBank.

Sequence alignment and editing were performed using the

Table 1
List of sampling sites for *M. peguana* in Malaysia.

Sampling site		Latitude, Longitude	Type of biotope	Number of samples	
				Adult	Juvenile
Mainland	1. Permatang Rawa (PR)	5° 22′ 09.9″ N, 100°26′57.1″ E	Housing area	10	1
	2. Ampang Jajar (AJ)	5° 08′ 43.5″ N, 100°30′09.9″ E	Recreational park	9	1
	3. Tasik Taiping (TL)	4°50′56.1″ N, 100°45′03.4″ E	Recreational park	12	1
	4. Taman Rimba Bukit Mertajam (HLBM)	5°21′31.2″ N, 100°29′36.1″ E	Lowland forest	7	1
	5. Batu Hampar (BH)	5°30′04.5″ N, E100°46′19.1″ E	Lowland forest	1	1
	6. Maxwell Hill (MH)	5°11′47.7″ N, E100°34′57.9″ E	Highland forest	2	0
Island	1. Spice garden (SG)	5°27′48.2″ N, E100°13′44.4″ E	Tropical spice garden	2	0
	2. Youth garden (YG)	5°25′ 33.4″ N,E100°18′ 04.5″ E	Recreational park	2	0
	3. Air Hitam Dam (AHD)	5°23′34.0″ N, E100°15′26.2″ E	Lowland forest	14	2
	4. Kebun Bunga (BG)	5°26′05.6″ N, E100°17′38.2″ E	Recreational park	13	1
	5. Kem Bina Negara (KBN)	5°18′04.3″ N, E100°11′05.2″ E	Coastal hill forest	21	0
	6. Tanjung Asam (TA)	5°16′59.7″ N, E100°12′33.4″ E	Coastal hill forest	5	0
	7. Taman Metropolitan Park (TMR)	5°20′45.1″ N, E100°16′20.9″ E	Recreational park	14	0
	8. Anjung Indah (AI)	5°21′01.3″ N, E100°15′08.7″ E	Highland reserved forest	17	0
	9. Penang Hill (PH)	5°25′27.8″ N, E100°16′08.6″ E	Highland forest	14	1
Total				143	9

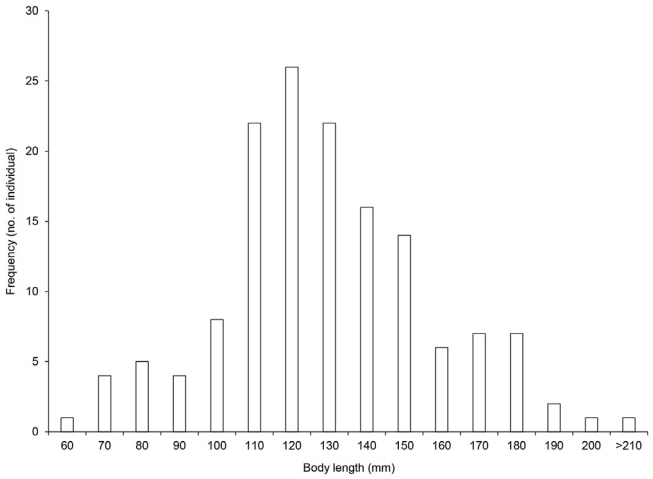


Fig. 1. Length frequency distribution of adult *Metaphire peguana* from Penang (n = 143 adults).

MEGA 6 software [14]. The aligned sequence was then applied to construct phylogenetic tree based on the Neighbor Joining (NJ) distance method, using Kimura 2-Parameter model [15] with 100 bootstrap replicates.

2.6. Analysis of soil physical and chemical parameters

Soil pH was measured using the pH-H_2O method [16]. In brief, 10 g of soil was weighed into a 50 mL centrifuge tube and 25 mL of deionized water was added and shaken on an orbital shaker for 2 h at 120 rpm. After 2 h, before opening the tube, hand shake once or twice and then pH was measured from the upper part of the suspension. The soil moisture content (wt%) was performed by the oven-dry method [16]. In brief, 5 g of soil sample was transferred to a tared dry aluminum foil boat and weighed to the nearest 0.001 g to obtain the wet weight (W_w). The sample was then dried at 105 °C to a constant weight (the dry weight, W_d), and the moisture content was then defined as $(W_d - W_w)/(W_w - \text{tare tin}) \times 100$.

The soil texture was determine accordingly based on the relative proportions of sand, silt and clay of the soil sample by referring to the soil textural triangle. The percentage of the respective soil type was obtained by using the settling method. Soil sample (without any stones, twigs and leaves) was added into the 1000 mL graduated cylinder until it was even with 500 mL line, gently tap the bottom of the cylinder on a firm surface to eliminate air spaces and followed by adding tap water to the 1000 mL line. The soil sample was then thoroughly mixed and allowed to stand for 15 min. After 15 min, the content of the graduated cylinder was slowly inverted and mixed for another 5 min. The graduated cylinder was then allowed to stand undisturbed for 24 h or longer until a clear layer of solution was observed at the top of settled soil in the graduated cylinder. The percentage of each fraction in the graduated cylinder was then determine and calculated as

$$\%\,sand = \frac{top\ of\ sand\ column,\ mL - bottom\ of\ sand\ column,\ mL}{Total\ volume\ of\ soil\ sample,\ mL} \times 100$$

$$\%\,silt = \frac{top\ of\ silt\ column,\ mL - bottom\ of\ silt\ column,\ mL}{Total\ volume\ of\ soil\ sample,\ mL} \times 100$$

$$\%\,clay = 100 - \%\,sand - \%\,silt$$

The soil samples were then grouped into textural classes according to USDA textural class on the basis of the percentages of sand, silt and clay by using a textural triangle to identify the correct textural class name.

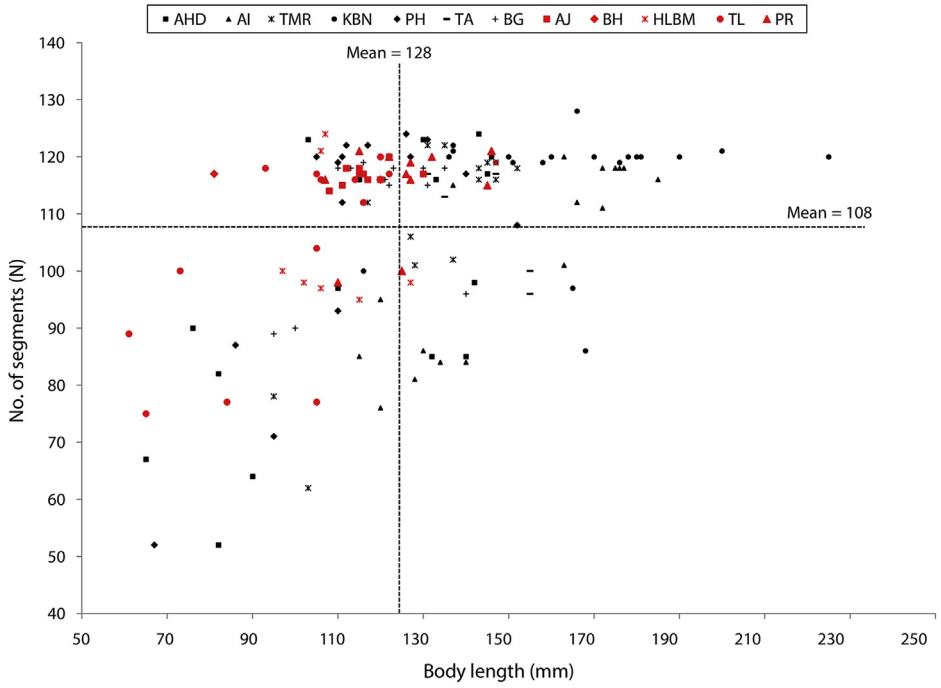


Fig. 2. Number of segments plotted against the body length of *M. peguana* collected from sampling sites. Black and red markers indicate *M. peguana* from the island and mainland, respectively. Site codes are as given in Table 1.

2.7. Statistical analysis

The data in percentage were arcsine transformed in order to improve the normality of the distribution of the data and then the transformed data were compared between sampling sites using one-way analysis of variance (ANOVA), two tailed T test and Kruskal-Wallis tests, performed with the MINITAB software. Data are shown as the mean ± one standard deviation unless indicated otherwise.

3. Results

3.1. Morphological traits of M. peguana

A total of 143 individuals of *M. peguana* were collected from the 15 sampling sites across Penang, Perak and Kedah state. The lengths of *M. peguana* from all sampling sites were normally distributed (Fig. 1). The length of 120 mm (n = 26) was the most abundant, followed by length of 110 mm and 130 mm (N = 22) while the only one individual was recorded for a length of 60 mm or > 200 mm, respectively (Fig. 1).

The relationship between the body length and the total number of segments of *M. peguana* is plotted in Fig. 2, where an apparent non-linear relationship between the body length and number of segments was found, with the number of segments approaching an asymptote at around 120 as the body length increased, and so a low correlation ($R^2 = 22\%$) was found between the body length and the total number of segments. The shortest length of *M. peguana* recorded in Penang was 61 mm, whereas the longest was about 225 mm, with a mean and median body length of *M. peguana* of 127.7 ± 28.6 mm and 126.5 mm, respectively.

In terms of the total number of segments, adult *M. peguana* (n = 143) had an average of 108.3 ± 16.3 segments while juveniles (n = 9) had a similar average number of segments of 108.1 ± 13.2. Thus, there was no significant difference between the mean number of segments for worms with and without clitella, even if newly hatched.

3.2. Geographical variability

3.2.1. Morphometrics of M. peguana

The body lengths and diameters of *M. peguana* were significantly different (P < 0.05) between the 10 sampling sites (from which ≥ five samples were taken), as shown in Fig. 3. The longest average body length (163.7 ± 24.7 mm) was recorded at KBN, while the widest average diameter (5.6 ± 0.9 mm) was at TA. The *M. peguana* from TL and AJ were the shortest (97.6 ± 20.7 mm) and thinnest (3.4 ± 0.4 mm) of all the sampling sites.

3.2.2. Morphological differences between mainland and island

The 10 sampling sites (from which ≥ 5 samples were taken) were clustered into groups based on the morphometric data and environmental parameters (Fig. 4). For the *M. peguana* data, 8 clusters are evident in the final partition. Generally, the *M. peguana* from 10 sampling sites were clustered into two main groups based on the disturbance (disturbed and undisturbed). Under undisturbed group, the group was divided into two subclusters, where KBN and TA population were more similar to each other than to AI in term of type of biotopes.

Under disturbed group, BG and TMR has the highest similarity level (91.42) where both localities were the recreational park at the island. TL and PH were clustered into the same group based on the highest similarity level in environmental parameters but yet both populations were highly separated into island (PH) and mainland (TL). AJ and PR were the mainland population with slightly

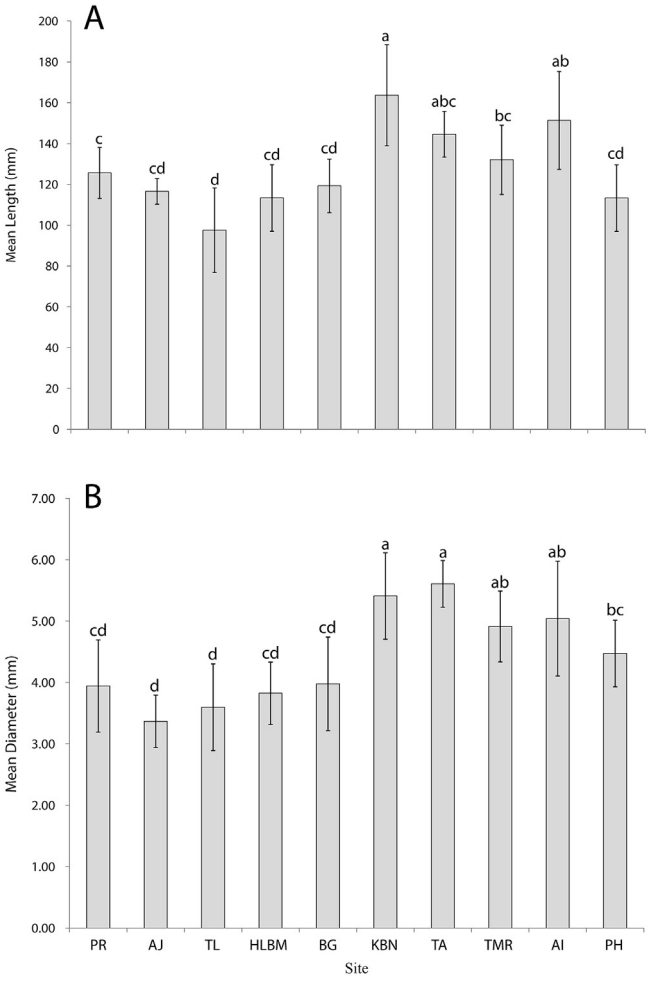


Fig. 3. Mean (A) length and (B) diameter of *M. peguana* collected from different sampling sites in Penang state. Different letters above bar chart indicate a significant difference (P < 0.05; one way ANOVA). Site codes are as given in Table 1.

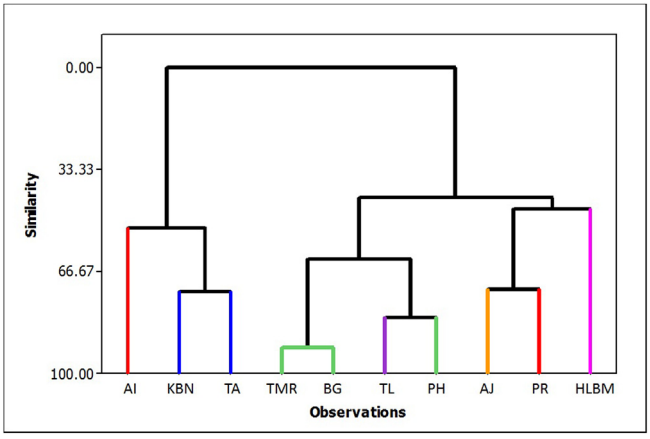


Fig. 4. Cluster of the sampling sites for *Metaphire peguana* based on morphometric data and environmental parameters. Site codes are as given in Table 1.

dissimilarity (27.52) based on soil type and type of biotope. HLBM the lowland forest was the lowest similarity among all the sampling localities.

In general, *M. peguana* from the island were significantly longer

Table 2
Mean body length, diameter and number of segments of *M. peguana* collected from the mainland (four sites) and island (six sites) of Penang state.

Morphometric	Mainland (mm)	Island (mm)	Statistics (P value)	CI
Length	112.5 ± 18.4	134.5 ± 29.5	0.01	22.06
Diameter	3.7 ± 0.69	4.8 ± 0.9	0.01	1.08
Number of segments	109.8 ± 13.0	107.6 ± 17.8	0.437	−2.13

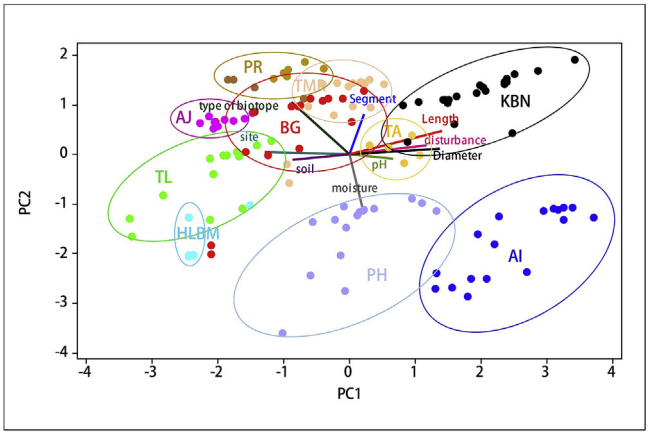


Fig. 5. PCA-CM analysis of the correlation between morphometric data and environmental parameters of the sampling sites. Site codes inside the ellipses are as given in Table 1.

Table 3
Mean body length and diameter (mean mm ± SD) and the number of segments of *M. peguana* collected from different types of biotopes. Means within a column followed by a different superscript letter are significantly different (P < 0.05;*****).

Type of biotopes	Length***** (mm)	Diameter***** (mm)	No. of segments
Housing area	125.7 ± 12.5^{bc}	3.9 ± 0.8^{c}	114.8 ± 8.1
Recreational park	116.1 ± 20.2^{c}	4.0 ± 0.9^{c}	109.0 ± 14.6
Lowland forest	113.4 ± 16.3^{bc}	3.8 ± 0.5^{c}	106.5 ± 12.4
Coastal hill forest	160.0 ± 23.8^{a}	5.5 ± 0.7^{a}	115.2 ± 10.2
Highland forest	133.5 ± 29.5^{b}	4.8 ± 0.9^{b}	104.6 ± 19.0

than those from the mainland (two sample T test, P = 0.01), by some 14.00–30.12 mm. Likewise, the diameters of *M. peguana* were also significantly greater in the island population than in the mainland (Table 2). However, there was no significant difference in the number of segments observed in *M. peguana* from the mainland and island Penang (Kruskal-Wallis test, P = 0.823).

3.2.3. Correlation between the morphometrics, the type of biotope and the soil texture, pH and moisture content

Metaphire peguana was found in different types of biotopes around Penang state, specifically in housing area, recreational park, lowland forest, coastal hill forest and highland forest. The first principal component has variance (eigenvalue) 3.0027 and accounts for 33.4% of the total variance. The coefficients listed under PC1 show the scores of each variables tested:

$$\begin{aligned} PC1 = {} & 0.473\ \text{Length} + 0.459\ \text{Diameter} + 0.392\ \text{Disturbance} \\ & + 0.219\ \text{pH} + 0.076\ \text{Segment} + 0.074\ \text{Moisture} \\ & - 0.289\ \text{Soil} - 0.294\ \text{Type of biotope} - 0.428\ \text{Site} \end{aligned}$$

The second component has variance (eigenvalue) 1.8839 and accounts for 20.9% of the data variability. This component is contrasting the morphometric data (length, diameter and segment), type of biotope and disturbance with the moisture, pH and type of soil to some extent. Together, the first two and three principal components represent 54.3% and 67.7%, respectively, of the total variability. The scatter plot provides this information visually (Fig. 5).

The *M. peguana* from the undisturbed or least disturbed areas within the same type of biotope had a longer average body length, with the coastal hill forest population having the significantly longest length (160.0 ± 23.8 mm) and widest diameter (5.5 ± 0.7 mm) among the biotopes, followed by highland forest, while the shortest length (113.4 ± 16.3 mm) and thinnest diameter (3.8 ± 0.5 mm) *M. peguana* were from the lowland forest (Table 3).

No significant difference in the size of *M. peguana* collected from the lowland forest and recreational park was found (Table 3). From observation, the microhabitat at the coastal hill forest where *M. peguana* was found looked different to the other sites. As shown in Fig. 6, *M. peguana* was mainly found freely moving under the leaf litter at the coastal hill forest (Fig. 6A), while *M. peguana* was burrowing in the soil at about 5–10 cm depth in the lowland forest (Fig. 6B), recreational park (Fig. 6C) and housing area (Fig. 6D). However, there was no significant difference in the number of segments observed in *M. peguana* from the same or different types of biotope, as well as between the mainland and island (Kruskal-Wallis test, P = 0.823).

In this study, *M. peguana* was found to be widely distributed in different types of soil texture, with the longest and widest average sized samples being found in loamy sand and silt loam, respectively, suggesting these soil types may be more suitable for large sized *M. peguana*. In contrast, silt may not an ideal soil texture for *M. peguana* as a habitat since the populations in this type of soil were the shortest and thinnest samples found (Table 4). A soil pH of 7.2 and a moisture content of ≤50% were associated with longer and wider worms with significantly higher number of segments (P < 0.05), and so these may be the most preferred soil parameters for *M. peguana* to undergo their life. Soil with a lower pH or higher moisture content revealed smaller average sized *M. peguana*.

3.2.4. Genetic haplotypes

The mitochondrial sequences of the *M. peguana* from all sampling location were composed of 658 base pairs (bp) of partial COI gene. The NJ tree of *M. peguana* showed very low genetic distances among the 10 geographical locations (Fig. 7). However, the haplotypes of *M. peguana* at the sampled locations clearly showed that the morphometric of *M. peguana* was obviously different in size specifically their length as well as the diameter.

4. Discussion

Geographic variations can have a crucial effect on the genetics and morphology of animal populations. *Metaphire peguana* from this study in Penang (Malaysia) had a length range of 51–225 mm, which was slightly shorter than that reported previously for *M. peguana* populations from Thailand at 95–220 mm [3] and the Philippines at 140–240 mm [4].

The *M. peguana* from Penang were significantly shorter and thinner at sites where the majority of them were found burrowing in the soil, while at the sites where they were found under leaf litter

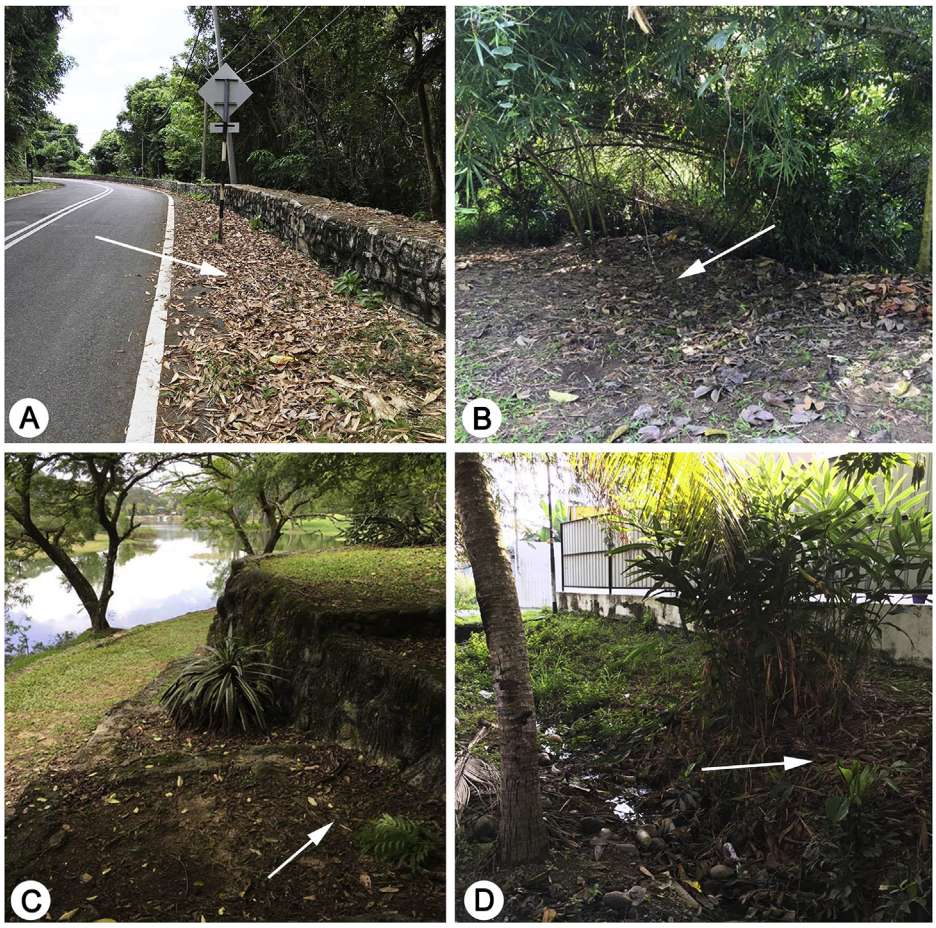


Fig. 6. Photographs showing examples of the different types of biotopes where *M. peguana* were found (arrow pointed). (A) coastal hill forest, (B) lowland forest, (C) recreational park and (D) housing area.

(coastal hill and highland forests) were much wider and longer. Such physical characteristics of *M. peguana* may impose size-dependent constraints on the type of habitat and the habits of the worms, due to the greater required pressure for burrowers than non-burrowers. Thus thinner bodies are likely advantage to overcome the problem of burrowing, especially in harder ground [17].

Different types of habitat were significantly correlated with the differences in the size of *M. peguana*. Basically *M. peguana* from the housing area, recreational forest and lowland forest was found burrowing in the soil at about 10 cm deep, while those from coastal hill and highland reserved forests were found under leaf litter. Thus the type of habitat potentially influenced the average size of *M. peguana* populations. As previously reported [18,19], different types of earthworm produce a different type of force, where a good circumferential muscle (and so a broader relaxed diameter) is necessary for crawling worms to squeeze in between rocks, litter and debris as well as allowing the worms to search and access new habitats, which results in larger sized worms. This notion is consistent with the free crawling *M. peguana* found under leaf litter in this study which had a significantly wider diameter compared to the thinner burrowing *M. peguana*. Another possible reason that the crawling *M. peguana* are larger in length and diameter is because they can more easily access new habitats or searching for food compared to the burrowing *M. peguana*. However, this hypothesis is yet to be supported.

Soil pH is a key parameter that is important for worms to grow, and it was among the first soil parameters studied for its effect on earthworm abundance [20]. Different species of earthworms have different pH preferences. For example, several species of *Bimastos* [21], *Dendrodrilus rubidus*, *Dendrobaena octaedra* and *Lumbricus rubellus* [22] are acid tolerant, while some endogeic earthworms clearly prefer a pH of 6–7 [23], and *Eisenia fetida* has been reported to prefer slightly alkaline soil with a pH between 7.0 and 8.0 [24].

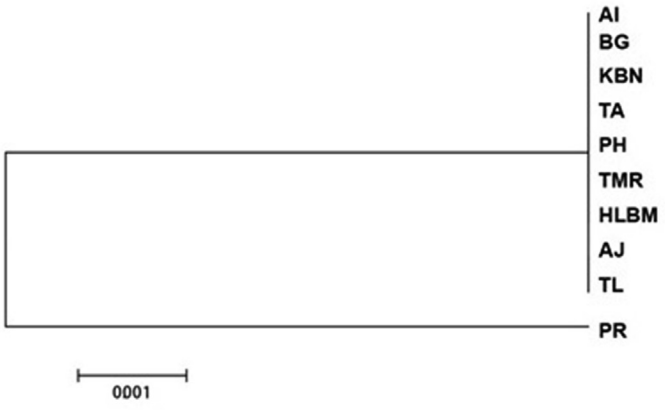
Soil moisture content is another parameter that can greatly influence earthworm activities, number and biomass, since water constitutes 75–90% of the body weight of earthworms [25] and so water loss must be minimized. It was reported that the largest numbers of earthworms occurred in soil with a moisture content between 12 and 30% [26] However, the apparent requirement of soil moisture for *M. peguana* in Penang would appear to be slightly higher ($\leq$50%), suggesting that the required soil moisture content may vary with earthworm species. For example soil moisture content as high as 80% was reported to be most favorable for *Eudrilus eugeniae* in waste management [27].

Low heterogeneity in population of *M. peguana* was also observed in Thailand as revealed by analysis of COI sequences and nuclear allozyme [5]. Morphometric variation across different types of biotopes, combined with relatively low levels of gene flow, is expected to flavor local adaptation of *M. peguana*. This is clearly indicated that *M. peguana* do not have very specific requirements to survive within multiple types of habitat.

Overall, the results of this study indicate that the size of *M. peguana* varies among populations in Penang and is mainly influenced by the habitat quality, such as type of biotope, soil texture and soil parameters.

Table 4
Mean body length and diameter (mean mm ± SD) and the number of segments of *M. peguana* collected from different soil physiological parameters where *M. peguana* were found. Means within a column followed by a different superscript letter are significantly different ($P < 0.05$;*****).

Parameters		Length***** (mm)	Diameter***** (mm)	No. of segments
Soil texture	Silt	112.6 ± 19.5^{c}	3.9 ± 0.8^{d}	109.8 ± 16.1^{a}
	Sandy clay loam	113.4 ± 16.3^{bc}	3.8 ± 0.5^{cd}	106.5 ± 12.4^{a}
	Sandy loam	135.8 ± 26.7^{ab}	4.5 ± 1.0^{bc}	105.8 ± 14.7^{a}
	Loamy sand	151.0 ± 26.8^{a}	5.2 ± 0.7^{b}	113.2 ± 14.0^{a}
	Silt loam	144.6 ± 11.2^{ab}	5.6 ± 0.4^{a}	108.6 ± 9.9^{a}
pH	6.7	113.4 ± 16.3^{ab}	3.8 ± 0.5^{b}	106.5 ± 12.4^{a}
	6.9	116.9 ± 15.4^{b}	3.9264 ± 0.8^{b}	110.1 ± 11.7^{a}
	7	132.1 ± 17.0^{ab}	4.9 ± 0.6^{ab}	108.0 ± 17.8^{a}
	7.1	131.6 ± 14.8^{ab}	4.5 ± 1.0^{ab}	112.9 ± 8.9^{a}
	7.2	142.7 ± 34.1^{a}	5.0 ± 0.9^{a}	112.8 ± 16.4^{a}
	7.3	125.2 ± 30.8^{ab}	4.2 ± 1.1^{b}	106.1 ± 15.6^{a}
Moisture*****	≤50	135.3 ± 26.2^{a}	4.5 ± 1.0^{a}	113.4 ± 11.7^{a}
	51–100	111.9 ± 24.9^{b}	4.3 ± 1.0^{a}	105.8 ± 18.5^{b}
	101–150	115.2 ± 11.5^{b}	3.6 ± 0.5^{b}	112.2 ± 9.6^{ab}



locality		Length (mm)	Diameter (mm)	total segment	setae between MP
Island	AI	175	5.06	118	13
	BG	135	5.1	118	9
	KBN	160	5.8	120	7
	TA	155	6.02	100	12
	PH	152	5.7	108	12
	TMR	131	5.18	122	11
	HLBM	97	4.11	100	12
mainland	AJ	122	2.7	120	8
	TL	120	4.57	120	13
	PR	125	5.03	100	10

Fig. 7. Bootstrap value of the corresponding NJ tree and the detail morphometric data for *M. peguana*. Site codes inside the figure are as given in Table 1. MP = Male Pole.

5. Conclusions

In conclusion, morphometric variations in *M. peguana* did not reflect any genetic difference. By contrast, we found that the environmental factors appear to play an important role in size and behavior of *M. peguana*. *M. peguana* from the island and undisturbed or least disturbed areas within the same type of biotope were significantly longer than those from the mainland and disturbed area. Morphometric variation across different types of biotopes, combined with relatively low levels of gene flow, is expected to favor local adaptation of *M. peguana*.

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