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ORIGINAL ARTICLE

Mitochondrial Genetic Diversity of the Freshwater Snail *Melanoides tuberculata*

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Abstract

According to geological history, Peninsular Malaysia and Borneo formed at different times and were once connected during Quaternary glaciations. To determine how this history has influenced phylogeography, our study examined the population genetic structure of the tropical freshwater gastropod *Melanoides tuberculata* across Peninsular Malaysia and Borneo using the sequences from mitochondrial DNA 16S rRNA and cytochrome oxidase subunit I genes (1168 bp). In total, 104 specimens were collected from seventeen populations. All mtDNA haplotypes were identified as belonging to two highly divergent lineages, and these lineages were almost allopatric in their distributions. Our study found that the freshwater fauna in Malaysia might be divided into four regions: northeast Peninsular Malaysia, northwest Peninsular Malaysia, south Peninsular Malaysia, and Borneo. The phylogeography of *M. tuberculata* in Malaysia was shaped by the landforms of Peninsular Malaysia and by the paleo-river systems in the Sunda continental shelf. In addition, our study found that these two lineages in Malaysia have invaded the globe. These results suggest that Malaysia is located in important shipping lanes throughout the world, and the populations of *M. tuberculate* might be widely distributed throughout the world by shipping.

Keywords Malaysia · *Melanoides tuberculate* · Population genetics · Snail · Sundaland

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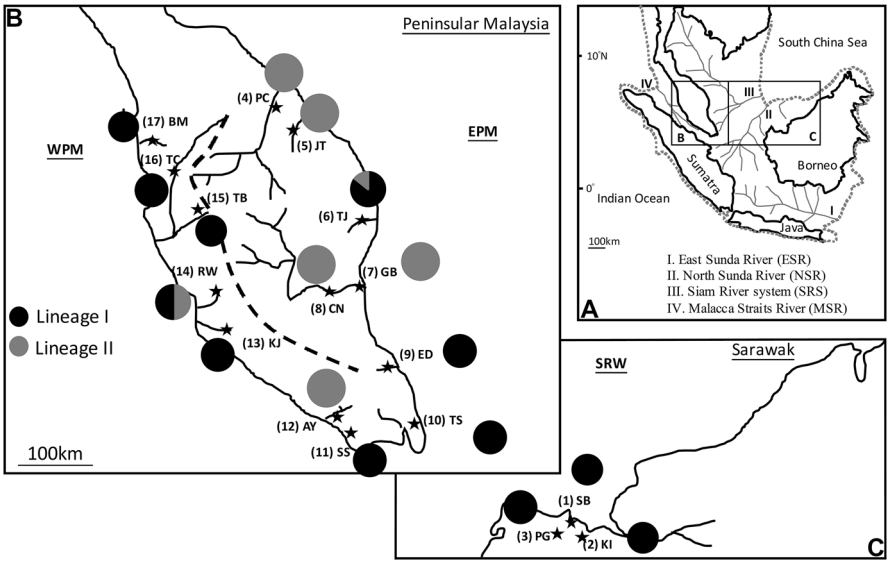

Fig. 1 Map showing the sampling localities of *Melanoides tuberculata* used in this study (asterisks). The pie charts are the frequencies of the mtDNA lineages (Fig. 3) in each population

Introduction

Peninsular Malaysia, Borneo, southern Thailand, southern Indo-China, Sumatra, Java, and the Sunda continental shelf (the present-day shallow sea) are defined as Sundaland in Paleogeology (Hanebuth et al. 2011). Sundaland is considered a biodiversity hotspot by the organization Conservation International based in Washington DC (Cox and Moore 2000). It has been documented that this high biodiversity is because of the complex geological history of this area and the effects of Pleistocene sea-level changes (Voris 2000). During Quaternary glaciations, the Sunda continental shelf was exposed by the sea-level dropping. At this time, four major drainage systems existed on the Sunda continental shelf (Fig. 1a; Voris 2000). The Siam River system (SRS) collected from the south to the tip of Peninsular Malaysia, and the North Sunda River system (NSR) collected from eastern Sumatra, western Borneo, and possibly south Peninsular Malaysia. These two rivers emptied into the present-day South China Sea. The East Sunda River (ESR) was located from the present-day Java Sea and eastward. The Malacca Straits River (MSR) drained northwestward between northern Sumatra and western Peninsular Malaysia. Many phylogeographic studies of freshwater species have shown that these paleo-river systems in the Sunda continental shelf caused gene flow among islands during Pleistocene glaciations (e.g., Inger and Chin 2002; McConnell 2004; De Bruyn et al. 2004; Ryan and Esa 2006).

In addition, the longitudinal Main Range of Peninsular Malaysia might play an important role in isolating populations on either side of the mountains. Yeo et al. (2007) proposed that two largely peninsular groups of the freshwater crab genus

Johora appeared to have fairly distinct distributions on either side of the Main Range of Peninsular Malaysia.

To determine how this complex system has influenced phylogeography, our study examines the genetic patterns of the tropical freshwater snail *Melanoides tuberculata* (Müller 1774) since it is restricted to freshwater systems and therefore provides opportunities for testing the influences of geological events on the distribution of genetic variations (e.g., Chen et al. 2007; Hsu et al. 2014). *Melanoides tuberculata*, red-rimmed Melania, is a tropical freshwater gastropod in the family Thiaridae. This taxon was originally described from India and is common to freshwaters within its native distributional range, which covers tropical regions, although it has been introduced in many other tropical and subtropical areas worldwide (e.g., Dundee and Pain 1977; Madsen and Frandsen 1989; Duggan 2002; Santos et al. 2007; Reeves et al. 2008; Lasso et al. 2009; Vázquez and Perera 2010). Thus, the objectives in our study are to (1) establish the DNA barcodes of *M. tuberculata* in Malaysia, (2) characterize the phylogeography of *M. tuberculata* in Malaysia, and (3) examine the relationships among populations of *M. tuberculata* in Malaysian accessions and other worldwide accessions available at GenBank. To achieve the above objectives, genetic variability was analyzed by the mitochondrial DNA (mtDNA) cytochrome oxidase subunit I (COI) and 16S rRNA (16S) genes. mtDNA sequences are usually analyzed in studies of animal phylogeography (e.g., Chen et al. 2007; Hsu et al. 2014). Among mtDNA genes, the COI gene is used in DNA barcodes (e.g., Folmer et al. 1994; Hebert et al. 2003), and 16S rRNA is used to resolve phylogenies (e.g., Guo et al. 2005; Liu et al. 2018).

Methods

Study Site and Taxon Sampling

The territories of Malaysia include two parts: East (Sarawak, Borneo of Malaysia) and West (Peninsular Malaysia) Malaysia, which are separated by the South China Sea. Peninsular Malaysia is the most southern region in Asia, and Borneo is a large island between Asia and Australia. To examine the relationships within and between Borneo and Peninsular Malaysia, our study sampled specimens in Malaysia.

In total, specimens were collected from seventeen populations (Fig. 1). These seventeen populations were divided into three regions, two regions in Peninsular Malaysia, i.e., western Peninsular Malaysia (WPM) and eastern Peninsular Malaysia (EPM), and one in Sarawak (SRW; Borneo part of Malaysia). A total of 104 specimens were collected (Table 1; Fig. 1). These samples were fixed and stored in 100% ethanol. All specimens were stored in the National Museum of National Science in Taiwan. Sequences of *M. tuberculata* from other regions were also directly obtained from GenBank for comparison (Tables S1 and S2). *Tarebia granifera* (COI: AY958760; 16S: EF382644) was used as an outgroup.

Table 1 Samples of *Melanoides tuberculata* used for mtDNA analyses, location, code, haplotype diversity (h), and nucleotide diversity (%, θ_π and θ_ω) (for sampling site number see Fig. 1)

Region	Drainage	Population	Code	Size	Latitude (N)	Longitude (E)	H	θ_π	θ_ω	Habitat
Sarawak (SRW)				**18**			**0.647**	**0.745**	**0.500**	
	Sarawak River	Sarawak River Barrage (1)	SB	6	1°34′24.33″	110°24′33.54″	0.000	0.000	0.000	ditch
		Kampong Iboi (2)	KI	6	1°31′9.24″	110°42′38.91″	0.000	0.000	0.000	ditch
		Pisang Garden (3)	PG	6	1°32′35.92″	110°19′24.46″	0.600	0.258	0.188	ditch
East of Peninsular Malaysia (EPM)				**43**			**0.849**	**5.795**	**2.792**	
	Kelantan River	Pulai Chondong (4)	PC	6	5°54′52.44″	102°12′8.02″	0.000	0.000	0.000	ditch
	Besut River	Jertih (5)	JT	6	5°44′17.64″	102°29′30.31″	0.333	0.029	0.038	ditch
	Dungun River	Tanjong Jara (6)	TJ	7	4°49′11.81″	103°25′9.89″	0.286	3.229	4.613	stream
	Pahang River	Gambang (7)	GB	6	3°41′59.99″	103°7′20.78″	0.533	0.046	0.038	stream
		Chini (8)	CN	6	3°23′23.02″	102°56′35.09″	0.333	0.057	0.075	ditch
	Endau River	Endau (9)	ED	6	2°36′56.36″	103°40′23.00″	0.000	0.000	0.000	ditch
	Johor River	Tanjong Surat (10)	TS	6	1°34′36.49″	104°7′9.18″	0.000	0.000	0.000	ditch
West of Peninsular Malaysia (WPM)				**43**			**0.672**	**4.846**	**2.872**	
	Lenek River	Sungai Suloh (11)	SS	5	1°46′55.75″	102°57′26.61″	0.000	0.000	0.000	ditch
		Sungai Ayam (12)	AY	7	1°47′1.94″	102°56′31.27″	0.571	0.049	0.035	stream
	Langat River	Kajang (13)	KJ	6	3°0′56.22″	101°46′24.33″	0.000	0.000	0.000	ditch
	Selangor River	Rawang (14)	RW	8	3°19′30.67″	101°33′34.97″	0.571	6.459	4.359	ditch
	Perak River	Tambun (15)	TB	6	4°37′9.44″	101°9′35.40″	0.000	0.000	0.000	ditch
		Tasik Chenderoh (16)	TC	6	4°58′22.06″	100°56′0.03″	0.000	0.000	0.000	ditch
	Juru River	Bukit Mertajam (17)	BM	5	5°21′27.69″	100°28′27.27″	0.000	0.000	0.000	stream
Total				**104**			**0.889**	**5.707**	**2.630**	

Bold values indicate the statistics in the region

Genetic Analyses

Genomic DNA was extracted from muscle tissue by a Tissue & Cell Genomic DNA Purification Kit (GeneMark-DP021). The COI and 16S genes were amplified using polymerase chain reaction (PCR) with primers adapted from Folmer et al. (1994) and Simon et al. (1994). The COI gene was amplified using primers LCO-1490 (5′-GGTCAACAAATCATAAGATATGG-3′) and HCO-2198 (5′-TAAACTTCAGGG TGACCAAAAAATCA-3′). The 16S gene was amplified using primers 16Sar (5′-GCCTGTTTAACAAAAACAT-3′) and 16Sbr (5′-CCGGTCTGAACTCAGATC AGATCACGT-3′). Each 50 µl PCR reaction mixture contained 5 ng of template DNA, 5 µl of 10 × reaction buffer, 5 µl of dNTP mix (10 mM), 5 pmol of each primer ,and 2 U of Taq polymerase (Promega, Madison, WI, U.S.A.). The PCR was programmed on an MJ Thermal Cycler as one cycle of denaturation at 94° C for 3 min and 35 cycles of denaturation at 94° C for 35 s, annealing at 50° C for 60 s and extension at 72° C for 1 min, followed by extension at 72° C for 7 min and storage at 4° C. The purified PCR products were run on an ABI 377 automated sequencer (Applied Biosystems, Foster City, CA, U.S.A.). The chromatograms were checked with CHROMAS software (Technelysium, AU).

The nucleotide sequences were aligned with Clustal X 1.81 (Thompson et al. 1997) and adjusted manually. The substitution model used for the phylogenetic reconstructions was generated in jModelTest 2 (Darriba et al. 2012) based on Akaike information criterion (AIC) scores. The HKY model (Hasegawa-Kishino-Yano) with gamma distribution (HKY+G) was selected as the best model of nucleotide substitutions. The maximum likelihood (ML) analyses used the programs in DAMBE v. 5.3.78 (Xia 2013) and MEGA 6 (Tamura et al. 2013). In MEGA, the outgroup was not used, and the ML topology was identified after an ML heuristic search conducted using the best NJ tree as starting tree. In DAMBE, *Tarebia granifera* was used as an outgroup, and the parameters were determined: base frequencies, transition/transversion ratio, search for best, and shape parameter of gamma distribution. For all phylogenetic analyses, all missing data were included, and the bootstrapping was performed with 1000 replications. Three data in Malaysia [658-bp COI sequences (KP284130-39), 510-bp 16S sequences (KP284118-28), and combined COI and 16S], and two data in the world (280-bp 16S sequences Table S1 and 580-bp COI sequences in Table S2) were examined using MEGA and DAMBE. The topologies were identical (results not shown).

The levels of intrapopulation genetic diversity were estimated with indices of haplotype diversity (h) (Nei and Tajima 1983) and nucleotide diversities (θ_π and θ_ω) (Jukes and Cantor 1969) in DnaSP 4.10.8 (Rozas et al. 2003). Comparing estimates of current (θ_π) and historical (θ_ω) genetic diversity provides insight into population dynamics over recent evolutionary history (Templeton 1993). Differences between these statistics indicate population decline (if $\theta_\pi < \theta_\omega$) or growth (if $\theta_\pi > \theta_\omega$). The existence of a phylogeographic structure was examined following the method of Pons and Petit (1996) by calculating two genetic differentiation indices (G_{ST} and N_{ST}) in DnaSP. If N_{ST} is larger than G_{ST}, the population differentiation is significant.

The nucleotide divergences among samples were estimated with pairwise uncorrected genetic distance in MEGA 6. Statistical Dispersal-Vicariance Analysis

(S-DIVA) v. 1.9, a program that complements DIVA, was employed to determine the statistical support for the ancestral range reconstructions (Ronquist 1997; Yu et al. 2010) and to determine the possible diversification scenarios of *M. tuberculata*. The tree file formats used in S-DIVA were generated by the program BEAST 1.8.0 (Drummond et al. 2013) with 10^7 MCMC steps, where the first 10% was burn-in. The analysis was performed using the 'maxareas = 3' option.

Results

Genetic Variations in Malaysia

A total of ten COI haplotypes (658 bp; KP284130-39) and eleven 16S haplotypes (510 bp; KP284118-28) were obtained for the 104 *M. tuberculata* specimens. The sequences were A+T rich (62.1% for 16S and 62.5% for COI). Our study used the combined dataset (COI+16S, hereafter mtDNA) to examine the phylogeny of *M. tuberculata*. In total, thirteen unique haplotypes were defined by 233 variable sites. Among these 13 haplotypes, eight haplotypes (H5-6 and H8-13; private haplotypes) were only displayed in one population, and others (H1–4 and H7; shared haplotypes) were shared by more than two populations (Table 2). The most widespread haplotypes were H1 and H7. H1 was distributed in four populations (KJ, TB, TC and BM) in WPM, and H7 was distributed in five populations (PC, JT, TJ, AY, and RW) in Peninsular Malaysia. The populations in EPM had the most private haplotypes (six haplotypes; H5, H9–13), and SRW and WPM only contained one private haplotype each, H6 and H8, respectively (Table 2).

The ML trees of 16S (Fig. 2a), COI (Fig. 2b) and combined data (Fig. 3) showed an identical topology. In the mtDNA phylogeny (Fig. 3), thirteen haplotypes were sorted into two major lineages, hereafter lineages I and II. Lineage I included most populations except for four populations (PC, JT, GB, and CN) in EPM and one population (AY) in WPM (Figs. 1 and 3; Table 2). Lineage II contained seven populations in Peninsular Malaysia, five populations (PC, JT, TJ, GB, and CN) in EPM and two populations (AY and RW) in WPM. These two lineages were only sympatrically distributed in two populations, TJ and RW. Lineage I included six haplotypes (H1–H6), and lineage II contained seven haplotypes (H7–H13). The two most widespread haplotypes, H1 and H7, were sorted into these two lineages, respectively (Table 2). Samples of each population were nested within one lineage, except for two populations, TJ and RW. Lineage I was separated into three haplogroups, Ia–Ic (Fig. 3; Table 2). These three haplogroups were allopatric. Haplogroup Ia was distributed in Borneo and south Peninsular Malaysia; Ib was only distributed in one population, SB, in the SRW region; and Ic was distributed in the WPM region. The sequence divergence between lineages I and II was 12.4%. Within lineage I, pairwise divergences among haplogroups Ia-Ic ranged from 1.5 to 2.4%.

The haplotype diversities (h) were remarkably low within the populations, ranging between 0.000 and 0.600 (Table 1); most populations displayed a single haplotype ($h = 0.000$). The nucleotide diversity (θ_π) in the population of each region was the highest in EPM (5.795) and the lowest in SRW (0.745) (Table 1). The estimates

Table 2 The haplotype frequencies in each population

Pop.	MtDNA lineage	N	Lineage I						Lineage II						
			H1	H2	H3	H4	**H5**	**H6**	H7	**H8**	**H9**	**H10**	**H11**	**H12**	**H13**
SRW	**Ia, Ib**	**18**													
SB (1)	Ib	6						**6**							
KI (2)	Ia	6		6											
PG (3)	Ia	6		3	3										
EPM	**Ia, II**	**43**													
PC (4)	II	6							6						
JT (5)	II	6							1		**5**				
TJ (6)	Ia, II	7				6			1						
GB (7)	II	6										**2**	**4**		
CN (8)	II	6												**4**	**2**
ED (9)	Ia	6					**6**								
TS (10)	Ia	6				6									
WPM	**Ia, Ic, II**	**43**													
SS (11)	Ia	5			5										
AY (12)	II	7							3	**4**					
KJ (13)	Ic	6	6												
RW (14)	Ia, II	8		4					4						
TB (15)	Ic	6	6												
TC (16)	Ic	6	6												
BM (17)	Ic	5	5												
		104	23	13	8	12	6	6	15	4	5	2	4	4	2

The lineage and cluster corresponded to the phylogeny analyses based on the mtDNA (Fig. 3). The solid indicated the private haplotype

Bold values indicate the information in the region and marked the private haplotype

Underline indicates the population code

Bold and underlined values indicate the sampling size for the private haplotype

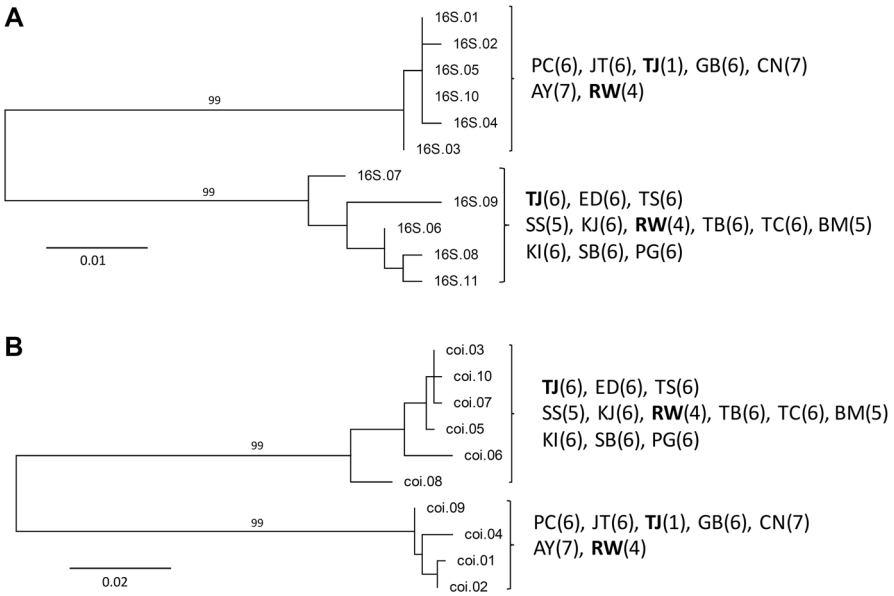

Fig. 2 The best maximum likelihood (ML) tree of *Melanoides tuberculata* in Malaysia with the HKY+G model based on COI sequences (**a**) and 16S sequences (**b**). The numbers at the nodes are bootstrap values

of the current (θ_π) and historical (θ_ω) genetic diversity per site for each sample indicated that most populations showed a pattern of growth ($\theta_\pi > \theta_\omega$; Table 1), and only three populations (JT, TJ and CN) in EPM displayed a pattern of decline ($\theta_\pi < \theta_\omega$). A comparison of the fixation indices, N_{ST} and G_{ST}, revealed that N_{ST} was much larger than G_{ST} (0.526 and 0.099, respectively). This result suggested a strong relationship between phylogeny and geography.

The S-DIVA analyses indicated that the ancestral areas of *M. tuberculata* in Malaysia were northern Peninsular Malaysia and Borneo (Fig. 3). The vicariance event separated Peninsular Malaysia into two groups, EPM and WPM. Within lineage II, the ancestral populations were distributed in EPM and then dispersed to WPM. Within lineage I, the ancestral populations were distributed in WPM and SRW and then divided into two groups: WPM (haplogroup Ic) and SRW (haplogroups Ia and Ib). Finally, the populations dispersed from SRW to EPM and WPM.

Global Genetic Variation

A total of 165 16S sequences were sorted into 23 haplotypes W01–W23, Table S1). These 23 haplotypes fell into three major phylogroups, A–C (Fig. 4). Phylogroup A included specimens from South America, Europe, Africa, and West and South Asia; phylogroup B was only distributed in French Polynesia; and phylogroup C contained specimens from North and South America, Africa, and South Asia. Phylogroup A was divided into two subphylogroups, A1 and A2. The divergence among these

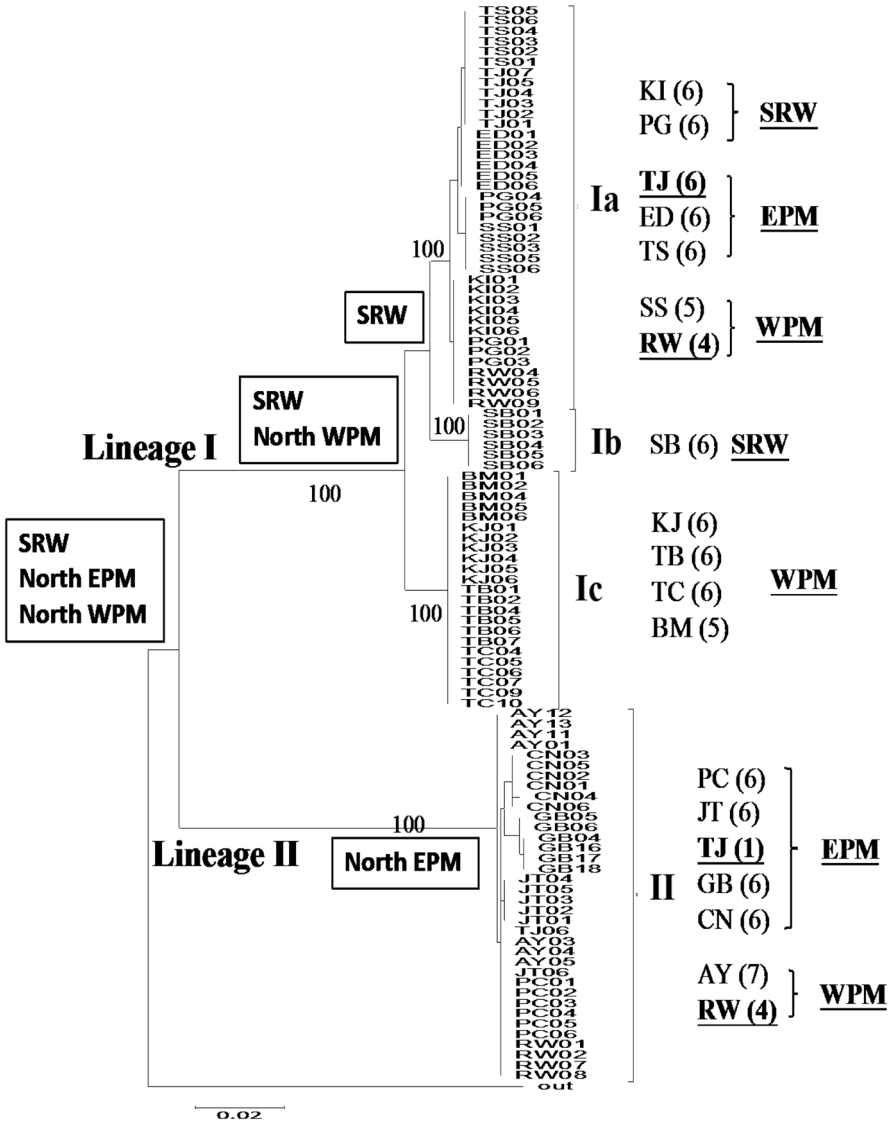

Fig. 3 The best maximum likelihood (ML) tree of *Melanoides tuberculata* in Malaysia with the HKY model based on mtDNA (COI+16S) sequences. The numbers at the nodes are bootstrap values. The ancestral distribution inferred using S-DIVA is given in the box above each node

three phylogroups ranged from 10.75% (between phylogroups A and C) to 16.81% (between phylogroups B and C), and that between subphylogroups A1 and A2 was 3.62%. Two lineages of *M. tuberculata* in Malaysia were sorted into phylogroups A1 and C, respectively.

A total of 176 COI sequences were sorted into 51 haplotypes (COI.01-COI.51, Table S2). These 51 haplotypes fell into three major phylogroups, I–III (Fig. 5).

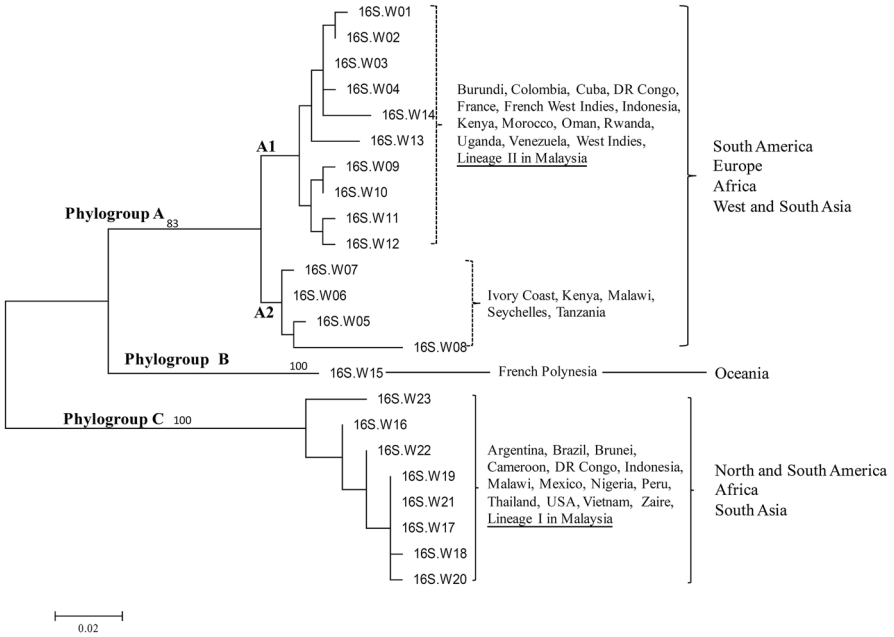

Fig. 4 The best maximum likelihood (ML) tree of *Melanoides tuberculata* in the world with the Tamura-3-parameter model based on 16S sequences. The numbers at the nodes are bootstrap values

Phylogroup I included specimens from Africa and Asia; phylogroup II only contained specimens from French Polynesia; and phylogroup III contained specimens from Africa and East Asia. Phylogroup I was divided into two subphylogroups, I.1 and I.2. The divergence among these three phylogroups ranged from 10.79% (between phylogroups I and II) to 13.70% (between phylogroups II and III). The two lineages of *M. tuberculata* in Malaysia were sorted into phylogroups I.1 and III, respectively.

Discussion

Spatial Genetic Variations of *M. tuberculata* in Malaysia

The sequence divergence between the two mtDNA lineages of *M. tuberculata* in Malaysia was 12.4%. Hsu et al. (2014) proposed that the genetic distance between two monophyletic lineages of *Semisulcospira libertina* in Taiwan was 12.20% and suggested that these two distant lineages of *S. libertina* would be two cryptic species. Köhler and Johnoson (2012) evaluated the utility of mtDNA COI for delimiting the species boundary of Australian land snails, *Amplirhagada*, and suggested that the genetic distance between the highest intraspecific and lowest interspecific groups was 6.00%. Likewise, Spencer et al. (2009) proposed that the mean Gastropoda interspecific genetic distance was 11.50% and 8.2% among sister taxa. Accordingly,

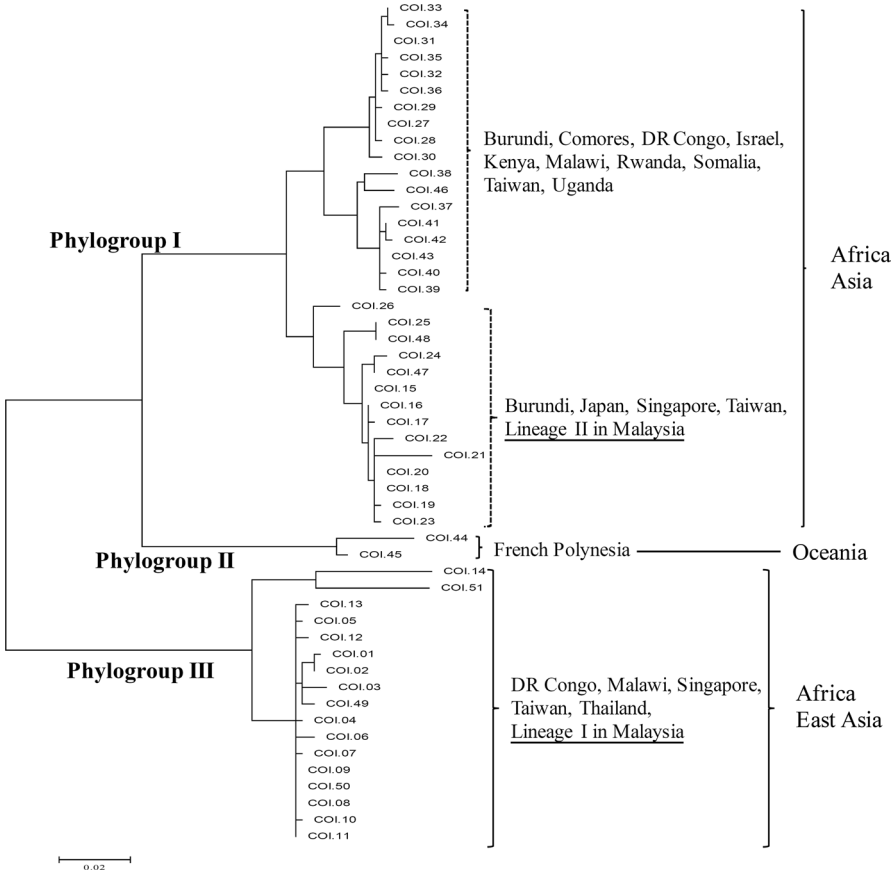

Fig. 5 The best maximum likelihood (ML) tree of *Melanoides tuberculata* in the world with the Tamura-Nei model based on COI sequences. The numbers at the nodes are bootstrap values

this study suggested that the two lineages of *M. tuberculata* in Malaysia had an independent evolutionary history similar to the colonization history of *S. libertina* in Taiwan (Chiu et al. 2017).

Lineage I was distributed in WPM, SRW, and southern EPM, excluding population AY, and lineage II was distributed in five populations in northern EPM and two populations in WPM (Figs. 1 and 3). The results of the S-DIVA analysis showed that the ancestral populations of *M. tuberculata* in Malaysia were distributed in northern Peninsular Malaysia and SRW and then separated into two groups, northern EPM and northern WPM+SRW (Fig. 3). Our study showed that the Main Range played an important role in shaping the genetic variation distribution of *M. tuberculata* in Peninsular Malaysia. A previous phylogeny and biogeography study of the freshwater crab genus *Johora* (Crustacea: Brachyura: Potamidae) showed that two largely peninsular groups of *Johora* appeared to have fairly distinct distributions on either side of the Main Range (Yeo et al. 2007). Based on studies of geochemistry, Azman

(2000) proposed that the Peninsular Malaysian granites have been grouped into two granite provinces, namely, Western and Eastern Belt granite. The geochemistry results might imply that these parts of Peninsular Malaysia have different geological histories. Thus, our study suggested that these two lineages of *M. tuberculata* might be divided into two independent evolutionary units by geological history and landforms.

Regarding the phylogeography of freshwater species in Malaysia, many previous studies have shown that the paleo-river systems in the Sunda continental shelf (Fig. 1a) shaped their genetic structure (e.g., Inger and Chin 2002; McConnell 2004; De Bruyn et al. 2004; Ryan and Esa 2006). Two haplotypes (H2 and H3; Table 2) were shared between Peninsular Malaysia and Borneo. This result indicated that these two pieces of land were connected recently. Ryan and Esa (2006) and Esa et al. (2008) also found that there were shared haplotypes of cyprinoids in these two regions, which supported the idea that Peninsular Malaysia and Borneo disconnected in recent glaciation. Zainudin et al. (2010) suggested that the population of *Hylarana erythraea* (Amphibia: Anura: Ranidae) in western Sarawak was more closely related to the population in Peninsular Malaysia than to that in eastern Sarawak. Therefore, our study suggested that the ice melted in postglacial areas, resulting in flooding of the Sunda continental shelf and contributing to the exchange of fauna between Peninsular Malaysia and Borneo.

Global Genetic Diversity of *M. tuberculata*

Melanoides tuberculata is native to northern Africa and southern Asia, but it has been introduced in many other tropical and subtropical areas worldwide (e.g., Duggan 2002; Santos et al. 2007; Reeves et al. 2008; Lasso et al. 2009; Vázquez and Perera 2010). Combining results from COI and 16S datasets (Figs. 4 and 5), our study suggested that the two lineages of *M. tuberculata* in Malaysia are distributed all over the world, including in Europe and America. This species is a well-known and successful worldwide invader. Moreover, Jenkins et al. (2007) also proposed that the populations of *Coptotermes gestroi* in Ohio and Australia likely originated from Singapore and Malaysia by shipping. The Strait of Malacca, a narrow stretch of water between Peninsular Malaysia and Sumatra, is one of the most important shipping lanes in the world. This strait is the main shipping channel between the Indian Ocean and the Pacific Ocean, linking major Asian economies such as India, China, Japan, Taiwan, and South Korea. Thus, the populations of *M. tuberculata* in Malaysia might be widely distributed throughout the world by shipping. However, this species is a freshwater snail. How exactly is shipping responsible for moving a freshwater snail? The literature (Van Damme and Lange 2017) suggests that the introduction of this species to other regions depends on aquarium trade and possibly movement with rice cultivation and spread by birds. However, our study proposed a query about this issue. If this species spreads by birds and movement with the rice cultivation, why is the population differentiation of *M. tuberculata* in Malaysia significant? Thus, our study suggests that Malaysia is located in important shipping lanes, and the populations of *M. tuberculata* might be widely distributed throughout the world

by shipping with the aquarium trade. Further, the literature (Van Damme and Lange 2017) suggests that this species is able to survive in relatively alkaline and saline waters. Therefore, this species might spread by many methods.

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Compliance with Ethical Standards

Conflict of interest The authors declare no conflict of interest.

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