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Intra- and Inter-specific Variation of Four *Acetes* Species (Crustacea: Decapoda: Sergestidae) Sampled along the West Coast of Peninsular Malaysia

(Variasi Intra- dan antara Spesies Empat Spesies *Acetes* (Crustacea: Decapoda: Sergestidae) disampel Sepanjang Pantai Barat Semenanjung Malaysia)

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ABSTRACT

The intra- and inter-specific variation of Acetes *shrimps were evaluated based on samples collected from in-shore catches and off-shore trawling around the west coast of Peninsular Malaysia. Species captured were identified as* Acetes indicus, A. serrulatus, A. japonicus and A. sibogae. *A region of the mitochondrial cytochrome c oxidase subunit I (COI) gene comprising 552 base pairs (bp) was amplified from 159* Acetes *specimens. The sequence alignment analysis generated phylogenetic trees which depicted the four major clades that were consistent with the species identified morphologically. These four species varied considerably for haplotype and nucleotide diversity, with* A. indicus *and* A. serrulatus *showing different demographic histories. Furthermore, the observation of two clades in the* A. indicus *and* A. sibogae *lineages, with relatively high levels of intraspecific divergence, suggests that cryptic diversity is possibly present in these two taxa. This study has contributed to the knowledge of the distribution patterns and molecular phylogenetics of four* Acetes *spp. in the Straits of Malacca.*

Keywords: Acetes; COI gene; cryptic species; Peninsular Malaysia; phylogenetic analysis

ABSTRAK

Variasi intra- dan antara spesies Acetes *dinilai berdasarkan sampel yang dikumpulkan daripada tangkapan di pantai dan tunda di luar pesisir pantai di sekitar pantai barat Semenanjung Malaysia. Spesies yang ditangkap dikenal pasti sebagai* Acetes indicus, A. serrulatus, A. japonicus *dan* A. sibogae. *Suatu kawasan gen sitokrom* c *oksidase mitokondria subunit I (COI) yang terdiri daripada 552 pasangan bes (bp) telah teramplifikasi daripada 159 spesimen* Acetes. *Analisis penjajaran jujukan menghasilkan pokok filogenetik yang menggambarkan empat klad utama adalah tekal dengan spesies yang telah dikenal pasti secara morfologi. Empat spesies ini sangat berbeza daripada segi kepelbagaian haplotip dan nukleotid dengan* A. indicus *dan* A. serrulatus *menunjukkan sejarah demografi yang berbeza. Tambahan pula, pemerhatian titisan dalam dua klad* A. indicus *dan* A. sibogae*, dengan tahap perbezaan intraspesies yang agak tinggi, menunjukkan bahawa kepelbagaian krip mungkin ada dalam dua taksa ini. Kajian ini telah menyumbang kepada pengetahuan tentang pola taburan dan molekul filogenetik empat* Acetes *spp. di Selat Melaka.*

Kata kunci: Acetes; analisis filogenetik; gen COI; Semenanjung Malaysia; spesies kriptik

INTRODUCTION

Acetes shrimps of the family Sergestidae (Decapoda) are small planktonic shrimps (10-40 mm in total length), which are locally known as '*Udang Geragau*' or '*Udang Baring*' (Omori 1978, 1975). Currently, seven out of 14 described *Acetes* species have been found within Malaysian coastal waters, namely *Acetes indicus*, *A. japonicus*, *A. serrulatus*, *A. vulgaris*, *A. sibogae*, *A. intermedius* and *A. erythraeus* (Amani et al. 2011a, 2011b, 2011c; Amin et al. 2011, 2010, 2009a, 2009b, 2009c, 2009d, 2008; Arshad et al. 2012, 2008, 2007; Longhurst 1970; Omori 1975, 1978; Pathansali 1966). Landings of *Acetes* species are confined mainly to the west coast of Peninsular Malaysia where 75% or more of the total landing occurs (DOF 2013). *Acetes* are known for their commercial importance in subsistence fisheries (Holthuis 1980; Omori 1978, 1975) and potential use as feed in agriculture and aquaculture (Deshmukh 1991; Job et al. 2006). These species also play important roles as both predators and prey in the food webs of coastal waters (Xiao & Greenwood 1993).

Previous studies on *Acetes* spp. focused mainly on their population dynamics, distribution, morphology, reproductive biology, morphometrics and lifecycles (Amani et al. 2011a, 2011b, 2011c; Amin et al. 2011, 2010, 2009a, 2009b, 2009c, 2009d, 2008; Arshad et al. 2012, 2008, 2007; Wong 2013; Wong et al. 2015). Presently, little is known about their genetic diversity spanning the common fishing grounds along the west coast of Peninsular Malaysia. To conserve the existing resources of these highly exploited species for long-term sustainable yields, information on the genetic diversity of *Acetes* populations is crucial for the assessment and management of wild stocks (Allendorf & Luikart 2006; Carvalho & Hauser 1994; Thorpe et al. 2000; Ward 2000; Ward & Grewe 1994).

The identification of *Acetes* species is commonly based on the global identification keys by Omori (1975) as this identification system applies to a vast geographical coverage and is also able to differentiate males from females at different stages of their life cycles (Wong 2013; Wong et al. 2015). Conversely, species identification studies using the mitochondrial cytochrome *c* oxidase subunit I (*COI*) gene have shown the usefulness of its sequence analysis in examining the phylogenetic and evolutionary relationships of decapod crustaceans such as penaeid shrimps (Baldwin et al. 1998), brachyuran crabs of the genus *Cancer* (Harrison & Crespi 1999), snapping shrimp genus *Alpheus* (Williams et al. 2001), *Farfantepenaeus* shrimps in Cuban waters (García-Machado et al. 2001), Western Pacific squat lobsters (Machordom & Macpherson 2004), European crayfish genus *Austropotamobius* (Trontelj et al. 2005), freshwater glass shrimp *Paratya australiensis* in eastern Australia (Cook et al. 2006), giant tiger prawn *Penaeus monodon* in Thai waters (Khamnamtong et al. 2009), *Melicertus kerathurus* populations in the Mediterranean Sea and eastern Atlantic Ocean (Pellerito et al. 2009), Western Mediterranean red shrimp *Aristeus antennatus* (Roldán et al. 2009) and Indo-West Pacific portunid crabs (Lai et al. 2010).

In this study, morphological identification based on the global identification keys of Omori (1975) and mtDNA *COI* sequence analyses were used to evaluate the genetic diversity and phylogenetic relationships among *Acetes* species in the west coast of Peninsular Malaysia.

Materials & Methods

Sample collection and identification

Acetes shrimps were sampled from inshore catches using push-nets and trawling activities at sea more than 5 nautical miles (nm) offshore along the west coast of Peninsular Malaysia (Figure 1), from August 2007 to October 2008. A global positioning system (GPS) was used to mark the

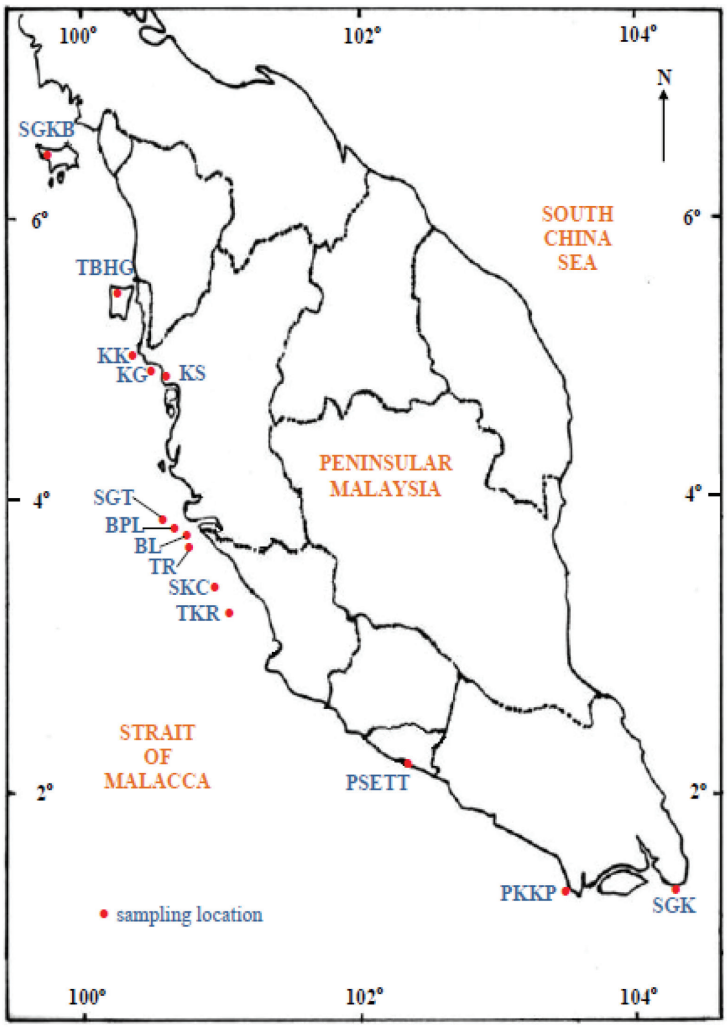

FIGURE 1. Map of Peninsular Malaysia showing the 14 sampling locations for this study The sampling locations are – SGKB: Sungai Kubang Badak; TBHG: Teluk Bahang; KK: Kuala Kurau; KG: Kuala Gula; KS: Kuala Sepetang; SGT: Sungai Tiang; BPL: Bagan Pasir Laut; BL: Bagan Lipas; TR: Teluk Rhu; SKC: Sekinchan; TKR: Tanjong Karang; PSETT: Portuguese Settlement; PKKP: Pulau Kukup; SGK: Sungai Kapal; indicated as (•)

geographical position of each sampling location (Table 1). Perak was the only state in which both in- and offshore samples were collected. The samples were preserved immediately in 70% ethanol (Merck, Germany) upon collection, followed by long-term storage in 95% ethanol as described by Lai et al. (2010), Wong (2013) and Wong et al. (2015). Fixation and preservation in ethanol was carried out to prevent degradation of DNA by enzymes upon death of the specimens as the latter would subsequently be used for DNA analyses (Black & Dodson 2003; Bucklin 2009; Díaz-Viloria et al. 2005; DiStefano et al. 1994; Wong 2013). The species and sexes of *Acetes* spp. were identified under a dissecting microscope (Leica ZOOM 2000™ , Model No. Z45V, Germany), according to the key characters described by Omori (1975) and Wong (2013) (Table 2).

DNA EXTRACTION, AMPLIFICATION AND SEQUENCING

DNA was extracted from 25 g of muscle sample, using i-genomic CTB DNA Extraction Mini Kit (iNtRON Biotechnology Inc., South Korea). Amplification of the 552 bp fragment from the 5'-end of mitochondrial DNA cytochrome *c* oxidase subunit I (*COI*) gene was performed using PCR (Saiki et al. 1988) with the primer pair LCO1490 (5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3') and HCO2198 (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3') (Folmer et al. 1994). Each PCR reaction mixture contained 2.5 µL of 10× PCR buffer (Vivantis), 1.5 mM of $MgCl_2$ (Vivantis), 50 µM of each dNTP (Vivantis), 1 unit (U) of *Taq* polymerase (Vivantis), 0.3 µM of each primer (1st BASE Pte. Ltd., Singapore), 2 µL of DNA template (50 ng) and adjusted to a final volume of 25 µL with deionised water. The PCR of COI gene was performed on an Eppendorf Mastercycler® Gradient (Eppendorf, Germany) with the following profile: Initial denaturation at 94°C for 60 s; five cycles at 94°C for 30 s, 45°C for 90 s and 72°C for 60 s; 35 cycles at 94°C for 30 s, 51°C for 90 s and 72°C for 60 s; followed by a final extension at 72°C for 5 min (Costa et al. 2007; Hebert et al. 2003). Prior to sequencing, PCR products were purified using the MEGAquick-spin™ PCR and Agarose Gel DNA Extraction System (iNtRON Biotechnology Inc., South Korea). Purified PCR products were sequenced from both directions, on an ABI Genetic Analyzer 3730 (Applied Biosystems).

SEQUENCE ANALYSIS

DNA sequence chromatograms were viewed and manually edited with Chromas LITE 2.01 (Technelysium Pty. Ltd., Australia). Homology search was performed with Basic Local Alignment Search Tool (BLAST; Altschul et al. 1990). Alignments of the COI sequences were performed in Molecular Evolutionary Genetics Analysis 4 (MEGA4; Tamura et al. 2007). The aligned nucleotide sequences were then translated into amino acid based on invertebrate mitochondrial genetic code. The sequence variation and base composition of the amplified sequences were analyzed using MEGA4 and DnaSP v5.10 (Librado & Rozas 2009). When homologous sequences from two individuals differed by one or more than one nucleotide, the sequences were considered as different haplotypes.

PHYLOGENETIC ANALYSES

Based on all aligned *COI* sequences, the phylogenetic relationships among haplotypes were examined by four phylogenetic methods to verify whether alternative topologies were supported by different tree-building methods. Prior to these analyses, the best-fit evolutionary model of nucleotide substitution was chosen using corrected Akaike Information Criterion (AIC; Hurvich & Tsai 1989; Sugiura 1978) in jModelTest 0.1.1 (Posada 2009, 2008).

The calculation of pairwise genetic distances within and among the four *Acetes* species and the Neighbour-

TABLE 1. Sampling locations of *Acetes* species along the west coast of Peninsular Malaysia

State	Sampling Location (Abbreviation)	Latitude	Longitude	Sampling Method
Kedah	Sungai Kubang Badak (SGKB)	6°23'58.75"N	99°43'32.21"E	In-shore
Pulau Pinang	Teluk Bahang (TBHG)	5°27'36.91"N	100°12'44.51"E	In-shore
Perak	Kuala Kurau (KK)	5°0'11.41"N	100°25'22.47"E	In-shore
Perak	Kuala Gula (KG)	4°55'0.35"N	100°27'39.54"E	In-shore
Perak	Kuala Sepetang (KS)	4°51'12.23"N	100°32'9.53"E	In-shore
Perak	Sungai Tiang (SGT)	3°55'9.28"N	100°36'15.02"E	Off-shore
Perak	Bagan Pasir Laut (BPL)	3°49'11.80"N	100°41'4.16"E	Off-shore
Perak	Bagan Lipas (BL)	3°45'48.83"N	100°44'18.62"E	Off-shore
Selangor	Teluk Rhu (TR)	3°42'47.86"N	100°45'11.12"E	Off-shore
Selangor	Sekinchan (SKC)	3°26'42.08"N	100°54'39.76"E	Off-shore
Selangor	Tanjong Karang (TKR)	3°19'48.37"N	101° 2'20.32"E	Off-shore
Malacca	Portuguese Settlement (PSETT)	2°10'57.14"N	102°15'57.91"E	In-shore
Johor	Pulau Kukup (PKKP)	1°19'5.39"N	103°26'37.77"E	In-shore
Johor	Sungai Kapal (SGK)	1°20'51.04"N	104°13'12.94"E	In-shore

(Wong 2013; Wong et al. 2015)

TABLE 2. Morphological characters, based on Omori (1975) and Wong (2013), which were used to (a) identify the species and (b) differentiate the sexes of *Acetes* species sampled from the west coast of Peninsular Malaysia

Morphology	Apex of Telson	Rostrum (no. of denticles)	Pairs of Pleopods (presence of procurved tooth)	Petasma (presence of pars astrigens)	Lower Antenullar Flagellum	3rd Pereiopod	3rd Thoracic Sternites
Sex	Males and females			Males only		Females only	
A. indicus	Triangular	Two	Present between bases of 1st pair	Absent	One clasping spine	Inner margin has sharply pointed projection	3rd and 4th sternite deeply channeled longitudinally
A. sibogae	Triangular	Two	Absent	Present. Capitulum has 1 large hook and often 1 small hook along outer margin.	Clasping spine extends beyond the end of second segment of the main branch	Distal inner margin ends in a projection	A pair of small protuberances on the anterior
A. japonicus	Triangular	None	Absent	Absent. Distal part of capitulum is bulb-like with numerous hooks	Two clasping spines, without triangular projection	Emargination of posterior margin is shallow	Without protuberances
A. serrulatus	Truncated	Two	Absent	Absent, without ventral projection, 1 large hook at the end	Two clapsing spines, triangular projection from the 1st segment	Tooth present on distal inner margin of the coax	Anterior margin of 4th sternite is convex

Joining (NJ; Saitou & Nei 1987) tree were based on the substitution model of Kimura's Two Parameter (K2P; Kimura 1980) and were constructed using MEGA4 in which the stabilities of the derived clusters in phylogenetic trees were accessed by 2000 replications of non-parametric bootstrapping (Felsenstein 1985).

The Maximum Parsimony (MP; Camin & Sokal 1965) tree was constructed from a heuristic search using Tree-Bisection-Reconnection (TBR) in Phylogeny Analysis Using Parsimony (PAUP* 4.0b10; Swofford 2002). Nodal support was accessed through non-parametric bootstrapping using the heuristic search option of 1000 replications with 10 random addition-sequence replicates. The Maximum Likelihood (ML; Felsenstein 1981) tree was constructed using the starting tree obtained by BioNJ (Gascuel 1997) and Nearest Neighbour Interchange (NNI; Jarvis et al. 1983) branch swapping arrangements in PhyML 3.0 (Guindon et al. 2005). The data set was bootstrapped for 1000 replications.

Bayesian Inference (BI) was performed using MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003) with substitution model parameters set to lset nst=6 rates=gamma and all priors were left default to allow estimation of the parameters from the data. Each BI was conducted three times to check for consistency of results. Two runs of four Metropolis-coupled Markov Chain Monte Carlo (MCMC) chains each (one cold chain and three heated chains, default temperature = 0.20) were run for four million generations (mcmc ngen = 4000000) and sampled every 1000th generations (sample freq = 1000). When the average standard deviation of split frequencies was less than 0.01, 25% of the samples were discarded as burn-in (sump burnin = 1000). The remaining trees were used to calculate the posterior probabilities (PP) and to produce the 50% majority-rule consensus tree after discarding burn-in samples in each analysis. Probabilities of 95% or higher were considered significant support. All the phylogenetic trees were rooted with *Sergestes similis* (GenBank Accession Number: DQ882152) as outgroup and displayed with TreeView 1.6.6 (Page 1996).

In addition, a haplotype network was constructed for *A. indicus* and *A. sibogae* using the TCS 1.13 software (Clement et al. 2000), which employs a 95% statistical parsimony method (Templeton et al. 1992). For the intraspecific variation, haplotype diversity (*h*; Nei 1987) and nucleotide diversity (π; Nei 1987) was computed using DnaSP v5 based on segregating sites (*S*).

Population structure for each species was carried out using Analysis of Molecular VAriance (AMOVA; Excoffier et al. 1992) to produce the pairwise Φ-statistics (Weir & Cockerham 1984) in Arlequin (Excoffier & Lischer 2010). The significant levels of the AMOVA and pairwise Φ-statistics were tested with 10000 permutations. When the overall AMOVA was statistically significant, a Mantel test (Mantel 1967) was performed in XLSTAT v.2010.3.06 to determine if genetic distance was due to geographical distance. Statistical significance was determined by 10000 permutations.

Demographic histories were investigated using the Neutrality Tests and Mismatch Distribution Analysis. Tajima's *D* (Tajima 1989), Fu's F_S (Fu 1997) and the R_2 (Ramos-Onsins & Rozas 2002) and their significance was tested with 10000 coalescent simulations (Hudson 1990) in DnaSP v5. Mismatch distribution was performed with Arlequin v3.5 and mismatch figures were created using DnaSP v5. The parameters of the mismatch distribution or demographic expansion before and after population growth (Θ_0 and Θ_1) and time since expansion, τ, expressed in units of mutational time (Rogers 1995; Rogers & Harpending 1992) were estimated using generalised non-linear least-squares approach (Schneider & Excoffier 1999). Their respective 95% confidence intervals (CI) were obtained by parametric bootstrapping with 10000 permutations. The fit between the observed and expected distributions under population growth was evaluated by the sum of square deviations (*SSD*; Schneider & Excoffier 1999) and Harpending's Raggedness Index (*r*; Harpending 1994) with 10000 bootstrap replicates.

Results

Species identification of *Acetes*

From a total of 159 specimens collected, four main *Acetes* species, namely, *Acetes indicus* (*n*=69), *A. serrulatus* (*n*=65), *A. japonicus* (*n*=13) and *A. sibogae* (*n*=12) were identified based on the key morphological characters described by Omori (1975) and Wong (2013). Males and females were identified by the presence of a pair of protuberances (genital coxae) between the third pereiopods and first pleopods, a petasma and lower antenullar flagellum with spine(s) in males but absent in females. The different species of *Acetes* were differentiated based on the apex of telson, petasma and antennular flagellum of males and third thoracic sternite of females (Table 2).

Cytochrome *C* oxidase subunit I (*COI*) gene

The 552 bp of the *COI* gene fragment (GenBank Accession Number: HQ630429-HQ630587) amplified in this study were obtained for 159 specimens and showed 46 haplotypes (Table 3): 11 haplotypes were identified for *A. indicus*, 31 haplotypes for *A. serrulatus*, two haplotypes for *A. japonicus* and two haplotypes for *A. sibogae*. From the multiple sequence alignment of 46 haplotypes, 167 variable sites were found, of which 144 and 23 were parsimony informative sites and singleton sites, respectively. No insertions or deletions (indels) were found. Most of the variations (139 sites, 83%) occurred at the third codon position, while 26 variable sites (16%) were at the first position. Only two variable sites (1%) were at the second position.

The mean nucleotide composition of each *Acetes* species is shown in Table 4, together with the base composition according to first, second and third codon position. The pattern of nucleotide substitution was biased in favour of

TABLE 3. List of specimens used in this study and their GenBank accession numbers

Species	Lab Identification (specimen no._sex of specimen[1]_sampling location[2])	Haplotype	GenBank Accession Number
Acetes indicus	AI9_f_BPL2	ai1	HQ630429
	AI14_m_SGT1	ai1	HQ630430
	AI15_f_SGT1	ai1	HQ630431
	AI26_f_PSETT4	ai1	HQ630432
	AI27_m_PSETT4	ai2	HQ630433
	AI28_m_BPL2	ai1	HQ630434
	AI29_m_PSETT4	ai3	HQ630435
	AI30_f_PSETT4	ai4	HQ630436
	AI31_m_PSETT4	ai4	HQ630437
	AI32_f_PSETT4	ai5	HQ630438
	AI33_m_PSETT4	ai4	HQ630439
	AI34_f_PSETT4	ai6	HQ630440
	AI35_m_SGT5	ai1	HQ630441
	AI36_f_SGT5	ai7	HQ630442
	AI37_m_SGT6	ai1	HQ630443
	AI38_f_SGT6	ai1	HQ630444
	AI39_m_BPL7	ai1	HQ630445
	AI40_f_BPL7	ai1	HQ630446
	AI41_m_BPL8	ai1	HQ630447
	AI43_m_BL9	ai1	HQ630448
	AI44_f_BL9	ai1	HQ630449
	AI45_m_BL10	ai4	HQ630450
	AI46_f_BL10	ai1	HQ630451
	AI47_m_SKC11	ai1	HQ630452
	AI49_m_TKR12	ai1	HQ630453
	AI50_f_TKR12	ai8	HQ630454
	AI52_f_TR13	ai1	HQ630455
	AI53_m_TKR14	ai1	HQ630456
	AI55_m_SKC15	ai1	HQ630457
	AI56_f_SKC15	ai1	HQ630458
	AI57_m_BPL16	ai1	HQ630459
	AI58_f_BPL16	ai1	HQ630460
	AI59_m_SGT17	ai1	HQ630461
	AI60_f_SGT17	ai1	HQ630462
	AI62_f_BL9	ai1	HQ630463
	AI63_m_BL10	ai1	HQ630464
	AI64_f_BL10	ai1	HQ630465
	AI65_m_SKC11	ai1	HQ630466
	AI66_f_SKC11	ai1	HQ630467
	AI67_m_SKC15	ai1	HQ630468
	AI68_f_SKC15	ai8	HQ630469
	AI69_m_TKR12	ai1	HQ630470
	AI70_f_TKR12	ai1	HQ630471
	AI72_f_TKR14	ai1	HQ630472
	AI73_m_TR13	ai1	HQ630473
	AI74_f_TR13	ai1	HQ630474
	AI75_m_TR13	ai9	HQ630475
	AI76_f_TR13	ai10	HQ630476
	AI77_m_KK19	ai4	HQ630477
	AI78_f_KK19	ai4	HQ630478
	AI79_m_KK19	ai4	HQ630479
	AI81_m_KK19	ai4	HQ630480
	AI82_f_KK19	ai4	HQ630481
	AI83_m_KG26	ai4	HQ630482
	AI84_f_KG26	ai4	HQ630483
	AI85_m_KG26	ai4	HQ630484

(continue)

Continued (TABLE 3)

Species	Lab Identification (specimen no._sex of specimen[1]_sampling location[2])	Haplotype	GenBank Accession Number
	AI86_f_KG26	ai4	HQ630485
	AI87_m_KG26	ai4	HQ630486
	AI88_f_KG26	ai4	HQ630487
	AI89_m_PKKP29	ai1	HQ630488
	AI90_f_PKKP29	ai1	HQ630489
	AI92f_PKKP29	ai1	HQ630490
	AI93_m_PKKP29	ai1	HQ630491
	AI94_f_PKKP29	ai11	HQ630492
	AI95_m_SGK30	ai1	HQ630493
	AI96_f_SGK30	ai1	HQ630494
	AI97_m_SGK30	ai1	HQ630495
	AI99_m_SGK30	ai1	HQ630496
	AI100_f_SGK30	ai1	HQ630497
Acetes serrulatus	AS3_m_BPL2	as1	HQ630498
	AS4_m_SGT1	as2	HQ630499
	AS5_f_BPL2	as2	HQ630500
	AS6_f_BPL2	as1	HQ630501
	AS7_m_BPL2	as3	HQ630502
	AS8_m_SGT1	as2	HQ630503
	AS9_m_SGT5	as4	HQ630504
	AS10_f_SGT5	as5	HQ630505
	AS11_m_SGT6	as1	HQ630506
	AS12_f_SGT6	as6	HQ630507
	AS13_m_BPL7	as7	HQ630508
	AS14_f_BPL7	as2	HQ630509
	AS15_m_BPL8	as2	HQ630510
	AS16_f_BPL8	as8	HQ630511
	AS17_m_BL9	as1	HQ630512
	AS18_f_BL9	as9	HQ630513
	AS19_m_BL10	as2	HQ630514
	AS20_f_BL10	as2	HQ630515
	AS21_m_SKC11	as1	HQ630516
	AS22_f_SKC11	as8	HQ630517
	AS23_m_TKR12	as10	HQ630518
	AS24_f_TKR12	as1	HQ630519
	AS25_m_TR13	as1	HQ630520
	AS26_f_TR13	as11	HQ630521
	AS27_m_SGT1	as12	HQ630522
	AS28_f_SGT1	as13	HQ630523
	AS30_f_BPL2	as1	HQ630524
	AS31_m_SGT5	as1	HQ630525
	AS32_f_SGT5	as14	HQ630526
	AS33_m_SGT6	as2	HQ630527
	AS34_f_SGT6	as15	HQ630528
	AS35_m_BPL7	as2	HQ630529
	AS36_f_BPL7	as16	HQ630530
	AS37_m_BPL8	as17	HQ630531
	AS38_f_BPL8	as18	HQ630532
	AS39_m_BL9	as19	HQ630533
	AS40_f_BL9	as1	HQ630534
	AS41_m_BL10	as20	HQ630535
	AS42_f_BL10	as1	HQ630536
	AS43_m_SKC11	as21	HQ630537
	AS44_f_SKC11	as1	HQ630538
	AS45_m_TKR12	as22	HQ630539
	AS46_f_TKR12	as23	HQ630540

(continue)

Continued (TABLE 3)

Species	Lab Identification (specimen no._sex of specimen[1]_sampling location[2])	Haplotype	GenBank Accession Number
	AS47_m_TR13	as1	HQ630541
	AS48_f_TR13	as24	HQ630542
	AS49_m_TKR14	as8	HQ630543
	AS50_f_TKR14	as25	HQ630544
	AS51_m_SKC15	as1	HQ630545
	AS52_f_SKC15	as1	HQ630546
	AS53_m_BPL16	as26	HQ630547
	AS54_f_BPL16	as27	HQ630548
	AS55_m_SGT17	as8	HQ630549
	AS56_f_SGT17	as7	HQ630550
	AS58_f_TR13	as8	HQ630551
	AS64_f_SKC15	as2	HQ630552
	AS69_m_PKKP29	as1	HQ630553
	AS70_f_PKKP29	as1	HQ630554
	AS71_m_PKKP29	as1	HQ630555
	AS72_f_PKKP29	as28	HQ630556
	AS73_m_PKKP29	as2	HQ630557
	AS75_m_SGK30	as1	HQ630558
	AS76_f_SGK30	as29	HQ630559
	AS77_m_SGK30	as30	HQ630560
	AS79_m_SGK30	as31	HQ630561
	AS80_f_SGK30	as18	HQ630562
Acetes japonicus	AJ1_m_TBHG	aj1	HQ630563
	AJ2_f_TBHG	aj2	HQ630564
	AJ3_m_TBHG	aj2	HQ630565
	AJ4_f_TBHG	aj2	HQ630566
	AJ5_m_TBHG	aj1	HQ630567
	AJ6_f_TBHG18	aj2	HQ630568
	AJ7_m_KG26	aj1	HQ630569
	AJ8_f_KG26	aj2	HQ630570
	AJ10_f_KG26	aj2	HQ630571
	AJ11_m_KG26	aj1	HQ630572
	AJ12_f_KG26	aj1	HQ630573
	AJ13_f_KK19	aj2	HQ630574
	AJ18_f_KK19	aj1	HQ630575
Acetes sibogae	Asi1_m_SGKB28	asi1	HQ630576
	Asi2_f_SGKB28	asi1	HQ630577
	Asi3_m_SGKB28	asi1	HQ630578
	Asi4_f_SGKB28	asi2	HQ630579
	Asi5_m_SGKB28	asi1	HQ630580
	Asi6_f_SGKB28	asi1	HQ630581
	Asi7_m_KS27	asi1	HQ630582
	Asi8_f_KS27	asi1	HQ630583
	Asi9_m_KS27	asi1	HQ630584
	Asi10_f_KS27	asi1	HQ630585
	Asi11_m_KS27	asi1	HQ630586
	Asi12_f_KS27	asi1	HQ630587

[1]f: female, m: male; [2]sampling location: refer to Table 1

122 transitions (Ts, 44 A↔G and 78 T↔C changes) over 95 transversions (Tv, 62 T↔A, 8 T↔G, 20 C↔A and 5 C↔G changes), yielding a Ts/Tv ratio of 1.28. Furthermore, from the 196 mutations, 194 (99%) were synonymous mutations and two (1%) were non-synonymous mutations. Non-synonymous mutations that resulted in amino acid substitutions occurred at sites 253, 301 and 434, resulting in a change from *leucine* to *methionine*, *alanine* to *serine*, *serine* to *threonine*, respectively. The substitutions resulted in a change of chemically similar amino acids. Overall, the pattern of base composition nucleotide substitution was similar among *Acetes* species.

TABLE 4. Base composition (%) of *COI* gene amplified for each *Acetes* species

	First codon				Second codon				Third codon				Overall				
	T	C	A	G	T	C	A	G	T	C	A	G	T	C	A	G	A+T
A. indicus	23.8	16.8	28.4	31.0	45.7	23.8	12.5	18.1	36.9	9.1	51.5	2.5	35.5	16.6	30.8	17.2	66.3
A. serrulatus	23.9	16.9	28.8	30.4	45.7	23.9	12.5	17.9	38.7	8.7	50.2	2.4	36.1	16.5	30.5	16.9	66.6
A. japonicus	20.7	19.6	28.8	31.1	45.7	23.9	12.5	17.9	33.2	19.6	44.3	3.0	33.2	21.0	28.5	17.3	61.7
A. sibogae	19.6	20.1	29.3	31.0	45.7	23.9	12.5	17.9	35.4	14.7	41.8	8.2	33.5	19.6	27.9	19.0	61.4
Overall	23.3	17.3	28.7	30.8	45.7	23.9	12.5	18.0	37.2	10.2	49.6	2.9	35.4	17.1	30.3	17.2	65.7

PHYLOGENETIC ANALYSES

Phylogenetic trees constructed based on Neighbour-Joining (NJ) and Maximum Likelihood (ML), Maximum Parsimony (MP) and Bayesian Inference (BI) are shown in Figures 2 and 3, respectively. NJ, ML, MP and BI consistently produced trees with the same overall topology, which are four major clades, namely, clade *ai*, *as*, *aj* and *asi* for *A. indicus*, *A. serrulatus*, *A. japonicus* and *A, sibogae*, respectively. The four major clades corresponded well to the four identified *Acetes* species based on morphological characters (Omori 1975; Wong

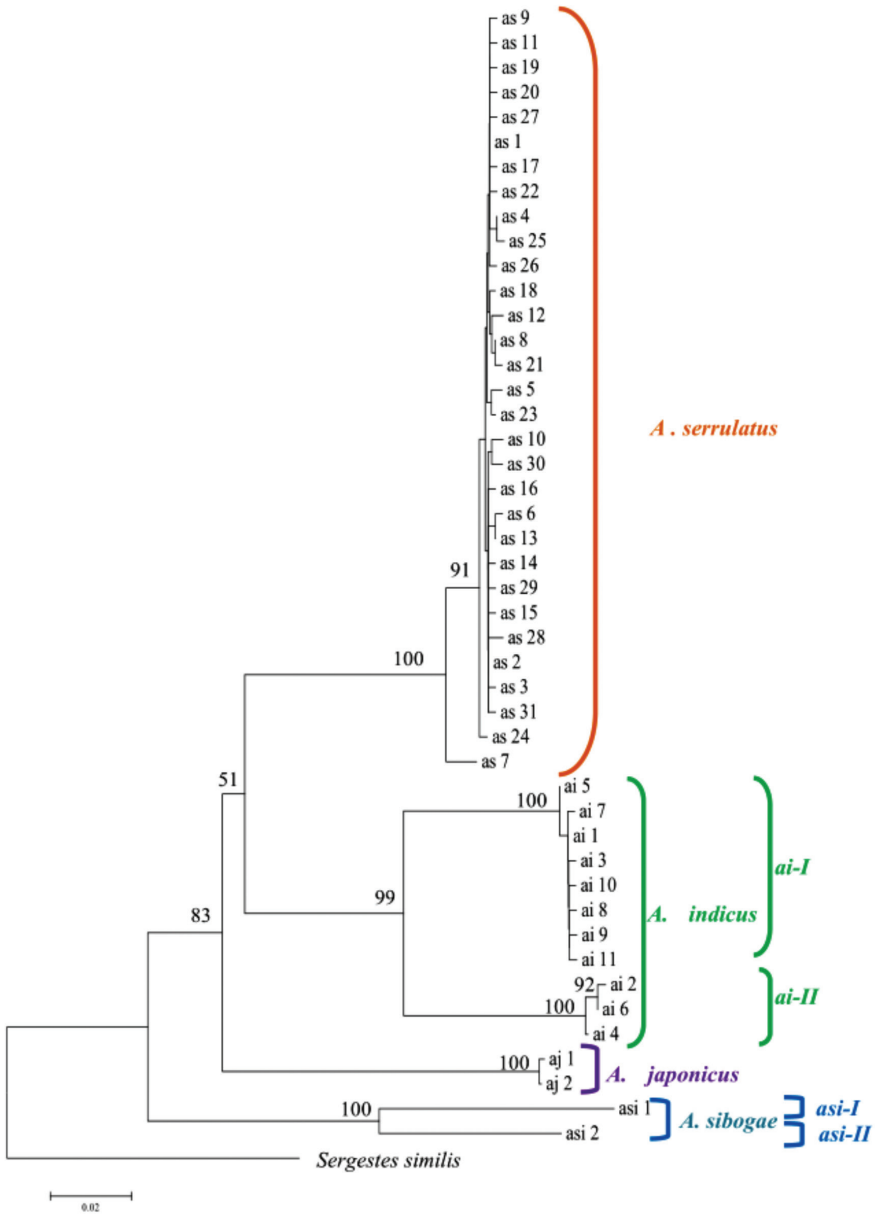

FIGURE 2. Neighbour-Joining (NJ) phylogram showing the relationships among *COI* mtDNA haplotypes of the *Acetes* species shrimps

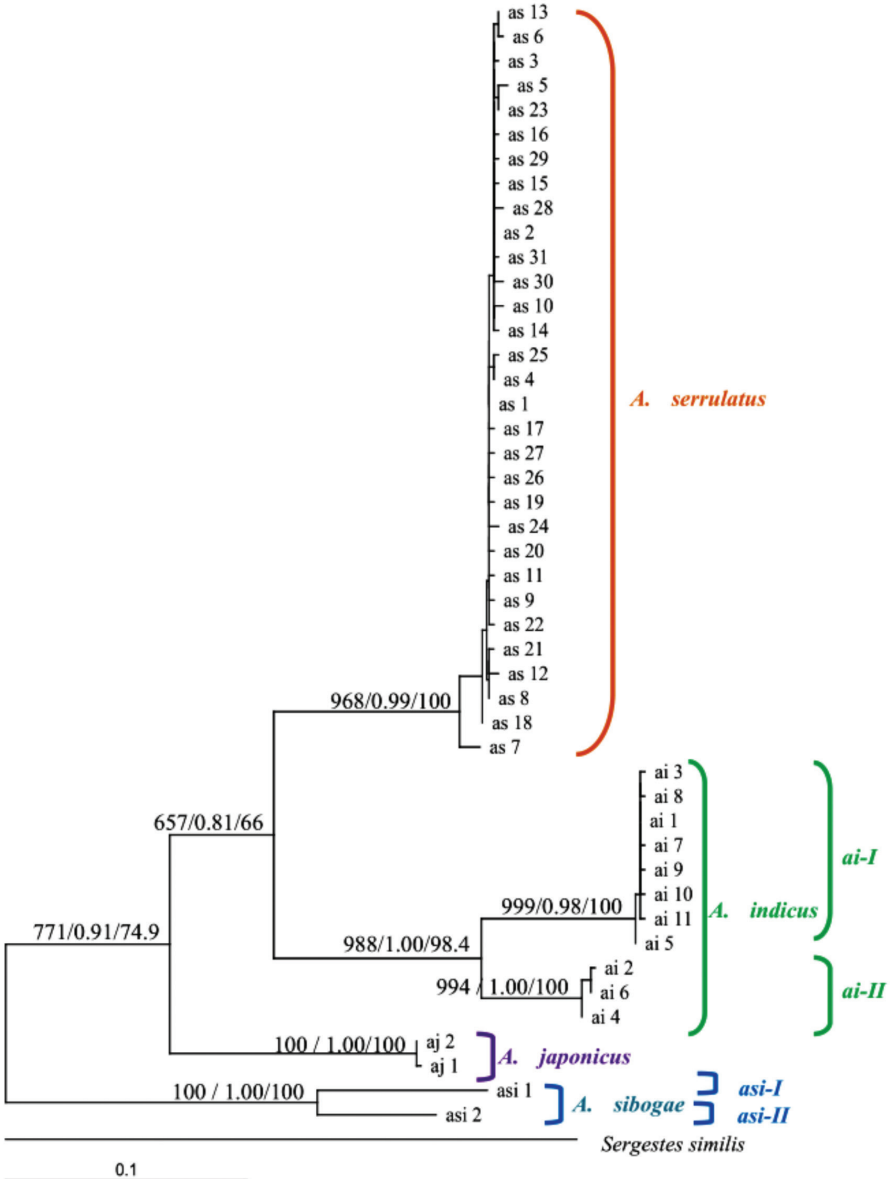

FIGURE 3. Maximum Likelihood (ML) tree from *COI* mtDNA haplotype data under the best-fitting model HKY+I+G selected by jModeltest

The parameters were as follows: model = HKY85, number of substitution types (nst) = 2, proportion of invariable sites (*p*-invar) = 0.6220, Transition/Transversion ratio = 4.2197 and gamma (γ) distribution shape parameter (α = 1.7320). The value at each node represents the bootstrap value (BS, %) for ML / posterior probability (PP) for BI / (BS%) for MP

2013). Each clade was strongly supported by high bootstrap (BS) values of 97-100% and posterior probability (PP) values of 0.99-1.00. Furthermore, *A. indicus* and *A. sibogae* were shown to cluster into two distinct clades, respectively.

The mean percentage of nucleotide sequence divergence (K2P) within and between *Acetes* species are summarized in Table 5. The interspecific variation ranged from 14.50-20.50%. This result indicates that *A. sibogae* was the most divergent among the four *Acetes* species, followed by *A. japonicus*, *A. serrulatus* and *A. indicus*. In addition, the two distinct clades of *A. indicus* and *A. sibogae* showed a mean sequence divergence value of 8.94% and 10.93%, respectively.

In the statistical parsimony haplotype network produced using TCS (Figure 4), both the *Acetes indicus* and *A. sibogae* formed two separate networks. For *A. indicus*, the clade *ai-II* haplotypes could not be parsimoniously connected to the *ai-I* clade network at the 95% significance criterion and the corresponding sequences were separated by at least 44 mutational steps from *ai-I* clade haplotypes. Similarly, the haplotype *asi2* was separated by 52 mutation steps from the haplotype *asi1*. For *A. japonicus*, both *aj1* and *aj2* haplotypes were connected. All haplotypes of *A. serrulatus*, with the exception of *as7* and *as25*, were connected to either one of the common haplotypes, *as1* and *as2*, with an overall

TABLE 5. Mean nucleotide sequence divergence (%) estimated with Kimura's Two Parameter (K2P), based on haplotypes only: (a) between and within *Acetes* species and outgroup, *Sergestes similis*; (b) between and within two distinct clades of *A. indicus*; (c) between and within two distinct clades of *A. sibogae*

Species	*A. indicus*	*A. serrulatus*	*A. japonicus*	*A. sibogae*	*S. similis* (outgroup)
Acetes indicus	4.08				
A. serrulatus	14.49	0.63			
A. japonicus	17.86	14.69	0.18		
A. sibogae	20.47	19.58	19.89	10.30	
S. similis (outgroup)	21.35	19.32	21.21	21.57	-

(b) Interclade variation of *A. indicus*

Clade	ai-I	ai-II
ai-I	0.32	
ai-II	8.94	0.36

(c) Interclade variation of *A. sibogae*

Clade	asi-I	asi-II
asi-I	-	
asi-II	10.30	-

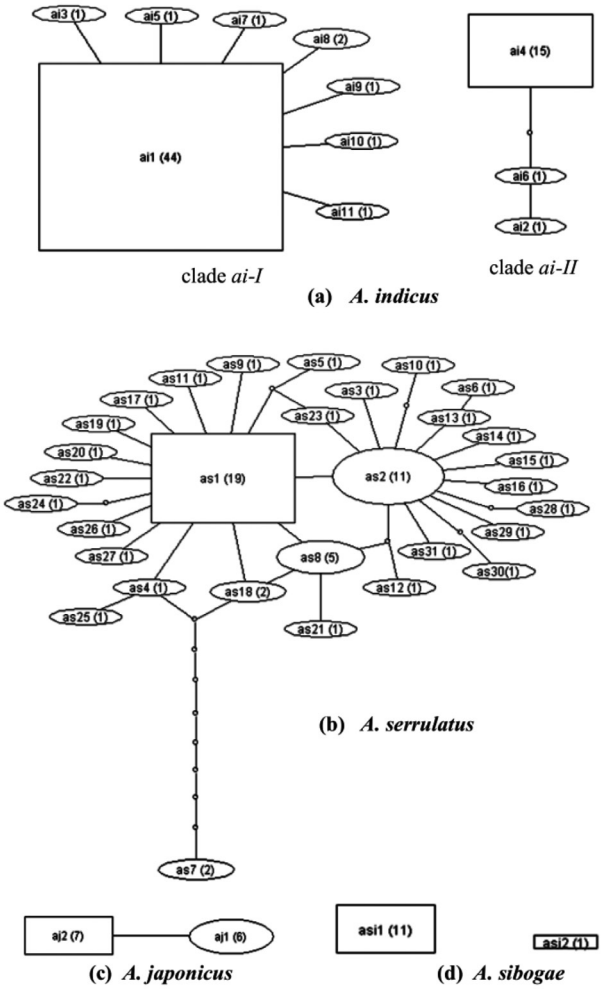

FIGURE 4. Parsimony network of (a) *A. indicus*, (b) *A. serrulatus*, (c) *A. japonicus* and (d) *A. sibogae* based on 552 bp of COI amplified in this study

Each oval represents a haplotype and the haplotype in a square has the highest outgroup probability. The size of the oval or square corresponds to the haplotype frequency. The haplotype abbreviations correspond to the haplotypes as reported in Table 2, and the number in parentheses corresponds to the frequency of the haplotype. Small circles indicate the number of mutational changes among haplotypes

appearance of the network resembling a radiating star-like shape (Figure 4).

There were 11 haplotypes detected for *A. indicus* (Table 6). The *ai1* haplotype was the most common and occurred in all but two locations, Kuala Gula and Kuala Kurau. The haplotype diversity for *A. indicus* was moderate ($h = 0.552$) while nucleotide diversity was high ($\pi = 0.031$) in the overall samples. However, when the two clades were analysed separately, low levels of both h (*ai-I* = 0.286, *ai-II* = 0.228) and π (*ai-I* = 0.001, *ai-II* = 0.001) were observed. *A. serrulatus* had 31 haplotypes in total with *as1* being the most common followed by *as2*, *as8*, *as7* and *as18*, respectively (Table 7). The overall haplotype diversity was high ($h = 0.886$) while overall nucleotide diversity was low ($\pi = 0.004$).

Both *A. japonicus* and *A. sibogae* had only two haplotypes each. Both haplotypes occurred in all four locations where *A. japonicus* was detected and the overall h was moderate (0.540) while π was low (0.001) (Table 8). The overall h and π was low for *A. sibogae* with one location having both haplotypes (Sungai Kubang Badak) while Kuala Sepetang had only one haplotype (Table 9).

The AMOVA results for each *Acetes* species are reported in Table 10. Only *A. indicus* showed significant population differentiation with 75.8% of the molecular variance due to variation among the sampling sites. There was also significant differentiation between the two clades *ai-I* and *ai-II* (99.2%). The pairwise Φ_{ST} was not significant for Kuala Kurau, Kuala Gula and the Portuguese Settlement when they were compared with one another. However, they were significant when each was compared with the other populations (Table 11). The Mantel Test indicated no correlation between Φ_{ST} estimates and geographical distribution ($r = 0.106$, $p>0.05$). Similar to AMOVA, the pairwise Φ_{ST} values were not significant for the other three *Acetes* species (detailed results not shown; Wong 2013).

All the Neutrality Tests were significant for *A. serrulatus* while *A. japonicus* was the only species that did not show any significance in these tests (Table 12). Although none of the Neutrality Tests were significant for the pooled *A. indicus* samples, the *ai-I* clade, which was analysed separately was significant for all the tests while the *ai-II* clade was significant only for R_2. Similarly, *A. sibogae* was only significant for Tajima's *D*.

A bimodal mismatch distribution was observed for *A. sibogae* ($SSD = 0.040$, $0.01< p<0.05$) (Figure 5). Similarly, a bimodal mismatch distribution was observed for the pooled samples of *A. indicus* ($SSD = 0.112$, $p>0.05$), which did not differ significantly from the distribution expected for population expansion. When the *ai-I* and *ai-II* clades were analysed separately, both showed a unimodal distribution that did not differ significantly from the distribution expected for population expansion (*ai-I*, $SSD = 0.005$, $p>0.05$; *ai-II*, $SSD = 0.040$, $p>0.05$), with peaks closer to zero (L-shaped distribution) (Figure 5). This pattern was also seen in *A. serrulatus* ($SSD = 0.004$, $p>0.05$) and *A. japonicus* ($SSD = 0.032$, $p>0.05$) (Figure 5).

TABLE 6. Haplotype compositions and summary of molecular diversity in *Acetes indicus* collected in this study

Haplo-type	Sampling Locations*											
	SGT	BPL	BL	KK	KG	TR	SKC	TKR	PSETT	PKKP	SGK	Total
ai1	7	7	6			3	6	5	1	4	5	44
ai2									1			1
ai3									1			1
ai4			1	5	6				3			15
ai5									1			1
ai6									1			1
ai7	1											1
ai8							1	1				2
ai9						1						1
ai10						1						1
ai11										1		1
n	8	7	7	5	6	5	7	6	8	5	5	69
S	1	0	45	0	0	2	1	1	47	1	0	50
N_{hap}	2	1	2	1	1	3	2	2	6	2	1	11
h	0.2500	0.0000	0.2857	0.0000	0.0000	0.7000	0.2857	0.3333	0.8929	0.4000	0.0000	0.5516
π	0.0005	0.0000	0.02323	0.0000	0.0000	0.0015	0.0005	0.0006	0.0450	0.0007	0.0000	0.0312

*Abbreviations for sampling locations: refer to Table 1

n: number of sequences; *S*: number of segregating sites; N_{hap}: number of haplotypes; *h*: haplotype diversity; and π: nucleotide diversity.

TABLE 7. Haplotype compositions and summary of molecular diversity in *Acetes serrulatus* collected in this study

Haplotype	Sampling locations*								Total
	SGT	BPL	BL	SKC	TKR	TR	PKKP	SGK	
as1	2	3	3	4	1	2	3	1	19
as2	3	4	2	1			1		11
as3		1							1
as4	1								1
as5	1								1
as6	1								1
as7	1	1							2
as8	1	1		1	1	1			5
as9			1						1
as10					1				1
as11						1			1
as12	1								1
as13	1								1
as14	1								1
as15	1								1
as16		1							1
as17		1							1
as18		1						1	2
as19			1						1
as20			1						1
as21				1					1
as22					1				1
as23					1				1
as24						1			1
as25					1				1
as26		1							1
as27		1							1
as28							1		1
as29								1	1
as30								1	1
as31								1	1
n	14	15	8	7	6	5	5	5	65
S	17	15	4	3	8	4	3	6	60
N_{hap}	11	10	5	4	6	4	3	5	31
h	0.9560	0.9143	0.8571	0.7143	1.0000	0.9000	0.7000	1.0000	0.8856
π	0.0062	0.0049	0.0021	0.0019	0.0052	0.0029	0.0025	0.0047	0.0042

*Abbreviations for sampling locations: refer to Table 1

n: number of sequences; *S*: number of segregating sites; N_{hap}: number of haplotypes; *h*: haplotype diversity; and π: nucleotide diversity

TABLE 8. Haplotype compositions and summary of molecular diversity in *Acetes japonicus* collected in this study

Haplotype	Sampling locations*			Total
	KK	KG	TBHG	
aj1	1	3	2	6
aj2	1	2	4	7
n	2	5	6	13
S	1	1	1	1
N_{hap}	2	2	2	2
h	1.0000	0.6000	0.5330	0.5386
π	0.0018	0.0011	0.0010	0.0010

*Abbreviations for sampling locations: refer to Table 1
n: number of sequences; *S*: number of segregating sites; N_{hap}: number of haplotypes;
h: haplotype diversity; and π: nucleotide diversity

TABLE 9. Haplotype compositions and summary of molecular diversity in *Acetes sibogae* collected in this study

Haplotype	Sampling locations*		Total
	SGKB	KS	
asi1	5	6	11
asi2	1	-	1
n	6	6	12
S	52	0	52
N_{hap}	2	1	2
h	0.3330	0.0000	0.1670
π	0.0314	0.0000	0.0157

*Abbreviations for sampling locations: refer to Table 1
n: number of sequences; *S*: number of segregating sites; N_{hap}: number of haplotypes;
h: haplotype diversity; and π: nucleotide diversity

TABLE 10. Analysis of Molecular Variance (AMOVA) for *Acetes indicus*, *A. serrulatus*, *A. japonicus* and *A, sibogae*

Analysis	Source of variation	d.f.	Sum of Squares	Variance Components[2]	Percentage of Variation	Fixation index (Φ_{ST})	*P* value
Acetes indicus	Among populations (Va)	10	721.343	10.97601	75.77	0.75768	0.00000 ± 0.00000
	Within populations (Vb)	58	203.601	3.51036	24.23		
		68	924.943	14.48637			
Acetes indicus	Among populations (Va)	7	6.902	-0.02362	-2.13	-0.02129	0.59881 ± 0.00491
(without KK, KG	Within populations (Vb)	42	47.586	1.13299	102.13		
and PSETT[1])		49	54.488	1.10937			
Acetes indicus	Among clade (Va)	1	573.519	22.37565	99.19	0.99188	0.00000 ± 0.00000
(clade ai-I and	Within clade (Vb)	67	12.278	0.18326	0.81		
ai-II)		68	585.897	22.55891			
Acetes serrulatus	Among populations (Va)	7	7.164	-0.02252	-1.91	-0.01912	0.78921 ± 0.00420
	Within populations (Vb)	57	68.437	1.20065	101.91		
		64	75.601	1.17812			
Acetes japonicus	Among populations (Va)	2	0.198	-0.05137	-20.28	-0.20285	0.76317 ± 0.00416
	Within populations (Vb)	10	3.046	0.30462	120.28		
		12	3.244	0.25325			
Acetes sibogae	Among populations (Va)	1	4.763	0.00000	0.00	0.00000	1.00000 ± 0.00000
	Within populations (Vb)	10	47.631	4.76314	100.00		
		11	52.395	4.76314			

[1]Sampling location, KK = Kuala Kurau, KG = Kuala Gula, PSETT = Portuguese Settlement
[2]Va, Vb, Vc are the associate covariance components

TABLE 11. Pairwise Φ_{ST} values (TrN + G=0.310) among *Acetes indicus* sampling populations calculated from *COI* sequences using Arlequin v3.5. Significance level (number of permutations: 10100): ns not significant; *0.01<*p*<0.05; **0.001< *p*<0.01; *** *p*<0.0001

Sampling population	SGT	BPL	BL	KK	KG	TR	SKC	TKR	PSETT	PKKP	SGK
SGT	–										
BPL	-0.01818 ns	–									
BL	0.01252 ns	-0.00000 ns	–								
KK	0.99772 ***	1.00000 **	0.80447 *	–							
KG	0.99791 ***	1.00000 ***	0.82013 **	0.00000 ns	–						
TR	0.04778 ns	0.06818 ns	-0.05644 ns	0.99427 **	0.99491 **	–					
SKC	0.00129 ns	0.00000 ns	0.00083 ns	0.99756 ***	0.99778 ***	0.02864	–				
TKR	0.00657 ns	0.02778 ns	-0.02271 ns	0.99737 **	0.99763 **	0.01268	-0.18052	–			
PSETT	0.56465 **	0.54739 **	0.29732 *	0.18745 ns	0.22064 ns	0.48839 *	0.54624 **	0.52149 **	–		
PKKP	0.01977 ns	0.07285 ns	-0.05229 ns	0.99716 **	0.99747 **	-0.00290	0.01372	0.00663	0.49273 *	–	
SGK	-0.06870 ns	0.00000 ns	-0.05528 ns	1.00000 **	1.00000 **	-0.00434	-0.05528	-0.03448	0.49346 *	0.00000	–

*Abbreviations for sampling locations: refer to Table 1

TABLE 12. Neutrality statistics (Tajima's D, Fu's F_s, Fu and Li's D^* and F^*, R_2), sum of square deviation (*SSD*) and Harpending's Raggedness index (r) were reported as well. Significance level: *$0.01<p<0.05$; **$0.001< p<0.01$; *** $p<0.001$; ^{ns} not significant

	Tajima's *D*	Fu's *Fs*	Fu and Li's *D**	Fu and Li's *F**	R_2	*SSD*	*r*
A. indicus, pooled	2.7155^{ns}	15.6730^{ns}	1.4841^{ns}	2.0928^{ns}	0.1715^{ns}	0.1116	0.2302
A. indicus, Clade ai-I	-2.1066**	-8.940***	-3.4682**	-3.5615**	-0.0460*	0.0051	0.2618
A. indicus, Clade ai-II	-1.0486^{ns}	-0.1260^{ns}	-0.0627^{ns}	-0.3736^{ns}	0.1075***	0.0399	0.6075
A. serrulatus	-2.0787**	-31.7964***	-3.5228**	-3.5664**	0.0314***	0.0041	0.0631
A. japonicus	1.4754^{ns}	1.2350^{ns}	0.7324^{ns}	1.0368^{ns}	0.2692^{ns}	0.0318	0.2959
A. sibogae	-2.2821***	11.772^{ns}	2.8994***	-3.1207***	0.2764^{ns}	0.0403*	0.7500

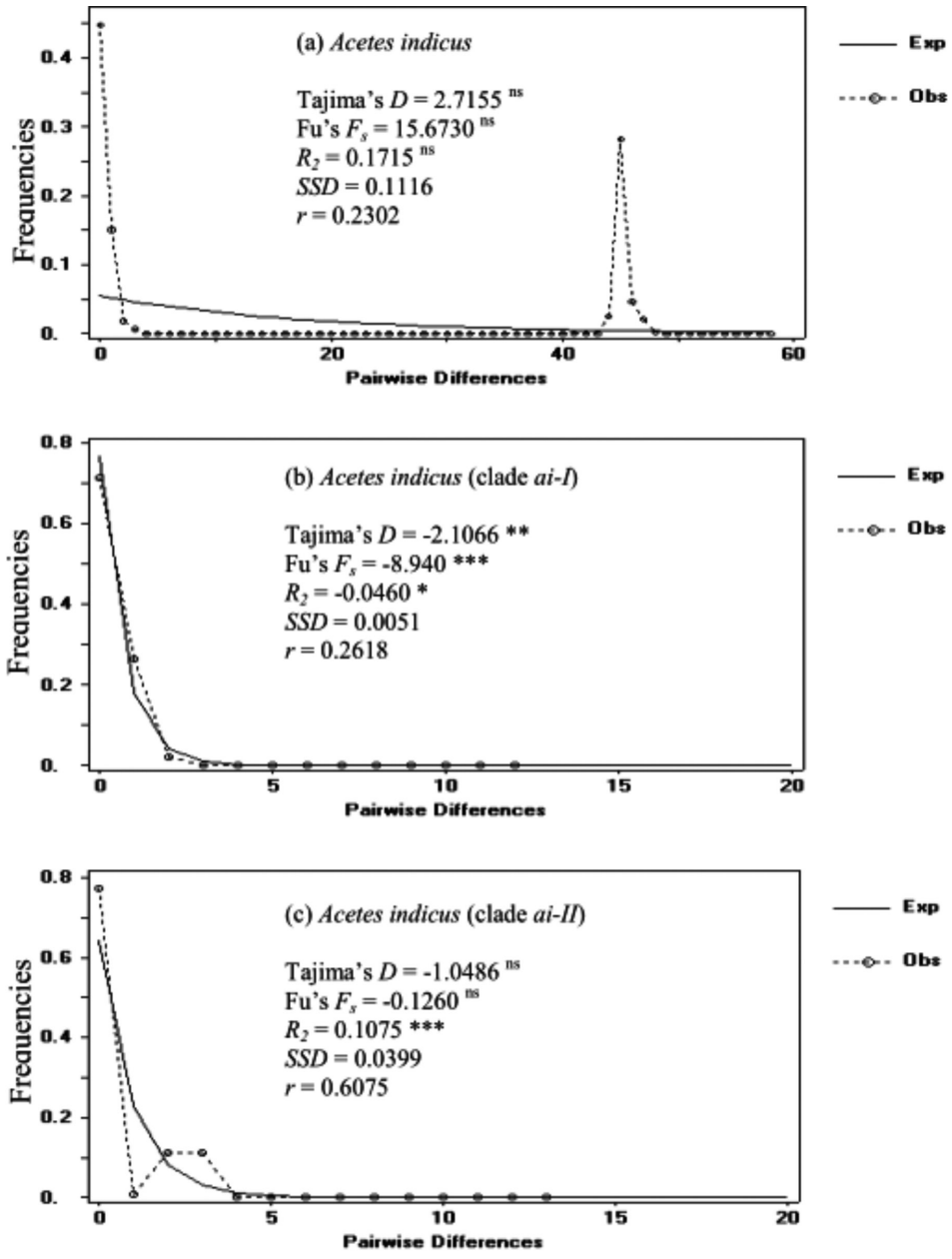

FIGURE 5. Mismatch distribution based on *COI* sequence from (a) *A. indicus*, (b) *A. indicus*, clade ai-I, and (c) *A. indicus*, clade ai-II. The graph represents the observed mismatch distribution from segregating sites of the aligned *COI* sequences. Dotted lines indicate the observed (Obs) distribution of mismatches, and solid lines show the expected (Exp) distribution under an expansion model. The numbers of pairwise differences are given on the horizontal axis and their frequencies on the vertical axis

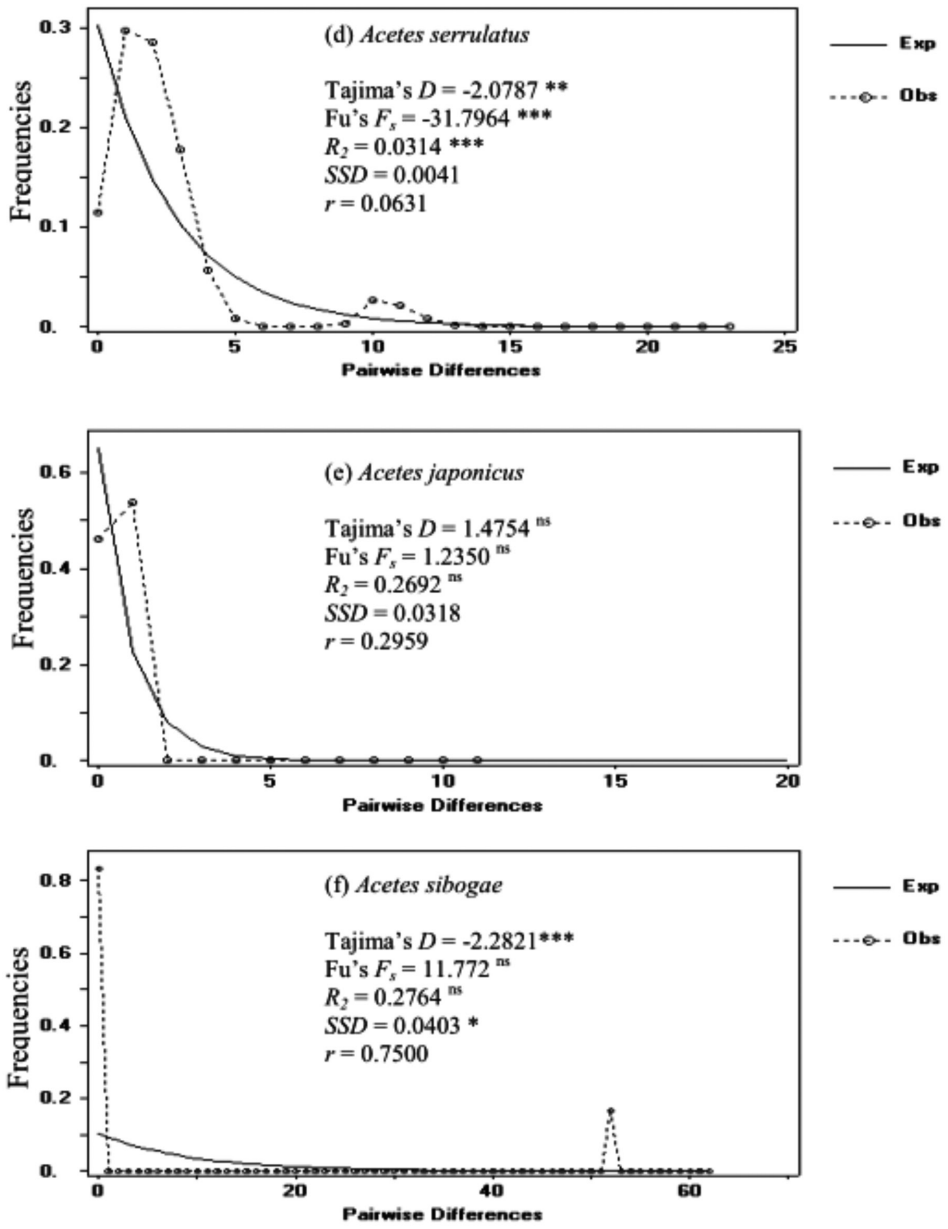

FIGURE 5 (*continuation*). Mismatch distribution based on *COI* sequence from (d) *A. serrulatus*, (e) *A. japonicus*, and (f) *A. sibogae*. The graph represents the observed mismatch distribution from segregating sites of the aligned *COI* sequences. Dotted lines indicate the observed distribution of mismatches, and solid lines show the expected distribution under an expansion model. The numbers of pairwise differences are given on the horizontal axis and their frequencies on the vertical axis

DISCUSSION

COI SEQUENCE VARIATION

High A+T content and positional biases, for example, slight bias against cytosine (17.3%) in the first position, in favour of thymine (45.7%) in the second position and substantial bias against guanine (2.9%) in the third position of mitochondrial *COI* gene fragment was found in all *Acetes indicus*, *A. serrulatus*, *A. japonicus* and *A. sibogae* individuals analysed in this study (Table 4). This pattern of base composition is similar to the *COI* gene region sequences in other groups of crustaceans, including *Raymunida* (Macpherson & Machordom 2001), Portunidae (Chu et al. 1999; Lai et al. 2010; Pfeiler et al. 2005), Alpheidae (Williams & Knowlton 2001; Williams et al. 2002, 2001), Gammaridae (Meyran et al. 1997), as well as some penaeid shrimp species (Baldwin et al. 1998; Maggioni et al. 2001; Quan et al. 2004; Tong et al. 2000; Zitari-Chatti et al. 2009).

With respect to the amino acid substitutions, *COI* is considered to be one of the most conservative genes in the mitochondrial genome (Black et al. 1997), thus only three amino acids substitution were detected in this study. The translation of the 552 bp of *COI* gene fragment

resulted in a sequence of 184 amino acids without in-frame stop codons or indels. Together with the patterns of base composition and base substitutions as discussed above, these observations showed that the *COI* gene fragment amplified in this study was not a nuclear mitochondrial pseudogenes (Numts) (Bensasson et al. 2001; Song et al. 2008; Zhang & Hewitt 1996) that have been reported in crustaceans, including in the snapping shrimp, *Alpheus* (Williams & Knowlton 2001; Williams et al. 2002).

INTERSPECIFIC VARIATION OF *ACETES* SPECIES

From the phylogenetic trees inferred from the COI sequence (Figures 2 & 3), it is evident that four distinct clades could be clearly identified using NJ, MP, ML and BI. All clades were monophyletic and supported by high BS and PP that corresponded with the four different *Acetes* species identified morphologically. This indicates that the COI molecular trees and species identification based on morphological characters provided by Omori (1975) and Wong (2013) are congruent. In addition, the aligned 552 bp of COI sequence showed a divergence range of 14.69-20.47% among the four Acetes species in this study (Table 5). This level of sequence divergence is similar to those reported in other shrimp genera such as *Penaeus* (8.0-24.0%; Baldwin et al. 1998) and *Metapenaeus* (6.1-19.9%; Tong et al. 2000), but is higher than those of the portunid sister groups (2-7%; Lai et al. 2010).

INTRASPECIFIC VARIATION OF *ACETES* SPECIES

Acetes indicus For the pooled samples, the moderate haplotype diversity and high nucleotide diversity is a good reflection of the abundance of this species within the west coast of Peninsular Malaysia (Table 6). This species was found in most sampling sites and had previously been reported to occur from the north-western region to the south of Peninsular Malaysia (Amani et al. 2011c; Amin et al. 2011, 2009a, 2009b, 2009c, 2009d; Fernandez-Leborans et al. 2009; Oh et al. 2010), mainly from in-shore catches. In this study, *A. indicus* was also caught from off-shore catches. However, the actual geographical range of dispersal of *A. indicus* is unknown.

The moderate haplotype diversity and high nucleotide diversity is also indicative of past evolutionary processes (Table 6), suggesting either secondary contact between historically isolated populations or stable populations with large, long term-effective population sizes (Grant & Bowen 1998). The secondary contact of historically isolated populations could have occurred due to the fluctuation of sea-levels in the regions around the Sunda and Sahul shelves in which low sea levels led to the formation of large land masses which partly isolated the Indian Ocean from the West Pacific and enclosed the South China Sea, Sulu Sea and Sulawesi Sea (Voris 2000). The central part of Indo-West Pacific area is reported as the geographical range of this species (Chan 1998; Holthius 1980; Omori 1975; Xiao & Greenwood 1993). The receding sea levels could have temporarily isolated the *A. indicus* populations occurring in these regions and restricted gene flow among the populations. Thus, the isolated *A. indicus* populations could have evolved separately and secondary contact occurred only during subsequent increase of the sea levels. Using the minimum and maximum nucleotide divergence between the two distinct clades of *A. indicus* (*ai-I* and *ai-II*) seen in the NJ tree (Figure 2) and the 1.40-3.00% per million years for *COI* divergence rates for decapod crustaceans on K2P distances (Table 5), we found a 2.98-6.39 million years ago (MYA) split between the clades indicating an early Pliocene to late Miocene divergence, thus supporting the geographical isolation episode mentioned earlier. The mixture of haplotypes found in the Bagan Lipas and the Portuguese Settlement populations may reflect secondary contact between the two clades (Table 6).

When the two clades were analysed separately, low haplotype diversity and nucleotide diversity were observed (Table 6). This pattern of low genetic diversity often reflects recent events of population bottleneck or founder effects by a single or a few mtDNA lineages (Grant & Bowen 1998). The NJ tree showed two deep clades for *ai-I* and *ai-II* (Figure 2), but shallow phylogeny within these two clades suggests population expansion after bottleneck (Slatkin & Hudson 1991). This hypothesis was also supported by the unimodal mismatch distribution, the non-significant value of sum of squared deviation (*SSD*) and Harpending's raggedness index (*r*) and the negative values of Tajima's *D* and Fu's F_S (Aris-Brosou & Excoffier 1996; Fu, 1997; Rand 1996; Tajima 1989) (Table 12; Figure 5a-5c).

The L-shaped mismatch distribution has been reported in other shrimp species indicating population expansion from a smaller initial population and recent bottleneck events (Frankham et al. 2002; Li et al. 2009; Pellerito et al. 2009; Rogers & Harpending 1992; Roldan et al. 2009). As many as 10 major Pleistocene sea-level fluctuation events, during which the Sunda Shelf (including the Straits of Malacca) was exposed had occurred with the latest being the last-glacial maximum, around 18000-20000 years ago during which the sea level dropped to about 120 m below the present level in Southeast Asia (Hanebuth et al. 2000; Pillans et al. 1998). These events could have caused local extinctions in the sampling area of this study. Based on the τ value computed using Arlequin and 1.4-3.0% mutation rates (Tables 5, 10 & 11), the estimates of the time since the most recent sudden population expansion for these two clades fall within the range of 97000-45000 years ago, coinciding with the Pleistocene era sea fluctuations. Therefore, for *A. indicus*, the genetic diversity seems to suggest secondary contact between historically isolated populations (Wong 2013).

Acetes serrulatus A high haplotype diversity and low nucleotide diversity were observed for this species (Table 7). Similar to *A. indicus*, the high haplotype diversity could be due to the occurrence of this species in both in- and offshore catches of a relatively large region from the central to the south of the west coast of Peninsular Malaysia.

Previous studies had only reported their occurrence along in-shore areas of the south of the west coast (Amin et al. 2011, 2009d; Oh et al. 2011). The high haplotype diversity and low nucleotide diversity combination usually suggests a population that had undergone population bottlenecks followed by rapid population growth and accumulation of mutations (Avise et al. 1984; Grant & Bowen 1998), which have also been noted in other marine species (Chen et al. 2004; Daemen et al. 2001; Kong et al. 2010; Liu et al. 2008; Maggio et al. 2009; Pellerito et al. 2009; Stockley et al. 2005).

The low nucleotide diversity reflects low genetic divergence among *A. serrulatus* individuals (Table 7). As reported for *A. indicus*, the shallow phylogeny of the NJ tree (Figure 2) is consistent with a population expansion event after a period of low effective population sizes caused by bottlenecks or founder effects (Slatkins & Hudson 1991). Negative and significant values of Tajima's *D* and Fu's F_S and significant R_2 indicate population expansion (Aris-Brosou & Excoffier 1996; Fu 1997; Ramos-Onsins & Rozas 2002; Rand 1996; Tajima 1989) (Table 12; Figure 5). The star-like radiating pattern of the haplotype network (Figure 4), unimodal mismatch distribution, and low non-significance of Harpending's Raggedness index (*r*) further supports a hypothesis of recent population expansion (Rogers & Harpending 1992; Slatkin & Hudson 1991). There is an excess of rare mutations seen here as excess in singletons suggests accumulation of mutations during the rapid population growth (Avise et al. 1984; Jorde et al. 2001; Rogers & Harpending 1992; Ramos-Onsins & Rozas 2002; Slatkins & Hudson 1991). As in *A. indicus* (Tables 5, 10 & 11), *A. serrulatus* appears to have also undergone late-Pleistocene expansion, due to the fluctuating sea levels (Wong 2013). The estimates of the time since the most recent population expansion event for *A. serrulatus* took place approximately 61000-28000 years ago, coinciding with rising sea levels during the late Pleistocene (Geyh et al. 1979; Hanebuth et al. 2000; Voris 2000).

Acetes japonicus AND *Acetes sibogae* The presence of these two species from the sampling sites in the northern waters of the west coast of Peninsular Malaysia is similar to that reported by Amani et al. (2011a, 2011b, 2011c), Amin et al. (2011, 2010, 2009c, 2009d), Arshad et al. (2012), Fernandez-Leborans et al. (2009), Hanamura (2007) and Panthansali (1966). However, we did not detect any of these two species at the southern region of the west coast during the sampling period of our study (Wong 2013; Wong et al. 2015). As such, with only three sample populations for *A. japonicus* and two populations for *A. sibogae*, demographic analyses and genetic diversity of these species were not conducted in detail to avoid inaccurate and biased reporting (Tables 8-10 & 12).

CRYPTIC DIVERSITY AND ITS IMPLICATIONS

Although morphologically defined species was congruent with the mtDNA in this study, it does not reveal all the variations that are present genetically, especially in the cryptic diversity possibly present in *A. indicus* and *A. sibogae*. Evidence for cryptic diversity comes from the extent of the genetic distance seen between clades within these two species (Wong 2013). Sequence divergence between clades ai-I and ai-II (8.94%) and clades asi-I and asi-II (10.30%) are lower than interspecific *COI* divergences of *Acetes* species in the current study (Table 5). Divergence values of similar magnitude have been noted in cryptic or sibling species (i.e. morphologically indistinguishable, but genetically distinct) of other decapod crustaceans (Bickford et al. 2007; Knowlton 1986; Pfenninger & Schwenk 2007). In particular, studies have reported a 6-8% divergence between two cryptic species of the kuruma shrimp, *Penaeus japonicus* (Tsoi et al. 2007, 2005), a 2-5% divergence between two sibling alpheid species, *Alpheus angulatus* and *A. armillatus* (Mathews et al. 2002), two morphologically indistinguishable clades within *Fenneropenaus* (*Penaeus*) *merguiensis* with an average divergence of 5% (Hualkasin et al. 2003) and 2-7% genetic divergence among sister groups of *Portunus* spp. (Lai et al. 2010).

Further support for cryptic diversity is shown by the *COI* haplotypes of *A. indicus* and *A. sibogae* which grouped into two disconnected statistical parsimony network at the 95% connection limit (Figure 4). As proposed by Chen et al. (2010) and Hart and Sunday (2007), statistical parsimony networks that are separated by more than the parsimony connection limit would indicate the presence of cryptic species. Hence, the high sequence divergence values and the disconnected parsimony network for *Acetes* suggest that cryptic taxa may be present in *A. indicus* and *A. sibogae* (Wong 2013). Cryptic species require special consideration in conservation planning especially for highly exploited resources. The likely presence of cryptic complexes within these *Acetes* species implies that more in-depth knowledge of location boundaries (if any) and other biotic and abiotic factors for each species would need to be considered for conservation efforts to ensure the long term sustainability of the *Acetes* fishing industry. Fishery activities beyond sustainable limits coupled with the presence of unknown cryptic species can lead to the disappearance of these resources (Thorpe et al. 2000).

Another implication of cryptic diversity is that previous reports of the life cycles of *Acetes* spp. may not reflect the true biological nature of these species, since those reports were based solely on morphological data (Amani et al. 2011a, 2011b, 2011c; Amin et al. 2011, 2010, 2009a, 2009b, 2009c, 2009d, 2008; Arshad et al. 2012, 2008, 2007) which is unable to differentiate among the cryptic complexes. Our study recommends that a thorough and detailed investigation should be carried out throughout the year with more sampling sites for both in- and offshore *Acetes* populations in order to elucidate the actual distribution and life cycles of each cryptic complex. This should also be coupled with nuclear gene studies (Wong 2013). Since mtDNA has a higher rate of evolution and thus more mutations than nuclear genes, it

is essential to have genealogical data from nuclear genes, which are inherited from both parents, to establish the status of these two clades. These studies would provide a better management plan for sustainable fishing over time.

In conclusion, this study presents evidence of the molecular phylogenetic relationships among four major *Acetes* species sampled from the west coast of Peninsular Malaysia. The four species were found to vary considerably for haplotype and nucleotide diversity, with *A. indicus* and *A. serrulatus* having different demographic histories. Furthermore, the observation of two clades within the *A. indicus* and *A. sibogae* lineages, with relatively high levels of intraspecific divergence, suggests that cryptic diversity may occur in these two taxa.

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REFERENCES

Allendorf, F.W. & Luikart, G. 2006. *Conservation and the Genetics of Populations*. Oxford: Blackwell Publishing, MA.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W. & Lipman, D.J. 1990. Basic local alignment search tool. *Journal of Molecular Biology* 215(3): 403-410.

Amani, A.A., Arshad, A., Amin, S.M.N. & Aziz, N.A.A. 2011a. Catch composition of a set bag net used for *Acetes* trapping in the estuarine waters of Kedah, Peninsular Malaysia. *Journal of Fisheries and Aquatic Science* 6(3): 279-284.

Amani, A.A., Amin, S.M.N., Arshad, A. & Rahman, M.A. 2011b. Population dynamics of sergestid shrimps *Acetes japonicus* in the estuary of Tanjung Dawai, Kedah, Malaysia. *Journal of Fisheries and Aquatic Science* 6(7): 751-760.

Amani, A.A., Amin, S.M.N. & Arshad, A. 2011c. Stomach contents of sergestid shrimp *Acetes japonicus* from the estuary of Tanjung Dawai, Peninsular Malaysia. *Journal of Fisheries and Aquatic Science* 6(7): 771-779.

Amin, S.M.N., Arshad, A., Siraj, S.S. & Bujang, J.S. 2011. Update on the species composition and distribution of sergestide shrimps (*Acetes* spp.) in Malaysian waters. *Journal of Fisheries and Aquatic Science* 6(7): 761-770.

Amin, S.M.N., Arshad, A., Siraj, S.S. & Japar, S.B. 2010. Reproductive seasonality and *maturation of the sergestid shrimp, Acetes japonicus (Decapoda: Sergestidae) in* coastal waters of Malacca, Peninsular Malaysia. *African Journal of Biotechnology* 9(45): 7747-7752.

Amin, S.M.N., Arshad, A., Bujang, J.S. & Siraj, S.S. 2009a. Age structure, growth, mortality and yield-per-recruit of sergestid shrmp, *Acetes indicus* (Decapoda: Sergestidae) from the coastal waters of Malacca, Peninsular Malaysia. *Journal of Applied Sciences* 9(5): 801-814.

Amin, S.M.N., Arshad, A., Bujang, J.S., Siraj, S.S. & Goddard, S. 2009b. Reproductive biology of the sergestid shrimp *Acetes indicus* (Decapoda: Sergestidae) in coastal waters of Malacca, Peninsular Malaysia. *Zoological Studies* 48(6): 753-760.

Amin, S.M.N., Arshad, A., Siraj, S.S. & Japar, S.B. 2009c. Population structure, growth, mortality and yield per recruit of sergestid shrimp, *Acetes japonicus* (Decapoda: Sergestidae) from the coastal waters of Malacca, Peninsular Malaysia. *Indian Journal of Marine Science* 38(1): 57-68.

Amin, S.M.N., Arshad, A., Siraj, S.S. & Bujang, J.S. 2009d. Population structure, growth and length - weight relationship of sergestid shrimps (*Acetes* spp.) from the coastal waters of Malacca, Peninsular Malaysia. *Sains Malaysiana* 38(2): 159-169.

Amin, S.M.N., Arshad, A., Zainal, Z., Idris, M.H., Siraj, S.S. & Japar, S.B. 2008. First distribution records of *Acetes intermedius* (Decapoda: Sergestidae) from the coastal waters of Bintulu, Sarawak: Population structure, length-weight and length-length relationship. *Journal of Sustainability Science and Management* 3(1): 74-83.

Aris-Brosou, S. & Excoffier, L. 1996. The impact of population expansion and mutation rate heterogeneity on DNA sequence polymorphism. *Molecular Biology and Evolution* 13(3): 494-504.

Arshad, A., Amin, S.M.N., Nuradiella, Y.L.Z., Cob, Z.C., Ara, R. & Aziz, D. 2012. Population characteristics of *Acetes japonicus* from the Kedah coastal waters of Peninsular Malaysia. *Journal of Fisheries and Aquatic Science* 7(2): 162-172.

Arshad, A., Amin, S.M.N., Yu, G.T., Oh, S.Y., Bujang, J.S. & Ghaffar, M.A. 2008. Population characteristics, length-weight and length-length relationships of *Acetes vulgaris* (Decapoda: Sergestidae) in the coastal waters of Pontian, Johor, Peninsular Malaysia. *Journal of Biological Sciences* 8(8): 1298-1303.

Arshad, A., Amin, S.M.N., Siraj, S.S. & Japar, S.B. 2007. New distribution records of sergestid shrimp, *Acetes intermedius* (Decapoda: Sergestidae) from Peninsular Malaysia with notes on its population characteristics. *Journal of Biological Sciences* 7(8): 1305-1313.

Avise, J., Neigel, J. & Arnold, J. 1984. Demographic influences on mitochondrial DNA lineage survivorship in animal populations. *Journal of Molecular Evolution* 20(2): 99-105.

Baldwin, J.D., Bass, A.L., Bowen, B.W. & Clark, W.H. 1998. Molecular phylogeny and biogeography of the marine shrimp *Penaeus*. *Molecular Phylogenetics and Evolution* 10(3): 399-407.

Bensasson, D., Zhang, D.X., Hartl, D.L. & Hewitt, G.M. 2001. Mitochondrial pseudogenes: Evolution's misplaced witnesses. *Trends in Ecology and Evolution* 16(6): 314-321.

Bickford, D., Lohman, D.J., Sodhi, N.S., Ng, P.K.L., Meier, R., Winker, K., Ingram, K.K. & Das, I. 2007. Cryptic species as a window on diversity and conservation. *Trends in Ecology & Evolution* 22(3): 148-155.

Black, M.B., Halanych, K.M., Maas, P.A.Y., Hoeh, W.R., Hashimoto, J., Desbruyères, D., Lutz, R.A. & Vrijenhoek, R.C. 1997. Molecular systematics of vestimentiferan tubeworms from hydrothermal vents and cold-water seeps. *Marine Biology* 130(2): 141-149.

Black. A.R. & Dodson, S.I. 2003. Ethanol: A better preservation technique for *Daphnia*. *Limnology and Oceanography: Methods* 1: 45-50.

Bucklin, A. 2009. CMarz sample collection protocol. Census of Marine Zooplankton. CMarz Barcoding Association. (http://www.cmarz.org/barcode/protocols/barcode_CMarZ_Sample_Protocol_jan06.html)

Camin, J. & Sokal, R. 1965. A method for deducing branching sequences in phylogeny. *Evolution* 19(3): 311-326.

Carvalho, G.R. & Hauser, L. 1994. Molecular genetics and the stock concept in fisheries. *Reviews in Fish Biology and Fisheries* 4(3): 326-350.

Chan, T.Y. 1998. Shrimps and prawns. In *FAO Species Identification Guide for Fishery Purposes. The Living Marine Resource of the Western Central Pacific, Vol. 2. Cephalopds, Crustaceans, Holothurians and Sharks*, edited by Carpenter, K.E. & Niem, V.H. Rome: FAO.

Chen, C.A., Ablan, M.C.A., Mcmanus, J.W., Bell, J.D., Tuan, V.S., Cabanban, A.S. & Shao, K.T. 2004. Population structure and genetic variability of six bar wrasse (*Thallasoma hardwicki*) in Northern South China Sea revealed by mitochondrial control region sequences. *Marine Biotechnology* 6(4): 312-326.

Chen, H., Strand, M., Norenburg, J.L., Sun, S., Kajihara, H., Chernyshev, A.V., Maslakova, S.A. & Sundberg, P. 2010. Statistical parsimony networks and species assemblages in cephalotrichid nemerteans (nemertea). *PLoS ONE* 5(9): e12885.

Chu, K.H., Tong, J. & Chan, T.Y. 1999. Mitochondrial cytochrome oxidase I sequence divergence in some Chinese species of *Charybdis* (Crustacea: Decapoda: Portunidae). *Biochemical Systematics and Ecology* 27(5): 461-468.

Clement, M., Posada, D. & Crandall, K.A. 2000. TCS: A computer program to estimate gene genealogies. *Molecular Ecology* 9(10): 1657-1659.

Cook, B.D., Baker, A.M., Page, T.J., Grant, S.C., Fawcett, J.H., Hurwood, D.A. & Hughes, J.M. 2006. Biogeographic history of an Australian freshwater shrimp, *Paratya australiensis* (Atyidae): The role life history transition in phylogeographic diversification. *Molecular Ecology* 15: 1083-1093.

Costa, F.O., deWaard, J.R., Boutillier, J., Ratnasingham, S., Dooh, R.T., Hajibabaei, M. & Hebert, P.D. 2007. Biological identifications through DNA barcodes: The case of the Crustacea. *Canadian Journal of Fisheries and Aquatic Sciences* 64(2): 272-295.

Daeman, E., Cross, T., Ollevier, F. & Volckaert, F.A.M. 2001. Analysis of the genetic structure of European eel (*Anguilla anguilla*) using microsattelite DNA and mtDNA markers. *Marine Biology* 139(4): 755-764.

Deshmukh, V.D. 1991. Utilisation of paste shrimp *Acetes*: A review. *Marine Fisheries Information Service Technical Extension Series* 110: 7-8.

Díaz-Viloria, N., Sánchez-Velasco, L. & Perez-Enriquez, R. 2005. Inhibition of DNA amplification in marine fish larvae preserved in formalin. *Journal of Plankton Research* 27(8): 787-792.

DiStefano, R.J., Roell, M.J., Wagner, B.A. & Decoske, J.J. 1994. Relative performances of four preservatives on fish and crayfish. *Transactions of the American Fisheries Society* 123: 817-823.

DOF. 2013. *Annual Fisheries Statistics*. Kuala Lumpur: Department of Fisheries Malaysia, Ministry of Agriculture.

Excoffier, L. & Lischer, H.E.L. 2010. Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* 10(3): 546-567.

Excoffier, L., Smouse, P.E. & Quattro, J.M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. *Genetics* 131(2): 479-491.

Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 39(4): 783-791.

Felsenstein, J. 1981. Evolutionary trees from DNA sequences: A maximum likelihood approach. *Journal of Molecular Evolution* 17(6): 368-376.

Fernandez-Leborans, G., Hanamura, Y., Siow, R. & Chee, P.E. 2009. Intersite epibiosis characterization on dominant mangrove crustacean species from Malaysia. *Contributions to Zoology* 78(1): 9-23.

Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. 1994. DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3(5): 294-299.

Frankham, R., Ballou, J.D. & Briscoe, D.A. 2002. *Introduction to Conservation Genetics*. Cambridge: Cambridge University Press.

Fu, Y.X. 1997. Statistical tests of neutrality against population growth, hitchhiking and background selection. *Genetics* 147: 915-925.

García-Machado, E., Robainas, A., Espinosa, G., Oliva, M., Páez, J., Verdecia, N. & Monnerot, M. 2001. Allozyme and mitochondrial DNA variation in Cuban populations of the shrimp *Farfantepenaeus notialis* (Crustacea: Decapoda). *Marine Biology* 138(4): 701-707.

Gascuel, O. 1997. BIONJ: An improved version of the NJ algorithm based on a simple model of sequence data. *Molecular Biology and Evolution* 14(7): 685-695.

Geyh, M.A., Steif, H. & Kudrass, H.R. 1979. Sea-level changes during the late Pleistocene and Holocene in the Strait of Malacca. *Nature* 278(5703): 441-443.

Grant, W.S. & Bowen, B.W. 1998. Shallow population in deep evolutionary lineages of marine fishes: Insights from sardines and anchovies and lessons for conservation. *Journal of Heredity* 89(5): 415-426.

Guindon, S., Lethiec, F., Duroux, P. & Gascuel, O. 2005. PHYML Online - a web server for fast maximum likelihood-based phylogenetic inference. *Nucleic Acids Research* 33(Suppl 2): W557-W559.

Hanamura, Y., Siow, R. & Chee, P.E. 2007. Abundance and spatio temporal distributions of hyper benthic crustaceans in the Merbok and Matang mangroves estuaries, Malaysia. In *Sustainable Production Systems of Aquatic Animals in Brackish Mangrove Areas* (2005), edited by Nakamura K. Japan International Research Center for Agriculture Sciences (JIRCAS).

Hanebuth, T., Stattegger, K. & Grootes, P.M. 2000. Rapid flooding of the Sunda Shelf: A late-glacial sea-level record. *Science* 288(5468): 1033-1035.

Harpending, H.C. 1994. Signature of ancient population growth in a low-resolution mitochondrial DNA mismatch distribution. *Human Biology* 66(4): 591-600.

Harrison, M.K. & Crespi, B.J. 1999. Phylogenetics of cancer crabs (Crustacea: Decapoda: Brachyura). *Molecular Phylogenetics and Evolution* 12(2): 186-199.

Hart, M.W. & Sunday, J. 2007. Things fall apart: Biological species form unconnected parsimony networks. *Biology Letters* 3(5): 509-512.

Hebert, P.D.N., Cywinska, A., Ball, S.L. & deWaard, J.R. 2003. Biological identifications through DNA barcodes. *Proceedings of the Royal Society London B* 270(1512): 313-321.

Holthuis, L.B. 1980. *FAO Species Catalogue Vol. 1. Shrimps and Prawns of the World: An Annotated Catalogue of Species of Interest to Fisheries*. Rome: FAO Fisheries Synopsis. 125(1): 62-67.

Hualkasin, W., Sirimontaporn, P., Chotigeat, W., Querci, J. & Phongdara, A. 2003. Molecular phylogenetic analysis of white prawns species and the existence of two clades in

Penaeus merguiensis. *Journal of Experimental Marine Biology and Ecology* 296(1): 1-11.

Hudson, R.R. 1990. Gene genealogies and the coalescent process. *Oxford Surveys in Evolutionary Biology* 7: 1-44.

Huelsenbeck, J.P. & Ronquist, F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17(8): 754-755.

Hurvich, C.M. & Tsai, C.L. 1989. Regression and time series model selection in small samples. *Biometrika* 76(2): 297-307.

Jarvis, J.P., Luedeman, J.K. & Shier, D.R. 1983. Comments on computing the similarity of binary trees. *Journal of Theoretical Biology* 100(3): 427-433.

Job, S., Buu, D. & Vincent, A. 2006. Growth and survival of the tiger tail seahorse, *Hippocampus comes*. *Journal of the World Aquaculture Society* 37(3): 322-327.

Jorde, L.B., Watkins, W.S. & Bamshad, M.J. 2001. Population genomics: A bridge from evolutionary history to genetic medicine. *Human Molecular Genetics* 10(20): 2199-2207.

Khamnamtong, B., Klinbunga, S. & Menasveta, P. 2009. Genetic diversity and geographic differentiation of the giant tiger shrimp (*Penaeus monodon*) in Thailand analyzed by mitochondrial *COI* sequences. *Biochemical Genetics* 47(1-2): 42-55.

Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 16(2): 111-120.

Knowlton, N. 1986. Cryptic and sibling species among the decapod crustacea. *Journal of Crustacean Biology* 6(3): 356-363.

Kong, X.Y., Li, Y.L., Shi, W. & Kong, J. 2010. Genetic variation and evolutionary demography of *Fenneropenaeus chinensis* populations, as revealed by the analysis of mitochondrial control region sequences. *Genetics and Molecular Biology* 33(2): 379-389.

Lai, J.C.Y., Ng, P.K.L. & Davie, P.J.F. 2010. A revision of the *Portunus pelagicus* (Linnaeus, 1758) species complex (Crustacea: Brachyura: Portunidae), with the recognition of four species. *The Raffles Bulletin of Zoology* 58(2): 199-237.

Li, Y.L., Kong, X.Y., Yu, Z.N., Kong, J., Ma, S. & Chen, L.M. 2009. Genetic diversity and historical demography of Chinese shrimp *Fenneropenaeus chinensis* in Yellow Sea and Bohai Sea based on mitochondrial DNA analysis. *African Journal of Biotechnology* 8(7): 1193-1202.

Librado, P. & Rozas, J. 2009. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25(11): 1451-1452.

Liu, S.Y., Kokita, T. & Dai, C.F. 2008. Population genetic structure of the neon damselfish (*Pomacentrus coelestis*) in the northwestern Pacific Ocean. *Marine Biology* 154(4): 745-753.

Longhurst, A.R. 1970. Crustacean resources. In *The Fish Resources of the Oceans,* edited by Gulland, J.A. *FAO Fisheries Technical Paper No. 97*. Rome: FAO. pp. 252-305.

Machordom, A. & Macpherson, E. 2004. Rapid radiation and cryptic speciation in squat lobsters of the genus *Munida* (Crustacea, Decapoda) and related genera in the South West Pacific: Molecular and morphological evidence. *Molecular Phylogenetics and Evolution* 33(2): 259-279.

Macpherson, E. & Machordom, A. 2001. Phylogenetic relationships of species of *Raymunida* (Decapoda: Glatheidae) based on morphology and mitochondrial cytochrome oxidase sequences, with the recognition of four new species. *Journal of Crustacean Biology* 21(3): 696-714.

Maggio, T., Lo Brutto, S., Cannas, R., Deiana, A.M. & Arculeo, M. 2009. Environmental features of deep-sea habitats linked to the genetic population structure of a crustacean species in the Mediterranean Sea. *Marine Ecology* 30(3): 354-365.

Maggioni, R., Rogers, A.D., Maclean, N. & D'Incao, F. 2001. Molecular phylogeny of western atlantic *Farfantepenaeus* and *Litopenaeus* shrimp based on mitochondrial 16S partial sequences. *Molecular Phylogenetics and Evolution* 18(1): 66-73.

Mantel, N. 1967. The detection of disease clustering and a generalised regression approach. *Cancer Research* 27: 209-220.

Mathews, L.M., Schubart, C.D., Neigel, J.E. & Felder, D.L. 2002. Genetic, ecological, and behavioural divergence between two sibling snapping shrimp species (Crustacea: Decapoda: *Alpheus*). *Molecular Ecology* 11(8): 1427-1437.

Meyran, J.C., Monnerot, M. & Taberlet, P. 1997. Taxonmic status and phylogenetic relationships of some species of the genus *Gammarus* (Crustacea, Amphipoda) deduced from mitochondrial DNA sequence. *Molecular Phylogenetics and Evolution* 8(1): 1-10.

Nei, M. 1987. *Molecular Evolutionary Genetics*. New York: Columbia University Press.

Oh, S.Y., Arshad, A., Japar, S.B., Nor Azwady, A.A. & Amin, S.M.N. 2011. Diet composition of sergestid shrimp *Acetes serrulatus* from the coastal waters of Kukup, Johor, Malaysia. *Journal of Fisheries and Aquatic Science* 6(7): 809-815.

Oh, S.Y., Arshad, A., Pang, S.P. & Amin, S.N. 2010. Catch composition of estuarine set bag net fishery in the coastal area of Pontian, Johor, Peninsular Malaysia. *Journal of Biological Sciences* 10(3): 247-250.

Omori, M. 1978. Zooplankton fisheries of the world: a review. *Marine Biology* 48(3): 199-205.

Omori, M. 1975. *The Systematics, Biogeography, and Fishery of Epipelagic Shrimps of the Genus* Acetes *(Crustacea, Decapoda, Sergestidae)*. Tokyo, Japan: Ocean Research Institute, University of Tokyo.

Page, R.D.M. 1996. Tree view: An application to display phylogenetic trees on personal computers. *Computer Applications in the Biosciences* 12(4): 357-358.

Pathansali, D. 1966. *Acetes* (Sergestidae) from Malay Peninsula. *Bulletin of the National Museum Singapore* 33(8): 59-63.

Pellerito, R., Arculeo, M. & Bonhomme, F. 2009. Recent expansion of Northeast Atlantic and Mediterranean populations of *Melicertus (Penaeus) kerathurus* (Crustacea: Decapoda). *Fisheries Science* 75(5): 1089-1095.

Pfeiler, E., Hurtado, L.A., Knowles, L.L., Torre-Cosío, J., Bourillón-Moreno, L., Márquez-Farías, J.F. & Montemayor-Lpoez, G. 2005. Population genetics of the swimming crab *Callinectes bellicosus* (Brachyura: Portunidae) from the eastern Pacific Ocean. *Marine Biology* 146(3): 559-569.

Pfenninger, M. & Schwenk, K. 2007. Cryptic animal species are homogenously distributed among taxa and biogeographical regions. *BMC Evolutionary Biology* 7: 121-126.

Pillians, B., Chappell, J. & Naish, T.R. 1998. A review of the Milankovitch climatic beat: Template for Plio-Pleistocene sea-level changes and sequence statigraphy. *Sedimentary Geology* 122(1-4): 5-21.

Posada, D. 2009. Selection of models of DNA evolution with jModelTest. In *Bioinformatic Analysis of DNA Sequences*, edited by Posada, D. New Jersey: Human Press. pp. 93-112.

Posada, D. 2008. jModelTest: Phylogenetic model averaging. *Molecular Biology and Evolution* 25(7): 1253-1256.

Quan, J., Zhuang, Z., Deng, J., Dai, J. & Zhang, Y.P. 2004. Phylogenetic relationships of 12 Penaeoidea shrimp species deduced from mitochondrial DNA sequences. *Biochemical Genetics* 42(9): 331-345.

Ramos-Onsins, S.E. & Rozas, J. 2002. Statistical properties of new neutrality tests against population growth. *Molecular Biology and Evolution* 19(12): 2092-2100.

Rand, D.M. 1996. Neutrality tests of molecular markers and connection between DNA polymorphism, demography, and conservation biology. *Conservation Biology* 10(2): 665-671.

Rogers, A.R. 1995. Genetic evidence for a Pleistocene population explosion. *Evolution* 49(4): 608-615.

Rogers, A.R. & Harpending, H.C. 1992. Population growth makes waves in the distribution of pairwise genetic differences. *Molecular Biology and Evolution* 9: 552-569.

Roldán, M.I., Heras, S., Patellani, R. & Maltagliati, F. 2009. Analysis of genetic structure of red shrimp *Aristeus antennatus* from the Western Mediterranean employing two mitochondrial regions. *Genetica* 136(1): 1-4.

Ronquist, F. & Huelsenbeck, J.P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19(12): 1572-1574.

Saiki, R., Gelfand, D., Stoffel, S., Scharf, S., Higuchi, R., Horn, G.T., Mullis, K.B. & Erlich, H.A. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* 239(4839): 487-491.

Saitou, N. & Nei, M. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 4(4): 406-425.

Schneider, S. & Excoffier, L. 1999. Estimation of past demographic parameters from the distribution of pairwise differences when mutation rates vary among sites: Application to human mitochondrial DNA. *Genetics* 152(3): 1079-1089.

Slatkin, M. & Hudson, R.R. 1991. Pairwise comparison of mitochondrial DNA sequences in stable and exponentially growing populations. *Genetics* 129(2): 555-562.

Song, H., Buhay, J.E., Whiting, M.F. & Crandall, K.A. 2008. Many species in one: DNA barcoding overestimates the number of species when nuclear mitochondrial pseudogenes are coamplified. *Proceedings of the National Academy of Sciences of the United States of America* 105(36): 13486-13491.

Stockley, B., Menezes, G., Pinho, M.R. & Rogers, A.D. 2005. Genetic population structure in the black-spot sea bream (*Pagellus bogaraveo* Brűnnich, 1768) from the NE Atlantic. *Marine Biology* 146(4): 793-804.

Sugiura, N. 1978. Further analysts of the data by Akaike's information criterion and the finite corrections. *Communications in Statistics, Theory and Methods* A7(1): 13-26.

Swofford, D. 2002. *PAUP*. Phylogenetic Analysis using Parsimony (*and other methods)*. Version 4. Massachusetts: Sinauer Associates.

Tajima, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* 123: 585-595.

Tamura, K., Dudley, J., Nei, M. & Kumar, S. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) Software Version 4.0. *Molecular Biology and Evolution* 24(8): 1596-1599.

Templeton, A.R., Crandall, K.A. & Sing, C.F. 1992. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. *Genetics* 132(2): 619-633.

Thorpe, J.P., Solé-Cava, A.M. & Watts, P.C. 2000. Exploited marine invertebrates: Genetics and fisheries. *Hydrobiologia* 420(1): 165-184.

Tong, J.G., Chan, T.Y. & Chu, K.H. 2000. A preliminary phylogenetic analysis of *Metapenaeopsis* (Decapoda: Penaeidae) based on mitochondrial DNA sequences of selected species from the Indo-West Pacific. *Journal of Crustacean Biology* 20(3): 541-549.

Trontelj, P., Machino, Y. & Sket, B. 2005. Phylogenetic and phylogeographic relationships in the crayfish genus *Austropotamobius* inferred from mitochondrial COI gene sequences. *Molecular Phylogenetics and Evolution* 34(1): 212-226.

Tsoi, K.H., Chan, T.Y. & Chu, K.H. 2007. Molecular population structure of the kuruma shrimp *Penaeus japonicus* species complex in western Pacific. *Marine Biology* 150(6): 1345-1364.

Tsoi, K.H., Wang, Z.Y. & Chu, K.H. 2005. Genetic divergence between two morphologically similar varieties of the kuruma shrimp *Penaeus japonicus*. *Marine Biology* 147(2): 367-379.

Voris, H.K. 2000. Maps of Pleistocene sea levels in Southeast Asia: Shorelines, river systems and time durations. *Journal of Biogeography* 27: 1153-1167.

Ward, R.D. 2000. Genetics in fisheries management. *Hydrobiologia* 420(1): 191-201.

Ward, R.D. & Grewe, P.M. 1994. Appraisal of molecular genetic techniques in fisheries. *Reviews in Fish Biology and Fisheries* 4(3): 300-325.

Weir, B.S. & Cockerham, C. 1984. Estimating F - statistics for the analysis of population structure. *Evolution* 38(6): 1358-1370.

Williams, S.T. & Knowlton, N. 2001. Mitochondrial pseudogenes are pervasive and often insidious in the snapping shrimp genus *Alpheus*. *Molecular Biology and Evolution* 18(8): 1484-1493.

Williams, S.T., Jara, J., Gomez, E. & Knowlton, N. 2002. The marine Indo-West Pacific break: Contrasting the resolving power of mitochondrial and nuclear genes. *Integrative and Comparative Biology* 42(5): 941-952.

Williams, S.T., Knowlton, N., Weigt, L.A. & Jara, J.A. 2001. Evidence for three major clades within the snapping shrimp genus *Alpheus* inferred from nuclear and mitochondrial gene sequence data. *Molecular Phylogenetics and Evolution* 20(3): 375-389.

Wong, B.Y. 2013. Genetic diversity and morphometric characterization of *Acetes* (Decapoda: Sergestidae) collected from the west coast of Peninsular Malaysia. Master of Science dissertation, Universiti Tunku Abdul Rahman, Malaysia (Unpublished). (http://eprints.utar.edu.my/802/).

Wong, B.Y., Ong, H.K.A. & Khoo, G. 2015. Length-weight relationships of *Acetes* spp. sampled along the west coast of Peninsular Malaysia. *Sains Malaysiana* 44(3): 379-386.

Xiao, Y. & Greenwood, J.G. 1993. The biology of *Acetes* (Crustacea: Sergestidae). In *Oceanography and Marine Biology: An Annual Review,* Vol. 31, edited by Ansell, A.D., Gibson, R.N. & Barnes, M. London: UCL Press.

Zhang, D.X. & Hewitt, G.M. 1996. Nuclear integrations: Challenges for mitochondrial DNA markers. *Trends in Ecology and Evolution* 11(6): 247-251.

Zitari-Chatti, R., Chatti, N., Fulgione, D., Caiazza, I., Aprea, G., Elouaer, A., Said, K. & Capriglione, T. 2009. Mitochondrial DNA variation in the caramote prawn *Penaeus* (*Melicertus*) *kerathurus* across a transition zone in the Mediterranean Sea. *Genetica* 136(3): 439-447.

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