GENETIC DIVERSITY AND MORPHOMETRIC CHARACTERIZATION OF *ACETES* (DECAPODA: SERGESTIDAE) COLLECTED FROM THE WEST COAST OF PENINSULAR MALAYSIA

WONG BOON YEE

MASTER OF SCIENCE

FACULTY OF ENGINEERING AND SCIENCE
UNIVERSITI TUNKU ABDUL RAHMAN
MAY 2013

GENETIC DIVERSITY AND MORPHOMETRIC CHARACTERIZATION OF *ACETES* (DECAPODA: SERGESTIDAE) COLLECTED FROM THE WEST COAST OF PENINSULAR MALAYSIA

By

WONG BOON YEE

A thesis submitted to the Faculty of Engineering and Science,
Universiti Tunku Abdul Rahman,
in partial fulfillment of the requirements for the degree of
Master of Science

May 2013

ABSTRACT

GENETIC DIVERSITY AND MORPHOMETRIC CHARACTERIZATION OF *ACETES* (DECAPODA: SERGESTIDAE) COLLECTED FROM THE WEST COAST OF PENINSULAR MALAYSIA

WONG BOON YEE

Acetes shrimps are in high demand for human consumption, as feed for livestock and as livefeeds in aquaculture. In Malaysia, they occur widely across the west coast of Peninsular Malaysia and are fished commercially in-shore (using traditional fishing gears) and in open waters via trawling activities. Previous morphometric studies of this genus based solely on in-shore catches. However, now the majority of *Acetes* landings are from off-shore trawling activities and thus morphometric data remain scarce. In addition, little is known about the genetic diversity and population structure of *Acetes*, which are crucial for the assessment and management of wild stocks.

Acetes shrimps were collected from both in-shore and off-shore areas around the west coast of Peninsular Malaysia. Species captured were identified as *Acetes indicus*, *A. serrulatus*, *A. japonicus* and *A. sibogae*, using the identification keys of Omori (1975b). Morphometric measurements (total length, TL; carapace length, CL; and wet weight, WW) were obtained from the samples. Significant differences in measurements were observed between the sexes, between in-shore and off-shore samples, and among species. TL–WW Relationships and CL–TL Relationships were also estimated.

Genetic diversity and population structure were described based on mitochondrial DNA sequence analysis. A region of the mitochondrial cytochrome *c* oxidase subunit I (*COI*) gene consisting of 552 base pairs (bp) was amplified from 159 *Acetes* specimens. Multiple sequence alignment analysis revealed 46 haplotypes representing *A. indicus* (11), *A. serrulatus* (31), *A. japonicus* (2), and *A. sibogae* (2). Sequence divergence among the four *Acetes* species ranged from 14.19% to 20.47% (mean = 8.23%). Neighbour-Joining, Maximum Parsimony, Maximum Likelihood and Bayesian Inference methods consistently revealed four distinct clades based on aligned *COI* gene fragment sequences. This agrees with the four described *Acetes* species that were identified using morphological keys. All clades were monophyletic and supported with high bootstrap values and high posterior probabilities. Besides that, cryptic diversity is present in at least two taxa (*A. indicus* and *A. sibogae*).

Overall haplotype and nucleotide diversity varied considerably among species. Analysis of Molecular Variance (AMOVA) showed significant differentiation among *A. indicus* populations, while no significant genetic differentiation was detected among populations of *A. serrulatus, A. japonicus* and *A. sibogae*. In addition, the combinations of haplotype and nucleotide diversity, neutrality tests and mismatch analysis suggested different demographic histories for *A. indicus* (*i.e.*, secondary contact between historically isolated populations) and *A. serrulatus* (*i.e.*, historical population bottlenecks followed by rapid population growth). Patterns in *A. serrulatus* and presence of two distinct lineages observed in *A. indicus* are suggestive of Pleistocene population expansions.

ACKNOWLEDGEMENT

First, I would like to thank my supervisor, Associate Professor Dr. Alan Ong Han Kiat and co-supervisor, Associate Professor Dr. Gideon Khoo for giving me the opportunity to complete this research under their supervision, while providing invaluable advices and guidance throughout the course of the research project, as well as during the thesis preparation.

My gratitude also goes to the Mr. Oong Ah Lim, Mr. Tan Eng Hock, Mr. Lee Boon Hock, Mr. Chan Hua Lim, Mr. Lim Chu Chin, Mr. Koh Kong Ho, Mr. Lim Hui Cheng, Mr. Chai Huat, Mr. Tho Kim Lai, Mr. Chai, Encik Mohammad Yatim Khamis, Mr. Shaaban and other people who have helped in the collection of specimens used in this study. My appreciation is also express to all laboratory assistants of Universiti Tunku Abdul Rahman (UTAR) for their support and helps that make this research success.

Furthermore, my deepest appreciation goes to my supportive family and friends. Once again, a deep sincere thanks Universiti Tunku Abdul Rahman (UTAR) for supporting this research financially.

APPROVAL SHEET

This dissertation/thesis entitled "**GENETIC DIVERSITY AND MORPHOMETRIC CHARACTERIZATION OF *ACETES* (DECAPODA: SRGESTIDAE) COLLECTED FROM THE WEST COAST OF PENINSULAR MALAYSIA"** was prepared by WONG BOON YEE and submitted as partial fulfillment of the requirements for the degree of Master of Science at Universiti Tunku Abdul Rahman.

Approved by:

_____
(Assoc. Prof. Dr. Alan Ong Han Kiat) Date: ……..
Supervisor
Department of Pre-clinical Sciences
Faculty of Medicine and Health Sciences
Universiti Tunku Abdul Rahman

_____
(Assoc. Prof. Dr. Gideon Khoo) Date: ……..
Co-supervisor
Department of Biological Science
Faculty of Science
Universiti Tunku Abdul Rahman

FACULTY OF ENGINEERING AND SCIENCE

UNIVERSITI TUNKU ABDUL RAHMAN

Date: ……..

SUBMISSION OF THESIS

It is hereby certified that ***WONG BOON YEE*** (ID No: ***07UEM08599***) has completed this thesis entitled " **GENETIC DIVERSITY AND MORPHOMETRIC CHARACTERIZATION OF *ACETES* (DECAPODA: SRGESTIDAE) COLLECTED FROM THE WEST COAST OF PENINSULAR MALAYSIA** " under the supervision of Assoc. Prof. Dr. Alan Ong Han Kiat (Supervisor) from the Department of Pre-clinical Sciences, Faculty of Medicine and Health Science, and Assoc. Prof. Dr. Gideon Khoo (Co-Supervisor) from the Department of Biological Science, Faculty of Science.

I understand that University will upload softcopy of my thesis in pdf format into UTAR Institutional Repository, which may be made accessible to UTAR community and public.

Yours truly,

_____

(WONG BOON YEE)

DECLARATION

I hereby declare that the dissertation is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UTAR or other institutions.

Name _____

Date _____

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AMOVA	:	Analysis of molecular variance
BI	:	Bayesian inference
CL	:	Carapace length
COI	:	Cytochrome *c* oxidase subunit (I) gene
DNA	:	Deoxyribonucleic acid
dNTPs	:	Deoxynucleotide triphosphate
EDTA	:	Ethylenediaminetetraacetic acid
EtBr	:	Ethidium bromide
K2P	:	Kimura 2 parameter
LLR	:	Length-length relationship
LWR	:	Length-weight relationship
LGM	:	Last Glacial Maximum
MEGA	:	Molecular Evolutionary Genetics Analysis
ML	:	Maximum likelihood
MP	:	Maximum parsimony
MYA	:	Million years ago
NJ	:	Neighbour-joining
r	:	Harpending's raggedness index
PCR	:	Polymerase Chain Reaction
SPSS	:	Statistical Packages for Social Science
TBE	:	Tris-borate-EDTA
TE	:	Tris-EDTA
TL	:	Total length

SSD	:	Sum of squared deviation
WW	:	Wet weight
bp	:	Base pair
M	:	Molar
mm	:	Millimetre
V	:	Volt
U.V.	:	Ultraviolet
ng	:	Nanogram
µL	:	Microlitre
nm	:	Nautical mile

CHAPTER 1.0

INTRODUCTION

Sergestid shrimps in the genus *Acetes* (Decapoda: Sergestidae) are small planktonic shrimps (10–40 mm in total length) locally known as '*Udang Geragau'* or '*Udang Baring'* (Omori, 1975b). Currently, seven (*A. indicus*, *A. japonicus*, *A. serrulatus*, *A. vulgaris*, *A. sibogae*, *A. intermedius* and *A. erythraeus*) out of 14 described *Acetes* species have been found within Malaysian coastal waters (Amani et al., 2011c; Amin et al., 2011; Longhurst, 1970; Pathansali, 1966). Landings of *Acetes* spp. are confined mainly to the west coast of Peninsular Malaysia where 75% or more of the total *Acetes* spp. landings occur (DOF, 2001-2010). Besides their commercial importance for human comsumption (Holthuis, 1980; Omori, 1978) and potential used as food organisms in agriculture and aquaculture (Deshmukh, 1991; Job et al., 2006), they play an important role as both predators and prey, in the food webs of coastal waters (Xiao and Greenwood, 1993).

Morphometric analyses are useful for species identification and may also suggest certain patterns of the life-cycle, while length-weight relationships (LWR) are useful for growth pattern evaluation (Anderson and Neumann, 1996; Jobling, 2002; Le Cren, 1951). In Malaysia, previous similar studies of *Acetes* spp. have focused on in-shore catches using traditional fishing gears (Amin et al., 2009b; Amin et al., 2010a; Amin et al., 2009c; Amin et al., 2011; Arshad et al., 2012; Arshad et al., 2007). However, the majority of *Acetes* landings are now from off-shore trawling activities (DOF, 2001-2010) and thus

morphometric data of these species remain scarce especially from around the off-shore fishing grounds.

While much is known about distribution, abundance and morphometrics of these commercially important *Acetes* species, little is known about their genetic diversity level and patterns and population structure. To conserve existing wild resources of these highly exploited species for long term sustainable yields, information on the genetic diversity and population structure of *Acetes* species will be crucial for the assessment and management of wild stocks (Allendorf and Luikart, 2006; Carvalho and Hauser, 1994; Thorpe et al., 2000; Ward, 2000; Ward and Grewe, 1994). Furthermore, phylogenetic relationships among *Acetes* spp. may shed light on the evolutionary relationships of these shrimps that remain largely unknown and external morphology is still the main criteria used for defining taxonomic status of *Acetes* spp.

Therefore, the objectives of this study are:

1. To identify the *Acetes* shrimps species sampled from in-shore and from off-shore trawling activities along the west coast of Peninsular Malaysia using morphological criteria detailed in Omori (1975b);
2. To assess morphometric variation between sexes of each species, among and between species, as well as between in-shore and off-shore catches,

3. To establish length-weight relationships suitable for use on *Acetes* shrimps from the west coast of Peninsular Malaysia and for growth pattern evaluation;
4. To infer phylogenetic relationships among the *Acetes* species identified via morphological analysis.
5. To assess levels and patterns of genetic variation in each species of, and determine if population structure is present in the sampled locations.

CHAPTER 2.0

LITERATURE REVIEW

2.1 *Acetes* shrimps

2.1.1 Morphology

Sergestid shrimps in the genus *Acetes* are small planktonic shrimps, with a body length ranging between 10–40 mm (Omori, 1975b). Their bodies are rather slender (Colefax, 1940), translucent or semi-translucent, with black eyes, and several pairs of red pigment spots on the base of the uropod and on the endopods of the uropods (Achuthankutty and Nair, 1976; Chan, 1998; Holthuis, 1980; Miquel, 1984; Okada, 1928; Omori, 1975b).

In Figure 2.1, a diagram of a typical male *Acetes* shrimps is presented with the parts labelled (Omori, 1975b). The rostrum is acute and short, lacking or with one or two dorsal denticles (Omori, 1975b), and, both hepatic and supraorbital spines are well developed (Colefax, 1940; Hansen, 1919). The compound eyes are stalked, nearly spherical and are heavily pigmented (Ball et al., 1986). In addition, the lower flagellum is short and composed of about ten to twelve joints, while the upper flagellum is very long, and thicker than the lower (Kishinouye, 1928).

The head (cephalon) region consists of five somites (Xiao and Greenwood, 1993), that include a pair of antennules (1st antennae), antennae (2nd antennae), mandibles, maxillules (1st maxillae) and maxillae (2nd maxillae). In addition, the thorax has eight somites: three pairs of maxillipeds and five pairs

of pereiopods (legs), the first three are chelated and the fourth and fifth pereiopod are absent except for a pair of protuberances (genital coxae) in males (Xiao and Greenwood, 1993).

The abdomen has six somites, the first five abdominal somites bear a pair of pleopods used for forward swimming, and the sixth with uropods and telson (Xiao and Greenwood, 1993). The uropods have a basal protopod and an inner endopod and outer exopod. Several pairs of red pigment spots which are considered to be phototactic organs by Okada (1928), occur on the base of the uropod while the other(s) occur on the endopods of the uropods (Achuthankutty and Nair, 1976; Okada, 1928; Omori, 1975b).

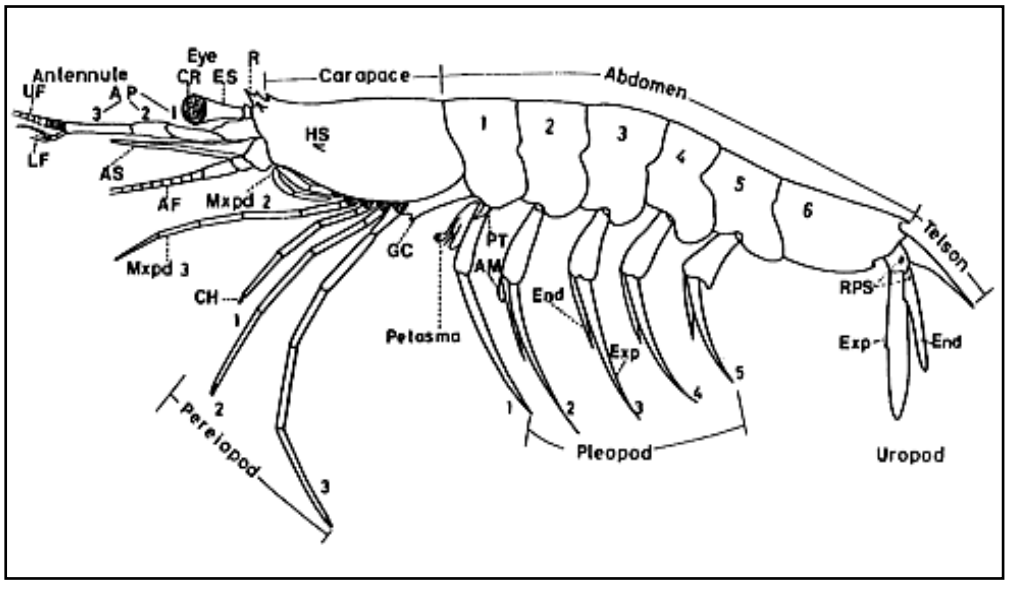


Figure 2.1: Diagram of a male *Acetes*. AM, appendix masculina; AF, antennal flagellum; AP, antennular peduncle; AS, antennal scale; CH, chela; CR, cornea; End, endopod; ES, eye stalk; Exp, exopod; GC, genital coxa; HS, hepatic spine; LF, lower flagellum; Mxpd, maxilliped; PT, procurved tooth; R, rostrum; RPS, red pigment spots; UF, upper flagellum (Omori, 1975b).

2.1.2 Classification

The genus *Acetes* is classified in the phylum Arthropoda, subphylum Crustacea, class Malacostraca, order Decapoda and family Sergestidae (De Grave et al., 2009; Martin and Davis, 2001). Since the genus was first raised by Milne Edwards (1830) based on a species captured from the mouth of Ganges in India, 22 species have been reported across the world (Table 2.1, Omori, 1975b). Currently however, only 14 distinct species are recognized (De Grave et al., 2009; Omori, 1975b) as some species names are considered to be synonyms (Burkenroad, 1934; Colefax, 1940; Hansen, 1919; Holthuis, 1959; Kemp, 1917; Pathansali, 1966; Rao, 1968).

Table 2.1: List of distinct species reported around the world and synonyms in the genus *Acetes*.

Distinct species	Synonym
Indo-West	
1. *Acetes chinensis* Hansen, 1919	
2. *Acetes erythraeus* Nobili, 1905	*Acetes* sp. Hansen, 1919
3. *Acetes indicus* H. Milne Edwards, 1830	*Acetes spiniger* Hansen, 1919
4. *Acetes intermedius* Omori, 1975	
5. *Acetes japonicus* Kishinouye, 1905	*Acetes disper* Hansen, 1919 *Acetes cochinensis* Rao, 1968
6. *Acetes johni* Nataraj, 1947	
7. *Acetes natalensis* Barnard, 1955	
8. *Acetes serrulatus* (Kröyer, 1859)	*Acetes insularis* Kemp, 1917
9a. *Acetes sibogae sibogae* Hansen, 1919	
9b. *Acetes sibogae australis* Colefax, 1940	*Acetes australis* Colefax, 1940
9c. *Acetes sibogae sibogalis* Achuthankutty and George, 1973	*Acetes sibogalis* Achuthankutty and George, 1973
10. *Acetes vulgaris* Hansen, 1919	
Pacific America	
11. *Acetes binghami* Burkenroad, 1934	
Atlantic America	
12a. *Acetes americanus americanus* Ortmann, 1893	*Acetes brasiliensis* Hansen, 1919
12b. *Acetes americanus carolinae* Hansen, 1933	*Acetes carolinae* Hansen, 1933
13. *Acetes marinus* Omori, 1975	
14. *Acetes paraguayensis* Hansen, 1919	

2.1.3 Identification Keys

Since the identification keys for males and females of *Acetes* are different, a method for identifying the sexes of *Acetes* is needed (Omori, 1975b). The unique characters used to identify the sexes of *Acetes* are the presence of a pair of protuberances (genital coxae) between the third pereiopods and first pleopods in males. In addition, a petasma and lower antenullar flagellum with spine(s) are observed in males, but are absent in females.

While many regional keys have been reported for *Acetes* species identification (Barnard, 1955; Chan, 1998; D'Incao and Martins, 2000; George, 1969; Hansen, 1919; Kemp, 1917; Miquel, 1984; Pathansali, 1966; Ravindranath, 1980), global keys reported by Omori (1975b) may be more suitable. As an example, the regional keys provided by Pathansali (1966) were able to identify six species of *Acetes*, including *A. indicus*, *A. erythraeus*, *A. japonicus*, *A, serrulatus*, *A. sibogae* and *A.vulgaris* collected in Peninsular Malaysia. Indeed, *Acetes* spp. collected from different sampling locations in the coastal waters of Peninsular Malaysia were identified as *A. indicus*, *A. serrulatus*, *A. japonicus*, *A. vulgaris*, and *A. intermedius* (Amin et al., 2011; Amin et al., 2008b; Arshad et al., 2007) based on Omori (1975b). *A. intermedius* will not able to be identified based on the regional keys provided by Pathansali (1966) and this illustrates a case where regional keys have limitations as they may not represent all morphological features present in some *Acetes* spp. occurring in a region. Apart from this, it should be noted that most keys provided by different authors only apply to adults as the taxonomy of *Acetes* larvae, postlarvae and juveniles has not been elucidated (Omori, 1975b).

Indeed, Kemp (1917) showed that when distinguishing *A. indicus*, *A. serrulatus* (= *A. insularis*), *A. erythraeus*, and *A. japonicus* adult males, the form of the petasma is the most reliable guide and the lower antennular flagellum is also a reliable character; while the third thoracic sternite offers distinctive characters in the females of each species. This is further confirmed in a study by Omori (1975b) of 14 *Acetes* species, where each species had a distinctive form of petasma and lower antennular flagellum in males, and a distinct third thoracic sternite in females. Apart from these differences, *Acetes* collected from Malaysian coastal waters (Amin et al., 2011; Amin et al., 2008b; Arshad et al., 2007) agrees well with descriptions of these three characters provided by Omori (1975b). This indicates the usefulness of these three characters for distinguishing *Acetes* species as well as the global species identification keys reported by Omori (1975b).

Apart from the characters above, the species may also be distinguished by each or combinations of the following characters: number of denticles on the rostrum behind the terminal point, size of the eye, proportional lengths of the three segments of the antennular peduncle, detailed structure of the basis (trochancter) and coxa of the third pereiopod, presence or absence of a procurved tooth between the bases of the first pair of pleopods, shape of the telson, and proportional length of the non-ciliated part of the outer margin of the exopod of the uropod to the entire margin (Omori, 1975b). For example, in the identification of *A. erythraeus*, *A. vulgaris, A. serrulatus*, and *A. japonicus* (= *A. dispar*) found in Singapore waters (Tham, 1955), *A. vulgaris* can be immediately separated from the other three species that occur there by

presence of a procurved tooth between the first pair of pleopods in both females and males. As with the other three species, no procurved spine is evident. Furthermore, males and females of the other three species can be easily separated by the shape of the petasma and lower antenullar flagellum, and the distinctive shape of the third thoracic sternite, respectively.

2.1.4 Phylogeny of *Acetes* based on Morphology

A phylogeny for *Acetes* species has been reported by Omori (1975b) that is based on morphological characters (Figure 2.2). Species in the genus *Acetes* can be divided into *erythraeus* and *japonicus* groups based on presence of distinctive morphological characteristics.

For females, a pair of conspicuous protuberances on the anterior part of the third thoracic sternite is present in the *erythraeus* group, but absent in the *japonicus* group (Omori, 1975b). For males, the anterior margin of the genital coxa is pointed and a petasma with a par astringens is observed in the *erythraeus* group (Omori, 1975b). Conversely, males of the *japonicus* group have a petasma without pars astringens and an anterior margin of the genital coxa that is rounded (Omori, 1975b). Interestingly, *Acetes indicus* can be distinguished initially from other species by possessing characters that are a mix of the *japonicus* and *erythraeus* groups, namely only one clasping spine on the lower antennular peduncle in the male (Omori, 1975b).

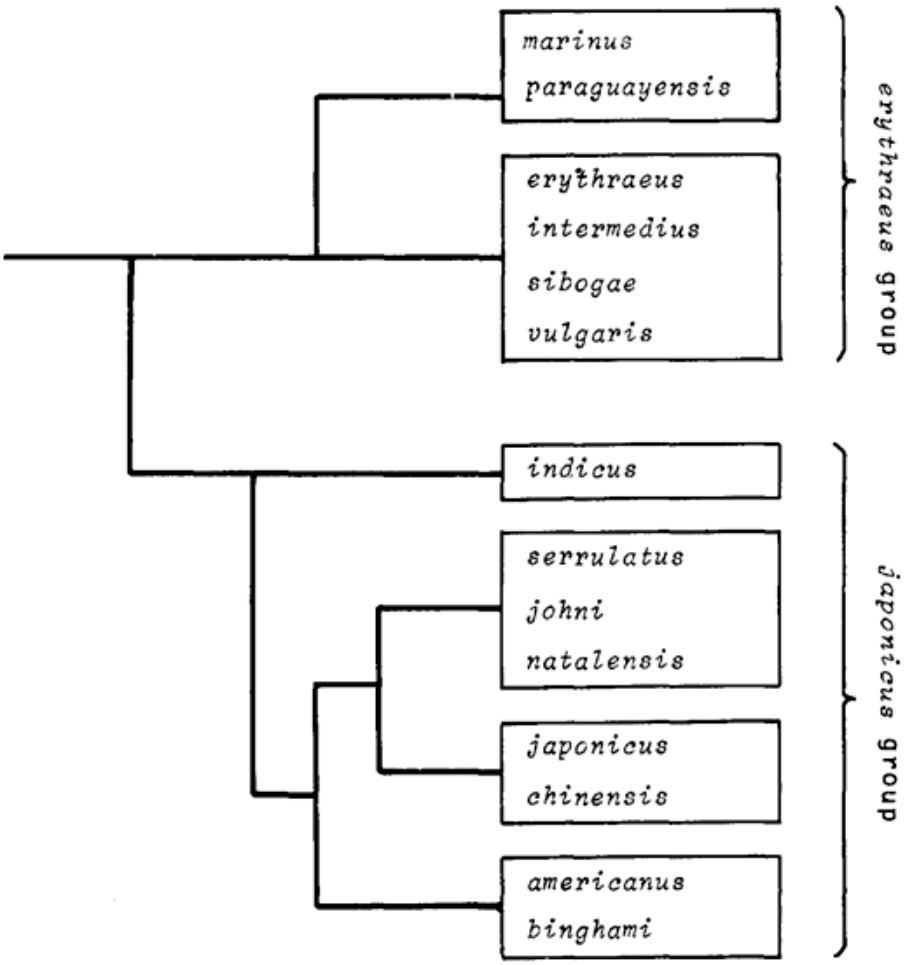


Figure 2.2: Diagram showing relationships of the genus *Acetes* based on morphological character following Omori (1975b).

2.1.5 Geographical Distributions

Acetes species are mainly distributed in estuarine and coastal waters in tropical and subtropical regions, and species are restricted to the Indo-West Pacific, Atlantic and eastern tropical Pacific Oceans (Omori, 1975a; b; 1977). Ten *Acetes* species are found in the Indo-West Pacific region and the Indo-Malay archipelago regions (Figure 2.3–2.5): *A. erythraeus*, *A. intermedius*, *A. vulgaris*, *A. sibogae*, *A. johni*, *A. natalensis*, *A. serrulatus*, *A. chinensis*, *A.*

indicus and *A. japonicus* (Barnard, 1955; Chullasorn and Martosubroto , 1986; George, 1969; Hansen, 1919; Johnson, 1965; Jones, 1969; Kemp, 1917; Kensley, 1971; Le Reste, 1970; Nobili, 1905; 1906; Omori, 1975b; 1978; Park et al., 2009; Pathansali, 1966; Pérez Farfante and Kensley, 1997; Ravindranath, 1980; Tirmizi and Ghani, 1982).

Another three species of *Acetes* are restricted to the Atlantic America (Figure 2.6): *Acetes americanus* (Allen et al., 2008; Calazans, 2002; Camp et al., 1998; Chace, 1972 ; Costa et al., 2003; Johnson and Allen, 2005; Joyce, 1966; Williams, 1965; 1969), *A. marinus* (Coelho and Ramos-Porto, 1984; D'Incao and Martins, 2000), and *A. paraguayensis* (Aldrich, 1962; Arrington and Winemiller, 2003; 2006; Magalhães, 1999; 2002; Melo Júnior, 2006). *A. paraguayensis* is the only *Acetes* species that occurs in freshwater (Collins and Williner, 2003; García-Dávila and Magalhães, 2003; Holthuis, 1959; Magalhães and Pereira, 2007; Rodríguez, 1982), while *A. binghami* is the only species found on the Pacific coast of America (Omori, 1975b; Pérez Farfante and Kensley, 1997). No species has been reported however, from the East Atlantic Mediterranean region or the islands of the Central Pacific (Hawaii and New Zealand).

In Malaysia, seven species of *Acetes* has been identified from coastal waters on both West and East Malaysia: *A. indicus*, *A. serrulatus*, *A. japonicus*, *A. sibogae, A. vulgaris*, *A. intermedius* and *A. erythraeus* (Amin et al., 2011; Fernandez-Leborans et al., 2009; Johnson, 1965; Longhurst, 1970; Pathansali, 1966; Tham, 1968).

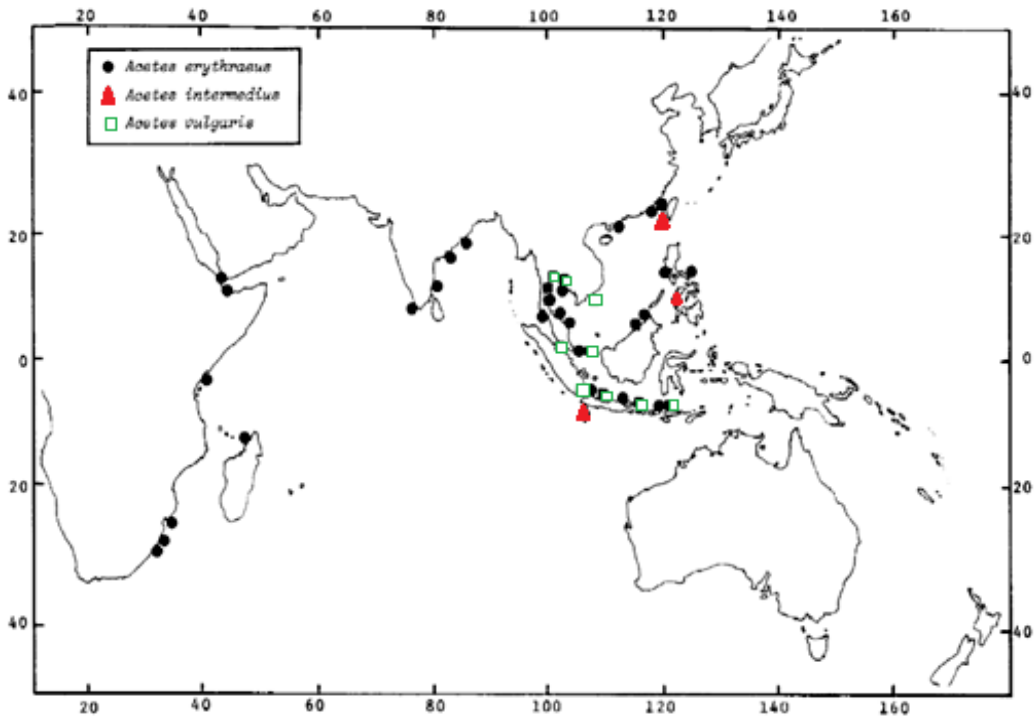


Figure 2.3: Distribution of *Acetes erythraeus, A. intermedius* and *A. vulgaris* (Omori, 1975b).

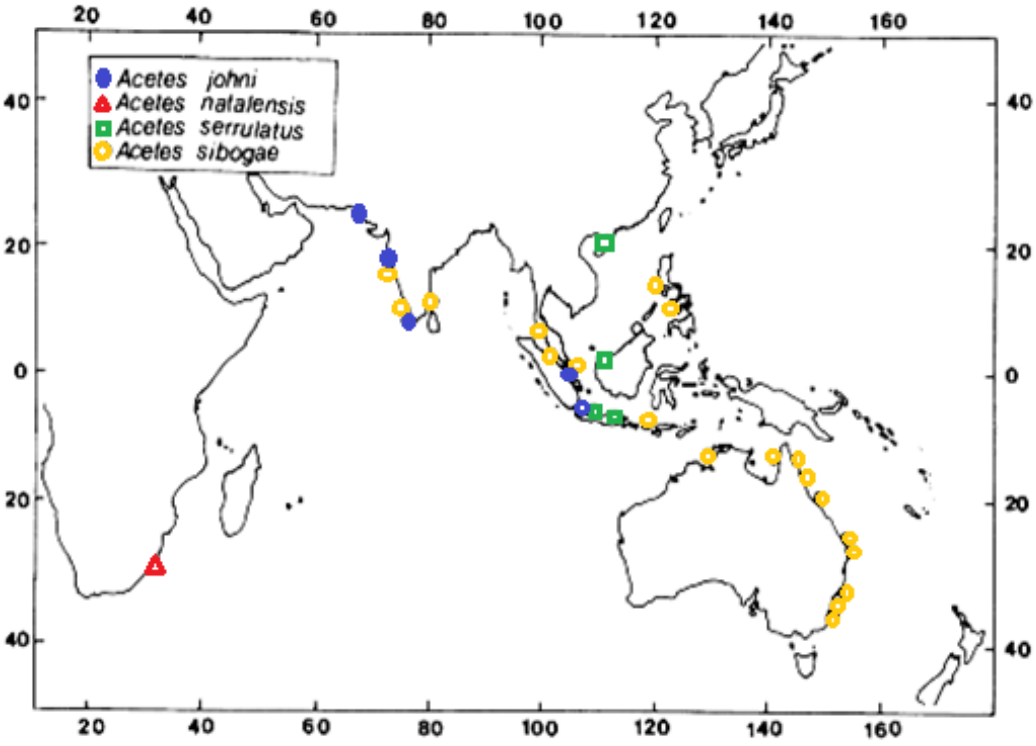


Figure 2.4: Distribution of *Acetes sibogae, A. johni, A. natalensis* and *A. serrulatus* (Xiao and Greenwood, 1993).

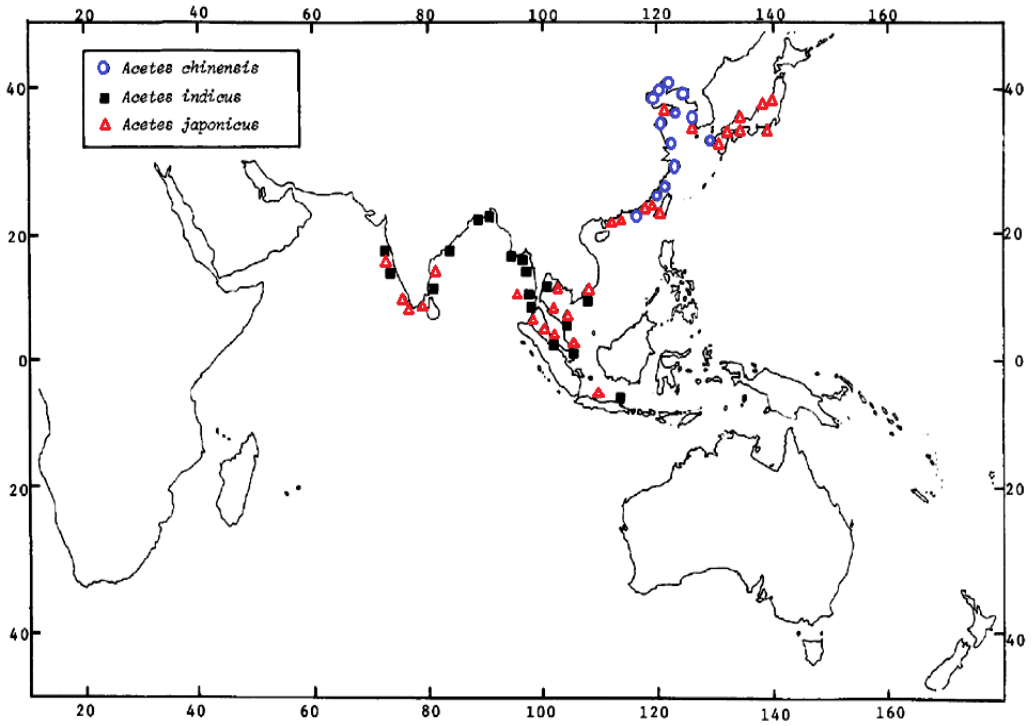


Figure 2.5: Distribution of *Acetes chinensis, A. indicus* and *A. japonicus* (Omori, 1975b).

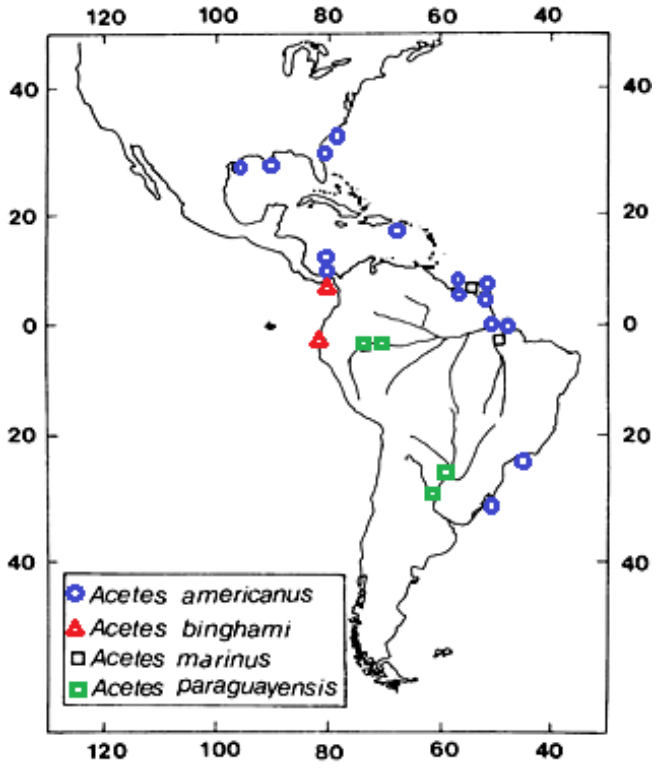


Figure 2.6: Distribution of *Acetes americanus*, *A. binghami*, *A. marinus*, *A. paraguayensis* (Xiao and Greenwood, 1993).

2.1.6 Ecology

2.1.6.1 Dispersal among Habitats

Organisms that undertake extensive migrations are believed to do so for a specific biological purpose. Some species of *Acetes* have been inferred to migrate between different habitats in order to complete their life cycles. A study by Ikematsu (1953) and Lei (1984) reported a spawning ground for *A. japonicus* in the innermost areas of the Ariake Sea (coastal waters of western Japan) and eastern coastal waters of Guangdong Province in China, respectively, but did not describe associated dispersal between regions. Feng *et al.* (1982) and Shi (1986) however, described in great detail, seasonal migration of *A. chinensis* in western areas of the Bohai Sea and in-shore waters in southern Zhejiang (East China Sea). In these two regions, shrimps which are concentrated mainly in wintering grounds from December to February, but move towards the shore and reach their spawning grounds (*i.e.*, shallow, in-shore area, coastal and estuaries) in spring (March to May). During summer and autumn (mid of May to August), they spawn in the spawning grounds and leave the in-shore area for wintering grounds at the end of autumn (September to November). Similar migratory patterns were also observed by Omori (1975a) in which the spawning of *A. japonicus* took place in the innermost areas of the Ariake Sea (Ikematsu, 1953), then post-larvae moved to deeper water in late autumn and remained there across the winter before returning in-shore in the early spring as adults.

In a study by Chiou *et al.* (2000), adults of *A. intermedius* were reported to migrate from estuaries to deeper off-shore waters when river discharges

increased due to heavy rainfall in the summer (*i.e.*, the southwest monsoon from May to October). This behavior may reduce competition for food between adults and their offspring. When the northeast monsoon began, *A. intermedius* then returned to estuaries. In addition, migrations may be affected by multiple environmental factors, including water temperature (Ikematsu, 1953; Jiang and Guo, 1983; Shi, 1986), rainfall and/or direction and intensity of wind (Chiou et al., 2000; Jiang and Guo, 1983).

2.1.6.2 Sex Ratio

In general, most sex ratios are reported to be close to 1: 1 (males: female) in nature (Fisher, 1930). The sex ratio of *Acetes* spp. however usually deviate from 1:1, with in general more females than males, as in *A. chinensis* in Laizhou Bay and the Bohai Sea (Zhang, 1992), Laizhou Bay and the Huanghe estuary (Zhong et al., 2001), western coast of Korea, Yellow Sea (Oh and Jeong, 2003), *A. chinensis* and *A. japonicus* in south western waters of Korea (Oh and Jeong, 2002) and *A. johni* in Karwar coast, India (Kakati et al., 1988). Similar sex ratio patterns have been observed in *A. intermedius*, *A. indicus* and *A. japonicus* in coastal waters of Klebang Besar, Malacca, Malaysia (Amin et al., 2009b; Amin et al., 2010b; Arshad et al., 2007). Alternatively, a higher proportion of males than females was observed for *A. sibogae* in western Australia (Hanamura, 1999), *A. vulgaris* in coastal waters of Pontian, Johor, Malaysia (Arshad et al., 2008) and *A. intermedius* in coastal waters of Bintulu, Sarawak, Malaysia (Amin et al., 2008b).

A skewed sex ratio can be related to many potential factors, including growth, relative mortality, and behavior of shrimp populations. As shown by Oh and Jeong (2003), the faster growth of females leads to biased proportions toward females (*i.e.*, the proportions of females increased logistically with carapace length), greater sizes can result in higher mesh-size selection and thus dominance in fishery catches. During the spawning season, females were more than males (Zhang, 1992). Females and males may also have different mortality rates after spawning, in which the lifespan of males can be shorter than females by 15–30 days (Lei, 1984). Both these factors can lead to the female-biased sex ratios. In addition, a skewed of sex ratio can result from 'spatial sexual segregation'. Female to male ratio of *A. chinensis* in Laizhou Bay and southern Pohai seems to increase logistically with total body length from slightly over 30% at a body length of about 6 mm to unity at a body length of about 34 mm (Zhang, 1992). In contrast larger proportions of males compared with females may be due to larger body size of females that makes them more vulnerable to starvation (*i.e.*, sensitive to food shortage due to large size) and predation (*i.e.*, less mobile). As a consequence, they may suffer a higher rate of mortality than males and this leads to a sex ratio skewed in favour of males (Berglund, 1981).

2.1.6.3 Life Span

Some authors have reported that some *Acetes* may spawn twice per year, producing two types of generation within a single year (Ikematsu, 1953; Lei, 1984; Otto and Jamieson, 2001; PICES, 1999). *Acetes* that hatch in autumn and live through the winter, then may die after spawning between late April or

May and June or July (*i.e.*, summer). The other cohort that hatches in early summer will then grow rapidly in the warmer season and spawns in early August (first summer generation). Shrimps that hatch from the first summer generation produce the second summer generation that lay eggs in late September or autumn. All spent shrimps die after spawning. Duration of the life of this shrimp therefore, is 9–10 months for the former generation and 2.5–3 months in the latter one. Similar patterns were reported by Yasuda *et al.* (1953), who observed two generation types in the life cycle, and the life spans of both generations were 10–11 months and 25–50 days, respectively.

2.1.7 Fisheries and Commercial Values of *Acetes*

The fishing grounds for *Acetes* are mostly located in the calm, muddy intertidal zone or waters shallower than 5m in depth (Omori, 1975b; 1978). As shown in Figure 2.7, *Acetes* fisheries operate mainly in Asia, and to a much lesser extent also in Africa and South America (Omori, 1975b; Xiao and Greenwood, 1993). *A. chinensis*, *A. serrulatus*, *A. erythraeus*, *A. japonicus*, *A. indicus*, *A. vulgaris* and *A. sibogae* from single or combine species commercial fisheries are undertaken in India, Thailand, Malaysia, Singapore, Indonesia, Philippines, China, Japan, Korea and Taiwan (Aravindakshan and Karbhari, 1988; Chikuni, 1985; Holthuis, 1980; Li et al., 1986; Macintosh, 2001; Macintosh et al., 2003; Mines et al., 1986; Omori, 1975b; Otto and Jamieson, 2001; Tham, 1968; Zhang, 1986). Smaller amounts of *Acetes* are also caught for local consumption in Myanmar, Vietnam, Sri Lanka, Bangladesh, (Omori, 1975b), Africa (Crosnier and Fourmanoir, 1962; Jiddawi and Öhman, 2002; Le Reste, 1970) and in South America (Holthuis, 1959; 1980).

According to Omori (1975b), the fishing season is aligned to the swarming season of the *Acetes*, where during this season, *Acetes* form conspicuous aggregations near the shore, and are fished mainly with push nets or fixed bag nets set near the shore against the flow of the tide (Omori, 1975b; Pillai, 1983; Ramamurthy and Muthu, 1969; Ruddle, 1986; Sehara and Kharbari, 1987). In addition, beach seine, purse seine, stake nets and boat seines are used (Jiddawi and Öhman, 2002; Khan, 1987; Omori, 1975b; Ramamurthy and Muthu, 1969; Wei et al., 1985). *Acetes* fishing is also carried out by offshore trawling as well (Deshmukh, 2004; FAO, 2001; Rao, 1988; Zynudheen et al., 2004).

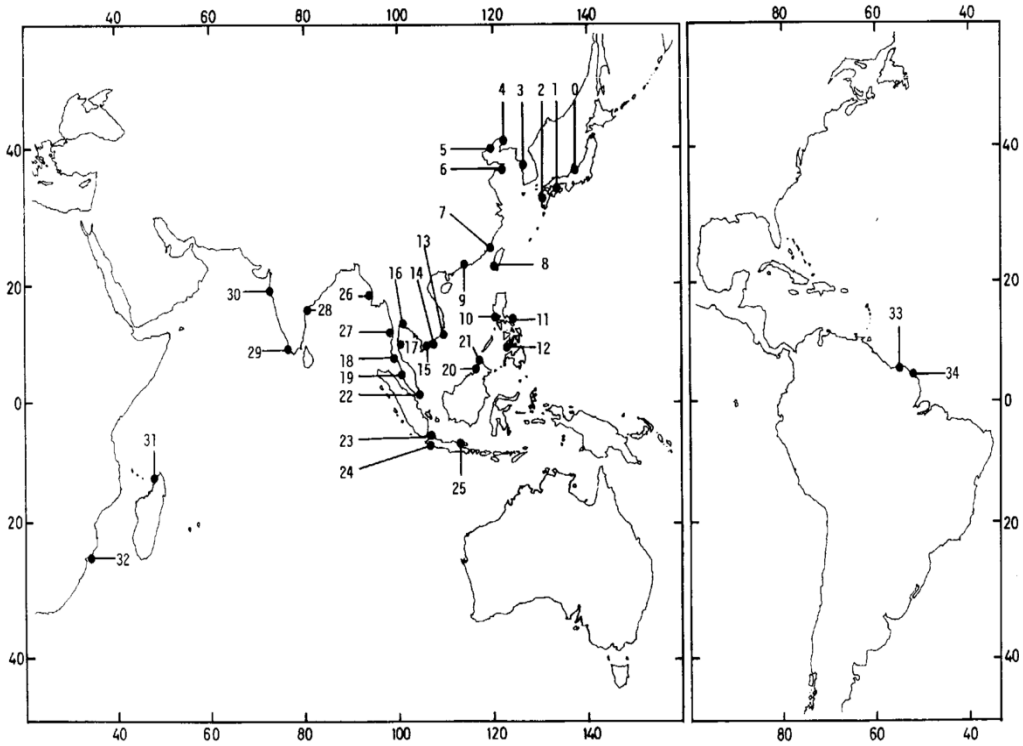


Figure 2.7: Major global fishing grounds of *Acetes* (Omori, 1975b). 0, Toyama Bay; 1, Seto Inland Sea; 2, Ariake Sea; 3, Kyŏnggi Bay; 4, Yingkow; 5, mouth of Luan River; 6, Shihtao; 7, Matsu Island; 8, Tungkiang; 9, Hong Kong; 10, Cavite; 11, Paracale; 12, Iloilo; 13, Nhatrang; 14, Vung Tau; 15, Bac Lieu; 16, Chonburi; 17, Choomporn; 18, Goh Pangi; 19, Penang; 20, Labuan; 21, Kudat; 22, Ponggol; 23, Jakarta; 24, Pelabuhan Ratu; 25, Surabaya; 26, Sandowa; 27, Mergui; 28, mouth of Godavari River; 29, Cochin; 30, Versova; 31, Ambaro Bay; 32, Lígamo; 33, Paramaribo; 34, Cayenne.

In both East and West Malaysia (Peninsular Malaysia), *Acetes* is locally known as '*Udang Baring'*, '*Udang Geragok'*, '*Udang Geragau'*, '*Udang Kepal*' or '*Bubok'*, respectively (Omori, 1975b). Although both *A. indicus* and *A. japonicus* are commonly exploited (Omori, 1975b), *A. serrulatus*, *A. eryhtraeus*, *A. sibogae*, *A. vulgaris* and *A. intermedius* are also present (landed during trawling) (Amin et al., 2011; Fernandez-Leborans et al., 2009; Johnson, 1965; Longhurst, 1970; Pathansali, 1966; Tham, 1968).

Landings of sergestid shrimp (*Acetes* spp.) have been recorded from both East and West Malaysia (DOF, 2001-2010). Landings of *Acetes* spp. are confined however mainly to the west coast of West Malaysia (comprising 75% or more and 85% or more of the total *Acetes* spp. catches in Malaysia and West Malaysia, respectively), with Perak and Selangor as the main fishing centres. Push nets, beach-seine, small purse seine, bag net or stake traps are the commonly used traditional fishing gears (DOF, 2001-2010; Longhurst, 1970; Omori, 1975b; Pathansali, 1966). *Acetes* are also harvested commercially in open waters, via trawling activities at more than 5 nautical miles (nm) off-shore (DOF, 2001-2010; FAO, 2000; Noh and Yew, 1995; Ogawa, 2004). It should be noted that the majority of the *Acetes* spp. landings in Malaysia were from the off-shore regions (DOF, 2001-2010).

Only a very small portion of *Acetes* catches are sold as fresh shrimp in Asian countries. The greater proportion are boiled, dried in the sun, dried after boiling and sometimes processed further by removing the carapace and fermented with salt (shrimp paste and shrimp sauce) or pickled in salt

(Deshmukh, 1991; Omori, 1975b; Yeap and Tan, 2003). Among these products, fermented shrimp paste ('*Xiajiang*' in China, '*Memtep'* in Vietnam, '*Gapi*', '*Ngapi'* in Mynmar, '*Trassi*', '*Terasi*' in Indonesia, '*Kapi'* in Thailand, '*Bagoong alamang'* in Philippines, '*Belacan*' or '*Belachan*' in Malaysia and Singapore) and sauce ('*Xiayou*' in China, '*Nam-pla*' or '*Nam-keow*' in Thailand) are highly desirable in China and South East Asia (Burkenroad, 1946; Deshmukh, 1991; Ling and Suriyong, 1954; Mabesa and Babaan, 1993; Omori, 1975b). Apart from '*Belacan*', *Acetes* shrimps landed can be dried also pickled in whole salt and fermented with cooked rice into a local delicacy known as '*Chincalok'*, '*Cencalok'*, '*Cincalok'* or '*Cincaluk'* (Abdullah and Idrus, 1978; Pathansali, 1966; Wan Daud, 1978; Yeap and Tan, 2003; Yeoh and Merican, 1978).

Apart from high demand for human consumption, *Acetes* spp. provide a major source of protein for coastal populations in Asia and East Africa (Holthuis, 1980; Omori, 1975a; b; 1978), *Acetes* spp. as a food organism also play an important role in agriculture and aquaculture. As examples, they are used for feed for livestock and poultry (Deshmukh, 1991; Raje, 1991), as food for feeding different larval stages in prawn hatcheries (Deshmukh, 1991; Pan and Chien, 2003), and as a live feed for broodstock (Job et al., 2006). Apart from this, they play an important role in the food webs of coastal waters, acting as predators feeding on a variety of foods (*i.e.,* detritus, diatoms, copepods, meroplankton of molluscs) and in turn constitute as prey for other fishes and predators (Deshmukh, 2003; Jaiswar and Chakraborty, 2005; Xiao and Greenwood, 1993).

2.2 Fixing and Preservation of Specimens

The process of fixing consists of killing an animal rapidly so that the specimen can retain their original shape, and also to prevent postmortem decay (autolysis and tissue degradation) (Huber, 1998; Pollock, 1998). Preservation is also required to protect and maintain the fixed-specimens from any degradation prior to further analysis (Huber, 1998; Martin, 2004; Pollock, 1998).

According to Rosenberg (2005), samples should be preserved using more than one method where one approach is optimal for morphologyical studies while the other is optimal for genetic analysis. This is due to different fixative and preservative have different levels of effectiveness when preserving specimens for morphological analysis vs DNA for genetic analysis. For example, formalin and ethanol are the preferred fixative and preservative for marine invertebrates (Pollock, 1998), but formalin is more effective than ethanol for preserving specimens for morphologyical analysis (Zimmermann et al., 2008) while an ethanol concentration of 95% or above is best for preserving DNA for genetic analysis (Rosenberg, 2005). Formalin is not suitable however for both fixing and preservation of crustaceans as it erodes the cuticle (Huber, 1998) and formalin preserved specimens often causes problems with DNA extraction, Polymerase Chain Reaction (PCR) and DNA sequencing due to DNA shearing (Díaz-Viloria et al., 2005; Zimmermann et al., 2008). Thus, it is suggested that fixing and preservation of crustaceans are done simultaneously, in which the live specimens are directly placed into 70–90% ethanol (Huber, 1998). A concentration of 95% or above is preferred for DNA sequencing, but concentrations above 80% may harden a specimen's tissues and cause them to

become brittle and difficult for morphological examination (Rosenberg, 2005). An ethanol concentration of 70% is preferred therefore for preserving both physical structure and DNA (Beaumont and Croucher, 2006; Dawson et al., 1998). As fluids within the specimen's tissues seep out during preservation, a ratio of at least 3:1 (3 parts of ethanol to 1 part of crustacean) is suggested to avoid excessive dilution (Martin, 2004; Rosenberg, 2005).

2.3 Length-Weight Relationships (LWRs)

Length-Weight Relationships (LWRs) are used to describe the relationships between length and weight mathematically, so that when one is known, the other can be predicted (Hile, 1936; Le Cren, 1951). Under field conditions, length measurements can be easier than weighing, due to wind and boat movement (Kimmerer et al., 2005). Thus, weight may be estimated from the length, if the LWR is known (Jobling, 2002; Martin-Smith, 1996). In addition, LWR is essential for estimating production and standing stock biomass from length (Anderson and Neumann, 1996; Binohlan and Pauly, 2000; Kimmerer et al., 2005), allows for conversion of growth-in-length equations to growth-in-weight for stock assessment (Le Cren, 1951), and to calculate indices of condition, *i.e.*, indicators of general "well being" or "fatness" of an the aquatic species (Anderson and Neumann, 1996; Bolger and Connolly, 1989; Jobling, 2002; Jones et al., 1999; Le Cren, 1951; Richter et al., 2000; Safran, 1992). In addition, data on length and weight are also useful for life-history and morphological comparisons among populations of the same species or comparisons between species (Ecoutin et al., 2005; Morato et al., 2001; Oliva-Paterna et al., 2009; Petrakis and Stergiou, 1995; Stergiou and Politou, 1995).

2.3.1 Parameter estimation

Generally, the relationship between length (L) and weight (W) can be expressed by the equation: $W = aL^b$ (Pauly, 1984; Schneider et al., 2000), where *a* and *b* are the parameters. Parameters *a* and *b* can be estimated using least squares linear regression on log-log transformed data: $\log W = \log a + b\log L$ (Pauly, 1984; Schneider et al., 2000).

2.3.1.1 Parameters *a* and *b*

Parameter *a* is the coefficient of the arithmetic form ($W = aL^b$) of LWR, and the intercept of the logarithmic form ($\log W = \log a + b\log L$) (Froese, 2006). Parameter *b* is the exponent of the arithmetic form and the slope of the regression line in the logarithmic form (Froese, 2006).

The value of *b* normally falls between 2.5 and 3.5 (Binohlan and Pauly, 2000; Pauly, 1984). From this, if the *b* value is equal or not significantly different from 3, it indicates isometric growth (*i.e.*, the shape does not change as the individual grows, or small individuals have the same shape and condition as large individuals). If however, the *b* values differ significantly from 3, it indicates either positive ($b > 3$) or negative ($b < 3$) allometric growth. Positive allometric growth (A+) either indicates that large specimens have increased in height or width more than in length (specimens become "plumper") due to large specimens in the sample being in better condition than small ones. Negative allometric growth (A-) indicates either that specimens have become more elongated (or "slimmer") with increase in length, or small specimens were in better nutritional condition at the time of sampling (Anderson and

Neumann, 1996; Froese, 2006; Froese and Pauly, 2011; Jobling, 2002). Therefore, some indication of the condition of the population can be obtained from the LWRs. On the other hand, values of $b < 2.5$ or $b > 3.5$ are often derived from samples with narrow size ranges (Froese, 2006; Froese and Pauly, 2011), or indicate an over-proportional increase in length relative to growth in weight and an over-proportional increase in weight relative to growth in length, respectively (Froese, 2006).

As suggested by few authors, the parameters *a* and *b* vary with the size range of the sample (Froese and Pauly, 2011). Thus, the use of LWR should strictly be limited to the size range applied when estimating regression parameters (Benedito-Cecilio et al., 1997; Dulčić and Kraljević, 1996; Froese and Pauly, 2011; Gonçalves et al., 1997; Morey et al., 2003; Muto et al., 2000; Petrakis and Stergiou, 1995; Xu and Abdul Ghaffar, 1995). Additionally, a number of factors are known to influence length or weight, including growth phase, season, stomach contents, maturity, sex, health and general fish condition and preservation techniques (Ajah and Nunoo, 2003; Froese, 2006; Kohler et al., 1995; Pauly, 1984; Tesch, 1971; Wetzel et al., 2005), that can directly influence the LWR parameters. The *b* value results from combined effects of one or more of the unaccounted factors. In the case of shrimps, significant differences in the length-weight relationship among sexes, species, seasons, sampled location and growth phase have been reported (Anderson and Lindner, 1958; Anger and Moreira, 1998; Cartaxana, 2003; Chu et al., 1995; Colloca, 2002; Company and Sardà, 2000; Papaconstantinou and Kapiris, 2003; Pérez-

Castañeda and Defeo, 2002; Primavera et al., 1998; Siegfried, 1980; Tosunoglu et al., 2007; Watson and Keating, 1989).

2.3.1.2 R^2, coefficient of determination

The coefficient of determination (R^2), which is the correlation coefficient squared (Pauly, 1984) was also estimated here. According to Motulsky and Christopoulus (2003), the value R^2 quantifies goodness of fit. R^2 is a fraction between 0.0 and 1.0, and has no units. R^2 can be interpreted from nonlinear regression very much like interpreting r^2 from linear regression. An r^2 value of 0.0 means that knowing X does not help in predicting Y, and there is no linear relationship between X and Y (Figure 2.8); when r^2 equals 1.0, all points lie exactly on a straight line with no scatter (Figure 2.8) and knowing X allows Y to be predicted perfectly (Motulsky and Christopoulos, 2003).

In addition, where F is significant ($P < 0.05$) in the analysis of variance (ANOVA), the R^2 is significantly different from zero. This means that one can assume there is a linear relationship between the predictor and the dependent variables and that the regression equation allows you to predict the dependent variable at greater than chance level (Foster, 2001)

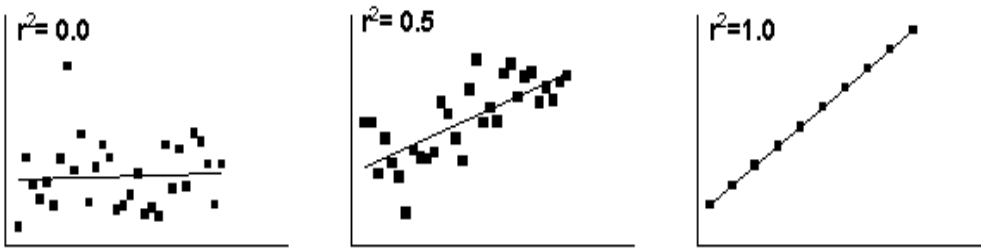


Figure 2.8: A measure of goodness-of-fit of linear regression, r^2 (Motulsky and Christopoulos, 2003).

2.4 Length-Length Relationships (LLRs)

Length-Length Relationships (LLRs) is the relationship between different types of lengths (e.g., CL vs. TL). LLRs linking first length type (L_1) and second length type (L_2) were determined using the least squares method to fit a simple linear regression analysis: $L_1 = a + bL_2$ (Binohlan et al., 2000), where *a* and *b* are the parameters. Sometimes, published LWRs are difficult to use, as they may be based on a length measurement type (e.g., carapace length) different from length measurements (expressed e.g., as total length). Thus, Length-Length Relationships (LLRs), which is the relationship between different type of lengths (e.g., CL vs. TL), is devised to facilitate conversion between length types (Binohlan et al., 2000; Binohlan and Pauly, 2000). Besides, LLRs are generally more important in comparative growth studies (Binohlan et al., 2000; Moutopoulos and Stergiou, 2002).

2.5 Mitochondrial DNA (mtDNA)

Animal mitochondrial DNA (mtDNA) is generally a small (15–20 kb) and circular genome containing 37 genes: 13 protein subunits of the enzymes of oxidative phosphorylation, 2 ribosomal RNA (rRNA) gene, and 22 transfer RNA (tRNA) genes (Boore, 1999) that code for subunits of enzymes functioning in electron transport, ATP synthesis or other proteins. It is popular as a genetic marker in population and evolutionary biology for several reasons: high copy number, maternal inheritance, lack of recombination, and a generally higher mutation rate than found in nuclear DNA (Avise et al., 1987; Harrison, 1989; Mitton, 1994; Moritz et al., 1987; Wilson et al., 1985). The relatively high copy number of mitochondria in tissues makes extraction of

mtDNA easier (Galtier et al., 2009; Moritz et al., 1987; Toon et al., 2009). Maternal mode of inheritance and lack of recombination result in an effective population size for mtDNA that is smaller than that of nuclear DNA (nDNA) (Moritz et al., 1987). Thus, mtDNA is more sensitive to change in population size than are nuclear genes (Wilson et al., 1985). Lack of recombination and maternal inheritance also simplify phylogeny reconstruction using mtDNA. Furthermore, some mtDNA genes evolves 5–10 times faster than the majority of genes encoded in the nuclear genome (Brown et al., 1979), this has led to its widespread use as a genetic marker for population-level studies (Moritz et al., 1987).

2.6 Cytochrome *c* Oxidase Subunit I (*COI*) Gene

Cytochrome *c* oxidase is the terminal enzyme in the respiratory chain of mitochondria and aerobic bacteria. It catalyzes electron transfer from cytochrome *c* to molecular oxygen, reducing the latter to water (Michel et al., 1998; Richter and Ludwig, 2003). In this process, the generation of transmembrane electrochemical gradient will drive ATP synthesis (Michel et al., 1998; Richter and Ludwig, 2003). Cytochrome *c* oxidase contains 13 subunits coded by both nuclear DNA (nDNA) and mitochondrial DNA (mtDNA): three large subunits (I, II, III) are coded in the mitochondrial genome, and the rest are coded the in nuclear genome (Capaldi, 1990). Among the three cytochrome *c* oxidase coding genes, cytochrome *c* oxidase subunit I (*COI*) gene is the most conserved (Hwang and Kim, 1999).

According to Hwang and Kim (1999, p. 215), "The highly conserved molecular markers and/or gene regions are useful for investigating phylogenetic relationships at higher categorical levels (deep branches of evolutionary history). On the other hand, the hypervariable molecular markers and/or gene regions are useful for elucidating phylogenetic relationships at lower categorical levels (recently diverged branches)". The appropriate categorical levels of commonly used molecular markers or gene regions in rDNA and animal mtDNA are shown in Figure 2.9. The *COI* is highly variable among species, thus making it a good candidate at lower levels (Toon et al., 2009) and has been proven to be useful in examining both phylogenetic relationships (at the species level) and population genetic variation among populations within species of decapod crustaceans (Baldwin et al., 1998; García-Machado et al., 2001; Harrison and Crespi, 1999; Khamnamtong et al., 2009; Machordom and Macpherson, 2004; Roldán et al., 2009; Shank et al., 1999; Trontelj et al., 2005).

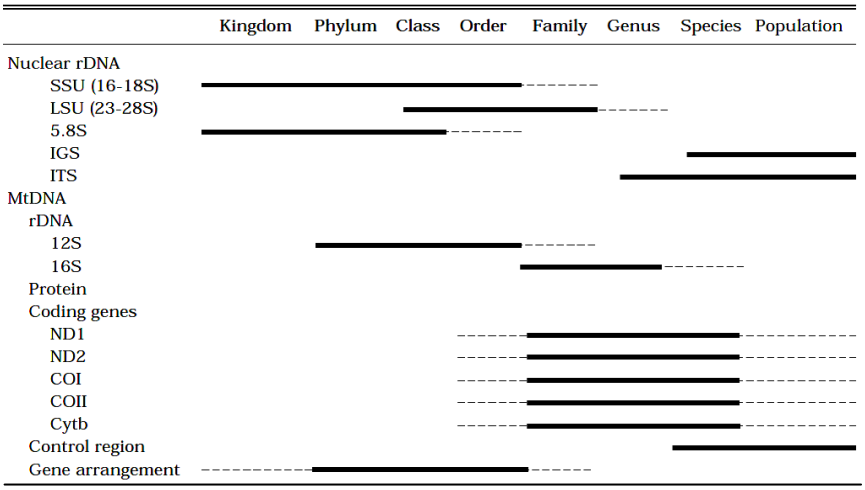


Figure 2.9: Applicable categorical levels of each molecular marker or gene region in rDNA and mtDNA. The bold lines indicate mainly applicable categorical levels of each molecular marker or gene region while dotted lines indicate less frequently applicable categorical levels (Hwang and Kim, 1999).

2.7 Nuclear Mitochondrial Pseudogenes (Numts)

Numts have been reported in a variety of organisms , including domestic cat (Lopez et al., 1994), birds (Sorenson and Quinn, 1998), humans and great apes (Thalmann et al., 2004) and crustaceans (Williams et al., 2002; Williams and Knowlton, 2001). They are known to be the copies of mtDNA fragment incorporated into the nuclear genome (Bensasson et al., 2001; Thalmann et al., 2004; Zhang and Hewitt, 1996) that can be easily coamplified with the mitochondrial orthologue by a conserved universal primer. Consequently, this may lead to incorrect species identification and an overestimation of the number of species (Song et al., 2008).

Symptoms of Numts contamination include, (1) PCR amplification that constantly produces more than one band or different bands, (2) sequence ambiguities (particularly if they are polymorphic sites, or if they are encountered when sequencing from both strands), double peak or background noise in sequence chromatogram, (3) unexpected insertions or deletions, frameshift mutation or stop codons, (4) nucleotide sequences obtained are radically different from those expected, or (5) phylogenetic analysis yields an unusual or contradictory tree topology (Bensasson et al., 2001; Song et al., 2008; Zhang and Hewitt, 1996). However, Numts can be avoided by purifying mitochondria before DNA extraction, long PCR amplification, using tissue that is rich in mtDNA relative to nuclear DNA, or by using taxon specific primers in PCR (Bensasson et al., 2001; Song et al., 2008; Sorenson and Quinn, 1998).

2.8 Patterns of Genetic Variation

In the marine environment, species with a planktonic larval phase are expected to possess a higher levels of dispersal potential and thus lower levels of genetic differentiation compared with species with direct or non-planktonic development (Arndt and Smith, 1998; Bernardi, 2000; Collin, 2001; Duffy, 1993; Hellberg, 1996; Hoskin, 1997; McMillan et al., 1992; Palumbi, 1992; Teske et al., 2007; Wilke and Davis, 2000). However, accumulated evidence has shown that marine species in general, are more genetically structured than predicted despite their high dispersal potential (Bay et al., 2004; Benzie, 1999; Benzie and Williams, 1997; Bird et al., 2007; Briggs, 1999; Palumbi, 1997; Richards et al., 2007). Thus, even while possession of a pelagic larvae phase provides a potential means of dispersal, successful migration of individuals is heavily dependent on whether the dispersing larvae can successfully survive, settle, mature, and then reproduce in the new environments (Hedgecock, 1986).

Several factors limiting actual movement by marine organisms with high dispersal potential have been reviewed: species, life-history traits, habitats, geographical distance, local environmental feature (temperature, salinity), ocean conditions and drafting processes (Azuma et al., 2008; Benzie et al., 2002; Brooker et al., 2000; Bulhões Arruda et al., 2009; Díaz-Jaimes et al., 2006; Donald et al., 2005; Gusmão et al., 2005; Khamnamtong et al., 2009; Palumbi, 1994; Pellerito et al., 2009; Tzeng et al., 2004; Zhan et al., 2009; Zitari-Chatti et al., 2009). These factors may have significant effects on dispersal potential of marine organisms. As an example, a significant correlation was observed between the genetic distance and geographical

distance in a study by Khamnamtong et al., (2009). Thus, the observed population structure in giant tiger shrimp, *Penaeus monodon* may be explained by the isolation by distance model (or geographical distance). On the other hand, *Farfantepenaeus notialis* collected from Batabano and Ana Maria Gulfs (that are less than 15 km apart), showed significant population differentiation which could might be explained by the presence of the Calzones Gulf (the deepest in Cuba) that prevent the movement of larvae and adults (García-Machado et al., 2001). More importantly, the population genetic structure may reflect historical gene flow that have produced present-day patterns of distribution and connectivity among populations rather than ongoing gene flow (Benzie, 1999; Palumbi, 1997).

2.9 Demographic History

For tropical marine species, one of the primary impacts of Pleistocene-era environmental fluctuation was the effects of sea levels dropping to 120 m below present during glacial maxima (Voris, 2000). This was particularly strong in tropical areas (*i.e.*, Indo-Australian Archipelago, IAA that are characterized by broad, shallow continental shelves that become exposed during low sea-level stands). As an example in Southeast Asia, parts of the Sunda Shelf was exposed when the sea-level dropped to about 120 m below the present sea-level (*i.e*., during the last-glacial maximum, around 18,000–20,000 years ago) (Hanebuth et al., 2000). In addition, Pleistocene sea-level fluctuations closed the Torres, Sunda and Malacca straits in the IAA more than 10 times over the past million years (Pillans et al., 1998), and on seven different occasions during the past 150,000 years alone (Voris, 2000).

These rapid changes in Pleistocene sea-levels allowed for restriction and expansion in species worldwide, that directly affecting population distributions and demographics (Hewitt, 2000; Hewitt, 1996), produced cycles of genetic isolation, secondary contact, and subsequent merging (Benzie, 1999). The species whose populations have been subject to the effects of such cycles may exhibit genetic signals characterised by high genetic diversities and/or complex geographical structures (Grant and Bowen, 1998). As in Grant and Bowen (1998), different combinations of small and large values for haplotype diversity (h) and nucleotide diversity (π) may indicate four different categories of demographic histories (Figure 2.10). The value of h varies between 0–1.0; π varies between 0 (no divergence) to over 10% for very deep divergence.

The first category consists of species with small values of $h < 0.5$ and $\pi < 0.5\%$). Recent population bottlenecks or founder events by single or a few mtDNA lineages are responsible for the low levels of genetic diversity (Atlantic cod, Carr et al., 1995; Atlantic cod, Pogson et al., 1995; Beaugregory damselfish, Shulman and Bermingham, 1995). The second category includes species with high h and low π ($h > 0.5$ and $\pi < 0.5\%$). This condition is attributed to population expansions event after a period of low effective population size caused by bottlenecks. This has been found in several other studies (six bar wrasse, Chen et al., 2004; European eel, Daemen et al., 2001; fleshy prawn, Kong et al., 2010; neon damselfish, Liu et al., 2008; red shrimp, Maggio et al., 2009; caramote prawn, Pellerito et al., 2009; black-spot sea bream, Stockley et al., 2005). Many of these species are believed to have originated in the Pliocene or early Pleistocene, but their mtDNA genealogies

coalesce at a more recent time scale, perhaps during the last few hundred thousand years. A third category consists of species with low *h* and high π, and characterizes populations with a few highly divergent haplotypes. This may result from secondary contact between isolated populations or by a strong bottleneck in a formerly large, stable population (Bermingham and Avise, 1986; Burton, 1986; Planes and Doherty, 1997). The fourth category consists of populations with large values of both *h* and π. The high level of divergence between haplotypes may be attributed to secondary contact between previously differentiated allopatric lineages (round mackerel, Borsa, 2003; trumpet worm, Jolly et al., 2004; cuttlefish, Kassahn et al., 2003; false clownfish, Nelson et al., 2000; scad mackerel, Perrin and Borsa, 2001; swimming crab, Pfeiler et al., 2005; scad mackerel, Rohfritsch and Borsa, 2005; ray-finned fish, Wang et al., 2008) or to a long evolutionary history in a large stable population (horse mackerel, Comesaña et al., 2008; red snapper, Garber et al., 2004; shovelnose guitarfish, Sandoval-Castillo et al., 2004; Japanese Spanish mackerel, Shui et al., 2009; redlip blenny, Shulman and Bermingham, 1995).

	h	
π	Small	Large
Small	1. Recent population bottleneck or founder event by single or a few mtDNA lineages.	2. Population bottleneck followed by rapid population growth and accumulation of mutations.
Large	3. Divergence between geographically subdivided populations.	4. Large stable population with long evolutionary history or secondary contact between differentiated lineages.

Figure 2.10: Interpreting haplotype and nucleotide diversities (sensu Grant and Bowen, 1998).

CHAPTER 3.0

MATERIALS AND METHODS

3.1 Specimens

Acetes shrimps were sampled from in-shore catches using push-nets and trawling activities at sea more than 5 nautical miles (nm) off-shore along the west coast of Peninsular Malaysia (Figure 3.1), from August 2007 to September 2008. A Global Positioning System (GPS) was used to indicate the geographical position of each sampling location (Table 3.1, Figure 3.2).

(a)

(b)

Figure 3.1: Sampling techniques used in this study: (a) push-net (b) trawling.

Table 3.1: Sampling locations of *Acetes* spp. collected 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^{o} 10’57.14’’N	102^{o}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

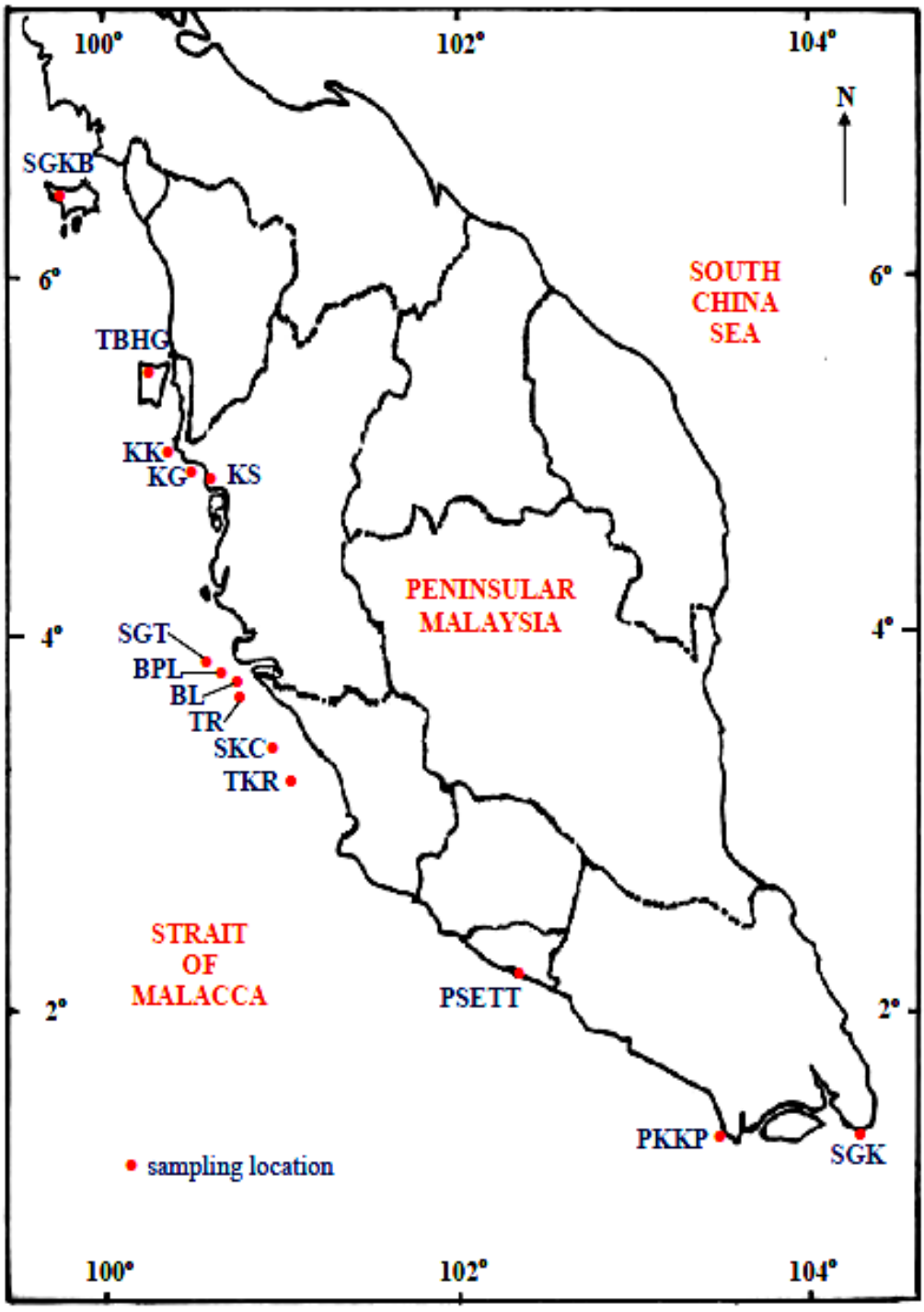


Figure 3.2: Map of Peninsular Malaysia showing the 14 locations (•) where *Acetes* were sampled for this study. The sampling locations are – SGKB: Sungai Kubang Badak; TBHG: Teluk Bahang; KK: Kuala Kurau; KG: Kuala Gula; KS: Kuala Sepetang; SGT: Sungai Tiang; BPL: Bagan Pasir Laut; BL: Bagan Lipas; TR: Teluk Rhu; SKC: Sekinchan; TKR: Tanjong Karang; PSETT: Portuguese Settlement; PKKP: Pulau Kukup; SGK: Sungai Kubang Badak.

3.2 Preservation of Specimens

The specimens were stored in 70% ethanol (Beaumont and Croucher, 2006; Dawson et al., 1998) at a ratio of 3:1 (*i.e.*, three parts of ethanol to one part of crustacean; Martin, 2004) for subsequent morphometric and DNA analyses. The 70% ethanol was prepared by diluting absolute ethanol (HmbG Chemicals, Germany) with deionised water (King and Porter, 2004). The specimens were then transported to the laboratory for further analysis.

3.3 Species and Sexes Identification

In the laboratory, species and sex of *Acetes* shrimp were identified under a Leica dissecting microscope (Leica ZOOM 2000™, Model No. Z45V, Germany). Identification was done by using the key characters detailed in Table 3.2 and Table 3.3 (Omori, 1975b), with the help of a number of figures (Figure 3.3–3.6)

Table 3.2: Key to the sexes of genus *Acetes.*

Sex
A pair of protuberances (genital coxae) between third pereiopods and first pleopods. Lower antennular flagellum with 1- 2 clasping spines or modification thereof. Petasma present on first pleopods ……..……...….Male
No protuberance in genital area. Lower antennular flagellum without spine. Petasma absent …….……..…….....……...…… Female

Table 3.3: Key to the species of the genus *Acetes*.

	Males
1	Anterior margin of genital coxa rounded. Petasma without pars astringen ……..…... 2 Anterior margin of genital coxa pointed. Petasma with pars astringens ……...…… 9
2	Rostrum without or with only 1 denticle behind terminal point. Lower antennular flagellum without large clasping spine ……. 3 Rostrum with 2 denticles behind terminal point. Lower antennular flagellum with 1–2 clasping spine…….…… 4
3	Rostrum with 1 denticle behind terminal point ……...…….…… *A. americanus* Rostrum without any denticles behind terminal point ……....…..…..…..… *A. binghami*
4	Procurved tooth present between bases of first pair of pleopods. Lower antennular flagellum with 1 clasping spine ……..…….....……..… *A. indicus* Procurved tooth absent. Lower antennular flagellum with 2 clasping spines …..……..….. 5
5	Lower antennular flagellum with triangular projection from upper end of first segment of main branch ……..…….…… 6 First segment of main branch of lower antennular flagellum without triangular projection ….....…… 8
6	Petasma with processus ventralis; capitulum cylindrical and elongated …..…. *A. natalensis* Petasma without processus ventralis; capitulum expanded on outer margin ……...…… 7
7	Capitulum of petasma with large ventral projection at right angles to long axis of pars media ……..…….……..... *A. johni* Capitulum of petasma without ventral projection; broad end cut off transversely and armed with 1 large hook ……...… *A. serrulatus*
8	Distal expanded part of capitulum of petasma cucumber-shaped; much longer than basal part of capitulum. Endopod of uropod with 4 – 8 red spots .....…. *A. chinensis* Distal expanded part of capitulum of petasma bulb-like; proportionally shorter than basal part of capitulum. Endopod of uropod with 1 red spot ……..…….…… *A. japonicus*
9	First segment of main branch of lower antennular flagellum with large swelling; clasping spine not reaching end of second segment of main branch. Apex of telson rounded or truncated ……...……10 First segment of main branch of lower antennular flagellum with small swelling; clasping spine extending beyond end of second segment of main branch. Apex of telson triangular ……...… 11
10	First segment of antennular peduncle longer than second and third segments combined. Petasma with rudimentary capitulum ……..…….…… *A. paraguayensis* First segment of antennular peduncle shorter than second and third segments combined. Petasma without capitulum …….....…… *A. marinus*
11	Procurved tooth present between bases of first pair of pleopods …….…….. 12 Procurved tooth absent …….....…..…… 13
12	First segment of antennular peduncle shorter than second and third segments combined. Capitulum of petasma with 3–5 subequally large hooks along outer margin … *A. intermedius* First segment of antennular peduncle longer than second and third segments combined. Capitulum of petasma with 1 large hook along outer margin …..... *A. erythraeus*
13	Lower antennular flagellum with 12 segments or less. Capitulum of petasma with 1 large and often 1 small hook along outer margin …….. *A. sibogae* Lower antennular flagellum with 17 segments or more. Capitulum of petasma with 3 large hooks along outer margin …….…….…… *A. vulgaris*

Table 3.3 continued: Key to the species of the genus *Acetes*.

	Females
1	Rostrum without denticle behind terminal point ……... *A. binghami* Rostrum with 1 denticle behind terminal point ……..… *A. americanus* Rostrum with 2 denticles behind terminal point ……. 2
2	Apex of telson rounded or truncated ……..……...……...… 3 Apex of telson triangular ……..…… 9
3	Third thoracic sternite produced posteriorly ……... 4 Third thoracic sternite not produced posteriorly …...……..……..… 7
4	Third thoracic sternite with paired protuberances. Exopod of uropod broad; 3.3 – 3.9 times as long as broad ……..……...……..... 5 Third thoracic sternite without protuberances. Exopod of uropod slender; 4.2 – 4.7 times as long as broad ……..……..… 6
5	Coxa of third pereiopod with large acute tooth ……..……...… *A. paraguayensis* Coxa of third pereiopod with small blunt tooth ……..……...… *A. marinus*
6	Emargination of posterior margin of third thoracic sternite deep; endopod of uropod with 4 – 8 red spots ……..……...……. *A. chinensis* Emargination of posterior margin of third thoracic sternite shallow; endopod of uropod with 1 red spot ……...… *A. japonicus*
7	Tooth absent on distal inner margin of coxa of third pereiopod ……..….. *A. natalensis* Tooth present on distal inner margin of coxa of third pereiopod ……... 8
8	Anterior margin of fourth thoracic sternite pointed laterally; median part broadly grooved ……..... *A. johni* Anterior margin of fourth thoracic sternite smooth and convex …….. *A. serrulatus*
9	Procurved tooth present between bases of first pair of pleopods ……...…….. 10 Procurved tooth absent ……..… 12
10	Inner margin of basis of third pereiopod with sharply pointed projection. Third and fourth thoracic sternites deeply channelled longitudinally …….. *A. indicus* Inner margin of basis of third pereiopod without sharply pointed projection. Third and fourth thoracic sternites not channelled longitudinally ……..……..……..… 11
11	First segment of antennular peduncle at most as long as second and third segments combined. Distal inner margin of basis of third pereiopod ending in blunt projection ……... *A. intermedius* First segment of antennular peduncle longer than second and third segments combined. Distal inner margin of basis of third pereiopod ending without projection ……..…… *A. erythraeus*
12	Lower antennular flagellum with 20 segments or less. Distal inner margin of basis of third pereiopod ending in projection. A pair of small protuberances on anterior part of third thoracic sternite ……..……...…… *A. sibogae* Lower antennular flagellum with 20 segments or more. Distal inner margin of basis of third pereiopod ending without projection. A pair of large protuberances on anterior part of third thoracic sternite ……..……..……... *A. vulgaris*

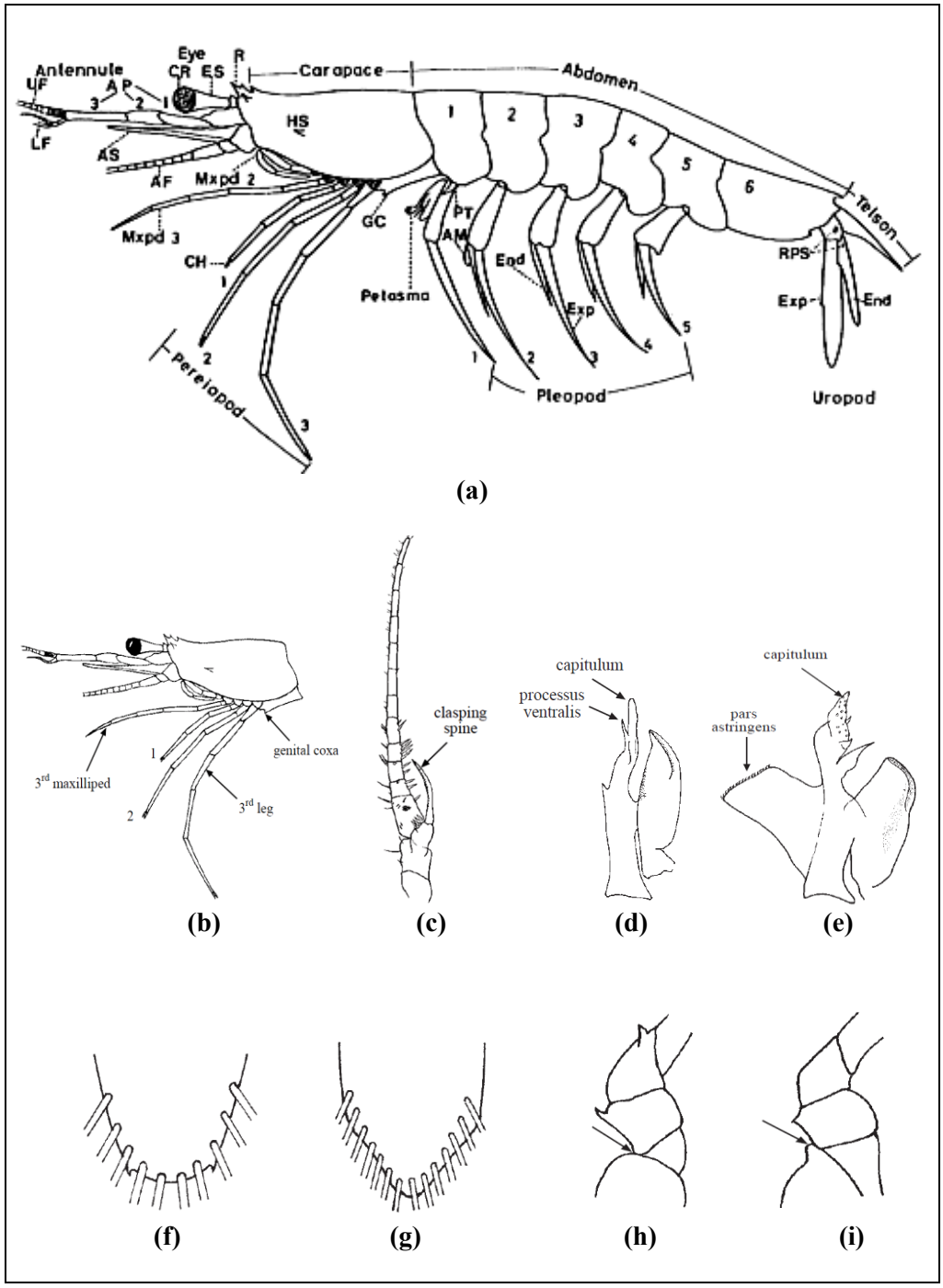


Figure 3.3: Diagram of *Acetes* with parts labelled (a) lateral view of a male *Acetes*. AM, appendix masculina; AF, antennal flagellum; AP, antennular peduncle; AS, antennal scale; CH, chela; CR, cornea; End, endopod; ES, eye stalk; Exp, exopod; GC, genital coxa; HS, hepatic spine; LF, lower flagellum; Mxpd, maxilliped; PT, procurved tooth; R, rostrum; RPS, red pigment spots; UF, upper flagellum (Omori, 1975b) (b) a pair of protuberance (genital coxae) in *Acetes* (c) lower antennular flagellum of *Acetes* (male) (d) the example of petasma without pars astringens in *Acetes* (male) (e) example of petasma with pars astringens in *Acetes* (male) (f) apex of telson rounded or truncated in *Acetes* (g) apex of telson triangular in *Acetes* (h) third thoracid sternite produced posteriorly in *Acetes* (female) (i) third thoracid sternite not produced posteriorly in *Acetes* (female) (Chan, 1998).

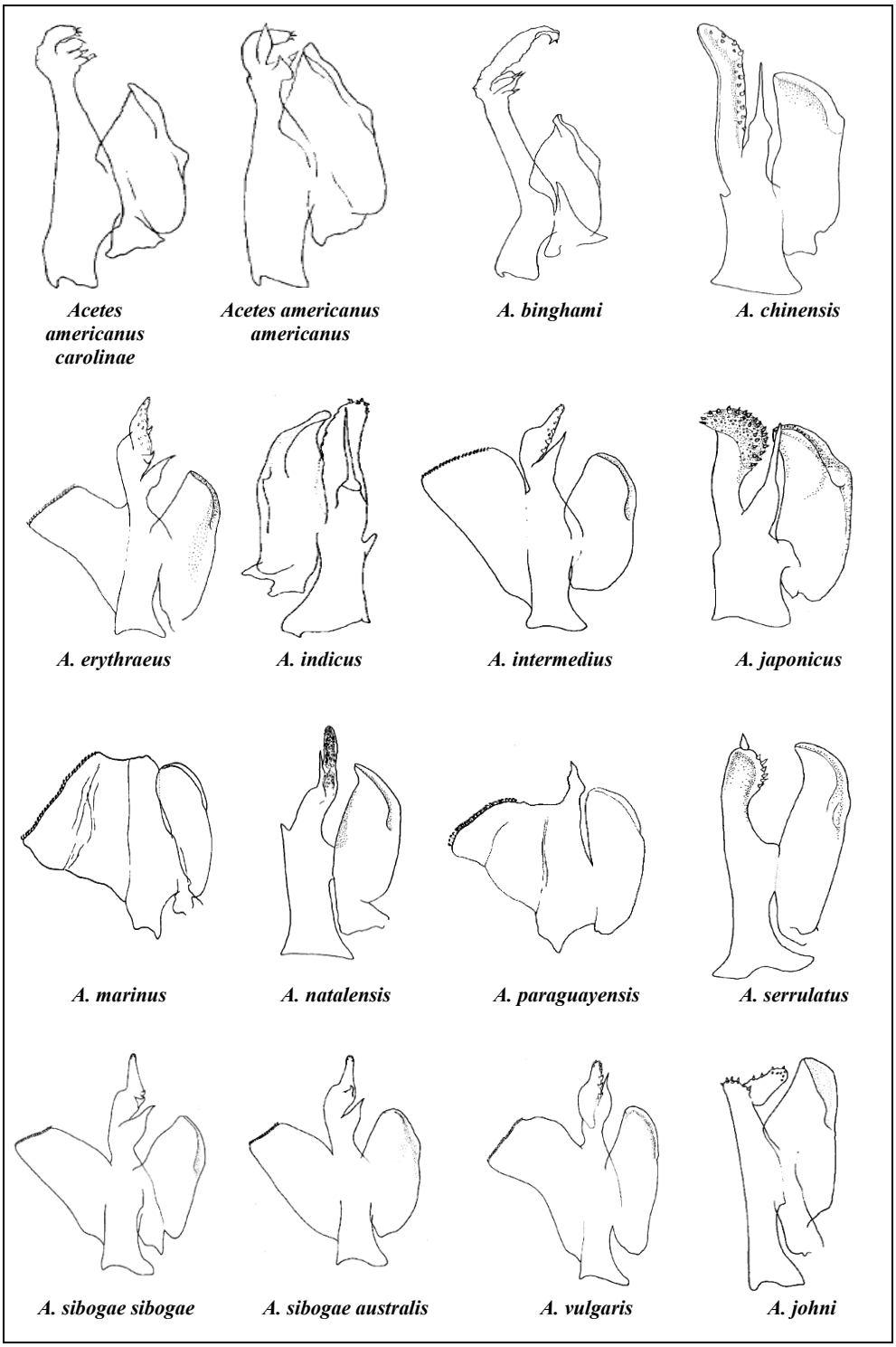


Figure 3.4: Examples of the petasma in the males of *Acetes* (Omori, 1975b).

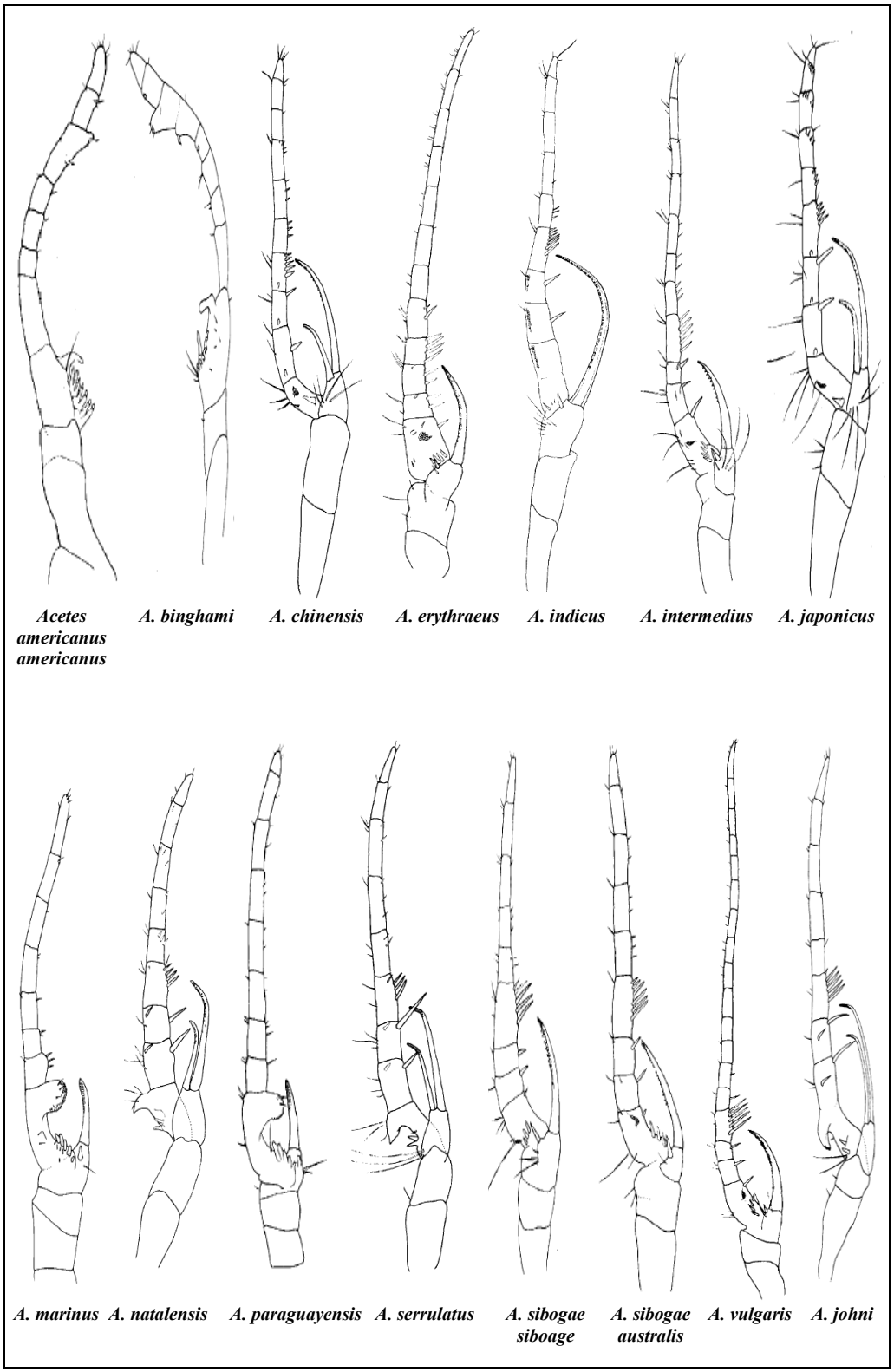


Figure 3.5: Examples of the lower antennular flagellum in the males of *Acetes* (Omori, 1975b).

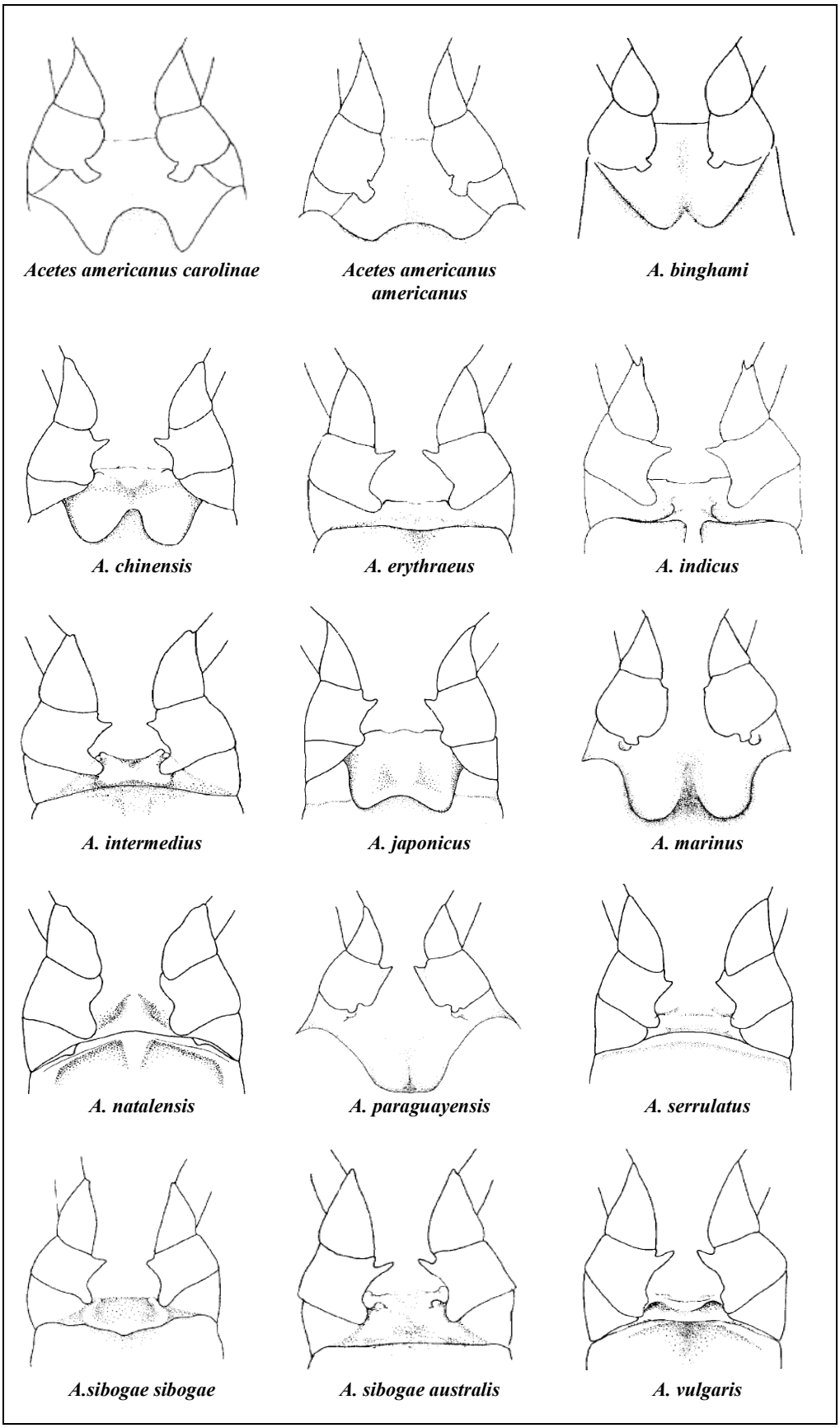


Figure 3.6: Examples of the third thoracic sternite in female *Acetes* (Omori, 1975b).

3.4 Morphometric Data Collection

A total of 1112 specimens from various sampling locations (Table 3.4) were measured using a dissecting microscope. Three morphometric measurements were obtained: total length (TL; Figure 3.7), carapace length (CL; Figure 3.7) and wet weight (WW). TL was measured along the dorsal surface from the anterior tip of the rostrum to the posterior end of the telson (Amin et al., 2009c; Amin et al., 2009d; Arshad et al., 2007; Arshad et al., 2008) to the nearest hundredth of a millimetre (0.01 mm) using a digital calliper. CL was measured as the shortest distance between the posterior margin of the orbit and the mid-dorsal posterior edge of the carapace (Amin et al., 2008b; Oh and Jeong, 2003; Oh et al., 2002), to the nearest hundredth of a millimetre (0.01 mm) using a digital calliper. Lastly, the shrimps were weighed after they were removed from ethanol and blotted dry on paper towels (Kuun et al., 1999; Oh et al., 1999). WW was then measured using an analytical balance (Adventurer™ Balances, Ohaus, USA) of 0.1 mg accuracy.

Table 3.4: The number of specimens (n) from the sampling locations used in the morphometric analyses.

Sampling locations (Abbreviation)	n
Sungai Kubang Badak (SGKB)	53
Teluk Bahang (TBHG)	52
Kuala Kurau (KK)	35
Kuala Gula (KG)	53
Kuala Sepetang (KS)	0*
Sungai Tiang (SGT)	200
Bagan Pasir Laut (BPL)	200
Bagan Lipas (BL)	100
Teluk Rhu (TR)	50
Sekinchan (SKC)	100
Tanjong Karang (TKR)	100
Portuguese Settlement (PSETT)	40
Pulau Kukup (PKKP)	74
Sungai Kapal (SGK)	55
Total	1112

*Sample collected in this study but not able to obtain measurements.

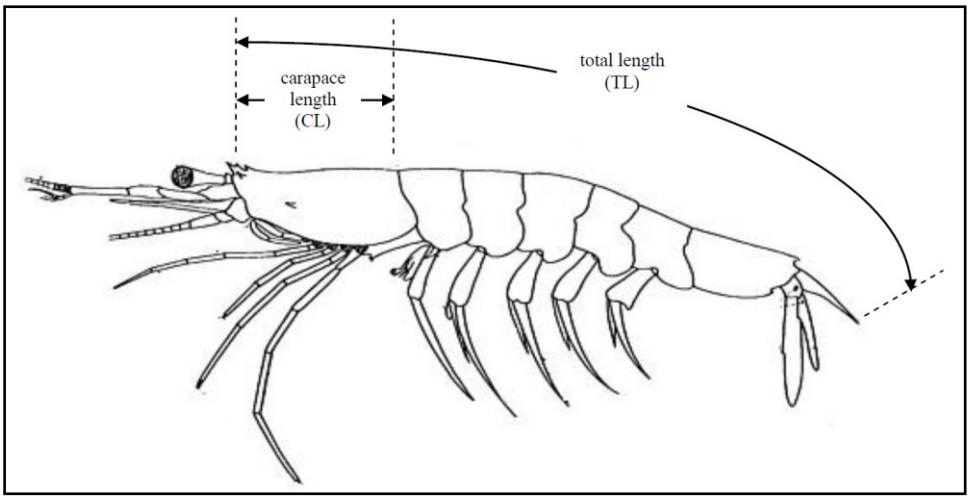


Figure 3.7: Measurements of total length (TL) and carapace length (CL) of *Acetes*.

3.5 Morphometric Data Analysis

Morphometric data analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 16.0. Descriptive statistics such as mean and standard deviation (S. D.) of each morphometric measurement were calculated. Prior to Independent Samples *t*-test (*i.e.*, to compare the means of each measurement between sexes, and between in-shore and off-shore samples) and ANOVA (*i.e.*, to compare the means of each measurement among species), the assumption of normality was evaluated using either Kolmogorov-Smirnov (K-S) or Shapiro-Wilks (S-W) *W* tests. The S-W *W* test was used when the sample size was less than 50, K-S test was used when there is 50 or more samples (Foster, 2001; O'Donoghue, 2010). In this study, the assumption of normality was violated, thus, non-parametric tests including Mann-Whitney *U*-test and Kruskal-Wallis *H*-test were used, respectively (Landau and Everitt, 2004; Leech et al., 2005; Marques de Sá, 2007; Morgan et al., 2004). If Kruskal-Wallis *H*-test was significant, pairwise comparisons among the groups was examined using Mann-Whitney *U*-test (Corder and Foreman, 2009)

with Bonferroni correction (Bland and Altman, 1995). As six comparisons were conducted, a Bonferronic corrected *P*-value of 0.0083 was used to determine significance based on an uncorrected *P*-value of 0.05. In addition, the size-frequency distribution of each measurement was plotted. Overall sex ratio (males: females) for each *Acetes* species was estimated and Chi-square test was employed to determine the differences in the occurrence of males and females over the sampling period.

3.6 Length-Weight Relationships (LWRs) and Length-Length Relationships (LLRs)

The relationship between length and weight was established by: $W = aL^b$ (Pauly, 1984; Schneider et al., 2000), where W is the weight, L is the length, *a* and *b* are the parameters. In this study, W was the Wet Weight (mg), and L was either the total length (TL, mm). Both the TL (independent variable) and WW (dependent variable) were log-transformed (Bird and -Prairie, 1985). The parameters *a* and *b* were estimated by least squares linear regression on log-log transformed data: $LogW = Log a + b LogL$ (Pauly, 1984; Schneider et al., 2000). The coefficient of determination (R^2) and the 95% confidence interval (CI) values for parameters *a* and *b* were estimated as well. The significance of the regression was evaluated with ANOVA, in order to test the null hypothesis (H_0: $\beta = 0$) that the slope of the regression line was not different from zero against the alternative hypothesis (H_A: $\beta \neq 0$), that the slope of the regression was significantly different from zero.

The null hypothesis of isometric growth, H_0: $b = 3$ was tested by Student's *t*-test with the following equation: $t_s = (b - 3) / S_b$, where t_s is the *t*-test value, *b*

is the slope, and S_b is the standard error of the slope (b), for $\alpha = 0.05$ (Sokal and Rohlf, 1987). When the t-test value was greater than the t critical value (Appendix A; Zar, 1999), the null hypothesis was rejected (Zar, 1999). This meant that the b value had deviated significantly from 3, and the growth type would be classified as positive allometric growth (when $b > 3$) or negative allometric growth (when $b < 3$). Conversely, when the t-test value was smaller than the t critical value, the growth was isometric ($b = 3$). The normal distribution (z-test) was also used to test the null hypothesis of isometric growth (Sokal and Rohlf, 1987).

The length-length relationships (LLRs) linking first length type (L_1) and second length type (L_2) were determined by the method of least squares to fit a linear regression analysis: $L_1 = a + bL_2$ (Binohlan et al., 2000), where a and b are the parameters of LLR. LLRs between TL and CL were established using a linear regression analysis of $TL = a + b$CL. The coefficient of determination (R^2) was estimated, and significance of the regression slope was evaluated with ANOVA.

3.7 Sample Preparation (DNA Extraction)

For each species that identified morphologically, a minimum of two and a maximum of 15 individuals (total: 159 individuals) from each sampling location were used in genetic analyses (Table 3.5). Initially, eight individuals of each species identified morphologically from each location were used for DNA extraction. However, due to the small size of the individuals (*i.e.*, limited material), some problems were encountered during DNA extraction or

the subsequent steps (*i.e.*, Polymerase Chain Reactions, sequencing) resulting in some sampling locations having less than 8 individuals. In the case of *A. serrulatus,* because more unique haplotype was found and in order to explore more, additional samples were added only for the sampling site that were sampled twice, which is Sungai Tiang (SGT) and Bagan Pasir Laut (BPL) each having 14 and 15 individuals respectively.

Ethanol-preserved *Acetes* specimens were first rinsed with deionised water and blotted onto tissue paper to eliminate as much ethanol as possible. Muscle tissue were scraped and transferred into 1.5 ml microcentrifuge tubes, then finely minced with scissors and tissue incubated at 55 ^{o}C for about 2 min to evaporate any extra ethanol (Martinez et al., 2006). DNA was then extracted from 25 g of sample with i-genomic CTB DNA Extraction Mini Kit (iNtRON Biotechnology, Inc., South Korea), according to the manufacturer's protocol (Appendix B) with some minor modifications in Step 8 (*i.e.*, incubation at room temperature was 20 min instead of 1 min). Finally, the DNA was eluted in 100 µL of buffer available from the kit (buffer CL) and stored at -20 ^{o}C.

Table 3.5: The number of specimens (n) used in the genetic analyses.

Sampling locations (Abbreviation)	Species			
	ai	*as*	*aj*	*asi*
Sungai Kubang Badak (SGKB)				6
Teluk Bahang (TBHG)			6	
Kuala Kurau (KK)	5		2	
Kuala Gula (KG)	6		5	
Kuala Sepetang (KS)				6
Sungai Tiang (SGT)	8	14		
Bagan Pasir Laut (BPL)	7	15		
Bagan Lipas (BL)	7	8		
Teluk Rhu (TR)	5	5		
Sekinchan (SKC)	7	7		
Tanjong Karang (TKR)	6	6		
Portuguese Settlement (PSETT)	8			
Pulau Kukup (PKKP)	5	5		
Sungai Kapal (SGK)	5	5		
Total	69	65	13	12

3.8 DNA Quantification

Gel electrophoresis was carried out by running 3 µL of eluate mixed with 1 µL of 6× loading dye solution (Fermentas) and 1 kb DNA ladder (Vivantis) were used as band-size markers in each gel. Gel electrophoresis was performed for 45 min at 90V through a 1% agarose gel (1st BASE), in 1× Tris-Borate-EDTA (TBE) buffer. The quality of the DNA was visualized under U.V. light and the images were captured using the GeneFlash Gel Documentation System (Syngene, Cambridge, U.K.). The gel was then stained with ethidium bromide (EtBr) for 30–45 min The quantity of double-stranded DNA was estimated by measuring the absorbance at 260/280 nm ratio with the SmartSpec™ Plus Spectrophotometer (Bio-Rad). 60 µL of 1× Tris-EDTA (TE) was used as blank. The sample used for quantification contained 1 µL of sample DNA that was mixed with 59 µL of 1× TE, resulting in a 60 times dilution of the DNA sample.

3.9 Polymerase Chain Reaction (PCR)

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. A negative

control consisting of a template-free reaction was included during all PCR amplifications to detect contamination.

3.10 PCR Thermal Regime

The PCR of *COI* gene was performed on an Eppendorf Mastercycler® Gradient (Eppendorf, Hamburg, 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).

3.11 Agarose Gel Electrophoresis

Gel electropheresis of the PCR product was then used to detect the presence of a single band. Five µL of each PCR product was mixed with 1 µL of 6× loading dye solution (Fermentas) and 100bp DNA ladder (Fermentas) were used as band-size markers in each gel. Gel electrophoresis was performed for 45 min at 90 V through a 2% agarose gel (1st BASE), in 1× TBE buffer. The results were visualized under U.V. light and images were captured using the GeneFlash Gel Documentation System (Syngene, Cambridge, U.K.) after staining the gel with ethidium bromide (EtBr).

3.12 Template Purification and Sequencing

Prior to sequencing, PCR products were purified using MEGAquick-spin™ PCR and Agarose Gel DNA Extraction System (iNtRON Biotechnology, Inc., South Korea) according to the manufacturer's protocol (Appendix C). Lastly,

the PCR product was eluted in 30 μL of buffer available from the kit and stored at -20 ^{o}C. Purified PCR products were out-sourced for sequencing.

3.13 DNA Sequence Alignment and Analysis

DNA sequence chromatograms were viewed with Chromas LITE 2.01 (Technelysium Pty. Ltd., Queensland, Australia). Homology search was carried out with the Basic Local Alignment Search Tool (BLAST; Altschul et al., 1990; Altschul et al., 1997) available on National Center for Biotechnology information (NCBI) website (Johnson et al., 2008). Alignments of the *COI* sequences were performed with CLUSTAL W (Thompson et al., 1994) in Molecular Evolutionary Analysis 4 (MEGA4; Tamura et al., 2007). The aligned nucleotide sequences were then translated into amino acid sequences based on the invertebrate mitochondrial genetic code (Appendix D) with EMBOSS Transeq (Rice et al., 2000). Sequence variation and base composition of the *COI* gene were analyzed using MEGA4 and DnaSP v5 (Librado and Rozas, 2009; Rozas et al., 2003). When homologous sequences from two individuals differed by one or more than one nucleotide, the sequences were considered as different haplotypes.

3.14 Nucleotide Substitution Model

Prior to phylogenetic analysis, the best-fit evolutionary model of nucleotide substitution was chosen using corrected Akaike Information Criterion (AICc; Hurvich and Tsai, 1989) in jModelTest 0.1.1 (Posada, 2008; 2009).

3.15 Phylogenetic Analyses

Based on all aligned *COI* sequences, the phylogenetic relationships among haplotypes were examined by four different phylogenetic methods to verify whether alternative topologies were supported by different tree-building methods. Neighbour-joining (NJ; Saitou and Nei, 1987) and Maximum Parsimony (MP; Camin and Sokal, 1965) were performed with MEGA4 and Phylogeny Analysis Using Parsimony (PAUP* 4.0b10; Swofford, 2002), respectively. Maximum Likelihood (ML; Felsenstein, 1981) and Bayesian Inference (BI) were performed with PhyML 3.0 (Guindon and Gascuel, 2003) on the ATGC Bioinformatics platform (http://www.atgc-montpellier.fr/phyml/; Guindon et al., 2005) and MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003), respectively. Stability of the derived clusters in phylogenetic trees were assessed by non-parametric bootstrapping (Felsenstein, 1985) and Bayesian analysis. All the phylogenetic trees were rooted with *Sergestes similis* (GenBank Accession Number: DQ882152) as outgroup and displayed with TreeView 1.6.6 (Page, 1996; 2002).

3.15.1 Neighbour-joining (NJ) Tree

An NJ tree, based on the Kimura's Two parameter's (Kimura, 1980) substitution model, was constructed using MEGA4. The clustering stability of the tree topology was verified by 2000 replications of non-parametric bootstrapping.

3.15.2 Maximum Parsimony (MP) Tree

MP analyses were conducted by assuming that all the 552 characters were of the 'unord' type and were weighted equally. The most parsimonious trees (MPTs) were generated using an heuristic search algorithm employing the tree-bisection-reconnection (TBR) as branch-swapping algorithm, with the steepest descent option not in effect and the 'MulTrees' option in effect. Ten stepwise random additions of taxa were used and nodes were collapsed when minimum branch length was zero. Nodal support was evaluated via nonparametric bootstrap analysis of 1000 replications with 10 random addition-sequence replicates per bootstrap replicate.

3.15.3 Maximum Likelihood (ML) Tree

ML tree was constructed using PhyML 3.0 (Guindon and Gascuel, 2003; Guindon et al., 2005). The data set was bootstrapped for 1000 replications using the parameter calculated by jModeltest: 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 starting tree was obtained using the BioNJ algorithm (Gascuel, 1997) and tree topologies were estimated by Nearest Neighbour Interchange (NNI; Jarvis et al., 1983) branch swapping arrangements. To further test if NNI could produce the best topology, Subtree Pruning and Regrafting (SPR; Hordijk and Gascuel, 2005) and a combination of NNI + SPR were performed. The program was set to optimize topology and branch length.

3.15.4 Bayesian Inference (BI)

The BI analysis was performed with substitution model parameters set to lset nst = 6 rates = gamma and all priors were left at default to allow estimation of the parameters from the data. Each BI anlaysis 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 between both simultaneous runs was less than 0.01, 25% of the samples (as recommended by Ronquist et al., 2005) 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. The mean, variance, and 95% credibility intervals were calculated from the set of substitution parameters.

3.16 Pairwise Genetic Distance and Time of Divergence

The pairwise genetic distances within and among the four *Acetes* species were calculated based on Kimura's Two Parameter's (K2P) substitution model. In this study, the range of divergence time in *Acetes* was estimated based on the minimum and maximum *COI* divergence rates that had been reported for decapod crustaceans on K2P distances: 1.40–3.00% per million years (Baldwin et al., 1998; Knowlton et al., 1993; Knowlton and Weigt, 1998; Schubart et al., 1998).

3.17 Intraspecific Analyses

3.17.1 DNA Polymorphisms

DNA polymorphisms from *COI* data were computed using DnaSP v5 according to the following features: segregating sites (*S*), the number of haplotypes (N_{hap}), haplotype (gene) diversity (*h*; Nei, 1987), and nucleotide diversity, Pi (π; Nei, 1987).

3.17.2 Haplotype Network

Haplotype network was constructed using the TCS 1.13 software (Clement et al., 2000) which employs a 95% statistical parsimony method (Templeton et al., 1992). The input data consisted of individual *COI* sequence. This program collapsed the sequences into haplotypes and produced a network linking unique haplotypes. It provided a 95% plausible branch connection between unique haplotypes, *i.e.*, it calculated the number of mutational steps by which pairwise haplotypes differ and computed the probability of parsimony for pairwise differences until the probability exceeded 0.95.

3.17.3 Analysis of Molecular Variance (AMOVA) and Pairwise Φ_{ST}

The population structure in each species was examined using AMOVA (Excoffier et al., 1992) in Arlequin v3.5 (Excoffier and Lischer, 2010). AMOVA was used to partition the total genetic variation into its variance components and to produce Φ-statistics (Φ_{ST}; Weir and Cockerham, 1984). Pairwise differences (pairwise Φ_{ST}) between pairs of populations were estimated to examine whether any two populations were genetically different

from each other. The significance levels of the results of AMOVA and pairwise Φ_{ST} were tested by 10,000 permutations.

3.17.4 Mantel Test

When the overall AMOVA was statistically significant, a Mantel test (Mantel, 1967) was performed in XLSTAT v. 2010. 3. 06 (Addinsoft^{TM}, New York, USA) to determine whether there was a relationship between genetic distance and geographical distance. Genetic distances (*i.e.* Φ_{ST} values based on TrN) were calculated from each pairwise comparison between sampling locations. Geographical distances were estimated as great circle distance between each pair of sampling locations. Statistical significance of Mantel test was determined by 10,000.

3.17.5 Neutrality Tests

Tajima's *D* (Tajima, 1989), Fu's F_s (Fu, 1997), and the R_2 (Ramos-Onsins and Rozas, 2002) statistic were generated using DnaSP v5. The significance of these tests was tested statistically using 10,000 coalescent simulations (Hudson, 1990) as implemented in DnaSP v5.

3.17.6 Mismatch Distribution Analysis

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: θ_0, θ_1 (before and after the population growth) and τ (time since expansion expressed in units of mutational time) (Rogers, 1995; Rogers and Harpending, 1992) were estimated using the

generalized nonlinear least-squares approach (Schneider and Excoffier, 1999), and their respective 95% confidence intervals (CI) were obtained by parametric bootstrapping (10000 permutations). The fit between the observed and expected distributions under population growth was evaluated by the sum of square deviations (*SSD*; Schneider and Excoffier, 1999) and Harpending's raggedness index (*r*; Harpending, 1994) with 10,000 bootstrap replicates.

If a unimodal distribution was observed, estimation of the time (*t*) since population expansion could be calculated using the formula: $\tau = 2ut$ (Rogers and Harpending, 1992), where τ is the mode of the mismatch distribution, expressed in units of mutational time, u is the mutation rate of the sequence under study (such as $u = 2\mu k$, where μ is the mutation rate per nucleotide and k is the number of nucleotides of the analyzed fragment) and t is the expansion time measured in *generations*. From the estimated average pairwise sequence divergence rate of *COI* reported for decapod crustaceans (1.40–3.00%; Baldwin et al., 1998; Knowlton et al., 1993; Knowlton and Weigt, 1998; Schubart et al., 1998), these rates (*i.e.,* the estimated single-lineage value for μ was $0.70 \times 10^{-8} - 1.50 \times 10^{-8}$) were used in this study. A generation time of 0.5 year was assumed for all analyses, based on the studies of Ikematsu (1953), Lei (1984) and Yasuda *et al.,* (1953).

CHAPTER 4.0

RESULTS

4.1 Sexes and Species Identification of *Acetes*

The bodies of *Acetes* individuals were translucent or semi-translucent, with black eyes and several pairs of red pigment spots on the base of the uropod with additional one(s) on the endopods of the uropods (Figure 4.1). The specimens were easily identified as *Acetes indicus*, *A. serrulatus*, *A. japonicus* and *A. sibogae*, based on the key characters described by Omori (1975b).

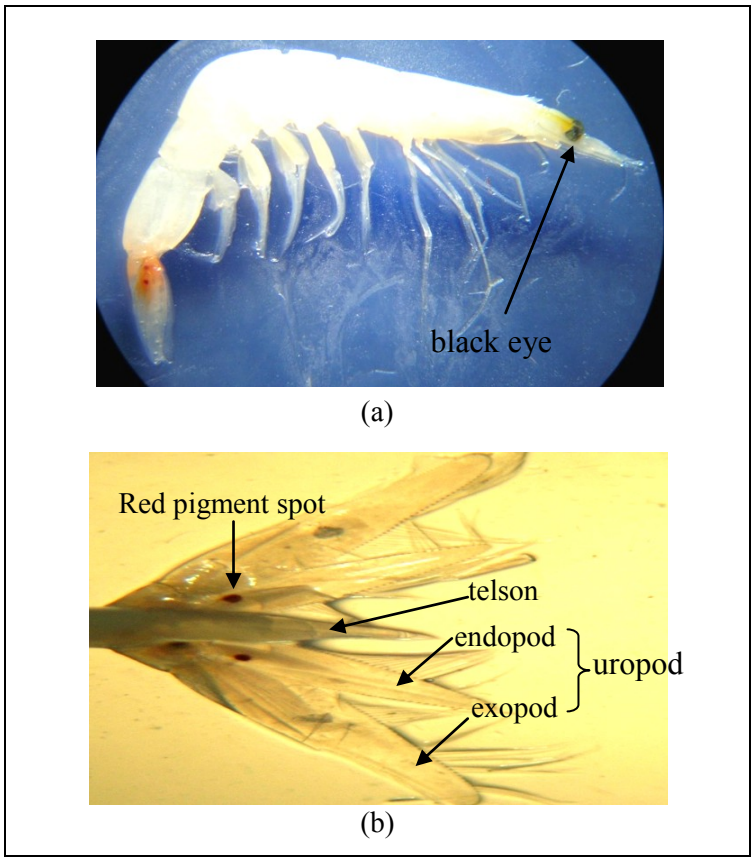


Figure 4.1: The body of *Acetes* under the Leica dissecting microscope (Leica ZOOM 2000™, Model No. Z45V). (a) Semi-translucent body with black eyes (magnification x5) (b) red pigment spots on the basic of uropod and on the endopods of the uropods (magnification x10).

4.1.1 *Acetes indicus* (Figure 4.2a)

In both males and females of *Acetes indicus*, the apex of the telson was triangular (Figure 4.2b) and the rostrum possessed two denticles behind the terminal point (Figure 4.2c). A procurved tooth was found between the bases of the first pair of pleopods in both males and females (Figure 4.2d). The petasma was without pars astringens (Figure 4.2e) and the lower antennular flagellum with one clasping spine (Figure 4.2f) was only present in males. In females, the inner margin of the basis of the third pereiopod had a sharply pointed projection, and, the third and fourth thoracic sternites were deeply channelled longitudinally (Figure 4.2g).

4.1.2 *Acets serrulatus* (Figure 4.3a)

The rostrum of *A. serrulatus* was had two denticles behind the terminal point, and the apex of the telson was truncated and bore on either corner a small tooth in both females and males (Figure 4.3b). In males, two clasping spines and a triangular projection from the upper end of the first segment of the main branch of the lower antennular flagellum were observed (Figure 4.3c). Besides, the petasma did not possess a pars astringen, or a precessus ventralis and the capitulum of the petasma was without ventral projection; and had one large hook at the end (Figure 4.3d). The third thoracic sternite was not produced posteriorly in females. The tooth present on the distal inner margin of the coxa of the third pereiopod, and the anterior margin of the fourth thoracic sternite was smooth and convex (Figure 4.3e).

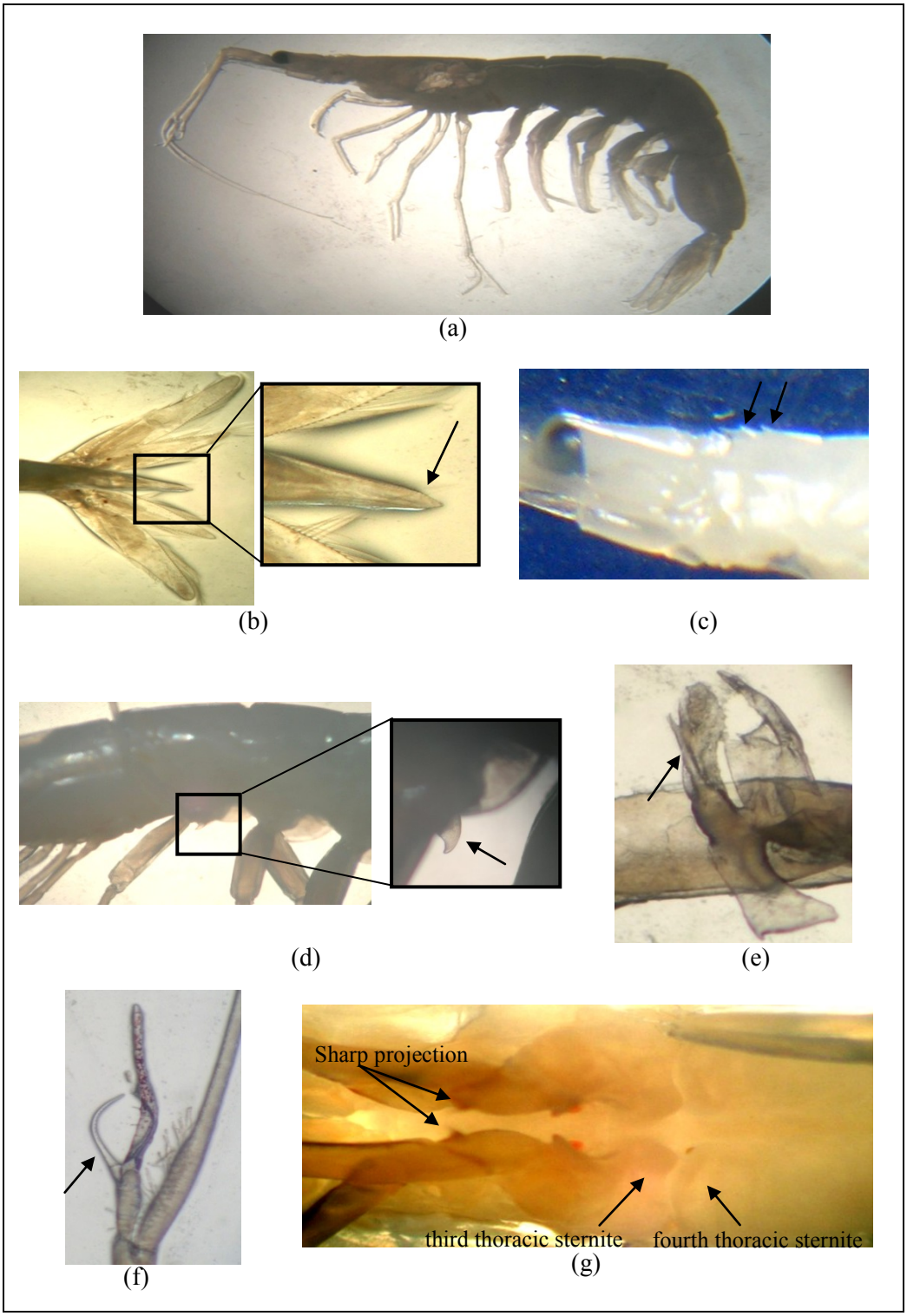


Figure 4.2: Morphological characters in each sex and species identification of *Acetes indicus* (a) body of *Acetes indicus* (male) (magnification x5) (b) apex of telson (male) (magnification x10, x50) (c) two denticles behind terminal point of the rostrum (female) (magnification x25) (d) procurve tooth (male) (magnification x25, x50) (e) petasma without pars astringens (male) (magnification x100) (f) lower antennular flagellum (male) (magnification x50) (g) sharp projection of basis of third pereiopod and, third and fourth thoracic sternites deeply channeled longitudinally (magnification x100).

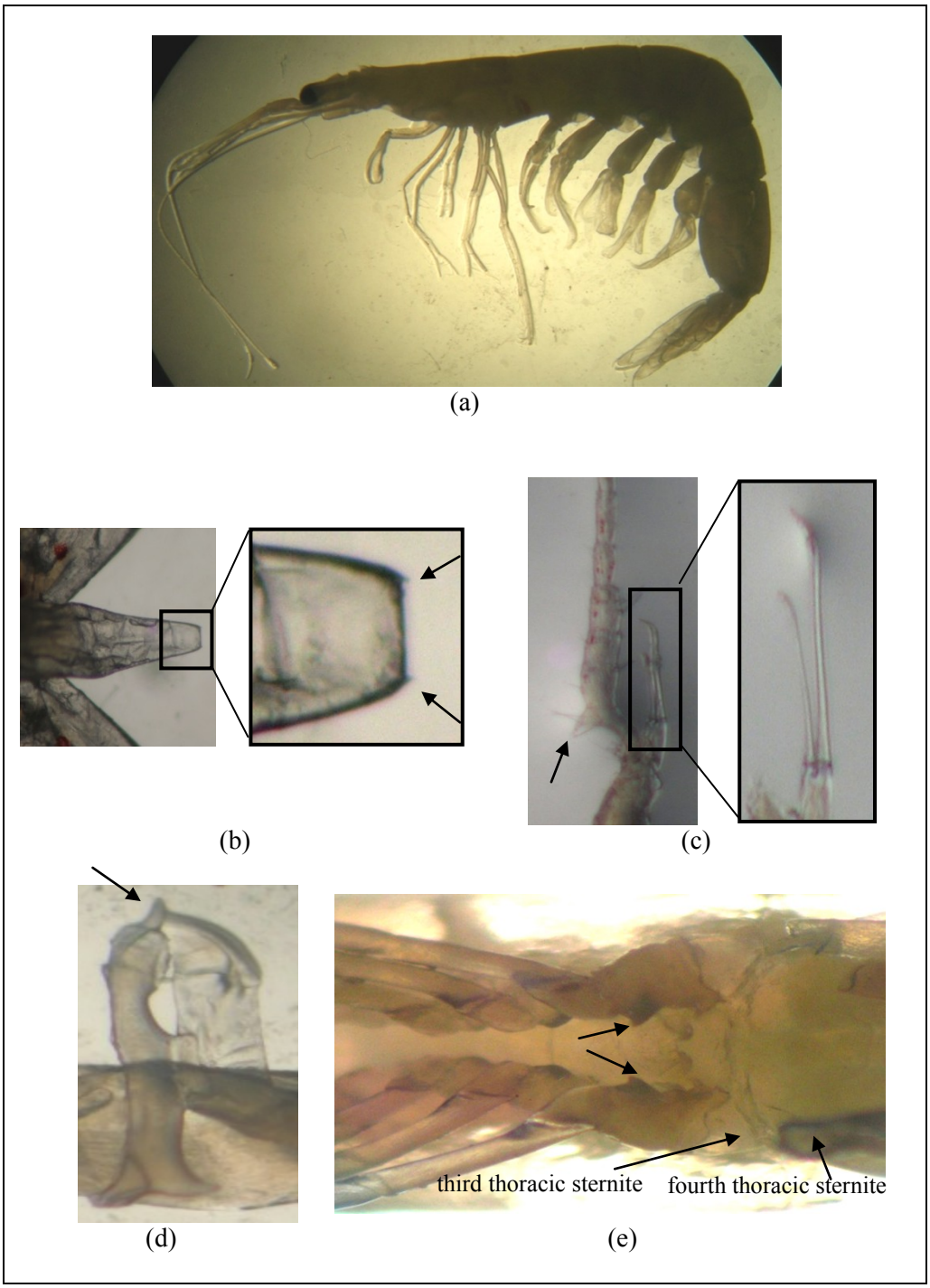


Figure 4.3: Morphological characters in each sex and species identification of *Acetes serrulatus* (a) body of *Acetes serrulatus* (male) (magnification x5) (b) apex of the telson (female) (magnification x10, x50) (c) lower antennular flagellum with triangular projection and two clasping spines (male) (magnification x50, x100) (d) petasma without pars astringen, without precessus ventralis and the capitulum of petasma without ventral projection; with one large hook at the end (male) (magnification x100) (e) third thoracic sternite not produced posteriorly, tooth present on distal inner margin of coxa of third pereiopod, and anterior margin of fourth thoracic sternite smooth and convex (female) (magnification x100).

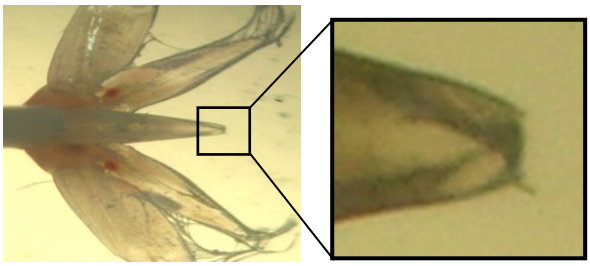
4.1.3 *Acetes japonicus* (Figure 4.4a)

Both males and females of *A. japonicus* possessed a rostrum with two denticles behind the terminal point and the apex of the telson was truncated (Figure 4.4b). In males, the first segment of the main branch of the lower antennular flagellum was without a triangular projection, and the lower antennular flagellum had two clasping spines (Figure 4.4c). In addition, the petasma did not possess a pars astringen, the distal expanded part of the capitulum of the petasma was bulb-like with numerous hooks (Figure 4.4d). In females, the third thoracic sternite was produced posteriorly and the emargination of the posterior margin of the third thoracic sternite was shallow (Figure 4.4e).

4.1.4 *Acetes sibogae* (Figure 4.5a)

Both males and females of *A. sibogae* possessed a rostrum with two denticles behind the terminal point, the apex of the telson was triangular (Figure 4.5b) and a procurved tooth was absent. In males, the lower antennular flagellum had 12 segments or less, and the first segment of the main branch of the lower antennular flagellum had a small swelling; and the clasping spine extended beyond the end of the second segment of the main branch (Figure 4.5c). The anterior margin of the genital coxae was pointed (Figure 4.5d). Furthermore, the petasma was with pars astringens, and the capitulum of the petasma had one large hook and often one small hook along the outer margin (Figure 4.5e). In females, the distal inner margin of the basis of the third pereiopod ended in a projection and a pair of small protuberances were observed on the anterior part of the third thoracic sternite (Figure 4.5f).

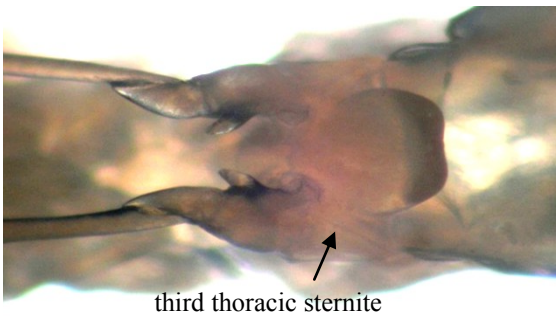
(a)



(b)

(c)

(d)



(e)

Figure 4.4: Morphological characters in each sex and species identification of *Acetes japonicus* (a) body of *Acetes japonicus* (male) (magnification x10) (b) the apex of telson (magnification x10, x100) (c) lower antennular flagellum (male) (magnification x100) (d) petasma (male) (magnification x100) (e) third thoracic sternite produced posteriorly and emargination of posterior margin of third thoracic sternite shallow (female) (magnification x100).

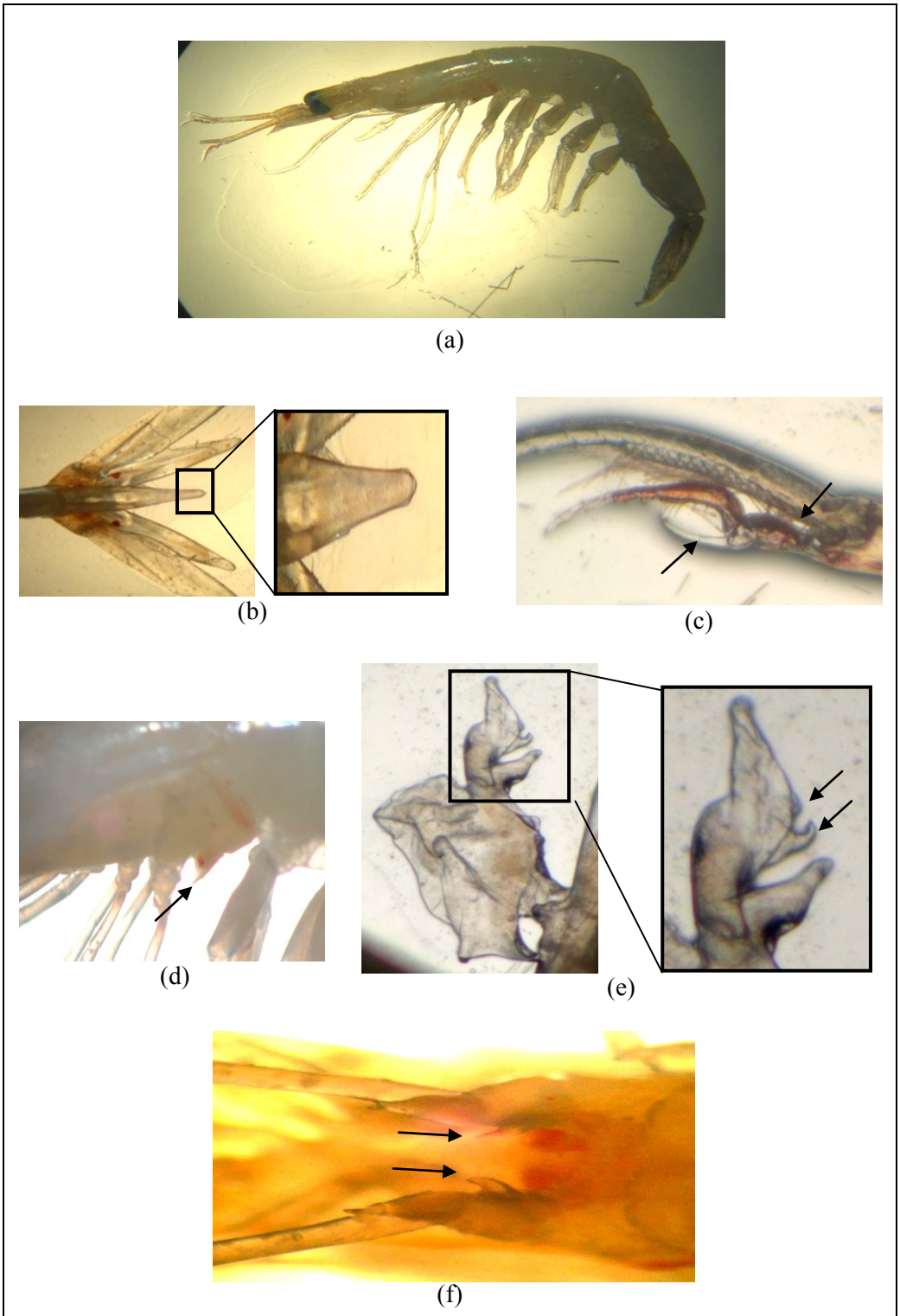


Figure 4.5: Morphological characters in each sex and species identification of *Acetes sibogae* (a) body of *Acetes sibogae* (male) (magnification x5) (b) apex of telson (magnification x10, x50) (c) lower antennular flagellum (male) (magnification x50) (d) Anterior margin of genital coxa (magnification x50) (e) petasma (male) (magnification x100, x200) (f) Distal inner margin of basis of third pereiopod ending in projection; a pair of small protuberances on anterior part of third thoracic sternite (female) (magnification x100).

4.2 Distribution

Distributions of the four *Acetes* species collected along the west coast of Peninsular Malaysia in this study are shown in Table 4.1. *Acetes japonicus* and *A. sibogae* were collected from the sampling locations of TBHG, KK, KG, KS and SGKB, KS, respectively, which were in contrast to the distribution of *A. indicus* and *A. serrulatus*.

4.3 Morphometric Analysis

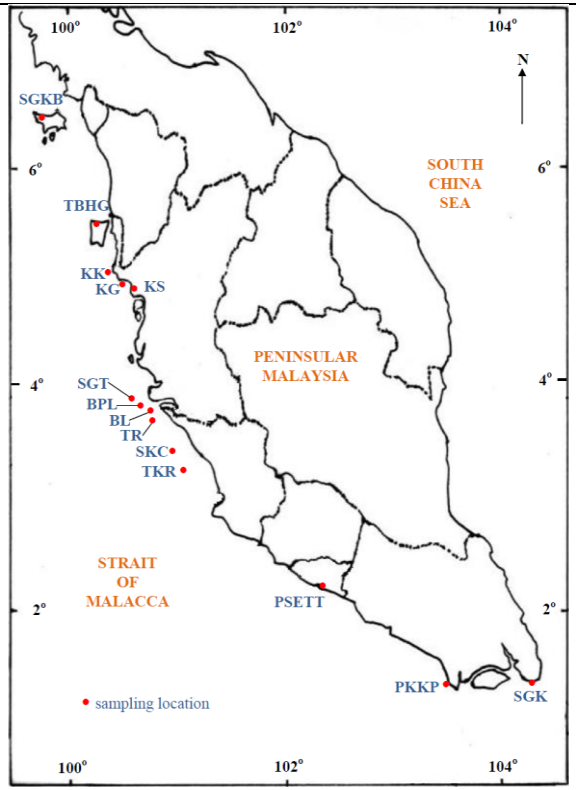
A total of 1112 *Acetes indicus* individuals (360 females, 264 males), *A. serrulatus* (194 females, 187 males), *A. japonicus* (49 females, 25 males) and *A. sibogae* (10 females, 43 males) were sampled and measured, between August of 2007 and October of 2008.

4.3.1 Population Structure

The total length (TL) range, mean total length (± S. D.), carapace length (CL) range, mean carapace length (± S. D.), wet weight (WW) range, and mean wet weight (± S. D.) of females and males of each species, are presented in Table 4.2. For each of the *Acetes* species, the mean of TL, CL and WW were significantly larger in females than in males (Mann-Whitney *U*-test; Table 4.2).

The size-frequency distributions for each measurement are illustrated separately for females and males of the four *Acetes* species in Figure 4.6. Females were dominant in the larger size classes, while males were more numerous at smaller size classes for each measurement.

Table 4.1: The distribution of four *Acetes* species sampled along the west coast of Peninsular Malaysia in this study.

Sampling location[a]		In-shore / Off-shore region	Species[b]			
			ai	*as*	*aj*	*asi*
	SGKB	In-shore				√ (53)
	TBHG	In-shore			√ (52)	
	KK	In-shore	√ (24)		√ (11)	
	KG	In-shore	√ (42)		√ (11)	
	KS	In-shore			√ (3)	√ (6)
	SGT	Off-shore	√ (135)	√ (65)		
	BPL	Off-shore	√ (115)	√ (85)		
	BL	Off-shore	√ (48)	√ (52)		
	TR	Off-shore	√ (14)	√ (36)		
	SKC	Off-shore	√ (61)	√ (39)		
	TKR	Off-shore	√ (65)	√ (35)		
	PSETT	In-shore	√ (40)			
	PKKP	In-shore	√ (45)	√ (29)		
	SGK	In-shore	√ (15)	√ (40)		

[a]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 Kubang Badak.
[b]*ai*: *Acetes indicus*; *as*: *A. serrulatus*; *aj*: *A. japonicus*; *asi*: *A. sibogae*. (The number of individual collected was given in parentheses).

Table 4.2: Total length (TL), carapace length (CL) and wet weight (WW) for males and females of *Acetes indicus*, *A. serrulatus*, *A. japonicus*, and *A. sibogae.*

Species	Sex	n	TL (mm)		Mann-Whitney *U* Test	*P*	CL (mm)		Mann-Whitney *U* Test	*P*	WW (mg)		Mann-Whitney *U* Test	*P*
			range	Mean ± S. D			range	Mean ± S. D			range	Mean ± S. D		
A.indicus	F	340	16.71 – 38.94	24.82 ± 0.24	14316.000	0.000	3.63 – 7.88	5.57 ± 0.77	7062.000	0.000	18.00 – 287.30	69.10 ± 41.72	13274.500	0.000
	M	264	15.07 – 29.52	19.88 ± 0.17			3.54 – 5.84	4.35 ± 0.42			14.90 – 110.40	34.10 ± 15.15		
A.serrulatus	F	194	15.28 – 26.55	20.99 ± 2.42	10661.000	0.000	3.46 – 5.74	4.63 ± 0.44	8432.000	0.000	14.20 – 70.00	39.83 ± 12.75	9795.500	0.000
	M	187	14.21 – 25.87	19.22 ±2.18			3.20 – 5.22	4.20 ± 0.37			12.10 – 49.90	29.65 ± 9.28		
A. japonicus	F	49	15.25 – 22.00	18.76 ± 1.79	136.500	0.000	2.71 – 4.28	3.52 ± 0.38	66.000	0.000	11.60 – 33.00	22.10 ± 5.44	100.500	0.000
	M	25	14.25 – 18.59	16.04 ± 0.96			2.40 – 3.28	2.78 ± 0.25			8.50 – 20.60	13.11 ±3.16		
A.sibogae	F	10	19.29 – 23.04	21.65 ± 1.27	67.500	0.001	3.50 – 4.55	4.20 ± 0.33	57.000	0.000	22.40 – 40.00	33.51 ± 6.59	61.500	0.000
	M	43	18.17 – 21.93	19.95 ± 1.12			3.19 – 4.13	3.69 ± 0.28			17.00 – 33.10	24.27 ± 4.56		

sex: F, female; M, male.
n: sample size
P: Significance value of Mann-Whitney *U* test

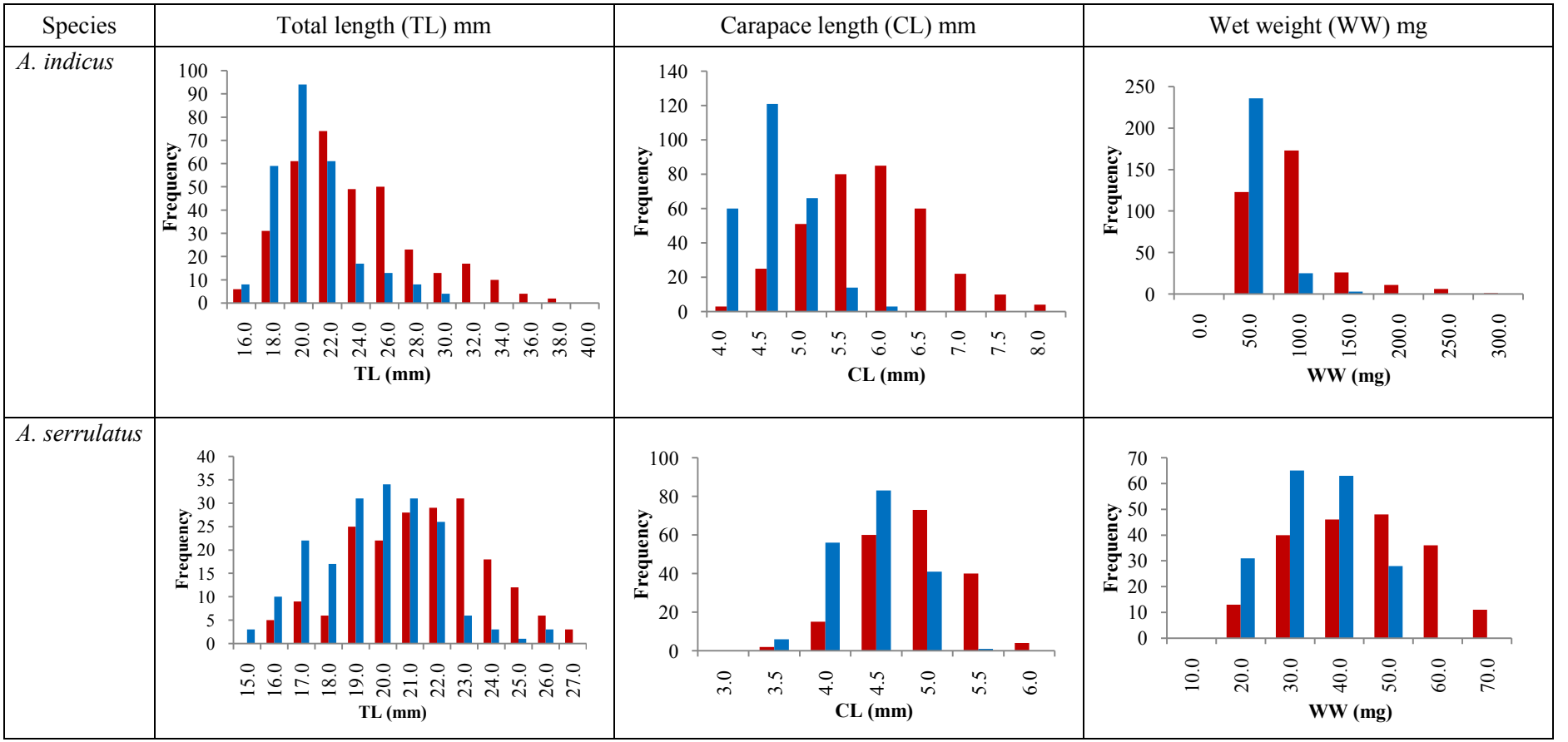


Figure 4.6: Size-frequency distribution of females and males of *Acetes indicus*, *A. serrulatus*, *A. japonicus* and *A. sibogae* sampled along the west coast of Peninsular Malaysia.

■ female ■ male

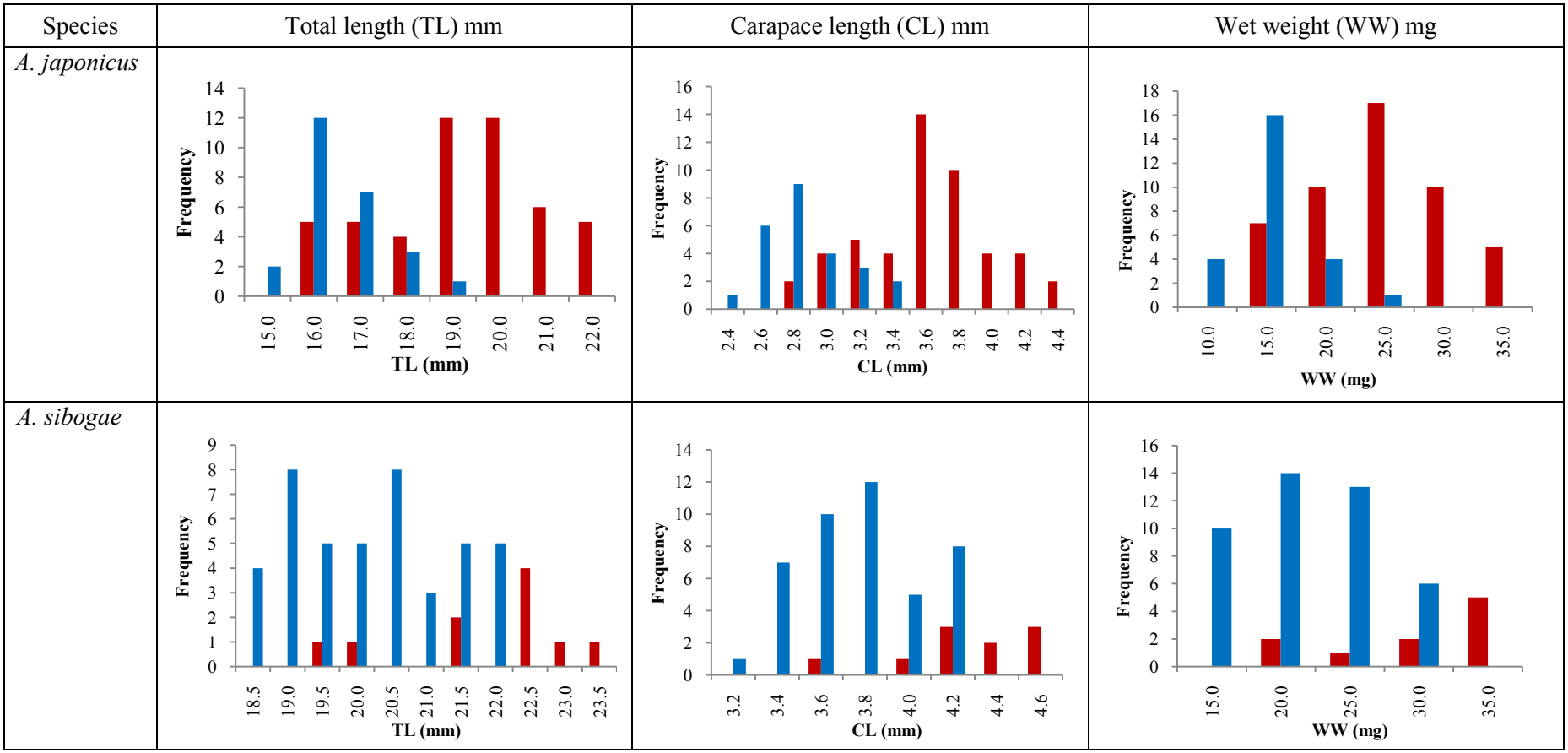


Figure 4.6 (continued): Size-frequency distribution of females and males of *Acetes indicus*, *A. serrulatus*, *A. japonicus* and *A. sibogae* sampled along the west coast of Peninsular Malaysia.

■ female ■ male

4.3.2 Sex Ratio

The overall sex ratio (males : females) for *A. indicus*, *A. serrulatus*, *A. japonicus* and *A. sibogae* in this study was found to be 1:1.29, 1:1.04, 1:1.96 and 1:0.23, respectively. Chi-square tests revealed that the total number of females was significantly greater than that of males for *A. indicus* (χ^2 = 9.563, df = 1, P = 0.002) and *A. japonicus* (χ^2 = 7.784, df = 1, P = 0.005) samples across the sampling period. This was in contrast to *A. sibogae* samples in which the total number of males was significantly higher than females (χ^2 = 20.547, df = 1, P = 0.000006). However, there was no significant difference between the total number of females and males (χ^2 = 0.129, df = 1, P = 0.720) for *A. serrulatus.*

The sex ratio (number of females/ total number of females and males) of *A. indicus*, *A. serrulatus*, *A. japonicus* and *A. sibogae* was plotted for the TL (mm), CL (mm), and WW (mg) in Figure 4.7. For *A. indicus*, the sex ratio by size class showed that the females outnumbered males at TL, CL, and WW greater than 24.00 mm, 5.50 mm, and 100.00 mg, respectively. The females of *A. serrulatus* outnumbered males at TL, CL, and WW greater than 22.00 mm, 5.00 mm and 45.00 mg, respectively. In *A. japonicus* and *A, sibgoae*, measurements of TL, CL, and WW also showed that females were larger than males, as in *A. indicus* and *A. serrulatus.* The females of *A. japonicus* and *A. sibogae* outnumbered males at TL, CL, and WW greater than 18.00 mm, 3.20 mm, 20.00 mg and 22,50 mm, 4.40 mm, 40.00 mg, respectively.

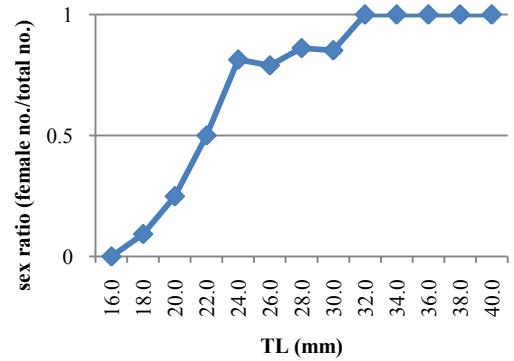
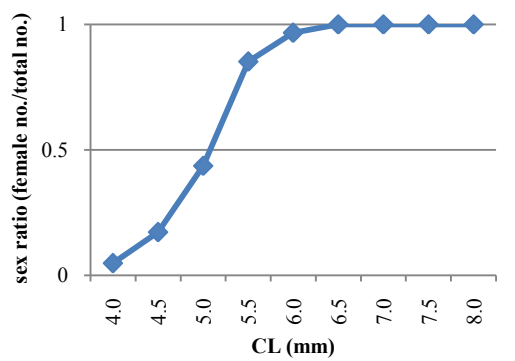
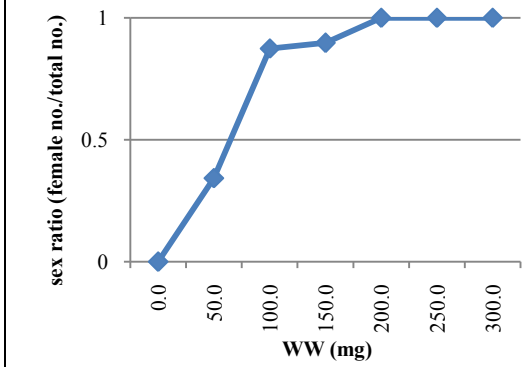
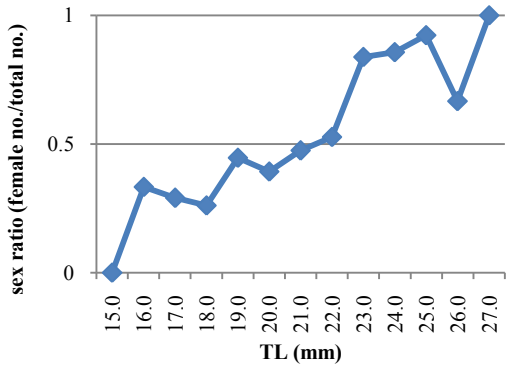
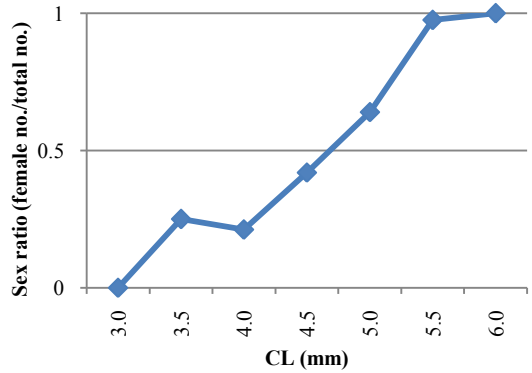
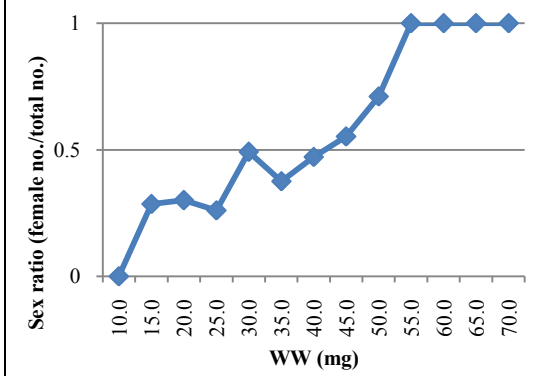
Species	Total length (TL) mm	Carapace length (CL) mm	Wet weight (WW) mg
A. indicus			
A. serrulatus			

Figure 4.7: Sex ratio (female no. / total no.) of *Acetes indicus*, *A. serrulatus*, *A. japonicus* and *A. sibogae* plotted for the total length (TL, mm), carapace length (CL, mm) and wet weight (WW, mg). The dotted-line indicates a ratio of 1:1 (females : males).

sex ratio (female no./total.no.)

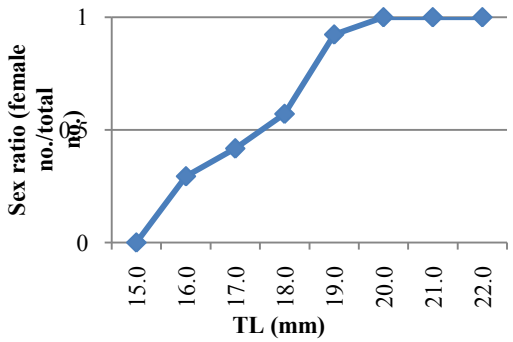
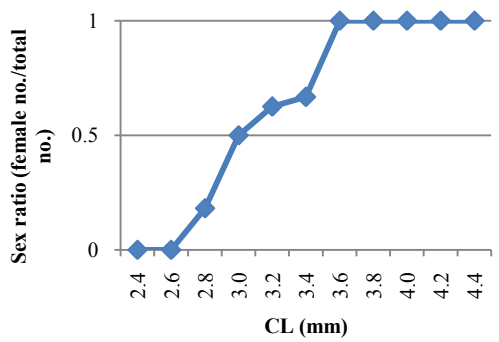
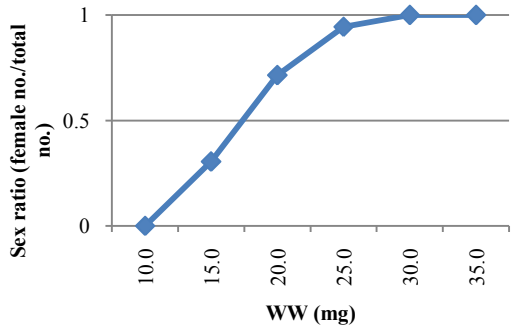
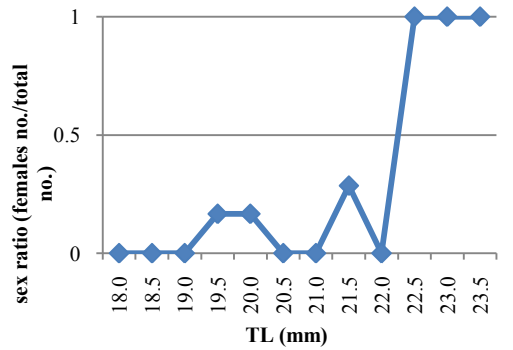
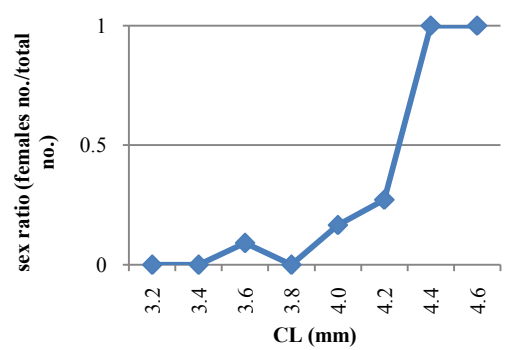
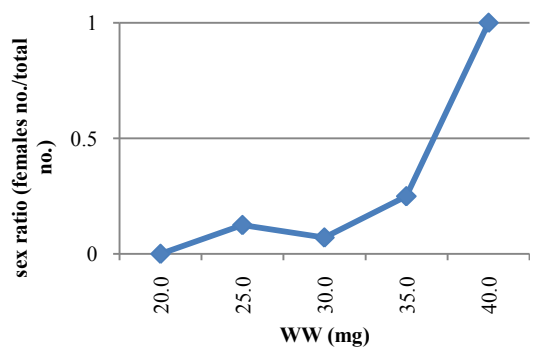
Species	Total length (TL) mm	Carapace length (CL) mm	Wet weight (WW) mg
A. japonicus			
A. sibogae			

Figure 4.7 (continued): Sex ratio (female no. / total no.) of *Acetes indicus*, *A. serrulatus*, *A. japonicus* and *A. sibogae* plotted for the total length (TL, mm), carapace length (CL, mm) and wet weight (WW, mg). The dotted-line indicates a ratio of 1:1 (females : males).

sex ratio (female no./total.no.)

4.3.3 In-shore and Off-shore Samples Comparison

The sampling locations for *A. indicus* and *A. serrulatus* were grouped as in-shore and off-shore. For females, males, and both sexes combined of *A. indicus*, the means of TL, CL and WW for in-shore samples were significantly ($P < 0.001$) smaller than off-shore samples. This was in contrast to the females, males, and both sexes combined for the *A. serrulatus* samples, in which case the means of TL and WW for in-shore sample were significantly larger than off-shore samples (Mann-Whitney *U*-test; Table 4.3). However, the mean of CL for *A. serrulatus* was larger than for in-shore samples, but was not significant ($P = 0.187$).

The size-frequency distributions showed that females, males, and both sexes combined for in-shore samples of *A. indicus* were dominant in the smaller size class, while offshore samples were dominant in the larger size-class for TL, CL, and WW (Figure 4.8). Females, males, and both sexes combined for off-shore samples of *A. serrulatus* were dominant in the larger size classes, while in-shore samples were numerous in the smaller size classes (except for CL).

Table 4.3: Results of Mann-Whitney *U*-test for the in-shore and off-shore samples of *Acetes indicus* and *A. serrulatus*. Test was conducted separately for females (F), males (M), and combined sexes (B). TL: total length; CL: carapace length; WW: wet weight.

Species	Sex	Region	n	TL (mm)		Mann-Whitney *U*-test	*P*	CL (mm)		Mann-Whitney *U*-test	*P*	WW (mg)		Mann-Whitney *U*-test	*P*
				range	Mean ± S. D			range	Mean ± S. D			range	Mean ± S. D		
Acetes indicus	F	In-shore	241	16.71 – 29.20	22.92 ± 2.60	3051.000	0.000	3.63 – 6.78	5.34 ± 0.67	5233.000	0.000	18.30 – 93.00	53.02 ±17.47	3618.000	0.000
		Off-shore	99	17.38 – 38.94	29.44 ± 4.78			4.80 – 7.88	6.12 ± 0.71			26.40 – 287.30	108.25 ± 55.60		
	M	In-shore	197	15.07 – 23.26	19.10 ± 1.68	3291.500	0.000	3.54 – 5.07	4.27 ± 0.33	4286.000	0.000	14.90 – 52.20	30.12 ± 7.92	3987.500	0.000
		Off-shore	67	15.32 – 29.52	22.20 ± 3.88			3.69 – 5.84	4.60 ± 0.54			15.00 – 110.40	45.81± 23.28		
	B	In-shore	436	15.07 – 29.20	21.20 ± 2.93	1590.000	0.000	3.54 – 6.78	4.86 ± 0.76	22464.000	0.000	14.90 – 93.00	42.72 ± 18.05	18343.000	0.000
		Off-shore	166	15.32 – 38.94	26.51 ± 5.68			3.69 – 7.88	5.51 ± 0.99			15.00 – 287.30	83.05 ± 54.74		
Acetes serrulatus	F	In-shore	39	18.81 – 26.55	23.89 ± 1.71	423.000	0.000	3.46 – 5.04	4.50 ± 0.38	2430.500	0.059	18.90 – 60.20	45.53 ± 10.03	1992.000	0.001
		Off-shore	155	15.28 – 24.07	20.25 ± 1.99			3.62 – 5.74	4.66 ± 0.45			14.20 – 70.00	38.39 ± 12.98		
	M	In-shore	30	19.52 – 25.87	22.06 ± 1.66	333.000	0.000	3.66 – 4.76	4.13 ± 0.30	2019.500	0.217	26.90 – 49.60	36.85 ± 6.82	1102.500	0.000
		Off-shore	157	14.21 – 22.21	18.68 ± 1.82			3.20 – 5.20	4.22 ± 0.38			12.10 – 49.90	28.28 ± 9.07		
	B	In-shore	69	18.81 – 26.55	23.10 ± 1.91	2179.500	0.000	3.46 – 5.04	4.34 ± 0.39	9670.500	0.187	18.90 – 60.20	41.76 ± 9.75	6172.000	0.000
		Off-shore	312	14.21 – 24.07	19.46 ± 2.06			3.20 – 5.74	4.44 ± 0.47			12.10 – 70.00	33.30 ± 12.26		

sex: F, female; M, male.
n: sample size
P: Significance value of Mann-Whitney *U*-test

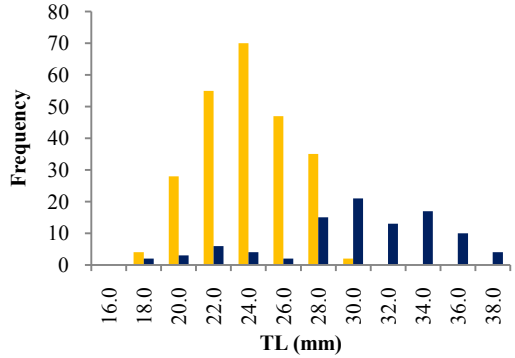
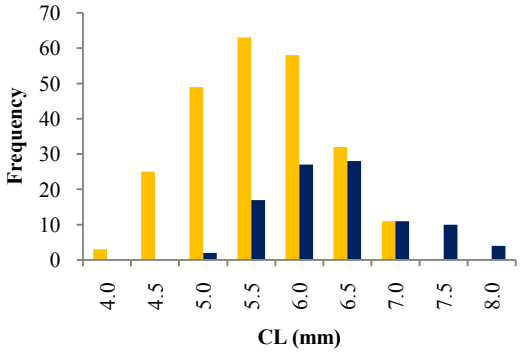
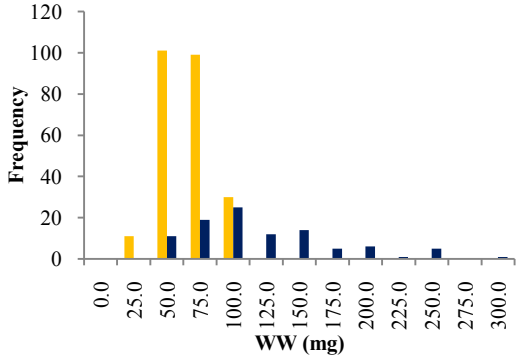
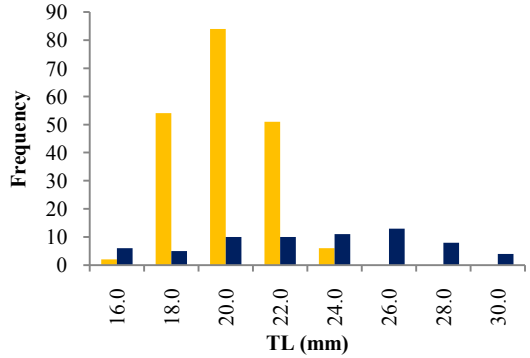
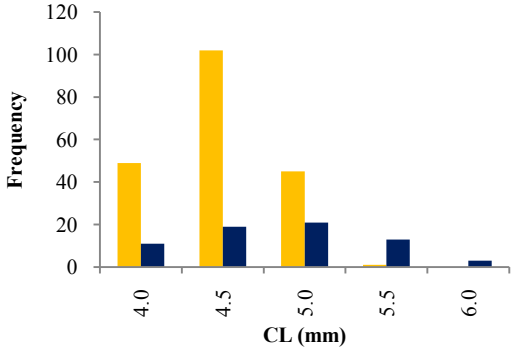
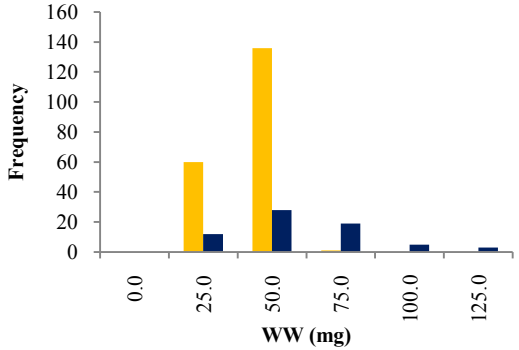
Species	Total length (TL) mm	Carapace length (CL) mm	Wet weight (WW) mg
A. indicus (females)			
A. indicus (males)			

Figure 4.8: Size-frequency distributions of females, males, and both sexes combined for the in-shore and off-shore samples of *Acetes indicus* and *A. serrulatus*, collected from the west coast of Peninsular Malaysia.

in-shore off-shore

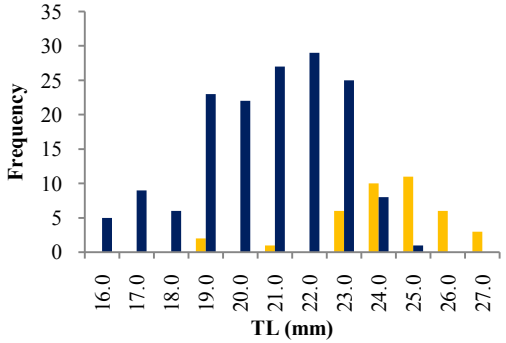
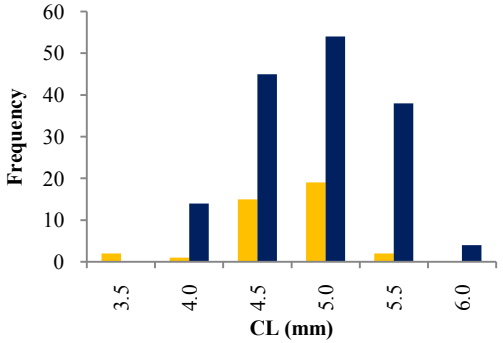
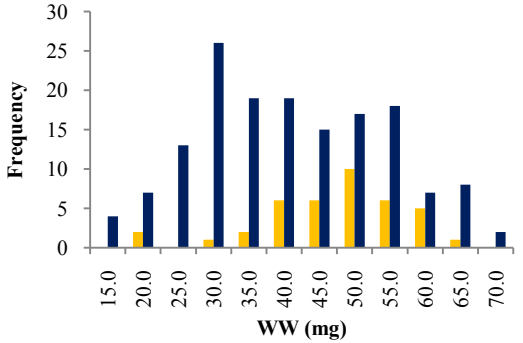
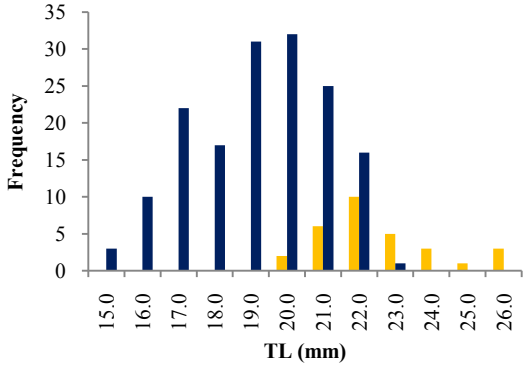
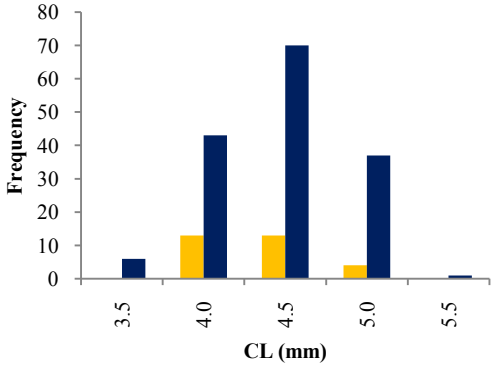
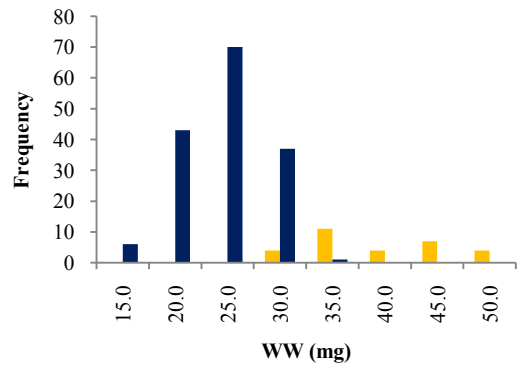
Species	Total length (TL) mm	Carapace length (CL) mm	Wet weight (WW) mg
A. serrulatus (females)			
A. serrulatus (males)			

Figure 4.8 (continued): Size-frequency distributions of females, males, and both sexes combined for the in-shore and off-shore samples of *Acetes indicus* and *A. serrulatus*, collected from the west coast of Peninsular Malaysia.
■ in-shore ■ off-shore

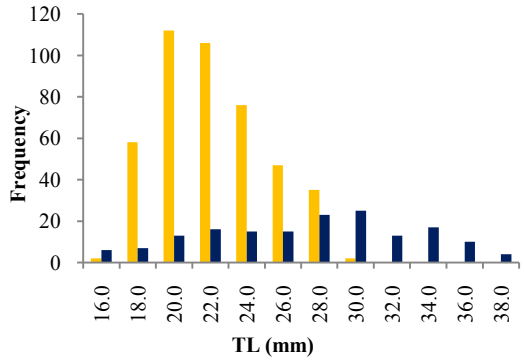
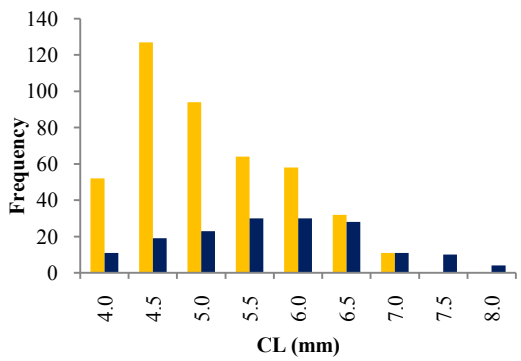
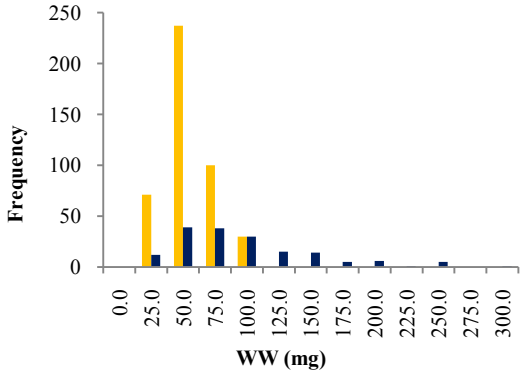
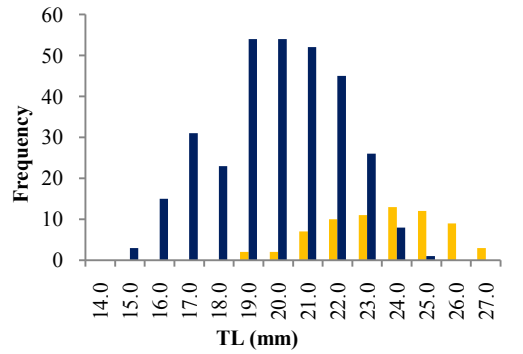
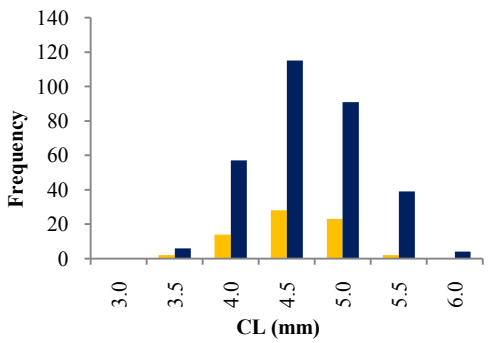
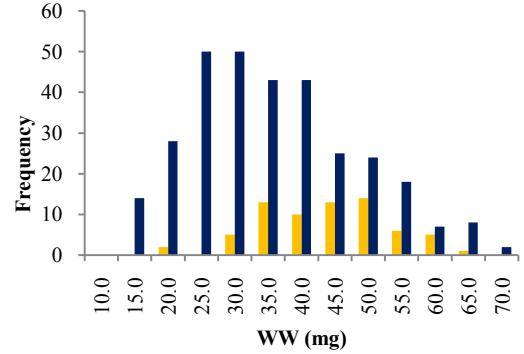
Species	Total length (TL) mm	Carapace length (CL) mm	Wet weight (WW) mg
A. indicus			
A. serrulatus			

Figure 4.8 (continued) Size-frequency distributions of females, males, and both sexes combined for the in-shore and off-shore samples of *Acetes indicus* and *A. serrulatus*, collected from the west coast of Peninsular Malaysia.
■ in-shore ■ off-shore

4.3.4 Comparison among Species

Since the assumption of normality was violated, a Kruskal-Wallis *H* test was used to test for significant differences among the four *Acetes* species for TL, CL, and WW morphometric measurements of. Significant differences ($P < 0.001$) were detected in the mean of the measurements among species (Table 4.4).

Among the four species, *A. indicus* showed the widest range for all three measurements and *A. serrulatus* has the second widest range of distribution (Figure 4.9). Both *A. japonicus* and *A. sibogae* showed narrower size ranges, and were smaller, compared with *A. indicus* and *A. serrulatus*, for all measurements, respectively (Figure 4.9).

Following the Kruskal-Wallis tests, pairwise comparison of each measurement using the Mann-Whitney *U*-tests revealed that significant differences existed between *Acetes* species, except for a few cases (Table 4.5). For all measurements (TL, CL, and WW), no significant difference were detected for the comparison between female samples of *A. serrulatus* and *A. sibogae* ($P = 0.320$, $P = 0.003$, and $P = 0.100$ respectively), and male samples of *A. indicus* and *A. serrulatus* ($P = 0.092$, $P = 0.001$, and $P = 0.019$ respectively). The comparison of the means of TL between *A. indicus* and *A. sibogae* was not significantly different for both female ($P = 0.012$) and male samples ($P = 0.186$). Also, comparison of the means of TL between the male samples of *A. serrulatus* and *A. sibogae* was not significantly different ($P = 0.022$).

Table 4.4: Results of Kruskal-Wallis *H*-test for the comparison among *Acetes indicus, A. serrulatus*, *A. japonicus*, *A. siboage.* The test was conducted for females (F), males (M), and combined sexes (B), separately. TL: total length; CL: carapace length; WW: wet weight.

Species	Sex	n	TL (mm)		Kruskal-Wallis *H*-Test (χ^2)	*P*	CL (mm)		Kruskal-Wallis *H*-Test (χ^2)	*P*	WW (mg)		Kruskal-Wallis *H* – Test (χ^2)	*P*
			range	Mean ± S. D			range	Mean ± S. D			range	Mean ± S. D		
A.indicus	F	340	16.71 – 38.94	24.82 ± 0.24	170.052	0.000	3.63 – 7.88	5.57 ± 0.77	299.384	0.000	18.00 – 287.30	69.10 ± 41.72	213.922	0.000
A.serrulatus	F	194	15.28 – 26.55	20.99 ± 2.42			3.46 – 5.74	4.63 ± 0.44			14.20 – 70.00	39.83 ± 12.75		
A. japonicus	F	49	15.25 – 22.00	18.76 ± 1.79			2.71 – 4.28	3.52 ± 0.38			11.60 – 33.00	22.10 ± 5.44		
A.sibogae	F	10	19.29 – 23.04	21.65 ± 1.27			3.50 – 4.55	4.20 ± 0.33			22.40 – 40.00	33.51 ± 6.59		
A.indicus	M	264	15.07 – 29.52	19.88 ± 0.17	59.156	0.000	3.54 – 5.84	4.35 ± 0.42	145.472	0.000	14.90 – 110.40	34.10 ± 15.15	90.551	0.000
A.serrulatus	M	187	14.21 – 25.87	19.22 ±2.18			3.20 – 5.22	4.20 ± 0.37			12.10 – 49.90	29.65 ± 9.28		
A. japonicus	M	25	14.25 – 18.59	16.04 ± 0.96			2.40 – 3.28	2.78 ± 0.25			8.50 – 20.60	13.11 ±3.16		
A.sibogae	M	43	18.17 – 21.93	19.95 ± 1.12			3.19 – 4.13	3.69 ± 0.28			17.00 – 33.10	24.27 ± 4.56		
A.indicus	B	604	15.07 – 38.94	22.66 ± 4.55	152.192	0.000	3.54 – 7.88	5.04 ± 0.08	364.868	0.000	14.90 – 287.30	53.80 ± 37.15	251.374	0.000
A.serrulatus	B	381	14.21- 26.55	20.12 ± 2.47			3.20 – 5.74	4.42 ± 0.46			12.10 – 70.00	34.83 ± 12.27		
A.japonicus	B	74	14.25 – 22.00	17.84 – 2.02			2.40 – 4.28	3.22 ± 0.49			8.50 – 33.30	19.06 ± 6.40		
A. sibogae	B	53	18.17 – 23.04	20.27 – 1.31			3.19 – 4.55	3.78 ± 0.35			17.00 – 40.00	26.01 ± 6.14		

sex: F, female; M, male.
n: sample size
P: Significance value of Kruskal-Wallis *H*-test

Table 4.5: Results on pairwise comparisons among *Acetes* species for TL (total length), CL (carapace length), and WW (wet weight). Tests were based on the Mann-Whitney *U*-test, with *P*-values being corrected according to the Bonferroni method ($P = 0.0083$) as six comparisons had to be conducted separately for females, males, and combined sexes of each species.

		TL (mm)				CL (mm)				WW (mg)			
	Species	1	2	3	4	1	2	3	4	1	2	3	4
Females	*1. A. indicus*												
	2. A.serrulatus	15607.000, $P = 0.000$				9529.000, $P = 0.000$				14796.500, $P = 0.000$			
	3. A. japonicus	1296.000, $P = 0.000$	2229.500, $P = 0.000$			61.500, $P = 0.000$	279.500, $P = 0.000$			415.000, $P = 0.000$	1037.500, $P = 0.000$		
	4. A. sibogae	910.000, $P = 0.012$	789.000, $P = 0.320$	45.000, $P = 0.000$		140.500, $P = 0.000$	434.500, $P = 0.003$	46.000, $P = 0.000$		433.000, $P = 0.000$	671.000, $P = 0.100$	50.000, $P = 0.000$	
	Species	1	2	3	4	1	2	3	4	1	2	3	4
Males	*1. A. indicus*												
	2. A.serrulatus	22385.000, $P = 0.092$				20337.000, $P = 0.001$				21486.500, $P = 0.019$			
	3. A. japonicus	399.000, $P = 0.000$	436.500, $P = 0.000$			0.000, $P = 0.000$	3.000, $P = 0.000$			90.000, $P = 0.000$	162.000, $P = 0.000$		
	4. A. sibogae	4962.000, $P = 0.186$	3122.500, $P = 0.022$	5.000, $P = 0.000$		957.000, $P = 0.000$	1125.000, $P = 0.000$	3.000, $P = 0.000$		2864.500, $P = 0.000$	2594.000, $P = 0.000$	27.000, $P = 0.000$	
	Species	1	2	3	4	1	2	3	4	1	2	3	4
Both	*1. A. indicus*												
	2. A.serrulatus	78425.000, $P = 0.000$				68178.000. $P = 0.000$				76283.000, $P = 0.000$			
	3. A. japonicus	6861.500, $P = 0.000$	6809.000, $P = 0.000$			793.000, $P = 0.000$	1176.000, $P = 0.000$			3056.000, $P = 0.000$	3634.000, $P = 0.000$		
	4. A. sibogae	11211.000, $P = 0.000$	9632.500, $P = 0.588$	671.000, $P = 0.000$		2265.000, $P = 0.000$	3842.000, $P = 0.000$	837.000, $P = 0.000$		5286.500, $P = 0.000$	5621.500, $P = 0.000$	907.500, $P = 0.000$	

Indicate non-significant result (Significance level, $P = 0.0083$)

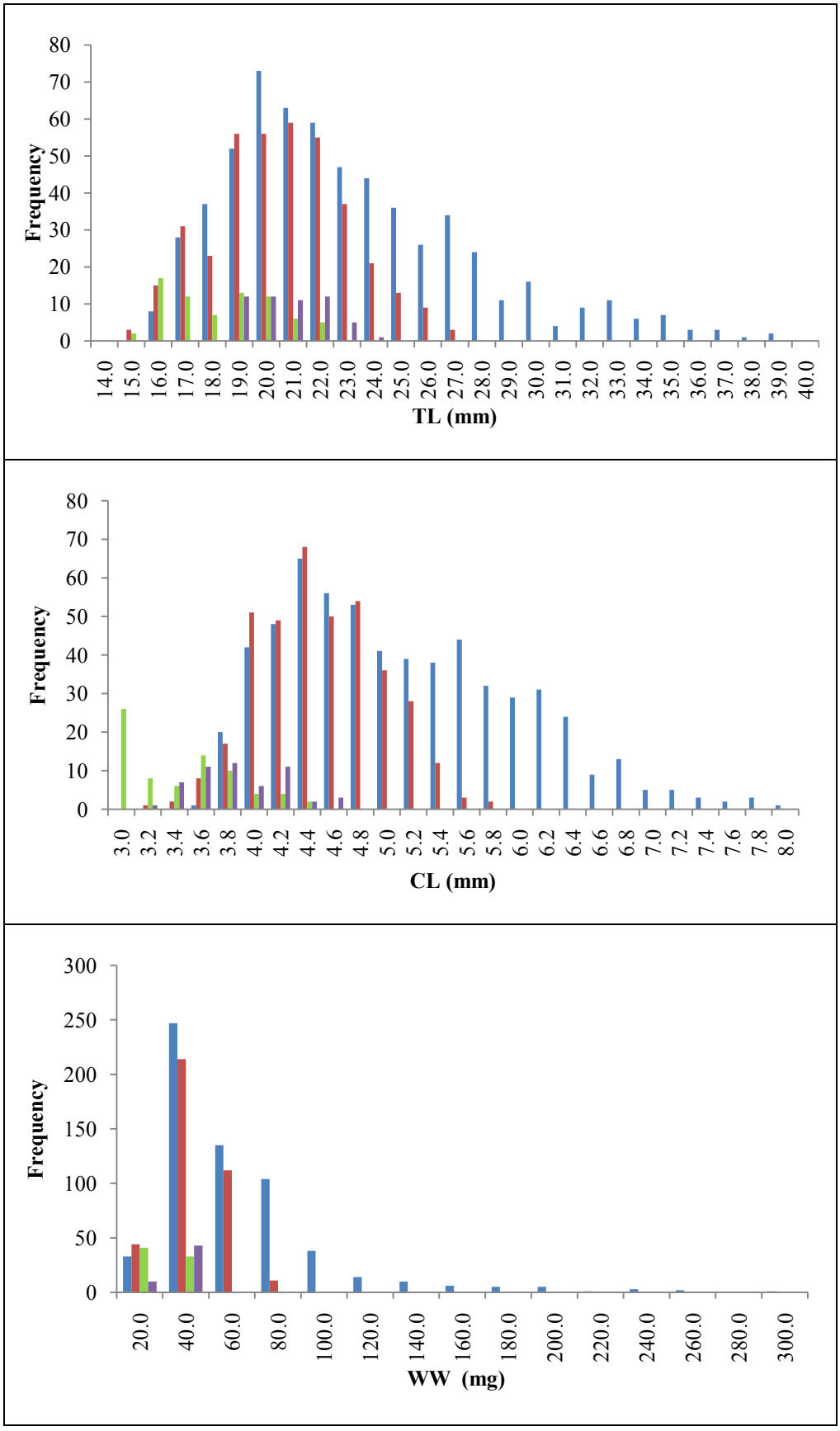


Figure 4.9: Size-frequency distributions of *Acetes indicus, A. serrulatus*, *A. japonicus*, *A. sibogae* collected form the west coast of Peninsular Malaysia.
■ *Acetes indicus* ■ *A. serrulatus* ■ *A. japonicus* ■ *A. sibogae*

4.4 Length-Weight Relationships (LWRs) and Length-Length Relationships (LLRs)

The LWRs were estimated for different groups (females and males for each species, combined sexes for each species, and in-shore and off-shore samples of *A. indicus* and *A. serrulatus*) in this study are shown in Table 4.6 and Table 4.7, respectively. The LWRs estimated in this study were significant ($P < 0.05$), with the coefficient of determination of $R^2 > 0.659$. LLRs were estimated as well with the same grouping as LWRs, (Table 4.8 and Table 4.9). LLRs were all significant ($P < 0.001$) with the coefficient of determination of $R^2 > 0.347$.

The estimated *b* values for LWRs ranged from 2.285 to 3.403 (Table 4.6 – 4.7). If the estimated *b* value is equal or not significantly different from the isometric value (3), it indicates isometric growth (the shape does not change as shrimps grow). However, if the *b* value is significantly higher or lower than 3, it indicates positive or negative allometric growth, respectively. Both *t*-test and normal distribution (*z*-test) gave similar results (Table 4.6 – 4.7). Generally, *A. indicus* and *A. serrulatus* demonstrated negative allometric growth for males, females, and in overall pooled data of both sexes (Table 4.6). As for *A. japonicus* and *A. sibogae*, the males showed an isometric growth type. Differences in growth patterns were, however, observed between males and females of *A. japonicus* whereas the pooled data of both sexes for *A. sibogae* showed positive allometric growth as compared with isometric growth, in the case of individual sexes (Table 4.6).

Since samples of *A. japonicus* and A. *sibogae* were not found from off-shore trawling catches, LWRs for only *A. indicus* and *A. serrulatus* for in-shore and

off-shore samples were estimated (Table 4.7). The in-shore samples of *A. indicus* showed isometric growth while off-shore samples showed isometric growth only for females and negative allometric growth for males and both sexes combined. For *A.serrulatus*, in-shore and off-shore samples exhibited isometric growth and positive allometric growth pattern, respectively, for females and when both sexes were combined (Table 4.7). Negative allometric and isometric growth pattern were also detected in males of in-shore and off-shore samples, respectively.

Table 4.6: Descriptive statistics and estimated parameters of the length-weight relationships of the four *Acetes* species collected along the west coast of Peninsular Malaysia.

Species	N	Sex	TL range (mm)	WW range (mg)	a	95 % CI of a	b (S.E.)	95 % CI of b	Growth (*t*-test value)	Growth (*z*-test value)	R^2
Acetes indicus	340	F	16.71 – 38.94	18.00 – 287.30	0.008	0.006 – 0.011	2.778 (0.045)	2.688 – 2.867	A- (4.933)	A- (4.933)	0.917 ***
	264	M	15.07 – 29.52	14.90 – 110.40	0.010	0.007 – 0.014	2.694 (0.053)	2.589 – 2.799	A- (5.773)	A- (5.773)	0.907***
	604	B	15.07 – 38.94	14.90 – 287.30	0.007	0.006 – 0.008	2.829 (0.029)	2.773 – 2.886	A- (5.896)	A- (5.896)	0.941 ***
Acetes serrulatus	194	F	15.28 – 26.55	14.20 – 70.00	0.013	0.007 – 0.023	2.637 (0.104)	2.431 – 2.843	A- (3.490)	A- (3.490)	0.769 ***
	187	M	14.21 – 25.87	12.10 – 49.90	0.010	0.006 – 0.016	2.699 (0.084)	2.533 – 2.865	A- (3.583)	A- (3.583)	0.847 ***
	381	B	14.21 – 26.55	12.10 – 70.00	0.009	0.006 – 0.013	2.749 (0.064)	2.623 – 2.875	A- (3.921)	A- (3.921)	0.829 ***
Acetes japonicus	49	F	15.25 – 22.00	11.60 – 33.00	0.017	0.006 – 0.050	2.432 (0.179)	2.072 – 2.791	A- (3.173)	A- (3.173)	0.798 ***
	25	M	14.25 – 18.59	8.50 – 20.60	0.002	0.0001 – 0.031	3.153 (0.473)	2.175 – 4.132	I (0.323)	I (0.323)	0.659 ***
	74	B	14.25 – 22.00	8.50 – 33.30	0.005	0.002 – 0.010	2.883 (0.139)	2.606 – 3.160	I (0.842)	I (0.842)	0.856 ***
Acetes sibogae	10	F	19.29 – 23.04	22.40 – 40.00	0.001	0.000 – 0.009	3.393 (0.315)	2.667 – 4.119	I (1.247)	I (1.247)	0.936 ***
	43	M	18.17 – 21.93	17.00 – 33.10	0.002	0.001 – 0.005	3.191 (0.162)	2.864 – 3.519	I (1.179)	I (1.179)	0.904 ***
	53	B	18.17 – 23.04	17.00 – 40.00	0.001	0.000 – 0.002	3.403 (0.130)	3.143 – 3.664	A+ (3.100)	A+ (3.100)	0.931 ***

N = number of individuals; Sex: F = female, M = male, B = female and male; TL = total length (mm); WW = wet weight (mg); Regression parameter: a = intercept, b = slope; CI = confidence interval; S. E. = standard error of the slope b; R^2: coefficient of determination; significance level: * $0.01<P<0.05$, ** $0.01<P<0.05$, *** $P < 0.001$.

Table 4.7: Descriptive statistics and estimated parameters of the length-weight relationships for the in-shore and off-shore samples of *A. indicus* and *A. serrulatus* collected along the west coast of Peninsular Malaysia.

Species		N	Sex	TL range (mm)	WW range (mg)	a	95 % CI of a	b (S.E.)	95 % CI of b	Growth (*t*-test value)	Growth (*z*-test value)	R^2
Acetes indicus	in-shore	241	F	16.71 – 29.20	18.00 – 93.00	0.006	0.004 – 0.009	2.911 (0.072)	2.770 – 3.052	I (1.236)	I (1.236)	0.874 ***
		197	M	15.07 – 23.26	14.90 – 52.20	0.007	0.004 – 0.011	2.836 (0.086)	2.666 – 3.005	I (1.907)	I (1.907)	0.848 ***
		438	B	15.07 – 29.20	14.90 – 93.00	0.005	0.004 – 0.007	2.940 (0.041)	2.858 – 3.021	I (1.463)	I (1.463)	0.921 ***
	off-shore	99	F	17.38 – 38.94	26.40 – 287.30	0.008	0.004 – 0.015	2.803 (0.104)	2.597 – 3.009	I (1.894)	I (1.894)	0.882 ***
		67	M	15.32 – 29.52	15.00 – 110.40	0.009	0.005 – 0.015	2.733 (0.090)	2.554 – 2.912	A- (2.967)	A- (2.967)	0.935
		166	B	15.32 – 38.94	15.00 – 287.30	0.006	0.004 – 0.009	2.867 (0.057)	2.755 – 2.979	A- (2.333)	A- (2.333)	0.940 ***
Acetes serrulatus	in-shore	39	F	18.81 – 26.55	18.90 – 60.20	0.001	0.0001 – 0.003	3.335 (0.176)	2.978 – 3.693	I (1.903)	I (1.903)	0.906 ***
		30	M	19.52 – 25.87	26.90 – 49.60	0.031	0.001 – 0.100	2.285 (0.181)	1.914 – 2.655	A- (3.950)	A- (3.950)	0.851 ***
		69	B	18.81 – 26.55	18.90 – 60.20	0.006	0.002 – 0.014	2.806 (0.125)	2.556 – 3.056	I (1.552)	I (1.552)	0.882 ***
	off-shore	155	F	15.28 – 24.07	14.20 – 70.00	0.002	0.001 – 0.003	3.326 (0.106)	3.116 – 3.536	A+ (3.075)	A+ (3.075)	0.865 ***
		157	M	14.21 – 22.21	12.10 – 49.90	0.003	0.002 – 0.005	3.157 (0.094)	2.972 – 3.342	I (1.670)	I (1.670)	0.880 ***
		312	B	14.21 – 24.07	12.10 – 70.00	0.002	0.001 – 0.003	3.305 (0.066)	3.175 – 3.435	A+ (4.621)	A+ (4.621)	0.889 ***

N = number of individuals; Sex: F = female, M = male, B = female and male; TL = total length (mm); WW = wet weight (mg); Regression parameter: a = intercept, b = slope; CI = confidence interval; S. E. = standard error of the slope b; R^2: coefficient of determination; significance level: * $0.01<P<0.05$, ** $0.01<P<0.05$,*** $P < 0.001$.

Table 4.8: Length-length relationships of the four *Acetes* species collected along the west coast of Peninsular Malaysia.

Species	Sex	N	Length-length relationships	R^2
A. indicus	F	340	TL = 5.045CL-3.267	0.740***
	M	264	TL = 5.596CL-4.473	0.705***
	B	604	TL = 4.636CL-0.684	0.798***
A. serrulatus	F	194	TL = 3.217CL+6.087	0.347***
	M	187	TL = 4.222CL+1.470	0.513***
	B	381	TL = 3.730CL+3.625	0.486***
A.japonicus	F	49	TL = 4.421CL+3.199	0.883***
	M	25	TL = 3.106CL+7.411	0.640***
	B	74	TL = 3.917CL+5.036	0.902***
A. sibogae	F	10	TL = 3.661CL+6.289	0.908***
	M	43	TL = 3.803CL+5.922	0.850***
	B	53	TL = 3.621CL+6.531	0.921***

N = number of individuals; Sex: F = female, M = male, B = female and male; significance level: * $0.01< P <0.05$, ** $0.01< P <0.05$, *** $P < 0.001$.

Table 4.9: Length-length relationships of the in-shore and off-shore samples of *A. indicus* and *A. serrulatus* collected along the west coast of Peninsular Malaysia.

Species	Sampling region	Sex	N	Length-length relationships	R^2
A. indicus	in-shore	F	241	TL = 3.678CL+3.281	0.883***
		M	197	TL = 4.367CL+0.455	0.722 ***
		B	438	TL = 3.683CL+3.309	0.906***
	off-shore	F	99	TL = 5.123CL-1.919	0.586***
		M	67	TL = 5.856CL-4,731	0.667***
		B	166	TL = 5.001CL-1.021	0.760***
A. serrulatus	in-shore	F	39	TL = 4.031CL+5.744	0.800***
		M	30	TL = 5.094CL+0.983	0.857***
		B	69	TL = 4.519CL+3.468	0.853***
	off-shore	F	155	TL = 3.736CL+2.830	0.727***
		M	157	TL = 4.453CL-0.101	0.869***
		B	312	TL = 3.923CL+2.049	0.815***

N = number of individuals; Sex: F = female, M = male, B = female and male; significance level: * $0.01< P <0.05$, ** $0.01< P <0.05$, *** $P < 0.001$.

4.5 DNA Analysis of *Acetes* samples

The images of EtBr-stained gels of DNA extract (Figure 4.10) and PCR products (Figure 4.11) were viewed under U.V. illumination. One band was visualized in the PCR products (~ 700bp).

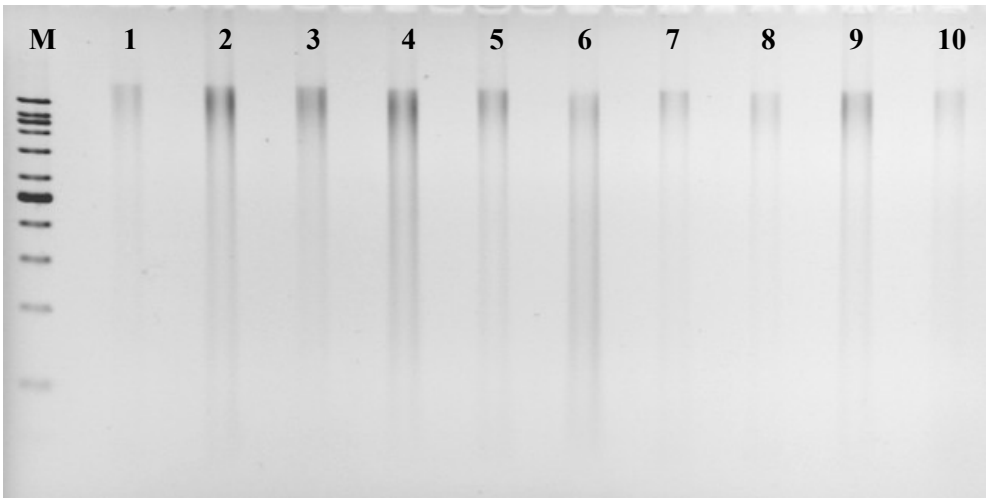


Figure 4.10: Gel electrophoresis of extracted DNA. A sample of the genomic DNA extracted from *Acetes indicus*. Lane M: 1 kb DNA ladders; Lane 1 – 10: DNA extracts. (1% Agarose gel, TBE buffer, 90V, 45 mins).

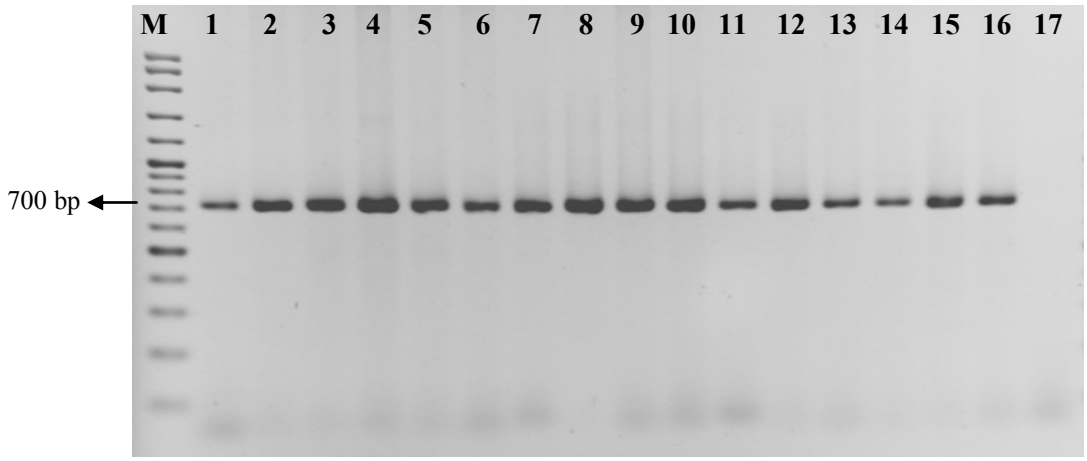


Figure 4.11: Gel electrophoresis of PCR products. A sample of the PCR products amplified from *Acetes indicus*. Lane M: 100bp DNA ladders; Lane 1 – 16: PCR products; Lane 17: negative control. (2% Agarose gel, TBE buffer, 90V, 45 mins).

4.6 Cytochrome *C* Oxidase Subunit I (*COI*) Gene

The 552 base pairs (bp) of the *COI* gene fragment (GenBank Accession Number: HQ630429–HQ630587) amplified in this study were obtained for 159 specimens and revealed 46 haplotypes: 11 haplotypes were identified for *Acetes indicus*, 31 haplotypes for *A. serrulatus*, two haplotypes for *A. japonicus*, and two haplotypes for *A. sibogae* (Appendix E). From the multiple sequence alignment of 46 haplotypes (Figure 4.12), 167 variable sites were found, of which 144 and 23 were parsimony informative sites and singleton sites, respectively. No insertions or deletion (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 4.10, together with the base composition according to first, second and third codon position. The pattern of nucleotide substitution was biased in favour of 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 (9%) 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 (see Appendix F). The substitutions resulted in chemically similar amino acids change. Overall, the pattern of base composition nucleotide substitution was similar among *Acetes* species.

```
[                                 11111 1111111111 1111111111 1112222222]
[           12333 3444556667 7788900111 2233344455 5666677788 8990011112]
[      1367925036 9238470392 5814325147 0925814836 7235814736 9281703792]
#ai_1  TAATATCAAA ACTTTTATTA ATATCTTGTA CTAATACTTA TACAACATTT TGTTACATAA
#ai_2  .......... .T...C.CC. ...CT..A.G T...C..CAT ...G.T...C .A.C...CTC
#ai_3  .......... .......... .......... .......... .......... .........T
#ai_4  .......... .T...C.CC. ...CT..A.G T...C..CAT ...G.T...C .A.C...CTT
#ai_5  .......... .......... .......... .......... .......... .A........
#ai_6  .......... .T...C.CC. ...CT..A.G T...C..CAT ...G.T...C .A.C...CTC
#ai_7  .......... .T........ .......... .......... .......... ..........
#ai_8  .......... .......... .......... .......... .......... ..........
#ai_9  .......... ...C...... .......... .......... .......... ..........
#ai_10 .......... .....C.... .......... .......... .......... ..........
#ai_11 .G........ .......... .......... .......... .......... ..........
#as_1  ......T... ..C....C.. T.TCT.CA.. T....T.CCT ..T......C CAC.GT....
#as_2  ......T... ..C....C.. T.TCT.CA.. T....T.CCT ..T......C CAC.GT....
#as_3  ......T... ..C....C.. T.TCT.CA.. T....T.CCT ..T......C CAC.GT....
#as_4  ......T... ..C....C.. T.TCT.CA.. T..G.T.CCT ..T......C CAC.GT....
#as_5  ......T... ..C....C.. T.TCT.CA.. T....T.CCT ..T......C CAC.GT....
#as_6  ......T... .AC....C.. T.TCT.CA.. T....T.CCT ..T......C CACCGT....
#as_7  ......T... ..C....C.. T.TCT.CA.. T..G.T.CCT ..T....... CAC..T....
#as_8  ......T... ..C....C.. T.TCT.CA.. T....T.CCT ..T......C CAC.GT....
#as_9  ......T... ..C....C.. T.TCT.CA.. T....T.CCT ..T......C CAC.GT....
#as_10 ......T... ..C....C.. T.TCT.CA.. T....T.CCT ..T......C CAC.GT....
#as_11 ......T... ..C....C.. T.TCT.CA.. T....T.CCT ..T.T....C CAC.GT....
#as_12 ......TG.. ..C....C.. T.TCT.CA.. T....T.CCT ..T......C CAC.GT....
#as_13 ......T... ..C....C.. T.TCT.CA.. T....T.CCT ..T......C CACCGT....
#as_14 ......T... ..C....C.. T.TCT.CA.. T....T.CCT ..T......C CAC.GT....
#as_15 ......T... .......C.. T.TCT.CA.. T....T.CCT ..T......C CAC.GT....
#as_16 ......T... ..C....C.. T.TCT.CA.. T....T.CCT ..T......C CAC..T....
#as_17 ......T... ..C....C.. T.TCT.CA.. T....T.CCT ..T......C CAC.GT....
#as_18 ......T... ..C....C.. T.TCT.CA.. T....T.CCT ..T......C CAC.GT....
#as_19 ......T... ..C....C.. T.TCT.CA.. T....T.CCT ..T......C CAC.GT....
#as_20 ......T... ..C....C.. T.TCT.CA.. T....T.CCT ..T......C CAC.GT....
#as_21 ......T... ..C....C.. T.TCT.CA.. T....T.CCT ..T......C CAC.GT.C..
#as_22 ......T... .TC....C.. T.TCT.CA.. T....T.CCT ..T......C CAC.GT....
#as_23 ......T... ..C....C.. T.TCT.CA.. T....T.CCT ..T......C CAC.GT....
#as_24 ......T... ..C....C.. T.TCT.CA.. T....T.CCT ..T......C CAC.GT....
#as_25 ......T... ..C....C.. T.TCT.CAA. T..G.T.CCT ..T......C CAC.GT....
#as_26 ......T... ..C....C.. T.TCT.CA.. T....T.CCT ..T......C .AC.GT....
#as_27 ......T... ..C....C.. T.TCT.CA.. T....T.CCT ..T......C CAC.GT....
#as_28 ......T... ..C....C.. T.TCT.CA.. T....T.CCT ..T..T...C CAC.GT....
#as_29 ......T... ..C....C.. T.TCT.CA.. T....T.CCT ..T....... CAC.GT....
#as_30 ......T... ..C....C.. T.TCT.CA.. T....T.CCT ..T......C CAC.GT.C..
#as_31 ......T.G. ..C....C.. T.TCT.CA.. T....T.CCT ..T......C CAC.GT....
#aj_1  ...CG.T..T C.C......C ....T..A.. TCT.CTTCCT CG....GC.C AA.CTT.CCC
#aj_2  ...CG.T..T C.C......C ....T..A.. TCT.CTTCCT CG....GC.C AA.CTT.CCC
#asi_1 C.TCTC.T.T TGC.CCG.CC GAC.TCCA.. T..G...C.T .G..TTT.AC .ACC...CT.
#asi_2 ..TCT..C.T TAC..CG.CC .AC...CA.G T.G..G.C.T ....T.T.AC .ACC..GC.G
```

Figure 4.12: Multiple sequence alignment of the 46 haplotypes identified from 159 specimens. Only variable sites in 552 bp of *COI* gene are shown. Haplotypes are named according to the species, *i.e*., ai – *Acetes indicus*; as – *A. serrulatus*; aj – *A. japonicus*; asi – *A. sibogae*. The same nucleotides with the haplotype ai1 are represented by dot.

```
[      2222222222 2222222222 2222233333 3333333333 3333333333 3333333333]
[      2223333444 4555566778 8899900000 1112223333 3445555666 7777888999]
[      5894578013 6235847392 5847801369 2581470367 9281478369 2589147036]
#ai_1  CACTTACTTA TACTTGAAAA TTTTTAGTAA TTTTATATTT ATTATCTCAA TACTAATTTT
#ai_2  ..T....G.. ...C.A.... CC.....A.G .C........ ....C.CT.. ...C......
#ai_3  .......... .......... .......... .......... .......... ..........
#ai_4  ..T....G.. ...C.A.... CC.....A.G .C........ ......CT.. ...C......
#ai_5  .......... .......... .......... .......... .......... ..........
#ai_6  ..T....G.. ...C.A.... CC.....A.G .C........ ......CT.. ...C......
#ai_7  .......... .......... .......... .......... .......... ..........
#ai_8  .......... .......... .......... .......... .......... ..........
#ai_9  .......... .......... .......... .......... .......... ..........
#ai_10 .......... .......... .......... .......... .......... ..........
#ai_11 .......... .......... .......... .......... .......... ..........
#as_1  T.T.CCTACT CT..AA.... A...CTTATT .A.A..TA.C .....T.A.G ..T.......
#as_2  T.T.CCTACT CT..AA.... A...CTTATT .A.A..TA.C .....T.A.. ..T.......
#as_3  T.T.CCTACT CT..AAG... A...CTTATT .A.A..TA.C .....T.A.. ..T.......
#as_4  T.T.CCTACT CT..AA.... A...CTTATT .A.A..TA.C .....T.A.G ..T.......
#as_5  T.T.CCTACT CT..AA.... A...CTTATT .A.A..TA.C .....T.A.G ..T.......
#as_6  T.T.CCTACT CT..AA.... A...CTTATT .A.A..TA.C .....T.A.. ..T.......
#as_7  T.T.CCTACT .T.CAA.... A...CTTATT .A.A..TA.. .....T.A.G ..T.......
#as_8  T.T.CCTACT CT..AA.... A...CTTATT .A.A..TA.C .....T.A.G ..T.......
#as_9  T.T.CCTACT CT..AA.... A...CTTATT .A.A..TA.C .....T.A.G ..T.......
#as_10 ..T.CCTACT .T..AA.... A...CTTATT .A.A..TA.C .....T.A.. ..T.......
#as_11 T.T.CCTACT CT..AA.... A...CTTATT .A.A..TA.C .....T.A.G ..T.......
#as_12 T.T.CCTACT CT..AA.... A...CTTATT .A.A..TA.C .....T.A.. ..T.......
#as_13 T.T.CCTACT CT..AA.... A...CTTATT .A.A..TA.C .....T.A.. ..T.......
#as_14 T.T.CTTACT CT..AA.... A...CTTATT .A.A..TA.C .....T.A.. ..T.......
#as_15 T.T.CCTACT CT..AA.... A...CTTATT .A.A..TA.C .....T.A.. ..T.......
#as_16 T.T.CCTACT CT..AA.... A...CTTATT .A.A..TA.C .....T.A.. ..T.......
#as_17 T.T.CCTACT CT..AA.... A...CTTATT .A.A..TA.C .....T.AGG ..T.......
#as_18 T.T.CCTACT CT..AA.... A...CTTATT .A.A..TA.C .....T.A.G ..T.......
#as_19 T.T.CCTACT CT..AA.... A...CTTATT .A.A..TA.C .....T.A.G ..T......C
#as_20 T.T.CCTACT CT..AA.... A...CTTATT .A.A..TA.C .....T.A.G ..T.......
#as_21 T.T.CCTACT CT..AA.... A...CTTATT .A.A..TA.C .....T.A.G ..T.......
#as_22 T.T.CCTACT CT..AA.... A...CTTATT .A.A..TA.C .....T.A.G ..T.......
#as_23 T.T.CCTACT CT..AA.... A...CTTATT .A.A..TA.C .....T.A.. ..T.......
#as_24 T.T.C.TACT CT..AA.... A...CTTATT .A.A..TA.C .....T.A.G ..T.......
#as_25 T.T.CCTACT CT..AA.... A...CTTATT .A.A..TA.C .....T.A.G ..T.......
#as_26 T.T.CCTACT CT..AA.... A...CTTATT .A.A..TA.C .....T.A.G ..T.......
#as_27 T.T.CCTACT CT..AA.... A...CTTATT .A.A..TA.C ...G.T.A.G ..T.......
#as_28 T.T.CCTACT CT..AA.... A...CTTATT .A.A..TA.C .....T.A.. ..T..G....
#as_29 T.T.CCTACT CT..AA.... A...CTTATT .A.A..TA.C .....T.A.. ..T.......
#as_30 T.T.CCTACT AT..AA.... A...CTTATT .A.A..TA.C .....T.A.. ..T.......
#as_31 T.T.CCTACT CT..AA.... A...CTTATT .A.A..TA.C .....T.A.. ..T.......
#aj_1  TTTCCTTAC. CG..CA.GC. .C.CCC.ATT CCCCT.TA.. ..CT...T.. C.T...CAC.
#aj_2  TTTCCTTAC. C...CA.GC. .C.CCC.ATT CCCCT.TA.. ..CT...T.. C.T...CAC.
#asi_1 ATTAC.TAC. A.AAATG.T. AG..C...C. C..ATCTAC. .A.GC..TGT .TT.G.A...
#asi_2 GT.CCGTA.G A.AGAC..TG ACC.C...TG ..CATATAC. GA....CTTT .TT...A...
```

Figure 4.12 (continued): Multiple sequence alignment of the 46 haplotypes identified from 159 specimens. Only variable sites in 552 bp of *COI* gene are shown. Haplotypes are named according to the species, *i.e.*, ai – *Acetes indicus*; as – *A. serrulatus*; aj – *A. japonicus*; asi – *A. sibogae*. The same nucleotides with the haplotype ai1 are represented by dot.

```
[      4444444444 4444444444 4444444444 4455555555 5555555]
[      0011222223 3444455555 6788889999 9900011223 3444445]
[      5814012394 5145701369 5106790235 8914736581 7036792]
#ai_1  AACTTTCTGG ATATAATATA ACTATATACT CTATTAATAA TTATCAT
#ai_2  ..TCAAG... .......... .T.G.G.... AC...C.... .C.C...
#ai_3  .......... .......... .......... .......... .......
#ai_4  ..TCAAG... .......... .T.G...... AC...C.... .C.C...
#ai_5  .......... .......... .......... .......... .......
#ai_6  ..TCAAG... .......... .T.G.G.... AC...C.... .C.C...
#ai_7  .......... .......... .......... .......... .......
#ai_8  G......... .......... .......... .......... .......
#ai_9  .......... .......... .......... .......... .......
#ai_10 .......... .......... .......... .......... .......
#ai_11 .......... .......... .......... .......... .......
#as_1  .GT.AAGGAC .....T.... .TC...CTTA A.....TA.T ....TGC
#as_2  .GT.AAGGAC .....T.... .TC...CTTA A.....TA.T ....TGC
#as_3  .GT.AAGGAC .....T.... .TC...CTTA A.....TA.T ....TGC
#as_4  .GT.AAGGAC .....T.... .TC...CTTA A.....TA.T ....TGC
#as_5  .TT.AAGGAC .....T.... .TC.C.CTTA A.....TA.T ....TGC
#as_6  .GT.AAGGAC .....T.... .TC...CTTA A.....TA.T ....TGC
#as_7  ..T.AAG.AC .....T.... .TC...CTTA A....CTA.T ....T.C
#as_8  .GT.AAGAAC .....T.... .TC...CTTA A.....TA.T ....TGC
#as_9  .GT.AAGGAC .....T.... .TC...CTTA A.G...TA.T ....TGC
#as_10 .GT.AAGGAC .....T.... .TC...CTTA A.....TA.T ....TGC
#as_11 .GT.AAGGAC .....T.... .TC...CTTA A.....TA.T ....TGC
#as_12 .GT.AAGAAC .....T.... .TC...CTTA A.....TA.T ....TGC
#as_13 .GT.AAGGAC .....T.... .TC...CTTA A.....TA.T ....TGC
#as_14 .GT.AAGGAC .....T.... .TC...CTTA A.....TA.T ....TGC
#as_15 .GT.AAGGAC .....T.... .TC...CTTA A.....TA.T ....TGC
#as_16 .GT.AAGGAC .....T.... .TC...CTTA A.....TA.T ....TGC
#as_17 .GT.AAGGAC .....T.... .TC...CTTA A.....TA.T ....TGC
#as_18 .GT.AAG.AC .....T.... .TC...CTTA A.....TA.T ....TGC
#as_19 .GT.AAGGAC .....T.... .TC...CTTA A.....TA.T ....TGC
#as_20 .GT.AAGGAC .....T.... .TC...CTTA A.....TAGT ....TGC
#as_21 .GT.AAGAAC .....T.... .TC...CTTA A.....TA.T ....TGC
#as_22 .GT.AAGGAC .....T.... .TC...CTTA A.....TA.T ....TGC
#as_23 .GT.AAGGAC .....T.... .TC.C.CTTA A.....TA.T ....TGC
#as_24 .GT.AAGGAC .....T.... .TC...CTTA A.....TA.T ....T.C
#as_25 .GT.AAGGAC .....T.... .TC...CTTA A.....TA.T ....TGC
#as_26 .GT.AAGGAC .....T.... .TC...CTTA A.....TA.T ....TGC
#as_27 .GT.AAGGAC .....T.... .TC...CTTA A.....TA.T ....TGC
#as_28 .GT.AAGGAC .....T.... .TC...CTTA A.....TA.T ....TGC
#as_29 .GT.AAGGAC .....T.... .TC...CTTA A.....TA.T ....TGC
#as_30 .GT.AAGGAC .....T.... .TC...CTTA A.....TA.T ....TGC
#as_31 .GT.AAGGAC .....T.... .TC...CTTA A.....TA.T ....TGC
#aj_1  ..T.AAGAAC .CTC..C... .......... T...G.TA.C .C.CT.C
#aj_2  ..T.AAGAAC .CTC..C... .......... T...G.TA.C .C.CT.C
#asi_1 .TT..AG.AC TC.CCCCGCG GTCGCT...A T..A.....T ACG.T..
#asi_2 .T...AG.AC T..CCTCG.C GT..CT..TA TC.A...G.T AC..T.C
```

Figure 4.12 (continued): Multiple sequence alignment of the 46 haplotypes identified from 159 specimens. Only variable sites in 552 bp of *COI* gene are shown. Haplotypes are named according to the species, *i.e.*, ai – *Acetes indicus*; as – *A. serrulatus*; aj – *A. japonicus*; asi – *A. sibogae*. The same nucleotides with the haplotype ai1 are represented by dot.

Table 4.10: Base composition (%) of *COI* gene amplified for each *Acetes* species.

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**

4.7 Phylogenetic Analyses

Phylogenetic trees constructed based on Neighbour-Joining (NJ) and Maximum Likelihood (ML), Maximum Parsimony (MP) and Bayesian Inference (BI) are shown in Figure 4.13 and Figure 4.14, respectively. NJ, ML, MP, and BI consistently produced trees with the same overall topology, which are four major clades, namely clade *ai*, *as*, *aj*, *asi* for *Acetes indicus*, *A. serrulatus*, *A. japonicus* and *A, sibogae*, respectively. The four major clades corresponding to the four identified *Acetes* species based on morphological features (key). Each clade was strongly supported by high bootstrap (BS) and posterior probability (PP), of 97–100% and 0.99–1.00 values, respectively. In addition, two distinct clades were observed in the *A. indicus* and *A. sibogae* samples, namely clade *ai-I* and *ai-II* and clade *asi-I* and *asi-II*, respectively.

4.7.1 Pairwise Genetic Distances and Time of Divergence

The mean percent nucleotide sequence divergence (K2P) within and between *Acetes* species are summarized in Table 4.11. The interspecific variation ranged from 14.50 to 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, *A. indicus* and *A. sibogae* showed 8.94% and 10.93% mean sequence divergence between the two distinct clade *ai-I* and *ai-II* and asi-*I* and *asi-II*, respectively (Table 4.11). In addition, time of divergence between species and between clades of *A. indicus* and *A. sibogae* was shown in Table 4.11 as well.

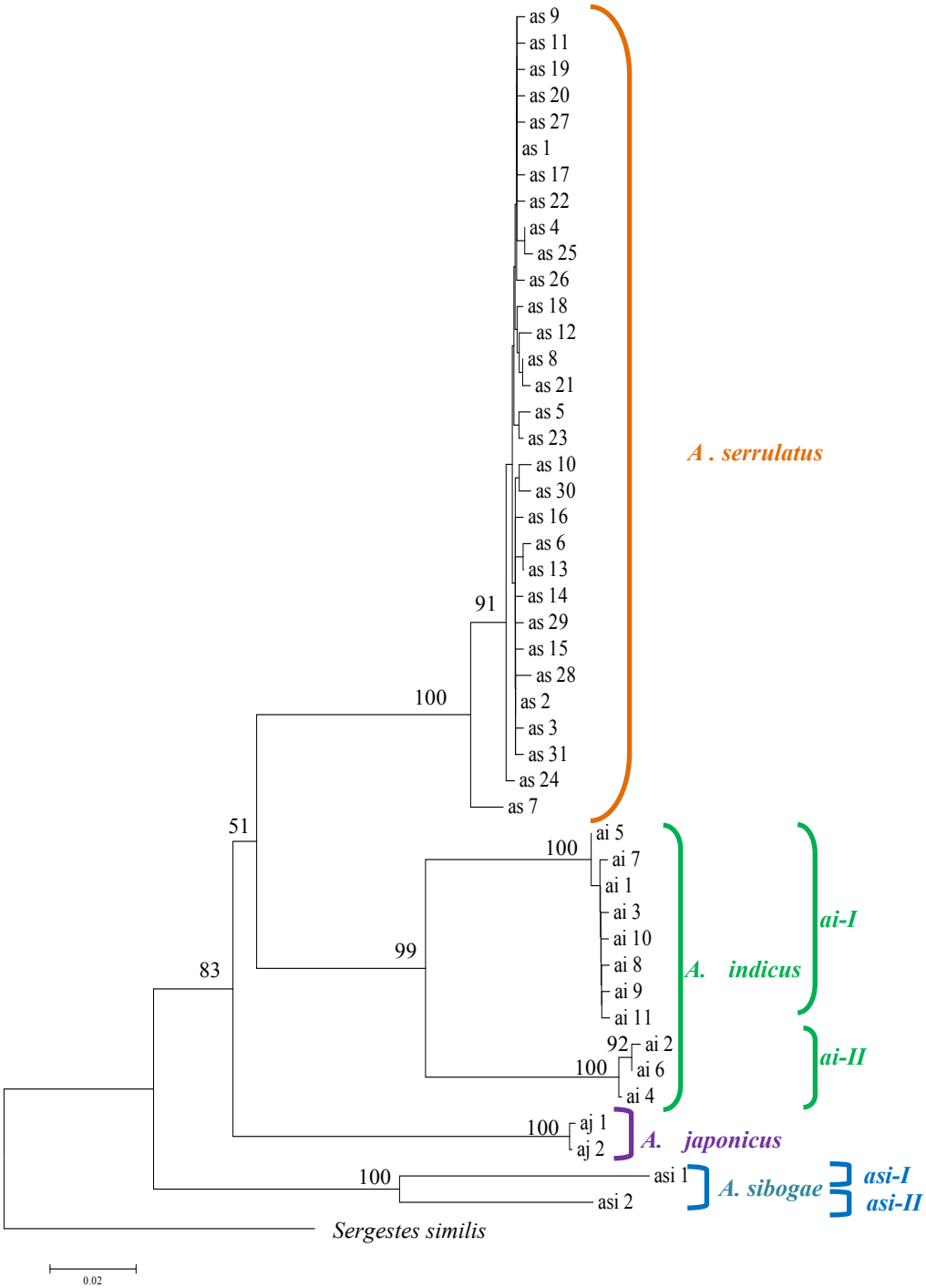


Figure 4.13: Neighbour-joining (NJ) phylogram (consensus tree) showing the relationship among *COI* mtDNA haplotypes of the *Acetes* sp. shrimp. Haplotypes are named according to the species (as – *A. serrulatus*; ai – *A. indicus*; aj – *A. japonicus;* asi – *A.sibogae*) and the corresponding number of haplotype. The value at each node represents the bootstrap value (%) based on 2000 pseudoreplicates.

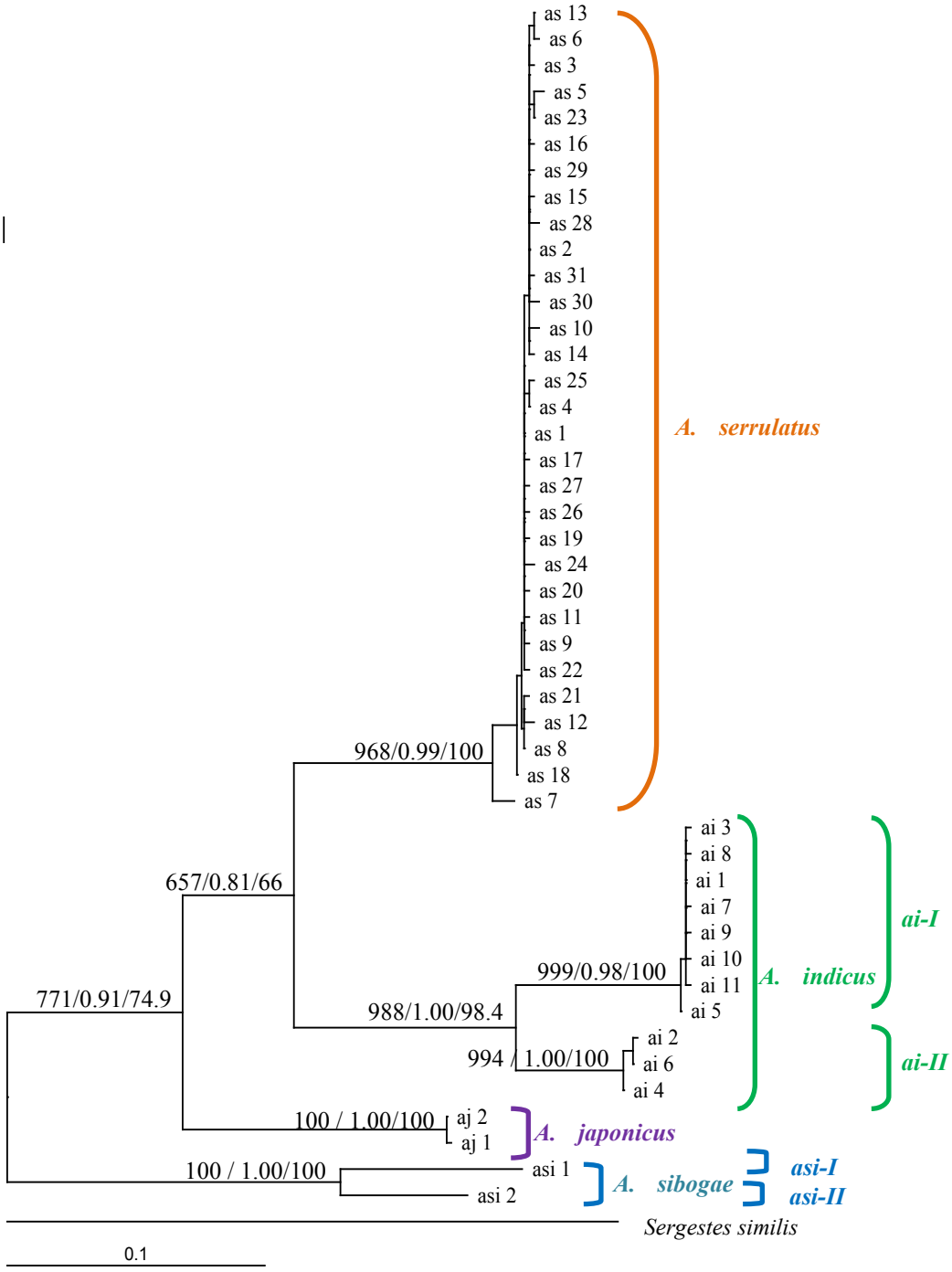


Figure 4.14: Maximum likelihood tree from *COI* mtDNA haplotype data under the best-fitting model HKY+I+G selected by jModeltest. The parameters were as follow: model = HKY85, number of substitution types (nst) = 2, proportion of invariable sites (*p*-invar) = 0.6220, Transition/Transversion ratio = 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 and BS (%) for MP.

Table 4.11: The mean nucleotide sequence divergence (%) estimated with Kimura's Two Parameter's, based on haplotypes only (a) Between and within *Acetes* species and outgroup, *Sergestes similis* (b) between and within two disctinct clade of *A. indicus* (c) between and within two disctinct clade of *A. sibogae.* The values in parentheses are the divergence time based on 1.40 % and 3.00 % sequence divergence rate, respectively, in million year ago (MYA).

(a) Interspecific variation

Species	*A. indicus*	*A. serrulatus*	*A. japonicus*	*A. sibogae*	*S. similis* (outgroup)
Acetes indicus	4.08 (2.91; 1.36)				
A. serrulatus	14.49 (10.35; 4.83)	0.63 (0.45; 0.21)			
A. japonicus	17.86 (12.76; 5.95)	14.69 (10.49; 4.89)	0.18 (0.130; 0.06)		
A. sibogae	20.47 (14.62; 6.82)	19.58 (13.98; 6.52)	19.89 (14.20; 6.63)	10.30 (7.36; 3.43)	
S. similis (outgroup)	21.35 (15.25; 7.12)	19.32 (13.80; 6.44)	21.21 (15.15; 7.07)	21.57 (15.41; 7.19)	-

(b) Interclade variation of *A. indicus*

Clade	*ai-I*	*ai-II*
ai-I	0.32 (0.23; 0.11)	
ai-II	8.94 (6.39; 2.98)	0.36 (0.26; 0.12)

(c) Interclade variation of *A. sibogae*

clade	*asi-I*	*asi-II*
asi-I	-	
asi-II	10.30 (7.36; 3.43)	-

4.8 Intraspecific Analyses

4.8.1 Haplotype Composition and Distribution, and DNA Polymorphism

The haplotype composition, segregating sites (*S*), number of haplotypes (N_{hap}), haplotype (gene) diversity (*h*), and nucleotide diversity (π) for each species are summarized in Tables 4.12 – 4.15.

For *A. indicus* (Table 4.12 and Figure 4.15), *ai1* was the most common haplotype but it was not detected in the KK (Kuala Kurau) and KG (Kuala Gula) populations. However, *ai4* was the only haplotype found in the latter two populations and it was also present in BL (Bagan Lipas) and PSETT (Portugues Settlement). Moderate level of haplotye diversity (h = 0.552) and high nucleotide diversity (π = 0.031) were observed in overall samples. In addition, low levels of *h and* π in the two distinct clades (*i.e.,* clade *ai-I*, h = 0.286, π = 0.001; clade *ai-II*, h = 0.228, π = 0.001), were observed.

For *A. serrulatus* (Table 4.13 and Figure 4.16)*,* all sites shared the most frequent haplotype, *i.e.*, *as1*, followed by *as2* and *as8*. Haplotype *as7* was shared by SGT (Sungai Tiang) and BPL (Bagan Pasir Lipas), while *as18* was shared by BPL (Bagan Pasir Lipas) and SGK (Sungai Kapal), respectively. The remaining haplotypes were found in only one locality and *as7* was relatively divergent from the rest of the haplotypes. The overall haplotype diversity (h = 0.886) was high, ranging from 0.700 in PKKP (Pulau Kukup) to 1.000 in TKR (Tanjong Karang) and SGK (Sungai Kapal). However, the overall nucleotide diversity (π) was low (0.004), ranging from 0.002 in SKC (Sekinchan) to 0.006 in SGT (Sungai Tiang), respectively.

For *A. japonicus* (Table 4.14 and Figure 4.17), both haplotypes *aj1* and *aj2* were shared by KK, KG and TBHG (Teluk Bahang). A moderate level of haplotye diversity (h = 0.540) and low level nucleotide diversity (π = 0.001) were observed. The *h* and π values were highest for KK, followed by KG and TBHG.

For *A. sibogae*, only 2 haplotypes were present in the 12 samples analysed, namely, *asi1* and *asi2* (Table 4.15 and Figure 4.18). Haplotype *asi1* occurred in both SGKB (Sungai Kubang Badak) and KS (Kuala Sepetang), while *asi2* was only observed in SGKB, but not in KS.

Table 4.12: Haplotype compositions and summary of molecular diversity in *Acetes indicus* collected in this study.

Haplotype	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	0.250	0.000	0.286	0.000	0.000	0.700	0.286	0.333	0.893	0.400	0.000	0.552
π	0.000	0.000	0.023	0.000	0.000	0.002	0.001	0.001	0.045	0.001	0.000	0.031

*Abbreviation of sampling locations refer to Table 3.1
n: number of sequences;
S: number of segregating sites;
N_{hap}: number of haplotypes;
h: haplotype diversity;
π: nucleotide diversity.

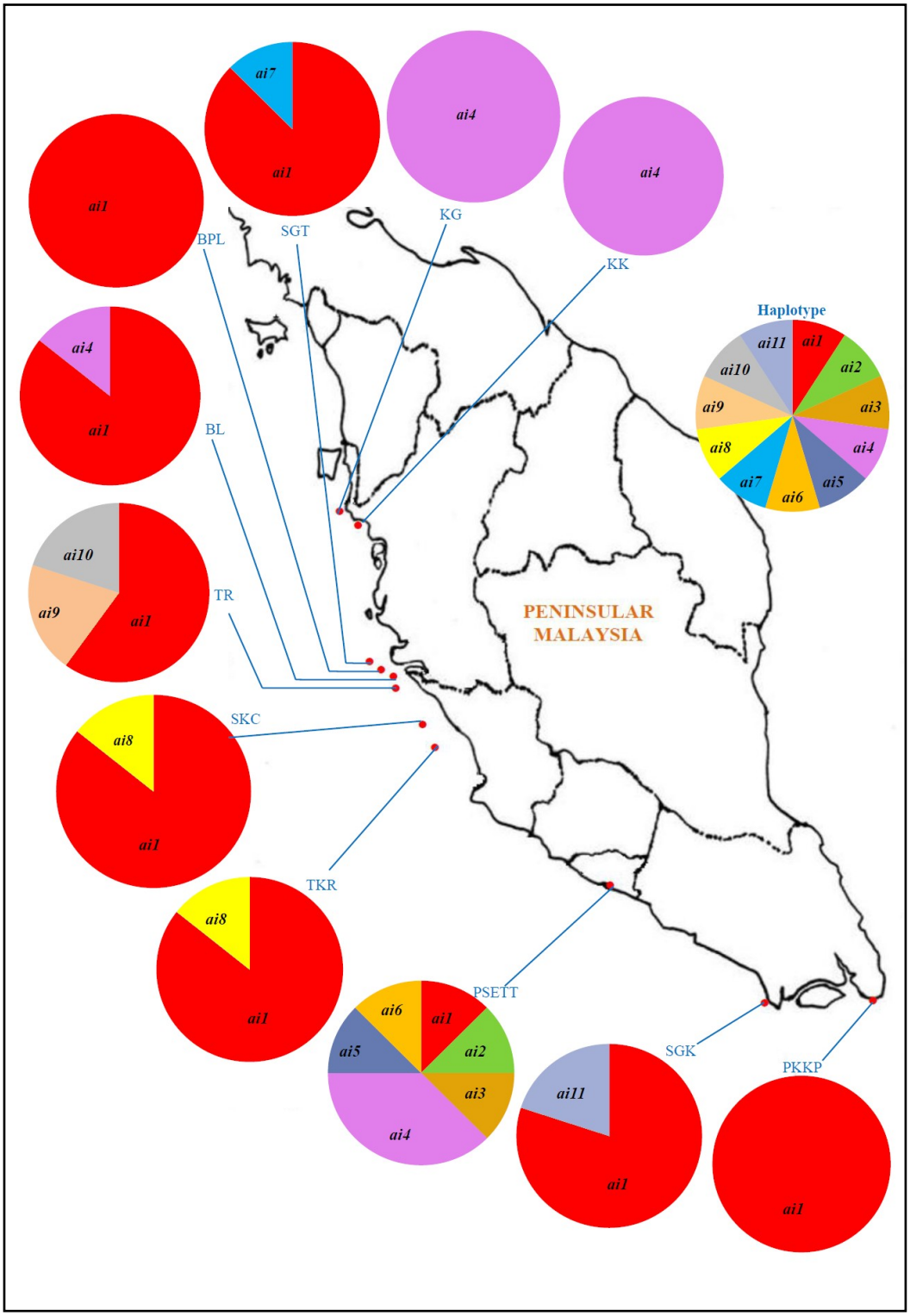


Figure 4.15 Haplotype distribution of *Acetes indicus* collected in this study.
• sampling locations

Table 4.13: Haplotype compositions and summary of molecular diversity in *Acetes serrulatus* collected in this study.

Haplotype	Sampling location*								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	0.956	0.914	0.857	0.714	1.000	0.900	0.700	1.000	0.886
π	0.006	0.005	0.002	0.002	0.005	0.003	0.003	0.005	0.004

*Abbreviation of sampling locations refer to Table 3.1
n: number of sequences;
S: number of segregating sites;
N_{hap}: number of haplotypes;
h: haplotype diversity;
π: nucleotide diversity.

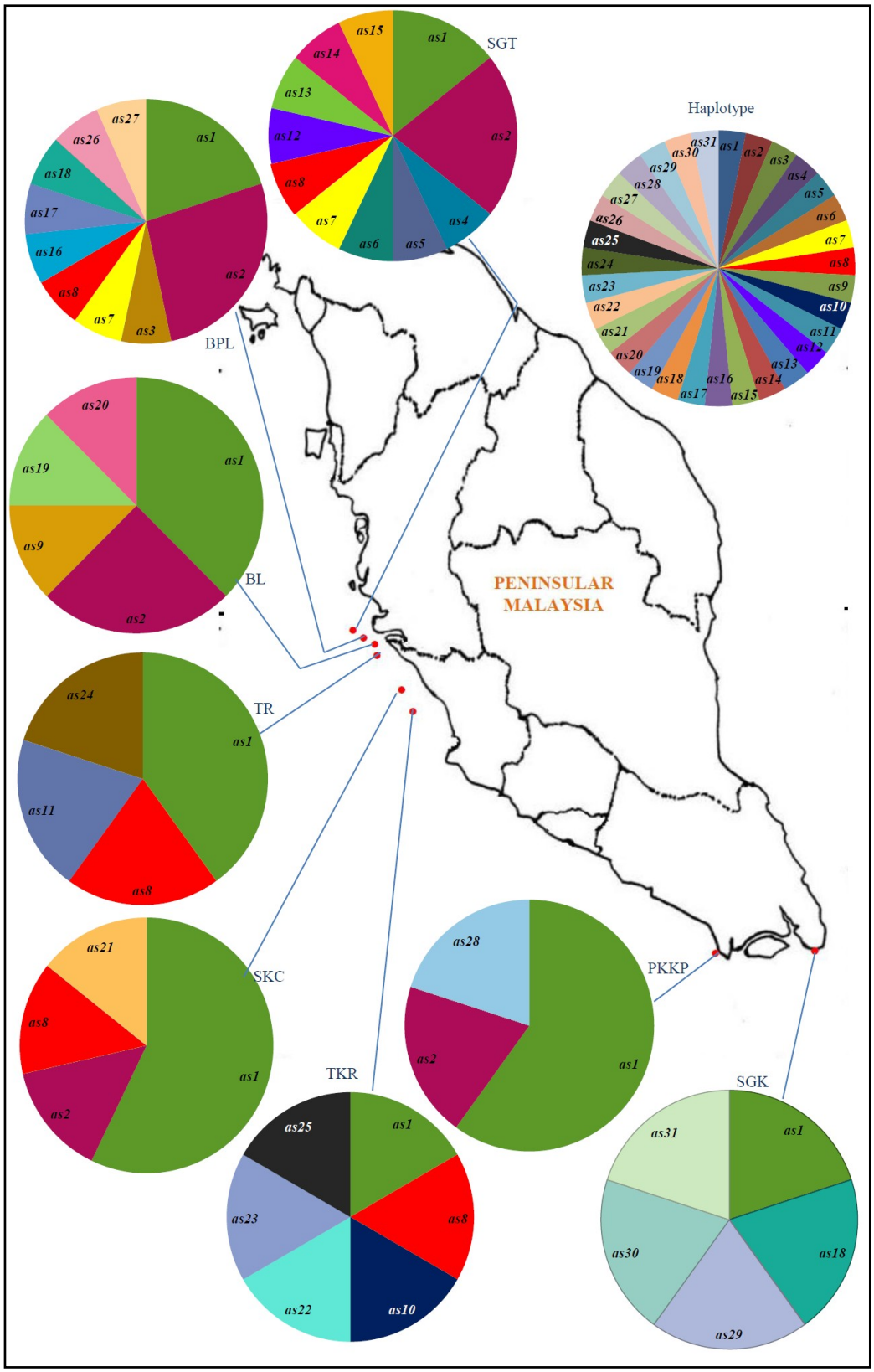


Figure 4.16 Haplotype distribution of *Acetes serrulatus* collected in this study.
• sampling locations

Table 4.14: Haplotype compositions and summary of molecular diversity in *Acetes japonicus* collected in this study.

Haplotype	Sampling location*			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	1.000	0.600	0.533	0.539
π	0.002	0.001	0.001	0.001

*Abbreviation of sampling locations refer to Table 3.1
n: number of sequences;
S: number of segregating sites;
N_{hap}: number of haplotypes;
h: haplotype diversity;
π: nucleotide diversity.

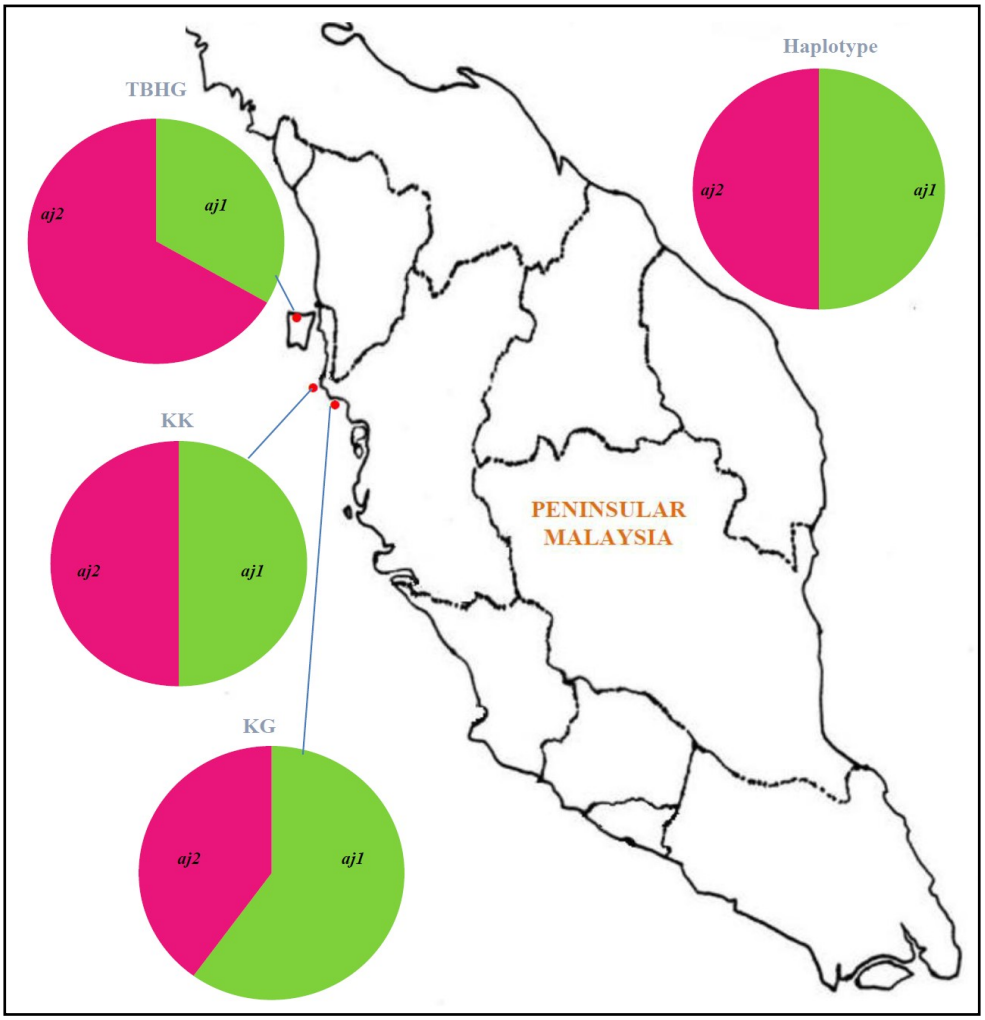


Figure 4.17 Haplotype distribution of *Acetes japonicus* collected in this study. • sampling locations

Table 4.15: The haplotype compositions and summary of molecular diversity in *Acetes sibogae* collected in this study.

Haplotype	Sampling location *		Total
	SGKB	KS	
asi1	5	6	11
asi2	1	-	1
n	6	6	12
S	52	0	52
N_{hap}	2	1	2
h	0.333	0.000	0.167
π	0.031	0.000	0.016

*Abbreviation of sampling locations refer to Table 3.1
n: number of sequences;
S: number of segregating sites;
N_{hap}: number of haplotypes;
h: haplotype diversity;
π: nucleotide diversity.

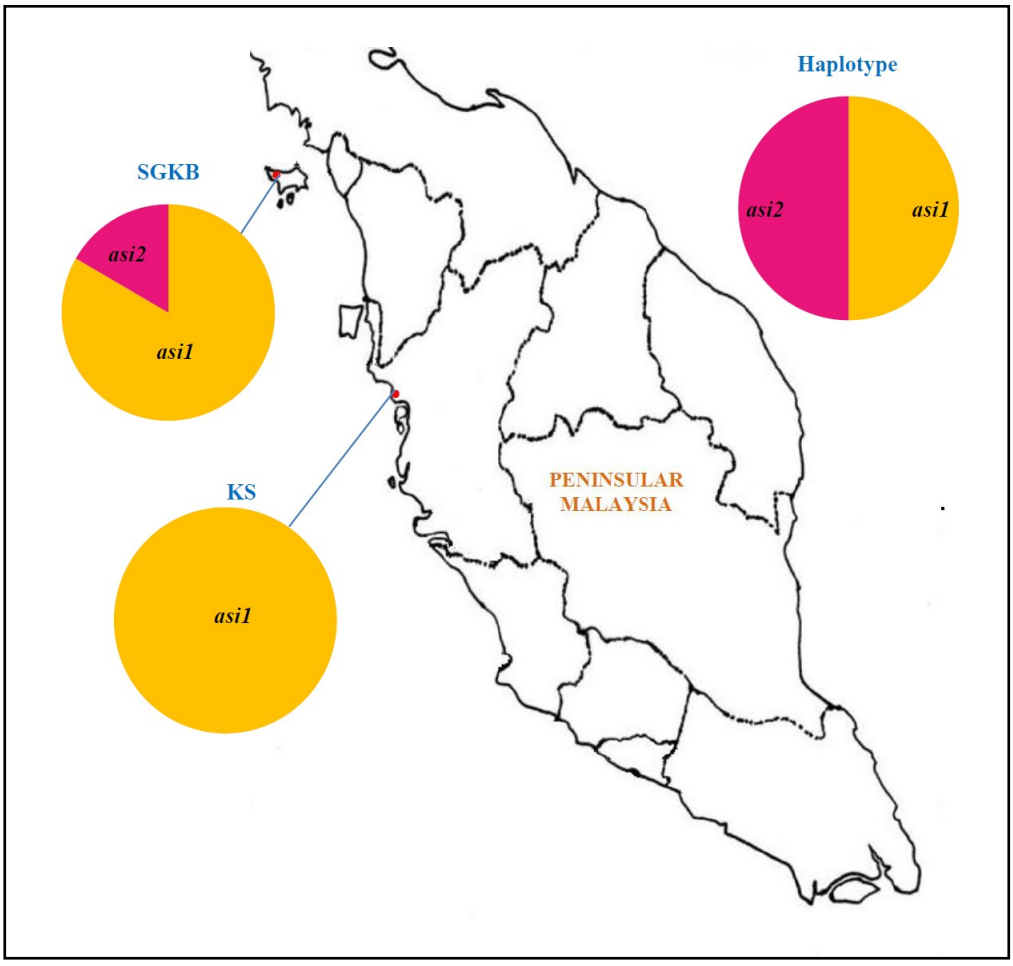


Figure 4.18 Haplotype distribution of *Acetes japonicus* collected in this study.
• sampling locations

4.8.2 Haplotype Network

In the statistical parsimony network computed in TCS (Figure 4.19), 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 according to a 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.

The network for *A. serrulatus* (Figure 4.18b) was unique, with sequences differing by as many as nine substitutions able to be connected in a parsimonious fashion with 95% probability. The network exhibited a star-like shape, with most haplotypes connected to a single common haplotype (*as1*).

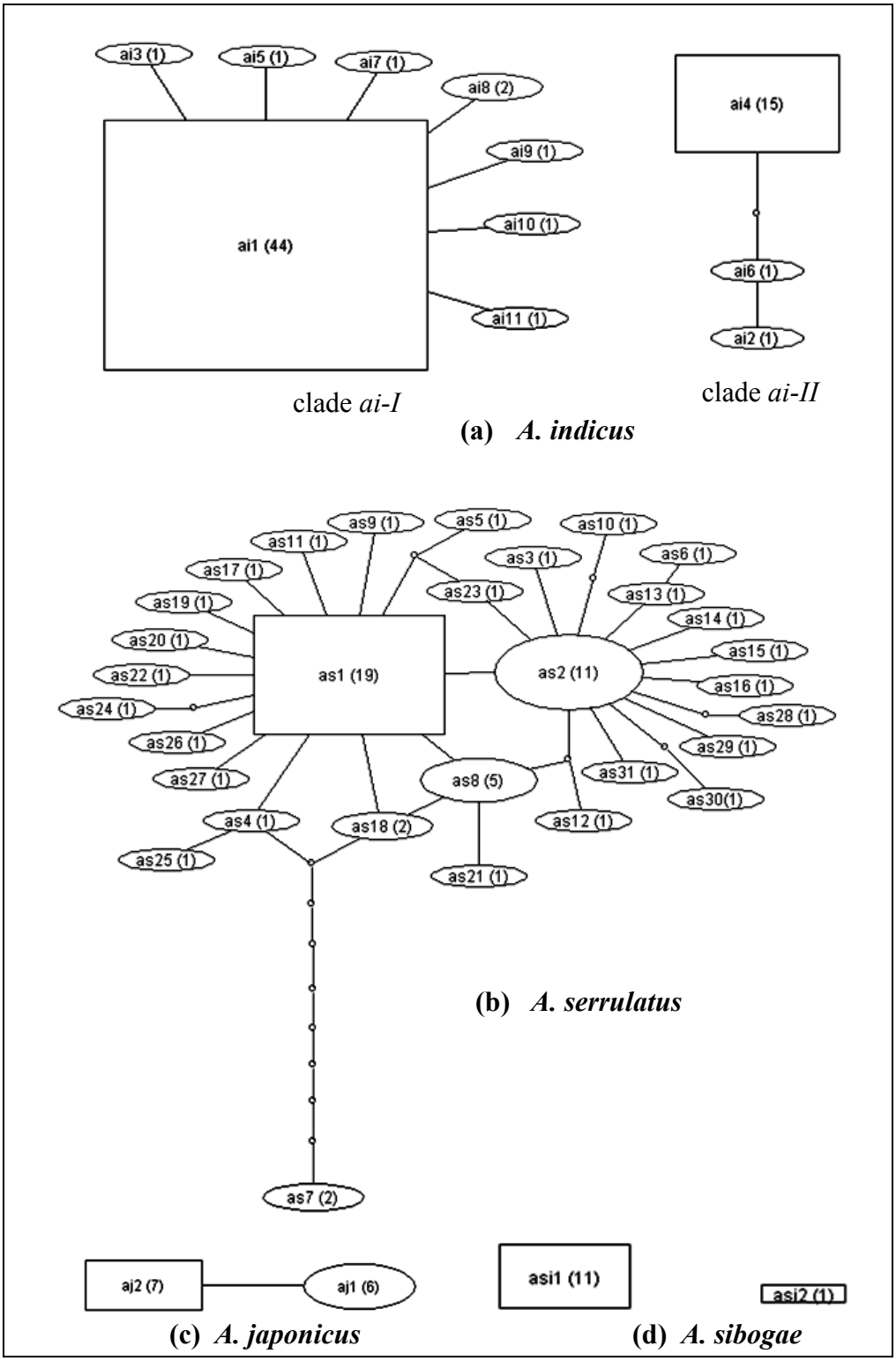


Figure 4.19: Parsimony network of (a) *A. indicus* (b) *A. serrulatus* (c) *A. japonicus* and (d) *A. sibogae* based on 552 bp of *COI* amplified in this study. Each oval represents a haplotype, and the haplotype in a square has the highest outgroup probability. The size of the oval or square corresponds to the haplotype frequency. The haplotype abbreviations correspond to the haplotypes as reported in Table 4.12–4.15, and the number in parentheses correspond to the frequency of the haplotype. Small circles indicate the number of mutational changes among haplotypes.

4.8.3 Population Structure

AMOVA results for each *Acetes* species are reported in Table 4.16. For *Acetes indicus*, significant population differentiation was observed (Φ_{ST} = 0.755; P = 0.000), with 75.50% of the molecular variance owing to variance among the sampling locations. Besides, AMOVA also revealed significant differentiation between the two clades (Φ_{ST} = 0.992; P = 0.000). Approximately 99.20% of the genetic variation was between clades *ai-I* and *ai-II* and variation within the clade explained the rest (0.81%). The pairwise Φ_{ST} statistics (Table 4.17) for KK and KG with other populations were high and statistically significant, with the exception of PSETT. In contrast, Φ_{ST} estimates between PSETT and all other populations were highly significant. All other pairwise comparisons showed low or negative Φ_{ST}, with no significant differences. The Mantel Test indicated that there was no correlation between Φ_{ST} estimatesand geographical distance (r = 0.106, P > 0.05).

For the remaining three species, AMOVA analysis (Table 4.16) revealed that 100% of the genetic variation was present within sampling populations and the overall Φ_{ST} value was not significant for *A. serrulatus* (Φ_{ST} = -0.0184; P = 0.785), *A. japonicus* (Φ_{ST} = - 0.203; P = 0.763) and *A. sibogae* (Φ_{ST} = 0.000; P = 1.000), respectively. The pairwise Φ_{ST} values (Tables 4.18 – 4.20) were generally low and were statistically significant (P > 0.05) for *A. serrulatus* (-0.062 – 0.110), *A. japonicus* (-0.504 – -0.060) and *A. sibogae* (0.000). In addition, none of the pairwise Φ_{ST} values for these three species showed significant results.

Table 4.16: 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	Percentage of variation	Fixation index	*P* value
Acetes indicus	Among populations	10	455.260	6.92165	75.46	$\Phi_{ST} = 0.755$	0.000 ± 0.000
	Within populations	58	130.537	2.25064	24.54		
		68	585.797	9.17229			
Acetes indicus	Among clade	1	573.519	22.37565	99.19	$\Phi_{ST} = 0.992$	0.000 ± 0.000
(clade ai-I and ai-II)	Within clade	67	12.278	0.18326	0.81		
		68	585.897	22.55891			
Acetes serrulatus	Among populations	7	7.059	- 0.02119	- 1.84	$\Phi_{ST} = - 0.0184$	0.785 ± 0.00402
	Within populations	57	66.987	1.17521	101.84		
		64	74.046	1.15402			
Acetes japonicus	Among populations	2	0.197	- 0.05115	- 20.28	$\Phi_{ST} = - 0.203$	0.763 ± 0.00402
	Within populations	10	3.033	0.30333	120.28		
		12	3.231	0.25218			
Acetes sibogae	Among populations	1	4.333	-0.00000	-0.00	$\Phi_{ST} = 0.000$	1.000 ± 0.00000
	Within populations	10	43.333	4.33333	100.00		
		11	47.667	4.33333			

Table 4.17: Pairwise Φ_{ST} values (pairwise difference) among *Acetes indicus* sampling populations calculated from *COI* sequences using Arlequin v 3.5.

Sampling population[1]	1 SGT	2 BPL	3 BL	4 KK	5 KG	6 TR	7 SKC	8 TKR	9 PSETT	10 PKKP	11 SGK
1 SGT											
2 BPL	-0.0182 ^{ns}										
3 BL	0.0139 ^{ns}	0.000 ^{ns}									
4 KK	0.997 ***	1.000 ***	0.804 *								
5 KG	0.997 ***	1.000 **	0.820 **	0.000 ^{ns}							
6 TR	0.0476 ^{ns}	0.0729 ^{ns}	-0.0574 ^{ns}	0.991 **	0.992 **						
7 SKC	0.00129 ^{ns}	0.000 ^{ns}	0.000 ^{ns}	0.996 **	0.997 ***	0.0318 ^{ns}					
8 TKR	0.00656 ^{ns}	0.0278 ^{ns}	-0.0232 ^{ns}	0.996 **	0.997 **	0.0157 ^{ns}	-0.181 ^{ns}				
9 PSETT	0.563 **	0.546 **	0.296 *	0.194 ^{ns}	0.227 ^{ns}	0.484 *	0.543 **	0.519**			
10 PKKP	0.0198 ^{ns}	0.0729 ^{ns}	-0.0523 ^{ns}	0.996**	0.996 **	-0.000 ^{ns}	0.0107 ^{ns}	0.00339 ^{ns}	0.489 *		
11 SGK	-0.0687 ^{ns}	0.000 ^{ns}	-0.0553 ^{ns}	1.000 **	1.000 **	-0.000 ^{ns}	-0.0553 ^{ns}	-0.0345 ^{ns}	0.492 *	-0.000 ^{ns}	

[1] Abbreviation for the sampling population as shown in Table 1
Significance level: ^{ns} not significant; *0.01<*P*<0.05; **0.001<P<0.01; ****P*<0.0001

Table 4.18: Pairwise Φ_{ST} values (pairwise difference) among *Acetes serrulatus* sampling populations calculated from *COI* sequences using Arlequin v 3.5.

Sampling population[1]	1 SGT	2 BPL	3 BL	4 SKC	5 TKR	6 TRHU	7 PKKP	8 SGK
1 SGT								
2 BPL	-0.0331 ^{ns}							
3 BL	0.0163 ^{ns}	-0.0185 ^{ns}						
4 SKC	0.0183 ^{ns}	-0.0176 ^{ns}	0.00943 ^{ns}					
5 TKR	-0.0487 ^{ns}	-0.0415 ^{ns}	-0.0141 ^{ns}	-0.0289 ^{ns}				
6 TRHU	0.0380 ^{ns}	0.00497 ^{ns}	0.0382^{ns}	-0.0404 ^{ns}	-0.0103 ^{ns}			
7 PKKP	-0.0491 ^{ns}	-0.0620 ^{ns}	-0.0459 ^{ns}	0.0360 ^{ns}	-0.0609 ^{ns}	0.0625 ^{ns}		
8 SGK	-0.0607 ^{ns}	-0.0482 ^{ns}	0.0525 ^{ns}	0.0622 ^{ns}	-0.0397 ^{ns}	0.110 ^{ns}	-0.0417 ^{ns}	

[1] Abbreviation for the sampling population as shown in Table 1
Significance level: ^{ns} not significant; *0.01<*P*<0.05; **0.001<*P*<0.01; ***P<0.0001

Table 4.19: Pairwise Φ_{ST} values (pairwise difference) among *Acetes japonicus* sampling populations calculated from *COI* sequences using Arlequin v 3.5.

Sampling population[1]	1 TBHG	2 KG	3 KK
1 TBHG			
2 KG	-0.0605 ^{ns}		
3 KK	-0.404 ^{ns}	-0.504 ^{ns}	

[1] Abbreviation for the sampling population as shown in Table 1
Significance level: ^{ns} not significant; *0.01<*P*<0.05; **0.001<*P*<0.01; ***P<0.0001

Table 4.20: Pairwise Φ_{ST} values (pairwise difference) among *Acetes sibogae* sampling populations calculated from *COI* sequences using Arlequin v 3.5.

Sampling population[1]	1 SGKB	2 KS
1 SGKB		
2 KS	-0.000 ^{ns}	

[1] Abbreviation for the sampling population as shown in Table 1
Significance level: *0.01<*P*<0.05; **0.001<*P*<0.01; ***P<0.0001; ^{ns} not significant.

4.8.4 Neutrality Tests and Mismatch Analysis

The neutrality statistics, mismatch distributions, Harpending's raggedness index (*r*), and sum of squared deviations (SSD) for *COI* data from *A. indicus*, *A. serrulatus*, *A. japonicus* and *A. siboage* are shown in Figure 4.20.

For *A. indicus* (Figure 4.20a), none of the neutrality tests showed significant results when the samples were pooled. However, when the neutrality test was applied separately to clades *ai-I* (Figure 4.20b) and *ai-II* (Figure 4.20c), all tests was significant for *ai-I*, while only R_2 was significant for *ai-II*. The neutrality tests applied to *A. serrulatus* (Figure 4.20d) gave significant results, in contrast with *A. japonicus* (Figure 4.20e). For *A. sibogae*, only Tajima's *D* was negative and highly significant (Figure 4.20f).

The mismatch distribution for *A. indicus* (Figure 4.20a) was clearly bimodal but did not differ significantly from the distribution expected under population expansion (*SSD* = 0.112, $P > 0.05$). However, a unimodal distribution (Figure 4.20b and c) that did not differ significantly from the distribution expected under population expansion was observed in each separate analysis for the two clades *ai-i* (*SSD* = 0.112, $P > 0.05$) and *ai-II* (*SSD* = 0.040, $P > 0.05$). Both clades *ai-I* and *ai-II* showed similar results in which the peak of the mismatch distribution for the number of nucleotide substitutions was close to zero (*i.e.*, L-shaped mismatch distribution). Similarly, this was noted for *A. serrulatus* (*SSD* = 0.004, $P > 0.05$) and *A. japonicus* (*SSD* = 0.032, $P > 0.05$) in Figure 4.20d and e. A bimodal mismatch distribution was observed for *A. sibogae* (Figure 4.20f).

Based on the τ value computed in Arlequin v3.5, and the 1.40% and a 3.00% mutation rates, the estimates of the time since the most recent sudden population expansion for *A. indicus* clades *ai-I* and clade *ai-II* was approximately 97,000 – 45,000 years ago, and for *A. serrulatus* was 61,000 – 28,000 years ago (Table 4.21).

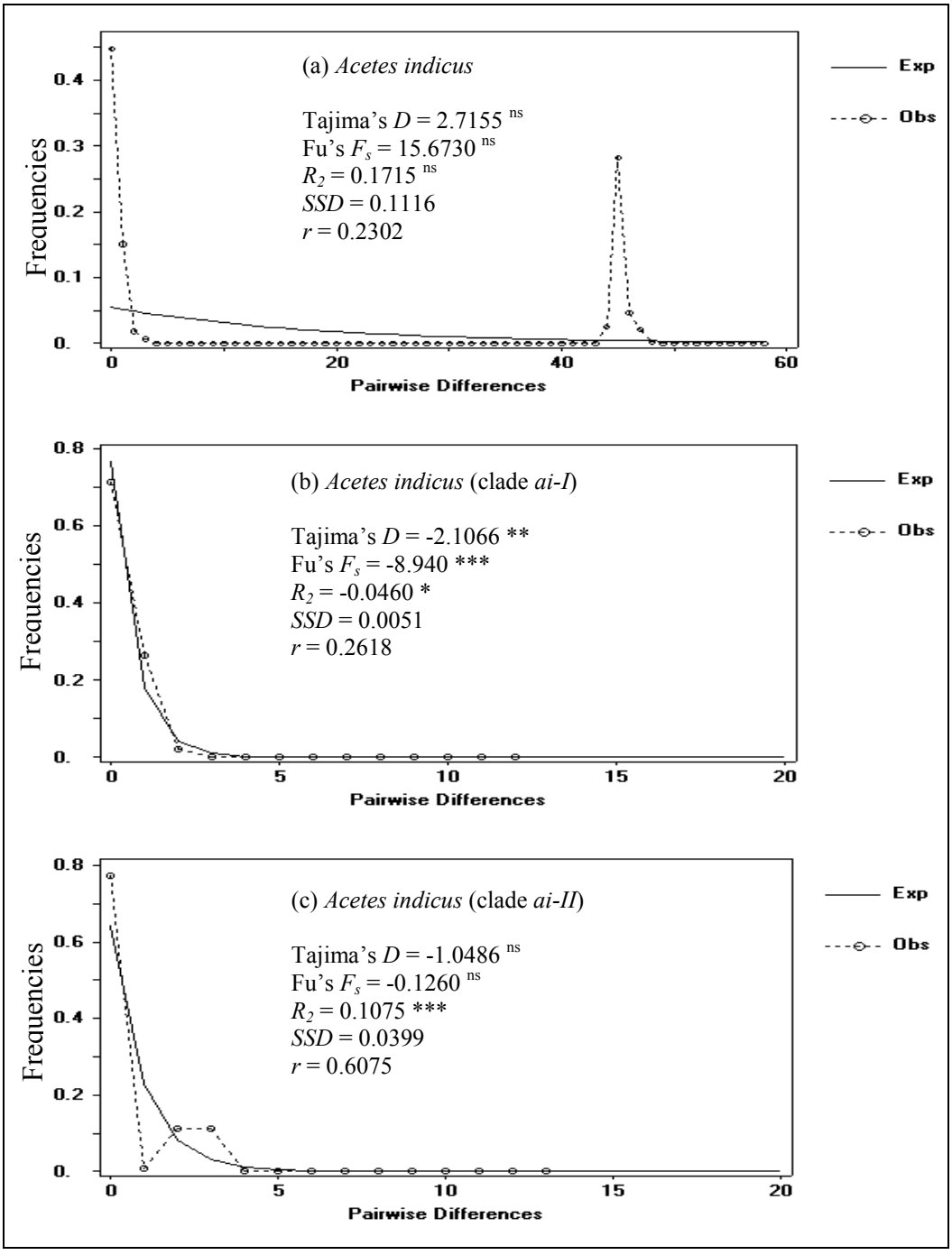


Figure 4.20: Mismatch distribution based on *COI* sequence from (a) *A. indicus* (b) *A. indicus*, clade *ai-I* (c) *A. indicus*, clade *ai-II* (d) *A. serrulatus* (e) *A. japonicus* (f) *A. sibogae*. The graph represents the observed mismatch distribution from segregating sites of the aligned *COI* sequences. Dotted lines show 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. Neutrality statistics (Tajima's *D*, Fu's F_s, R_2), sum of square deviation (*SSD*) and Harpending's Raggedness index (*r*) were reported as well. (*0.01<*P*<0.05; **0.001<P<0.01; ***P<0.001; ^{ns}, not significant)

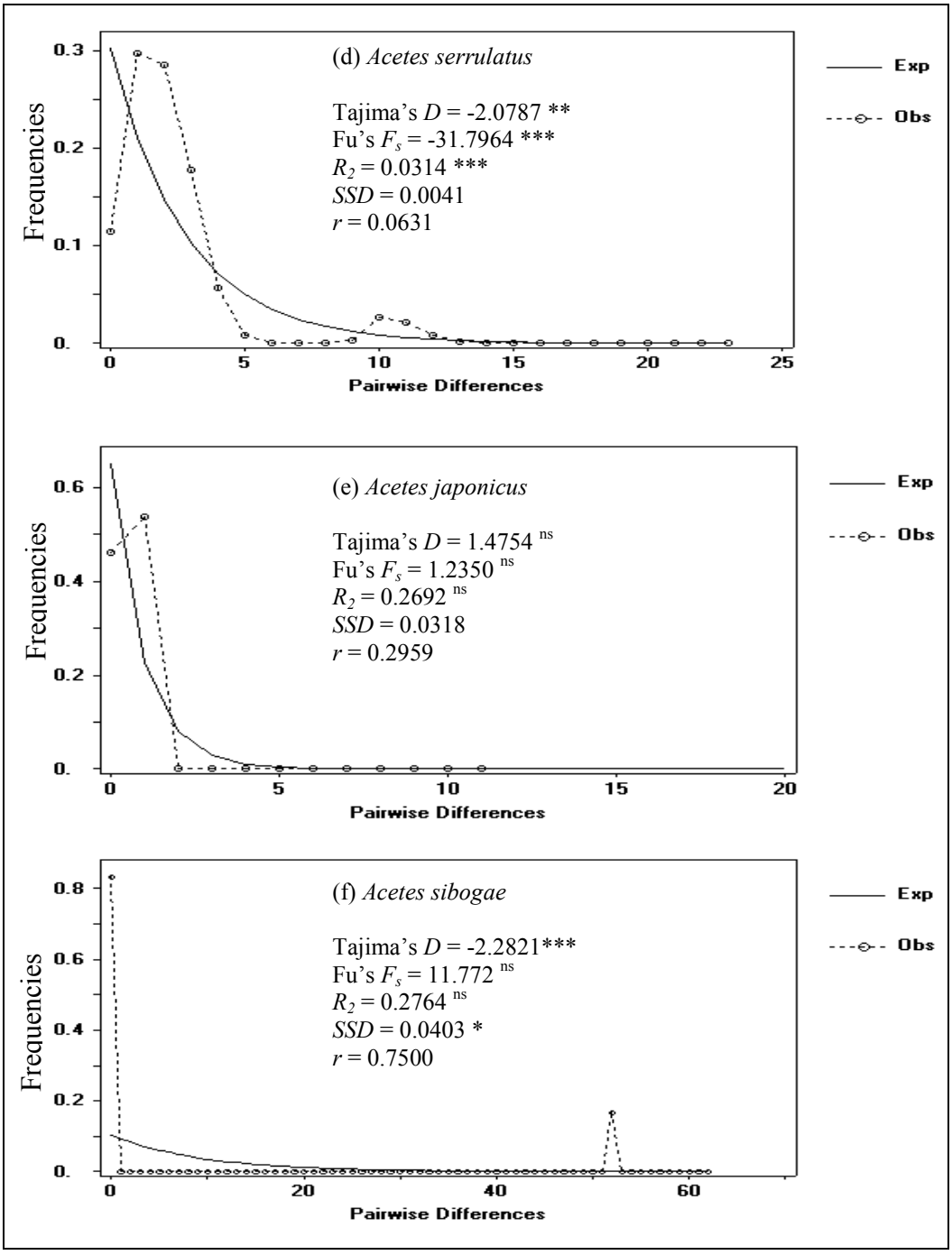


Figure 4.20 (continued): Mismatch distribution based on *COI* sequence from (a) *A. indicus* (b) *A. indicus*, clade *ai-I* (c) *A. indicus*, clade *ai-II* (d) *A. serrulatus* (e) *A. japonicus* (f) *A. sibogae*. The graph represents the observed mismatch distribution from segregating sites of the aligned *COI* sequences.Dotted lines show 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. Neutrality statistics (Tajima's *D*, Fu's F_s, R_2), sum of square deviation (*SSD*) and Harpending's Raggedness index (*r*) were reported as well. (*0.01<*P*<0.05; **0.001<P<0.01; ***P<0.001; ^{ns}, not significant).

Table 4.21: Result of mismatch distribution for *Acetes indicus* and *A. serrulatus*. Parameters of population expansion obtained from mismatch distribution analyses include: age of expansion in units of mutational time (τ), mutation parameter before (θ_0) and after (θ_1) the expansion in units of mutational time, age of expansion (t) in years before present (B. P.) calculated using 1.40 % and 3.00 % pairwise sequence divergence rate [95% confidence interval].

`

Species	n	Parameters estimated under the sudden expansion model					t * (based on 1.40 % mutation rate)	t * (based on 3.00 % mutation rate)
		Mismatch observed mean	Mismatch observed variance	Tau	θ_0	θ_1		
Acetes indicus (clade *ai-I*)	52	0.306 [0.000 – 1.551]	0.253	3.000 [0.000 – 3.500]	0.000 [0.000 – 0.007]	0.421 [0.000 – 99999.000]	97,049 [0.000 – 113,225]	45,290 [0.000 – 52,838]
A. indicus (clade *ai-II*)	17	0.559 [0.000 – 1.000]	1.137	3.000 [0.498 – 3.500]	0.000 [0.000 – 0.217]	0.203 [0.000 – 99999.000]	97,049 [16,165 – 113,225]	45,290 [7,518 – 52,838]
A. serrulatus	65	2.314 [1.445 – 2.487]	5.719	1.908 [1.246 – 2.549]	0.004 [0.000 – 0.438]	99999.000 [6.314 – 99999.000]	61,724 [40,308 – 82,460]	28,804 [18,810 – 38,481]

n: sample size;
t*: expansion time based on two generation per year

CHAPTER 5.0

DISCUSSION

5.1 Sex and Species Identification

The sexes of *Acetes* sp. were distinguished prior to morphological species identification. A pair of protuberances (genital coxae) between the third pereiopods and first pleopods in males is a unique character for sex identification. In addition, a petasma and lower antenullar flagellum with spine(s) were observed in males, but were absent in females. Specimens obtained for this study could be easily classified as *Acetes indicus*, *A. serrulatus*, *A. japonicus* and *A. sibogae*, based on agreement with key characters described by Omori (1975b) for the four species, respectively (Figure 4.2–4.5). This is similar to the studies conducted by Amin *et al.,* (2011), Arshad *et al.,* (2007), and Amin *et al.,* (2008b), in which *Acetes* spp. collected from Malaysian waters could all be differentiated into *A. intermedius*, *A. indicus*, *A. japonicus*, *A. intermedius*, *A. vulgaris*, and *A. serrulatus* based on key taxonomic characters identified by Omori (1975b).

The four *Acetes* species all showed distinctive form and structure for three distinguishing morphological characters – the structure of the petasma and the lower antennular flagellum in males, and the third thoracic sternite (basic of third leg) in females (Figure 4.2–4.5). Observations here agreed with those of Amin *et al.,* (2011), Kemp (1917), Omori (1975b) and Tham (1955), and so these three morphological characters are considered to be reliable and distinctive specific identification characters for each taxon.

5.2 Distributions of *Acetes* species

A. indicus was identified from most locations sampled in the current study. This agrees with findings of previous studies, where *A. indicus* was reported from the north-western regions of Peninsular Malaysia, including from estuarine waters of Merbok River, Kedah (Amani et al., 2011c), Kuala Gula (Amin et al., 2011), Matang mangroves in Perak (Fernandez-Leborans et al., 2009), to the south-western regions: coastal waters of Klebang Besar in Malacca (Amin et al., 2009a; Amin et al., 2009b; Amin et al., 2010a; Amin et al., 2008a; Amin et al., 2009c) and Pontian in Johor (Oh et al., 2010). It should be noted that the samples collected from earlier studies were from in-shore regions and sampling had not been conducted from off-shore regions. Importantly, in the current study, *A. indicus* was identified for the first time from off-shore regions at SGT, BPL, BL, TR, SKC, and TKR (Table 4.1).

For *A. serrulatus*, samples were identified from the central-western and the south-western regions of Peninsular Malaysia (Table 4.1). Previously, this species was reported in the in-shore waters of Pontian (Amin et al., 2011; Oh et al., 2010) and Kukup (Oh et al., 2011) in the south-western region of Peninsular Malaysia. Both are in-shore region as well. Thus, in current study, *A. serrulatus* is reported for the first time to also be present in off-shore regions in central-western Peninsular Malaysia.

Presence of *A. japonicus* and *A. sibogae* in the north-west of Peninsular Malaysia in the current study (*i.e.,* SGKB, KS, and TBHG) (Table 4.1) is similar to reports of the same species in coastal and estuarine waters of Perlis,

Penang, Kedah (Amani et al., 2011a; Amani et al., 2011b; Amani et al., 2011c; Amin et al., 2011; Arshad et al., 2012; Pathansali, 1966) and Merbok and Matang mangrove estuaries in Perak (Fernandez-Leborans et al., 2009; Hanamura, 2007; Hanamura et al., 2007). Although, *A. japonicus* had been reported in the south-western region, including from Klebang Besar in Malacca (Amin et al., 2010a; Amin et al., 2008a; Amin et al., 2009c; Amin et al., 2009d) and Pontian in Johor (Oh et al., 2010), it was not detected at the south-western region (*i.e.,* PSETT, PKKP, and SGK) in the current study that may be due to a different sampling strategy. As an example, samplings of *Acetes* spp. were done fortnightly from April 2006 to March 2007 (Amin et al., 2008a) and monthly from June to November, 2007 (Oh et al., 2010), but only sampled once in PSETT, PKKP and SGK throughout the sampling period in current study. In addition, a relatively lower sample size of *Acetes* spp. in current study, (*i.e.*, 40, 74, and 65 in PSETT, PKKP, and SGK, respectively) as compared to a total of 804 specimens of *Acetes* spp. in Oh et al., (2010). So, it is very possible that with our sampling frequency and low sample size might affected the possibility of detecting *A. japonicus* in this study.

5.3 Morphometric Analysis

5.3.1 Size Dimorphism with Sex

From the comparison of measurements between females and males of each species (Table 4.2), females were significantly larger than males, suggesting sexual dimorphism for adult size. These results is in agreement with the earlier studies on other local populations of *Acetes* species (*Acetes indicus*, Amin et al., 2009a; *A. japonicus*, Amin et al., 2009d; *A. intermedius*, Arshad et al.,

2007; *A. chinensis*, Oh and Jeong, 2003), as well as in some other shrimp species, including the Jack-knife shrimp, *Haliporoides sibogae* (Ohtomi and Matsuoka, 1998), Pacific white shrimp, *Litopenaeus vannamei* (Moss and Moss, 2006), brown shrimp, *Artemesia longinaris* (Castilho et al., 2007) and white prawn, *Palaemon longirostris* (Cartaxana, 2003). Although the implications for sexual dimorphism in *Acetes* are still unknown, several potential explanations have been proposed. A parallel situation is evident in the sculptured shrimp (*Sclerocrangon boreas*), where onset of sexual maturity occurs earlier in the males than in the females potentially leading to smaller body size of males (Sainte-Marie et al., 2006). For the penaeid shrimp, *Trachysalambria curvirostris*, fecundity of females increases exponentially with body size, and the larger body size of females has been inferred by Yamada *et al.*, (2007) to be an adaptation to increase overall egg production. This is similar to reports for the Baltic prawn (*Palaemon adspersus*) and Mediterranean prawn (*P. squilla*), where larger body size of females provides for higher egg carrying capacity (Berglund, 1981). Conversely, the smaller body size observed in males provides some advantages by potentially reducing the costs of locomotion (*i.e.*, enhanced ability of finding and inseminating as many females as possible) and predation pressure (Berglund, 1981).

5.3.2 Sex Ratio

According to Xiao and Greenwood (1993), the sex ratio of *Acetes* spp. commonly deviates from a 1:1 ratio, in which females in general seem to occur more frequently in catches than do males. In the current study, the sex ratio of four *Acetes* species all deviated significantly from 1:1 as well. Sex ratios for *A.*

indicus, *A. serrulatus*, and *A. japonicus* favoured of females over the sampling period, while that of *A. sibogae* was favoured of males. Earlier studies showing that higher proportions of females were also observed for *A. indicus, A. intermedius*, *A. japonicus*, *A. chinensis* (Amin et al., 2009b; Amin et al., 2010b; Arshad et al., 2007; Oh and Jeong, 2002; Oh and Jeong, 2003; Zhong et al., 2001). In contrast, a male-biased sex ratio in *A. sibogae* also noted for *A. vulgaris* in Pontian, Johor, Malaysia (Arshad et al., 2008), and for *A. intermedius* in Bintulu, Sarawak, Malaysia (Amin et al., 2008b).

A skewed sex ratio can be related to a number of potential factors, including differential growth rates, mortality, and behavior of the different sexes in shrimp populations. As shown by Oh and Jeong (2003), faster growth rate of females can bias proportions toward females in catches (*i.e*., the proportions of females increased logistically with carapace length), because their greater average size results in higher mesh-size selection and thus dominance in fishery catches. During the spawning season, females may be more common than males (Zhang, 1992) due to different mortality rates between the sexes after spawning. The life span is also shorter in males compared with females by 15–30 days (Lei, 1984). Both of these situations can lead to higher abundances of females. In addition, a skewed sex ratio may result from 'spatial sexual segregation' (Xiao and Greenwood, 1993), as has been reported for the female to male ratio of *A. chinensis* in Laizhou Bay and southern Pohai that appear to increase logistically with total body length from slightly over 30% at a body length of about 6 mm to unity at a body length of about 34 mm (Zhang, 1992).

5.3.3 Comparison between In-shore and Off-shore Samples

Some species of *Acetes* have been shown to migrate between different habitats (*i.e.*, in-shore and off-shore) in order to complete their life cycle (Xiao and Greenwood, 1993) as demonstrated for *A. japonicus* in the Ariake Sea, Japan (Omori, 1975b), *A. chinensis* in the western areas of the Bohai Sea (Feng et al., 1982) and in-shore waters of southern Zhejiang in China (Shi, 1986). Shrimps apparently move shoreward to spawning grounds (*i.e.* shallow, in-shore areas, coasts and estuaries) for spawning, and subsequently leave in-shore areas to wintering grounds (*i.e.*, deep waters, off-shore areas) at the end of autumn. Thus, the significant differences between in-shore and off-shore samples observed here for *A. indicus* and *A. serrulatus* observed in this study (Table 4.3) could result from temporal migrations of *Acetes* species between different habitats. The size ranges of *A. indicus* collected from in-shore areas were generally smaller than off-shore areas and this may reflect the fact that the young *A.indicus* adults and juveniles that were heading to deeper coastal waters after growing up in estuaries and mangrove swamps. Conversely, since the sizes of *A. serrulatus* collected in-shore were larger than off-shore samples, the in-shore samples could be gravid or fertilized adult females that were heading to estuaries and mangroves in order to lay their eggs. In addition, migration between in-shore and off-shore habitats might be affected by food availability and environmental factor (*i.e.*, rainfall) as well (Chiou et al., 2000). The adults of *A. intermedius* were reported to migrate from estuaries to deeper off-shore in the summer (*i.e.*, when river discharges increased due to heavy rainfall) and this behavior may reduce competition for food between adults and their offspring.

5.3.4 Comparison among Species

In this study, size range of *A. indicus* was the largest of the species examined here (Figure 4.9, Table 4.4) and was also within the size range reported for *A. indicus* individuals collected from Klebang Besar near Malacca in Malaysia (Amin et al., 2009a; Amin et al., 2009b). Pathansali (1966) and Holthuis (1980) reported that *A. japonicus* was the smallest of the *Acetes* recorded in Peninsular Malaysia, as was the same here. To date, data on morphometric variation in *Acetes* spp. is only available from local populations of Malacca (Amin et al., 2010a) that reported significant differences among *A. japonicus*, *A. intermedius* and *A. indicus* population detected for the measurements of total length and carapace length.

5.4 Length-Weight Relationships (LWRs) and Length-Length Relationships (LLRs)

Hypothesis testing of *b* value against isometric growth use the *t*-test instead of normal distribution (*z*-test) on similar studies not only shrimp and prawn (Abohweyere and Williams, 2008; Arshad et al., 2007; Cartaxana, 2003; Diaz et al., 2001; Gökoğlu et al., 2008; Mgaya and Teikwa, 2003; Pérez-Castañeda and Defeo, 2002; Ragonese et al., 1997; Thessalou-Legaki and Kiortsis, 1997; Xu and Abdul Ghaffar, 1995), but also fish (Anastasopoulou et al., 2006; Aslan et al., 2004; Ayoade and Ikulala, 2007; Barbieri et al., 1994; Bektas et al., 2008; Joyeux et al., 2009; Kallianiotis et al., 2005; Mata et al., 2008; Morey et al., 2003; Patimar et al., 2009; Santos et al., 2002; Torcu-Koç et al., 2006; Veiga et al., 2009). In addition, Sokal and Rohlf (1987) and Zar (1999) also recommend the use of the *t*-test for such studies.

The *b* value is used in LWRs as an indicator of growth type (*i.e.*, to determine whether deviation from isometric growth had occurred) and it normally falls between 2.5 and 3.5 (Binohlan and Pauly, 2000; Pauly, 1984). In this study, the estimated *b* values of LWR ranged from 2.432 to 3.403 (Table 4.6). This is similar to earlier studies of LWR of *Acetes* spp. in the coastal waters of Malaysia (Amani et al., 2011b; Amin et al., 2009b; Amin et al., 2009d; Amin et al., 2008b; Arshad et al., 2012; Arshad et al., 2007; Arshad et al., 2008) and in some other geographical locations (Deshmukh, 2002; Ikeda and Raymont, 1989; Lei, 1988; Uye, 1982; Zafar et al., 1998a; Zafar et al., 1998b; Zafar et al., 1997), in which *b* values for the genus *Acetes* ranged from 2.155 to 3.472. Values of $b < 2.5$ or $b > 3.5$ are often derived from samples with narrow size ranges (Froese, 2006; Froese and Pauly, 2011). This pattern can also indicate either an over-proportional increase in length relative to growth in weight ($b < 2.5$) or an over-proportional increase in weight relative to growth in length ($b > 3.5$) (Froese, 2006).

Differences in LWR between the in-shore and off-shore groups of both species (Table 4.7) could be due to presence of at least two cohorts representing two annual generations with different life stages. This is because *Acetes* species are known to have spawning peaks twice a year (Amin et al., 2009d; Oh and Jeong, 2003), and undergo seasonal migration between shallow in-shore and deeper off-shore waters at different life stages (Chiou et al., 2000).

Variation in *a* and *b* vary with the size range of the samples (Froese and Pauly, 2011). Thus, the use of LWR should strictly be limited to the size ranges

applied when estimating regression parameters (Dulčić and Kraljević, 1996; Froese and Pauly, 2011; Gonçalves et al., 1997; Morey et al., 2003; Muto et al., 2000; Petrakis and Stergiou, 1995).

5.5 *COI* Sequence Variation

High A+T content and positional biases, e.g., 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*, *A. sibogae* individuals analysed in this study (Table 4.10). This pattern of base composition is similar to the *COI* gene region sequences in other groups of crustaceans, including Raymunidae (Macpherson and Machordom, 2001), Portunidae (Chu et al., 1999; Pfeiler et al., 2005), Bresiliidae (Shank et al., 1999), 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) and thus only three amino acids substitution was 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 and Hewitt, 1996) that have been reported in crustaceans, including in the snapping shrimp, *Alpheus* (Williams et al., 2002; Williams and Knowlton, 2001).

5.6 Interspecific Variation of *Acetes* sp. and Cryptic Diversity

From the phylogenetic trees inferred from the *COI* sequence (Figure 4.13–4.14), it is evident that four distinct clades could be clearly identified from NJ, MP, ML and BI. All clades were monophyletic and supported by high BS and PP that correspond with the four different *Acetes* species identified morphologically using the taxonomic keys of Omori (1975b). This proves that the *COI* molecular trees and species identification based on morphological characters provided by Omori (1975b) are congruent. Besides that, the aligned 552 bp of *COI* sequence showed a divergence range of 14.69% to 20.47% among the four *Acetes* species in current study (Table 4.11). This level of sequence divergence is similar to those reported in *Penaeus* (8.00–24.00%; Baldwin et al., 1998), and *Metapenaeus* (6.10–19.90%; Tong et al., 2000).

In general, morphologically defined species stand up well to molecular characterisation, but do not reveal all of the variation that is present, implying that cryptic diversity is present in at least two taxa (*i.e.*, *A. indicus* and *A. sibogae*). Evidence for cryptic diversity comes from the extent of the genetic distance between clades. Although sequence divergence between clades *ai-I* and *ai-II* (*i.e.,* 8.94%) and clades *asi-I* and *asi-II* (*i.e.,* 10.30%) remain lower than interspecific *COI* – divergences of *Acetes* species in current study (Table 4.11), however, these divergences of similar magnitude have been considered

of a cryptic or sibling species (*i.e.,* morphologically indistinguishable, but genetically distinct) (Bickford et al., 2007; Knowlton, 1986; Pfenninger and Schwenk, 2007) in other decapods species. For example, 6–8% between two cryptic species of kuruma shrimp, *Penaeus japonicus* (Tsoi et al., 2007; Tsoi et al., 2005), 2–5% between two sibling *Alpheus* species, *A. angulatus* and *A. armillatus* (Mathews et al., 2002). Besides that, indication of cryptic speciation was also revealed via *COI* sequence analysis of two morphologically indistinguishable clades within *Fenneropenaus (Penaeus) merguiensis* that had average sequence divergences of 5% (Hualkasin et al., 2003). Alternatively, the *COI* haplotypes grouped into two disconnected statistical parsimony network at the 95% connection limit. As suggested by Hart and Sunday (2007) and Chen et al., (2010), statistical parsimony networks separated by more than the parsimony connection limit would be indicating the presence of cryptic species. So, while it is not really possible to recognise cryptic taxa formally using mtDNA marker (due to maternal inheritance), both the evidence would suggested that there are cryptic taxa present in *A. indicus* and *A. sibogae*, and they have been evolving independently for a significantly period of evolutionary time (Table 4.11).

5.7 Intraspecific Variation Analysis of *Acetes* sp.

5.7.1 Patterns of Genetic Differentiation

The actual magnitude and geographical range of dispersal in *Acetes* is unknown. However it may be relatively high as the species in the genus pass through long planktonic larval stages (*i.e.*, about 6 weeks) before metamorphosing to the more benthic juvenile stage (Rao, 1968), followed by

subadult and adult stage. Lower levels of genetic differentiation would be expected in the marine environment, as species with planktonic larval phases are assumed to possess higher levels of dispersal potential coupled with an absence of physical barriers to movement between ocean basins or adjacent continental margins (Arndt and Smith, 1998; Bernardi, 2000; Collin, 2001; Duffy, 1993; Hellberg, 1996; Hoskin, 1997; McMillan et al., 1992; Palumbi, 1992; Wilke and Davis, 2000).

Acetes indicus does not conform to this pattern and shows genetic differentiation among its sampling populations. Results of AMOVA analysis revealed significant population differentiation among populations (Table 4.16). High and significant pairwise population differentiation was found between PSETT, KK, KG and other sampling populations (Table 4.17). In contrast, non-significant population differentiation among the sampling populations was observed in *A. serrulatus*, *A. japonicus* and *A. sibogae* with none of the pairwise comparisons showing significant differentiation (Table 4.18–4.20). Moreover, some of the Φ_{ST} values are negative, indicating that the variation within samples was greater than the variation between populations (Lessios et al., 1998). However, the result of *A. japonicus and A. sibogae* may be biased due to the low number of populations analysed using AMOVA (*i.e.* three populations in *A. japonicus* and two populations in *A. sibogae*) and low sequence numbers in pairwise Φ_{ST} (*i.e.* two sequences in KK) (Fitzpatrick, 2009).

The difference in genetic differentiation patterns among species suggests that they may have different demographic histories (McMillen-Jackson and Bert, 2003). Due to the limited number of *A. japonicus* and *A. sibogae* specimens, demographic history was only discussed for *A. indicus* and *A. serrulatus.*

5.7.2 Demographic History of *Acetes indicus*

For *A. indicus*, the observed genetic structure appears to reflect the historical gene flow between geographically separated populations rather than on-going gene flow (Benzie, 1999; Palumbi, 1997). The moderate haplotype diversity (h = 0.552) and high nucleotide diversity (π = 3.121%) (Table 4.12) is indicative of past evolutionary processes, suggesting either to secondary contact between historically isolated populations or stable populations with large, long-term effective population sizes (Grant and Bowen, 1998).

According to Voris (2000), the region around the Sunda and Sahul shelves experienced fluctuations of sea-levels in the past, and during low sea-levels, more land was exposed and thus forming broad geographical barriers that partly isolated the Indian Ocean from the West Pacific and enclosed the South China Sea, Sulu Sea, and Sulawesi Sea. Sea-level changes may have temporarily isolated populations belonging to species occurring in this region and restricted the gene flow among some populations. Consequently, the fragmented subpopulations may have evolved separately in the South China Sea and the Indian Ocean or Arabian Sea, forming distinct clades among the isolated regions. When sea levels rose, the sea waters reconnected, allowing secondary contact between the distinct clades. As noted from the geographical

distribution of *A. indicus* which occurs in the central part of Indo-West Pacific (Chan, 1998; Holthuis, 1980; Omori, 1975b; Xiao and Greenwood, 1993), this species most probably experienced such a scenario in the past. Two distinct clades of *A. indicus* (*ai-I* and *ai-II*) obtained by Neighbour-joining (NJ) tree and the amount of nucleotide divergence between clades *ai-I* and *ai-II* indicate early Pliocene to late Miocene divergence (*i.e.*, 2.98 – 6.39 MYA). This suggests a long-term historical isolation of *A. indicus* populations. The mixture of haplotypes found in the BL (Bagan Lipas) and PSETT (Portuguese Settlement) populations may reflect secondary contact between clades *ai-I* and *ai-II*.

During the last-glacial maximum (LGM), around 18,000–20,000 years ago, the sea level dropped to about 120 m below the present level in Southeast Asia (Hanebuth et al., 2000). This exposed parts of the Sunda Shelf, including the Straits of Malacca where the samplings for this study were conducted. The LGM would have caused local extinctions in the area, hence limiting the distribution of *A. indicus* in the Straits of Malacca. Furthermore, over the past million years, there have been as many as 10 major Pleistocene sea-level fluctuation events, during which a large part of the Sunda Shelf and the Straits of Malacca were exposed (Pillans et al., 1998). These sea level fluctuations may have contributed to local extinction events, of which the latest might have taken place during the LGM. Therefore, the sampling sites might not contain large and stable populations. This indirectly supports the presence of differentiated populations which became connected again when sea levels rose after the LGM, *i.e.* secondary contact.

It is worth noting here, however, that when clades *ai-I* and *ai-II* were analysed separately, low haplotype diversity and nucleotide diversity (clade *ai-I*: h = 0.286 and π = 0.05%; clade *ai-II*: h = 0.228, π = 0.10%) were observed (Table 4.13). Low genetic variability can often reflects recent events of population bottleneck events or founder effects by a single or a few mtDNA lineages (Grant and Bowen, 1998). Although the NJ tree showed two deep clades for the whole *COI* data, both clades *ai-I* and *ai-II* showed shallow phylogeny which is consistent with a population expansion after bottleneck (Slatkin and Hudson, 1991). This is supported by both the mismatch analysis and neutrality test statistics (Figure 4.20b and c). A unimodal mismatch distribution with a steeper wave that was observed for both clades (*i.e.,* L-shaped) (Figure 4.20b and c) has been reported in other shrimp studies, including in Chinese shrimp, *Fenneropenaeus chinensis* (Li et al., 2009), red shrimp, *Aristeus antennnatus* (Roldán et al., 2009) and karamote prawn, *Melicertus* (*Penaeus*) *kerathurus* (Pellerito et al., 2009), and such a pattern is indicative of population expansions from a smaller initial population and recent bottlenecks (Frankham et al., 2002; Rogers and Harpending, 1992). The population expansion for each clade is further supported by the non-significant value of sum of squared deviation (*SSD*) and Harpending's raggedness index (r) (clade *ai-I*: *SSD* = 0.112, $P > 0.05$; r = 0.2618, $P > 0.05$ and clade *ai-II*: *SSD* = 0.0399, $P > 0.05$; r = 0.6075, $P > 0.05$) (Figure 4.20b and c). Furthermore, Tajima's D and Fu's F_s statistics are sensitive to the factors such as bottlenecks or population expansion that tends to drive Tajima's D and Fu's F_s estimates towards more negative values (Aris-Brosou and Excoffier, 1996; Fu, 1997; Rand, 1996; Tajima, 1989), as seen in clades *ai-I* and *ai-II*. However, only the R_2 test

showed a significant value as it is a more sensitive test statistic for detecting population growth with small sample sizes (Ramos-Onsins and Rozas, 2002). It has been inferred that the main clades seemed to be generated by population expansion and isolation with change of the sea levels and/or sea environment such as water temperature or currents during glacial periods in Pleistocene in many marine invertebrates and vertebrates (Palumbi, 1994; Uthicke and Benzie, 2003; Wang et al., 2008). In such a case, a strait, which was once closed as a land bridge or isthmus in glacial periods, likely remained as a border separating distinct lineages. Subsequently, once climatic conditions were restored, contact was resumed between the differentiated populations. This model could support an hypothesis of "secondary contact between historically isolated populations" in *A. indicus*.

5.7. 3 Demographic History of *Acetes serrulatus*

High haplotype diversity (h = 0.886) and low nucleotide diversity (π = 0.42%) were noted in the 552 bp of *COI* gene fragment among the *A. serrulatus* in this study (Table 4.13). Similar observations were found in several other marine species, such as the six bar wrass, *Tallasoma hardwicki* (Chen et al., 2004), European eel, *Anguilla Anguilla* (Daemen et al., 2001), neon damselfish,, *Pomacentrus coelestis*, (Liu et al., 2008), sea spotted bream, *Pagellus bogaraveo* (Stockley et al., 2005), as well as penaeid shrimp, including Chinese shrimp, *Fenerropenaeus chinensis* (Kong et al., 2010), red shrimp, *Aristeus antennatus* (Maggio et al., 2009), and karamote prawn, *Melicertus* (*Penaeus*) *kerathurus* (Pellerito et al., 2009). Such a combination of genetic variability suggests that the populations of *A. serrulatus* probably underwent

'population bottlenecks followed by rapid population growth and accumulation of mutations' (Avise et al., 1984; Grant and Bowen, 1998).

The low π value reflects the low divergence between individuals (as suggested also by the short branch lengths on the NJ phylogenetic trees) together with the shallow phylogeny of the NJ tree (Figure 4.13–4.14) is consistent with a population expansion event after a period of low effective population sizes caused by bottlenecks or founder effects (Slatkin and Hudson, 1991). Negative and significant value of Tajima's D (D = -2.0787, $P < 0.01$), Fu's F_s (F_s = -31.7964, $P < 0.001$) and significant value of R_2 (R_2 = 0.0314, $P < 0.001$) (Figure 4.20d) indicated population expansion events (Aris-Brosou and Excoffier, 1996; Fu, 1997; Ramos-Onsins and Rozas, 2002). Moreover, the network revealed by TCS (*i.e.*, can be viewed as star-like shape pattern), unimodal mismatch distribution (*SSD*; $P < 0.05$), low and non-significant of Harpending's Raggedness index (r) further support recent population expansion (Rogers and Harpending, 1992; Slatkin and Hudson, 1991).

Population expansion usually generates an excess of rare mutations and therefore, an excess of singletons (Avise et al., 1984; Jorde et al., 2001; Ramos-Onsins and Rozas, 2002; Rogers and Harpending, 1992; Slatkin and Hudson, 1991). This is observed in the high h, which was due to the presence of many haplotypes, of which 26 out of 31 (84 %) were rare and represented by single individuals (Table 4.13). Besides, the star-like shape network (Figure 4.19) with few frequent haplotypes and a large number of haplotypes separated by only one or few mutations (*i.e.* most of the segregating sites were

singletons–17 out of the 31 variable site sites were singleton variable sites), are compatible with this expectation. These findings would, add support to the hypothesis of population expansion in *A. serrulatus*.

As seen in *A. indicus*, it is suggested that population of *A. serrulatus* also undergone late-Pleistocene expansion, possibly related with the rising of the sea-level (Table 4.11). This is similar to the pink shrimp, *Farfantepenaeus duorarum* (McMillen-Jackson and Bert, 2004), whiskered velvet shrimp, *Metapenaeopsis barbata* (Chu et al., 2012) and fleshy prawn, *Fenneropenaeus chinensis* (Kong et al., 2010). During the Pleistocene sea-level fluctuation period of the past 150,000 years (Voris, 2000), sea levels fluctuated repeatedly and would not be optimal for *Acetes* shrimps. As an example, the Sunda Shelf including Malacca Straits was exposed and the disappearance of habitat had restricted *A. serrulatus* to the relatively limited areas, and this could have resulted in population bottlenecks. When the conditions improved (*i.e*., the rising of sea-level), the populations that retreated to the surrounding refugia during unfavourable conditions would rapidly expand their ranges. So, geographic ranges and population sizes of these *A. serrulatus* may have changed (Hewitt, 1996), resulting in the genetic signatures of population expansion that we observed. The estimates of the time since the most recent population expansion event for populations of *A. serrulatus* took place approximately 61,000–28,000 years ago (Table 4.11), suggest consistency with a sea level rise since the late Pleistocene (Geyh et al., 1979; Hanebuth et al., 2000; Voris, 2000).

CHAPTER 6.0

CONCLUSIONS

In this study, *Acetes* spp. collected from the in-shore and off-shore regions of the west coast of Peninsular Malaysia were morphologically identified as *Acetes indicus*, *A. serrulatus, A. japonicus*, and *A. sibogae*. Morphometric analysis showed significant differences between the sexes of each species, between in-shore and off-shore catches of *A. indicus* and *A. serrulatus*, and among and between the four species. Length-weight relationships estimated in this study were significant and provided the first reference on morphometric data and LWRs for *A. indicus* and *A. serrulatus* obtained from the off-shore regions of Peninsular Malaysia. For interspecific variation among species, high genetic divergence was observed (14.69%–20.47%). Four distinct clades were consistently produced from Neighbour-joining, Maximum Pasimony, Maximum Likelihood and Bayesian Inference trees, supported by high bootstrap and posterior probabilities that corresponded with the four different *Acetes* species identified morphologically. Besides that, cryptic diversity is present in *A. indicus* and *A. sibogae*. Furthermore, significant genetic differentiation was found among populations of *A. indicus* but such differentiation was not supported in the other three species. The differences between the patterns of genetic differentiation, combination of neutrality tests and mismatch analysis suggest that *A. indicus* and *A. serrulatus* may have had different demographic histories, which are secondary contact between historically isolated populations, and historical population bottlenecks followed by rapid population growth, respectively. The estimated time since

expansion for both clades of *A. indicus* and *A. serrulatus* was 97,000– 45,000 years ago and 61,000–28,000 years ago, respectively.

REFERENCES

Abdullah, M. I. & Idrus, A. Z. 1978. The fish processing industry in Peninsular Malaysia. *In:* Proceedings of the 18th session of the Indo-Pacific Fishery Commission, Manila, Philippines, March 8–17, 1978. Section 3, Symposium on Fish Utilization Technology and Marketing in the IPFC Region, 1978. 45-60.

Abohweyere, P. O. & Williams, A. B. 2008. Length-weight relationship and condition factor of *Macrobrachium macrobrachion* in the Lagos-Lekki Lagoon System, Nigeria. *Research Journal of Biological Sciences,* 3(11)**,** 1333-1336.

Achuthankutty, C. T. & Nair, S. A. 1976. A new species of sergestid shrimp, *Acetes orientalis* (Crustacea: Decapoda, Sergestidae) from Goa, Central West Coast of India. *Hydrobiologia,* 48(3)**,** 233-239.

Ajah, P. O. & Nunoo, F. K. E. 2003. The effects of four preservation methods on length, weight and condition factor of the clupeid *Sardinella aurita* Val. 1847. *Journal of Applied Ichthyology,* 19(6)**,** 391-393.

Aldrich, F. A. 1962. Results of the Catherwood Foundation Peruvian Amazon Expedition. The distribution of *Acetes paraguayensis* Hansen (Crustacea; Decapoda). *Notulae Naturae of the Academy of Natural Science of Philadelphia,* 351**,** 1-7.

Allen, D. M., Ogburn-Matthews, V., Buck, T. & Smith, E. M. 2008. Mesozooplankton Responses to Climate Change and Variability in a Southeastern U.S. Estuary (1981–2003). *Journal of Coastal Research: Special Issue 55 - Research & Monitoring of NERRS Aquatic Ecosystems [Kennish]***,** 95-110.

Allendorf, F. W. & Luikart, G. 2006. *Conservation and the genetics of populations,* Malden, Mass, Blackwell Publishing.

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.

Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D. J. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research,* 25(17)**,** 3389-3402.

Amani, A. A., Amin, S. M. N. & Arshad, A. 2011a. 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.

Amani, A. A., Amin, S. M. N., Arshad, A. & Aminur Rahman, M. 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., Arshad, A., Amin, S. M. N. & Aziz, N. a. A. 2011c. 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.

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., Ismail, N. H., Idris, M. H., Bujang, J. S. & Siraj, S. S. 2010a. Morphometric variation among the three species of genus *Acetes* (Decapoda: Sergestidae) in the coastal waters of Malacca, Peninsular Malaysia. *Pertanika Journal of Tropical Agricultural Science,* 33(2)**,** 341-347.

Amin, S. M. N., Arshad, A., Shamsuddin, S. B., Bujang, J. S. & Siraj, S. S. 2008a. Catch per unit effort of estuarine push net with emphasis on occurence and abundance of *Acetes* shrimps in the coastal waters of Malacca, Peninsular Malaysia. *Pertanika Journal of Science & Technology,* 16(2)**,** 281-289.

Amin, S. M. N., Arshad, A., Siraj, S. S. & Bujang, J. S. 2009c. 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., 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. 2009d. 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. & Japar, S. B. 2010b. 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., Zainal, Z., Idris, M. H., Siraj, S. S. & Japar, S. B. 2008b. 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.

Anastasopoulou, A., Yiannopoulos, C., Megalofonou, P. & Papaconstantinou, C. 2006. Distribution and population structure of the *Chlorophthalmus agassizi* (Bonaparte, 1840) on an unexploited fishing ground in the Greek Ionian Sea. *Journal of Applied Ichthyology,* 22(6)**,** 521-529.

Anderson, R. O. & Neumann, R. M. 1996. Chapter 15: Length, weight, and associated structural indices. *In:* MURPHY, B. R. & WILLIS, D. W. (eds.) *Fisheries Techniques, 2nd Edition.* Bethesda, Maryland: American Fisheries Society.

Anderson, W. W. & Lindner, M. J. 1958. Length-weight relation in the common or white shrimp, *Penaeus setiferus*. *U.S. Fish and Wildlife Service, Special Scientific Report - Fisheries No. 256***,** 1-13.

Anger, K. & Moreira, G. S. 1998. Morphometric and reproductive traits of tropical caridean shrimps. *Journal of Crustacean Biology,* 18(4)**,** 823-838.

Aravindakshan, M. & Karbhari, J. P. 1988. *Acetes* shrimp fishery of Bombay coast. *Marine Fisheries Information Service Technical and Extension Series,* 80**,** 28-29.

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.

Arndt, A. & Smith, M. J. 1998. Genetic diversity and population structure in two species of sea cucumber: differing patterns according to mode of development. *Molecular Ecology,* 7(8)**,** 1053-1064.

Arrington, D. A. & Winemiller, K. O. 2003. Diel changeover in sandbank fish assemblages in a neotropical floodplain river. *Journal of Fish Biology,* 63(2)**,** 442-459.

Arrington, D. A. & Winemiller, K. O. 2006. Habitat affinity, the seasonal flood pulse, and community assembly in the littoral zone of a Neotropical floodplain river. *Journal of the North American Benthological Society,* 25(1)**,** 126-141.

Arshad, A., Amin, S. M. N., Nuradiella, Y. L. Z., Cob, Z. C., Ara, R. & Aziz, D. 2012. Population characteristics of *A. 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., Siraj, S. S. & Japar, S. B. 2007. New distribution records of sergestid shrimp, *Acetes intermedius* (Decapoda: Sergestidae) from Peninsular Malaysia with notes on it population characteristics. *Journal of Biological Sciences,* 7(8)**,** 1305-1313.

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.

Aslan, M., Yildirim, A. & Bektas, S. 2004. Length-weight relationship of brown trout, *Salmo trutta* L., inhabiting Kan Stream, Coruh Basin, North-Eastern Turkey. *Turkish Journal of Fisheries and Aquatic Sciences,* 4**,** 45-48.

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.

Avise, J. C., Arnold, J., Ball, R. M., Bermingham, E., Lamb, T., Neigel, J. E., Reeb, C. A. & Saunders, N. C. 1987. Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. *Annual Review of Ecology and Systematics,* 18(1)**,** 489-522.

Ayoade, A. A. & Ikulala, A. O. O. 2007. Length weight relationship, condition factor and stomach contents of *Hemichromis bimaculatus*, *Sarotherodon melanotheron* and *Chromidotilapia guentheri* (Perciformes: Cichlidae) in Eleiyele Lake, Southwestern Nigeria. *Revista de Biologia Tropical International Journal of Tropical Biology and Conservation,* 55(3-4)**,** 969-977.

Azuma, N., Kunihiro, Y., Sasaki, J., Mihara, E., Mihara, Y., Yasunaga, T., Jin, D.-H. & Abe, S. 2008. Genetic variation and population structure of hair crab (*Erimacrus isenbeckii*) in Japan inferred from mitochondrial DNA sequence analysis. *Marine Biotechnology,* 10(1)**,** 39-48.

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.

Ball, E. E., Kao, L. C., Stone, R. C. & Land, M. F. 1986. Eye structure and optics in the pelagic shrimp *Acetes sibogae* (Decapoda, Natantia Sergestidae) in relation to light--dark adaption and natural history. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences,* 313(1160)**,** 251-270.

Barbieri, L. R., Chittenden Jr., M. E. & Jones, C. M. 1994. Age, growth, and mortality of Atlantic croaker, *Micropogonias undulatus*, in the Chesapeake Bay region, with a discussion of apparent geographic changes in population dynamics. *Fishery Bulletin,* 92**,** 1-12.

Barnard, K. H. 1955. Additions to the fauna-list of South African Crustacea and Pycnogonida. *Annals of the South African Museum,* 43**,** 1-107.

Bay, L. K., Choat, J. H., Van Herwerden, L. & Robertson, D. R. 2004. High genetic diversities and complex genetic structure in an Indo-Pacific tropical reef fish (*Chlorurus sordidus*): evidence of an unstable evolutionary past? *Marine Biology,* 144(4)**,** 757-767.

Beaumont, A. R. & Croucher, T. 2006. Limited stock structure in UK populations of the brown shrimp, Crangon crangon, identified by morphology and genetics. *Journal of the Marine Biological Association of the United Kingdom,* 86(5)**,** 1107-1112.

Bektas, S., Arslan, M. & Yildirim, A. 2008. Spatial and temporal changes in length–weight relationships of *Barbus escherichii* (Steindachner, 1897) from the upper Çoruh River Basin, Turkey. *Journal of Applied Ichthyology,* 24(6)**,** 713-714.

Benedito-Cecilio, E., Agostinho, A. A. & Carnelós-Machado Velho, R. C. 1997. Length-weight relationship of fishes caught in the Itaipu Reservoir, Paraná, Brazil. *NAGA, The ICLARM Quarterly,* 20(3 & 4)**,** 57-61.

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

Benzie, J. a. H. 1999. Genetic structure of coral reef organisms: Ghosts of dispersal past. *American Zoologist,* 39(1)**,** 131-145.

Benzie, J. a. H., Ballment, E., Forbes, A. T., Demetriades, N. T., Sugama, K., Haryanti & Moria, S. 2002. Mitochondrial DNA variation in Indo-Pacific populations of the giant tiger prawn, *Penaeus monodon*. *Molecular Ecology,* 11(12)**,** 2553-2569.

Benzie, J. a. H. & Williams, S. T. 1997. Genetic structure of giant clam (*Tridacna maxima*) populations in the West Pacific is not consistent with dispersal by present-day ocean currents. *Evolution,* 51(3)**,** 768-783.

Berglund, A. 1981. Sex dimorphism and skewed sex ratios in the prawn Species *Palaemon adspersus* and *P. Squilla*. *Oikos,* 36(2)**,** 158-162.

Bermingham, E. & Avise, J. C. 1986. Molecular zoogeography of freshwater fishes in the southeastern United States. *Genetics,* 113(4)**,** 939-965.

Bernardi, G. 2000. Barrier to gene flow in *Embiotoca jackson*i, a marine fish lacking a pelagic larval stage. *Evolution,* 54(1)**,** 226-237.

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.

Binohlan, C., Froese, R. & Pauly, D. 2000. The Length-Length Table. *In:* FROESE, R. & PAULY, D. (eds.) *FishBase 2000: concepts, design and data sources.* Los Baños, Laguna, Philippines: International Center for Living Aquatic Resources Management.

Binohlan, C. & Pauly, D. 2000. The Length-Weight Table. *In:* FROESE, R. & PAULY, D. (eds.) *FishBase 2000: concepts, design and data sources.* Los Baños, Laguna, Philippines: International Center for Living Aquatic Resources Management.

Bird, C. E., Holland, B. S., Bowen, B. W. & Toonen, R. J. 2007. Contrasting phylogepgraphy in three endemic Hawaiian limpets (*Cellana* spp.) with similar life histories. *Molecular Ecology,* 16(15)**,** 3173-3186.

Bird, D. F. & -Prairie, Y. T. 1985. Practical guidelines for the use of zooplankton length-weight regression equation. *Journal of Plankton Research,* 7(6)**,** 955-960.

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.

Bland, J. M. & Altman, D. G. 1995. Multiple significance tests: the Bonferroni method. *BMJ,* 310(6973)**,** 170.

Bolger, T. & Connolly, P. L. 1989. The selection of suitable indices for the measurement and analysis of fish condition. *Journal of Fish Biology,* 34(2)**,** 171-182.

Boore, J. L. 1999. Animal mitochondrial genomes. *Nucleic Acids Research,* 27(8)**,** 1767-1780.

Borsa 2003. Genetic structure of round scad mackerel *Decapterus macrosoma* (Carangidae) in the Indo-Malay archipelago. *Marine Biology,* 142(3)**,** 575-581.

Briggs, J. C. 1999. Coincident biogeographic patterns: Indo-West Pacific ocean. *Evolution,* 53(2)**,** 326-335.

Brooker, A. L., Benzie, J. a. H., Blair, D. & Versini, J. J. 2000. Population structure of the giant tiger prawn *Penaeus monodon* in Australian waters, determined using microsatellite markers. *Marine Biology,* 136(1)**,** 149-157.

Brown, W. M., George, M. & Wilson, A. C. 1979. Rapid evolution of animal mitochondrial DNA. *Proceedings of the National Academy of Sciences,* 76(4)**,** 1967-1971.

Bulhões Arruda, C. C., Beasley, C. R., Vallinoto, M., Do Socorro Marques-Silva, N. & Tagliaro, C. H. 2009. Significant genetic differentiation among populations of *Anomalocardia brasiliana* (Gmelin, 1791): A bivalve with planktonic larval dispersion. *Genetics and Molecular Biology,* 32(2)**,** 423-430.

Burkenroad, M. D. 1934. The Penaeidae of Louisiana with a discussion of their world relationship. *Bulletin of the American Museum of Natural History,* 68(2)**,** 61-143.

Burkenroad, M. D. 1946. The Development of Marine Resources in Indonesia. *The Far Eastern Quarterly,* 5(2)**,** 189-199.

Burton, R. S. 1986. Evolutionary consequences of restricted gene flow among natural populations of the copepod, *Trigriopus californicus*. *Bulletin of Marine Science,* 39(2)**,** 526-535.

Calazans, D. 2002. Seasonal larval composition and abundance of shrimps in the surrpunding area of Pato Lagoon Mouth. *Nauplius,* 10(2)**,** 111-120.

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

Camp, D. K., Lyons, W. G. & Perkins, T. H. 1998. Checklists of selected shallow-water marine invertebrates of Florida. *Florida Marine Research Institute Technical Reports TR-3.*

Capaldi, R. A. 1990. Structure and function of cytochrome *c* oxidase. *Annual Review of Biochemistry,* 59(1)**,** 569-596.

Carr, S. M., Snellen, A. J., Howse, K. A. & Wroblewski, J. S. 1995. Mitochondrial DNA sequence variation and genetic stock structure of Atlantic cod (*Gadus morhua*) from bay and offshore locations on the Newfoundland continental shelf. *Molecular Ecology,* 4(1)**,** 79-88.

Cartaxana, A. 2003. Growth of the prawn *Palaemon longirostris* (Decapoda, Palaemonidae) in Mira River and estuary, SW Portugal. *Journal of Crustacean Biology,* 23(2)**,** 251-257.

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

Castilho, A. L., Costa, R. C., Fransozo, A. & Boschi, E. E. 2007. Reproductive pattern of the South American endemic shrimp *Artemesia longinaris* (Decapoda: Penaeoidea) off São Paulo State, Brazil. *Revista de Biologia Tropical International Journal of Tropical Biology and Conservation,* 55(1)**,** 39-48.

Chace, F. A., Jr. 1972 The shrimps of the Smithsonian-Bredin Caribbean Expeditions with a summary of the West Indian shallow-water species (Crustacea: Decapoda: Natantia). *Smithsonian Contributions to Zoology* 98**,** 1-179.

Chan, T. Y. 1998. Shrimps and Prawns. *In:* CARPENTER, K. E. & NIEM, V. H. (eds.) *FAO species identification guide for fishery purposes. The living marine resources of the Western Central Pacific. Volume 2. Cephalopods, crustaceans, holothurians and sharks.* Rome: FAO.

Chen, C. A., Ablan, M. C. A., Mcmanus, J. W., Diepernk Bell, J., 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 sssemblages in cephalotrichid nemerteans (Nemertea). *PLoS ONE,* 5(9)**,** e12885.

Chikuni, S. 1985. The fish resources of tthe northwest Pacific. *FAO Fisheries Technical Paper No. 266.* Rome: FAO.

Chiou, W.-D., Wu, C.-C. & Cheng, L.-Z. 2000. Spatio-temporal distribution of sergestid shrimp *Acetes intermedius* in the coastal waters of southwestern Taiwan. *Fisheries Science,* 66(6)**,** 1014-1025.

Chu, K. H., Chen, Q. C., Huang, L. M. & Wong, C. K. 1995. Morphometric analysis of commercially important penaeid shrimps from the Zhujiang estuary, China. *Fisheries Research,* 23(1-2)**,** 83-93.

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.

Chu, T.-J., Wang, D., Huang, H.-L., Lin, F.-J. & Tzeng, T.-D. 2012. Populatiion structure and historical demography of the whiskered velvet shrimp (*Metapenaeopsis barbata*) off China and Taiwan inferred from the mitochondrial control region. *Zoological Studies,* 51(1)**,** 99-107.

Chullasorn, S. & Martosubroto, P. 1986. Distribution and important biological features of coastal fish resources in Southeast Asia. *FAO Fisheries Techinal Paper No. 128.* Rome: FAO.

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

Coelho, P. A. & Ramos-Porto, M. 1984. Camarões de água doce do Brasil: Distribuição geográfica. *Revista Brasileira de Zoologia,* 2(6)**,** 405-410.

Colefax, A. N. 1940. An Australian species of *Acetes* (Crustacea, Macrura, Sergestidae), with remarks on the distribution and literature of the genus. *Records of the Australian Museum,* 20(5)**,** 341-353.

Collin, R. 2001. The effects of mode of development on phylogeography and population structure of North Atlantic *Crepidula* (Gastropoda: Calyptraeidae). *Molecular Ecology,* 10(9)**,** 2249-2262.

Collins, P. A. & Williner, V. 2003. Feeding of *Acetes paraguayensis* (Nobili) (Decapoda: Sergestidae) from the Parana River, Argentina. *Hydrobiologia,* 493(1-3)**,** 1-6.

Colloca, F. 2002. Life cycle of the deep-water pandalid shrimp *Plesionika edwardsii* (Decapoda: Caridea) in the central Mediterranean Sea. *Journal of Crustacean Biology,* 22(4)**,** 775-783.

Comesaña, A. S., Martínez-Areal, M. T. & Sanjuan, A. 2008. Genetic variation in the mitochondrial DNA control region among horse mackerel (*Trachurus trachurus*) from the Atlantic and Mediterranean areas. *Fisheries Research,* 89(2)**,** 122-131.

Company, J. B. & Sardà, F. 2000. Growth parameters of deep-water decapod crustaceans in the Northwestern Mediterranean Sea: a comparative approach. *Marine Biology,* 136(1)**,** 79-90.

Corder, G. W. & Foreman, D. I. 2009. *Nonparamteric statistics for non-statisticians: a step by step approach,* Hoboken, New Jersey, John Wilye & Sons, Inc.

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

Costa, R. C. D., Fransozo, A., Melo, G. a. S. & Freire, F. a. D. M. 2003. An illustrated key for dendrobranchiata shrimps from the northern coast of São Paulo State, Brazil. *Biota Neotropica,* 3(1-12).

Crosnier, A. & Fourmanoir, P. 1962. La tsivakihiny. *Le Naturaliste Malgache,* 13**,** 87-88.

D'incao, F. & Martins, S. T. S. 2000. Brazilian species of the genera *Acetes* H. Milne Edwards, 1830 and *Peisos* Burkenroad, 1945 (Decapoda: Sergestidae). *Journal of Crustaceans Biology,* 20(2)**,** 78-86.

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

Dawson, M. N., Raskoff, K. A. & Jacobs, D. K. 1998. Field preservation of marine invertebrate tissue for DNA analyses. *Molecular Marine Biology and Biotechnology,* 7(2)**,** 145-152.

De Grave, S., Pentcheff, N. D., Ahyong, S. T., Chan, T.-Y., Crandall, K. A., Dworschak, P. C., Felder, D. L., Feldmann, R. M., Fransen, C. H. J. M., Goulding, L. Y. D., Lemaitre, R., Low, M. E. Y., Martin, J. W., Ng, P. K. L., Schweitzer, C. E., Tan, S. H., Tshudy, D. & Wetzer., R. 2009. A classification of living and fossil genera of decapod crustaceans. *Raffles Bulletin of Zoology, Supplement Series No. 21***,** 1-109.

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

Deshmukh, V. D. 2002. Biology of *Acetes indicus* Milne Edwards in Bombay waters. *Indian Journal of Fisheries,* 49(4)**,** 379-388.

Deshmukh, V. D. 2003. Non-penaeid shrimps. *In:* MOHAN JOSEPH, M. & JAYAPRAKASH, A. A. (eds.) *Status of exploited marine fishery resources of India.* Kochi: CMFRI.

Deshmukh, V. D. 2004. Non-penaeid shrimps. *In:* MOHAN JOSEPH, M. & JAYAPRAKASH, A. A. (eds.) *Status of exploited marine fishery resources of India.* Kochi: CMFRI.

Díaz-Jaimes, P., Barbosa-Saldaña, M. D. L. & Uribe-Alcocer, M. 2006. Allozyme variation in eastern Pacific brown shrimp *Farfantepenaeus californiensis* populations. *Fisheries Science,* 72(3)**,** 696-698.

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.

Diaz, G. A., Smith, S. G., Serafy, J. E. & Ault, J. S. 2001. Allometry of the growth of pink shrimp *Farfantepenaeus duorarum* in a subtropical bay. *Transactions of the American Fisheries Society,* 130(2)**,** 328-335.

Dof 2001-2010. *Annual fisheries statistics*, Department of Fisheries Malaysia, Ministry of Agriculture, Kuala Lumpur.

Donald, K. M., Kennedy, M. & Spencer, H. G. 2005. Cladogenesis as the result of long-distance rafting events in South Pacific topshells (Gastropoda, Trochidae) *Evolution,* 59(8)**,** 1701-1711.

Duffy, J. E. 1993. Genetic population structure in two tropical sponge-dwelling shrimps that differ in dispersal potential. *Marine Biology,* 116(3)**,** 459-470.

Dulčić, J. & Kraljević, M. 1996. Weight-length relationships for 40 fish species in the eastern Adriatic (Croatian waters). *Fisheries Research,* 28(3)**,** 243-251.

Ecoutin, J. M., Albaret, J. J. & Trape, S. 2005. Length-weight relationships for fish populations of a relatively undisturbed tropical estuary: The Gambia. *Fisheries Research,* 72(2-3)**,** 347-351.

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)**,** 564-567.

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.

Fao 2000. Report of the four GEF/UNEP/FAO Regional Workshops on Reducing the Impact of Tropical Shrimp Trawl Fisheries. Lagos, Nigeria, 15-17 December 1999. Puntareñas, Costa Rica, 15-17 January 2000. Teheran, Iran, 28 February-1 March 2000. Denpasar, Bali, Indonesia, 6-8 March 2000. *FAO Fisheries Report No. 627.* Rome: FAO.

Fao 2001. Tropical shrimp fisheries and their impact on living resources. Shrimp fisheries in Asia: Bangladesh, Indonesia and the Philippines; in the Near East: Bahrain and Iran; in Africa: Cameroon, Nigeria and the United Republic of Tanzania; in Latin America: Colombia, Costa Rica, Cuba, Trinidad and Tobago, and Venezuela. *FAO Fisheries Circular. No. 974.* Rome: FAO.

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

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

Feng, Z.-Q., Sun, J.-H., Yang, G.-B. & An, X.-S. 1982. A discussion of *Acetes chinensis* resources in the western areas of Bohai Sea and their rational use. [In Chinese]. *Transactions of Oceanology and Limnology,* 4**,** 62-68.

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

Fisher, R. A. 1930. *The genetical theory of natural selection,* Oxford, Oxford University Press.

Fitzpatrick, B. M. 2009. Power and sample size for nested analysis of molecular variance. *Molecular Ecology,* 18(19)**,** 3961-3966.

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.

Foster, J. J. 2001. *Data analysis using SPSS for Window version 8 to 10: A beginner's guide,* London, Sage Publications.

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

Froese, R. 2006. Cube law, condition factor and weight–length relationships: history, meta-analysis and recommendations. *Journal of Applied Ichthyology,* 22(4)**,** 241-253.

Froese, R. & Pauly, D. 2011. FishBase. World Wide Web electronic publication. http://www.fishbase.org, Version (06/2011).

Fu, Y. X. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics,* 147(2)**,** 915-925.

Galtier, N., Nabholz, B., Glémin, S. & Hurst, G. D. D. 2009. Mitochondrial DNA as a marker of molecular diversity: a reappraisal. *Molecular Ecology,* 18(22)**,** 4541-4550.

Garber, A. F., Tringali, M. D. & Stuck, K. C. 2004. Population structure and variation in red snapper (*Lutjanus campechanus*) from the Gulf of Mexico and Atlantic Coast of Florida as determined from mitochondrial DNA control region sequence. *Marine Biotechnology,* 6(2)**,** 175-185.

García-Dávila, C. R. & Magalhães, C. 2003. Taxonomic revision of the freshwater shrimps (Crustacea: Decapoda: Palaemonidae, Sergestidae) from the Peruvian Amazonia *Acta Amazonica,* 33(4)**,** 663-686.

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.

George, M. J. 1969. II Systematics - Taxonomic consideration and general distribution. *In:* JONES, S. (ed.) *CMFRI Bulletin No.14, Prawn fisheries of India.* Mandapam Camp: CMFRI.

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

Gökoğlu, M., Kaya, Y., Deval, M. C. & Tosunoğlu, Z. 2008. Some biological barameters of the erythrean mantis shrimp, *Erugosquilla massavensis* (Kossmann, 1880) (Stomatopoda, Squillidae) in the Northeastern Mediterranean (Turkish Waters). *Crustaceana,* 81(1)**,** 35-42.

Gonçalves, J. M. S., Bentes, L., Lino, P. G., Ribeiro, J., Canário, A. V. M. & Erzini, K. 1997. Weight-length relationships for selected fish species of the small-scale demersal fisheries of the south and south-west coast of Portugal. *Fisheries Research,* 30(3)**,** 253-256.

Grant, W. & Bowen, B. 1998. Shallow population histories 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. & Gascuel, O. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology,* 52(5)**,** 696-704.

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.

Gusmão, J., Lazoski, C. & Solé-Cava, A. M. 2005. Population genetic structure of Brazilian shrimp species (*Farfantepenaeus* sp., *F. brasiliensis*, *F. paulensis* and *Litopenaeus schmitti*: Decapoda: Penaeidae). *Genetics and Molecular Biology,* 28(1)**,** 165-171.

Hanamura, Y. 1999. Occurence of *Acetes sibogae* Hansen (Crustacea: Decapoda: Sergestidae) in Western Australia, with notes on the northern Australian population. *Records of the Western Australian Museum,* 19**,** 465-468.

Hanamura, Y. 2007. Ecological characteristics of mangrove hyperbenthos in the northwest coast of Peninsular Malaysia. *Japan International Research Center for Agricultural Sciences (JIRCAS) Annual Report 2007***,** 32-33.

Hanamura, Y., Siow, R. & Chee, P. E. 2007. Abundance and spatio temporal distributions of hyperbenthic crustaceans in the Merbok and Matang mangrove estuaries, Malaysia. *In:* NAKAMURA, K. (ed.) *Sustainable Production Systems of Aquatic Animals in Brackish Mangrove Areas (2005), Japan International Research Center for Agricultural Sciences (JIRCAS) Working Report 56.*

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.

Hansen, H. J. 1919. *Sergestidæ of the Siboga expedition,* Leiden, Late E. J. Brill.

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.

Harrison, R. G. 1989. Animal mitochondrial DNA as a genetic marker in population and evolutionary biology. *Trends in Ecology & Evolution,* 4(1)**,** 6-11.

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 of London. Series B: Biological Sciences,* 270(1512)**,** 313-321.

Hedgecock, D. 1986. Is gene flow from pelagic larval dispersal important in the adaptation and evolution of marine invertebrates? *Bulletin of Marine Science,* 39(2)**,** 550-564.

Hellberg, M. E. 1996. Dependence of gene flow on geographic distance in two solitary corals with different larval dispersal capabilities. *Evolution,* 50(3)**,** 1167-1175.

Hewitt, G. 2000. The genetic legacy of the Quaternary ice ages. *Nature,* 405(6789)**,** 907-913.

Hewitt, G. M. 1996. Some genetic consequences of ice ages, and their role in divergence and speciation. *Biological Journal of the Linnean Society,* 58(3)**,** 247-276.

Hile, R. 1936. Age and growth of the Cisco, *Leucichthys artedi* (Le Sueur), in the lakes of the northeastern highlands, Wisconsin. *Bulletion of the Bureau of Fisheries,* 48(19)**,** 211-317.

Holthuis, L. B. 1959. The Crustacea Decapoda of Suriname (Dutch Guiana). *Zoologische Verhandelingen uitgegeven door het Rijksmuseum Van Natuurlijke Historie Te Leiden,* 44(1)**,** 1-296.

Holthuis, L. B. 1980. FAO species catalogue. Vol. 1. Shrimps and prawns of the world. An annotated catalogue of species of interest to fisheries. *FAO Fisheries Synopsis No. 125, vol. 1.* Rome: FAO.

Hordijk, W. & Gascuel, O. 2005. Improving the efficiency of SPR moves in phylogenetic tree search methods based on maximum likelihood. *Bioinformatics,* 21(24)**,** 4338-4347.

Hoskin, M. G. 1997. Effects of contrasting modes of larval development on the genetic structures of populations of three species of prosobranch gastropods. *Marine Biology,* 127(4)**,** 647-656.

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.

Huber, J. T. 1998. The importance of voucher specimens, with practical guidelines for preserving specimens of the major invertebrate phyla for identification. *Journal of Natural History,* 32(3)**,** 367-385.

Hudson, R. R. 1990. Gene genealogies and the coalescent process. *In:* FUTUYMA, D. J. & ANTONOVICS, J. D. (eds.) *Oxford Surveys in Evolutionary Biology.* New York: Oxford University Press.

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.

Hwang, U.-W. & Kim, W. 1999. General properties and phylogenetic utilities of nuclear ribosomal DNA and mitochondrial DNA commonly used in molecular systematics. *The Korean Journal of Parasitology,* 37(4)**,** 215-228.

Ikeda, T. & Raymont, J. K. B. 1989. Preliminary studies on the intermoult period and growth of the pelagic shrimp *Acetes sibogae australis* from a Tropical Sea. *Bulletin of Plankton Society of Japan,* 36(1)**,** 11-18.

Ikematsu, W. 1953. On the life-history of *Acetes japonicus* Kishinouye, in Ariake Sea. *Bulletin of the Japanese Society of Scientific Fisheries,* 19(6)**,** 771-780.

Jaiswar, A. K. & Chakraborty, S. K. 2005. *Acetes*, the preferred food of fishes along the northwest coast of India. *Indian Journal of Fisheries,* 52(2)**,** 215-219.

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.

Jiang, R. & Guo, B. 1983. Meteorological factors affecting shrimp yield along the coast of Fujian Province. [In Chinese]. *Marine Science Bulletin,* 2(1)**,** 69-74.

Jiddawi, N. S. & Öhman, M. C. 2002. Marine fisheries in Tanzania. *AMBIO: A Journal of the Human Environment,* 31(7)**,** 518-527.

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.

Jobling, M. 2002. Environmental factors and rates of development and growth. *In:* HART, P. J. B. & REYNOLDS, J. D. (eds.) *Handbook of Fish Biology and Fisheries Volume 1 (Fish Biology).* Malden, MA: Blackwell Publishing.

Johnson, D. S. 1965. A review of the brackish water prawns of Malaya. *Bulletin of the National Museum Singapore,* 33(2)**,** 7-11.

Johnson, M., Zaretskaya, I., Raytselis, Y., Merezhuk, Y., Mcginnis, S. & Madden, T. L. 2008. NCBI BLAST: a better web interface. *Nucleic Acids Research,* 36(suppl 2)**,** W5-W9.

Johnson, W. S. & Allen, D. M. 2005. *Zooplankton of the Atlantic and Gulf coasts: a guide to their identification and ecology,* Baltimore, Johns Hopkins University Press.

Jolly, M. T., Jollivet, D., Gentil, F., Thiébaut, E. & Viard, F. 2004. Sharp genetic break between Atlantic and English Channel populations of the polychaete *Pectinaria koreni*, along the North coast of France. *Heredity,* 94(1)**,** 23-32.

Jones, R. E., Petrell, R. J. & Pauly, D. 1999. Using modified length-weight relationships to assess the condition of fish. *Aquacultural Engineering,* 20(4)**,** 261-276.

Jones, S. 1969. The prawn fishery resources of India. *FAO Fisheries Report No. 57.* Rome: FAO.

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.

Joyce, E. A., Jr. 1966. *Acetes* shrimp on the Florida east coast. *Quarterly Journal of the Florida Academy of Sciences,* 29(1)**,** 38.

Joyeux, J. C., Giarrizzo, T., Macieira, R. M., Spach, H. L. & Vaske Jr, T. 2009. Length–weight relationships for Brazilian estuarine fishes along a latitudinal gradient. *Journal of Applied Ichthyology,* 25(3)**,** 350-355.

Kakati, V. S., Telang, K. Y. & Dinesh, C. K. 1988. On the fishery of *Acetes johni* at Karwar and Tadri. *Marine Fisheries Information Service Technical and Extension Series,* 85**,** 13.

Kallianiotis, A., Torre, M. & Argyri, A. 2005. Age, growth, mortality, reproduction and feeding habits of the striped seabream, *Lithognathus mormyrus* (Pisces: Sparidae) in the coastal waters of Thracian Sea, Greece. *Scientia Marina,* 69(3)**,** 391-404.

Kassahn, K. S., Donnellan, S. C., Fowler, A. J., Hall, K. C., Adams, M. & Shaw, P. W. 2003. Molecular and morphological analyses of the cuttlefish *Sepia apama* indicate a complex population structure. *Marine Biology,* 143(5)**,** 947-962.

Kemp, S. 1917. Notes on Crustacea Decapoda in the Indian Museum, VIII. The genus *Acetes*, Milne-Edwards. *Records of the Indian Museum,* 13(7)**,** 43-58.

Kensley, B. F. 1971. The family Sergestidae in the waters around southern Africa (Crustacea, Decapoda, Natantia). *Annals of The South African Museum,* 57(10)**,** 215-264.

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.

Khan, M. Z. 1987. A note on the dol-net fishery off Jaffrabad (Gujarat) with special reference to Bombay duck, from 1979-80 to 1981-82. *Indian Journal of Fisheries,* 34(2)**,** 188-192.

Kimmerer, W., Avent, S. R., Bollens, S. M., Feyrer, F., Grimaldo, L. F., Moyle, P. B., Nobriga, M. & Visintainer, T. 2005. Variability in Length–Weight Relationships used to estimate biomass of estuarine fish from survey data. *Transactions of the American Fisheries Society,* 134(2)**,** 481 - 495.

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.

King, J. R. & Porter, S. D. 2004. Recommendations on the use of alcohols for preservation of ant specimens (Hymenoptera, Formicidae). *Insectes Sociaux,* 51(2)**,** 197-202.

Kishinouye, K. 1928. Notes on the Sergestidae. *Proceedings of the Imperial Academy of Japan,* 4(3)**,** 125-127.

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

Knowlton, N., Weigt, L., Solórzano, L., Mills, D. & Bermingham, E. 1993. Divergence in proteins, mitochondrial DNA, and reproductive compatibility across the Isthmus of Panama. *Science,* 260(5114)**,** 1629-1632.

Knowlton, N. & Weigt, L. A. 1998. New dates and new rates for divergence across the Isthmus of Panama. *Proceedings of the Royal Society of London. Series B: Biological Sciences,* 265(1412)**,** 2257-2263.

Kohler, N. E., Casey, J. G. & Turner, P. A. 1995. Length-weight relationships for 13 species of sharks from the western North Atlantic. *Fishery Bulletin,* 93(2)**,** 412-418.

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.

Kuun, P., Pakhomov, E. A. & Mcquaid, C. D. 1999. Morphometric relationships of the caridean shrimp *Nauticaris marionis* Bate, 1888 at the Prince Edward Islands (Southern Ocean). *Polar Biology,* 22(3)**,** 216-218.

Landau, S. & Everitt, B. S. 2004. *A Handbook of Statistical Analyses using SPSS,* Boca Raton, Florida, Chapman & Hall / CRC Press LLC.

Le Cren, E. D. 1951. The Length-Weight Relationship and seasonal cycle in gonad weight and condition in the perch (*Perca fluviatilis*). *Journal of Animal Ecology,* 20(2)**,** 201-219.

Le Reste, L. 1970. Biologie de *Acetes erythraeus* (Sergestidae) dans une baie du N. W. de Madagascar (Baie d'Ambaro). *Cahiers O. R. S. T. O. M (Office de la Recherche Scientifique et Technique Outre-Mer Océanographique,* 8(2)**,** 35-56.

Leech, N. L., Barrett, K. C. & Morgan, G. A. 2005. *SPSS for Intermediate Statictics: Use and Interpretation (Second Edition),* Mahwah, New Jersey, Lawrence Erlbaum Associates.

Lei, M. 1984. Studies on the biology of *Acetes japonicus* Kishinouye in the eastern coastal waters of Guangdong Province, China. *Trophic Oceanography,* 3(4)**,** 41-51.

Lei, M. 1988. *Studies in the biology of Acetes japonicus Kishinouye in the eastern coastal waters of Guangdong Province, China,* Beijing, Ocean Press.

Lessios, H. A., Kessing, B. D. & Robertson, D. R. 1998. Massive gene flow across the world's most potent marine biogeographic barrier. *Proceedings of the Royal Society of London. Series B: Biological Sciences,* 265(1396)**,** 583-588.

Li, X.-J., Dai, T.-S. & Wu, C.-W. 1986. Shrimp resources along the coast of north Zhejiang province. [In Chinese]. *Journal of Zhejiang college of Fisheries,* 5(1)**,** 13-20.

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.

Ling, S. W. & Suriyong, M. K. 1954. Notes on the utilization of zooplankton for food in Thailand. *Symposium on Marine and Freshwater Plankton in the Indo-Pacific.* Bangkok: Indo-Pacific Fisheries Council, UNESCO-FAO.

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:* GULLAND, J. A. (ed.) *The fish resources of the oceans. FAO Fisheries Technical Paper No. 97.* Rome: FAO.

Lopez, J. V., Yuhki, N., Masuda, R., Modi, W. & O'brien, S. J. 1994. *Numt*, a recent transfer and tandem amplification of mitochondrial DNA to the nuclear genome of the domestic cat. *Journal of Molecular Evolution,* 39(2)**,** 174-190.

Mabesa, R. C. & Babaan, J. S. 1993. Fish fermentation technology in the Philippines. *In:* LEE, C.-H., STEINKRAUS, K. H. & ALAN REILLY, P. J. (eds.) *Fish Fermentation Technology.* Korea: United Nations University Press.

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.

Macintosh, D. J. 2001. The Ranong Biosphere Reserve: A Living Experiment in Coastal Zone Management, Education and Research (Part 2). *AARM Newsletter,* 6(1)**,** 2-3.

Macintosh, D. J., Ashton, E. C. & Tansakul, V. 2003. Utilisation and Knowledge of Biodiversity in the Ranong Biosphere Reserve, Thailand. *ITCZM Monograph No. 7.*

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.

Magalhães, C. 1999. Diversity and abundance of decapods crustaceans in the rio Tahuamanu and rio Manuripi basins. *In:* CHENOFF, B. & WILINK, P. W. (eds.) *A Biological Assessment of the Aquatic Ecosystems of the Upper Rio Orthon Basin, Pando, Bolivia. Conservation Internation Rapid Assessment Program, Bulletin of Biological Assessment 15.* Washington, D. C.

Magalhães, C. 2002. A rapid assessment of the decapod fauna in the Rio Tahuamanu and Rio Manuripi Basins, with new records of shrimps and crabs for Bolivia (Crustacea, Decapoda, Palaemonidae, Sergestidae, Trichodactylidae). *Revista Brasileira de Zoologia,* 19(4)**,** 1091-1103.

Magalhães, C. & Pereira, G. 2007. Assessment of the decapod crustacean diversity in the Guayana Shield region aiming at conservation decisions. *Biota Neotropica,* 7(2)**,** 111-124.

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 generalized regression approach. *Cancer Research,* 27(2 Part 1)**,** 209-220.

Marques De Sá, J. P. 2007. *Applied Statistics using SPSS, STATISTICA, MATHLAB and R,* New York, Springer Berlin Heidelberg.

Martin-Smith, K. M. 1996. Length/weight relationships of fishes in a diverse tropical freshwater community, Sabah, Malaysia. *Journal of Fish Biology,* 49(4)**,** 731-734.

Martin, J. W. 2004. *Collection, preservation and archival storage of crustaceans* [Online]. Natural History Museum of Los Angeles Country. Available: http://clade.ansp.org/malacology/people/rosenberg/archiving/taxa/crustaceans.html [Accessed July 5 2007].

Martin, J. W. & Davis, G. E. 2001. *An updated Classification of the Recent Crustacea,* Los Angeles, CA, Natural History Museum of Los Angeles County.

Martinez, I., Aschan, M., Skjerdal, T. & Aljanabi, S. M. 2006. The genetic structure of *Pandalus borealis* in the Northeast Atlantic determined by RAPD analysis. *Ices Journal of Marine Science,* 63(5)**,** 840-850.

Mata, A. J., Morales, J. & Márquez, L. 2008. Weight–length relationships for 26 demersal fish species of the Spanish South-Atlantic coastal waters. *Journal of Applied Ichthyology,* 24(3)**,** 330-333.

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.

Mcmillan, W. O., Raff, R. A. & Palumbi, S. R. 1992. Population genetic consequences of developmental evolution in sea urchins (Genus *Heliocidaris*). *Evolution,* 46(5)**,** 1299-1312.

Mcmillen-Jackson, A. L. & Bert, T. M. 2003. Disparate patterns of population genetic structure and population history in two sympatric penaeid shrimp species (*Farfantepenaeus aztecus* and *Litopenaeus setiferus*) in the eastern United States. *Molecular Ecology,* 12(11)**,** 2895-2905.

Mcmillen-Jackson, A. L. & Bert, T. M. 2004. Genetic diversity in the mtDNA control region and population structure in the pink shrimp *Farfantepenaeus duorarum*. *Journal of Crustacean Biology,* 24(1)**,** 101-109.

Melo Júnior, M. D. 2006. Dynamic patterns of transport and migration of zooplankton at Catuama Inlet (Pernambuco, Brazil), with emphasis on the planktonic Decapoda. *Biota Neotropica,* 6(3)**,** 1.

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.

Mgaya, Y. D. & Teikwa, E. D. 2003. Abundance and reproductive biology of the penaeid prawns of Bagamoyo coastal waters, Tanzania. *Western Indian Ocean Journal of Marine Science,* 2(2)**,** 117-126.

Michel, H., Behr, J., Harrenga, A. & Kannt, A. 1998. Cytochrome *c* oxidase: structure and spectroscopy. *Annual Review of Biophysics and Biomolecular Structure,* 27(1)**,** 329-356.

Milne Edwards, H. 1830. Description des genres Glaucothoé, Sicyonie, Sergeste et Acète, de l'ordre des Crustacés Décapodes. *Annales des Sciences Naturelles: comprenant La physiologie animale et végétale, l'anatomie comparée des deux règnes, la zoologie, la botanique, la mineralogy, et la géologie,* 19**,** 333-352.

Mines, A. N., Smith, I. R. & Pauly, D. 1986. An overview of the fisheries of San Miguel Bay, Philippines. *In:* MACLEAN, J. L., DIZON, L. B. & HISILLO, L. Y. (eds.) *The First Asian Fisheries Forum.* Manila, Philippines: Asian Fisheries Society.

Miquel, J. C. 1984. Shrimps and Prawns. *In:* FISHER, W. & BIANCHI, G. (eds.) *FAO species identification sheets for fisheries purposes. Western Indian Ocean (Fishing Area 51).* Rome: FAO.

Mitton, J. B. 1994. Molecular approaches to population biology. *Annual Review of Ecology and Systematics,* 25(1)**,** 45-69.

Morato, T., Afonso, P., Lourinho, P., Barreiros, J. P., Santos, R. S. & Nash, R. D. M. 2001. Length-weight relationships for 21 coastal fish species of the Azores, north-eastern Atlantic. *Fisheries Research,* 50(3)**,** 297-302.

Morey, G., Moranta, J., Massutí, E., Grau, A., Linde, M., Riera, F. & Morales-Nin, B. 2003. Weight-length relationships of littoral to lower slope fishes from the western Mediterranean. *Fisheries Research,* 62(1)**,** 89-96.

Morgan, G. A., Leech, N. L., Gloeckner, G. W. & Barrett, K. C. 2004. *SPSS for Introductory Statistics Use and Interpretation (Second Edition),* Mahwah, New Jersey, Lawrence Erlbaum Associates.

Moritz, C., Dowling, T. E. & Brown, W. M. 1987. Evolution of animal mitochondrial DNA: relevance for population biology and systematics. *Annual Review of Ecology and Systematics,* 18(1)**,** 269-292.

Moss, D. R. & Moss, S. M. 2006. Effects of gender and size on feed acquisition in the Pacific white shrimp *Litopenaeus vannamei*. *Journal of the World Aquaculture Society,* 37(2)**,** 161-167.

Motulsky, H. J. & Christopoulos, A. 2003. *Fitting models to biological data using linear and nonlinear regression. A practical guide to curve fitting.,* San Diego CA, GraphPad Software Inc.

Moutopoulos, D. K. & Stergiou, K. I. 2002. Length–weight and length–length relationships of fish species from the Aegean Sea (Greece). *Journal of Applied Ichthyology,* 18(3)**,** 200-203.

Muto, E. Y., Soares, L. S. H. & Rossi-Wongtschowski, C. L. D. B. 2000. Length-weight relatinoship of marine fish species off São Sebastião system, São Paulo, Southeastern Brazil. *NAGA, The ICLARM Quarterly,* 23(4)**,** 27-29.

Nelson, J. S., Hoddell, R. J., Chou, L. M., Chan, W. K. & Phang, V. P. E. 2000. Phylogeographic structure of false clownfish, *Amphiprion ocellaris*, explained by sea level changes on Sunda shelf. *Marine Biology,* 137(4)**,** 727-736.

Nobili, G. 1905. Diagnoses préliminaires de 34 espèces et variétés nouvelles, et de 2 genres nouveaux de Décapodes de la Mer Rouge. *Bulletin du Muséum d'Histoire Naturelle,* 11**,** 393-416.

Nobili, G. 1906. *Faune carcinologique de la Mer Rouge. Décapodes et Stomatopodes,* Paris, Masson et Cie.

Noh, K. M. & Yew, T. S. 1995. A bioeconomic analysis of the East Johore prawn fishery. *Pertanika Journal of Social Sciences & Humanities,* 3(1)**,** 31-45.

O'donoghue, P. 2010. *Research methods for sport performance analysis,* New York, Taylor & Francis.

Ogawa, Y. 2004. Marine fisheries management and utilization of fishing ground in Malaysia. *Japan Agricultural Research Quarterly,* 38(3)**,** 209-212.

Oh, C.-W., Hartnoll, R. G. & Nash, R. D. M. 1999. Population dynamics of the common shrimp, *Crangon crangon* (L.), in Port Erin Bay, Isle of Man, Irish Sea. *ICES Journal of Marine Science: Journal du Conseil,* 56(5)**,** 718-733.

Oh, C.-W. & Jeong, I.-J. 2002. Fisheries biology of shrimps in the south western waters of Korea. *Journal of the Korean Fisheries Society,* 35(3)**,** 223-230.

Oh, C.-W. & Jeong, I.-J. 2003. Reproduction and population dynamics of *Acetes chinensis* (Decapoda : Sergestidae) on the western coast of Korea, Yellow Sea. *Journal of Crustacean Biology,* 23(4)**,** 827-835.

Oh, C.-W., Suh, H.-L., Park, K.-Y., Ma, C.-W. & Lim, H.-S. 2002. Growth and reproductive biology of the freshwater shrimp *Exopalaemon modestus* (Decapoda : Palaemonidae) in a lake of Korea. *Journal of Crustacean Biology,* 22(2)**,** 357-366.

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. M. 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.

Ohtomi, J. & Matsuoka, T. 1998. Reproduction and growth of Jack-knife shrimp, *Haliporoides sibogae*, off south-western Kyushu, Japan. *Fisheries Research,* 38(3)**,** 271-281.

Okada, Y. K. 1928. Note on the tail-organs of *Acetes*. *The Annals and Magazine of Natural History,* 10(1)**,** 308-310.

Oliva-Paterna, F. J., Torralva, M. & Carvalho, E. D. 2009. Length–weight relationships for 20 species collected in the Jurumirim reservoir (Paranapanema Basin, Brazil). *Journal of Applied Ichthyology,* 25(3)**,** 360-361.

Omori, M. 1975a. The biology of pelagic shrimps in the ocean. *In:* FREDERICK, S. R. & MAURICE, Y. (eds.) *Advances in Marine Biology*. Academic Press.

Omori, M. 1975b. *The systematics, biogeography, and fishery of epipelagic shrimps of the genus Acetes (Crustacea, Decapoda, Sergestidae),* Tokyo, Japan, Ocean Research Institute, University of Tokyo.

Omori, M. 1977. Distribution of warm water epiplanktonic shrimps of the genera *Lucifer* and *Acetes* (Macrura, Penaeidae, Sergestidae). *Proceedings of the symposium on warm water zooplankton (Special publication of UNESCO/NIO)*. Goa, India: National Institute of Oceanography.

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

Otto, R. S. & Jamieson, G. S. 2001. *Commercially important crabs, shrimps and lobsters of the North Pacific Ocean,* Sidney, British Columbia, North Pacific Marine Science Organization (PICES).

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

Page, R. D. M. 2002. *Visualizing phylogenetic trees using TreeView*, John Wiley & Sons, Inc.

Palumbi, S. R. 1992. Marine speciation on a small planet. *Trends in Ecology & Evolution,* 7(4)**,** 114-118.

Palumbi, S. R. 1994. Genetic divergence, reproductive isolation, and marine speciation. *Annual Review of Ecology and Systematics,* 25(1)**,** 547-572.

Palumbi, S. R. 1997. Molecular biogeography of the Pacific. *Coral Reefs,* 16(5)**,** S47-S52.

Pan, C.-H. & Chien, Y.-H. 2003. Concentration and composition of astaxanthin in black tiger prawn *Penaeus monodon* postlarvae fed *Artemia* sp. nauplii or mauxia shrimp *Acetes intermedius*. *Journal of the World Aquaculture Society,* 34(1)**,** 57-65.

Papaconstantinou, C. & Kapiris, K. 2003. The biology of the giant red shrimp (*Aristaeomorpha foliacea*) at an unexploited fishing ground in the Greek Ionian Sea. *Fisheries Research,* 62(1)**,** 37-51.

Park, W., Ma, C., Hong, S. Y. & Lee, K. 2009. Distribution and abundance of planktonic shrimps in the southern sea of Korea during 1987-1991. *Fisheries and Aquatic Science,* 12(3)**,** 240-248.

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

Patimar, R., Ownagh, E., Jafari, N. & Hosseini, M. 2009. Intrabasin variation in allometry coefficients of Lenkoran *Capoeta capoeta gracilis* (Keyserling, 1861) in the Gorganroud basin, southeast Caspian Sea, Iran. *Journal of Applied Ichthyology,* 25(6)**,** 776-778.

Pauly, D. 1984. Fish population dynamics in tropical waters: a manual for use with programmable calculators. *ICLARM Studies and Reviews 8.* Manila, Philippines: International Center for Living Aquatic Resources Management.

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.

Pérez-Castañeda, R. & Defeo, O. 2002. Morphometric relationships of penaeid shrimps in a coastal lagoon: Spatio-temporal variability and management implications. *Estuaries and Coasts,* 25(2)**,** 282-287.

PéRez Farfa nte, I. & Kensley, B. F. 1997. *Penaeoid and sergestoid shrimps and prawns of the world. Keys and diagnoses for the families and genera,* Paris, Editions du Muséum, 1997.

Perrin, C. & Borsa, P. 2001. Mitochondrial DNA analysis of the geographic structure of Indian scad mackerel in the Indo-Malay archipelago. *Journal of Fish Biology,* 59(5)**,** 1421-1426.

Petrakis, G. & Stergiou, K. I. 1995. Weight-length relationships for 33 fish species in Greek waters. *Fisheries Research,* 21(3-4)**,** 465-469.

Pfeiler, E., Hurtado, L. A., Knowles, L. L., Torre-Cosío, J., Bourillón-Moreno, L., Márquez-Farías, J. F. & Montemayor-López, 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.

Pices 1999. Annual Report 1999. North Pacific Marine Science Organization (PICES). Eighth meeting, Vladivostok, Russia, October 8-17, 1999. Sidney, British Columbia: North Pacific Marine Science Organization (PICES).

Pillai, S. K. 1983. Some observations on dol (bag) net fishery at Sassoon dock, Bombay. *Journal of the Bombay Natural History Society,* 82(3)**,** 331-340.

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

Planes, S. & Doherty, P. J. 1997. Genetic and color interactions at a contact zone of *Acanthochromis polyacanthus*: A marine fish lacking pelagic larvae. *Evolution,* 51(4)**,** 1232-1243.

Pogson, G. H., Mesa, K. A. & Boutilier, R. G. 1995. Genetic population structure and gene flow in the Atlantic Cod *Gadus morhua*: A comparison of allozyme and nuclear RFLP Loci. *Genetics,* 139(1)**,** 375-385.

Pollock, L. W. 1998. *A practical guide to the marine animals of northeastern North America,* New Brunswick, New Jersey, and London, Rutgers University Press.

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

Posada, D. 2009. Selection of models of DNA evolution with jModelTest. *In:* POSADA, D. (ed.) *Bioinformatics for DNA Sequence Analysis.* Humana Press.

Primavera, J. H., Parado-Estepa, F. D. & Lebata, J. L. 1998. Morphometric relationship of length and weight of giant tiger prawn *Penaeus monodon* according to life stage, sex and source. *Aquaculture,* 164(1-4)**,** 67-75.

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.

Ragonese, S., Bertolino, F. & Bianchini, M. L. 1997. Biometric relationships of the red shrimp, *Aristaeomorpha foliacea* Risso 1827, in the Strait of Sicily (Mediterranean Sea). *Scientia Marina,* 61(3)**,** 367-377.

Raje, S. G. 1991. Disposal of catch from 'dol' net at Versova, Bombay. *Marine Fisheries Information Service, Technical and Extension Series,* 113**,** 18-20.

Ramamurthy, S. & Muthu, M. S. 1969. Prawn fishing methods. *In:* JONES, S. (ed.) *CMFRI Bulletin No.14, Prawn fisheries of India.* Mandapam Camp: CMFRI.

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 the connection between DNA polymorphism, demography, and conservation biology. *Conservation Biology,* 10(2)**,** 665-671.

Rao, G. S. 1988. Exploitation of prawn resources by trawlers off Kakinada with note on the stock assessment of commercially important species. *Indian Journal of Fisheries,* 35(3)**,** 140-155.

Rao, P. V. 1968. A new species of shrimp, *Acetes cochinensis* (Crustacea: Decapoda, Sergestidae) from southwest coast of India with an account of its larval development. *Journal of the Marine Biological Association of India,* 10(2)**,** 298-320.

Ravindranath, K. 1980. Shrimps of the genus *Acetes* H. Milne Edwards (Crustacea, Decapoda, Sergestidae) from the estuarine system of river Krishna. *Proceedings of The Indian Academy of Sciences (Animal Science),* 89(3)**,** 253-273.

Rice, P., Longden, I. & Bleasby, A. 2000. EMBOSS: The European Molecular Biology Open Software Suite. *Trends in Genetics,* 16(6)**,** 276-277.

Richards, V. P., Thomas, J. D., Stanhope, M. J. & Shivji, M. S. 2007. Genetic connectivity in the Florida reef system: comparative phylogeography of commensal invertebrates with contrasting reproductive strategies. *Molecular Ecology,* 16(1)**,** 139-157.

Richter, H., Lückstädt, C., Focken, U. L. & Becker, K. 2000. An improved procedure to assess fish condition on the basis of length-weight relationships. *Archive of Fishery and Marine Research,* 48(3)**,** 226-235.

Richter, O. M. H. & Ludwig, B. 2003. Cytochrome *c* oxidase — structure, function, and physiology of a redox-driven molecular machine. *Reviews of Physiology, Biochemistry and Pharmacology.* Springer Berlin Heidelberg.

Rodríguez, G. 1982. Fresh-water shrimps (Crustacea, Decapoda, Natantia) of the Orinoco basin and the Venezuelan Guayana. *Journal of Crustacean Biology,* 2(3)**,** 378-391.

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

Rogers, A. R. & Harpending, H. 1992. Population growth makes waves in the distribution of pairwise genetic differences. *Molecular Biology and Evolution,* 9(3)**,** 552-569.

Rohfritsch, A. & Borsa, P. 2005. Genetic structure of Indian scad mackerel *Decapterus russelli*: Pleistocene vicariance and secondary contact in the Central Indo-West Pacific Seas. *Heredity,* 95(4)**,** 315-326.

Roldán, M. I., Heras, S., Patellani, R. & Maltagliati, F. 2009. Analaysis 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.

Ronquist, F., Huelsenbeck, J. P. & Van Der Mark, P. 2005. *MrBayes v. 3.1 Manual* [Online]. Available: http://mrbayes.csit.fsu.edu/wiki/index.php/Manual [Accessed June 1 2007].

Rosenberg, G. 2005. *Methods of preservation for marine samples* [Online]. Natural History Museum of Los Angeles Country. Available: http://clade.ansp.org/malacology/people/rosenberg/archiving/method/methods.html [Accessed July 5 2007].

Rozas, J., Sánchez-Delbarrio, J. C., Messeguer, X. & Rozas, R. 2003. DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics,* 19(18)**,** 2496-2497.

Ruddle, K. 1986. The supply of marine fish species for fermentation in Southeast Asia. *Bulletin of the National Museum of Ethnology,* 11(4)**,** 997-1036.

Safran, P. 1992. Theoretical analysis of the weight-length relationship in fish juveniles. *Marine Biology,* 112(4)**,** 545-551.

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

Sainte-Marie, B., Bérubé, I., Brillon, S. & Hazel, F. 2006. Observations on the growth of the sculptured shrimp *Sclerocrangon boreas* (Decapoda: Caridea). *Journal of Crustacean Biology,* 26(1)**,** 55-62.

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

Sandoval-Castillo, J., Rocha-Olivares, A., Villavicencio-Garayzar, C. & Balart, E. 2004. Cryptic isolation of Gulf of California shovelnose guitarfish evidenced by mitochondrial DNA. *Marine Biology,* 145(5)**,** 983-988.

Santos, M. N., Gaspar, M. B., Vasconcelos, P. & Monteiro, C. C. 2002. Weight–length relationships for 50 selected fish species of the Algarve coast (southern Portugal). *Fisheries Research,* 59(1–2)**,** 289-295.

Schneider, J. C., Laarman, P. W. & Gowing, H. 2000. Length-weight relationships. Chapter 17. *In:* SCHNEIDER, J. C. (ed.) *Manual of fisheries survey methods II: with periodic updates.* Michigan Department of Natural Resources, Fisheries Special Report 25, Ann Arbor.

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.

Schubart, C. D., Diesel, R. & Hedges, S. B. 1998. Rapid evolution to terrestrial life in Jamaican crabs. *Nature,* 393(6683)**,** 363-365.

Sehara, D. B. S. & Kharbari, J. P. 1987. A study on 'Dol' net fishery at selected centres in Northwest coast with special reference to costs and returns. *Marine Fisheries Information Service Technical and Extension Series,* 78**,** 1-15.

Shank, T. M., Black, M. B., Halanych, K. M., Lutz, R. A. & Vrijenhoek, R. C. 1999. Miocene radiation of deep-sea hydrothermal vent shrimp (Caridea: Bresiliidae): Evidence from mitochondrial cytochrome oxidase subunit I. *Molecular Phylogenetics and Evolution,* 13(2)**,** 244-254.

Shi, R.-D. 1986. The distribution, migration and generation of *Acetes chinensis* Hansen in the inshore waters of southern Zhejiang. [In Chinese]. *Donghai Marine Science,* 4(1)**,** 56-61.

Shui, B.-N., Han, Z.-Q., Gao, T.-X., Miao, Z.-Q. & Yanagimoto, T. 2009. Mitochondrial DNA variation in the East China Sea and Yellow Sea populations of Japanese Spanish mackerel *Scomberomorus niphonius*. *Fisheries Science,* 75(3)**,** 593-600.

Shulman, M. J. & Bermingham, E. 1995. Early life histories, ocean currents, and the population genetics of carribean reef fishes. *Evolution,* 49(5)**,** 897-910.

Siegfried, C. A. 1980. Seasonal abundance and distribution of *Crangon franciscorum* and *Palaemon macrodactylus* (Decapoda: Caridea) in the San Francisco Bay-Delta. *Biological Bulletin,* 159(1)**,** 177-192.

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

Sokal, R. R. & Rohlf, F. J. 1987. *Introduction to biostatistics,* New York, Freeman.

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,* 105(36)**,** 13486-13491.

Sorenson, M. D. & Quinn, T. W. 1998. Numts: A challenge for avian systematics and population biology. *The Auk,* 115(1)**,** 214-221.

Stergiou, K. I. & Politou, C.-Y. 1995. Biological parameters, body length-weight and length-height relationships for various species in Greek Waters. *NAGA, The ICLARM Quarterly,* 18(2)**,** 42-45.

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

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

Tajima, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics,* 123(3)**,** 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.

Tesch, W. 1971. Age and growth. *In:* RICKER, W. E. (ed.) *Methods for assessment of fish production in fresh waters, 2nd edition.* Oxford: Blackwell Scientific Publications Ltd.

Teske, P., Papadopoulos, I., Zardi, G., Mcquaid, C., Edkins, M., Griffiths, C. & Barker, N. 2007. Implications of life history for genetic structure and migration rates of southern African coastal invertebrates: planktonic, abbreviated and direct development. *Marine Biology,* 152(3)**,** 697-711.

Thalmann, O., Hebler, J., Poinar, H. N., Pääbo, S. & Vigilant, L. 2004. Unreliable mtDNA data due to nuclear insertions: a cautionary tale from analysis of humans and other great apes. *Molecular Ecology,* 13(2)**,** 321-335.

Tham, A. K. 1955. The shrimp industry of Singapore. *Proceedings of the Indo-Pacific Fisheries Council,* 5(2-3)**,** 145-155.

Tham, A. K. 1968. Unit stocks of shrimps and prawns in the IPFC region and unit fisheries exploiting them. *FAO Fisheries Report No. 57.* Rome: FAO.

Thessalou-Legaki, M. & Kiortsis, V. 1997. Estimation of the reproductive output of the burrowing shrimp *Callianassa tyrrhena*: a comparison of three different biometrical approaches. *Marine Biology,* 127(3)**,** 435-442.

Thompson, J. D., Higgins, D. G. & Gibson, T. J. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research,* 22(22)**,** 4673-4680.

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

Tirmizi, N. M. & Ghani, N. A. 1982. New distributional records for three species of *Acetes* (Decapoda, Sergestidae). *Crustaceana,* 42(1)**,** 44-53.

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.

Toon, A., Finley, M., Staples, J. & Crandall , K. A. 2009. Decapod Phylogenetics and Molecular Evolution. *In:* MARTIN, W. J., CRANDALL, K. A. & FELDER, D. L. (eds.) *Decapod Crustacean Phylogenetics.* Boca Raton, FL, USA: CRC Press: Taylor & Francis Group

Torcu-Koç, H., Erdogan, Z. & Treer, T. 2006. A review of length–weight relationships of fishes from freshwaters of Turkey. *Journal of Applied Ichthyology,* 22(4)**,** 264-270.

Tosunoglu, Z., Ozaydin, O. & Deval, M. C. 2007. Morphometric relationships of length-length and length-weight in *Parapenaeus longirostris* (Lucas, 1846) (Decapoda, Penaeidae). *Crustaceana,* 80(10)**,** 1253-1259.

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.

Tzeng, T.-D., Yeh, S.-Y. & Hui, C.-F. 2004. Population genetic structure of the kuruma prawn (*Penaeus japonicus*) in East Asia inferred from mitochondrial DNA sequences. *ICES Journal of Marine Science: Journal du Conseil,* 61(6)**,** 913-920.

Uthicke, S. & Benzie, J. a. H. 2003. Gene flow and population history in high dispersal marine invertebrates: mitochondrial DNA analysis of *Holothuria nobilis* (Echinodermata: Holothuroidea) populations from the Indo-Pacific. *Molecular Ecology,* 12(10)**,** 2635-2648.

Uye, S.-I. 1982. Length-weight relationships of important zooplankton from the Inland Sea of Japan. *Journal of the Oceanographical Society of Japan,* 38(3)**,** 149-158.

Veiga, P., Machado, D., Almeida, C., Bentes, L., Monteiro, P., Oliveira, F., Ruano, M., Erzini, K. & Gonçalves, J. M. S. 2009. Weight–length relationships for 54 species of the Arade estuary, southern Portugal. *Journal of Applied Ichthyology,* 25(4)**,** 493-496.

Voris, H. K. 2000. Maps of Pleistocene sea levels in Southeast Asia: shorelines, river systems and time durations. *Journal of Biogeography,* 27(5)**,** 1153-1167.

Wan Daud, W. J. B. 1978. Processing of traditional fish products in Malaysia with special reference to the State of Trengganu. *In:* Proceedings of the 18th session of the Indo-Pacific Fishery Commission, Manila, Philippines, March 8–17, 1978 Section 3. Symposium on Fish Utilization Technology and Marketing in the IPFC Region, 1978. 7-11.

Wang, M., Zhang, X., Yang, T., Han, Z., Yanagimoto, T. & Gao, T. 2008. Genetic diversity in the mtDNA control region and population structure in the *Sardinella zunasi* Bleeker *African Journal of Biotechnology,* 7(24)**,** 4384-4392.

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.

Watson, R. A. & Keating, J. A. 1989. Velvet shrimps (*Metapenaeopsis* spp.) of Torres Strait, Queensland, Australia. *Asian Fisheries Science,* 3(1)**,** 45-56.

Wei, S.-S., Wang, R.-X. & Qu, X.-Z. 1985. Experiment on hole-in-belly staked net in shrimp catching of Shandong inshore. [In Chinese]. *Journal of Fisheries of China,* 9(2)**,** 131-141.

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

Wetzel, M. A., Leuchs, H. & Koop, J. H. E. 2005. Preservation effects on wet weight, dry weight, and ash-free dry weight biomass estimates of four common estuarine macro-invertebrates: no difference between ethanol and formalin. *Helgoland Marine Research,* 59(3)**,** 206-213.

Wilke, T. & Davis, G. M. 2000. Infraspecfic mitochondrial sequence diversity in *Hydrobia ulvae* and *Hydrobia ventrosa* (Hydrobiidae: Rissooidea: Gastropoda): Do their different life-histories affect biogeographic patterns and gene flow? *Biological Journal of the Linnean Society,* 70(1)**,** 89-105.

Williams, A. B. 1965. Marine decapod crustaceans of the Carolinas. *Fishery Bulletin of the United States Fish and Wildlife Service,* 65(1)**,** 1-298.

Williams, A. B. 1969. A ten-year study of meroplankton in North Carolina estuaries: cycles of occurrence among penaeidean shrimps. *Chesapeake Science,* 10(1)**,** 36-47.

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. 2001. Mitochondrial pseudogenes are pervasive and often insidious in the snapping shrimp genus *Alpheus Molecular Biology and Evolution,* 18(8)**,** 1484-1493.

Wilson, A. C., Cann, R. L., Carr, S. M., George, M., Gyllensten, U. B., Helm-Bychowski, K. M., Higuchi, R. G., Palumbi, S. R., Prager, E. M., Sage, R. D. & Stoneking, M. 1985. Mitochondrial DNA and two perspectives on evolutionary genetics. *Biological Journal of the Linnean Society,* 26(4)**,** 375-400.

Xiao, Y. & Greenwood, J. G. 1993. The biology of *Acetes* (Crustacea: Sergestidae). *In:* ANSELL, A. D., GIBSON, R. N. & BARNES, M. (eds.) *Oceanography and Marine Biology: An Annual Review, volume 31.* London: UCL Press.

Xu, X. & Abdul Ghaffar, A. R. 1995. Statistical relations between lengths and weights of green tiger prawns, *Penaeus semisulcatus*, in Kuwait Waters. *Asian Fisheries Science,* 8(2)**,** 121-131.

Yamada, R., Kodama, K., Yamakawa, T., Horiguchi, T. & Aoki, I. 2007. Growth and reproductive biology of the small penaeid shrimp *Trachysalambria curvirostris* in Tokyo Bay. *Marine Biology,* 151(3)**,** 961-971.

Yasuda, J., Takamori, S. & Nishina, S. 1953. Study on ecology of Akiami (*Acetes japonicus* Kishinouye) and its multiplication and protection. *Bulletin of the Naikai Regional Fisheries Research Laboratory,* 4**,** 1-19.

Yeap, S. E. & Tan, S. M. 2003. Issues facing the traditional fish products industry in Southeast Asia. *JIRCAS International Symposium Series,* 11**,** 115-121.

Yeoh, Q. L. & Merican, Z. 1978. Processing of non-commercial and low-cost fish in Malaysia. *In:* LINDQVIST, O. V., ed. Proceedings of the 18th session of the Indo-Pacific Fishery Commission, Manila, Philippines, March 8–17, 1978 Section 3. Symposium on Fish Utilization Technology and Marketing in the IPFC Region, 8-17 March 1978 1978. 572-580.

Zafar, M., Mustafa, M. G. & Amin, S. M. N. 1998a. Population dynamics of *Acetes chinensis* in the Kutubdia Channel of Bangladesh coastal waters. *Indian Journal of Fisheries,* 45(2)**,** 121-127.

Zafar, M., Mustafa, M. G. & Amin, S. M. N. 1998b. Studies on age and growth, length-weight relationship and relative condition factor of two *Acetes* shrimps from Bangladesh coast. *The Chittagong University Journal of Science,* 22(II)**,** 109-116.

Zafar, M., Mustafa, M. G., Amin, S. M. N. & Akhter, S. 1997. Studies on population dynamics of *Acetes indicus* from Bangladesh Coast. *Journal of National Oceanography and Maritime (NOAMI),* 14(1-2)**,** 1-13.

Zar, J. H. 1999. *Biostatistical analysis,* Upper Saddle River, N.J., Prentice Hall.

Zhan, A., Hu, J., Hu, X., Zhou, Z., Hui, M., Wang, S., Peng, W., Wang, M. & Bao, Z. 2009. Fine-scale population genetic structure of Zhikong Scallop (*Chlamys farreri*): do local marine currents drive geographical differentiation? *Marine Biotechnology,* 11(2)**,** 223-235.

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

Zhang, M.-H. 1986. Studies on the methods of forecasting fishing conditions for *Acetes chinensis* near the Yellow River mouth. [In Chinese]. *Transactions of Oceanology and Limnology,* (4)**,** 68-76.

Zhang, M.-H. 1992. Reproductive characteristics of *Acetes chinensis* in Bohai Bay and Laizhou Bay. [In Chinese]. *Transactions of Oceanology and Limnology,* 2**,** 58-67.

Zhong, C., Zeng, X., Ren, Y. & Shen, H. 2001. Study on the fishery biology of the *Acetes chinensis* Hansen caught in coastal waters of Laizhou Bay and Huanghe Estuary. *Transactions of Oceanology and Limnology,* 1**,** 31-36.

Zimmermann, J., Hajibabaei, M., Blackburn, D., Hanken, J., Cantin, E., Posfai, J. & Evans, T. 2008. DNA damage in preserved specimens and tissue samples: a molecular assessment. *Frontiers in Zoology,* 5(1)**,** 1-13.

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.

Zynudheen, A. A., Ninan, G., Sen, N. & Badonia, R. 2004. Utilitzation of trawl bycatch in Gujarat (India). *NAGA, WorldFish Center Quaterly,* 27(3 & 4)**,** 20-23.

Appendix A

Critical values of the *t* Distribution (Zar, 1999)

ν	$\alpha(2)$: 0.50 $\alpha(1)$: 0.25	0.20 0.10	0.10 0.05	0.05 0.025	0.02 0.01	0.01 0.005	0.005 0.0025	0.002 0.001	0.001 0.0005
1	1.000	3.078	6.314	12.706	31.821	63.657	127.321	318.309	636.619
2	0.816	1.886	2.920	4.303	6.965	9.925	14.089	22.327	31.599
3	0.765	1.638	2.353	3.182	4.541	5.841	7.453	10.215	12.924
4	0.741	1.533	2.132	2.776	3.747	4.604	5.598	7.173	8.610
5	0.727	1.476	2.015	2.571	3.365	4.032	4.773	5.893	6.869
6	0.718	1.440	1.943	2.447	3.143	3.707	4.317	5.208	5.959
7	0.711	1.415	1.895	2.365	2.998	3.499	4.029	4.785	5.408
8	0.706	1.397	1.860	2.306	2.896	3.355	3.833	4.501	5.041
9	0.703	1.383	1.833	2.262	2.821	3.250	3.690	4.297	4.781
10	0.700	1.372	1.812	2.228	2.764	3.169	3.581	4.144	4.587
11	0.697	1.363	1.796	2.201	2.718	3.106	3.497	4.025	4.437
12	0.695	1.356	1.782	2.179	2.681	3.055	3.428	3.930	4.318
13	0.694	1.350	1.771	2.160	2.650	3.012	3.372	3.852	4.221
14	0.692	1.345	1.761	2.145	2.624	2.977	3.326	3.787	4.140
15	0.691	1.341	1.753	2.131	2.602	2.947	3.286	3.733	4.073
16	0.690	1.337	1.746	2.120	2.583	2.921	3.252	3.686	4.015
17	0.689	1.333	1.740	2.110	2.567	2.898	3.222	3.646	3.965
18	0.688	1.330	1.734	2.101	2.552	2.878	3.197	3.610	3.922
19	0.688	1.328	1.729	2.093	2.539	2.861	3.174	3.579	3.883
20	0.687	1.325	1.725	2.086	2.528	2.845	3.153	3.552	3.850
21	0.686	1.323	1.721	2.080	2.518	2.831	3.135	3.527	3.819
22	0.686	1.321	1.717	2.074	2.508	2.819	3.119	3.505	3.792
23	0.685	1.319	1.714	2.069	2.500	2.807	3.104	3.485	3.768
24	0.685	1.318	1.711	2.064	2.492	2.797	3.091	3.467	3.745
25	0.684	1.316	1.708	2.060	2.485	2.787	3.078	3.450	3.725
26	0.684	1.315	1.706	2.056	2.479	2.779	3.067	3.435	3.707
27	0.684	1.314	1.703	2.052	2.473	2.771	3.057	3.421	3.690
28	0.683	1.313	1.701	2.048	2.467	2.763	3.047	3.408	3.674
29	0.683	1.311	1.699	2.045	2.462	2.756	3.038	3.396	3.659
30	0.683	1.310	1.697	2.042	2.457	2.750	3.030	3.385	3.646
31	0.682	1.309	1.696	2.040	2.453	2.744	3.022	3.375	3.633
32	0.682	1.309	1.694	2.037	2.449	2.738	3.015	3.365	3.622
33	0.682	1.308	1.692	2.035	2.445	2.733	3.008	3.356	3.611
34	0.682	1.307	1.691	2.032	2.441	2.728	3.002	3.348	3.601
35	0.682	1.306	1.690	2.030	2.438	2.724	2.996	3.340	3.591
36	0.681	1.306	1.688	2.028	2.434	2.719	2.990	3.333	3.582
37	0.681	1.305	1.687	2.026	2.431	2.715	2.985	3.326	3.574
38	0.681	1.304	1.686	2.024	2.429	2.712	2.980	3.319	3.566
39	0.681	1.304	1.685	2.023	2.426	2.708	2.976	3.313	3.558
40	0.681	1.303	1.684	2.021	2.423	2.704	2.971	3.307	3.551
41	0.681	1.303	1.683	2.020	2.421	2.701	2.967	3.301	3.544
42	0.680	1.302	1.682	2.018	2.418	2.698	2.963	3.296	3.538
43	0.680	1.302	1.681	2.017	2.416	2.695	2.959	3.291	3.532
44	0.680	1.301	1.680	2.015	2.414	2.692	2.956	3.286	3.526
45	0.680	1.301	1.679	2.014	2.412	2.690	2.952	3.281	3.520
46	0.680	1.300	1.679	2.013	2.410	2.687	2.949	3.277	3.515
47	0.680	1.300	1.678	2.012	2.408	2.685	2.946	3.273	3.510
48	0.680	1.299	1.677	2.011	2.407	2.682	2.943	3.269	3.505
49	0.680	1.299	1.677	2.010	2.405	2.680	2.940	3.265	3.500
50	0.679	1.299	1.676	2.009	2.403	2.678	2.937	3.261	3.496
52	0.679	1.298	1.675	2.007	2.400	2.674	2.932	3.255	3.488
54	0.679	1.297	1.674	2.005	2.397	2.670	2.927	3.248	3.480
56	0.679	1.297	1.673	2.003	2.395	2.667	2.923	3.242	3.473
58	0.679	1.296	1.672	2.002	2.392	2.663	2.918	3.237	3.466
60	0.679	1.296	1.671	2.000	2.390	2.660	2.915	3.232	3.460

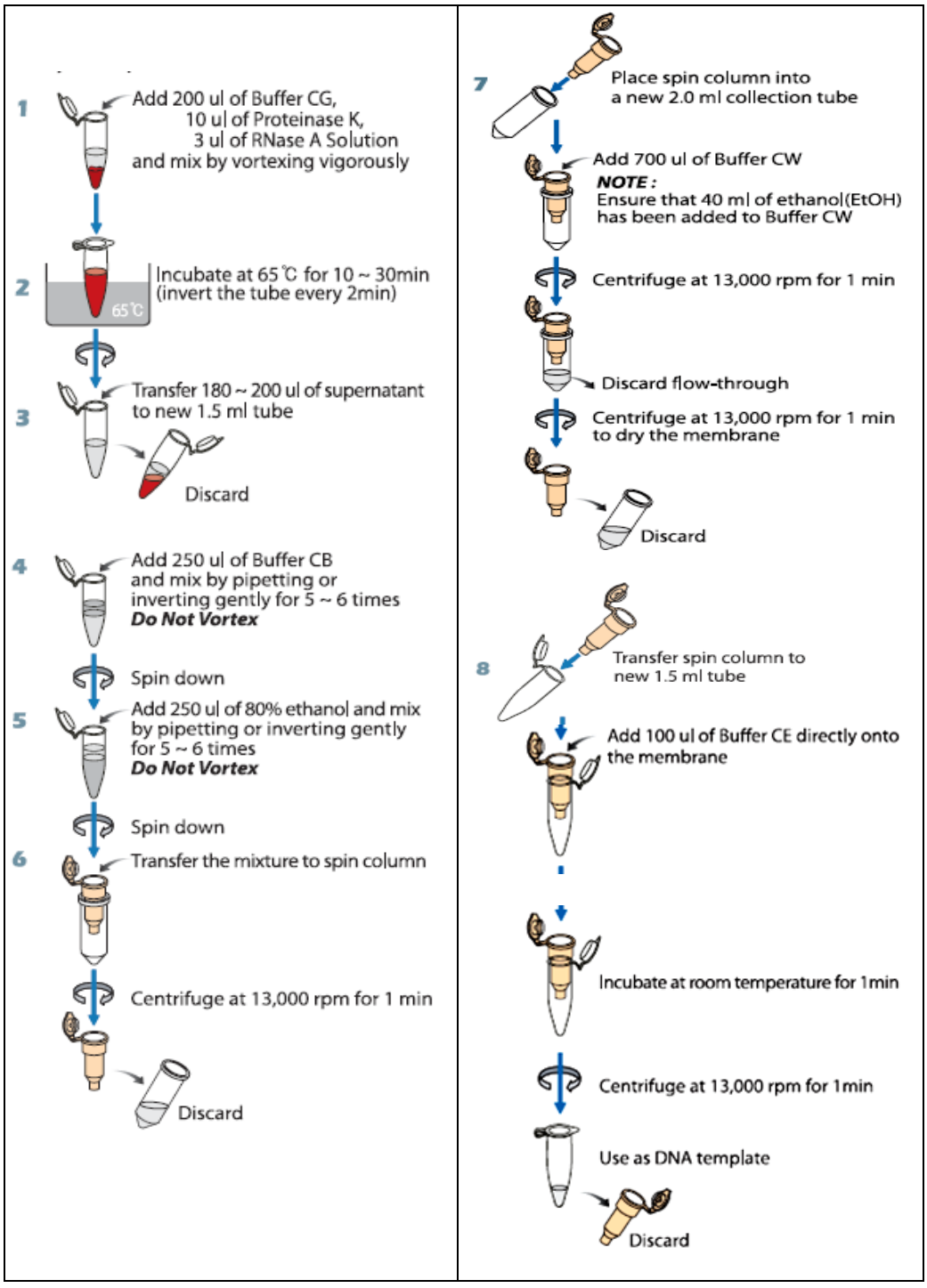
Appendix A (continued)

Critical values of the *t* Distribution (Zar, 1999)

ν	$\alpha(2)$: 0.50 $\alpha(1)$: 0.25	0.20 0.10	0.10 0.05	0.05 0.025	0.02 0.01	0.01 0.005	0.005 0.0025	0.002 0.001	0.001 0.0005
62	0.678	1.295	1.670	1.999	2.388	2.657	2.911	3.227	3.454
64	0.678	1.295	1.669	1.998	2.386	2.655	2.908	3.223	3.449
66	0.678	1.295	1.668	1.997	2.384	2.652	2.904	3.218	3.444
68	0.678	1.294	1.668	1.995	2.382	2.650	2.902	3.214	3.439
70	0.678	1.294	1.667	1.994	2.381	2.648	2.899	3.211	3.435
72	0.678	1.293	1.666	1.993	2.379	2.646	2.896	3.207	3.431
74	0.678	1.293	1.666	1.993	2.378	2.644	2.894	3.204	3.427
76	0.678	1.293	1.665	1.992	2.376	2.642	2.891	3.201	3.423
78	0.678	1.292	1.665	1.991	2.375	2.640	2.889	3.198	3.420
80	0.678	1.292	1.664	1.990	2.374	2.639	2.887	3.195	3.416
82	0.677	1.292	1.664	1.989	2.373	2.637	2.885	3.193	3.413
84	0.677	1.292	1.663	1.989	2.372	2.636	2.883	3.190	3.410
86	0.677	1.291	1.663	1.988	2.370	2.634	2.881	3.188	3.407
88	0.677	1.291	1.662	1.987	2.369	2.633	2.880	3.185	3.405
90	0.677	1.291	1.662	1.987	2.368	2.632	2.878	3.183	3.402
92	0.677	1.291	1.662	1.986	2.368	2.630	2.876	3.181	3.399
94	0.677	1.291	1.661	1.986	2.367	2.629	2.875	3.179	3.397
96	0.677	1.290	1.661	1.985	2.366	2.628	2.873	3.177	3.395
98	0.677	1.290	1.661	1.984	2.365	2.627	2.872	3.175	3.393
100	0.677	1.290	1.660	1.984	2.364	2.626	2.871	3.174	3.390
105	0.677	1.290	1.659	1.983	2.362	2.623	2.868	3.170	3.386
110	0.677	1.289	1.659	1.982	2.361	2.621	2.865	3.166	3.381
115	0.677	1.289	1.658	1.981	2.359	2.619	2.862	3.163	3.377
120	0.677	1.289	1.658	1.980	2.358	2.617	2.860	3.160	3.373
125	0.676	1.288	1.657	1.979	2.357	2.616	2.858	3.157	3.370
130	0.676	1.288	1.657	1.978	2.355	2.614	2.856	3.154	3.367
135	0.676	1.288	1.656	1.978	2.354	2.613	2.854	3.152	3.364
140	0.676	1.288	1.656	1.977	2.353	2.611	2.852	3.149	3.361
145	0.676	1.287	1.655	1.976	2.352	2.610	2.851	3.147	3.359
150	0.676	1.287	1.655	1.976	2.351	2.609	2.849	3.145	3.357
160	0.676	1.287	1.654	1.975	2.350	2.607	2.846	3.142	3.352
170	0.676	1.287	1.654	1.974	2.348	2.605	2.844	3.139	3.349
180	0.676	1.286	1.653	1.973	2.347	2.603	2.842	3.136	3.345
190	0.676	1.286	1.653	1.973	2.346	2.602	2.840	3.134	3.342
200	0.676	1.286	1.653	1.972	2.345	2.601	2.839	3.131	3.340
250	0.675	1.285	1.651	1.969	2.341	2.596	2.832	3.123	3.330
300	0.675	1.284	1.650	1.968	2.339	2.592	2.828	3.118	3.323
350	0.675	1.284	1.649	1.967	2.337	2.590	2.825	3.114	3.319
400	0.675	1.284	1.649	1.966	2.336	2.588	2.823	3.111	3.315
450	0.675	1.283	1.648	1.965	2.335	2.587	2.821	3.108	3.312
500	0.675	1.283	1.648	1.965	2.334	2.586	2.820	3.107	3.310
600	0.675	1.283	1.647	1.964	2.333	2.584	2.817	3.104	3.307
700	0.675	1.283	1.647	1.963	2.332	2.583	2.816	3.102	3.304
800	0.675	1.283	1.647	1.963	2.331	2.582	2.815	3.100	3.303
900	0.675	1.282	1.647	1.963	2.330	2.581	2.814	3.099	3.301
1000	0.675	1.282	1.646	1.962	2.330	2.581	2.813	3.098	3.300
∞	0.6745	1.2816	1.6449	1.9600	2.3263	2.5758	2.8070	3.0902	3.2905

Appendix B

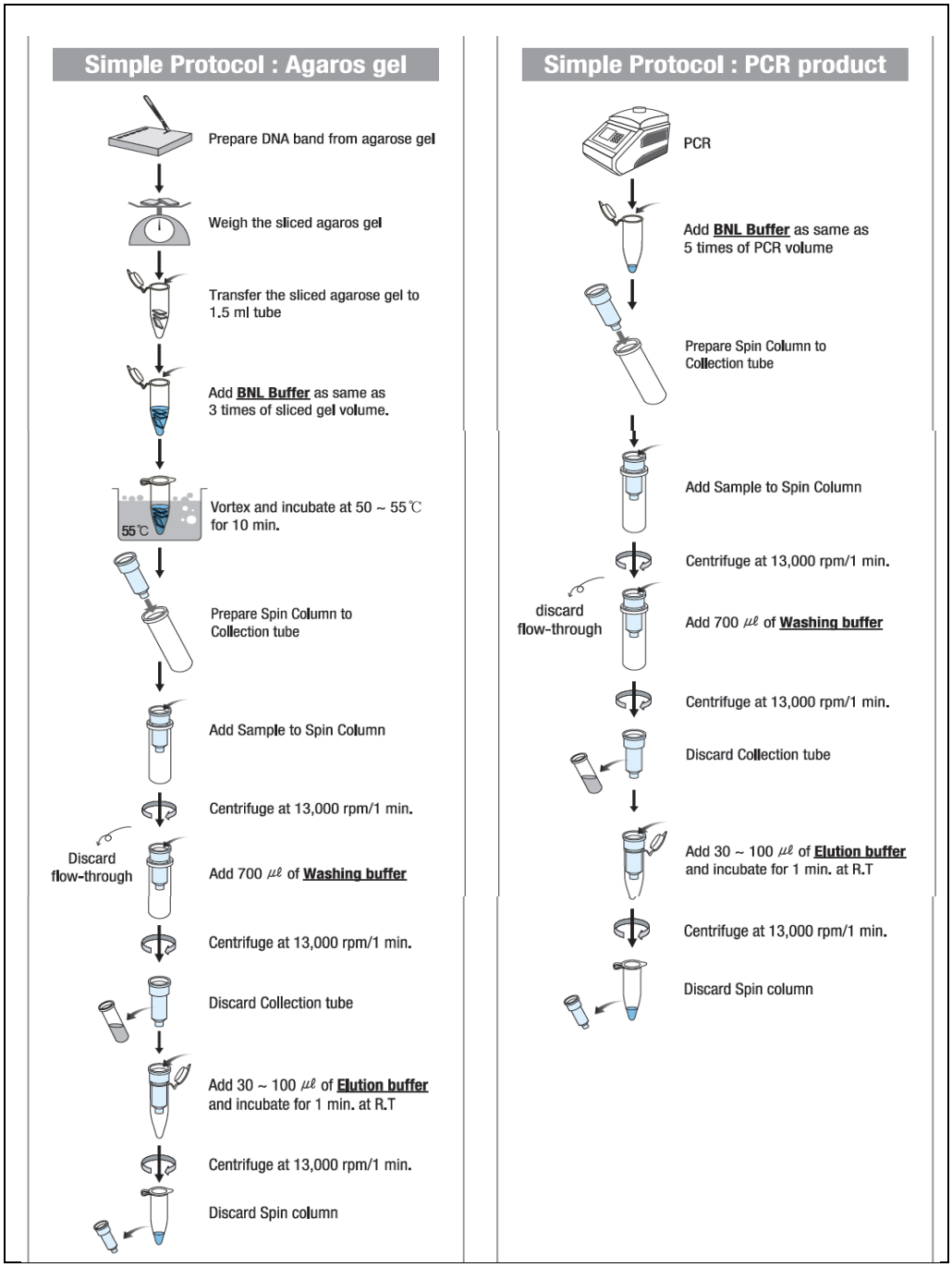
Extraction of genomic DNA with i-genomic CTB DNA Extraction Mini Kit (iNtRON Biotechnology, Inc., South Korea)



Buffer CG: Lysis buffer; Buffer CB: Binding buffer; Buffer CW: Washing buffer. Buffer CW are supplied as concentrate, add 40 ml of ethanol (96 ~ 100%) before use. Buffer CE is finally 10 mM Tris-HCl (pH 8.0). RNase A solution (20 mg/ml, stored at -20 ^{o}C) is completely free of Dnase activity. Proteinase K solution (20 mg/ml, -20 ^{o}C): after thawing, freshly use.

Appendix C

Purification of PCR products with MEGAquick-spinTM PCR and Agarose Gel DNA Extraction System (iNtRON Biotechnology, Inc., South Korea)



Washing Buffer is supplied as concentrate. Add 40 ml (50 columns) or 160 ml (250 columns) per each bottles of ethanol (96 ~ 100%) according to the bottle label before use. All buffers (BNL buffer, Washing buffer and Elution buffer) are stored at room temperature.

Appendix D

Invertebrate Mitochondrial Genetic Code (Librado and Rozas, 2009; Rozas et al., 2003).

UUU Phe	UCU Ser	UAU Tyr	UGU Cys
UUC Phe	UCC Ser	UAC Tyr	UGC Cys
UUA Leu	UCA Ser	UAA ***	UGA Trp
UUG Leu	UCG Ser	UAG ***	UGG Trp
CUU Leu	CCU Pro	CAU His	CGU Arg
CUC Leu	CCC Pro	CAC His	CGC Arg
CUA Leu	CCA Pro	CAA Gln	CGA Arg
CUG Leu	CCG Pro	CAG Gln	CGG Arg
AUU Ile	ACU Thr	AAU Asn	AGU Ser
AUC Ile	ACC Thr	AAC Asn	AGC Ser
AUA Met	ACA Thr	AAA Lys	AGA Ser
AUG Met	ACG Thr	AAG Lys	AGG Ser
GUU Val	GCU Ala	GAU Asp	GGU Gly
GUC Val	GCC Ala	GAC Asp	GGC Gly
GUA Val	GCA Ala	GAA Glu	GGA Gly
GUG Val	GCG Ala	GAG Glu	GGG Gly

Phe: Phenylalanine; Leu: Leucine; Ile: Isoleucine; Met: Methionine; Val: Valine; Ser: Serine; Pro: Proline; Thr: Threonine; Ala: Alanine; Tyr: Tyrosine; ***: Stop codon; His: Histidine; Gln: Glutamine; Asn: Asparagine; Lys: Lysine; Asp: Asparagine; Glu: Glutamic acid; Cys: Cysteine; Trp: Tryptophan; Arg: Arginine; Gly: Glycine

Appendix E

List of specimens used in this study and Genbank accession number. [1]f:female, m:male; [2]sampling location: refer to Table 3.1

Species	Lab Identification no. (specimens no, sexes of specimens[1], sampling location[2])	Haplotype	Genbank Accession No.
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	ai2	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
	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
	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

Appendix F

Translation of *COI* into amino acids

```
[               1111111111222222222233333333334444444444555555555566]
[      12345678901234567890123456789012345678901234567890123456789012]
#ai_1_ LSLIIRAELGQPGSLIGDDQIYNVVVTAHAFIMIFFMVMPIMIGGFGNWLVPLMLGAPDMAF
#ai_2  ............................................................
#ai_3  ............................................................
#ai_4  ............................................................
#ai_5  ............................................................
#ai_6  ............................................................
#ai_7  ............................................................
#ai_8  ............................................................
#ai_9  ............................................................
#ai_10 ............................................................
#ai_11 ............................................................
#as_1  ............................................................
#as_2  ............................................................
#as_3  ............................................................
#as_4  ............................................................
#as_5  ............................................................
#as_6  ............................................................
#as_7  ............................................................
#as_8  ............................................................
#as_9  ............................................................
#as_10 ............................................................
#as_11 ............................................................
#as_12 ............................................................
#as_13 ............................................................
#as_14 ............................................................
#as_15 ............................................................
#as_16 ............................................................
#as_17 ............................................................
#as_18 ............................................................
#as_19 ............................................................
#as_20 ............................................................
#as_21 ............................................................
#as_22 ............................................................
#as_23 ............................................................
#as_24 ............................................................
#as_25 ............................................................
#as_26 ............................................................
#as_27 ............................................................
#as_28 ............................................................
#as_29 ............................................................
#as_30 ............................................................
#as_31 ............................................................
#aj_1  ............................................................
#aj_2  ............................................................
#asi_1 ............................................................
#asi_2 ............................................................
```

Appendix F (continued)

Translation of *COI* into amino acids

```
[                                                  1111111111111111111111111]
[      6666666777777777788888888889999999999000000000011111111112222 2]
[      3456789012345678901234567890123456789012345678901234567890123 4]
#ai_1_ PRMNNMSFWMLPPSLTLLLSSGLVESGVGTGWTVYPPLAAGIAHAGASVDLGIFSLHLAGVS
#ai_2  .......................................................................
#ai_3  .......................................................................
#ai_4  .......................................................................
#ai_5  .......................................................................
#ai_6  .......................................................................
#ai_7  .......................................................................
#ai_8  .......................................................................
#ai_9  .......................................................................
#ai_10 .......................................................................
#ai_11 .......................................................................
#as_1  ....................................S..................................
#as_2  ....................................S..................................
#as_3  ....................................S..................................
#as_4  ....................................S..................................
#as_5  ....................................S..................................
#as_6  ....................................S..................................
#as_7  ....................................S..................................
#as_8  ....................................S..................................
#as_9  ....................................S..................................
#as_10 ....................................S..................................
#as_11 ....................................S..................................
#as_12 ....................................S..................................
#as_13 ....................................S..................................
#as_14 ....................................S..................................
#as_15 ....................................S..................................
#as_16 ....................................S..................................
#as_17 ....................................S..................................
#as_18 ....................................S..................................
#as_19 ....................................S..................................
#as_20 ....................................S..................................
#as_21 ....................................S..................................
#as_22 ....................................S..................................
#as_23 ....................................S..................................
#as_24 ....................................S..................................
#as_25 ....................................S..................................
#as_26 ....................................S..................................
#as_27 ....................................S..................................
#as_28 ....................................S..................................
#as_29 ....................................S..................................
#as_30 ....................................S..................................
#as_31 ....................................S..................................
#aj_1  .......................................................................
#aj_2  .......................................................................
#asi_1 ....................M..................................................
#asi_2 ....................M..................................................
```

Appendix F (continued)

Translation of *COI* into amino acids

```
[      1111111111111111111111111111111111111111111111111111111111111111]

[      2222233333333334444444444555555555566666666667777777777888888]
[      5678901234567890123456789012345678901234567890123456789012345678]
#ai_1_ SILGAVNFMTTVINMRSMGMSMDRLPLFVWAVFITALLLLLSLPVLAGAITMLLTDRNLN
#ai_2  ..........................................................
#ai_3  ..........................................................
#ai_4  ..........................................................
#ai_5  ..........................................................
#ai_6  ..........................................................
#ai_7  ..........................................................
#ai_8  ..........................................................
#ai_9  ..........................................................
#ai_10 ..........................................................
#ai_11 ..........................................................
#as_1  ..................T.......................................
#as_2  ..................T.......................................
#as_3  ..................T.......................................
#as_4  ..................T.......................................
#as_5  ..................T.......................................
#as_6  ..................T.......................................
#as_7  ..................T.......................................
#as_8  ..................T.......................................
#as_9  ..................T.......................................
#as_10 ..................T.......................................
#as_11 ..................T.......................................
#as_12 ..................T.......................................
#as_13 ..................T.......................................
#as_14 ..................T.......................................
#as_15 ..................T.......................................
#as_16 ..................T.......................................
#as_17 ..................T.......................................
#as_18 ..................T.......................................
#as_19 ..................T.......................................
#as_20 ..................T.......................................
#as_21 ..................T.......................................
#as_22 ..................T.......................................
#as_23 ..................T.......................................
#as_24 ..................T.......................................
#as_25 ..................T.......................................
#as_26 ..................T.......................................
#as_27 ..................T.......................................
#as_28 ..................T.......................................
#as_29 ..................T.......................................
#as_30 ..................T.......................................
#as_31 ..................T.......................................
#aj_1  ..................T.......................................
#aj_2  ..................T.......................................
#asi_1 ..................T.......................................
#asi_2 ..................T.......................................
```