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TITLE: Sequence Divergence of Mitochondrial DNA among Freshwater Prawn, Genus *Macrobrachium* in Basins of the Central, Western and Eastern Parts of Thailand

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THESIS

SEQUENCE DIVERGENCE OF MITOCHONDRIAL DNA AMONG FRESHWATER PRAWN, GENUS *Macrobrachium* IN BASINS OF THE CENTRAL, WESTERN AND EASTERN PARTS OF THAILAND

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A Thesis Submitted in Partial Fulfillment of
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Thanaporn Pinpart 2010: Sequence Divergence of Mitochondrial DNA among Freshwater Prawn, Genus *Macrobrachium* in Basin of Central, Western and Eastern Parts of Thailand. Master of Science (Genetics), Major Field: Genetics, Department of Genetics. Thesis Advisor: Ms. Lertluk Ngernsiri, Ph.D. 127 pages.

Freshwater prawns of the genus *Macrobrachium* from the provinces in central, western and eastern parts of Thailand were morphologically classified into 10 species namely, *M. assamese*, *M. lanchesteri*, *M. rosenbergii*, *M. tratense*, *M. sintangense*, *M. forcipatum*, *M. dienbienphuense*, *M. hirsutimanus*, *M. niphanae* and *M. sp3*. Their complete nucleotide sequences of *16S rRNA* gene were determined. The gene sequences ranged from 1300-1310 bp. The average evolutionary distance within and between species were 0.000-0.026 and 0.023-0.351 respectively. There is inconsistency between morphological features and genetic divergence in the species *M. assamense*. The species showed very little variation and formed a very well-supported clade with *M. lanchesteri*. Species in the *M. pillimanus* group, *M. forcipatum*, *M. dienbienphuense* and *M. hirsutimanus* formed a different clade and are closer to *M. lanchesteri* and *M.rosenbergii*. Thus, at least 8 *Macrobrachium* species in this study were supported species status by molecular evidence. Moreover, species specific marker using multiplex PCR for 3 species, *M. hirsutimanus*, *M. rosenbergii* and *M. lanchesteri* were successfully developed.

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Thanaporn Pinpart
June, 2010

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LIST OF ABBREVIATIONS

bp	base pair
DNA	deoxyribonucleic acid
dNTP	deoxynucleotide triphosphate
EtBr	ethidium bromide
kb	kilobase
L	Liter
mg	milligrams
min	minute
ML	Maximum Likelihood
MP	Maximum Parsimony
mtDNA	mitochondrial DNA
NJ	Neighbor joining
PCR	polymerase chain reaction
rRNA	ribosomal RNA
s	second
TS	transition
TV	transversion
µg	microgram
µl	microliter
µM	micromolar
V	Vlot

SEQUENCE DIVERGENCE OF MITOCHONDRIAL DNA AMONG FRESHWATER PRAWN, GENUS *Macrobrachium* IN BASINS OF THE CENTRAL, WESTERN AND EASTERN PARTS OF THAILAND

INTRODUCTION

Freshwater prawns of the genus *Macrobrachium* (Crustacea: Decapoda: Palaemonidea) are very successful settlement in the rivers, ponds, lakes, waterfalls and estuaries. The genus is the most diverse crustacean genera and distributes throughout the tropical and subtropical regions (Jayachandran, 2001). In Thailand, twenty-five species were reported in Thailand (Cai *et al.*, 2004). Some species are known as an important economically aquaculture species, e.g. *M. rosenbergii* due to its big size, high growth rate, disease resistance and good taste. For this reason, the market demand increase in several countries. In Thailand, freshwater prawns of this genus are the one kind of food for Thai people, since they have high protein food source including calcium which are inexpensive and easy to find.

There have been many problems associated with the classification of *Macrobrachium*, especially at a species level. The systematic studies of *Macrobrachium* species have been based on external morphological characters for example the rostrum and/or the 2nd pereiopod. However these characters are influenced by environmental parameters in some species (Dimmock *et al.*, 2004), making species identifation difficult.

Currently, *16S rRNA* gene of mitochondrial DNA was found extremely useful for studying taxonomic questions and phylogenetic relationship within a number of decapods crustacean groups. The *16S rRNA* gene contains both fast and slow evolving regions and can provide useful information across a broad taxonomic spectrum from the population to the family level (Crandall and Fizpatrick, 1996; Murphy and Austin, 2002). Therefore, molecular genetics approaches is applied to resolve systematic ambiguous in this genus.

OBJECTIVES

1. To sequence the *16S rRNA* gene from freshwater prawns, genus *Macrobrachium* collected from the basin of central, western and eastern parts of Thailand.

2. To study phylogenetic relationship of the genus by using *16S rRNA* gene sequences.

3. To developed species specific marker using multiplex polymerase chain reaction.

LITERATURE REVIEW

The Genus *Macrobrachium*

Freshwater prawn of the genus *Macrobrachium* has been classified by Taxonomy Browser (http://www.ncbi.nlm.nih.gov/Taxonomy/taxonomyhome.html/, 2009) as below:

Superkingdom	Eukaryota
Kingdom	Metazoa
Phylum	Arthropoda
Subphylum	Crustacea
Class	Malacostraca
Order	Decapoda
Suborder	Pleocyemata
Infraorder	Caridea
Superfamily	Palaemonoidea
Family	Palaemonidae
Genus	*Macrobrachium*

The genus is of the great interest to fishery science, especially in the production of freshwater crustaceans. More than a half of the freshwater productions of crustaceans are prawns in genus *Macrobrachium* such as giant freshwater prawn, *M. rosenbergii* and oriental river prawn, *M. nipponense*. Since the good taste and providing nutrition rises the market demand and production of the species as showed in Figure 1.

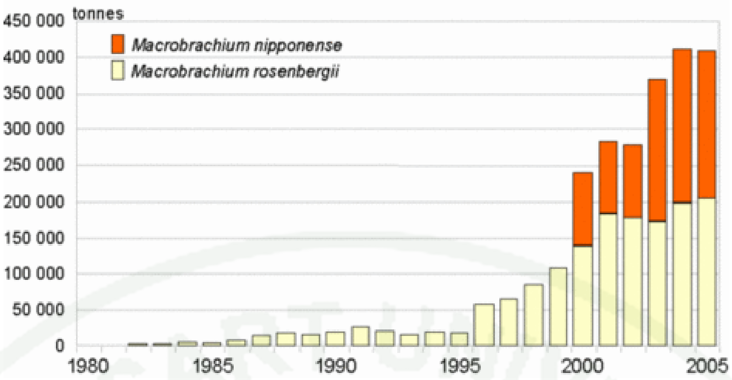

Figure 1 Total production of freshwater prawn, genus *Macrobrachium*.

Source: Food and Agriculture Organization (2007)

Other non commercial species are also important in ecosystem. They are used as bio-indicators in freshwater environment or biomonitors in presence of pollutants in freshwater resources, especially heavy metal contamination, because they are filter feeder and accumulate pollutants in their tissue (Chukwu and Lawson, 2009, Piyatiratitivorakul *et al*., 1998). Moreover, the genus is very diverse. The genus *Macrobrachium* is composed of about 200 species of prawns which is the largest number of species in all Palaemonid genera. Their distributions are pantropical covering the lowlands of Africa; Asia; Oceania; North, Central and South America. (Valencia and Campos, 2007).

Macrobrachium are widely studied in several regions such as China, India, Southeast Asia and Oceania including Thailand. In 1901, *Macrobrachium lanchesteri* was originally described from southern Thailand (Lanchester, 1901). In 1989, Sokita and Takeda described a new species of freshwater prawn, *M. niphanae*, collected from Nang-Rong waterfall in Nakhonnayok province since 1987. Naiyanetr (1998) overviewed the records of 740 species of Crustacean fauna in Thailand from consists of 14 *Macrobrachium* species. Naiyanetr (2001) described a new species, *M. sirindhon*, from the northern Thailand. So far, twenty five species of *Macrobrachium* has been reported. Among them, three new species were included, namely *M. thai*, *M.*

tratense and *M. dolotum*.The key for classification Thai *Macrobrachium* species was also presented (Cai *et al*., 2004).

The central, western and eastern parts of Thailand have a lot of water resources and high level in species diversity. According to literature since 1998, fourteen species of *Macrobrachium* were reported in Thailand (Naiyanetr, 1998). Among them, ten species could be found in central, western and eastern parts. Subsequently, twenty-five species of *Macrobrachium* were reported in Thailand, of which twenty-five species, sixteen species were reported that could be found in central, western and eastern parts of Thailand (Cai *et al*., 2004).

In Thailand, the region of central basin is the broad alluvial plain of the Chao Phraya River. This region is the most important district because over 90% of the area is used for agriculture due to soil fruitful and abundant water resources. The main rivers in central part of Thailand are Chao Phraya River, Pa Sak River, Noi River, Sakae Krang River, Lopburi River, Tha Chin River and Mae Klong River.

In western part of Thailand, the region is bordering between Thailand and Myanmar. The geography of this region is characterized by high mountains and steep river valleys. Water and minerals are the important natural resources of this region. So it contains many of the country’s major dams. The important rivers in this region are Khwae Noi, Khwae Yai and Mae Klong River.

Eastern part of Thailand is located between the Sankambeng Range that forms the border of the Northeastern plateau to the north and the Gulf of Thailand to the south. The geography of the region is characterized by short mountain ranges altering with small basins of short rivers which drain into the Gulf of Thailand. The important water resources in this region are Bang Pakong River, Nakhon Nayok River and Bang Pra Reservoir.

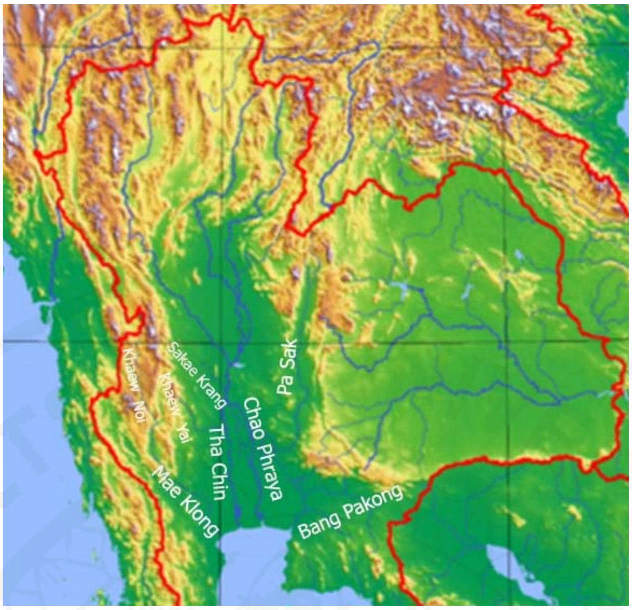

Figure 2 Map of major rivers of the basin of central (Chao Phraya and Pa Sak), western (Mae Klong, Khaew Yai and Khaew Noi), eastern (Bang Pakong) parts of Thailand.

Source: adapted from http://en.wikipedia.org/wiki_River_systems_of_Thailand

The important traits for classification of *Macrobrachium* are the rostrum, and/or the 2nd pereiopod, carapace and telson (Holthis, 1950), body length, carapace length, finger length and palm length (Figure 1).

The body of adult prawn is divided into two main parts, a cephalothorax (head) and an abdomen (tail) as shown in Figure 2. In detail, the body composes of twenty segments. Fourteen segments are fused together and invisible under a large dorsal and lateral shied, called the carapace. The carapace is hard and smooth. At the end of the carapace appears a rostrum. There are spines present in the position of brachiostegal, hepatic and the basal of antenna which was called brachiostegal spine, hepatic spine and antennal spine respectively. Presence of these spines is characteristic of the genus. The front portion of the cphalothorax or cephalon supports:

- the stalked eyes

- the first antennae
- the second antennae
- the mandibles, which are short and hard, are used to grind food
- the first maxillae, which are plate-like , hidden below the second maxillae, use to transfer food into the mouth
- the second maxillae, which are similar to the first maxillae but have an additional function (promoting the respiratory function)

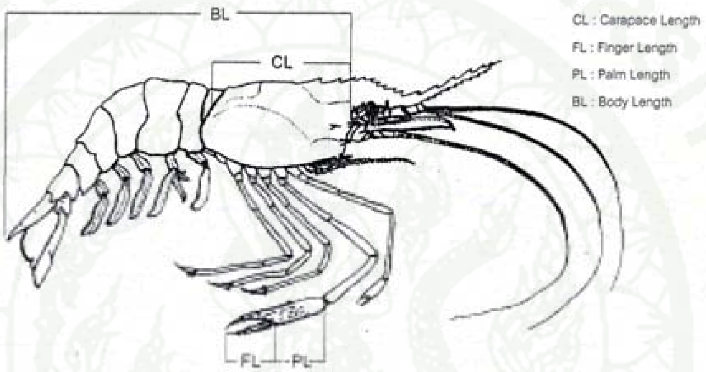

Figure 3 Length measurement of *Macrobrachium*

Source: Noiwangklang (2001)

The rear portion of the cephalothorax, called thorax, is formed by eight fused segments, each segment has easily visible pairs of appendages. These appendages include three sets of maxillipeds and five pairs of pereiopods, as follow:

- the first and second maxillipeds are similar to the first and second maxillae and function as mouthpart
- the third maxillipeds, which are also mouthparts but look rather like legs
- the first and the second legs (pereiopods), which have pincers (chelae). These legs are also called chelipeds. The fist legs are slender but the second pair is much stronger than any leg.

- the third, fourth and fifth legs (pereiopods), which are much shorter than the second chilipeds. These pereiopods have no pincers, and are called walking legs.

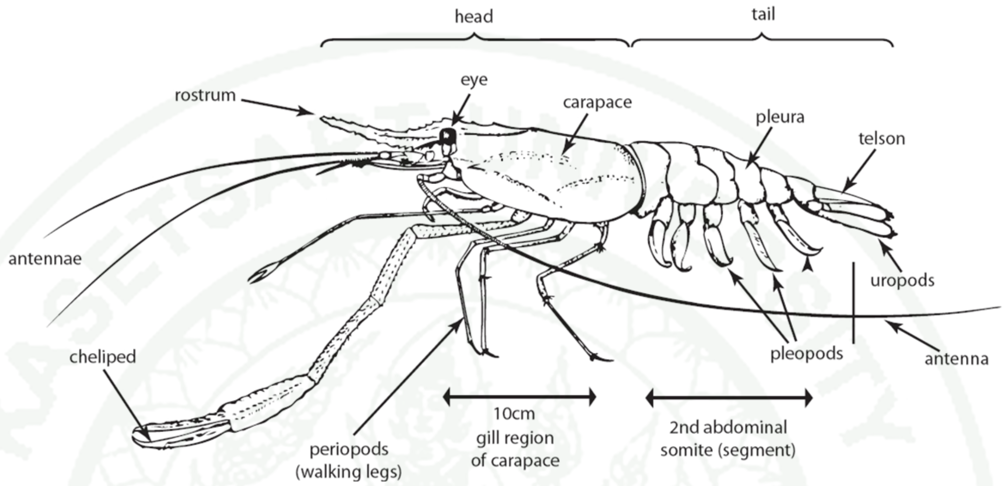

Figure 4 External anatomy of freshwater prawn (*M. rosenbergii*)

Source: Nandlal and Pickering (2005)

The left and right second cheliped of *Macrobrachium* spp. can be equal, subequal or unequal. The tail or abdomen is very clearly divided into six segments, each segment carrying a pair of appendages known as pleopod or swimmerets. The first five pairs of swimmeret are soft. In females, the swimmerets are attachment sites for holding cluster of their eggs (New, 2002).

Jayachandran (2001) suggested that this genus can be grouped into two categories based on the fallowing characters:

1. Equal or unequal second chelipeds.
2. Nature of telson, and its extension in relation to outer spinous process of uropodal exopod.
3. Rough or smooth nature of carapace and second chelipeds.
4. Habitat in which they occur, such as estuaries, rivers, freshwater

impoundment etc.

The first category includes species characterized by:

1. Equal or subequal second chelipeds.
2. Comparatively long telson, its distal end generally extending beyond level of outer lateral spinous process of uropodal exopod.
3. Generally smooth carapace or rarely with sparse prickles.
4. Distribution range from middle stretches of the rivers to estuaries.

The second category is characterized by:

1. Unequal second chelipeds.
2. Short telson its distal end not extending to level of outer lateral spinous process of uropodal exopod.
3. Generally scrobrous carapace.
4. Distribution range from the middle stretch of the rivers to hill tops, very rarely in estuaries.

In the part *Macrobrachium* was studied by using traditional morphological characters but it is still unstable and controversial. Because some traits can chance so much during larva's growth and quite variable between geographically distant populations, making species identification difficult and misidentification easy to ouccur. These facts can lead to overestimation in a species-level. Currently, molecular genetics approaches such as molecular phylogenetic prosper quickly and effectively. Therefore this molecular method can help to resolve systematic ambiguous in the genus.

Molecular phylogenetic

Molecular phylogenetic is the use of the structure of molecules to gain information on the organism's evolutionary relationship. Every living organism contains DNA, RNA and proteins. Closely related organisms usually have a high degree of agreement in these substances. In contrast, distantly related organisms usually show the different pattern of molecular materials, especially in conserved sequence such as mitochondrial DNA. Molecular phylogeny uses these data to generate relationship tree that is known as phylogenetic tree. Currently, molecular phylogenetic has been widely employed to show the relationship or evolution of various organisms. Moreover, molecular phylogenetic can be applied to other applications, for example DNA barcoding where the species of an individual organism is identified by using small sections of mitochondrial DNA. Molecular phylogenetic also effect on traditional biological classification. Because the advance in molecular biology and bioinformatic working with DNA, RNA and proteins can be done quickly and easily, lead to gain a lot of information for analysis in evolutionary relationship.

The phylogeny based on DNA needs the utilization of certain mathematical models or algorithms. The most popular algorithms of choice have been Neighbor Joining (NJ), Maximum Parsimony (MP) and Maximum Likelihood (ML) (Nei and Kumar, 2000).

The neighbor joining (NJ) is a distance matrix method which used to compare the taxa. In this method, the evolutionary distances are calculated in pairs of individuals (or pairwise) in sample and phylogenetic tree will be constructed from these evolutionary distances. The principle of NJ is to find pairs of operational taxonomic units (OTU or neighbor) that minimize the total branch length at each stage of clustering of OTUs starting with a starlike tree (Saitou and Nei, 1987) namely, neighbors are defined as two different taxa that are connected by a single node in an unrooted tree. It is important to note that the starting tree in the NJ method is unrooted and in a star formation where every taxa is connected to the center of the tree. From this star tree, the length of each branch on the tree (determined by the distance computed for all the pairs of taxa) is compared and neighbors are put together based on their branch lengths (Towler, 2002).

The maximum parsimony (MP) method has been used since 1966 (Hennig, 1966). The MP method based on the assumption the most likely tree is the one that requires the fewest number of changes. The MP method only uses information from variable sites for analysis. The sites which have no variation between taxa will be eliminated form analysis. The advantage of MP method is model-free. It is based on sheared and derived characters. It tries to provide information from the ancestral sequences and evaluates different tree. Disadvantage of MP method is do not use all the sequence information, does not correct.

The maximum likelihood (ML) was first used in 1967 (Cavalli-Sforza and Edwards, 1967). The ML method tries to infer and evolutionary tree by finding the tree that maximizes the probability of observing the data in that tree. The ML method is a well established statistical method (Nei and Kumar, 2000) so when used the ML method it need a model to work. In phylogenetics, this model describes how the various rates and probabilities of a nucleotide substitution will occur. For example, the standard software used in for determining a model will tests 56 different models for the use in ML analysis. When the ML method is computing the probability of a given tree or topology, the accurateness of this computation based on how well the chosen model fits the data set. If a model is chosen that does not fit the data well, the tree will be flawed (Towler, 2002).

However, the molecular data may not reflect phylogenetic relationship in case sequences have experienced full substitution saturation or multiple hit occurred. So the substitution saturation needs to be tested (Xia *et al.*, 2003) before use.

Mitochondrial DNA (mtDNA)

Presently, the molecular data or DNA sequences using in animal phylogeny studies have been based on either the mitochondrial DNA (mtDNA) or the nuclear ribosomal gene family. Although the role of mtDNA sequences in taxonomy and phylogenetic studies has become argumentative because the awareness that phylogenetic inference based on single molecule alone will not always be sufficient to answer the many questions. The marker suggests misleading patterns of variation; specifically, phylogenies that are inconsistent with derived from nuclear gene sequences in the context of species relationships among closely related taxa (Rubinoff and Holland, 2005; William *et al.*, 2004). However, analysis of mtDNA sequences data has been used extensively to study the evolutionary relationships both within and among species. Mitochondrial DNA offers a particularly rich source of markers for the study of closely related taxa because of the very low rate of recombination (Piganeau *et al.*, 2004), effective haploidy in DNA sequences, maternal inheritance, simple genetic structure and organization, mosaic molecule with faster and slower evolving DNA regions allowing the design of conserved primers and addressing of phylogenetic questions at various taxonomic levels (Zhang and Hewitt, 1996). In higher animals, the mtDNA is one of the most well characterized components of the genome, and comprises approximately 16 kb. There are thirteen genes provide instructions for making enzymes involved in production of energy, twenty-two genes provide instructions for making transfer RNAs (tRNAs) and the remaining genes provide instructions for making ribosomal RNAs (rRNAs) (Boore, 1999). Moreover, mtDNA have high copy number make them easy to isolate.

There are several genes in mitochondrial genome are used in phylogenetic studies, especially within crustacean systematic such as *cytochrome oxidase subunit* I (COI), *12S ribosomal RNA* (*12S rRNA*) and *16S ribosomal RNA* (*16S rRNA*) as shown in Table 1. Presently, no published studies exist and several mtDNA studies based on *16S rRNA* gene are in progress.

Table 1 Molecular phylogenetic studies within Crustacea at the species level and higher with studies grouped by taxa. Genes studied include nuclear *18S rRNA*, mitochondrial *12S*- and *16S rRNAs*, and protein-coding mitochondrial *cytochrome oxidase c subunit* I (COI) gene fragments (Wetzer, 2001).

Taxon	Hierarchical Level	Description	Gene	Reference
Branchiopoda: Cladocera	Species	cryptic endemism of *Daphnia*	*16S rRNA*	Taylor *et al.* (1998)
Maxillopoda: Copepoda	Species	intraspecific and interspecific patterns (Calanoida)	*16S rRNA*	Bucklin *et al.* (1992)
Maxillopoda: Copepoda	Species	species of *Calanus* (Calanoida)	*16S rRNA*	Bucklin *et al.* (1995)
Malacostraca: Brachyura	Family	Phylogeny of brachyuran families	*16S rRNA*	Schubart *et al.* (2000a)
Malacostraca: Anomura	Infraorder	king crabs and hermit crabs	*16S rRNA*	Cunningham *et al.* (1992)
Malacostraca: Decapoda	Genus	Australian crayfish (Parastacidae)	*16S rRNA*	Crandall *et al.* (1995)
Malacostraca: Decapoda	Genus	*Euastacus and Astacopsis*	*16S rRNA*	Lawler and Crandall (1998)
Malacostraca: Decapoda	Species	*Euastacus* relationships (Parastacidae)	*16S rRNA*	Ponniah and Hughes (1998)
Malacostraca: Decapoda	Subgenus	crayfish (Cambaridae)	*16S rRNA*	Crandall and Fizpatrick (1996)
Malacostraca: Decapoda	Species	Ozark crayfishes (Cambaridae)	*16S rRNA*	Crandall (1998)
Malacostraca: Decapoda	Species	divergence and zoogeography of mole crabs (Hippidae)	*16S rRNA*	Tam *et al.* (1996)
Malacostraca: Decapoda	genus/subgenus	fiddler crabs (Ocypodidae)	*16S rRNA*	Sturmbauer *et al.* (1996)
Malacostraca: Decapoda	species	cryptic invasion of *Carcinus* (Carcinidae)	*16S rRNA*	Geller *et al.* (1997)

Table 1 (continuted)

Taxon	Hierarchical Level	Description	Gene	Reference
Malacostraca: Decapoda	species/subspecies	species/subspecies differentiation of *Panulirus argus* (Palinuridae)	*16S rRNA*	Sarver *et al*. (1998)
Malacostraca: Euphausiacea	Species	relationships of krill	*16S rRNA*	Patarnello *et al*. (1996)
Malacostraca: Brachyura	Species	sequence variation in stone crabs *Menippe adina* and *M. mercernaria*	*16S rRNA*	Schneider *et al*. (1998)
Malacostraca: Brachyura	subfamily/genus	relationships of Ocypodidae	*12S rRNA* and *16S rRNA*	Kitaura *et al*. (1998)
Malacostraca: Decapoda	Species	species of *Sesarma* (Grapsidae)	*16S rRNA*	Schubart and Cuesta (1998a)
Malacostraca: Decapoda	Species	Jamaican grapsid crabs (Grapsidae)	*16S rRNA* and COI	Schubart *et al*. (1998b)
Malacostraca: Decapoda	subfamily/genus	phylogeny of Grapsoidea	*16S rRNA*	Schubart *et al*. (2000b)
Malacostraca: Amphipoda	genus/species	deepsea Lysianassidae	*16S rRNA*	France and Kocher (1996)
Malacostraca: Mysidacea	Family	Lophogastrida	*16S rRNA*	Casanova *et al*. (1998)
Malacostraca: Isopoda	genus/species	phylogenetic relationship among oniscids	*16S rRNA*	Michel-Salzat and Bouchon (2000)
Malacostraca: Isopoda	genus/species	phylogeny and biogeography of serolids	*16S rRNA* and *18S rRNA*	Held (2000)

The ribosomal RNA (rRNA) is the most conserved gene in cells (Smit *et al.*, 2007). For this reason, genes encode the rRNA are sequenced to identify an organism's taxonomic group, calculate related group and estimate rates of species divergence (Cole *et al.*, 2003). The *16S rRNA* or large subunit are used as DNA marker in molecular systematic. For example, the grapsoid crab which are best known from the marine intertidal and supratidal was reclassified. The traditional classification Grapsoidea subdivided into four subfamilies (Grapsinae, Plagusiinae, Sesarminea and Varuninae). From the study of patial *16S rRNA* suggested reclassification of some generas, *Cyclograpsus* and *Chasmagnathus* which traditional classification placed within the genera Sesarminea due to *16S rRNA* showed that both of these genera are significantly closer to most Varuninae genera than Sesarminea. These finding was supported by the basis of zoeal morphology (Schubart and Cuesta, 1998a; Schubart *et al.*, 2000b).

In addition, *16S rRNA* was used to resolved phylogenetic relationship between two western Atlantic prawns, genera *Farfantepenaeus* and *Litopenaeus.* The pink shrimp or morphotypeII from the northeastern of Brazilian coast which was usually identifed as *F. subtilis* was investigated. Based on *16S rRNA* partial sequence, *F. subtilis* and the morphotype II showed a little level of genetic divergence within population (0.002 and 0.004 respectively). While the genetic divergence of morphotype II that was compared with the other well characterized in genus *Farfantepenaeus* showed level in the range 0.04-0.06. The difference of genetic divergence within population and between species indicated that *F. subtilis* and morphotype II have reproductive isolation (Maggioni *et al.*, 2001).

The *16S rRNA* gene can be as useful information across a broad taxonomic spectrum from the population to the family level. The *16S rRNA* was used to proof the inconsistencies in classification of five species of Australian palaemonid, *Macrobrachium intermedium* (Stimpson), *M. australiense* (Holthius), *M. atactum* (Riek), *M. rosenbergii* (de Man) and *Palaemon serenus* (Heller). Sequence divergence between the two inland *Macrobrachium* species, *M. atactum* and *M. australiense*, was 2% whereas the mean of sequence divergence between the two inland *Macrobrachium* and the estuarine *M. intermedium* was 24%. In contrast, there was only show 9% sequence divergence between *M. intermedium* and *Palaemon serenus* and 10% between inland *Macrobrachium* species and *M. rosenbergii*. This results revealed *M. intermedium* formed a very well-supported clade with *P. serenus*, distinct from *M. australiense*, *M. atactum* and *M. rosenbergii* (Murphy and Austin, 2002). Owing to the large among of morphological divergence of *M. australiense*, this species has proven to be taxonomically difficult and consisted of three separate species, each with subsequent subspecies (*M. australiense australiense*, *M. australiense cristatum*, *M. atactum atactum*, *M. atactum sobrinum* and *M. adscitum* subspecies). The *16S rRNA* region was used to investigate the genetic relationship between twelve populations of these species, across inland eastern and northern part of Australia. In addition, four other Australian prawn species were also analyed to show that the variation of *16S rRNA* among *M. australiense* haplotypes were much less than among *Macrobrachium* species. The results indicated that it is in fact a single species (Murphy *et al.*, 2004).

Species Specific Marker

The taxonomic identification of animal show importance in several isuues such as law enforcement for food control and safety (Martín *et al.*, 2007; Ortea *et al.*, 2010; Rehbein *et al.*, 2002), identify endangered and protected species in wildlife (Ali *et al.*, 1999; Malisa *et al.*, 2005; Petersen *et al.*, 2007), identify crime and cratatophy victim, determined pedregree of seed or livestock (Erhardt and Weimann, 2007). The majority of molecular markers used nowadays are PCR-based marker e.g. amplified fragment length polymorphism (AFLPs), random amplified polymorphic DNA (RAPD), single strand conformation polymorphisms (SSCPs), polymerase chain reaction-restriction fragment length plyomorphism (PCR-RFLP), microsatellite markers and species-specific PCR.

The species-specific PCR is a powerful method for identification in genus and species level (Matsuki *et al.*, 2003; Quinteiro *et al.*, 2001). Gernerally, the species-specific primers were designed and combined together using multiplex polymerase chain reaction or multiplex PCR.

Multiplex PCR is a modification of PCR which consist of multiple primer sets in the single PCR mixture. Multiplex PCR aims to produce varying sizes of product that are specific to different DNA sequences. This method was first described in 1988 as a method to detect deletions in the dystrophin gene (Chamberlain *et al.*, 1988). Currently, multiplex PCR was used as a useful tool for several aspects e.g. detect deletion in gene, analysis of microsatellites and SNPs (Hayden *et al.*, 2008) and species identification in several organisms, including bacteria, fungi, mollusks, crustacean, fish and mammals (Amicucci *et al.*, 2000; Asensio, 2008; Marshall *et al.*, 2007; Nakamura *et al.*, 2009; Pfannebecker and Fröhlich, 2008). This method has been proven that it is a fast, accurate and repeatable method. Therefore, multiplex PCR can be an appropriate alternative to morphological species identification when species co-occur with closely relate taxa and have few distinguishing physical traits (Ray *et al.*, 2002).

Macrobrachium is a genus that notoriously difficult to identify by conservative systematic traditions becaus the dominant traits such as the rostrum and/or the second pereiopod can be change so much and so gradually during their growth (Holthis, 1950) and are influenced by environmental parameters (Dimmock *et al.*, 2004). Thus, species specific marker can be use as a powerful tool for species identification of *Macrobrachium* in the future.

MATERIALS AND METHODS

Materials

1. Freshwater prawns

Freshwater prawns of the genus *Macrobrachium* were collected from provinces in three parts of Thailand including central; Ayutthaya (Ay), Uthaithani (Ut), Saraburi (Sb) and Nakhonprathom (Np), western; Tak (Tk), Kanchanaburi (Kc) and Ratchaburi (Rb) and eastern; Nakhonnayok (Ny), Chachoengsao (Cs) and Chonburi (Cb).

2. Kit for DNA extraction

Genomic DNA Extraction Mini Kit (tissue) (RBC Bioscience, Taiwan)

3. Kit for purification

PCR Fragments Extraction Kit (Geneaid Biotech Ltd.)

HyYield^{TM} Gel/PCR DNA Fragments Extraction Kit (RBC Bioscience, Taiwan)

4. Chemicals and Reagents

100 mM dATP, dCTP, dGTP, and dTTP (Fermentas Life Sciences, USA)

Absolute ethanol (C_2H_5OH) (Merck, Germany)

Glacial acetic acid (CH_3COOH) (Merck, Germany)

Agarose I^{TM} (Amresco, USA)

DNA marker: GeneRuler^{TM} DNA Ladder Mix (Fermentas Life Sciences, USA)

Ethidium bromind (EtBr) (AppliChem, Germany)

Ethylene diamine tetraacetic acid, disodium salt dihydrate (EDTA) (Merck, Germany)

Tris(hydroxy methyl)aminomethane (Research Organics, USA)

Ultrapure water (Gibgo, USA)

5. Enzyme for polymerase chain reaction

Taq polymerase (Fermentas Life Sciences, USA)

6. Equipments for analysis

Autoclave: Model SS-325 (Tommy Seiko, Japan)

Autopipette: Pipetteman (Gilson, France)

Centrifuge, microcentrifuge: Model Spectrafuge 16M

Electrophoresis unit: MiniRun Gel Electrophoresis System

Cooling and heating block: Model CHB-100

Magnetic stirrer

Weighing machine

Microwave

Olympus SZ40 for species classification

Thermal Cycler (Biometra TPersonal)

UVP Biodoc-It Imaging System

Vortex mixer

Computer, All data analysis was performed on Personal Computer, Intel(R) Core(TM)2 Duo CPU 2.66 GHz, 1.00 GB of RAM, running Windows XP operating system and connect with internet.

7. Software programs for analysis

Oligos (developed by Dr. Ruslan Kalendar, Institute of Biotechnology, Helsinki. http://www.biocenter.helsinki.fi)

Blastn (Basic Local Alignment Search Tool-nucleotide)

CAP3 (Huang and Madan, 1999)
ClustalW (Chenna *et al.*, 2003)
DnaSP version 4.10 (Rozas *et al.*, 2003)
MEGA version 3.1(Kumar *et al.*, 2004)
MODELTEST (Posada and Crandall, 1998)
PAUP* beta version 4.0 (Swofford, 1998)
TREEVIEW version 1.6.6 (Page, 1996)

Methods

1. Sample collection

Freshwater prawns were collected from the natural water resources in the provinces of central (Ayutthaya, Uthaithani, Saraburi and Nakhonprathom), western (Tak, Kanchanaburi and Ratchaburi) and eastern (Nakhonnayok Chachoengsao and Chonburi). All samples were preseverd in 70% ethanol and were identified using the dichotomous key of Cai *et al*. (2004) and Jayachandran (2001).

The important traits to taxonomy are:

1. the feature of rostrum such as rostrum convex, straight or upturn
2. the feature of the second pereiopod such as presence or absence of dense velvety pubescence, the position that pubescence present, equal, subequal or unequal in length and similar or dissimilar in form
3. the feature of carpus such as shape and length of carpus

2. DNA extraction

Genomic DNA was extracted from muscle tissue using Genomic DNA Extraction Mini Kit (tissue) according to the manufacturer's protocol (RBC Bioscience, Taiwan)

3. Amplification of *16S ribosomal RNA* gene and sequencing

3.1 Primers design

Based on the complete mitochondrial sequence of *M. rosenbergii* (NC 006880) and *Halocaridina rubra* (NC 008413), the oligonucleotide primers were designed by a computer program, Oligos. Amplification of 5' region of *16S rRNA* was carried out using ML16_LF1or ML16_LF2 and ML16_LR1. In interior of *16S rRNA* using ML16_MF1 and ML16_MR1 and the 3' region using ML16_RF1 or ML16_RF2 and ML16_RR1 or ML16_RR2. The expected size of PCR product was approximately 700 bp. The sequences and the positions of all primers were shown in Table 2 and Figure 4 respectively.

Table 2 Sequences of nucleotide primers

Primer's name	Nucleotide sequence	Tm (^{o}C)
ML16_LF1	5'- CTACTTTAT(c/t)AGGGCCCTTACG	55.7
ML16_LF2	5'- ATGTAAAGAAAGCTACAGAAACCA	52.6
ML16_LR1	5'- TCTTTTAATTGGAGGCTTGAATG	55.4
ML16_MF1	5'- TTAATTCAACATCGAGGTCGCA	55.3
ML16_MR1	5'- (g/a)GTGGGCTTAAAATCAGCCA	55.5
ML16_RF1	5'- TTTGATAAACAGGCGGGGA(c/t)	55.4
ML16_RF2	5'- TGCTACCTTCGCACGGTCAA	55.4
ML16_RR1	5'-TGTATTGGAAA(g/a)TGCA(t/c)CTAGGA	52.5
ML16_RR2	5'- TTACGCTGGAGGGATTGCC	55.6

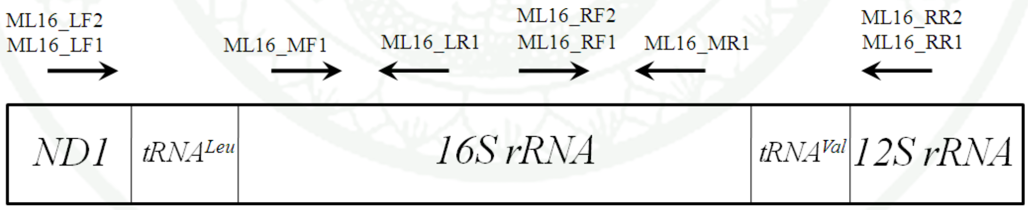

Figure 5 Diagram shows the position of all primers for *16S rRNA* amplification.

3.2 PCR reaction

The PCR reaction was prepared in 0.5 ml PCR-tube (Sorenson Bioscience, USA). The reaction mixture, total volume of 50 µl, consisted of 10X *Taq* buffer 5 µl, 25 mM $MgCl_2$ 5 µl, 2 mM dNTPs mix 4 µl, 10 µM primer forward (which no degenerate base) 4 µl, 10 µM primer reverse (which no degenerate base) 4 µl, *Taq* DNA polymerase (Fermentas Life Sciences, USA) 0.5 µl (2.5 unit), total DNA 3 µl (50-100 ng) and ultrapure water 24.5 µl. The reaction mixtures were amplified in Biometra TPersonal Thermal Cycler (Labrepco, USA). The PCR cycling profile consisted of initial denaturation at 94^{o}C for 2 min, followed by 35 cycles of 94^{o}C for 30 sec, 50-55^{o}C (depending on the species of sample) for 30 sec, 72^{o}C for 1 min and final extension at 72^{o}C for 5 min.

The PCR product was mixed with 6X loading buffer and the sample mixture was loaded into the wells of the submerged 1% agarose gel. The electrophoresis was carried out in 1X Tris Acetate EDTA (TAE) running buffer at 100V for 25 min. After finishing, the gel was stained in ethidium bromide (EtBr) solution for 10 min and destained by submerging in an excessive amount of water for 2 min. The nucleic acid bands were visualized under UV light.

3.3 Nucleotide sequencing

PCR products were purified by using PCR DNA Fragments Extraction Kit (Geneaid Biotech Ltd., Taiwan) according to the manufacturer's protocol. The DNA fragments were then sent to sequence at DNA sequencing unit, Macrogen Inc. (South Korea). All of the nucleotide sequences results from sequencing unit were checked with GenBank database by Blastn program (http://www.ncbi.nlm.nih.gov/) for confirmation that they were really *16S rRNA* sequences. The sequence assembly program, CAP3 (http://pbil.univ-lyon1.fr/cap3.php), was used to assemble all of overlapping PCR fragments into one contig strand of *16S rRNA* gene. Then all contigs were aligned for removing nucleotide margin and determine the actual region of *16S rRNA*.

4. Phylogenetic analysis

The nucleotide sequences of all samples were performed by ClustalW1.83 (Chenna *et al*., 2003) to generated the input files formats (especially *.fasta and *.nxs) for data analyses.

4.1 Number of nucleotide substitutions

Number of transition (TS), transversion (TV), nucleotide difference (TS+TV) and proportion of TS and TV were computed by MEGA 3.1 (Kumar *et al*., 2004).

4. 2 Substitution saturation test

Substitution saturation was determined by plotting graphs. Transitions, transversions and number of difference were plotted against pairwise evolutionary distance (Hanada *et al*., 2004; Miya *et al*., 2003).

4.3 Selection of nucleotide substitution model

The best-fit model of nucleotide substitution was performed by Modeltest (Posada and Crandall, 1998). This model will be used in calculation of evolutionary distance and construction phylogenetic tree by maximum likelihood method.

4.4 Nucleotide divergence and genetic distance

Nucleotide divergence (d_A) is the number of net nucleotide substitution between two populations or species. This value is estimated by equation

$$d_A = d_{XY} - (d_X + d_Y) / 2$$

when d_{XY} is the average number of nucleotide substitutions per site between populations (or species) X and Y

d_X is the average number of nucleotide substitutions in populations (or species) X (d_X; nucleotide diversity).

d_Y is the average number of nucleotide substitutions in populations (or species) Y (d_Y; nucleotide diversity).

Nucleotide divergence was calculated from DnaSP.

Genetic distance is value that means the extent of genetic differences between two populations or taxa. As evolutionary distance, genetic distance will be calculated under appropriate model of nucleotide substitution which plays a significant role in molecular phylogenetic.

4.5 Phylogenetic tree construction with Neighbour joining method (NJ)

Pairwise genetic distance and NJ tree was performed by PAUP* beta version 4.0 (Swofford, 1998). The reliability of infer tree will be evaluated by 1,000 bootstaps. Consensus tree was constructed by 50% majority rule consensus method.

4.6 Phylogenetic tree construction with Maximum Parsimony method (MP)

This method was performed by PAUP* beta version 4.0. Algorithm of heuristic search was used for finding the MP tree. Only parsimony informative site were used in analysis and defined gap as missing data. A provisional MP tree was constructed by using stepwise addition algorithm with random option. Tree bisection reconnection (TBR) was algorithm of branch swapping which was used in this method. The reliability of inferred tree was tested by bootstrap test (1000 replications).

4.7 Phylogenetic tree construction with Maximum Likelihood method (ML)

ML tree was performed under the best-fit model of substitution that was evaluated by Modeltest (Posada and Crandall, 1998). The best topology was found by using heuristic search, a provisional tree was constructed by using stepwise addition algorithm with random option. Tree bisection reconnection (TBR) was algorithm of branch swapping which was used in this method. The reliability of inferred tree was tested by bootstrap test (100 replications).

5. Species Specific Marker

Based on the *16S rRNA* sequences alignment of *Macrobrachium* spp., the specific primer for identification three species, namely *M. hirsutimanus*, *M. rosenbergii* and *M. lanchesteri*, were designed by Oligos. Each primer was named Mh_16speF2, Mh_16speR2, MRO_16speF1, MRO_16speR1, ML_16speF3 and ML_16speR1. The expected size of PCR product of *M. hirsutimanus*, *M. lanchesteri* and *M. rosenbergii* were approximately 439, 508 and 569 bp respectively.

The PCR reaction was prepared in 0.5 ml PCR-tube. The reaction mixture, 25 µl, total volume consisted of 10X *Taq* buffer 2.5 µl, 25 mM $MgCl_2$ 1.0 µl, 2 mM dNTPs mix 2 µl, 10 µM, Mh_16speF2 2 µl, Mh_16speR2 2 µl, MRO_16speF1 2 µl, MRO_16speR1 2 µl, ML_16speF3 2 µl, ML_16speR1 2 µl, *Taq* DNA polymerase (Fermentas Life Sciences, USA) 0.25 µl (2.5 unit), total DNA 0.5 µl (50-100 ng) and ultrapure water 6.75 µl. The reaction mixtures were amplified in Biometra TPersonal Thermal Cycler (Labrepco, USA). The PCR cycling profile consisted of initial denaturation at 94^{o}C for 2 min, followed by 35 cycles of 94^{o}C for 30 sec, 52^{o}C for 30 sec, 72^{o}C for 1 min and final extension at 72^{o}C for 5 min.

The obtained PCR product was mixed with 6X loading buffer and the sample mixture was loaded into the wells of the submerged 2% agarose gel. The electrophoresis was carried out in 1X TAE running buffer at 100V for 30 min. After finishing, the gel was stained in ethidium bromide (EtBr) solution for 10 min and destained by submerging in an excessive amount of water for 2 min. The nucleic acid bands were visualized under UV light.

RESULTS AND DISCUSSIONS

1. Numbers of prawn species

Nine species and 1cryptic species, including *M. assamense*, *M. lanchesteri*, *M. rosenbergii*, *M. tratense*, *M. sintangense*, *M. forcipatum*, *M. dienbienphuense*, *M. hirsutimanus*, *M. niphanae* and *M. sp3* respectively.

2. External morphology classification

1.1 *M. assamense* (Figure 6A and B)

1. Major second petyiopod with dense velvety pubescence
2. Pubescence present on fingers, finger with longitudinal groove
3. Velvety pubescence present throughout length of fingers, rostrum straight

Figure 6 (A) *M. assamense* (B) Feature of finger of *M. assamense*. Finger with longitudinal groove and velvety pubescence present throughout length of fingers

Figure 6 (continued)

1.2 *M. lacchesteri* (Figure 7)

1. Major second pereiopod without pubescence
2. Carpus distinctly longer than chela
3. Rostrum upturned, reaching distinctly beyond end of scaphocerite

Figure 7 *M. lanchesteri*

1.3 *M. rosenbergii* (Figure 8)

1. Carpus as long as or shorter than chela
2. Capus of major second pereiopod longer than plam but shorter than chela
3. Rostrum upturned, fingers of second pereiopods with teeth on cutting edges.
4. Rostrum with more than 10 ventral teeth

Figure 8 *M. rosenbergii*

1.4 *M. tratense* (Figure 9)

1. Major second pereiopod without pubescence
2. Carpus as long as or shorter than chela
3. Carpus of major second pereiopod longer than plam but shorter than chela
4. Rostrum straingth, no visible crest, reach to end of scaphocerite

Figure 9 *M. tratense*

1.5 *M. sintangense* (Figure 10)

1. Major second pereiopod with dense velvety pubescence
2. Pubescence present only on fingers
3. Finger without longitudinal groove, velvety present only proximal half

Figure 10 *M. sintangense*

1.6 *M. forcipatum* (Figure 11A and B)

1. Major second pereiopod with dense velvety pubescence
2. Pubescence present on both palm and fingers
3. Carpus cup-shaped
4. Cutting edges of fingers of second peiopod with 7-10 teeth

Figure 11 (A) *M. forcipatum* (B) Pubescence present on both palm and fingers. And cutting edges of fingers of second peiopod with 7-10 teeth

1.7 *M. hirsutimanus* (Figure 12A and B)

1. Major second pereiopod with dense velvety pubescence
2. Pubescence present on both palm and fingers
3. Carpus cup-shaped
4. Female second pereiopod much smaller than that of male in form

Figure 12 *M. hirsutimanus* (A) male (B) female

1.8 *M.dienbienphuense* (Figure 13)

1. Major second pereiopod with dense velvety pubescence
2. Pubescence present on both palm and fingers

3. Carpus elongated

Figure 13 *M. dienbienphuense*

1.9 *M. niphanae* (Figure 14A and B)

1. Major second pereiopod with dense velvety pubescence
2. Pubescence present only on merus and/or carpus

Figure 14 (A) *M. niphanae* (B) Pubescence present only on merus and/or carpus

Figure 14 (continuted)

1.10 *M. sp*3. (Figure 15A, B and C)

1. Major second pereiopod without pubescence
2. Carpus shorter than chela
3. Carpus as long as plam and finger
4. Rostrum straight

Figure 15 (A) *M. sp3.* (B) Rostrum straight

Figure 15 (continuted)

According to the morphological species concept, species is a group of individual or populations with the same or similar morphological characters (Mayr, 1964). In this study, there are nine species have different morphological characters according to the key for the freshwater prawns of the genus *Macrobrachium* of Thailand that was established by Cai *et al*. In accordance with this key, the pubescence was the exactly morphological feature used to characterize categories of *Macrobrachium* species. The *Macrobrachium* were separated into two groups. Group one was *Macrobrachium* which pubescence present on major second pereiopod. Another group was without pubescence present on major second pereiopod. Thus *M. assamense* was separated from *M. lanchesteri* by clearly presence of pubescence on major second pereiopod and finger with longitudinal groove. In this study, *M. assamense* was a single species by morphological species concept. Likewise, *M. niphanae* was separated from the other species by pubescence that present only on merus and/or carpus. In this study, *M.sp3* has morphology quite similar to *M. niphanae* but they absolutely have no pubescence on major second pereiopod.

3. Phylogenetic analysis

1.1 The *16S rRNA* sequences

The total length and base composition of *16S rRNA* gene of all *Macrobrachium* samples and other organisms were showed in Table 3. The sequence length of *16S rRNA* gene of *Macrobrachium* spp. ranged between 1300-1310 bp. The mean total nucleotide composition was found to be A= 40.02%, C=21.05%, G=9.54% and T=29.38%. The percentage of A+T nucleotides was 69.17%. This AT bias agreed with the study of other malacostracan mitochondrial genome (Miller *et al.*, 2004; Shen *et al.*, 2007; Wilson *et al.*, 2000). The *16S rRNA* full length sequences of *Macrobrachium* spp. were aligned for comparison with these of other arthropods sequences and portion of *Escherichia coli* ribosomal RNA (Gutell and Fox, 1998; Zwieb *et al.*, 1981) (Figure 16-21). The other arthropods sequences used for comparisons included 4 crustaceans and 6 insects namely *Penaeus monodon* (NC_002184), *Scylla serrata* (NC_012565), *S. tranquebarica* (NC_012567), *S. olivacea* (NC_012569), *Bombyx mandarina* (NC_003395), *B. mori* (NC_002355), *Anopheles gambiae* (NC_002084), *A. quadrimaculatus* (NC_000875), *Apis mellifera* (NC_001566), *Drosophila melanogaster* (NC_001709), *D. yakuba* (NC_001322)(Clary and Wolstenholme, 1985). All conserve Domains were further aligned in a structural format according to the structural map of *23S rRNA* (Egebjerg *et al.*, 1990). The result showed that *16S rRNA* sequences of *Macrobrachium* spp. were very similar to the *16S rRNA* sequences of several arthropods. The highest sequence identity was found in certain region which presumably corresponded to functional and/or structural portions of the *16S rRNA*. Some of these sequences are universally conserved among eukaryotic *16S rRNA* and *E. coli* e.g. nucleotide position 561-578 at the junction between Domain I and II of *E. coli* (Figure 16), much of Domain V corresponding to the Peptidyl Transferase Center (Figure 20) and the factor binding site, alpha-sarcin loop of Domain VI (Figure 21) which is essential for GTP-ase catalyzed step in translation. Comparison between crustaceans and insects found that there were highly conserved regions for most of Domain II, IV, V and alpha-sarcin loop of Domain VI. The remainings; most of Domain I, portions of Domain II, Domain III, portions of Domain IV and Domain VI are highly variable in both sequence and length. This result agreed with feature of *16S rRNA* of gypsy moth (*Lymantria dispar*) and some spiders (Davis *et al.*, 1994; Masts, 2000; Smith and Bond, 2003). Interestingly, region which presumably corresponded to functional of

GTPase Center in Domain II (Figure 17) is quite conserved in rank of organism's group but highly variable among group.

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M.assamense (Kc01)      -------------------------------ATAGTTGTTTATTATTAATTGAATTTGTT 29
M.assamense (Kc02)      -------------------------------ATAGTTGTTTATTATTAATTGAATTTGTT 29
M.lanchesteri (Rb01)    -------------------------------ATAGTTGTTTATTATTAATTGAATTTGTT 29
M.lanchesteri (Np01)    -------------------------------ATAGTTGTTTATTATTAATTGAATTTGTT 29
M.lanchesteri (Rb02)    -------------------------------ATAGTTGTTTATTATTAATTGAATTTGTT 29
M.lanchesteri (Np02)    -------------------------------ATAGTTGTTTATTATTAATTGAATTTGTT 29
M.lanchesteri (Ay03)    -------------------------------ATAGTTGTTTATTATTAATTGAATTTGTT 29
M.lanchesteri (Cs01)    --------------------------------TAGTTGTTTATTATTAATTGAATTTGTT 28
M.tratense (KC01)       ---------------------------------AATTAAGTATTATT--ATGATATGTTG 25
M.tratense (Ut01)       -------------------------------AAAATTAAGTATTATT--ATGATATGTTG 27
M.forcipatum (TK01)     -------------------------------AGGATTAAG-GTTATTCGTTTTGGTTTTG 28
M.hirsutimanus (Rb01)   -------------------------------GGGGTTAAGTGTTATTTATTTTGGTTTTG 29
M.hirsutimanus (Rb02)   -------------------------------GGGGTTAAGTGTTATTTATTTTGGTTTTG 29
M.niphanae (Kc01)       -------------------------------AAAGTTAAGTATTATT-ATTGTTTTGGTG 28
M.niphanae (Np01)       -------------------------------AAAGTTGAGTATTATT-ATTGTTTTGGTG 28
M.niphanae (Np02)       -------------------------------AAAGTTAAGTATTATT-ATTGTTTTGGTG 28
M.sintangense (Cb02)    -------------------------------AAAGTTAAATATTATT--ATTTTATGTTG 27
M.sintangense (Cb01)    ------------------------------------TAAATATTATT--ATTTTATGTTG 22
M.hirsutimanus (Sb01)   -------------------------------GGGGTTAAGTGTTATTTATTTTGGTTTTG 29
M.hirsutimanus (Sb02)   -------------------------------GGGGTTAAGTGTTATTTATTTTGGTTTTG 29
M.hirsutimanus (Sb03)   -------------------------------GGGGTTAAGTGTTATTTATTTTGGTTTTG 29
M.dienbienphuense(Ny01) -------------------------------AAGGTAATGTGTTATTCATTTTGATTTTG 29
M.hirsutimanus (Ny01)   -------------------------------GGGGTTAAGTGTTATTTATTTTGGTTTTG 29
M.hirsutimanus (Ny02)   -------------------------------GGGGTTAAGTGTTATTTATTTTGGTTTTG 29
M.sp3 (Np02)            -------------------------------AAAGATAAGTATTATT-ATTATTTTAGTG 28
M.sp3 (Np01)            -------------------------------AAAATTAAGTATTATT-ATTATTTTAGTG 28
M.niphanae (Ny01)       -------------------------------AAAGTTAAGTATTATT-ATTGTTTTGGTG 28
M.niphanae (Ny04)       ------------------------------------AAAGTAATTTT-TTTGTTGGGGTG 23
M.niphanae (Ny03)       -------------------------------AAAGATAAGTATTATT-ATTGTTTTGGTG 28
M.niphanae (Ny02)       -------------------------------AAAGATAAGTATTATT-ATTGTTTGGGTG 28
M.dienbienphuense(Ny02) -------------------------------AAGGTAATGTGTTATTCATTTTGATTTTG 29
M.rosenbergii(NC_006880)--------------------------------TAGTTGTTTATTATTAGTGGGGTTTGTG 28
M.rosenbergii (Np01)    -------------------------------ATAGTTGTTTATTATTAGTGGGCTTTGTG 29
P.monodon               -----------------------------------TTAAATCTTATT-AATGTAATCATG 24
S.serrata               --------------------------------------GAAATTTTAAATTATTGCTTTA 22
S.tranquebarica         --------------------------------------TATATTTTAAATTATTGCTTAG 22
S.olivacea              --------------------------------------GTTATTTTGATTTATTGCTTAT 22
B.mandarina             ------------------------AGGGGTCATAAATAAGAAAAAATTAAT-AAATTAAT 35
B.mori                  ------------------------GGGGGTTATAAATAAGAAAAAATTAAT-AAATTAAT 35
An.gambiae              ---------------------------------TAATAATTATTTATTAAT-TATTTTTT 26
An.quadrimaculatus      ---------------------------------TAAAATTTATTTATTAAT-TATTTTTT 26
Ap.mellifera            TAATTAAAATTATTAATAAAATTATTTGGTTATATTTATATATATATATATATATATATA 60
D.melanogaster          -------------------------------GTTAGTTTTTATTTATTAAT-TTTTATTA 28
D.yakuba                --------------------------------TTATATTTTATTTATTAAT-TTTAATTA 27

Escherichia coli        <-----------------------------------------------------------

                        <-------------------- E. coli Domain I-------------------- >
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Figure 16 Alignment of *16S rRNA* of *Macrobrachium* spp. compared with other arthropods and *E. coli*; Domain I. Initial alignment was done by ClustaW. Final matching was done manually. The sequences begin at the 5' ends of the genes. Stars indicated conserve regions between *Macrobrachium* spp. and other arthropods. Underlined segments indicated conserve regions between arthropods and *E. coli*.

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M.assamense (Kc01)      TAT---AGTTTAATTAGAT-----AAAAAATTAAT-----TAATTTTGTTGCTTAGTATT 76
M.assamense (Kc02)      TAT---AGTTTAATTAGAT-----AAAAAATTAAT-----TAATTTTGTTGCTTAGTATT 76
M.lanchesteri (Rb01)    TAT---AGTTTAATTAGAT-----AAAAAATTAAT-----TAATTTTGTTGCTTAGTATT 76
M.lanchesteri (NP01)    TAT---AGTTTAATTAGAT-----AAAAAATTAAT-----TAATTTTGTTGCTTAGTATT 76
M.lanchesteri (Rb02)    TAT---AGTTTAATTAGAT-----AAAAAATTAAT-----TAATTTTGTTGCTTAGTATT 76
M.lanchesteri (Np02)    TAT---AGTTTAATTAGAT-----AAAAAATTAAT-----TAATTTTGTTGCTTAGTATT 76
M.lanchesteri (Ay03)    TAT---AGTTTAATTAGAT-----AAAAAATTAAT-----TAATTTTGTTGCTTAGTATT 76
M.lanchesteri (Cs01)    TAT---AGTTTAATTAGAT-----AAAAAATTAAT-----TAATTTTGTTGCTTAGTATT 75
M.tratense (Kc01)       ATTTAAAGTTAAGTTTTTT-----AAAAAATTAAT-----AGATTTTGTTTTTTAGTATA 75
M.tratense (Ut01)       ATTTAAAGTTAAGTTTTTT-----AAAAAATTAAT-----AGATTTTGTTTTTTAGTATA 77
M.forcipatum (Tk01)     TTTGTGTGTTTATTTTTAT-----AAAAAATTAGT-----AAGTTTTGATTATTAGTATG 78
M.hirsutimanus (Rb01)   TTT-TGTGTTTATTTTCAT-----AAAAAATTAGT-----AAGTTTTGGTTATTAGTATG 78
M.hirsutimanus (Rb02)   TTT-TGTGTTTATTTTCAT-----AAAAAATTAGT-----AAGTTTTGGTTATTAGTATG 78
M.niphanae (Kc01)       GTT-TGTGTTTATTTTTAT-----AAAAAATTAAT-----GAGTTTTGTTTTTTAGTATG 77
M.niphanae (Np01)       GTT-TGTGTTTATTTTTAT-----AAAAAATTAAT-----GAGTTTTGTTTTTTAGTATG 77
M.niphanae (Np02)       GTT-TGTGTTTATTTTTAT-----AAAAAATTAAT-----GAGTTTTGTTTTTTAGTATG 77
M.sintangense (Cb02)    ATTTAAAGTTAAGTTTTTT-----AAAAAATTAAT-----AGGTTTTGTTTTTCAGTATG 77
M.sintangense (Cb01)    ATTTAAAGTTAAGTTTTTT-----AAAAAATTAAT-----AGGTTTTGTTTTTCAGTATG 72
M.hirsutimanus (Sb01)   TTT-TGTGTTTATCTTCAT-----AAAAAATTAGT-----AAGTTTTGGTTATTAGTATG 78
M.hirsutimanus (Sb02)   TTT-TGTGTTTATCTTCAT-----AAAAAATTAGT-----AAGTTTTGGTTATTAGTATG 78
M.hirsutimanus (Sb03)   TTT-TGTGTTTATCTTCAT-----AAAAAATTAGT-----AAGTTTTGGTTATTAGTATG 78
M.dienbienphuense(Ny01) GTT-TGTGTTTATTTTTAT-----AAAAAATTAGT-----AAGTTTTGGTTATTAGTATG 78
M.hirsutimanus (Ny01)   TTT-TGTGTTTATTTTCAT-----AAAAAATTAGT-----AAGTTTTGGTTATTAGTATG 78
M.hirsutimanus (Ny02)   TTT-TGTGTTTATTTTCAT-----AAAAAATTAGT-----AAGTTTTGGTTATTAGTATG 78
M.sp3 (Np02)            TTT-TGTGTTTATTTTTAT-----AAAAAATTAAT-----GAGTTTTGTTTTTTAGTATG 77
M.sp3 (Np01)            TTT-TGTGTTTATTTTTAT-----AAAAAATTAAT-----GAGTTTTGTTTTTTAGTATG 77
M.niphanae (Ny01)       GTT-TATGTTTATTTTTAT-----AAAAAATTAAT-----GAGTTTTGTTTTTTAGTATG 77
M.niphanae (Ny04)       GTG-TGCGTTTATTTTTAA-----AAAAATATAGA-----GAGTTTTGTTTTTTTATATG 72
M.niphanae (Ny03)       TTT-TATGTTTATTTTTAT-----AAAAAATTAAT-----GAGTTTTGTTTTTTAGTATG 77
M.niphanae (Ny02)       GTG-TGTGTTTATTTTTAT-----AAAAAAATAGA-----GAGTTTTGTTTTTTTGTCTG 77
M.dienbienphuense(Ny02) GTT-TGTGTTTATTTTTAT-----AAAAAATTGGT-----AAGTTTTGGTTATTAGTATG 78
M.rosenbergii(NC_006880)TGT---AGTTTAATTACAT-----AAAAAATTAAT-----TGATTTTGTTGATTAGTATT 75
M.rosenbergii (Np01)    TGT---AGTTTAATTACAT-----AAAAAATTAAT-----TGATTTTGTTGATTAGTATT 76
P.monodon               GTTTTCTATATATTAAGAAGT--AAAAAAATTATTTT-TGAAATGTATTTTTTTAGTAGA 81
S.serrata               AT--AAAATTTAGTTTTAAAAATGTAAAAATTAATAT---AAAAATAAATTAGTAAT-TA 76
S.tranquebarica         AT--AAAAGTTAGTTTTAAGT-TATAAAAATTAATAT---AAGAAAAATTTAGTAAT-TA 75
S.olivacea              TTTTAAAGAATACTAGAATAGATACAAAAATTAATA----AAAAGGGTATTAGTAAT-TA 77
B.mandarina             TTTTAATAAAAGAATTTTTGA---GGTTAAATTAT---TTTAATGGGGGGTTAAAGTAAT 89
B.mori                  TTTTAATGAAAGAATTTTTAA---GATTAAATTAT---TTTAATGGGGGGTTAAAGTAAT 89
An.gambiae              TTTATTTATAATTATTAATAA---AAATAATTT-----TATTTTTTATAGTTTTAGTAAT 78
An.quadrimaculatus      TTTATTT--GATTATTAATAA---AAATAATTT-----TAAATTTAATAGTTTTAGTAAT 76
Ap.mellifera            TATATATATATATATTAATAA---TAATAATTTATTTTTTTTTTTTAAAGTTTATAAAGA 117
D.melanogaster          TTTTTTA--AAAAATTATTAG---AAATAACTA-----TAAAATTTAAAGTTTTAGTATT 78
D.yakuba                TTTTATA--TAAAAATATTAG---AAATAACTA-----TAAATTTAAAAGTTTTAGTATT 77
                                                     *  *
Escherichia coli        --------------------E. coli 1-446 --------------------------

                        <------------------------- Domain I------------------------->
```

Figure 16 (continuted)

```
M.assamense (Kc01)      TTTTAGAAGTTTAGT----TTGAAATTATAGTTAATAAGTACAGTAA--------TGGAA 124
M.assamense (Kc02)      TTTTAGAAGTTTAGT----TTGAAATTATAGTTAATAAGTACAGTAA--------TGGAA 124
M.lanchesteri (Rb01)    TTTTAGAAGTTTAGT----TTGAAATTATAGTTAATAAGTACAGTAA--------TGGAA 124
M.lanchesteri (Np01)    TTTTAGAAGTTTAGT----TTGAAATTATAGTTAATAAGTACAGTAA--------TGGAA 124
M.lanchesteri (Rb02)    TTTTAGAAGTTTAGT----TTGAAATTATAGTTAATAAGTACAGTAA--------TGGAA 124
M.lanchesteri (Np02)    TTTTAGAAGTTTAGT----TTGAAATTATAGTTAATAAGTACAGTAA--------TGGAA 124
M.lanchesteri (Ay03)    TTTTAGAAGTTTAGT----TTGAAATTATAGTTAATAAGTACAGTAA--------TGGAA 124
M.lanchesteri (Cs01)    TTTTAGAAGTTTAGT----TTGAAATTATAGTTAATAAGTACAGTAA--------TGGAA 123
M.tratense (Kc01)       ATAAAGAATTTTATT----TAGAGATTATAGTTAATGAGTACAGTAA--------TGGAA 123
M.tratense (Ut01)       ATAAAGAATTTTATT----TAGAGATTATAGTTAATGAGTACAGTAA--------TGGAA 125
M.forcipatum (Tk01)     TTAGAGAATTTTATT----CGAGAATTATAGTTAATAAGTACAGTAA--------TGGAA 126
M.hirsutimanus (Rb01)   TTAAAGAATTTTGTT----TAAGGATAATAGTTAATAAGTACAGTAA--------TGGGA 126
M.hirsutimanus (Rb02)   TTAAAGAATTTTGTT----TAAGGATAATAGTTAATAAGTACAGTAA--------TGGGA 126
M.niphanae (Kc01)       TTATAGAATTTGATTTTGATTG--ATTATAGTTAATAAGTACAGTGA--------TGGAA 127
M.niphanae (Np01)       TTATAGAATTTGATTTTGATTG--ATTATAGTTAATAAGTACAGTGA--------TGGAA 127
M.niphanae (Np02)       TTATAGAATTTGATTTTGATTG--ATTATAGTTAATAAGTACAGTGA--------TGGAA 127
M.sintangense (Cb02)    ATAAAGAATTTTATT----TAAAGATTATAGCTAATAAGTACAGTGA--------CGGAA 125
M.sintangense (Cb01)    ATAAAGAATTTTATT----TAAAGATTATAGCTAATAAGTACAGTGA--------CGGAA 120
M.hirsutimanus (Sb01)   TTAAAGAATTTTGTT----TAAGGATAATAGTTAATAAGTACAGTAA--------TGGGA 126
M.hirsutimanus (Sb02)   TTAAAGAATTTTGTT----TAAGGATAATAGTTAATAAGTACAGTAA--------TGGGA 126
M.hirsutimanus (Sb03)   TTAAAGAATTTTGTT----TAAGGATAATAGTTAATAAGTACAGTAA--------TGGGA 126
M.dienbienphuense(Ny01) TTAAAGAATTTTGTT----TGAGAGTTATAGTTAATAAGTACAGTAG--------TGGAA 126
M.hirsutimanus (Ny01)   TTAAAGAATTTTGTT----TAAGGATAATAGTTAATAAGTACAGTAA--------TGGGA 126
M.hirsutimanus (Ny02)   TTAAAGAATTTTGTT----TAAGGATAATAGTTAATAAGTACAGTAA--------TGGGA 126
M.sp3 (Np02)            TTATAGAATTTGATTGTGATTGTGATTATAGTTAATAAGTACAGTGA--------TGGAA 129
M.sp3 (Np01)            TTATAGAATTTGATTGTGATTGTGATTATAGTTAATAAGTACAGTGA--------TGGAA 129
M.niphanae (Ny01)       TTATAGAATTTGATTTTTATTG--ATTATAGTTAATAAGTACAGTGA--------TGGAA 127
M.niphanae (Ny04)       TTATAGAATTTGATTGTGATTGTGATTATAGTTAATAAGTACAGTGA--------TGGAA 124
M.niphanae (Ny03)       TTATAGAATTTGATTTTTATTG--ATTATAGTTAATAAGTACAGTGA--------TGGAA 127
M.niphanae (Ny02)       TTATAGAATTTGATTGT--TTGTGATTATAGTTAATAAGTATAGTGA--------TGGAA 127
M.dienbienphuense(Ny02) TTAAAGAATTTTGTT----TGAGAGTTATAGTTAATAAGTACAGTAG--------TGGAA 126
M.rosenbergii(NC_006880)CTGTAGAATTTTGTTA---TTAAGGTTATAGTTAATGAGTACAGTGA--------TGGAA 124
M.rosenbergii (Np01)    CTGTAGAATTTTGTTA---TTAAGGTTATAGTTAATGAGTACAGTGA--------TGGAA 125
P.monodon               GTGAATCGAAATATTTTATAATAAATTATAGACTA-AAGTACTGTAAAGGAAAGATGAAA 140
S.serrata               ATAATGAATTTTTTGT---AATAGGCAATAGAGCAGAAGTACTGAAC-------AGGAAA 126
S.tranquebarica         ATAATAAAATTTATTA---AATGGGCAATAGGGTA-AAGTACTGAAT-------AGGAAA 124
S.olivacea              ATAAAGAAATTTATTT---GTTAG-CGATAGAGTATAAGTACTGAAT-------GGGAAA 126
B.mandarina             TTTTATTGAAA--AAATTTATAATTTTATAGTTTATTTGTATTGTGAAGGAATTTTGAAA 147
B.mori                  TTTTATTGAAA--AAATTTATAAATTTATAGTTTATTTGTATTGTGGGGGAATTTTGAAA 147
An.gambiae              TTATAATGAAA--TAATAATTTTAATTATAGTGTATTAGTATTTTAAAAGAATATTGAAA 136
An.quadrimaculatus      TTATAATGAAA--TAATAATTTTAATTATAGTGTATTAGTATTTTAAAAGAATATTGAAA 134
Ap.mellifera            TTTTATTTAGGTTAAATTAATTATTTTGAAATAATTTAAAATTTTATAG----TTTAAAG 173
D.melanogaster          GTTTAAAGAAA--AAATAATTTTAATAATAGTTTATTAGTATTGTAAAAGAAAATTGAAA 136
D.yakuba                GTTTAAAGAAA--AAATAATTTTAATAATAGTATATTAGTATTGTAAAAGAAAATTGAAA 135
                         *                           *          *
Escherichia coli        -------------------------> ATAGTGAACCAGTA<------------------

                        <-------------------------Domain I-------------------------->
```

Figure 16 (continuted)

```
M.assamense (Kc01)      ATATTATTAAA-----TTAATT----AAAAGTAGTATTAAATTTTCGTACCTTTTGTATC 175
M.assamense (Kc02)      ATATTATTAAA-----TTAATT----AAAAGTAGTATTAAATTTTCGTACCTTTTGTATC 175
M.lanchesteri (Rb01)    ATATTATTAAA-----TTAATT----AAAAGTAGTATTAAATTTTCGTACCTTTTGTATC 175
M.lanchesteri (Np01)    ATATTATTAAA-----TTAATT----AAAAGTAGTATTAAATTTTCGTACCTTTTGTATC 175
M.lanchesteri (Rb02)    ATATTATTAAA-----TTAATT----AAAAGTAGTATTAAATTTTCGTACCTTTTGTATC 175
M.lanchesteri (Np02)    ATATTATTAAA-----TTAATT----AAAAGTAGTATTAAATTTTCGTACCTTTTGTATC 175
M.lanchesteri (Ay03)    ATATTATTAAA-----TTAATT----AAAAGTAGTATTAAATTTTCGTACCTTTTGTATC 175
M.lanchesteri (Cs01)    ATATTATTAAA-----TTAATT----AAAAGTAGTATTAAATTTTCGTACCTTTTGTATC 174
M.tratense (Kc01)       TTATTT-GGTA-----TTTTGT----TAAAGTAGTGTTGGGGTTTCGTACCTTTTGTATC 173
M.tratense (Ut01)       TTATTT-GGTA-----TTTTGT----TAAAGTAGTGTTGGGGTTTCGTACCTTTTGTATC 175
M.forcipatum (Tk01)     GTGTT--GAAA-----TTTGAG----AAAAGTAGTGTTGGAATTTCGTACCTTTTGTATC 175
M.hirsutimanus (Rb01)   GCATT--GAAA-----TTTGGG----AGAAGTAGTGTTGGATACTCGTACCTTTTGTATC 175
M.hirsutimanus (Rb02)   GCATT--GAAA-----TTTGGG----AGAAGTAGTGTTGGATACTCGTACCTTTTGTATC 175
M.niphanae (Kc01)       TTATTT-AAAA-----TTTAAG----TAAAGTAGTGGTTGGTTTTCGTACCTTTTGTATC 177
M.niphanae (Np01)       TTTTTT-AAAA-----TTTTAG----TAAAGTAGTGGTTGGTTTTCGTACCTTTTGTATC 177
M.niphanae (Np02)       TTATTT-AAAA-----TTTAAG----TAAAGTAGTGGTTGGTTTTCGTACCTTTTGTATC 177
M.sintangense (Cb02)    TTATTT-AATA-----TTTTAT----TAAAGTAGTGTTTGATTTTCGTACCTTTTGTATC 175
M.sintangense (Cb01)    TTATTT-AATA-----TTTTAT----TAAAGTAGTGTTTGATTTTCGTACCTTTTGTATC 170
M.hirsutimanus (Sb01)   GCATT--GAAA-----CTTGGG----AGAAGTAGTGTTGGATACTCGTACCTTTTGTATC 175
M.hirsutimanus (Sb02)   GCATT--GAAA-----CTTGGG----AGAAGTAGTGTTGGATACTCGTACCTTTTGTATC 175
M.hirsutimanus (Sb03)   GCATT--GAAA-----TTTGGG----AGAAGTAGTGTTGGATACTCGTACCTTTTGTATC 175
M.dienbienphuense(Ny01) GTGTT--GAAA-----TTTAGG----AGAAGTAGTGTTGAAATTTCGTACCTTTTGTATC 175
M.hirsutimanus (Ny01)   GCATT--GAAA-----CTTGGG----AGAAGTAGTGTTGGATACTCGTACCTTTTGTATC 175
M.hirsutimanus (Ny02)   GCATT--GAAA-----CTTGGG----AGAAGTAGTGTTGGATACTCGTACCTTTTGTATC 175
M.sp3 (Np02)            TTATTT-AAAA-----TTTAAG----TAAAGTAGTAGTTGGTTTTCGTACCTTTTGTATC 179
M.sp3 (Np01)            TTATTT-AAAA-----TTTAAG----TAAAGTAGTAGTTGGTTTTCGTACCTTTTGTATC 179
M.niphanae (Ny01)       TTATTT-AAAA-----TTTAAG----TAAAGTAGTGGTTGGTTTTCGTACCTTTTGTATC 177
M.niphanae (Ny04)       TTATTT-AAAA-----TTTAAG----TAAAGTAGTGGTTGGTTTTCGTACCTTTTGTATC 174
M.niphanae (Ny03)       TTATTT-AAAA-----TTTAAG----TAAAGTAGTGGTTGGTTTTCGTACCTTTTGTATC 177
M.niphanae (Ny02)       TTATTT-AAAA-----TTTAAG----TAAAGTAGTGGTTGGTTTTCGTACCTTTTGTATC 177
M.dienbienphuense(Ny02) GTGTT--GAAA-----TTTAGG----AGAAGTAGTGTTGAAATTTCGTACCTTTTGTATC 175
M.rosenbergii(NC_006880)GTTTTATTGAA-----TTAGGT----TAAAGTAGCGTTGAGTTTTCGTACCTTTTGTATC 175
M.rosenbergii (Np01)    GTTTTATTGAA-----TTAGGT----TAAAGTAGCGTTGAGTTTTCGTACCTTTTGTATC 176
P.monodon               TAATTTGAAAAATGTATTAATA----AAAAGTAAAGTTAAATTCTTGTACCTTGTGTATT 196
S.serrata               TAAAAGTAAGA--AATTAAAAAATATAAA-GTAAATTTAAATTTTTGTACCTTGTGTATC 183
S.tranquebarica         TAAAATTAAGA--GATTTTAAAGTAAAAAAGTAAATTTAAATTTTTGTACCTTGTGTATC 182
S.olivacea              TTAAAGTTAAATGATTTTTAAAACAAAAAAGTAGATTTAAATTTTTGTACCTTGTGTATC 186
B.mandarina             TAATATGAA----ATTTAATTATTTTAAAAGTAAATTTTATTTATTGTATCTTGTGTATC 203
B.mori                  TAATATGAA----ATTTAATTATTTTAAAAGTAAATTTTATTTATTGTATCTTGTGTATC 203
An.gambiae              TAATTTGAA----AAATTTTTAATTTTTAAGAAAATTTAATTTATTGTACCTTGTGTATC 192
An.quadrimaculatus      TAATTTGAA----AAATTTTTATTTTAAAAGAAAATTTAATTTATTGTACCTTGTGTATC 190
Ap.mellifera            TAATGTAAATGA-AAATTTAAATGTGAAAAAGTAGAATAATTTTTTGTACCTTTTGTATC 232
D.melanogaster          TAATTTGAA----AAAATTTTATTTTAAAAGAAAATTTAATTTATTGTACCTTGTGTATC 192
D.yakuba                TAATTTGAA----AAAATTTTATTTTAAAAGAAAATTTAATTTATTGTACCTTGTGTATC 191
                                                     *        *      * *** *** *****

Escherichia coli        ------461-560-------------------------------> GTACCTTTTGTATA
                                                                      [---(561-578)--

                        <--------------------- E. coli Domain I------------------->
```

Figure 16 (continuted)

```
M.assamense (Kc01)      AGGGGTAAACTAAGTTGACATGTTAATTAT-GG-AGTCCCGAAACAAGGTGAGCTA-AAT 232
M.assamense (Kc02)      AGGGGTAAACTAAGTTGACATGTTAATTAT-GG-AGTCCCGAAACAAGGTGAGCTA-AAT 232
M.lanchesteri (Rb01)    AGGGGTAAACTAAGTTGACATGTTAATTAT-GG-AGTCCCGAAACAAGGTGAGCTA-AAT 232
M.lanchesteri (Np01)    AGGGGTAAACTAAGTTGACATGATAATTAT-GG-AGTCCCGAAACAAGGTGAGCTA-AAT 232
M.lanchesteri (Rb02)    AGGGGTAAACTAAGTTGACATGTTAATTAT-GG-AGTCCCGAAACAAGGTGAGCTA-AAT 232
M.lanchesteri (Np02)    AGGGGTAAACTAAGTTGACATGTTAATTAT-GG-AGTCCCGAAACAAGGTGAGCTA-AAT 232
M.lanchesteri (Ay03)    AGGGGTAAACTAAGTTGACATGTTAATTAT-GG-AGTCCCGAAACAAGGTGAGCTA-AAT 232
M.lanchesteri (Cs01)    AGGGGTAAACTAAGTTGACATGTTAATTAT-GG-AGTCCCGAAACAAGGTGAGCTA-AAT 231
M.tratense (KC01)       AGGGGTATACTATATAATTATGTTATTTAT-AA-GATCCCGAAACAAGGAGAGTTA-AAT 230
M.tratense (Ut01)       AGGGGTATACTATATAATTATGTTATTTAT-AA-GATCCCGAAACAAAGAGAGTTA-AAT 232
M.forcipatum (Tk01)     AGGGGTTAACTATTTTAGCGTGTTAGTTAT-GG-GGTCCCGAAACAAGAAGAGCTAGGGT 233
M.hirsutimanus (Rb01)   AGGGATTAACTATTTTAGCATGTTAGTTGT-GA-AATCCCGAAATAAGAAGAGCTAGGAT 233
M.hirsutimanus (Rb02)   AGGGATTAACTATTTTAGCATGTTAGTTGT-GA-AATCCCGAAATAAGAAGAGCTAGGAT 233
M.niphanae (Kc01)       AGGGGTTAACTATATAGTTATGTTAATTAT-AA-TATCCCGAAACAAAGAGAGTTAGGAT 235
M.niphanae (Np01)       AGGGGTTAACTATATAGTTATATTAATTAT-AA-TATCCCGAAACAAAGAGAGTTAGAAT 235
M.niphanae (Np02)       AGGGGTTAACTATATAGTTATATTAATTAT-AA-TATCCCGAAACAAAGAGAGTTAGAAT 235
M.sintangense (Cb02)    AGGGGTAAACTATGTAGTTATGTTAGTTTT-GA-GGTCCCGAAACAAGGAGAGTTA-AAT 232
M.sintangense (Cb01)    AGGGGTAAACTATGTAGTTATGTTAGTTTT-GA-GGTCCCGAAACAAGGAGAGTTA-AAT 227
M.hirsutimanus (Sb01)   AGGGATTAACTATTTTAGCATGTTAGTTGT-GA-AATCCCGAAATAAGAAGAGCTAGGAT 233
M.hirsutimanus (Sb02)   AGGGATTAACTATTTTAGCATGTTAGTTGT-GA-AATCCCGAAATAAGAAGAGCTAGGAT 233
M.hirsutimanus (Sb03)   AGGGATTAACTATTTTAGCATGTTAGTTGT-GA-AATCCCGAAATAAGAAGAGCTAGGAT 233
M.dienbienphuense (Ny01)AGGGGTTAACTATTTTAGCGTGCTAGTTAT-GG-GGTCCCGAAACAAGAAGAGCTAGGGT 233
M.hirsutimanus (Ny01)   AGGGATTAACTATTTTAGCATGTTAGTTGT-GA-AATCCCGAAATAAGAAGAGCTAGGAT 233
M.hirsutimanus (Ny02)   AGGGATTAACTATTTTAGCATGTTAGTTGT-GA-AATCCCGAAATAAGAAGAGCTAGGAT 233
M.sp3 (Np02)            AGGGGTTAACTATATAGTTATGTTAATTAT-AA-TATCCCGAAACAAAGAGAGTTAGAAT 237
M.sp3 (Np01)            AGGGGTTAACTATATAGTTATGTTAATTAT-AA-TATCCCGAAACAAAGAGAGTTAGAAT 237
M.niphanae (Ny01)       AGGGGTTAACTATATAGTTATGTTAATCAT-AA-TATCCCGAAACAAAGAGAGTTAGGAT 235
M.niphanae (Ny04)       GGGGGTTAACTATATAGTTATGTTAATTAT-AA-TATCCCGAAACAAAGAGAGTTAGAAT 232
M.niphanae (Ny03)       AGGGGTTAACTATATAGTTATGTTAATCAT-AA-TATCCCGAAACAAAGAGAGTTAGGAT 235
M.niphanae (Ny02)       AGGGGTTAACTATATAGTTATGTTAATCAT-AA-TATCCCGAAACAAAGAGAGTTAGGAT 235
M.dienbienphuense (Ny02)AGGGGTTAACTATTTTAGCGTGCTAGTTAT-GG-GGTCCCGAAACAAGAAGAGCTAGGGT 233
M.rosenbergii(NC_006880)AGGGGTGAACTAAATTGGTATATTAGTTAT-GG-AGTCCCGAAACAAAGAGAGCTA-GGT 232
M.rosenbergii (Np01)    AGGGGTGAACTAAATTGGTATATTAGTTAT-GG-AGTCCCGAAACAAAGAGAGCTA-GGT 233
P.monodon               AGGGAAAATCAAAATAATCTTATTATTTAAAGA-AATCCCGAAAGAAAAAGAGCTA--TT 253
S.serrata               AGGGATAATTAAAATTAGACATAAA-ATATGTT-GATCCCGAAATAGAGATAGCTA--AT 239
S.tranquebarica         AGGGATAATCAAAATTATTCATAAATAAATGCT-AATCCCGAAATAAAGATAGCTA--AT 239
S.olivacea              AGGGATAATTAAAATTATTCATAGA-AGATGAA-GATCCCGAAATAAAGATAGCTA--AT 242
B.mandarina             AGAGTTTATTAAAAATAATTTTAT--TTTTAAAGTTTCTCGAATTTAAAAGAGTTAATTA 261
B.mori                  AGAGTTTATTAAAAATAATTTTAT--TTTTAAAGTTTCTCGAATTTAAAAGAGTTAATTA 261
An.gambiae              AGGGTTTATTAAATAATAAATTATTATAATAATTTTTCTCGAATTTTAAAGATTTAATTA 252
An.quadrimaculatus      AGGGTTTATTAAATAAATAATTATTATATTAATTTTTCTCGAATTTTAAAGATTTAATTA 250
Ap.mellifera            AGGGTTGATTAAAATTTATTTAATGATTTTAAA-TATCTCGAAAATAAGGGAGCTAAATT 291
D.melanogaster          AGTGTTTATTAAATAAAAAAATAA--ATATTTATTTTTCTCGATTTTAAAAGAGTTAATAT 250
D.yakuba                AGCGTTTATTAAATAAAAAAATAA--ATATTTATTTTTCTCGATTTTAAAAGAGTTAATAT 249
                         * *       *                        ** ***         *  **
Escherichia coli        ATGG<------------------------------- 579-744 --------------
                        ----]
                        ----><------------------------ Domain II-------------------->
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Figure 17 Alignment of *16S rRNA* of *Macrobrachium* spp. compared with other arthropods and *E. coli*; Domain II.

```
M.assamense (Kc01)      TTTGTTGCTTTTTT-ACGTGGTAAAGTGATTTGTAACGAATCTTTAGTGGTGATATGTTA 291
M.assamense (Kc02)      TTTGTTGCTTTTTT-ACGTGGTAAAGTGATTTGTAACGAATCTTTAGTGGTGATATGTTA 291
M.lanchesteri (Rb01)    TTTGTTGCTTTTTT-ACGTGGTAAAGTGATTTGTAACGAATCTTTAGTGGTGATATGTTA 291
M.lanchesteri (Np01)    TTTGTTGCTTTTTT-ACGTGGTAAAGTGATTTGTAACGAATCTTTAGTGGTGATATGTTA 291
M.lanchesteri (Rb02)    TTTGTTGCTTTTTT-ACGTGGTAAAGTGATTTGTAACGAATCTTTAGTGGTGATATGTTA 291
M.lanchesteri (Np02)    TTTGTTGCTTTTTT-ACGTGGTAAAGTGATTTGTAACGAATCTTTAGTGGTGATATGTTA 291
M.lanchesteri (Ay03)    TTTGTTGCTTTTTTTACGTGGTAAAGTGATTTGTAACGAATCTTTAGTGGTGATATGTTA 292
M.lanchesteri (Cs01)    TTTGTTGCTTTTTT-ACGTGGTAAAGTGATTTGTAACGAATCTTTAGTGGTGATATGTTA 290
M.tratense (Kc01)       TTTATTGCTTCTCT-ACGTTGTAAAGTAGTCTGTAAAA-GTTTTTAGTGGTGATATGTTA 288
M.tratense (Ut01)       TTTATTGCTTCTCT-ACGTTGTAAAGTAGTCTGTAAAA-GTTTTTAGTGGTGATATGTTA 290
M.forcipatum (Tk01)     TT-GTTACTTTTCT-TCGTTGTAGAGAGGTTTGTAAAGTATTCTTAGTGATGATATGTCA 291
M.hirsutimanus (Rb01)   TTTATTACTTTTCT-TCGTTGCAGAGAGGTTTGCAAGGTATTCTTAGTGGTGATATGTTA 292
M.hirsutimanus (Rb02)   TTTATTACTTTTCT-TCGTTGCAGAGAGGTTTGCAAGGTATTCTTAGTGGTGATATGTTA 292
M.niphanae (Kc01)       TTCATTTCTTTTTA-TCGTTGTAGAGAGGTTTGTAAAGGATTTCTAGTAGTGATATGTTA 294
M.niphanae (Np01)       TTCATTTCTTTTTT-TCGTTGTAGAGAGGTTTGTAAAGGATTTCTAGTAGTGATATGTTA 294
M.niphanae (Np02)       TTCATTTCTTTTTA-TCGTTGTAGAGAGGTTTGTAAAGGATTTCTAGTAGTGATATGTTA 294
M.sintangense (Cb02)    TTTATTGCTTCTCT-ACGTTGTAAAGTAGTTTGGAAAA-GTTTTTAGTGGTGATATGTTA 290
M.sintangense (Cb01)    TTTATTGCTTCTCT-ACGTTGTAAAGTAGTTTGGAAAA-GTTTTTAGTGGTGATATGTTA 285
M.hirsutimanus (Sb01)   TTTATTACTTTTCT-TCGTTGCAGAGAGGTTTGCAAGGTATTCTTAGTGGTGATATGTTA 292
M.hirsutimanus (Sb02)   TTTATTACTTTTCT-TCGTTGCAGAGAGGTTTGCAAGGTATTCTTAGTGGTGATATGTTA 292
M.hirsutimanus (Sb03)   TTTATTACTTTTCT-TCGTTGCAGAGAGGTTTGCAAGGTATTCTTAGTGGTGATATGTTA 292
M.dienbienphuense(Ny01) TT-GTTACTTTTCT-TCGTTGTAGAGAGGTTTGTAAAATATTCTTAGTGGTGATATGTCA 291
M.hirsutimanus (Ny01)   TTTATTACTTTTCT-TCGTTGCAGAGAGGTTTGCAAGGTATTCTTAGTGGTGATATGTTA 292
M.hirsutimanus (Ny02)   TTTATTACTTTTCT-TCGTTGCAGAGAGGTTTGCAAGGTATTCTTAGTGGTGATATGTTA 292
M.sp3 (Np02)            TTTATTTCTTTTTA-TCGTTGTAAAGAGGTTTGTAAAGGATTTCTAGTAGTGATATGTTA 296
M.sp3 (Np01)            TTTATTTCTTTTTA-TCGTTGTAAAGAGGTTTGTAAAGGATTTCTAGTAGTGATATGTTA 296
M.niphanae (Ny01)       TTCATTTCTTTTTA-TCGTTGTAGAGAGGTTTGTAAAGGATTTCTAGTAGTGATATGTTA 294
M.niphanae (Ny04)       TTTATTTCTTTTTA-TCGTTGTAAAGAGGTTTGTAAAGGATTTCTAGTAGTGATATGTTA 291
M.niphanae (Ny03)       TTCATTTCTTTTTA-TCGTTGTAGAGAGGTTTGTAAAGGATTTCTAGTAGTGATATGTTA 294
M.niphanae (Ny02)       TTTATTTCTTTTTA-TCGTTGTAGAGAGGTTTGTAAAGGATTTCTAGTAGTGATATGTTA 294
M.dienbienphuense(Ny02) TT-GTTACTTTTCT-TCGTTGTAGAGAGGTTTGTAAAATATTCTTAGTGGTGATATGTCA 291
M.rosenbergii(NC_006880)TTTGTTGCTTTTTT-ACGTGGTAAAGTGATTTGTAATGGGTCTCTAGTGGTGATATGTTA 291
M.rosenbergii (Np01)    TTTGTTGCTTTTTT-ACGTGGTAAAGTGATTTGTAATGGATCTCTAGTGATGATATGTTA 292
P.monodon               ATAAATATATAGTTTTCGTATTAATGAAATTATCAAATTTATACTAGTAGTGAAATGCTA 313
S.serrata               ATTATAATTAATTTTTTGTAGCATAAAAATTTATAATATAATATTAAAGATGAAATGTCA 299
S.tranquebarica         ATTATAATTAATTTTTTGTAGCATAAAAATTTATAATATAGTATTAAAGATGAAATGTCA 299
S.olivacea              ATTGTAATTAGTTTTTTGTAACATAAAAATTTATAATATAATATTAAAGATGAAATGTCA 302
B.mandarina             ATTAATAATAAGTTATTGTAGTATAAATATTTTAAATAATTAATTTGAAATGAAATGTTA 321
B.mori                  ATTAATAATAAGTTATTGTAGTATAAATATTTTAAATAATTAATTTGAAATGAAATGTTA 321
An.gambiae              TATATAAA--AGTTATTGTGGAATAACTATTTTAAATATGTAATTAGAAATGAAATGTTA 310
An.quadrimaculatus      TATATGAA--AGTTATTGTGGAATAACTATTTTACATATATAATTAGAAATGAAATGTTA 308
Ap.mellifera            TATATTAT--TTTTTTTGTAGCAATAAAATTATAAATATAGATTTAGAAATTAAATGTTA 349
D.melanogaster          AATATTTA--AGTTAATGTGATAAAATTATTTATAATATTATATTAGAAATGAAATGTTA 308
D.yakuba                AATATTTA--AGTTAATGTGACAAAATTATTTATAATATTATATTAGAAATGAAATGTTA 307
                                          **   *      *     *        *     * * ***  *
Escherichia coli         ---------------->GTTGAAAA<--------753-778-------->TGAAAGG<--

                        <-------------------------- Domain II---------------------->
```

Figure 17 (continued)

```
M.assamense (Kc01)      GGCGATCTTTGTA-TATCTGGTTTTTCGTTAAATGAATTTAATTT---TGCATTAAG--- 344
M.assamense (Kc02)      GGCGATCTTTGTA-TATCTGGTTTTTCGTTAAATGAATTTAATTT---TGCATTAAG--- 344
M.lanchesteri (Rb01)    GGCGATCTTTGTA-TATCTGGTTTTTCGTTAAATGAATTTAATTT---TACATTAAG--- 344
M.lanchesteri (Np01)    GGCGATCTTTGTA-TATCTGGTTTTTCGTTAAATGAATTTAATTT---TGCATTGAG--- 344
M.lanchesteri (Rb02)    GGCGATCTTTGTA-TATCTGGTTTTTCGTTAAATGAATTTAATTT---TGCATTAAG--- 344
M.lanchesteri (Np02)    GGCGATCTTTGTA-TATCTGGTTTTTCGTTAAATGAATTTAATTT---TGCATTAAG--- 344
M.lanchesteri (Ay03)    GGCGATCTTTGTA-TATCTGGTTTTTCGTTAAATGAATTTAATTT---TGCATTAAG--- 345
M.lanchesteri (Cs01)    GGCGATCTTTGTA-TATCTGGTTTTTCGTTAAATGAATTTAATTT---TGCATTAAG--- 343
M.tratense (Kc01)       GTCGGTCTTTGTA-TATCTGGTTTTTTACGAAATGAATTTAATTT---TGTACTAAA--- 341
M.tratense (Ut01)       GTCGGTCTTTGTA-TATCTGGTTTTTTACGAAATGAATTTAATTT---TGTACTAAA--- 343
M.forcipatum (Tk01)     GTCGATTCTTGTA-TATCTGGTTCTTTATTAAATAAATTTAATTT---TGCACTGGG--- 344
M.hirsutimanus (Rb01)   GTCGATTTTTGTA-TATCTGGTTCCTTATTAAATAAATTTAATTT---TACACCTGG--- 345
M.hirsutimanus (Rb02)   GTCGATTTTTGTA-TATCTGGTTCCTTATTAAATAAATTTAATTT---TACACCTGG--- 345
M.niphanae (Kc01)       ATCGGTCTTTGTA-TATCTGGTTTTTCGTTAAATAAATTTAATTT---TACACTAAG--- 347
M.niphanae (Np01)       ATCGGTCTTTGTA-TATCTGGTTTTTCGTTAAATAAATTTAATTT---TACACTAAG--- 347
M.niphanae (Np02)       ATCGGTCTTTGTA-TATCTGGTTTTTCGTTAAATAAATTTAATTT---TACACTAAG--- 347
M.sintangense (Cb02)    GTCGGTCTTTGTA-TATCTGGTTTTTTATGAAATGAATTTAATTT---TTTGCTAGA--- 343
M.sintangense (Cb01)    GTCGGTCTTTGTA-TATCTGGTTTTTTATGAAATGAATTTAATTT---TTTGCTAGA--- 338
M.hirsutimanus (Sb01)   GTCGATTTTTGTA-TATCTGGTTCCTTATTAAATAAATTTAATTT---TACACCTGG--- 345
M.hirsutimanus (Sb02)   GTCGATTTTTGTA-TATCTGGTTCCTTATTAAATAAATTTAATTT---TACACCTGG--- 345
M.hirsutimanus (Sb03)   GTCGATTTTTGTA-TATCTGGTTCCTTATTAAATAAATTTAATTT---TACACCTGG--- 345
M.dienbienphuense(Ny01) GTCGATTCTTGTA-TATCTGGTTCCTTATTAAATAAATTTAATTT---TGCACTGAG--- 344
M.hirsutimanus (Ny01)   GTCGATTTTTGTA-TATCTGGTTCCTTATTAAATAAATTTAATTT---TACACCTGG--- 345
M.hirsutimanus (Ny02)   GTCGATTTTTGTA-TATCTGGTTCCTTATTAAATAAATTTAATTT---TACACCTGG--- 345
M.sp3 (Np02)            ATCGGTCTTTGTA-TATCTGGTTCTTCGTTAAATAAATTTAATTT---TACACTAAG--- 349
M.sp3 (Np01)            ATCGGTCTTTGTA-TATCTGGTTCTTCGTTAAATAAATTTAATTT---TACACTAAG--- 349
M.niphanae (Ny01)       ATCGGTCTTTGTA-TATCTGGTTTTTCGTTAAATAAATTTAATTT---TACACTAAG--- 347
M.niphanae (Ny04)       ATCGGTCTTTGTA-TATCTGGTTCTTCGTTAAATAAATTTAATTT---TACACTAAG--- 344
M.niphanae (Ny03)       ATCGGTCTTTGTA-TATCTGGTTTTTCGTTAAATAAATTTAATTT---TACACTAAG--- 347
M.niphanae (Ny02)       ATCCGTCTTTGTA-TATCTGGTTCTTCGTTAAATAAATTTAATTT---TACCCTAAG--- 347
M.dienbienphuense(Ny02) GTCGATTCTTGTA-TATCTGGTTCCTTATTAAATAAATTTAATTT---TGCACTGAG--- 344
M.rosenbergii(NC_006880)GTCGATTTTTGTA-TATCTGGTTTTCCGTTAAATGAATTTAATTT---TGTGTCAAG--- 344
M.rosenbergii (Np01)    GTCGATTTTTGTA-TATCTGGTTTTCCGTTAAATGAATTTAATTT---TGTGTCAAG--- 345
P.monodon               ATCG-TTTTTCA-TATCTGGTTATTGGTGAATTAAATTTAATTTGATTATATTGAGGTA 371
S.serrata               AACGAGCTTTATAATATCTGGTTTTTTAGGAATAAAATATAATTTA--ATTATTGAA--- 354
S.tranquebarica         AACGAGCTTTATAATATCTGGTTTTTTAGGAATAAAATATAATTTA--ATTATTGAA--- 354
S.olivacea              AACGAGTTTTATAATATCTGGTTTTTTAGGAATAAAATATAATTTG--ATTATTGAA--- 357
B.mandarina             TTCGTTTTTAAATATATCTAGTTTTTATAGAAAAAAATTTAATTTTTTATTAATTAA--- 378
B.mori                  TTCGTTTTTAAATATATCTAGTTTTTATAGAAAAAAATTTAATTTTTTATTAATTAG--- 378
An.gambiae              ATCGTTTTAAAATATATCTAGTTTTTTAAGAAATGAATTTAATTTAG-CTTATTTAT--- 366
An.quadrimaculatus      ATCGTTTTAAAATATATCTAGTTTTTTAAGAAATAAATTTAATTTAGGTTTATTTAT--- 365
Ap.mellifera            AACGAACTTATTAATATCTAGTTTTTTTAGAAATAAATTTAATTTAGATATAATTAT--- 406
D.melanogaster          TTCGTTTTTAAAGGTATCTAGTTTTTTAAGAAATAAATTTAATTTAGAAATTATAAA--- 365
D.yakuba                TTCGTTTTTAAAGGTATCTAGTTTTTTAAGAAATAAATTTAATTTAGAAATTATAAA--- 364
                          *           ***** ***       **   *** ******
Escherichia coli        ---------->AGATAGCTGGTTCTCCCCGAAA<--------------------------

                        <-------------------------- Domain II----------------------->
```

Figure 17 (continued)

```
M.assamense (Kc01)      -TTAAAA--TT-AACTTGATAGT-TATGGTAGGAGGAATGAGCTTCTTATTAGT---TTT 396
M.assamense (Kc02)      -TTAAAA--TT-AACTTGATAGT-TATGGTAGGAGGAATGAGCTTCTTATTAGT---TTT 396
M.lanchesteri (Rb01)    -TTAAAA--TT-AACTTGATAGT-TATGGTAGGAGGAATGAGCTTCTTATTAGT---TTT 396
M.lanchesteri (Np01)    -TTAAAA--TT-AACTTGATAGT-TATGGTAGGAGGAATGAGCTTCTTATTAGT---TTT 396
M.lanchesteri (Rb02)    -TTAAAA--TT-AACTTGATAGT-TATGGTAGGAGGAATGAGCTTCTTATTAGT---TTT 396
M.lanchesteri (Np02)    -TTAAAA--TT-AACTTGATAGT-TATGGTAGGAGGAATGAGCTTCTTATTAGT---TTT 396
M.lanchesteri (Ay03)    -TTAAAA--TT-AACTTGATAGT-TATGGTAGGAGGAATGAGCTTCTTATTAGT---TTT 397
M.lanchesteri (Cs01)    -TTAAAA--TT-AACTTGATAGT-TATGGTAGGAGGAATGAGCTTCTTATTATT---TTT 395
M.tratense (Kc01)       -TCTAAT--TGTGGTTTAGTGAG-AATGGTAGGAGGAATAAGCTTCTTATCTGA---TGT 394
M.tratense (Ut01)       -TCTAAT--TGTGGTTTAGTGAG-AATGGTAGGAGGAATAAGCTTCTTATCTGA---TGT 396
M.forcipatum (Tk01)     -TTTTAA--TTTAATTTGGTAGC-GAGGGTAGGAGGAATAAGCTTCTTATCTTT---TAT 397
M.hirsutimanus (Rb01)   -TTTAAA--CTTAATTTGGTAGT-AAGGGTAGGAGGAATGAGCTTCTTATCTTT---TAT 398
M.hirsutimanus (Rb02)   -TTTAAA--CTTAATTTGGTAGT-AAGGGTAGGAGGAATGAGCTTCTTATCTTT---TAT 398
M.niphanae (Kc01)       -TTTTAA--TTTAATTTAGTAGT-AATGGTAGGAGGAATGAGCTTCTTATCTTT---TGT 400
M.niphanae (Np01)       -TTTTAA--TTTTATTTATTAGT-AATGGTAGGAGGAATGAGCTTCTTATCTTT---TGT 400
M.niphanae (Np02)       -TTTTAA--TTTAATTTATTAGT-AATGGTAGGAGGAATGAGCTTCTTATCTTT---TGT 400
M.sintangense (Cb02)    -TCTTAT--TGTGGTTTAGTAAG-AAGGGTAGGAGGAATAAGCTTCTTATCTGA---TGT 396
M.sintangense (Cb01)    -TCTTAT--TGTGGTTTAGTAAG-AAGGGTAGGAGGAATAAGCTTCTTATCTGA---TGT 391
M.hirsutimanus (Sb01)   -TTTAAA--CTTAATTTGGTAGT-AAGGGTAGGAGGAATGAGCTTCTTATCTTT---TAT 398
M.hirsutimanus (Sb02)   -TTTAAA--CTTAATTTGGTAGT-AAGGGTAGGAGGAATGAGCTTCTTATCTTT---TAT 398
M.hirsutimanus (Sb03)   -TTTAAA--CTTAATTTGGTAGT-AAGGGTAGGAGGAATGAGCTTCTTATCTTT---TAT 398
M.dienbienphuense(Ny01) -TTTTAA--TTTAATTTGGTAGT-GAGGGTAGGAGGAATAAGCTTCTTATCTTT---TAT 397
M.hirsutimanus (Ny01)   -TTTAAA--CTTAATTTGGTAGT-AAGGGTAGGAGGAATGAGCTTCTTATCTTT---TAT 398
M.hirsutimanus (Ny02)   -TTTAAA--CTTAATTTGGTAGT-AAGGGTAGGAGGAATGAGCTTCTTATCTTT---TAT 398
M.sp3 (Np02)            -TTTTAA--TTTAATTTAGTAGT-AATGGTAGGAGGAATGAGCTTCTTATCTTT---TGT 402
M.sp3 (Np01)            -TTTTAT--TTTAATTTAGTAGT-AATGGTAGGAGGAATGAGCTTCTTATCTTT---TGT 402
M.niphanae (Ny01)       -TTTTAA--TTTAATTTAGTAGT-AATGGTAGGAGGAATGAGCTTCTTATCTTT---TGT 400
M.niphanae (Ny04)       -TTTTAA--TTTAATTTAGTAGT-AAGGGTAGGAGGAATGAGCTTCTTATCTTT---TGT 397
M.niphanae (Ny03)       -TTTTAA--TTTAATTTAGTAGT-AATGGTGGGAGGAATGAGCTTCTTATCTTT---TGT 400
M.niphanae (Ny02)       -TTTTAA--CTTAATTTAGTAGT-AATGGTAGGAGGAATGAGCTTCTTATCTTT---TGT 400
M.dienbienphuense(Ny02) -TTTTAA--TTTAATTTGGTAGT-GAGGGTAGGAGGAATAAGCTTCTTATCTTT---TAT 397
M.rosenbergii(NC_006880)-TTAGAG--TTTAATTTGATAGT-TACGATAGGAGGAATGAGCTTCTTATCTGT---TGT 397
M.rosenbergii (Np01)    -TTAAAG--TTTAATTTGATAGT-TACGATAGGAGGAATGAGCTTCTTATCTGT---TGT 398
P.monodon               ATCAAAAGCTTTAATATAATA---AAGAATAGGAGGGAAAAGCTTCTTGTTCTAACATAG 428
S.serrata               -ATAAAA---TAAGTTCAATATTTAAGGATAGGAGGGAAAAGCTTCTTAT-ACTAATAAA 409
S.tranquebarica         -ATAAAA---TAGATTCAATAATTAAGGATAGGAGGGAAAAGCTTCTTAT-TCTAATAAA 409
S.olivacea              -ATGAAA---TAAGTTCAATATTAAAGGGTAGGAGGGACGAGCTTCTTATCACTATAAAA 413
B.mandarina             --ATTTT---TTATTAA----TT--AAATTAATAAAAATTATATTTTTAATAAAA--TAA 425
B.mori                  --ATTTT---TTATTAA----TT--AAATTAATAAAAAT-ATATTTTTAATAAAA--TAA 424
An.gambiae              --TTTAT---TAAGTTA----AT--TTTTTAATTTAA-TAATTAAATAA-AGTAA--TAT 411
An.quadrimaculatus      --TTTAT---TAG--TA----TT--TTTTTAATTTAA-TAAATGGATAATAATAA--TAT 409
Ap.mellifera            --TTTAG---TAAATTAAATTTT--ATTTTAATTTAT-TAATTT--TTATATTAA--AAA 454
D.melanogaster          --TTTAC---TTAATTA----TT--TATTTAATTAAT-TAATTT-ATAATTTTAA--TGT 410
D.yakuba                --TTTAT---TTAATTA----TT--TATTTAATTAAA-TAATTT-ATAATTTTAA--TAT 409
                                                      *          *     *
Escherichia coli        ------------------------------822-1056-----------------------

                       <-------------------------- Domain II------------------------->
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Figure 17 (continuted)

```
M.assamense (Kc01)       TTT--GTAAAGTGTTT-------TATTAGATATCATTTGAAGTGGGCTTAAAATCAGCCA 447
M.assamense (Kc02)       TTT--GTAAAGTGTTT-------TATTAGATATCATTTGAAGTGGGCTTAAAATCAGCCA 447
M.lanchesteri (Rb01)     TTT--GTAAAGTGTTT-------TATTAGATATCATTTGAAGTGGGCTTAAAATCAGCCA 447
M.lanchesteri (Np01)     TTT--GTAAAGTGTTT-------TATTAGATATCATTTGAAGTGGGCTTAAAATCAGCCA 447
M.lanchesteri (Rb02)     TTT--GTAAAGTGTTT-------TATTAGGTATCATTTGAAGTGGGCTTAAAATCAGCCA 447
M.lanchesteri (Np02)     TTT--GTAAAGTGTTT-------TATTAGATATCATTTGAAGTGGGCTTTAAATCAGCCA 447
M.lanchesteri (Ay03)     TTT--GTAAAGTGTTT-------TATTAGATATCATTTGAAGTGGGCTTAAAATCAGCCA 448
M.lanchesteri (Cs01)     TTT--GTAAAGTGTTT-------TATTAGATATCATTTGAAGTGGGCTTAAAATCAGCCA 446
M.tratense (Kc01)        TTTT-GTTAAGGACTT-------TATTGTAAGTCGTTATAGGTGGGCTTAAAGTCAGCCA 446
M.tratense (Ut01)        TTTT-GTTAAGGACTT-------TATTGTAAGTCGTTATAGGTGGGCTTAAAGTCAGCCA 448
M.forcipatum (Tk01)      TTT--GTTGAACGTTTTGG----TTGTGTTATCCGTTTTAGGTGGGCTTAAGGTCAGTCA 451
M.hirsutimanus (Rb01)    TTT--ATCGAACATTT-AG----TTGTGTGACTCGTTTTAGGTGGGCTTAAAGTCAGCCA 451
M.hirsutimanus (Rb02)    TTT--ATCGAACATTT-AG----TTGTGTGACTCGTTTTAGGTGGGCTTTAAGTCAGCCA 451
M.niphanae (Kc01)        TTT--ATTAAACATTT--A----TTTAATATTTCATTTTAAGTGGGCTTAAAATCAGCCA 452
M.niphanae (Np01)        TTT--ATTAAACATTT--A----TTTAATATTTCGTTTTAAGTGGGCTTTAAATCAGCCC 452
M.niphanae (Np02)        TTT--ATTAAACATTT--A----TTTAATATTTCGTTTTAAGTGGGCTTAAAATCAGCCA 452
M.sintangense (Cb02)     TTTT-GTTAAGGACTT-------CTTTGTAAGTCGTTATAGGTGGGCTTAAAATCAGCTA 448
M.sintangense (Cb01)     TTTT-GTTAAGGACTT-------CTTTGTAAGTCGTTATAGGTGGGCTTAAAATCAGCTA 443
M.hirsutimanus (Sb01)    TTT--ATCGAACATTT-AG----TTGTGTGACTCGTTTTAGGTGGGCTTAAAGTCAGCCA 451
M.hirsutimanus (Sb02)    TTT--ATCGAACATTT-AG----TTGTGTGACTCGTTTTAGGTGGGCTTAAAGTCAGCCA 451
M.hirsutimanus (Sb03)    TTT--ATCGAACATTT-AG----TTGTGTGACTCGTTTTAGGTGGGCTTAAAGTCAGCCA 451
M.dienbienphuense(Ny01)  TTT--ATTGAGCGTTTTGG----TTGTGTTATCCGTTTTAGGTGGGCTTAAGGTCAGCCA 451
M.hirsutimanus (Ny01)    TTT--ATCGAACATTT-AG----TTGTGTGACTCGTTTTAGGTGGGCTTAAAGTCAGCCA 451
M.hirsutimanus (Ny02)    TTT--ATCGAACATTT-AG----TTGTGTGACTCGTTTTAGGTGGGCTTAAAGTCAGCCA 451
M.sp3 (Np02)             TTT--ATTAAACATTT--A----TTTAATATTTCGTTTTAAGTGGGCTTAAAATCAGCCA 454
M.sp3 (Np01)             TTT--ATTAAACATTT--A----TTTAATATTTCGTTTTAAGTGGGCTTAAAATCAGCCA 454
M.niphanae (Ny01)        TTT--ATTAAACATTT--A----TTTAATATTTCATTTTAAGTGGGCTTAAAATCAGCCA 452
M.niphanae (Ny04)        TTT--ATTAAACATTT--A----TTTAATATTTCGTTTTAAGTGGGCTTAAAATCACCCC 449
M.niphanae (Ny03)        TTT--ATTAAACATTT--A----TTTAATATTTCATTTTAAGTGGGCTTAAAATCAGCCA 452
M.niphanae (Ny02)        TTT--ATTAAACCTTT--A----TTTAATATTTCGTTTTAAGTGGGCTTAAAATCACCCC 452
M.dienbienphuense(Ny02)  TTT--ATTGAGCGTTTTGG----TTGTGTTATCCGTTTTAGGTGGGCTTAAGGTCAGCCA 451
M.rosenbergii(NC_006880) TTT--GTTAAGCGTCT-------TATTAGTTTTCATCTGAGGTGGGCTTAAAATCAGCCA 448
M.rosenbergii (Np01)     TTT--GTTAAGCGTCT-------TATTAGTTTTCATTTGAGGTGGGCTTAAAATCAGCCA 449
P.monodon                GTGT-GTTATGATATAATG----AATAATTATTTTATTGAATTAGGCTTAAAAACAGCTA 483
S.serrata                ATTAAATAATAGGATAAATAGTCATGAAAGATAAATGTTTTCTAAGCTTAAAAGTAGTTA 469
S.tranquebarica          GTGAAACAATAGAATTAAAAATTTTAAATAA--AATATTTTCTAAGCTTAAAAGTAGCTA 467
S.olivacea               ATCAAACAATAGGGTTGAG---TTGAAAAAATTAGTGTTTTCTAGGCTTAAAAGTAGCTA 470
B.mandarina              TTTAAGGGATAATCTTTAAA--TTAAATTTTTATAATAATTTTATTAAATGAAAAATTTA 483
B.mori                   TTTAAGGGATAATCTTTAAA--TTAAAATTTTATAATAATTTTATTAAAAAAGAAATTTA 482
An.gambiae               TTTAAGGGATGAGCTTTAAA--ATAAAATTTTATATTTTTTATAATTTTTAAATAAAT-A 468
An.quadrimaculatus       TTTAAGGGATTAGCTTTAAA--ATAAATTTTTATATTTTTAGTAATTTTAAAATAAAT-A 466
Ap.mellifera             TTTATGGGATAATCTATAAA--TTTAAGATAAAAATTTAATTTTAATTTTTAATG--TTA 510
D.melanogaster           TTTATGGGATAAGCTATAAA--ATAAATTTTTAAAAATATTAAATAGATTTAATAAAT-A 467
D.yakuba                 TTTATGGGATAAGCTATAAA--ATAAATTTTTAAAAATAATAAATAAATTTAATAAAT-A 466
                          *
Escherichia coli         ------------------------------------------------------->AT-G
                                                                               [----
                         <------------------------ Domain II------------------------>
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Figure 17 (continuted)

```
M.assamense (Kc01)       TCTTAGTATAACGTTAAAGT-AATATTGAATTTT--TTTTATATTTATAAAATA--CTTG 502
M.assamense (Kc02)       TCTTAGTATAACGTTAAAGT-AATATTGAATTTT--TTTTATATTTATAAAATA--CTTG 502
M.lanchesteri (Rb01)     TCTTAGTATAACGTTAAAGT-AATATTGAATTTT--TTTTATATTTATAAAATA--CTTG 502
M.lanchesteri (Np01)     TCTTAGTATAACGTTAAAGT-AATATTGAATTTT--TTTTATATTTATAAAATA--CTTG 502
M.lanchesteri (Rb02)     TCTTAGTATAACGTTAAAGT-AATATTGAATTTT--TTTTATATTTATAAAATA--CTTG 502
M.lanchesteri (Np02)     TCTTAGTATAACGTTAAAGT-AATATTGAATTTT--TTTTATATTTATAAAATA--CTTG 502
M.lanchesteri (Ay03)     TCTTAGTATAACGTTAAAGT-AATATTGAATTTT--TTTTATATTTATAAAATA--CTTG 503
M.lanchesteri (Cs01)     TCTTAGTATAACGTTAAAGT-AATATTGAATTTT--TTTTATATTTATAAAATA--CTTG 501
M.tratense (Kc01)        TCTTAGTATAACGTTAAAGT-AATAACGATTTGTATTTATAT--TTTTAAAGTG--TCTT 501
M.tratense (Ut01)        TCTTAGTATAACGTTAAAGT-AATAACGATTTGTATTTATAT--TTTTAAAGTG--TCTT 503
M.forcipatum (Tk01)      TCTTGATATAACGTTAAAGT-AATGTCGG-TTGT-TATTTTTATTTTTAAAATG--TTTG 506
M.hirsutimanus (Rb01)    TCTTAATATAACGTTAAAGT-AATGTCGAATTGT-TGTTTATTTTTTTAAAATG--TTTG 507
M.hirsutimanus (Rb02)    TCTTAATATAACGTTAAAGT-AATGTCGAATTGT-TGTTTATTTTTTTAAAATG--TTTG 507
M.niphanae (Kc01)        TCTTAGTATAACGTTAAAGT-AATATTGGTTGTT-AGTTTTTATTTTTAAATTG--TTTG 508
M.niphanae (Np01)        TCTTAGTATAACGTTAAAGT-AATATCGGTTGTT-GGTTTTTATTTTTAAATTG--TTTG 508
M.niphanae (Np02)        TCTTAGTATAACGTTAAAGT-AATATCGGTTGTT-GGTTCTTATTTTTAAATTG--TTTG 508
M.sintangense (Cb02)     TCTTAGTATAACGTTAAAGT-AATAATGATTTATATGTATATATTTTTAAAGTG--TCTT 505
M.sintangense (Cb01)     TCTTAGTATAACGTTAAAGT-AATAATGATTTATATGTATATATTTTTAAAGTG--TCTT 500
M.hirsutimanus (Sb01)    TCTTAATATAACGTTAAAGT-AATGTCGAATTGT-TGTTTATTTTTTTAAAATG--TTTG 507
M.hirsutimanus (Sb02)    TCTTAATATAACGTTAAAGT-AATGTCGAATTGT-TGTTTATTTTTTTAAAATG--TTTG 507
M.hirsutimanus (Sb03)    TCTTAATATAACGTTAAAGT-AATGTCGAATTGT-TGTTTATTTTTTTAAAATG--TTTG 507
M.dienbienphuense(Ny01)  TCTTGATATAACGTTAAAGT-AATGTCGG-TTGT-TATTTTTATTTTTAAAATG--TTTG 506
M.hirsutimanus (Ny01)    TCTTAATATAACGTTAAAGT-AATGTCGAATTGT-TGTTTATTTTTTTAAAATG--TTTG 507
M.hirsutimanus (Ny02)    TCTTAATATAACGTTAAAGT-AATGTCGAATTGT-TGTTTATTTTTTTAAAATG--TTTG 507
M.sp3 (Np02)             TCTTAGTATAACGTTAAAGT-AATATCGGTTGTT-GGTTCTTATTTTTAAATTG--TTTG 510
M.sp3 (Np01)             TCTTAGTATAACGTTAAAGT-AATATCGGTTGTT-GGTTCTTATTTTTAAATTG--TTTG 510
M.niphanae (Ny01)        TCTTAGTATAACGTTAAAGT-AATATTGGTTGTT-AGTTTTTATTTTTAAATTG--TTTG 508
M.niphanae (Ny04)        TCTTAGTATAACGTTAAAGT-AATATCGGTTGTT-GGTTCTTATTTTTAAATTG--TTTG 505
M.niphanae (Ny03)        TCTTATTATAACGTTAAAGT-AATATTGGTTGTT-AGTTTTTATTTTTAAATTG--TTTG 508
M.niphanae (Ny02)        TCTTAGTATAACGTTAAAGT-AATATTGGTTGTT-AGTTTTTATTTTTAAATTG--TTTG 508
M.dienbienphuense(Ny02)  TCTTGATATAACGTTAAAGT-AATGTCGG-TTGT-TATTTTTATTTTTAAAATG--TTTG 506
M.rosenbergii(NC_006880)TCTTGGTATAACGTTAGAGT-AATGTTGAGGTTT--TTTTATTTTTATAAGGTG--TTTG 503
M.rosenbergii (Np01)     TCTTGGTATAACGTTAGAGT-AATATTGAGGTTT--TTTTATTTTTATAAGGTG--TTTG 504
P.monodon                TATTAATAAAACGTTCTAGTTAATATAATTTTTCTTTATTATTTTAATAAAATGAATCTA 543
S.serrata                TGAAATTAAAGTGTTCTAATTTAGATTTAAAATAAAACAACAATTTATTATTCC--TTTA 527
S.tranquebarica          TGAAATTAAAGTGTTCTAATTTAGATTTAAAA--AAATAACGATTTATTATATC--TTTG 523
S.olivacea               TGAAATTAAAGTGTTCTAATTTAGATCTAAGA--GAATAATAATTTATCAGGTC--TTT- 525
B.mandarina              TATAATTTATATTGTTAATA-AATTTTA-TTTTA---TTATAAATAATT--TTA--TTAA 534
B.mori                   TATAATTTATATTGTTAATA-AATTTTA-TTTTA---TTATAAATAATT--TTA--CTAA 533
An.gambiae               TAAGCTTAAAAATAGCTATT-ATTAATAAATTTG---TTATAATTTATT--TTT--TATA 520
An.quadrimaculatus       TAAGCTTAAAAATAGCTATT-ATTGATAATTTTG---TTATAATTTATT--TTT--TAAA 518
Ap.mellifera             TAGAGATAATATTTTTCTTT-AATTTTAATAATT---TAATAATTTAATATTTA--TAAA 564
D.melanogaster           TATGCTTAGAATTAGCAATT-ATTAAAAAATGTG---TTATAATTTATT--TTA--TAAA 519
D.yakuba                 TATGCTTAGAATTAGCAATT-ATTAAAAAATGTG---TTATAATTTATT--TTA--TAAA 518
                         *     *                                     *
Escherichia coli         TTGGC TTAGAAGCA GCCATC-ATTTAAAGAAAGCG--TAATAGCTCA<------------
                         --------------- GTPase Center------------------]
                         <------------------------ Domain II---------------------- >
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Figure 17 (continuted)

```
M.assamense (Kc01)      CTTTT-ATTAGCGAAAAATGTTATTTATTGTTTATACAACAGTTAAAATGTTTATATAAG 561
M.assamense (Kc02)      CTTTT-ATTAGCGAAAAATGTTATTTATTGTTTATACAACAGTTAAAATGTTTATATAAG 561
M.lanchesteri (Rb01)    CTTTT-ATTAGCGAAAAATGTTATTTATTGTTTATACAACAGTTAAAATGTTTATATAAG 561
M.lanchesteri (Np01)    CTTTT-ATTAGCGAAAAATGTTATTTATTGTTTATACAACAGTTAAAATGTTTATATAAG 561
M.lanchesteri (Rb02)    CTTTT-ATTAGCGAAAAATGTTATTTATTGTTTATACAACAGTTAAAATGTTTATATAAG 561
M.lanchesteri (Np02)    CTTTT-ATTAGCGAAAAATGTTATTTATTGTTTATACAACAGTTAAAATGTTTATATAAG 561
M.lanchesteri (Ay03)    CTTTT-ATTAGCGAAAAATGTTATTTATTGTTTATACAACAGTTAAAATGTTTATATAAG 562
M.lanchesteri (Cs01)    CTTTT-ATTAGCGAAAAATGTTATTTATTGTTTATACAACAGTTAAAATGTTTATATAAG 560
M.tratense (Kc01)       GCTATTTTTAGTAAAAAATTTCATTTATTATTTAGTTGGAAGATATAGTGTTTATATAAG 561
M.tratense (Ut01)       GCTATTTTTAGTAAAAAATTTCATTTATTATTTAGTTGGAAGATATAGTGTTTATATAAG 563
M.forcipatum (Tk01)     TTGTTATCTAGTAAGGAATTTTGTTTATTGTTTAAATGAAAGATAAAATGTTTATATAAG 566
M.hirsutimanus (Rb01)   TTACTGTCTAGTAGGGAATTTTATCTATTGTTTAAATAAAAGATAAAGTGTTTGTATAAG 567
M.hirsutimanus (Rb02)   TTACTGTCTAGTAGGGAATTTTATCTATTGTTTAAATAAAAGATAAAGTGTTTGTATAAG 567
M.niphanae (Kc01)       TTGAT-TTTAGCGAAGAGTTTTATTTACTGTTTAAGTGAAAGATAAAATGTTTATATAAG 567
M.niphanae (Np01)       TTGAT-TTTAGCGAAGAGTTTTATTTACTGTTTAAGTGAAAGATAAAATGTTTATATAAG 567
M.niphanae (Np02)       TTGAT-TTTAGCGAAGAGTTTTATTTACTGTTTAAGTGAAAGATAAAATGTTTATATAAG 567
M.sintangense (Cb02)    GCTATTTTTAGTAAAAAGTTTCATTTATTGTTAAGGTGGAAGATATAGTGTTTATATAAG 565
M.sintangense (Cb01)    GCTATTTTTAGTAAAAAGTTTCATTTATTGTTAAGGTGGAAGATATAGTGTTTATATAAG 560
M.hirsutimanus (Sb01)   TTACTGTCTAGTAGGGAATTTTATCTATTGTTTAAATAAAAGATAAAGTGTTTGTATAAG 567
M.hirsutimanus (Sb02)   TTACTGTCTAGTAGGGAATTTTATCTATTGTTTAAATAAAAGATAAAGTGTTTGTATAAG 567
M.hirsutimanus (Sb03)   TTACTGTCTAGTAGGGAATTTTATCTATTGTTTAAATAAAAGATAAAGTGTTTGTATAAG 567
M.dienbienphuense(Ny01) TTGTTATCTAGTAAGGAATTTTATTTATTGTCTAAATGAAAGATAAAATGTTTATATAAG 566
M.hirsutimanus (Ny01)   TTACTGTCTAGTAGGGAATTTTATCTATTGTTTAAATAAAAGATAAAGTGTTTGTATAAG 567
M.hirsutimanus (Ny02)   TTACTGTCTAGTAGGGAATTTTATCTATTGTTTAAATAAAAGATAAAGTGTTTGTATAAG 567
M.sp3 (Np02)            TTGAT-TTTAGCGAAGAGTTTTATTTATTGTTTAAGTGAAAGATAAAATGTTTATATAAG 569
M.sp3 (Np01)            TTGAT-TTTAGCGAAGAGTTTTATTTACTGTTTAAGTGAAAGATAAAATGTTTATATAAG 569
M.niphanae (Ny01)       TTGAT-TTTAACGAAGAGTTTTATTTACTGTTTAAGTGAAAGATAAAATGTTTATATAAG 567
M.niphanae (Ny04)       TTGAT-TTTAGCGAAGAGTTTTATTTACTGTTTAAGTGAAAGATAAAATGTTTATATAAG 564
M.niphanae (Ny03)       TTGAT-TTTAACGAAGAGTTTTATTTACTGTTTAAGTGAAAGATAAAATGTTTATATAAG 567
M.niphanae (Ny02)       TTGAT-TTTAACGAAGAGTTTTATTTACTGTTTAAGTGAAAGATAAAATGTTTATATAAG 567
M.dienbienphuense(Ny02) TTGTTATCTAGTAAGGAATTTTATTTATTGTCTAAATGAAAGATAAAATGTTTATATAAG 566
M.rosenbergii(NC_006880)CTTTT-ATTAGCGGGAAATGCCGTTTATTGATTAAGCGGCAGTTAAAATGTTTATATAAG 562
M.rosenbergii (Np01)    CTTTT-ATTAGCGGGAAATGCCGTTTATTGATTAAGCGGCAGTTAAAATGTTTATATAAG 563
P.monodon               ACTTGTAGTAGCCAATATTTATGTTAAGTAAAATACCATAAGTTATAATGATTTTATTAG 603
S.serrata               GTAAA--GTACTAAAATATTAAATTTAATATTAAATTTTAAGAAGAAATGATTATATAAG 585
S.tranquebarica         GTAAT--GTACTAAAATATTAAATTCAATATTAAATTTTAAGAAAAAATGATTGTATAAG 581
S.olivacea              GTAAA--GTACTAAAATATTAAATTTAATATTAAATTTTAAGAAAAAATGATTTTATAAG 583
B.mandarina             TAAA--AAAATTTAA--TTTTA-TTTAATTTTTTATATAAAAATAAA---TTTTAATTTA 586
B.mori                  TAGA--AAAATTTAA--TTTTA-TTTAATTTTTTATATAAAAATAAA---TTTTAATTTA 585
An.gambiae              AAAA--ATTATTTAA--TTTAAATTAAATTATTTATTAAAATTTAAA---TTTTAATAAT 573
An.quadrimaculatus      TAAA--ATTATTTAG--TTTAAATTAAATTTTTTATTAAAATTTAAA---TTTTAATTAA 571
Ap.mellifera            TTAATAATGATTTAAATTTATATTTTATTTTTCTATAAAAATTTATA---TTTTAATTAA 621
D.melanogaster          TTAA--ATTATTTA---TTAAATTTTAATTATTTATTAAAATGTTAG---TTTTAATTAT 571
D.yakuba                TTAA--ATTATTTA---TTAAATTTTAATTATTTATTAAAATATTAA---TTTTAATATT 570
                                 *        *    *  * *           *          **  **
Escherichia coli        ---------------------------1104-1247-------------------------

                       <-------------------------- Domain II------------------------>
```

Figure 17 (continuted)

```
M.assamense (Kc01)      TAGAATTTTAAGT--TATTATTAATTT---TG-----AATTATTA--TTTATTTTTTAAA 609
M.assamense (Kc02)      TAGAATTTTAAGT--TGTTATTAATTT---TG-----AATTATTA--TTTATTTTTTAAA 609
M.lanchesteri (Rb01)    TAGAATTTTAAGT--TGTTATTAATTT---TG-----AATTATTA--TTTATTTTTTAAA 609
M.lanchesteri (Np01)    TAGAATTTTAAGT--TGTTATTAATTT---TG-----AATTATTA--TTTATTTTTTAAA 609
M.lanchesteri (Rb02)    TAGAATTTTAAGT--TGTTATTAATTT---TG-----AATTATTA--TTTATTTTTTAAA 609
M.lanchesteri (Np02)    TAGAATTTTAAGT--TGTTATTAATTT---TG-----AATTATTA--TTTATTTTTTAAA 609
M.lanchesteri (Ay03)    TAGAATTTTAAGT--TGTTATTAATTT---TG-----AATTATTA--TTTATTTTTTAAA 610
M.lanchesteri (Cs01)    TAGAATTTTAAGT--TGTTATTAATTT---TG-----AATTATTA--TTTATTTTTTAAA 608
M.tratense (Kc01)       TATAGTTAAAGTT--TTTTTA-AAATT---TT-----GAGTGAA---GAAATTTTTTAAG 607
M.tratense (Ut01)       TATAGTTGAAGTT--TTTTTA-AAATT---TT-----GAGTGAA---GAAATTTTTTAAG 609
M.forcipatum (Tk01)     TATGATTAAAGTT--TGTTTTTACGT----TT-----GAGTGAAA--TTTAGTGTTTAAT 613
M.hirsutimanus (Rb01)   TATAATTAAAGTT--TGTTTTTATGT----TT-----AAGTGAAA--TTAAGTATTTAGT 614
M.hirsutimanus (Rb02)   TATAATTAAAGTT--TGTTTTTATGT----TT-----AAGTGAAA--TTAAGTATTTAGT 614
M.niphanae (Kc01)       TATAATTAAAGTT--TGTTTTTAGTT----TT-----AAGTGAG---TTGGTTATTTAAT 613
M.niphanae (Np01)       TATAATTAAAGTT--TGTTTTTAATT----TT-----AAGTGAG---TTGGTTATTTAAT 613
M.niphanae (Np02)       TATAATTAAAGTT--TGTTTTTAATT----TT-----AAGTGAG---TTGGTTATTTAAT 613
M.sintangense (Cb02)    TATGATTGAAGTT--TTTTTTTAAATC---TT-----GAGTGGA---AAAGGTTTTTAAG 612
M.sintangense (Cb01)    TATGATTGAAGTT--TTTTTTTAAATC---TT-----GAGTGGA---AAAGGTTTTTAAG 607
M.hirsutimanus (Sb01)   TATAATTAAAGTT--CGTTTTTATGT----TT-----AAGTGAAA--TTAAGTATTTAGT 614
M.hirsutimanus (Sb02)   TATAATTAAAGTT--CGTTTTTATGT----TT-----AAGTGAAA--TTAAGTATTTAGT 614
M.hirsutimanus (Sb03)   TATAATTAAAGTT--CGTTTTTATGT----TT-----AAGTGAAA--TTAAGTATTTAGT 614
M.dienbienphuense(Ny01) TATGATTATAGTT--TGTTTTTATGT----TT-----GAGTAAAA--TTTAGTGTTTAAT 613
M.hirsutimanus (Ny01)   TATAATTAAAGTT--CGTTTTTATGT----TT-----AAGTGAAA--TTAAGTATTTAGT 614
M.hirsutimanus (Ny02)   TATAATTAAAGTT--CGTTTTTATGT----TT-----AAGTGAAA--TTAAGTATTTAGT 614
M.sp3 (Np02)            TATAATTAAAGTT--TGTTTTTAATT----TT-----AAGTGAG---TTGGTTATTTAAT 615
M.sp3 (Np01)            TATAATTAAAGTT--TGTTTTTAATT----TT-----AAGTGAG---TTGGTTATTTAAT 615
M.niphanae (Ny01)       TATAATTAAAGTT--TGTTTTTAGTT----TT-----AAGTGAG---TTGGTTATTTAAT 613
M.niphanae (Ny04)       TATAATTAAAGTT--TGTTTTTAATT----TT-----AAGTGAG---TTGGTTATTTAAT 610
M.niphanae (Ny03)       TATAATTAAAGTT--TGTTTTTAGTT----TT-----AAGTGAG---TTGGTTGTTTAAT 613
M.niphanae (Ny02)       TATAATTAAAGTT--TGTTTTTAGTT----TT-----AAGTGAG---TTGGTTATTTAAT 613
M.dienbienphuense(Ny02) TATGATTATAGTT--TGTTTTTATGT----TT-----GAGTAAAA--TTTAGTGTTTAAT 613
M.rosenbergii(NC_006880)TAGAATTG-AAGT--TAATTTTTATTT---TA-----GAGTGTTA--TTTGATTTTTAAG 609
M.rosenbergii (Np01)    TAGAATTG-AAGT--TAATTTTTATTT---TA-----GAGTGTTA--TTTGTTTTTTAAG 610
P.monodon               TAGAAATACATTTG-TGTAAATAGATAACATT-----AAATAATC--TATATATTTTTAT 655
S.serrata               TAAAATTTAAATTTGTAGAAATAATTTTTTTACAAAAAAATAAAATATTAGAAATTAAA- 644
S.tranquebarica         TAAAATT-AAGTTTGTA-AAATAATTTTTTCT----AAAATGAAATATTAGAAATTAAAA 635
S.olivacea              TAAAGTATTAATTTGTATAAG--GTTTCTCT-----AAAATAAAATAATAAAGAT--AAA 634
B.mandarina             ATAAATTAAATTA--TAATGATAAAATTAGTAAAATTTA---ATTAATAAAAATATTAAT 641
B.mori                  ATAAATTAAATTA--TAATGATAAAATTAGTAAAATTTATTAATTAATAAAATTATTAAT 643
An.gambiae              AAAAATTTAGTAA--TTATGATAAAATTAGTA-----TATAAATTTATATAA--AGTAAT 624
An.quadrimaculatus      AAAAATTTATTAA--TTATGATAAAATTAGTA-----TATTAGTTTATAAAA--TGTAAT 622
Ap.mellifera            TAAAATATAGAAA--TAGTGATTAGATTAGTAATAA-AGTTATATTTTATAA-TGATATT 677
D.melanogaster          TAAAATTAAGTAA--TAATGATAAAATTAGTA-----TATAATAATGTTAAA--ATTAAT 622
D.yakuba                TAAAATTAAGTAA--TAATGATAGAATTAGTA-----TATAATATTGTTAAA--ATAAAT 621

Escherichia coli       --------->GTGC--GAATGCTGACATAAGTA-----ACGATAA <----1276-1647--

                      <----- Domain II---------><------------- Domain III---------->
```

Figure 18 Alignment of *16S rRNA* of *Macrobrachium* spp. compared with other arthropods and *E. coli*; Domain III.

```
M.assamense (Kc01)      AATTTAT---GGAGAGAAGTTAAAAAGGAACTCGGCAAAT--TTTATCCCCGCCTGTTTA 664
M.assamense (Kc02)      AATTTAT---GGAGAGAAGTTAAAAAGGAACTCGGCAAAT--TTTATCCCCGCCTGTTTA 664
M.lanchesteri (Rb01)    AATTTAT---GGAGAGAAGTTAAAAAGGAACTCGGCAAAT--TTTATCCCCGCCTGTTTA 664
M.lanchesteri (Np01)    AATTTAT---GGAGAGAAGTTAAAAAGGAACTCGGCAAAT--TTTATCCCCGCCTGTTTA 664
M.lanchesteri (Rb02)    AATTTAT---GGAGAGAAGTTAAAAAGGAACTCGGCAAAT--TTTATCCCCGCCTGTTTA 664
M.lanchesteri (Np02)    AATTTAT---GGAGAGAAGTTAAAAAGGAACTCGGCAAAT--TTTATCCCCGCCTGTTTA 664
M.lanchesteri (Ay03)    AATTTAT---GGAGAGAAGTTAAAAAGGAACTCGGCAAAT--TTTATCCCCGCCTGTTTA 665
M.lanchesteri (Cs01)    AATTTAT---GGAGAGAAGTTAAAAAGGAACTCGGCAAAT--TTTATCCCCGCCTGTTTA 663
M.tratense (Kc01)       TGTT-------AAGAGTCGTTTAAAAGGAACTCGGCAAAT--TTTATCTCCGCCTGTTTA 658
M.tratense (Ut01)       TGTT-------AAGAGTCGTTTAAAAGGAACTCGGCAAAT--TTTATCTCCGCCTGTTTA 660
M.forcipatum (Tk01)     GGTC-------GAGGGGTGCTAAGAAGGAATTCGGCAAAT--ATTATCTCCGCCTGTTTA 664
M.hirsutimanus (Rb01)   GGAC-------GAGAGGTGCATGGAAGGAATTCGGCAAAT--ACTACCTCCGCCTGTTTA 665
M.hirsutimanus (Rb02)   GGAC-------GAGAGGTGCATGGAAGGAATTCGGCAAAT--ACTACCTCCGCCTGTTTA 665
M.niphanae (Kc01)       ATTT-------GTTTGGTGTTTAAAAGGAACTCGGCAAAT--TTTGCCTCCGCCTGTTTA 664
M.niphanae (Np01)       ATTT-------GTTTGGTGTTTAAAAGGAACTCGGCAAAT--TTTGCCTCCGCCTGTTTA 664
M.niphanae (Np02)       ATTT-------GTTTGGTGTTTAAAAGGAACTCGGCAAAT--TTTGCCTCCGCCTGTTTA 664
M.sintangense (Cb02)    TATT-------AAAAGTCGTTTAAAAGGAACTCGGCAAAT--TTTATCTCCGCCTGTTTA 663
M.sintangense (Cb01)    TATT-------AAAAGTCGTTTAAAAGGAACTCGGCAAAT--TTTATCTCCGCCTGTTTA 658
M.hirsutimanus (Sb01)   GGAC-------GAGAGGTGCCTGGAAGGAATTCGGCAAAT--ACTACCTCCGCCTGTTTA 665
M.hirsutimanus (Sb02)   GGAC-------GAGAGGTGCCTGGAAGGAATTCGGCAAAT--ACTACCTCCGCCTGTTTA 665
M.hirsutimanus (Sb03)   GGAC-------GAGAGGTGCCTGGAAGGAATTCGGCAAAT--ACTACCTCCGCCTGTTTA 665
M.dienbienphuense(Ny01) AGCC-------GAGGGGTGCTAAGAAGGAATTCGGCAAAT--ATTATCTCCGCCTGTTTA 664
M.hirsutimanus (Ny01)   GGAC-------GAGAGGTGCATGGAAGGAATTCGGCAAAT--ACTACCTCCGCCTGTTTA 665
M.hirsutimanus (Ny02)   GGAC-------GAGAGGTGCATGGAAGGAATTCGGCAAAT--ACTACCTCCGCCTGTTTA 665
M.sp3 (Np02)            ATTT-------GTTTGGTGTTTAAAAGGAACTCGGCAAAT--TTTGCCTCCGCCTGTTTA 666
M.sp3 (Np01)            ATTT-------GTTTGGTGTTTAAAAGGAACTCGGCAAAT--TTTGCCTCCGCCTGTTTA 666
M.niphanae (Ny01)       ATTT-------GTTTGGTGTTTAAAAGGAACTCGGCAAAT--TTTGCCTCCGCCTGTTTA 664
M.niphanae (Ny04)       ATTT-------GTTTGGTGTTTAAAAGGAACTCGGCAAAT--TTTGCCTCCGCCTGTTTA 661
M.niphanae (Ny03)       ATTT-------GTTTGGTGTTTAAAAGGAACTCGGCAAAT--TTTGCCTCCGCCTGTTTA 664
M.niphanae (Ny02)       ATTT-------GTTTGGTGTTTAAAAGGAACTCGGCAAAT--TTTGCCTCCGCCTGTTTA 664
M.dienbienphuense(Ny02) AGCC-------GAGGGGTGCTAAGAAGGAATTCGGCAAAT--ATTATCTCCGCCTGTTTA 664
M.rosenbergii(NC_006880)TAGTTT-----GAGAAGCGCTTAGAAGGAACTCGGCAAAT--TTTGTCCCCGCCTGTTTA 662
M.rosenbergii (Np01)    TAGTTT-----GAGAGGCGCTTAGAAGGAACTCGGCAAAT--TTTGTCCCCGCCTGTTTA 663
P.monodon               TTTT-------TTTAGGTATAATTAAGGAACTCGGCAAAT--ACTACTTTTGCCTGTTTA 706
S.serrata               TTATAAATTTATAATGAAATAAAAAAGGAACTCGGCAAAA--AAATTTCTTGCCTGTTTA 702
S.tranquebarica         TTATAAATTTGTTATGGAATAAAAAAGGAACTCGGCAAAA--AAAATTCTTGCCTGTTTA 693
S.olivacea              TTATATATAAATTATGGAACAGAAAAGGAACTCGGCAAAT--AAATTTCTTGCCTGTTTA 692
B.mandarina             TTATTTG----ATTAATTATAAAAAAGGAATTCGGCAAAT-ATTTATATTCACTTGTTTA 696
B.mori                  TTATTTA----ATTAATTATAAAAA-GGAATTCGGCAAAT-ATTTA-ATTC-CTTGTTTA 695
An.gambiae              TAATTTG----ATAGGTTTTAATGA-AGAATTCGGCAAATTAAATATATTCACCTGTTTA 679
An.quadrimaculatus      TTATTTG----ATAGGTTTTAATGA-AGAATTCGGCAAATTAAATATATTCACCTGTTTA 677
Ap.mellifera            TTGTATT----ATATAATTTAAAGG---AATTCGGCAAAT--ATATTATTCACCTGTTTA 728
D.melanogaster          TTATATG----AAAAGTTTAAATAA-AGAATTCGGCAAA--AATAATATTCGCCTGTTTA 675
D.yakuba                TTTTATG----AAAAGTTTAAATAA-AGAATTCGGCAAA--AATAATGTTCGCCTGTTTA 674
                                                    ** ********             * ******
Escherichia coli        -->TGAG----AGAACTCGGGTGAA-GGAACTAGGCAAAATG<-----------------

                       <------------------------- Domain IV---------------------- >
```

Figure 19 Alignment of *16S rRNA* of *Macrobrachium* spp. compared with other arthropods and *E. coli*; Domain IV.

```
M.assamense (Kc01)      TCAAAAACATGTC-TGTGTGAATTAATTATAAAGTCTAGCCTGCCCACTGATTTA----T 719
M.assamense (Kc02)      TCAAAAACATGTC-TGTGTGAATTAATTATAAAGTCTAGCCTGCCCACTGATTTA----T 719
M.lanchesteri (Rb01)    TCAAAAACATGTC-TGTGTGAATTAATTATAAAGTCTAGCCTGCCCACTGATTTA----T 719
M.lanchesteri (Np01)    TCAAAAACATGTC-TGTGTGAATTAATTATAAAGTCTAGCCTGCCCACTGATTTA----T 719
M.lanchesteri (Rb02)    TCAAAAACATGTC-TGTGTGAATTAATTATAAAGTCTAGCCTGCCCACTGATTTA----T 719
M.lanchesteri (Np02)    TCAAAAACATGTC-TGTGTGAATTAATTATAAAGTCTAGCCTGCCCACTGATTTA----T 719
M.lanchesteri (Ay03)    TCAAAAACATGTC-TGTGTGAATTAATTATAAAGTCTAGCCTGCCCACTGATTTA----T 720
M.lanchesteri (Cs01)    TCAAAAACATGTC-TGTGTGAATTAATTATAAAGTCTAGCCTGCCCACTGATTTA----T 718
M.tratense (Kc01)       TCAAAAACATGTC-TGTGTGTGTGAGTTATAAAGTCTAGCCTGCCCACTGATTAA----C 713
M.tratense (Ut01)       TCAAAAACATGTC-TGTGTGTGTGAGTTATAAAGTCTAGCCTGCCCACTGATTAA----C 715
M.forcipatum (Tk01)     TCAAAAACATGTC-TGTGTGAATTAGTTATAAAGTCTAGCCTGCCCACTGATTG-----T 718
M.hirsutimanus (Rb01)   TCAAAAACATGTC-TGTGTGAATTAGTTATAAAGTCTAGCCTGCCCACTGATTG-----T 719
M.hirsutimanus (Rb02)   TCAAAAACATGTC-TGTGTGAATTAGTTATAAAGTCTAGCCTGCCCACTGATTG-----T 719
M.niphanae (Kc01)       TCAAAAACATGTC-TGTGTGAATTAGTTATAAAGTCTAGCCTGCCCACTGATTA-----T 718
M.niphanae (Np01)       TCAAAAACATGTC-TGTGTGAATTAGTTATAAAGTCTAGCCTGCCCACTGATTA-----T 718
M.niphanae (Np02)       TCAAAAACATGTC-TGTGTGAATTAGTTATAAAGTCTAGCCTGCCCACTGATTA-----T 718
M.sintangense (Cb02)    TCAAAAACATGTC-TGTGTGAATTAGTTATAAAGTCTAGCCTGCCCACTGATTGA----T 718
M.sintangense (Cb01)    TCAAAAACATGTC-TGTGTGAATTAGTTATAAAGTCTAGCCTGCCCACTGATTGA----T 713
M.hirsutimanus (Sb01)   TCAAAAACATGTC-TGTGTGAATTAGTTATAAAGTCTAGCCTGCCCACTGATTG-----T 719
M.hirsutimanus (Sb02)   TCAAAAACATGTC-TGTGTGAATTAGTTATAAAGTCTAGCCTGCCCACTGATTG-----T 719
M.hirsutimanus (Sb03)   TCAAAAACATGTC-TGTGTGAATTAGTTATAAAGTCTAGCCTGCCCACTGATTG-----T 719
M.dienbienphuense(Ny01) TCAAAAACATGTC-TGTGTGAATTAGTTATAAAGTCTAGCCTGCCCACTGATTG-----T 718
M.hirsutimanus (Ny01)   TCAAAAACATGTC-TGTGTGAATTAGTTATAAAGTCTAGCCTGCCCACTGATTG-----T 719
M.hirsutimanus (Ny02)   TCAAAAACATGTC-TGTGTGAATTAGTTATAAAGTCTAGCCTGCCCACTGATTG-----T 719
M.sp3 (Np02)            TCAAAAACATGTC-TGTGTGAATTAGTTATAAAGTCTAGCCTGCCCACTGATTA-----T 720
M.sp3 (Np01)            TCAAAAACATGTC-TGTGTGAATTAGTTATAAAGTCTAGCCTGCCCACTGATTA-----T 720
M.niphanae (Ny01)       TCAAAAACATGTC-TGTGTGAATTAGTTATAAAGTCTAGCCTGCCCACTGATTA-----T 718
M.niphanae (Ny04)       TCAAAAACATGTC-TGTGTGAATTAGTTATAAAGTCTAGCCTGCCCACTGATTA-----T 715
M.niphanae (Ny03)       TCAAAAACATGTC-TGTGTGAATTAGTTATAAAGTCTAGCCTGCCCACTGATTA-----T 718
M.niphanae (Ny02)       TCAAAAACATGTC-TGTGTGAATTAGTTATAAAGTCTAGCCTGCCCACTGATTA-----T 718
M.dienbienphuense(Ny02) TCAAAAACATGTC-TGTGTGAATTAGTTATAAAGTCTAGCCTGCCCACTGATTG-----T 718
M.rosenbergii(NC_006880)TCAAAAACATGTCCTGTGTGAATTAGTTATAAAGTCTAGCCTGCCCACTGATTTA----T 718
M.rosenbergii (Np01)    TCAAAAACATGTC-TGTGTGAATTAGTTATAAAGTCTAGCCTGCCCACTGATTTA----T 718
P.monodon               TCAAAAACATGTC-TATATGATTGTTATATAAAGTCTAGCCTGCCCACTGAATTATT--T 763
S.serrata               TCAAAAACATGTC-TATTTGAGGTTATT-TATAGTCTAGCCTGCTCACTGACAAAAAGTG 760
S.tranquebarica         TCAAAAACATGTC-TATTTGAAATTATA-TATAGTCTAGCCTGCTCACTGACAAAGA-TG 750
S.olivacea              TCAAAAACATGTC-TATTAGAAGGTGTT-TATAGTCTAGCCTGCTCACTGACAAGAA-TG 749
B.mandarina             TCAAAAACATGTC-TTTTTGTTAATAATTTAAAGTCTAATCTGCCCACTGATATA----T 751
B.mori                  TCAAAAACATGTC-TTTTTGTTAATAATTTAAAGTCTAATCTGCCCACTGATATA----T 750
An.gambiae              ACAAAAACATGTC-TTTTTGTATTTTATTTAAAGTCTAGCCTGCCCACTGAGT------T 732
An.quadrimaculatus      ACAAAAACATGTC-TTTTTGTATTTTATTTAAAGTCTAGCCTGCCCACTGAAA------T 730
Ap.mellifera            TCAAAAACATGTC-CTGTTGATTATAATTTTAGGTC-GATCTGCTCAATGAATTA----T 782
D.melanogaster          ACAAAAACATGTC-TTTTTGAATTATATATAAAGTCTAACCTGCCCACTGAAAAT----T 730
D.yakuba                ACAAAAACATGTC-TTTTTGAATTATATATAAAGTCTAACCTGCCCACTGAAAAT----T 729
                         ************      *         *   ***    **** ** ***
Escherichia coli        -------------------------1682-2009--------------------------

                       <-------------------------- Domain IV------------------------>
```

Figure 19 (continuted)

```
M.assamense (Kc01)      T-----A 719CCGCGGTAATTTGACCGTGCGAAGGT-AGCÿÿCATGTCTAGTCTTTTAA 775
M.assamense (Kc02)      TTA---AAGGGCCGCGGTAATTTGACCGTGCGAAGGT-AGCATAATCAGTAGTCTTTTAA 775
M.lanchesteri (Rb01)    TTA---AAGGGCCGCGGTAATTTGACCGTGCGAAGGT-AGCATAATCAGTAGTCTTTTAA 775
M.lanchesteri (Np01)    TTA---AAGGGCCGCGGTAATTTGACCGTGCGAAGGT-AGCATAATCAGTAGTCTTTTAA 775
M.lanchesteri (Rb02)    TTA---AAGGGCCGCGGTAATTTGACCGTGCGAAGGT-AGCATAATCAGTAGTCTTTTAA 775
M.lanchesteri (Np02)    TTA---AAGGGCCGCGGTAATTTGACCGTGCGAAGGT-AGCATAATCAGTAGTCTTTTAA 775
M.lanchesteri (Ay03)    TTA---AAGGGCCGCGGTAATTTGACCGTGCGAAGGT-AGCATAATCAGTAGTCTTTTAA 776
M.lanchesteri (Cs01)    TTA---AAGGGCCGCGGTAATTTGACCGTGCGAAGGT-AGCATAATCAGTAGTCTTTTAA 774
M.tratense (Kc01)       TTA---AAGGGCCGCGGTATTTTGACCGTGCGAAGGT-AGCATAGTCAGTAGTCTTTTAA 769
M.tratense (Ut01)       TTA---AAGGGCCGCGGTATTTTGACCGTGCGAAGGT-AGCATAGTCAGTAGTCTTTTAA 771
M.forcipatum (Tk01)     TTA---AAGGGCCGCGGTAATTTGACCGTGCGAAGGT-AGCATAGTCAGTAGTCTTTTAA 774
M.hirsutimanus (Rb01)   TTA---AAGGGCCGCGGTAATTTGACCGTGCGAAGGT-AGCATAGTCAGTAGTCTTTTAA 775
M.hirsutimanus (Rb02)   TTA---AAGGGCCGCGGTAATTTGACCGTGCGAAGGT-AGCATAGTCAGTAGTCTTTTAA 775
M.niphanae (Kc01)       TTA---AAGGGCCGCGGTAATTTGACCGTGCGAAGGT-AGCATAGTCAGTAGTCTTTTAA 774
M.niphanae (Np01)       TTA---AAGGGCCGCGGTAATTTGACCGTGCGAAGGT-AGCATAGTCAGTAGTCTTTTAA 774
M.niphanae (Np02)       TTA---AAGGGCCGCGGTAATTTGACCGTGCGAAGGT-AGCATAGTCAGTAGTCTTTTAA 774
M.sintangense (Cb02)    TTA---AAGGGCCGCGGTATTTTGACCGTGCGAAGGT-AGCATAGTCAGTAGTCTTTTAA 774
M.sintangense (Cb01)    TTA---AAGGGCCGCGGTATTTTGACCGTGCGAAGGT-AGCATAGTCAGTAGTCTTTTAA 769
M.hirsutimanus (Sb01)   TTA---AAGGGCCGCGGTAATTTGACCGTGCGAAGGT-AGCATAGTCAGTAGTCTTTTAA 775
M.hirsutimanus (Sb02)   TTA---AAGGGCCGCGGTAATTTGACCGTGCGAAGGT-AGCATAGTCAGTAGTCTTTTAA 775
M.hirsutimanus (Sb03)   TTA---AAGGGCCGCGGTAATTTGACCGTGCGAAGGT-AGCATAGTCAGTAGTCTTTTAA 775
M.dienbienphuense(Ny01) TTA---AAGGGCCGCGGTAATTTGACCGTGCGAAGGT-AGCATAGTCAGTAGTCTTTTAA 774
M.hirsutimanus (Ny01)   TTA---AAGGGCCGCGGTAATTTGACCGTGCGAAGGT-AGCATAGTCAGTAGTCTTTTAA 775
M.hirsutimanus (Ny02)   TTA---AAGGGCCGCGGTAATTTGACCGTGCGAAGGT-AGCATAGTCAGTAGTCTTTTAA 775
M.sp3 (Np02)            TTA---AAGGGCCGCGGTAATTTGACCGTGCGAAGGT-AGCATAGTCAGTAGTCTTTTAA 776
M.sp3 (Np01)            TTA---AAGGGCCGCGGTAATTTGACCGTGCGAAGGT-AGCATAGTCAGTAGTCTTTTAA 776
M.niphanae (Ny01)       TTA---AAGGGCCGCGGTAATTTGACCGTGCGAAGGT-AGCATAGTCAGTAGTCTTTTAA 774
M.niphanae (Ny04)       TTA---AAGGGCCGCGGTAATTTGACCGTGCGAAGGT-AGCATAGTCAGTAGTCTTTTAA 771
M.niphanae (Ny03)       TTA---AAGGGCCGCGGTAATTTGACCGTGCGAAGGT-AGCATAGTCAGTAGTCTTTTAA 774
M.niphanae (Ny02)       TTA---AAGGGCCGCGGTAATTTGACCGTGCGAAGGT-AGCATAGTCAGTAGTCTTTTAA 774
M.dienbienphuense(Ny02) TTA---AAGGGCCGCGGTAATTTGACCGTGCGAAGGT-AGCATAGTCAGTAGTCTTTTAA 774
M.rosenbergii(NC_006880)TTA---AAGGGCCGCGGTAATTTGACCGTGCGAAGGT-AGCATAGTCAGTAGTCTTTTAA 774
M.rosenbergii (Np01)    TTA---AAGGGCCGCGGTAATTTGACCGTGCGAAGGT-AGCATAGTCAGTAGTCTTTTAA 774
P.monodon               TTA---AAGGGCCGCGGTATACTGACCGTGCGAAGGT-AGCATAATCATTAGTCTTTTAA 819
S.serrata               TTA---AATAGCCGCGGTATTTTGACCGTGCAAAGGT-AGCATAATCATTAGTTTTTTAA 816
S.tranquebarica         TTA---AATAGCCGCGGTATTTTGACCGTGCAAAGGT-AGCATAATCATTAGTTTTTTAA 806
S.olivacea              TTA---AATAGCCGCGGTATTTTGACCGTGCAAAGGT-AGCATAATCATTAGTTTTTTAA 805
B.mandarina             TTATTAAAGGGCTGCAGTATTTTGACTGTACAAAGGT-AGCATAATCATTAGTCTTTTAA 810
B.mori                  TTATTAAAGGGCTGCAGTATTTTGACTGTACAAAGGT-AGCATAATCATTAGTCTTTTAA 809
An.gambiae              TTA---AAGGGCCGCGGTATTTTGACCGTGCGAAGGT-AGCATAATCAATAGTCTTTTAA 788
An.quadrimaculatus      TTA---AAGGGCCGCGGTATTTTGACCGTGCGAAGGT-AGCATAATCAATAGTCTTTTAA 786
Ap.mellifera            TTT--AAATAGCTGCAGTATGTTGACTGTACAAAGGT-AGCATAATAAATTGTCTTTTAA 839
D.melanogaster          TTA---AATGGCCGCAGTATTTTGACTGTGCAAAGGTTAGCTAAATCATTAGTCTTTTAA 787
D.yakuba                TTA---AATGGCCGCAGTATTTTGACTGTGCAAAGGT-AGCATAATCATTAGTCTTTTAA 785
                        **    **  ** ** ***   **** ** * ***** ***  * * * * ** ******
Escherichia coli        ------------------------1682-2009---------------------------

                       <-------------------------- Domain IV------------------------>
```

Figure 19 (continuted)

```
M.assamense (Kc01)      TTGGAGGCTTGAATGAATGGTTGGACGAGGGATAAACTGTCTCTTTTA----CGGTGTTT 831
M.assamense (Kc02)      TTGGAGGCTTGAATGAATGGTTGGACGAGGGATAAACTGTCTCTTTTA----CGGTGTTT 831
M.lanchesteri (Rb01)    TTGGAGGCTTGAATGAATGGTTGGACGAGGGATAAACTGTCTCTTTTA----CGGTGTTT 831
M.lanchesteri (Np01)    TTGGAGGCTTGAATGAATGGTTGGACGAGGGATAAACTGTCTCTTTTA----CGGTGTTT 831
M.lanchesteri (Rb02)    TTGGAGGCTTGAATGAATGGTTGGACGAGGGATAAACTGTCTCTTTTA----CGGTGTTT 831
M.lanchesteri (Np02)    TTGGAGGCTTGAATGAATGGTTGGACGAGGGATAAACTGTCTCTTTTA----CGGTGTTT 831
M.lanchesteri (Ay03)    TTGGAGGCTTGAATGAATGGTTGGACGAGGGATAAACTGTCTCTTTTA----CGGTGTTT 832
M.lanchesteri (Cs01)    TTGGAGGCTTGAATGAATGGTTGGACGAGGGATAAACTGTCTCTTTTA----CGGTGTTT 830
M.tratense (Kc01)       TTGAAGGCTTGAATGAAAGGTTGGACGAGGGATAGGCTGTCTCTTTTA----TGAGATTT 825
M.tratense (Ut01)       TTGAAGGCTTGAATGAAAGGTTGGACGAGGGATAGGCTGTCTCTTTTA----TGAGATTT 827
M.forcipatum (TK01)     TTGAAGGCTTGAATGAAGGGTTGGACGAGGGATAATCTGTCTCTTTTG----CAGTGTTT 830
M.hirsutimanus (Rb01)   TTGAAGGCTTGAATGAAGGGTTGGACGAGGGGTAAGCTGTCTCCTTTG----CAGTGTTT 831
M.hirsutimanus (Rb02)   TTGAAGGCTTGAATGAAGGGTTGGACGAGGGGTAAGCTGTCTCCTTTG----CAGTGTTT 831
M.niphanae (Kc01)       TTGAAGGCTTGAATGAAAGGTTGGACGAGGGGTAAGCTGTCTCTTTTA----CAATATTT 830
M.niphanae (Np01)       TTGAAGGCTTGAATGAAAGGTTGGACGAGGGGTAAGCTGTCTCTTTTA----CACTATTT 830
M.niphanae (Np02)       TTGAAGGCTTGAATGAAAGGTTGGACGAGGGGTAAGCTGTCTCTTTTA----CAATATTT 830
M.sintangense (Cb02)    TTGAAGGCTTGAATGAAAGGTTGGACGAGGGATAGGCTGTCTCTTTTA----TGAGGTTT 830
M.sintangense (Cb01)    TTGAAGGCTTGAATGAAAGGTTGGACGAGGGATAGGCTGTCTCTTTTA----TGAGGTTT 825
M.hirsutimanus (Sb01)   TTGAAGGCTTGAATGAAGGGTTGGACGAGGGGTAAGCTGTCTCCTTTG----CAGTGTTT 831
M.hirsutimanus (Sb02)   TTGAAGGCTTGAATGAAGGGTTGGACGAGGGGTAAGCTGTCTCCTTTG----CAGTGTTT 831
M.hirsutimanus (Sb03)   TTGAAGGCTTGAATGAAGGGTTGGACGAGGGGTAAGCTGTCTCCTTTG----CAGTGTTT 831
M.dienbienphuense(Ny01) TTGGAGGCTTGAATGAAGGGTTGGACGAGGGATAATCTGTCTCCTTTG----CAGTGTTT 830
M.hirsutimanus (Ny01)   TTGAAGGCTTGAATGAAGGGTTGGACGAGGGGTAAGCTGTCTCCTTTG----CAGTGTTT 831
M.hirsutimanus (Ny02)   TTGAAGGCTTGAATGAAGGGTTGGACGAGGGGTAAGCTGTCTCCTTTG----CAGTGTTT 831
M.sp3 (Np02)            TTGAAGGCTTGAATGAAAGGTTGGACGAGGGGTAAGCTGTCTCTTTTA----CAATATTT 832
M.sp3 (Np01)            TTGAAGGCTTGAATGAAAGGTTGGACGAGGGGTAAGCTGTCTCTTTTA----CAATATTT 832
M.niphanae (Ny01)       TTGAAGGCTTGAATGAAAGGTTGGACGAGGGGTAAGCTGTCTCTTTTA----CAATATTT 830
M.niphanae (Ny04)       TTGAAGGCTTGAATGAAAGGTTGGACGAGGGGTAAGCTGTCTCTTTTA----CAATATTT 827
M.niphanae (Ny03)       TTGAAGGCTTGAATGAAAGGTTGGACGAGGGGTAAGCTGTCTCTTTTA----CAATATTT 830
M.niphanae (Ny02)       TTGAAGGCTTGAATGAAAGGTTGGACGAGGGGTAAGCTGTCTCTTTTA----CAATATTT 830
M.dienbienphuense(Ny02) TTGGAGGCTTGAATGAAGGGTTGGACGAGGGATAATCTGTCTCCTTTG----CAGTGTTT 830
M.rosenbergii(NC_006880)TTGGAGGCTTGAATGAATGGTTGGACGAGGGATAAGCTGTCTCCTTAG----CGGCGTTT 830
M.rosenbergii (Np01)    TTGGAGGCTTGAATGAATGGTTGGACGAGGGATAAACTGTCTCCTTAG----CGGCGTTT 830
P.monodon               TTGAAGGCTTGTATGAATGGTTGGACAAAAAGTAATCTGTCTCAGTTA----TAATAGTT 875
S.serrata               TTGGAAACTTGTATGAATGGTTGGACAAAGAAAAGACTGTCTCTTTTA----TTAAAATT 872
S.tranquebarica         TTGGAAACTTGTATGAATGGTTGGACAAAGAAAAAGCTGTCTCTTTTA----TTAAAATT 862
S.olivacea              TTGAAAACTTGTATGAATGGTTGGACAAAGAAAAAGCTGTCTCTTCTA----TTAAAATT 861
B.mandarina             TTGGTGACTTGTATGAAAGATTGGATGAAATATAAACTGTCTCTTTTATAAAAAAAG-TT 869
B.mori                  TTGGTGACTTGTATGAAAGATTGGATGAAATATAAACTGTCTCTTTTATAAAAAAAAATT 869
An.gambiae              TTGAAGGCTGGTATGAATGGTTGAATGAGATATATACTGTTTT-TTTA-AAATTTATATA 846
An.quadrimaculatus      TTGAAGGCTGGTATGAATGGTTGAATGAGATATATACTGTTTT-TTTA-AAATTTTTATA 844
Ap.mellifera            TTGAAGAATTGTATGAAAGAATTAATGAAATAATAACTGTCTC---TA-AATTATTAAAT 895
D.melanogaster          TTGAAGGCTGGAATGAATGGTTGGACGAAATATTAACTGTTTCATTTA-AAATTTTTATA 846
D.yakuba                TTGAAGGCTGGAATGAATGGTTGGACGAAATATTAACTGTTTCATTTA-AATTTAAAATA 844
                        ***     * * ***** *  *  *  *        **** *
Escherichia coli        ------------------------1682-2009------------------------> GT

                        <------------------------ Domain IV---------------------><-
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Figure 19 (continuted)

```
M.assamense (Kc01)      GAATTTAACTTTTGAGTGAAAAGGCTCAAATTGTTTAATGGGACGATAAGACCCCTATAA 891
M.assamense (Kc02)      GAATTTAACTTTTGAGTGAAAAGGCTCAAATTGTTTAATGGGACGATAAGACCC-TATAA 890
M.lanchesteri (Rb01)    GAATTTAACTTTTGAGTGAAAAGGCTCAAATTGTTTAATGGGACGATAAGACCC-TATAA 890
M.lanchesteri (Np01)    GAATTTAACTTTTGAGTGAAAAGGCTCAAATTGTTTAATGGGACGATAAGACCC-TATAA 890
M.lanchesteri (Rb02)    GAATTTAACTTTTGAGTGAAAAGGCTCAAATTGTTTAATGGGACGATAAGACCC-TATAA 890
M.lanchesteri (Np02)    GAATTTAACTTTTGAGTGAAAAGGCTCAAATTGTTTAATGGGACGATAAGACCC-TATAA 890
M.lanchesteri (Ay03)    GAATTTAACTTTTGAGTGAAAAGGCTCAAATTGTTTAATGGGACGATAAGACCC-TATAA 891
M.lanchesteri (Cs01)    GAATTTAACTTTTGAGTGAAAAGGCTCAAATTGTTTAATGGGACGATAAGACCC-TATAA 889
M.tratense (Kc01)       GAATTTAACTTTTGAGTGAAAAGGCTCAAATTATTTAGTGGGACGATAAGACCC-TATAA 884
M.tratense (Ut01)       GAATTTAACTTTTGAGTGAAAAGGCTCAAATTATTTAGTGGGACGATAAGACCC-TATAA 886
M.forcipatum (Tk01)     GAATTTAACTTTTGAGTGAAAAGGCTCAAATTGCTTAGTGGGACGATAAGACCC-TATAA 889
M.hirsutimanus (Rb01)   GAATTTAACTTTTGAGTGAAAAGGCTTAAATTATTTAGTGGGACGATAAGACCC-TATAA 890
M.hirsutimanus (Rb02)   GAATTTAACTTTTGAGTGAAAAGGCTTAAATTATTTAGTGGGACGATAAGACCC-TATAA 890
M.niphanae (Kc01)       GAATTTAACTTTTAAGTGAAAAGGCTTAAATTATTTAGTGGGACGATAAGACCC-TATAA 889
M.niphanae (Np01)       GAATTTAACTTTTAAGTGAAAAGGCTTAAATTATTTAGTGGGACGATAAGACCC-TATAA 889
M.niphanae (Np02)       GAATTTAACTTTTAAGTGAAAAGGCTTAAATTATTTAGTGGGACGATAAGACCC-TATAA 889
M.sintangense (Cb02)    GAATTTAACTTTTGAGTGAAAAGGCTCAGATTGTTTAGTGGGACGATAAGACCC-TATAA 889
M.sintangense (Cb01)    GAATTTAACTTTTGAGTGAAAAGGCTCAGATTGTTTAGTGGGACGATAAGACCC-TATAA 884
M.hirsutimanus (Sb01)   GAATTTAACTTTTGAGTGAAAAGGCTTAAATTGTTTAGTGGGACGATAAGACCC-TATAA 890
M.hirsutimanus (Sb02)   GAATTTAACTTTTGAGTGAAAAGGCTTAAATTGTTTAGTGGGACGATAAGACCC-TATAA 890
M.hirsutimanus (Sb03)   GAATTTAACTTTTGAGTGAAAAGGCTTAAATTGTTTAGTGGGACGATAAGACCC-TATAA 890
M.dienbienphuense(Ny01) GAATTTAACTTTTGAGTGAAAAGGCTCAAATTATTTAGTGGGACGATAAGACCC-TATAA 889
M.hirsutimanus (Ny01)   GAATTTAACTTTTGAGTGAAAAGGCTTAAATTGTTTAGTGGGACGATAAGACCC-TATAA 890
M.hirsutimanus (Ny02)   GAATTTAACTTTTGAGTGAAAAGGCTTAAATTGTTTAGTGGGACGATAAGACCC-TATAA 890
M.sp3 (Np02)            GAATTTAACTTTTAAGTGAAAAGGCTTAAATTATTTAGTGGGACGATAAGACCC-TATAA 891
M.sp3 (Np01)            GAATTTAACTTTTAAGTGAAAAGGCTTAAATTATTTAGTGGGACGATAAGACCC-TATAA 891
M.niphanae (Ny01)       GAATTTAACTTTTAAGTGAAAAGGCTTAAATTATTTAGTGGGACGATAAGACCC-TATAA 889
M.niphanae (Ny04)       GAATTTAACTTTTAAGTGAAAAGGCTTAAATTATTTAGTGGGACGATAAGACCC-TATAA 886
M.niphanae (Ny03)       GAATTTAACTTTTAAGTGAAAAGGCTTAAATTATTTAGTGGGACGATAAGACCC-TATAA 889
M.niphanae (Ny02)       GAATTTAACTTTTAAGTGAAAAGGCTTAAATTATTTAGTGGGACGATAAGACCC-TATAA 889
M.dienbienphuense(Ny02) GAATTTAACTTTTGAGTGAAAAGGCTCAAATTATTTAGTGGGACGATAAGACCC-TATAA 889
M.rosenbergii(NC_006880)GAATTTAACTTTTGAGTGAAAAGGCTCAAATTGTTTAGGGGGACGATAAGACCC-TATAA 889
M.rosenbergii (Np01)    GAATTTAACTTTTGAGTGAAAAGGCTCAAATTGTTTAGGGGGACGATAAGACCC-TATAA 889
P.monodon               GAACTTAACTTTTAAGTGAAAAGGCTTAAATACTTTAAGGGGACGATAAGACCC-TATAA 934
S.serrata               GAAATTAACTTCTAAGTGAAAAGGCTTAGATTTTCTAGAGGGACGATAAGACCC-TATAA 931
S.tranquebarica         GAAATTAACTTCTAAGTGAAAAGGCTTAGATTTTCTAGAGGGACGATAAGACCC-TATAA 921
S.olivacea              GAAATTAACTTCTAAGTGAAAAGGCTTAGATTTTCTAGAGGGACGATAAGACCC-TATAA 920
B.mandarina             GAATTTAATTTTTTAATTAAAAAGTTAAAATTATTTAAAAAGACGAGAAGACCC-TATAG 928
B.mori                  GAATTTAATTTTTTAATTAAAAAGTTAAAATTATTTAAAAAGACGAGAAGACCC-TATAG 928
An.gambiae              GAACTTTATTTTTTAGTTAAAAAGCTAAAATTTAATTAAAAGACGAGAAGACCC-TATAG 905
An.quadrimaculatus      GAACTTTATTTTTTAGTTAAAAAGCTAAAATTTAATTAAAGGACGAGAAGACCC-TATAG 903
Ap.mellifera            GAATTTAAATTTTTAGTTAAAATTCTAAAATTTAATTATGGGACGATAAGACCC-TATAG 954
D.melanogaster          GAATTTTATTTTTTAGTCAAAAAGCTAAAATTTATTTAAAAGACGAGAAGACCC-TATAA 905
D.yakuba                GAATTTTATTTTTTAGTCAAAAAGCTAAAATTAATTTAAAAGACGAGAAGACCC-TATAA 903
                        *** ** * ** * * * ****   * * **    *     ***** ******* ****
Escherichia coli        GAAATTGAACTCGCTGTGAAGATGCAGTGTACCCGCGGCAAGACGGAAAGACCC-CGTGA

                        <------------------------- Domain V------------------------ >
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Figure 20 Alignment of *16S rRNA* of *Macrobrachium* spp. compared with other arthropods and *E. coli*; Domain V.

```
M.assamense (Kc01)      AACTTAATA--TAATCTAGTTAATACTTGCGATT--TAGGTAA-AAAGGAGTTTTGTTTA 946
M.assamense (Kc02)      AACTTAATA--TAATCTAGTTAATACTTGCGATT--TAGGTAA-AAAGGAGTTTTGTTTA 945
M.lanchesteri (Rb01)    AACTTAATA--TAATCTGGTTAATACTTGCGATT--TAGGTAA-AAAGGAGTTTTGTTTA 945
M.lanchesteri (Np01)    AACTTAATA--TAATCTAGTTAATACTTGCGATT--TAGGTAA-AAAGGAGTTTTGTTTA 945
M.lanchesteri (Rb02)    AACTTAATA--TAATCTAGTTAATACTTGCGATT--TAGGTAA-AAAGGAGTTTTGTTTA 945
M.lanchesteri (Np02)    AACTTAATA--TAATCTAGTTAATACTTGCGATT--TAGGTAA-AAAGGAGTTTTGTTTA 945
M.lanchesteri (Ay03)    AACTTAATA--TAATCTAGTTAATACTTGCGATT--TAGGTAA-AAAGGAGTTTTGTTTA 946
M.lanchesteri (Cs01)    AACTTAATA--TAATCTAGTTAATACTTGCGATT--TAGGTAA-AAAGGAGTTTTGTTTA 944
M.tratense (Kc01)       AACTTTATA--TAAAAGTAACTTGGTTTATGATT--TAGGTGA-AAAGTAGCTTTGTTGG 939
M.tratense (Ut01)       AACTTTATA--TAAGAGTAACTTGGTTTATGATT--TAGGTGA-AAAGTAGCTTTGTTGG 941
M.forcipatum (Tk01)     AACTTAATA--TGGGTTTGGCTTGATTTGCGATTAGTGGGTGA-AAAGTAATTTTGTTTA 946
M.hirsutimanus (Rb01)   AACTTAATA--TAGGTTTAGCTTGATTTGCAATTA-TGGGTGA-AAAGTGGTTTTGTCTA 946
M.hirsutimanus (Rb02)   AACTTAATA--TAGGTTTAGCTTGATTTGCAATTA-TGGGTGA-AAAGTGGTTTTGTCTA 946
M.niphanae (Kc01)       AACTTAATA--TAGGTTTGACTTAATTTTATAAAA-TGGGTGATAAAGTGATTTTGTTTA 946
M.niphanae (Np01)       AACTTAATA--TAGGCTTGATTTAATTTTATAAAA-TGAGTGATAAAGTGATTTTGTCTA 946
M.niphanae (Np02)       AACTTAATA--TAGGCTTGATTTAATTTTATAAAA-TGAGTGATAAAGTGATTTTGTCTA 946
M.sintangense (Cb02)    AACTTTATA--TAAAAGTAGTTTGGTTTATAATT--TAAGTAA-AAAGTAACTTTGCTAA 944
M.sintangense (Cb01)    AACTTTATA--TAAAAGTAGTTTGGTTTATAATT--TAAGTAA-AAAGTAACTTTGCTAA 939
M.hirsutimanus (Sb01)   AACTTAATA--TAGGTTTAGCTTGATTTGCAATTA-TGGGTGA-AAAGTGGTTTTGTTTA 946
M.hirsutimanus (Sb02)   AACTTAATA--TAGGTTTAGCTTGATTTGCAATTA-TGGGTGA-AAAGTGGTTTTGTTTA 946
M.hirsutimanus (Sb03)   AACTTAATA--TAGGTTTAGCTTGATTTGCAATTA-TGGGTGA-AAAGTGGTTTTGTTTA 946
M.dienbienphuense(Ny01) AACTTAATA--TGGGTGTGGCTTGATTTGCGATTAGTAGGTGA-AAAGTAATTTTGTTTG 946
M.hirsutimanus (Ny01)   AACTTAATA--TAGGTTTAGCTTGATTTGCAATTA-TGGGTGA-AAAGTGGTTTTGTTTA 946
M.hirsutimanus (Ny02)   AACTTAATA--TAGGTTTAGCTTGATTTGCAATTA-TGGGTGA-AAAGTGGTTTTGTTTA 946
M.sp3 (Np02)            AACTTAATA--TAGGTTTGACTTAATTTTATAAAA-TGGGTGATAAAGTGATTTTGTTTA 948
M.sp3 (Np01)            AACTTAATA--TAGGTTTGACTTAATTTTATAAAA-TGGGTGATAAAGTGATTTTGTTTA 948
M.niphanae (Ny01)       AACTTAATA--TAGGTTTGACTTAATTTTATAAAA-TGGGTGATAAAGTGATTTTGTTTA 946
M.niphanae (Ny04)       AACTTAATA--TAGGTTTGACTTAATTTTATAAAA-TGGGTGATAAAGTGATTTTGTTTA 943
M.niphanae (Ny03)       AACTTAATA--TAGGTTTGACTTAATTTTATAAAA-TGGGTGATAAAGTGATTTTGTTTA 946
M.niphanae (Ny02)       AACTTAATA--TAGGTTTGACTTAATTTTATAAAA-TGGGTGATAAAGTGATTTTGTTTA 946
M.dienbienphuense(Ny02) AACTTAATA--TGGGTGTGGCTTGATTTGCGATTAGTAGGTGA-AAAGTAATTTTGTTTG 946
M.rosenbergii(NC_006880)AACTTAATA--TAATTTAGGCTTAACTTGCGATG--TGGGTGA-AAAGTAGTTTTGTCTG 944
M.rosenbergii (Np01)    AACTTAATA--TAATTTAGGCTTAACTTGCGATG--TGGGTGA-AAAGTAGTTTTGTCTG 944
P.monodon               AACTTAACA--ATAATTTGATTAAATTATAAATTG-TTAGTAT-AACTTGATTTTAATTA 990
S.serrata               AGCTTCATAAGTTAAATAAATTAGA-TGGATTTT--TAGAATA-AAAGTAAATTTAGTAA 987
S.tranquebarica         AGCTTTATAAATTAAGTAAGTTAGAACAAATTTTA-TAGAATA-AAGGTAAATTTAATAT 979
S.olivacea              AGCTTTATAGATTGAGTGGATTAAA-CCAATTTTT-TAGAATA-AAAGTAAATTTGGAAG 977
B.mandarina             AGTTTTATA-ATTTATTT--TGTAAAT-ATTTT-A--TTTTTATATTTTTATAATTTTAA 981
B.mori                  AGTTTTATA-ATTTATTT--TGTAAAT-ATTTTTA--TTTTTATATTTTTATAATTTTAA 982
An.gambiae              ATCTTTAT--TTTTATAAATTATAAATTATAAAGA--ATTTTAAAATTTATATTTTAAAT 961
An.quadrimaculatus      ATCTTTAT--TTTTATAAATTATAAATTATAAAGA--ATTTTAAAATTTATATTTTAAAT 959
Ap.mellifera            AATTTAAT---TTAATTT--TTAAATTTATTTATA--ATTTTAATAAATATATTTAAGAT 1007
D.melanogaster          ATCTTTATA-TTTTTTTTTATTTTAATTATATAGA--TTAATTAAATTTTAATAAATAAA 962
D.yakuba                ATCTTTATA-TTTTATTT-ATTTTAATTATAAAGA--TTAATTTAATTTTAATAAATTAA 959
                        *  ** *
Escherichia coli        ACCTT<---------------------------2076-2264------------------

                         <------------------------- Domain V---------------------->
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Figure 20 (continuted)

```
M.assamense (Kc01)      G-ATTATATTTCGTTGGGGAGATGAAGATATAA---TGTATAACTGTCTATTGA-ATAAT 1001
M.assamense (Kc02)      G-ATTATATTTCGTTGGGGAGATGAAGATATAA---TGTATAACTGTCTATTGA-ATAAT 1000
M.lanchesteri (Rb01)    G-ATTATATTTCGTTGGGGAGATGAAGATATAA---TGTATAACTGTCTATTGA-ATAAT 1000
M.lanchesteri (Np01)    G-ATTATATTTCGTTGGGGAGATGAAGATATAA---TGTATAACTGTCTATTGA-ATAAT 1000
M.lanchesteri (Rb02)    G-ATTATATTTCGTTGGGGAGATGAAGATATAA---TGTATAACTGTCTATTGA-ATAAT 1000
M.lanchesteri (Np02)    G-ATTATATTTCGTTGGGGAGATGAAGATATAA---TGTATAACTGTCTATTGA-ATAAT 1000
M.lanchesteri (Ay03)    G-ATTATATTTCGTTGGGGAGATGAAGATATAA---TGTATAACTGTCTATTGA-ATAAT 1001
M.lanchesteri (Cs01)    G-ATTATATTTCGTTGGGGAGATGAAGATATAA---TGTATAACTGTCTATTGA-ATAAT 999
M.tratense (Kc01)       G-TTTATATTTCGTTGGGGAGATGTAGATATAA---TATATAACTGTCTGTTTA-ATTTA 994
M.tratense (Ut01)       G-TTTATATTTCGTTGGGGAGATGTAGATATAA---TATATAACTGTCTGTTTA-ATTTA 996
M.forcipatum (Tk01)     G-CTCATATTTTGTTGGGGAGATGTGGATATAA---TGTGTAACTGTCTGTTGA-ATTTA 1001
M.hirsutimanus (Rb01)   GGCTTATATTTTGTTGGGGAGATGTAGATATAA---TGTGTAACTGTCTGTTGA-ATTTA 1002
M.hirsutimanus (Rb02)   GGCTTATATTTTGTTGGGGAGATGTAGATATAA---TGTGTAACTGTCTGTTGA-ATTTA 1002
M.niphanae (Kc01)       G-TTTATATTTTGTTGGGGCGATATAGATATAA---TATGTAACTGTCTATTAA-ATTTA 1001
M.niphanae (Np01)       G-TTTGTATTTTGTTGGGGCGATATAGATATAA---TATGTAACTGTCTATTAA-ATTTA 1001
M.niphanae (Np02)       G-TTTGTATTTTGTTGGGGCGATATAGATATAA---TATGTAACTGTCTATTAA-ATTTA 1001
M.sintangense (Cb02)    A-TTTATATTTCGTTGGGGAGATGTAGATATAA---TGTGTAACTGTCTGTTTA-ATTTG 999
M.sintangense (Cb01)    A-TTTATATTTCGTTGGGGAGATGTAGATATAA---TGTGTAACTGTCTGTTTA-ATTTG 994
M.hirsutimanus (Sb01)   GGCTTATATTTTGTTGGGGAGATGTAGATATAA---TGTGTAACTGTCTGTTGA-ATTTA 1002
M.hirsutimanus (Sb02)   GGCTTATATTTTGTTGGGGAGATGTAGATATAA---TGTGTAACTGTCTGTTGA-ATTTA 1002
M.hirsutimanus (Sb03)   GGCTTATATTTTGTTGGGGAGATGTAGATATAA---TGTGTAACTGTCTGTTGA-ATTTA 1002
M.dienbienphuense(Ny01) G-CTCATATTTTGTTGGGGAGATGTGGATATAA---TGTGTAACTGTCTGTTGA-ATTTA 1001
M.hirsutimanus (Ny01)   GGCTTATATTTTGTTGGGGAGATGTAGATATAA---TGTGTAACTGTCTGTTGA-ATTTA 1002
M.hirsutimanus (Ny02)   GGCTTATATTTTGTTGGGGAGATGTAGATATAA---TGTGTAACTGTCTGTTGA-ATTTA 1002
M.sp3 (Np02)            G-TTTATATTTTGTTGGGGCGATATAGATATAA---TATGTAACTGTCTATTAA-ATTTA 1003
M.sp3 (Np01)            G-TTTATATTTTGTTGGGGCGATATAGATATAA---TATGTAACTGTCTATTAA-ATTTA 1003
M.niphanae (Ny01)       G-TTTATATTTTGTTGGGGCGATATAGATATAA---TATGTAACTGTCTATTAA-ATTTA 1001
M.niphanae (Ny04)       G-TTTGTATTTTGTTGGGGCGATATAGATATAA---TATGTAACTGTCTATTAA-ATTTA 998
M.niphanae (Ny03)       G-TTTATATTTTGTTGGGGCGATATAGATATAA---TATGTAACTGTCTATTAA-ATTTA 1001
M.niphanae (Ny02)       G-TTTATATTTTGTTGGGGCGATATAGATATAA---TATGTAACTGTCTATTAA-ATTTA 1001
M.dienbienphuense(Ny02) G-CTCATATTTTGTTGGGGAGATGTGGATATAA---TGTGTAACTGTCTGTTGA-ATTTA 1001
M.rosenbergii(NC_006880)G-TTTATATTTCGTTGGGGAGATGAAGATATAA---TGAGTAACTGTCTATAAA-ATTTT 999
M.rosenbergii (Np01)    G-TTTATATTTCGTTGGGGAGATGAAGATATAA---TGAGTAACTGTCTATAAA-ATTTT 999
P.monodon               A-TGTTTGTTGCGTTGGGGCGACGGGAATATAA---TTAGTAACTGTTCTTAAATATTTT 1046
S.serrata               G---CTTATTTAGTTGGGGCGACAAGGGTATAAAATTAAATAACTG-CTATTAAGGTTAG 1043
S.tranquebarica         A---TTTATTTGGTTGGGGCGACAATGGTATAAA-TTAAATAACTG-CTATTAAAATTAA 1034
S.olivacea              A---TTTATTTGGTTGGGGCGACAAATGTATAATGTTAAATAACTG-CTATTAAATTTAA 1033
B.mandarina             A----TTATTTTGTTGGGGTGATAAAAAAATTT----AATTAACTTTTTTTAATA-TTAT 1032
B.mori                  A----TTATTTTGTTGGGGTGATAAAAAAATTT----AATTAACTTTTTTTAATA-GTTT 1033
An.gambiae              A----AAATTTTACTGGGGTGGTATTAAAATTA----AATAAACTTTTATTATTT-ATTT 1012
An.quadrimaculatus      A----AAATTTTACTGGGGTGGTATTAAAATTT----AATAAACTTTTATTTTTA-TTTA 1010
Ap.mellifera            T----AAATTTGATTGGGAGGATTGGTAAATTT----AATAAACTTTATTTAAGA-TTTA 1058
D.melanogaster          A----ATATTTTATTGGGGTGATATTAAAATTT----AAAAAACTTTTAATTTTTTAAAA 1014
D.yakuba                A----ATATTTTATTGGGGTGATATTAAAATTT----AAAAAACTTTTAATTTTA-AAAA 1010
                                **    ****  *        **          ****     *
Escherichia coli        ----------------------------> TAA----AGAGTAAC<---------------

                        <-------------------------- Domain V------------------------>
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Figure 20 (continued)

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M.assamense (Kc01)      AT----AATGCTAATTAGAGTT-TGATCCTTC-TTTGT---GGATTAAAAGAATAAGTTA 1052
M.assamense (Kc02)      AT----AATGCTAATTAGAGTT-TGATCCTTC-TTTGT---GGATTAAAAGAATAAGTTA 1051
M.lanchesteri (Rb01)    AT----AATGCTAATTAGAGTT-TGATCCTTC-TTTGT---GGATTAAAAGAATAAGTTA 1051
M.lanchesteri (Np01)    AT----AATGCTAATTAGAGTT-TGATCCTTC-TTTGT---GGATTAAAAGAATAAGTTA 1051
M.lanchesteri (Rb02)    AT----AATGCTAATTAGAGTT-TGATCCTTC-TTTGT---GGATTAAAAGAATAAGTTA 1051
M.lanchesteri (Np02)    AT----AATGCTAATTAGAGTT-TGATCCTTC-TTTGT---GGATTAAAAGAATAAGTTA 1051
M.lanchesteri (Ay03)    AT----AATGCTAATTAGAGTT-TGATCCTTC-TTTGT---GGATTAAAAGAATAAGTTA 1052
M.lanchesteri (Cs01)    AT----AATGCTAATTAGAGTT-TGATCCTTC-TTTGT---GGATTAAAAGAATAAGTTA 1050
M.tratense (Kc01)       AT----AGCTGTAGTTAGTGTG-TGATCCTTC-TTTGT---GGATTAAAAGTATAAGTTA 1045
M.tratense (Ut01)       AT----AGCTGTAGTTAGTGTG-TGATCCTTC-TTTGT---GGATTAAAAGTATAAGTTA 1047
M.forcipatum (Tk01)     AT----AGTTATGATTAGTGTG-TGATCCTTCATTTGT---GGATTAGGAGAATAAGTTA 1053
M.hirsutimanus (Rb01)   AT----AGTTATGATTAGTGTG-TGATCCTTCATTTGT---GGATTAAGAGAATAAGTTA 1054
M.hirsutimanus (Rb02)   AT----AGTTATGATTAGTGTG-TGATCCTTCATTTGT---GGATTAAGAGAATAAGTTA 1054
M.niphanae (Kc01)       AT----AATTATAATTAGTTTT-TGATCCTAC-TTTGT---GGATTGAAAGAATAAGTTA 1052
M.niphanae (Np01)       AT----AATTATAATTAGTTTT-TGATCCTTC-TTTGT---GGATTGGAAGAATAAGTTA 1052
M.niphanae (Np02)       AT----AATTATAATTAGTTTT-TGATCCTTC-TTTGT---GGATTAGAAGAATAAGTTA 1052
M.sintangense (Cb02)    AT----AATTGTAGTTAGTGTA-TGATCCTTC-TTTGT---GGATTAAAAGTATAAGTTA 1050
M.sintangense (Cb01)    AT----AATTGTAGTTAGTGTA-TGATCCTTC-TTTGT---GGATTAAAAGTATAAGTTA 1045
M.hirsutimanus (Sb01)   AT----AGTTATGATTAGTGTG-TGATCCTTCATTTGT---GGATTAAGAGAATAAGTTA 1054
M.hirsutimanus (Sb02)   AT----AGTTATGATTAGTGTG-TGATCCTTCATTTGT---GGATTAAGAGAATAAGTTA 1054
M.hirsutimanus (Sb03)   AT----AGTTATGATTAGTGTG-TGATCCTTCATTTGT---GGATTAAGAGAATAAGTTA 1054
M.dienbienphuense(Ny01) AT----AGTTATAATTAGTGTA-TGATCCTTCATTTGT---GGATTAGGAGAATAAGTTA 1053
M.hirsutimanus (Ny01)   AT----AGTTATGATTAGTGTG-TGATCCTTCATTTGT---GGATTAAGAGAATAAGTTA 1054
M.hirsutimanus (Ny02)   AT----AGTTATGATTAGTGTG-TGATCCTTCATTTGT---GGATTAAGAGAATAAGTTA 1054
M.sp3 (Np02)            AT----AATTATAATTAGTTTT-TGATCCTAC-TTTGT---GGATTGAAAGAATAAGTTA 1054
M.sp3 (Np01)            AT----AATTATAATTAGTTTT-TGATCCTAC-TTTGT---GGATTGAAAGAATAAGTTA 1054
M.niphanae (Ny01)       AT----AATTATAATTAGTTTT-TGATCCTAC-TTTGT---GGATTGAAAGAATAAGTTA 1052
M.niphanae (Ny04)       AT----AATTATAATTAGTTTT-TGATCCTAC-TTTGT---GGATTGAAAGAATAAGTTA 1049
M.niphanae (Ny03)       AT----AATTATAATTAGTTTT-TGATCCTAC-TTTGT---GGATTGAAAGAATAAGTTA 1052
M.niphanae (Ny02)       AT----AATTATAATTAGTTTT-TGATCCTAC-TTTGT---GGATTGAAAGAATAAGTTA 1052
M.dienbienphuense(Ny02) AT----AGTTATAATTAGTGTA-TGATCCTTCATTTGT---GGATTAGGAGAATAAGTTA 1053
M.rosenbergii(NC_006880)AT----AGCATTGACTAGAATT-TGATCCTTC-CTTGG---GGATTAGGAGAATAAGTTA 1050
M.rosenbergii (Np01)    AT----AGCATTGACTAGAATT-TGATCCTTC-CTTGG---GGATTAGGAGAATAAGTTA 1050
P.monodon               ATTAACAAGTATAATTGAAGAA-TAATTGATCCTTTATTAAAGATTAAAAGATTAAGTTA 1105
S.serrata               ACAATTATATTTGGTTGGA-TTGTAATTGATC-CTCGCTATGGATTAAAAGATTAAGTTA 1101
S.tranquebarica         ACAATTATATTTGATTAAAATTATAATTGATC-CTTATTATGGATTAAAAGATCAAGTTA 1093
S.olivacea              ACAATTATATTTGATAAAAATT-TAAATGATC-CTTAATAAAGATTAAAAGATCAAGTTA 1091
B.mandarina             AC----ATTAATAAGTGGTTAAATGATCCAATTTTAT----TGATTAAAAGAAAAAATTA 1084
B.mori                  AC----ATTAATAAGTGATTAAATGATCCAATTTTAT----TGATTAAAAGAAAAAATTA 1085
An.gambiae              AC----ATTGATTTATGAATAAAAGATCCTGTTTTAT----GGATTAAAAATTTAAGTTA 1064
An.quadrimaculatus      AC----ATTGATTTATGAATTTAAGATCCTGTATTAT----GGATTAAAAAATTAAGTTA 1062
Ap.mellifera            AT----TTTGATATAAAGATAATTTTTTGAATTATAAATTGTAATTAAAAGAATAAATTA 1114
D.melanogaster          AC----ATAAATTTATGAATATTTGATCCATTAATAA----TGATTAAAAAATTAAGTTA 1066
D.yakuba                AC----ATTAATTTATGAATAATTGATCCATTAATAA----TGATTAAAAAATTAAGTTA 1062
                        *          *                      *        ***   *    ** ***
Escherichia coli        ----- 2276-2421 ------------------------->CTCAACGGATAAAGGTA

                        <------------------------- Domain V---------------------- >
```

Figure 20 (continuted)

```
M.assamense (Kc01)      CTTTAGGGATAACAGCGTAATTTTCTTTGAGAGTTCTTATCGACAAGAGTAGTTGCGACC 1112
M.assamense (Kc02)      CTTTAGGGATAACAGCGTAATTTTCTTTGAGAGTTCTTATCGACAAGAGTAGTTGCGACC 1111
M.lanchesteri (Rb01)    CTTTAGGGATAACAGCGTAATTTTCTTTGAGAGTTCTTATCGACAAGAGTAGTTGCGACC 1111
M.lanchesteri (Np01)    CTTTAGGGATAACAGCGTAATTTTCTTTGAGAGTTCTTATCGACAAGAGTAGTTGCGACC 1111
M.lanchesteri (Rb02)    CTTTAGGGATAACAGCGTACTTTTCTTTGAGAGTTCTTATCCACAAGAGTAGTTGCGACC 1111
M.lanchesteri (Np02)    CTTTAGGGATAACAGCGTAATTTTCTTTGAGAGTTCTTATCGACAAGAGTAGTTGCGACC 1111
M.lanchesteri (Ay03)    CTTTAGGGATAACAGCGTAATTTTCTTTGAGAGTTCTTATCGACAAGAGTAGTTGCGACC 1112
M.lanchesteri (Cs01)    CTTTAGGGATAACAGCGTAATTTTCTTTGAGAGTTCTTATCGACAAGAGTAGTTGCGACC 1110
M.tratense (Kc01)       CTTTAGGGATAACAGCGTGATTTTCTTTGAGAGTTCCTATCGACAAGAGTAGTTGCGACC 1105
M.tratense (Ut01)       CTTTAGGGATAACAGCGTGATTTTCTTTGAGAGTTCCTATCGACAAGAGTAGTTGCGACC 1107
M.forcipatum (Tk01)     CTTTAGGGATAACAGCGTGATTTTCTTTGAGAGTTCTTATCGACAAGAGTAGTTGCGACC 1113
M.hirsutimanus (Rb01)   CTTTAGGGATAACAGCGTAATTTTCTTTGAGAGTTCTTATCGACAAGAGTAGTTGCGACC 1114
M.hirsutimanus (Rb02)   CTTTAGGGATAACAGCGTAATTTTCTTTGAGAGTTCTTATCGACAAGAGTAGTTGCGACC 1114
M.niphanae (Kc01)       CTTTAGGGATAACAGCGTGATTTTCTTTGAGAGTTCTTATCGACAAGAGTAGTTGCGACC 1112
M.niphanae (Np01)       CTTTAGGGATAACAGCGTGATTTTCTTTGAGAGTTCTTATCGACAAGAGTAGTTGCGACC 1112
M.niphanae (Np02)       CTTTAGGGATAACAGCGTGATTTTCTTTGAGAGTTCTTATCGACAAGAGTAGTTGCGACC 1112
M.sintangense (Cb02)    CTTTAGGGATAACAGCGTGATTTTCTTTGAGAGTTCTTATCGACAAGATTAGTTGCGACC 1110
M.sintangense (Cb01)    CTTTAGGGATAACAGCGTGATTTTCTTTGAGAGTTCTTATCGACAAGATTAGTTGCGACC 1105
M.hirsutimanus (Sb01)   CTTTAGGGATAACAGCGTAATTTTCTTTGAGAGTTCTTATCGACAAGAGTAGTTGCGACC 1114
M.hirsutimanus (Sb02)   CTTTAGGGATAACAGCGTAATTTTCTTTGAGAGTTCTTATCGACAAGAGTAGTTGCGACC 1114
M.hirsutimanus (Sb03)   CTTTAGGGATAACAGCGTAATTTTCTTTGAGAGTTCTTATCGACAAGAGTAGTTGCGACC 1114
M.dienbienphuense(Ny01) CTTTAGGGATAACAGCGTGATTTTCTTTGAGAGTTCTTATCGACAAGAGTAGTTGCGACC 1113
M.hirsutimanus (Ny01)   CTTTAGGGATAACAGCGTAATTTTCTTTGAGAGTTCTTATCGACAAGAGTAGTTGCGACC 1114
M.hirsutimanus (Ny02)   CTTTAGGGATAACAGCGTAATTTTCTTTGAGAGTTCTTATCGACAAGAGTAGTTGCGACC 1114
M.sp3 (Np02)            CTTTAGGGATAACAGCGTGATTTTCTTTGAGAGTTCTTATCGACAAGAGTAGTTGCGACC 1114
M.sp3 (Np01)            CTTTAGGGATAACAGCGTGATTTTCTTTGAGAGTTCTTATCGACAAGAGTAGTTGCGACC 1114
M.niphanae (Ny01)       CTTTAGGGATAACAGCGTGATTTTCTTTGAGAGTTCTTATCGACAAGAGTAGTTGCGACC 1112
M.niphanae (Ny04)       CTTTAGGGATAACAGCGTGATTTTCTTTGAGAGTTCTTATCGACAAGAGTAGTTGCGACC 1109
M.niphanae (Ny03)       CTTTAGGGATAACAGCGTGATTTTCTTTGAGAGTTCTTATCGACAAGAGTAGTTGCGACC 1112
M.niphanae (Ny02)       CTTTAGGGATAACAGCGTGATTTTCTTTGAGAGTTCTTATCGACAAGAGTAGTTGCGACC 1112
M.dienbienphuense(Ny02) CTTTAGGGATAACAGCGTGATTTTCTTTGAGAGTTCTTATCGACAAGAGTAGTTGCGACC 1113
M.rosenbergii(NC_006880)CTTTAGGGATAACAGCGTGATTTTCTTTGAGAGTTCTTATCGACAAGAGTAGTTGCGACC 1110
M.rosenbergii (Np01)    CTTTAGGGATAACAGCGTGATTTTCTTTGAGAGTTCTTATCGACAAGAGTAGTTGCGACC 1110
P.monodon               CTTTAGGGATAACAGCGTAATCTTCTTTGAGAGTCCTCATCGACAAGAAGGTTTGCGACC 1165
S.serrata               CTTTAGGGATAACAGCGTAATTTCTTTTAAGAGTTCTTATCAAAGGAGAAGTTTGCGACC 1161
S.tranquebarica         CTTTAGGGATAACAGCGTAATTTCTTTTAAGAGTTCTTATCAAAGAAGAAGTTTGCGACC 1153
S.olivacea              CTTTAGGGATAACAGCGTAATTTCTTTTAAGAGTTCTTATCAAAGAAGGAGTTTGCGACC 1151
B.mandarina             CCTTAGGGATAACAGCGTAATTTTTCTTTTTAGTTCAAATAAAAAGAAAAGTTTGCGACC 1144
B.mori                  CCTTAGGGATAACAGCGTAATTTTTTTTTTTAGTTCAAATAAAAAGAAAAGTTTGCGACC 1145
An.gambiae              CCTTAGGGATAACAGCGTAATTTTTTTAGAGAGTTCATATCGATAAAAAAGATTGCGACC 1124
An.quadrimaculatus      CCTTAGGGATAACAGCGTAATTTTTTTAGAGAGTTCATATCGATAAAAAAGATTGCGACC 1122
Ap.mellifera            CCTTAGGGATAACAGCGTAATATCTTTTGATAGATCATATAGATAAAGATGTTTGCGACC 1174
D.melanogaster          CTTTAGGGATAACAGCGTAATTTTTTTTGGAGAGTTCATATCGATAAAAAAGATTGCGACC 1126
D.yakuba                CTTTAGGGATAACAGCGTAATTTTTTTTGGAGAGTTCATATCGATAAAAAAGATTGCGACC 1122
                        * ****************  * *   *    **  *  **  *         ********

Escherichia coli        CTCCGGGGATAACAGGCTGATACCGCCCAAGAGTTCATATCGACGGCGGTGTTTGGCACC

                       <------------------------ Domain V---------------------- >
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Figure 20 (continuted)

```
M.assamense (Kc01)      TCGATGTTGAATTAAAATTTCAGCT-AAGTGTAGCCGTTTAGCTAGT-AGGTCTGTTCGA 1170
M.assamense (Kc02)      TCGATGTTGAATTAAAATTTCAGCT-AAGTGTAGCCGTTTAGCTAGT-AGGTCTGTTCGA 1169
M.lanchesteri (Rb01)    TCGATGTTGAATTAAAATTTCAGCT-AAGTGTAGCCGTTTAGCTAGT-AGGTCTGTTCGA 1169
M.lanchesteri (Np01)    TCGATGTTGAATTAAAATTTCAGCT-AAGTGTAGCCGTTTAGCTAGT-AGGTCTGTTCGA 1169
M.lanchesteri (Rb02)    TCGATGTTGAATTAAAATTTCAGCT-AAGTGTAGCCGTTTAGCTAGT-AGGTCTGTTCGA 1169
M.lanchesteri (Np02)    TCGATGTTGAATTAAAATTTCAGCT-AAGTGTAGCCGTTTAGCTAGT-AGGTCTGTTCGA 1169
M.lanchesteri (Ay03)    TCGATGTTGAATTAAAATTTCAGCT-AAGTGTAGCCGTTTAGCTAGT-AGGTCTGTTCGA 1170
M.lanchesteri (Cs01)    TCGATGTTGAATTAAAATTTCAGCT-AAGTGTAGCCGTTTAGCTAGT-AGGTCTGTTCGA 1168
M.tratense (Kc01)       TCGATGTTGAATTAAAGTTTCAGTT-AGGTGTAGCCGTCTAGCTGGT-AGGTCTGTTCGA 1163
M.tratense (Ut01)       TCGATGTTGAATTAAAGTTTCAGTT-AGGTGTAGCCGTCTAGCTGGT-GGGTCTGTTCGA 1165
M.forcipatum (Tk01)     TCGATGTTGAATTAAAGTTTCAGCT-AGGTGTAGCCGCTTAGCTAGT-GGGTCTGTTCGA 1171
M.hirsutimanus (Rb01)   TCGATGTTGAATTAAAGTTTCAGCT-AGGTGTAGCCGTTTAGCTGGT-AGGTCTGTTCGA 1172
M.hirsutimanus (Rb02)   TCGATGTTGAATTAAAGTTTCAGCT-AGGTGTAGCCGTTTAGCTGGT-AGGTCTGTTCGA 1172
M.niphanae (Kc01)       TCGATGTTGAATTAAAATTTCAGTT-AGGTGTAGCCGTTTAGCTGGT-GGGTCTGTTCGA 1170
M.niphanae (Np01)       TCGATGTTGAATTAAAATTTCAGTT-AGGTGTAGCCGTTTAGCTGGT-GGGTCTGTTCGA 1170
M.niphanae (Np02)       TCGATGTTGAATTAAAATTTCAGTT-AGGTGTAGCCGTTTAGCTGGT-GGGTCTGTTCGA 1170
M.sintangense (Cb02)    TCGATGTTGAATTAAAGTTTCAGTT-AGGTGTAGCCGTCTATCAGGT-GGGTCTGTTCGA 1168
M.sintangense (Cb01)    TCGATGTTGAATTAAAGTTTCAGTT-AGGTGTAGCCGTCTATCAGGT-GGGTCTGTTCGA 1163
M.hirsutimanus (Sb01)   TCGATGTTGAATTAAAGTTTCAGCT-AGGTGTAGCCGTTTAGCTGGT-AGGTCTGTTCGA 1172
M.hirsutimanus (Sb02)   TCGATGTTGAATTAAAGTTTCAGCT-AGGTGTAGCCGTTTAGCTGGT-AGGTCTGTTCGA 1172
M.hirsutimanus (Sb03)   TCGATGTTGAATTAAAGTTTCAGCT-AGGTGTAGCCGTTTAGCTGGT-AGGTCTGTTCGA 1172
M.dienbienphuense(Ny01) TCGATGTTGAATTAAAGTTTCAGTT-AGGTGTAGCCGCTTAGCTAGT-GGGTCTGTTCGA 1171
M.hirsutimanus (Ny01)   TCGATGTTGAATTAAAGTTTCAGCT-AGGTGTAGCCGTTTAGCTGGT-AGGTCTGTTCGA 1172
M.hirsutimanus (Ny02)   TCGATGTTGAATTAAAGTTTCCGCT-AGGTGTAGCCGTTTAGCTGGT-AGGTCTGTTCGA 1172
M.sp3 (Np02)            TCGATGTTGAATTAAAATTTCAGTT-AGGTGTAGTCGTTTAGCTGGT-GGGTCTGTTCGA 1172
M.sp3 (Np01)            TCGATGTTGAATTAAAATTTCAGTT-AGGTGTAGTCGTTTAGCTGGT-GGGTCTGTTCGC 1172
M.niphanae (Ny01)       TCGATGTTGAATTAAAATTTCAGTT-AGGTGTAGCCGTTTAGCTGGT-GGGTCTGTTCGA 1170
M.niphanae (Ny04)       TCGATGTTGAATTAAAATTTCAGTT-AGGTGTAGCCGTTTAGCTGGT-GGGTCTGTTCGA 1167
M.niphanae (Ny03)       TCGATGTTGAATTAAAATTTCAGTT-AGGTGTAGCCGTTTAGCTGGT-GGGTCTGTTCGA 1170
M.niphanae (Ny02)       TCGATGTTGAATTAAAATTTCAGTT-AGGTGTAGCCGTTTAGCTGGT-GGGTCTGTTCGA 1170
M.dienbienphuense(Ny02) TCGATGTTGAATTAAAGTTTCAGTT-AGGTGTAGCCGCTTAGCTAGT-GGGTCTGTTCGA 1171
M.rosenbergii(NC_006880)TCGATGTTGAATTAAAATTTCAGCT-AGGTGTAGCCGTTTAGCTGGT-GGGTCTGTTCGA 1168
M.rosenbergii (Np01)    TCGATGTTGAATTAAAATTTCAGCT-AGGTGTAGCCGTTTAGCTGGT-GGGTCTGTTCGA 1168
P.monodon               TCGATGTTGAATTAAGGTATCCTTA-TAATGCAGCAGTTACAAAGGA-AGGTCTGTTCGA 1223
S.serrata               TCGATGTTGAATTAAAATGTCTTTA-TAGTGCAGAAGCTATAAAAGA-AGGTCTGTTCGA 1219
S.tranquebarica         TCGATGTTGAATTAAAATGTCTTTA-TAGTGCAGAAGCTATAAAAGA-AGGTCTGTTCGA 1211
S.olivacea              TCGATGTTGAATTAAAATGTCTTTA-TAGTGCAGCAGCTATAAAAGA-AGGTCTGTTCGA 1209
B.mandarina             TCGATGTTGGATTAAGATAAAATTT-AAATGTAGAAGTTTAAAATTT-TGATCTGTTCGA 1202
B.mori                  TCGATGTTGGATTAAGATAAAATTT-AAATGTAGAAGTTTAAAATTT-TGATCTGTTCGA 1203
An.gambiae              TCGATGTTGGATTAAGAGTTATTTTTAGGTGTAGAAGTTTAAAGTTT-AGGTCTGTTCGA 1183
An.quadrimaculatus      TCGATGTTGGATTAAGAGTTATTTTTAGGTGTAGAAGTTTAAAGTTT-AGGTCTGTTCGA 1181
Ap.mellifera            TCGATGTTGAATTAAGATGAAAATT-AGGCGCAGTAGTTTAAGTTTTTAAGTCTGTTCGA 1233
D.melanogaster          TCGATGTTGGATTAAGATATAATTTTGGGTGTAGCCGTTCAAATTTT-AAGTCTGTTCGA 1185
D.yakuba                TCGATGTTGGATTAAGATATAATTTTGGGTGTAGCCGTTCAAATTTT-AAGTCTGTTCGA 1181
                        ********* *****               * **  *              ********
Escherichia coli        TCGATGTCGGCTCATCACATCCTGGGGCT-GAAGTAGGTCCCAAGGGTATGGCTGTTCGC

                        <-------------------------- Domain V----------------------->
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Figure 20 (continuted)

```
M.assamense (Kc01)      CCTTTAAAATTTTACATGATTTGAGTTCAAACCGGTGTGAGCCAGGTTGGTTTCTATCTT 1230
M.assamense (Kc02)      CCTTTAAAATTTTACATGATTTGAGTTCAAACCGGTGTGAGCCAGGTTGGTTTCTATCTT 1229
M.lanchesteri (Rb01)    CCTTTAAAATTTTACATGATTTGAGTTCAAACCGGTGTGAGCCAGGTTGGTTTCTATCTT 1229
M.lanchesteri (Np01)    CCTTTAAAATTTTACATGATTTGAGTTCAAACCGGTGTGAGCCAGGTTGGTTTCTATCTT 1229
M.lanchesteri (Rb02)    CCTTTAAAATTTTACATGATTTGAGTTCAAACCGGTGTGAGCCAGGTTGGTTTCTATCTT 1229
M.lanchesteri (Np02)    CCTTTAAAATTTTACATGATTTGAGTTCAAACCGGTGTGAGCCAGGTTGGTTTCTATCTT 1229
M.lanchesteri (Ay03)    CCTTTAAAATTTTACATGATTTGAGTTCAAACCGGTGTGAGCCAGGTTGGTTTCTATCTT 1230
M.lanchesteri (Cs01)    CCTTTAAAATTTTACATGATTTGAGTTCAAACCGGTGTGAGCCAGGTTGGTTTCTATCTT 1228
M.tratense (Kc01)       CCTTTAAAGCTTTACATGATTTGAGTTCAAACCGGTGTGAGCCAGGTTGGTTTCTATCTT 1223
M.tratense (Ut01)       CCTTTAAAGCTTTACATGATTTGAGTTCAAACCGGTGTGAGCCAGGTTGGTTTCTATCTT 1225
M.forcipatum (Tk01)     CCCTTAAAATTTTACATGATTTGAGTTCAAACCGGTGTGAGCCAGGTTGGTTTCTATCTT 1231
M.hirsutimanus (Rb01)   CCTTTAAAATTTTACATGATTTGAGTTCAAACCGGTGTGAGCCAGGTTGGTTTCTATCTT 1232
M.hirsutimanus (Rb02)   CCTTTAAAATTTTACATGATTTGAGTTCAAACCGGTGTGAGCCAGGTTGGTTTCTATCTT 1232
M.niphanae (Kc01)       CCTTTAAAATTTTACATGATTTGAGTTCAAACCGGTGTGAGCCAGGTTGGTTTCTATCTT 1230
M.niphanae (Np01)       CCTTTAAAATTTTACATGATTTGAGTTCAAACCGGTGTGAGCCAGGTTGGTTTCTATCTT 1230
M.niphanae (Np02)       CCTTTAAAATTTTACATGATTTGAGTTCAAACCGGTGTGAGCCAGGTTGGTTTCTATCTT 1230
M.sintangense (Cb02)    CCTTTAAAACTTTACATGATTTGAGTTCAAACCGGTGTGAGCCAGGTTGGTTTCTATCTT 1228
M.sintangense (Cb01)    CCTTTAAAACTTTACATGATTTGAGTTCAAACCGGTGTGAGCCAGGTTGGTTTCTATCTT 1223
M.hirsutimanus (Sb01)   CCTTTAAAATTTTACATGATTTGAGTTCAAACCGGTGTGAGCCAGGTTGGTTTCTATCTT 1232
M.hirsutimanus (Sb02)   CCTTTAAAATTTTACATGATTTGAGTTCAAACCGGTGTGAGCCAGGTTGGTTTCTATCTT 1232
M.hirsutimanus (Sb03)   CCTTTAAAATTTTACATGATTTGAGTTCAAACCGGTGTGAGCCAGGTTGGTTTCTATCTT 1232
M.dienbienphuense(Ny01) CCTTTAAAATTTTACATGATTTGAGTTCAAACCGGTGTGAGCCAGGTTGGTTTCTATCTT 1231
M.hirsutimanus (Ny01)   CCTTTAAAATTTTACATGATTTGAGTTCAAACCGGTGTGAGCCAGGTTGGTTTCTATCTT 1232
M.hirsutimanus (Ny02)   CCTTTAAAATTTTACATGATTTGAGTTCAAACCGGTGTGAGCCAGGTTGGTTTCTATCTT 1232
M.sp3 (Np02)            CCTTTAAAATTTTACATGATTTGAGTTCAAACCGGTGTGAGCCAGGTTGGTTTCTATCTT 1232
M.sp3 (Np01)            CCTTTAAAATTTTACATGATTTGAGTTCAAACCGGTGTGAGCCAGGTTGGTTTCTATCTT 1232
M.niphanae (Ny01)       CCTTTAAAATTTTACATGATTTGAGTTCAAACCGGTGTGAGCCAGGTTGGTTTCTATCTT 1230
M.niphanae (Ny04)       CCTTTAAAATTTTACATGATTTGAGTTCAAACCGGTGTGAGCCAGGTTGGTTTCTATCTT 1227
M.niphanae (Ny03)       CCTTTAAAATTTTACATGATTTGAGTTCAAACCGGTGTGAGCCAGGTTGGTTTCTATCTT 1230
M.niphanae (Ny02)       CCTTTAAAATTTTACATGATTTGAGTTCAAACCGGTGTGAGCCAGGTTGGTTTCTATCTT 1230
M.dienbienphuense(Ny02) CCTTTAAAATTTTACATGATTTGAGTTCAAACCGGTGTGAGCCAGGTTGGTTTCTATCTT 1231
M.rosenbergii(NC_006880)CCTTTAAAATTTTACATGATTTGAGTTCAAACCATTGTGAGCCAGGTTGGTTTCTATCTT 1228
M.rosenbergii (Np01)    CCTTTAAAATTTTACATGATTTGAGTTCAAACCGGTGTGAGCCAGGTTGGTTTCTATCTT 1228
P.monodon               CCTTTAAATCCTTACATGATTTGAGTTCAGACCGGCGTGAGCCAGGTCGGTTTCTATCTC 1283
S.serrata               CCTTTAATTTTTTACATGATTTGAGTTCAGACCGGCGTAAGCCAGGTCGGTTTCTATCTT 1279
S.tranquebarica         CCTTTAATTTTTTACATGATTTGAGTTCAGACCGGCGTAAGCCAGGTCGGTTTCTATCTT 1271
S.olivacea              CCTTTAATTTTTTACATGATTTGAGTTCAGACCGGCGTAAGCCAGGTCGGTTTCTATCTT 1269
B.mandarina             TCATTAAAATCTTACATGATCTGAGTTCAAACCGGTGTGAGCCAGGTTGGTTTCTATCTT 1262
B.mori                  TCATTAAAATCTTACATGATCTGAGTTCAAACCGGTGTGAGCCAGGTTGGTTTCTATCTT 1263
An.gambiae              CCTTTGAATTCTTACATGATCTGAGTTCAAACCGGCGTAAGCCAGGTTGGTTTCTATCTT 1243
An.quadrimaculatus      CCTTTAGATTCTTACATGATCTGAGTTCAAACCGGCGTAA--CAGGTTGGTTTCTATCCT 1239
Ap.mellifera            CTTTTAAAATCTTACATGATTTGAGTTCAAATCGACGTAAGTCAGATTGGTTTCTATCTA 1293
D.melanogaster          CTTTTAAATTCTTACATGATCTGAGTTCAAACCGGTGTAAGCCAGGTTGGTTTCTATCTT 1245
D.yakuba                CTTTTAAATTCTTACATGATCTGAGTTCAAACCGGTGTAAGCCAGGTTGGTTTCTATCTT 1241
                           **      ********* ******** * *   ** *  *** * **********
Escherichia coli        CATTTAAAGTGGTACGCGAGCTGGGTTTAGAACGTCGTGAGACAGTTCGGTCCCTATCTG

                        <-------------------------- Domain V----------------------->
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Figure 20 (continuted)

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M.assamense (Kc01)      TTATTGGTTTAA-AAATGGCTTTAGTACGAAAGGATTGAGCTTTTAAGATAGTCTTT-GA 1288
M.assamense (Kc02)      TTATTGGTTTAA-AAATGGCTTTAGTACGAAAGGATTGAGCTTTTAAGATAGTCTTT-GA 1287
M.lanchesteri (Rb01)    TTATTGGTTTAA-AAATGGCTTTAGTACGAAAGGATTGAGCTTTTAAGATAGTCTTT-GA 1287
M.lanchesteri (Np01)    TTATTGGTTTAA-AAATGGCTTTAGTACGAAAGGATTGAGCTTTTAAAATAGTCTTT-GA 1287
M.lanchesteri (Rb02)    TTATTGGTTTAA-AAATGGCTTTAGTACGAAAGGATTGAGCTTTTAAGATAGTCTTT-GA 1287
M.lanchesteri (Np02)    TTATTGGTTTAA-AAATGGCTTTAGTACGAAAGGATTGAGCTTTTAAGATAGTCTTT-GA 1287
M.lanchesteri (Ay03)    TTATTGGTTTAA-AAATGGCTTTAGTACGAAAGGATTGAGCTTTTAAGATAGTCTTT-GA 1288
M.lanchesteri (Cs01)    TTATTGGTTTAA-AAATGGCTTTAGTACGAAAGGATTGAGCTTTTAAGATAGTCTTT-GA 1286
M.tratense (Kc01)       TTACGGGTTTAA-AAATGGCTTTAGTACGAAAGGATTGGGCTTTTGGGATAATCTTAGTT 1282
M.tratense (Ut01)       TTATGGGTTTAA-AAATGGCTTTAGTACGAAAGGATTGAGCTTTTGGGATAGTCTTAGTT 1284
M.forcipatum (Tk01)     TTATGTGTTAGG-GAGGGGCTTTAGTACGAAAGGATTGAGTCCTTGGGATAATTCTTATT 1290
M.hirsutimanus (Rb01)   TTATATGTTAAT-GAGTGGTCTTAGTACGAAAGGATTAGGCTTCTGGGATAGTTCCT-TT 1290
M.hirsutimanus (Rb02)   TTATATGTTAAT-GAGTGGTCTTAGTACGAAAGGATTAGGCTTCTGGGATAGTTCCT-TT 1290
M.niphanae (Kc01)       TTATAAGTTTAA-AAATGACTTTAGTACGAAAGGATTGAGTCGTTAAGATAGTCTTT-TT 1288
M.niphanae (Np01)       TTATGAGTTTAA-AAGTGGCTTTAGTACGAAAGGATTAAGTCGTTAAGATAGTCTTT-TT 1288
M.niphanae (Np02)       TTATGAGTTTAA-AAGTGGCTTTAGTACGAAAGGATTAAGTCGTTAAGATAGTCTTT-TT 1288
M.sintangense (Cb02)    TTACAGGTTTAA-AAATAGCTTTAGTACGAAAGGATTGGGCTTTTGGGATAGTCTTAGTT 1287
M.sintangense (Cb01)    TTACAGGTTTAA-AAATAGCTTTAGTACGAAAGGATTGGGCTTTTGGGATAGTCTTAGTT 1282
M.hirsutimanus (Sb01)   TTATATGTTAAT-GAGTGGTCTTAGTACGAAAGGATTAGGCTTCTGGGATAGTTCCT-TT 1290
M.hirsutimanus (Sb02)   TTATATGTTAAT-GAGTGGTCTTAGTACGAAAGGATTAGGCTTCTGGGATAGTTCCT-TT 1290
M.hirsutimanus (Sb03)   TTATATGTTAAT-GAGTGGTCTTAGTACGAAAGGATTAGGCTTCTGGGATAGTTCCT-TT 1290
M.dienbienphuense(Ny01) TTATTGATCGGG-AAGGGGCTTTAGTACGAAAGGATTGGGCTCTTGGGATAGTTCTTATT 1290
M.hirsutimanus (Ny01)   TTATATGTTAAT-GAGTGGTCTTAGTACGAAAGGATTAGGCTTCTGGGATAATTCCT-TT 1290
M.hirsutimanus (Ny02)   TTATATGTTAAT-GAGTGGTCTTAGTACGAAAGGATTAGGCTTCTGGGATAGTTCCT-TT 1290
M.sp3 (Np02)            TTATAAGTTTAA-AAATGACTTTAGTACGAAAGGATTGAGTCGTTAAGATAGTCTTT-TT 1290
M.sp3 (Np01)            TTATAAGTTTAA-AAATGACTTTAGTACGAAAGGATTGAGTCGTTAAGATAGTCTTT-TT 1290
M.niphanae (Ny01)       TTATAAGTTTAA-AAATGACTTTAGTACGAAAGGATTGAGTCGTTAAGATAGTCTTT-TT 1288
M.niphanae (Ny04)       TTATGAGTTTAA-AAGTGGCTTTATTACGAAAGGATTAAGTCGTTAAGATAGTCTTT-TT 1285
M.niphanae (Ny03)       TTATAAGTTTAA-AAATGACTTTAGTACGAAAGGATTGAGTCGTTAAGATAGTCTTT-TT 1288
M.niphanae (Ny02)       TTATAAGTTTAA-AAATGACTTTAGTACGAAAGGATTGAGTCGTTAAGATAGTCTTT-TT 1288
M.dienbienphuense(Ny02) TTATTGATCGGG-AAGGGGCTTTAGTACGAAAGGATTGGGCTCTTGGGATAGTTCTTATT 1290
M.rosenbergii(NC_006880)TTAGTGGTTAAA-AAATGGCTTTAGTATGAAAGGATTGAGCTTTTGGGATAATCTTT-GT 1286
M.rosenbergii (Np01)    TTAGTGGTTAAA-AAATGGCTTTAGTACGAAAGGATTGAGCTTTTGGGATAGTCTTT-AT 1286
P.monodon               TTAATTTTATTA-TAATTACTTTAGTACGAAAGGATTAAGTAGTTAAAATAATTTTATTT 1342
S.serrata               CTAGAAAGAAAATAAATTATTTTAGTACGAAAGGATTGAATAATTAAAATTTTTTT--TA 1337
S.tranquebarica         CTAGAGGAAAATAAAATTACTTTAGTACGAAAGGATTAAGTAATTAAAATTTTTTTAGTA 1331
S.olivacea              CTAGGAAGGAATCGAATTACTTTAGTACGAAAGGATTAGGTAGTTAAAATTTTTTTAATA 1329
B.mandarina             TTAATATTTATA----ATATTTTAGTACGAAAGGATTA-AATATTACAATTAT-ATTGAA 1316
B.mori                  TTAATATTTATA----ATATTTTAGTACGAAAGGATTA-AATATTACAATTAT-ATTGAG 1317
An.gambiae              TAATAAATTATT----ATATTGTAGTACGAAAGGACCT-AATATAAAAAATATAATTTTA 1298
An.quadrimaculatus      TAATTAATTATT----ATATTGTAGTACGAAAGGACCT-AATATAAAAAATATAATTTTA 1294
Ap.mellifera            TAATAAAATATT----AATTAATTGTACGAAAGGACTTTAATTTTTAAATTATTTATAAA 1349
D.melanogaster          TAAAAAATTATG----ATATTTTAGTACGAAAGGACCA-AATATCAAAATAATTATATTT 1300
D.yakuba                TAAAAAATTATA----ATATTTTAGTACGAAAGGACCA-AATATTAAAATAATTATATTT 1296
                          *                   *  ** *******             *   *
Escherichia coli        <----2619-2652------ TAGTACGAGAGGACC<-----------------------
                                            [--Sarcin loop--]
                        <------------------------- Domain VI----------------------->
```

Figure 21 Alignment of *16S rRNA* of *Macrobrachium* spp. compared with other arthropods and *E. coli*; Domain VI.

```
M.assamense (Kc01)      ACAAGATACACTTTAATGG----------------------------------------- 1307
M.assamense (Kc02)      ACAAGATACACTTTAATGG----------------------------------------- 1306
M.lanchesteri (Rb01)    ACAAGATACACTTTAATGG----------------------------------------- 1306
M.lanchesteri (Np01)    ACAAGATACACTTTAATGG----------------------------------------- 1306
M.lanchesteri (Rb02)    ACAAGATACACTTTAATGG----------------------------------------- 1306
M.lanchesteri (Np02)    ACAAGATACACTTTAATGG----------------------------------------- 1306
M.lanchesteri (Ay03)    ACAAGATACACTTTAATGG----------------------------------------- 1307
M.lanchesteri (Cs01)    ACAAGATACACTTTAATGG----------------------------------------- 1305
M.tratense (Kc01)       TAAGGATAAACTTTAATGA----------------------------------------- 1301
M.tratense (Ut01)       TAAAGATACATTTTAATGA----------------------------------------- 1303
M.forcipatum (Tk01)     TTGAGATAAACTTTAAATGA---------------------------------------- 1310
M.hirsutimanus (Rb01)   TTGAGATGGTCTTTAAATGA---------------------------------------- 1310
M.hirsutimanus (Rb02)   TTGAGATGGTCTTTAAATGA---------------------------------------- 1310
M.niphanae (Kc01)       TCAAGATGAATATTAATTG----------------------------------------- 1307
M.niphanae (Np01)       TTAAGATGAATATTAATGG----------------------------------------- 1307
M.niphanae (Np02)       TTAAGATGAATATTAATGG----------------------------------------- 1307
M.sintangense (Cb02)    ATAAGATAAACTTTAATGA----------------------------------------- 1306
M.sintangense (Cb01)    ATAAGATAAACTTTAATGA----------------------------------------- 1301
M.hirsutimanus (Sb01)   TTGAGATGGTCTTTAAATGA---------------------------------------- 1310
M.hirsutimanus (Sb02)   TTGAGATGGTCTTTAAATGA---------------------------------------- 1310
M.hirsutimanus (Sb03)   TTGAGATGGTCTTTAAATGA---------------------------------------- 1310
M.dienbienphuense(Ny01) TTGAGATAAACTTTAAATGG---------------------------------------- 1310
M.hirsutimanus (Ny01)   TTGAGATGGTCTTTAAATGA---------------------------------------- 1310
M.hirsutimanus (Ny02)   TTGAGATGGTCTTTAAATGA---------------------------------------- 1310
M.sp3 (Np02)            TCAAGATGAATATTAATTG----------------------------------------- 1309
M.sp3 (Np01)            TCAAGATGAATATTAATTG----------------------------------------- 1309
M.niphanae (Ny01)       TCAAGATTAATATTAATTG----------------------------------------- 1307
M.niphanae (Ny04)       TTAAGATGAATATTAATGG----------------------------------------- 1304
M.niphanae (Ny03)       TCAAGATTAATATTAATTG----------------------------------------- 1307
M.niphanae (Ny02)       TCAAGATTAATATTAATTG----------------------------------------- 1307
M.dienbienphuense(Ny02) TTGAGATAAACTTTAAATGG---------------------------------------- 1310
M.rosenbergii(NC_006880)ATAAGATAAACTTTAATGG----------------------------------------- 1305
M.rosenbergii (Np01)    GTAAGATAAACTTTAATGG----------------------------------------- 1305
P.monodon               ATATGATTAATTTTAATTTATTT------------------------------------- 1365
S.serrata               AAAGAAATTAGTTGAAAC------------------------------------------ 1355
S.tranquebarica         ATTGGAAT---------------------------------------------------- 1339
S.olivacea              ATTGAAAT---------------------------------------------------- 1337
B.mandarina             TTATAT-TGAATTTTATTAAGTTTTATTTAAATTATTAATTATTTATATAATTTATTTAT 1375
B.mori                  TTGTGT-TGAATTTTATTAAGTTTTATTTAAATTAGTAATTATTT---TAATTTATTTAT 1373
An.gambiae              TT-TAAATGAAAATTATTAAAATAATTT-------------------------------- 1325
An.quadrimaculatus      AT-TAATTGAAAATTATTAATATTATTT-------------------------------- 1321
Ap.mellifera            TTAATAATAAATATATTATATA-------------------------------------- 1371
D.melanogaster          TT-TATAAGAATATTATTAATATAAA---------------------------------- 1325
D.yakuba                TTATATAAGAATATTATTAATATATAATAA------------------------------ 1326

Escherichia coli        ------------------------ 2668-2904-------------------------->

                       <------------------------- Domain VI------------------------>
```

Figure 21 (continuted)

3.2 Multiple alignment

The total numbers of sites for alignment were 1,340 sites with 73 gaps, 731 invariable sites and 554 variable sites. The polymorphic sites of all samples were showed in Table 4. There were 438 parsimony informative sites and 116 singleton variable sites were observed.

3.3 Substitution saturation test

The results of substitution saturation test were shown in Figure 22. The number of transitional (TS) and transversional (TV) differences and number of all nucleotide substitution (TS+TV) from pairwise comparison increased with increasing evolutionary distance. It means that substitution saturation dose not occured in this region, so it can reflect phylogenetic relationship of the samples (Miya *et al.*, 2003).

3.4 Best-fit model of nucleotide substitution

The best-fit model which was performed by Modeltest, selected from hLTRs (hierarchical Likelihood Ratio tests) was TrN+I+G (Tamura-Nei+I+G). Tamura-Nei model is under assumption variable base frequencies ($\pi_A \neq \pi_C \neq \pi_G \neq \pi_T$), equal transversion frequencies and variable transition frequencies. Four base frequencies of *16S rRNA* were $\pi_A = 0.4168$, $\pi_C = 0.2005$, $\pi_G = 0.0803$ and $\pi_T = 0.3025$ respectively. User-specified substitution rate matrix was

	A	C	G	T
A	-	1.0000	5.6838	1.0000
C	1.0000	-	1.000000	7.4982
G	5.6838	1.000000	-	1.0000
T	1.0000	7.4982	1.0000	-

Proportion of invariable sites (I) was 0.4320. The sequences of *16S rRNA* had rate heterogeneity which shape parameter (α) was 1.9657.

Table 3 Total length of *16S rRNA* and nucleotide composition of *Macrobrachium* spp. and other organisms

Name	Total length	Nucleotide composition (%)						Nucleotide bias	
		A	C	G	T	A+T	C+G	$\chi^2_{df=3}$	P
M. assamense (Kc01)	1,307	39.63	18.90	9.56	31.91	71.54	28.46	21.49	$P<0.001$
M. assamense (Kc02)	1,305	39.66	18.99	9.50	31.85	71.52	28.48	21.54	$P<0.001$
M. lanchesteri (Rb01)	1,306	39.66	18.99	9.50	31.85	71.52	28.48	21.54	$P<0.001$
M. lanchesteri (Np01)	1,306	39.59	18.99	9.50	31.93	71.52	28.48	21.49	$P<0.001$
M. lanchesteri (Rb02)	1,306	39.66	18.99	9.65	31.70	71.36	28.64	21.27	$P<0.001$
M. lanchesteri (Np02)	1,306	39.74	18.99	9.50	31.78	71.52	28.48	21.59	$P<0.001$
M. lanchesteri (Ay03)	1,307	39.71	18.98	9.49	31.83	71.54	28.46	21.60	$P<0.001$
M. lanchesteri (Cs23)	1,307	39.77	18.93	9.50	31.80	71.57	28.43	21.66	$P<0.001$
M. tratense (Kc01)	1,301	39.59	21.45	9.45	29.52	69.10	30.90	19.50	$P<0.001$
M. tratense (Ut01)	1,303	39.68	21.49	9.36	29.47	69.15	30.85	19.69	$P<0.001$
M. forcipatum (Tk01)	1,310	39.47	23.97	9.77	26.79	66.26	33.74	17.82	$P<0.001$
M. hirsutimanus (Rb01)	1,310	39.01	23.05	10.15	27.79	66.79	33.21	17.13	$P<0.001$
M. hirsutimanus (Rb02)	1,310	39.08	23.05	10.15	27.71	66.79	33.21	17.20	$P<0.001$
M. hirsutimanus (Sb02)	1,310	38.86	23.13	10.38	27.63	66.49	33.51	16.64	$P<0.001$
M. hirsutimanus (Sb03)	1,310	38.86	23.13	10.38	27.63	66.49	33.51	16.64	$P<0.001$
M. hirsutimanus (Sb01)	1,310	38.93	23.13	10.31	27.63	66.57	33.44	16.82	$P<0.001$
M. hirsutimanus (Ny01)	1,310	38.93	23.05	10.23	27.79	66.72	33.28	16.95	$P<0.001$
M. hirsutimanus (Ny02)	1,310	38.93	23.13	10.31	27.63	66.57	33.44	16.82	$P<0.001$

Table 3 (continuted)

Name	Total length	Nucleotide composition (%)						Nucleotide bias	
		A	C	G	T	A+T	C+G	$\chi^2_{df=3}$	P
M. niphanae (Kc01)	1,307	41.78	19.74	8.65	29.84	71.61	28.39	24.00	*P<0.001*
M. niphanae (Np01)	1,307	42.16	19.97	8.88	29.00	71.16	28.84	23.83	*P<0.001*
M. niphanae (Np02)	1,307	41.70	19.82	8.80	29.69	71.39	28.62	23.61	*P<0.001*
M. niphanae (Ny03)	1,307	41.85	19.43	8.72	29.99	71.84	28.16	24.19	*P<0.001*
M. niphanae (Ny02)	1,304	41.26	20.32	8.97	29.45	70.71	29.29	22.52	*P<0.001*
M. niphanae (Ny01)	1,307	41.93	19.43	8.72	29.92	71.84	28.16	24.27	*P<0.001*
M. niphanae (Ny04)	1,307	41.47	19.82	9.18	29.53	71.00	29.00	22.76	*P<0.001*
M. sintangense (Cb02)	1,306	39.89	21.13	9.27	29.71	69.60	30.40	20.26	*P<0.001*
M. sintangense (Cb01)	1,301	39.97	21.14	9.30	29.59	69.56	30.44	20.26	*P<0.001*
M. dienbienphuense (Ny01)	1,310	39.39	23.82	9.85	26.95	66.34	33.66	17.67	*P<0.001*
M. dienbienphuense (Ny02)	1,310	39.39	23.89	9.85	26.87	66.26	33.74	17.66	*P<0.001*
M. sp3 (Np02)	1,309	41.71	19.48	8.63	30.18	71.89	28.11	24.18	*P<0.001*
M. sp3 (Np01)	1,309	41.79	19.40	8.79	30.02	71.81	28.19	24.05	*P<0.001*
M. rosenbergii (NC_006880)	1,305	38.77	23.60	10.35	27.28	62.38	33.95	16.47	*P<0.001*
M. rosenbergii (Np01)	1,305	38.77	23.45	10.35	27.43	62.22	33.79	16.51	*P<0.001*
Exopalaemon carinicauda (NC_012566)	1,296	38.70	17.90	10.30	33.10	71.80	28.28	20.79	*P<0.001*
Penaeus monodon	1,365	38.02	15.46	9.67	36.85	53.48	25.13	24.82	*P<0.001*
Litopenaeus stylirostris	1,369	34.97	18.11	9.90	37.02	71.99	28.01	20.78	*P<0.001*
Eriocheir sinensis	1,311	38.22	15.03	7.55	39.21	77.42	22.58	31.22	*P<0.001*

Table 3 (continuted)

Name	Total length	Nucleotide composition (%)						Nucleotide bias	
		A	C	G	T	A+T	C+G	$\chi^2_{df=3}$	P
Scylla serrata	1,355	35.06	15.28	8.19	41.48	76.53	23.47	29.98	*P<0.001*
Anopheles quadrimaculatus	1,321	43.94	11.89	5.91	38.26	82.20	17.80	42.83	*P<0.001*
Bombyx mori	1,375	44.32	18.11	9.90	37.02	84.35	15.65	48.37	*P<0.001*
Drosophila melanogaster	1,325	42.26	15.03	7.55	39.21	82.94	17.06	43.99	*P<0.001*
Recticulitermes hageni	1,315	46.84	20.46	9.66	23.04	69.89	30.11	29.98	*P<0.001*
Tigriopus californicus	1,020	36.08	12.75	15.88	35.29	71.37	28.63	18.48	*P<0.001*

Table 4 Polymorphic sites of all *Macrobrachium* samples

Samples	Haplotype	Nucleotide position							
						1111	1111111111	1112222222	2222222223
		11111	1122222222	3333334445	5666666666	7777770033	3455556667	7780123366	7778899990
		1234901237	8901345679	3457890233	6012345789	0134565601	7014570141	4619618969	0171424590
M. assamense (Kc01)	A01	-CCAAGTGTT	TGTTCAAAGC	CTTAAAGTCG	CAGCCATTTT	AACCAACCAT	ATTTACAAGT	GTTCCATTTT	TTAAAACTAT
M. assamense (Kc02)	A02	-.........	....	....	....	....	....	....	....
M. lanchesteri (Rb01)	A03	-.........	....	....	....	....	....	....	....
M. lanchesteri (Np01)	A04	-.........	....	T.........	....	....	....	....	....
M. lanchesteri (Rb02)	A05	-.........	....	....	....	....	....	....G.G...	....
M. lanchesteri (Np02)	A06	-.........	....	....	....	....	....	....	....
M. lanchesteri (Ay03)	A02	-.........	....	....	....	....	....	....	....
M. lanchesteri (Cs01)	A07	-.........	....	....	....	....	....	....	....
M. tratense (Kc01)	A08	-T.....T.C	.TAA.T...T	.CC....C..	....	....CG..GC	...C..G..C	A.C..G.CA.	.....C.A.C
M. tratense (Ut01)	A09	-T...A....	.TAA.T....	.CC.......	....	....C...GC	..CC..G..C	A.C..G.CA.	.....C.A.C
M. forcipatum (Tk01)	A10	T.AT...T..	CAAAT..GAT	.CC.GGA...	CCCCC	T..AC.....	G.C....G.C	..C....C.C	C....C.A..
M. hirsutimanus (Rb01)	A11	T.AT..ACC.	CAAA-.GGA.	.CCG...CT.	.GA...CCA.	T..AT.....	...C.....C	..C......C	.....C.A..
M. hirsutimanus (Rb02)	A12	T.AT..ACC.	CAAA-.GGA.	.CCG...CT.	.GA...CCA.	T..AT.....	...C.....C	..C......C	.....C.A..
M. hirsutimanus (Sb02)	A13	T.AT..ACC.	CAAA-.GGA.	.CCG...CT.	.GA...CCA.	T..AT.....	...C.....C	..C......C	.....C.A..
M. hirsutimanus (Sb03)	A13	T.AT..ACC.	CAAA-.GGA.	.CCG...CT.	.GA...CCA.	T..AT.....	...C.....C	..C......C	.....C.A..
M. hirsutimanus (Sb01)	A14	T.AT..ACC.	CAAA-.GGA.	.CCG...CT.	.GA...CCA.	T..AT.....	...C.....C	..C......C	.....C.A..
M. hirsutimanus (Ny01)	A15	T.AT..ACC.	CAAA-.GGAT	.CCG...CT.	.GA...CCA.	T..AT.....	...C.....C	..C......C	.....C.A..
M. hirsutimanus (Ny02)	A16	T.AT..ACC.	CAAA-.GGA.	.CCG...CT.	.GA...CCA.	T..AT.....	...C.....C	.GC......C	.....C.A..
M. dienbienphuense (Ny01)	A17	C.AT...T..	CAAAT..GA.	.CC.G..C..	.....CC.CC	CGT.......	..C....G.C	A.C....C.C	C....T.A..

Table 4 (continuted)

Samples	Haplotype	Nucleotide position							
						1111	1111111111	1112222222	2222222223
		11111	1122222222	3333334445	5666666666	7777770033	3455556667	7780123366	7778899990
		1234901237	8901345679	3457890233	6012345789	0134565601	7014570141	4619618969	0171424590
M. dienbienphuense (Ny02)	A18	C.AT...T..	CAAAT..GA.	.CC.G..C..	.....CC.CC	CGT.......	..C....G.C	A.C....C.C	C....T.A..
M. niphanae (Ny03)	A19	-.A.TA.TA.	..AA-.....	....CGA...	...T......	...TT.....	..CC.....C	A......C..	.C..T.AA..
M. niphanae (Ny04)	A20	-.A.TA.TA.	..AA-.....	....CGA...	...T......	...TT.....	..CC.....C	A......C..	.C..T.AA..
M. niphanae (Kc01)	A21	-.A.TA.TC.	..AA-.....	....CGA...	...T......	...TT.....	..CC.....C	A......C..	.C..T.AA..
M. niphanae (Np01)	A22	-...TA.TC.	.AAA-.....	....CGA.T.	......C...	...TC.....	..CC.....C	A......C..	CC....AA..
M. niphanae (Np02)	A23	-...TA.TC.	.AAA-.....	....CGA.T.	......C...	...TC.....	..CC.....C	A......C..	C.....AA..
M. niphanae (Ny02)	A24	-...TA.TC.	.AAA-.....	....CGA.T.	A.....C...	...TC.....	..CC.....C	A......C..	.C..T.AA..
M. niphanae (Ny01)	A25	-.A.TA.TA.	..AA-.....	....CGA...	...T......	...TT.....	..CC.....C	A......C..	.C..T.AA..
M. sp3 (Np02)	A26	-.A.TA.TC.	..AA-.....	....CGA...	...T......	...TT.....	..CC....AC	A......C..	.C..T.AA..
M. sp3 (Np01)	A27	-.A.TA.TC.	..AA-.....	....CGA...	...T......	...TT.....	.GCC....AC	A......C..	.C..T.AA..
M. sintangense (Cb02)	A28	-T.....T..	.A.A.T....	.CC....C..	....T.....	....TG..G.	..CCTAG..C	A.CA...CA.	.....T.A.C
M. sintangense (Cb01)	A28	-T.....T..	.A.A.T....	.CC....C..	....T.....	....TG..G.	..CCTAG..C	A.CA...CA.	.....T.A.C
M. rosenbergii (NC_006880)	A29	-......T..	.A.A.....T	.CC......A	....	T....CAT..	..CC.....C	.......C.C	C.CG..T.G.
M. rosenbergii (Np01)	A30	-......T..	.ACAT.....	.CC.......	....	T....C....	..CC.....C	.......C.C	C.CG..T.G.

Table 4 (continued)

Samples	Haplotype	Nucleotide position							
		3333333333	3333333333	3333333333	3333333334	4444444444	4444444444	4444444445	5555555556
		0000011111	1233444456	6666666777	7888999990	0000000001	1111445555	7888999990	0023579992
		1345601267	9901012640	1345789456	7478235670	1234567890	1238560157	0789012364	5826596788
M. assamense (Kc01)	A01	TGCATATTCA	TTACTTCTGT	ATCTAAAACT	CTCTAACGCG	TATTAACTAG	ATTTATACTG	CCACCGTAAT	TTACTTATAT
M. assamense (Kc02)	A02	....	....	....	....	....	....	....	....
M. lanchesteri (Rb01)	A03	....	....	....	....	.......C..	....	....	....
M. lanchesteri (Np01)	A04	....	....	....	....	....	....	....	....
M. lanchesteri (Rb02)	A05	....	....	....	....	....	....	....	....
M. lanchesteri (Np02)	A06	....	....	....	....	....	....	....	....
M. lanchesteri (Ay03)	A02	....	....	....	....	....	....	....	....
M. lanchesteri (Cs01)	A07	....	....	....	....	....	....	....	....
M. tratense (Kc01)	A08	.CAGCTAAA.	C..T.A....	.A.CC..G..	AC.....ATA	CCAAGTTACT	T..A.C.T..	.TCT.A...C	C.TTCAG.TC
M. tratense (Ut01)	A09	.CAGCTAAA.	C..T.A....	.A.CC..G..	AC.....ATA	CCAAGTTACT	C..A.C.T..	.TCT.A...C	C.TTCAG.TC
M. forcipatum (Tk01)	A10	CTA.CTAA..	CC..CA..A.	GG......T.	AC.C.....A	.CAAGC.A.A	CCC..CG...	....T.C..A	..CTC..C-C
M. hirsutimanus (Rb01)	A11	CTA.CTAA..	CC...A..A.	.G.C.G...C	AC.C..T..A	.CAAGCTA.A	CC...C.T.A	....T.C.GC	.CCTC..C-C
M. hirsutimanus (Rb02)	A12	CTA.CTAA..	CC...A..A.	.G.C.G...C	AC.C..T..A	.CAAGCTA.A	CC...C.T.A	....T.C.GC	.CCTC..C-C
M. hirsutimanus (Sb02)	A13	CTA.CTAA..	CC...A..A.	.G.C.....C	AC.C..T..A	.CAAGCTA.A	CC...C...A	....T.C.GC	.CCTC..C-C
M. hirsutimanus (Sb03)	A13	CTA.CTAA..	CC...A..A.	.G.C.....C	AC.C..T..A	.CAAGCTA.A	CC...C...A	....T.C.GC	.CCTC..C-C
M. hirsutimanus (Sb01)	A14	CTA.CTAA..	CC...A..A.	.G.C.....C	AC.C..T..A	.CAAGCTA.A	CC...C...A	....T.C.GC	.CCTC..C-C
M. hirsutimanus (Ny01)	A15	CTA.CTAA..	CC...A..A.	.G.C.....C	AC.C..T..A	.CAAGCTA.A	CC...C...A	....T.C.GC	.CCTC..C-C
M. hirsutimanus (Ny02)	A16	CTA.CTAA..	CC...A..A.	.G.C.....C	AC.C..T..A	.CAAGCTA.A	CC...C...A	....T.C.GC	.CCTC..C-C
M. dienbienphuense (Ny01)	A17	.TA.CTAA..	CC..CA..A.	GG.C....T.	AC.......A	.CAAGC.ACA	CCC..C.T..	....T.C.GA	..C.C..C-C
M. dienbienphuense (Ny02)	A18	.TA.CTAA..	CC..CA..A.	GG.C....T.	AC.......A	.CAAGC.ACA	CCC..C.T..	....T.C.GA	..C.C..C-C

Table 4 (continued)

Samples	Haplotype	Nucleotide position							
		3333333333	3333333333	3333333333	3333333334	4444444444	4444444444	4444444445	5555555556
		0000011111	1233444456	6666666777	7888999990	0000000001	1111445555	7888999990	0023579992
		1345601267	9901012640	1345789456	7478235670	1234567890	1238560157	0789012364	5826596788
M. niphanae (Ny03)	A19	.TA..TAAT.	.C.T.ATGA.	.A......TC	AC.CTTATAA	.TAAGT.A.A	CC...C.T.A	TT.TT....C	.CTTC...-C
M. niphanae (Ny04)	A20	.TA..TAAT.	.C.T.ATGA.	.A......TC	AC.CTTATAA	.TAAGT.A.A	CC...C.T.A	TT.TT....C	.CTTC...-C
M. niphanae (Kc01)	A21	.TA..TAAT.	.C.T.ATGA.	.A......TC	AC.CTTATAA	.TAAGT.A.A	CC...C.T.A	TT.TT....C	.CTTC...-C
M. niphanae (Np01)	A22	.TA..TAAT.	.C.T.ATGAC	.A...G..TC	ACTCTTATAA	.TAA.T.A..	CC...C.T.A	TT.GT....C	.CTTC...-C
M. niphanae (Np02)	A23	.TA..TAAT.	.C.T.ATGAC	.A...G..TC	ACTCTTATAA	.TAA.T.A..	CC...C.T.A	TT.TT....C	.CTTC...-C
M. niphanae (Ny02)	A24	.TA..TAAT.	.C.T.ATGAC	.A......TC	AC.CTTATAA	.TAAGT.A.A	CC...C.T.A	TT.TT....C	.CTTC...-C
M. niphanae (Ny01)	A25	.TA..TAAT.	.C.T.ATGA.	.A......TC	AC.CTTATAA	.TAAGT.A.A	CC...C.T.A	TT.TT....C	.CTTC...-C
M. sp3 (Np02)	A26	.TA..TAAT.	.C.T.ATGA.	.A......TC	AC.CTTATAA	.TAAGT.A.A	CC...C.T.A	TT.TT....C	.CTTC...-C
M. sp3 (Np01)	A27	.TA..TAAT.	.C.T.ATGA.	.A......TC	AC.CTTATAA	.TAAGT.A.A	CC...C.T.A	TT.TT....C	.CTTC...-C
M. sintangense (Cb02)	A28	.CA..CAAA.	CC...A....	.AT.T.GGT.	A.T...TATA	CCAA.CTACT	T..A.C..C.	..CT.A...C	C.TTCA..CC
M. sintangense (Cb01)	A28	.CA..CAAA.	CC...A....	.AT.T.GGT.	A.T...TATA	CCAA.CTACT	T..A.C..C.	..CT.A...C	C.TTCA..CC
M. rosenbergii (NC_006880)	A29	CATGC.AATT	.CT.......	.A.C.G....	AC.CC.....	.TAAGC...A	....CC....	..G...CTGC	....C....C
M. rosenbergii (Np01)	A30	CATGC.AATT	.CT.......	.A.C.G....	AC.CC.....	.TAAGC...A	....CC....	..G...CTG.	....C....C

Table 4 (continuted)

Samples	Haplotype	Nucleotide position							
		6666666666	6666666666	7777777777	7777777777	7777777777	7777777777	7777777777	7777777788
		3336667788	8999999999	0000000111	1122222222	3333333444	4444555667	7777888888	8899999900
		0235780118	9012456789	0126789057	8901234579	0245679123	5689234391	4789012456	7924567912
M. assamense (Kc01)	A01	ATTGATAAGT	TTAATTCTCT	CCAATTTTAA	TAAATAATAT	CAATTATATA	CTAATTCTTT	AGTTGTAAAA	CAATAACTTT
M. assamense (Kc02)	A02	....	....	....	....	........C.	....	....	....
M. lanchesteri (Rb01)	A03	....	....	....	....	........C.	....	....	....
M. lanchesteri (Np01)	A04	....	....	....	....	........C.	....	....	....
M. lanchesteri (Rb02)	A05	....	....	....	....	........C.	....	....	....
M. lanchesteri (Np02)	A06	....	....	....	....	........C.	....	....	....
M. lanchesteri (Ay03)	A02	....	....	....	....	........C.	....	....	....
M. lanchesteri (Cs01)	A07	....	....	....	....	........C.	....	....	....
M. tratense (Kc01)	A08	CCAA......	.A..GA....	TA.-CAC...	.TTC.T-CCC	A.T..TA.A.	-CTTC.A.CA	TTCCAAC...	T...G.A...
M. tratense (Ut01)	A09	CCAA......	.A..GA....	TA.-CAC...	.TTC.T-CCC	A.T..TA.A.	-CTCC.A.CA	TTCCAAC...	T...G.A...
M. forcipatum (Tk01)	A10	...A...TAC	...GAC.C..	.G.-CCA.CC	.....TTCCC	-.CG..A.C.	ACTT.CA...	TT.CA.T...	...C..A.CC
M. hirsutimanus (Rb01)	A11	...AG.GTAC	CATGAC....	.GT-CCACTC	.T...TTCC.	-.CA..A.C.	ACTT..ACC.	TT..A.T...	..G...A.CC
M. hirsutimanus (Rb02)	A12	...AG.GTAC	CATGAC....	.GT-CCACTC	.T...TTCC.	-.CA..A.C.	ACTT..ACC.	TT..A.T...	..G...A.CC
M. hirsutimanus (Sb02)	A13	...AG.GTAC	CAGGAC....	.GT-CCACTC	.T...TTCC.	-.CA..A.CG	ACTT..ACC.	TT..A.T...	..G...A.CC
M. hirsutimanus (Sb03)	A13	...AG.GTAC	CAGGAC....	.GT-CCACTC	.T...TTCC.	-.CA..A.CG	ACTT..ACC.	TT..A.T...	..G...A.CC
M. hirsutimanus (Sb01)	A14	...AG.GTAC	CAGGAC....	.GT-CCACTC	.T...TTCC.	-.CA..A.CG	ACTT..ACC.	TT..A.T...	..G...A.CC
M. hirsutimanus (Ny01)	A15	...AG.GTAC	CATGAC....	.GT-CCACTC	.T...TTCC.	-.CA..A.CG	ACTT..ACC.	TT..A.T...	..G...A.CC
M. hirsutimanus (Ny02)	A16	...AG.GTAC	CATGAC....	.GT-CCACTC	.T...TTCC.	-.CA..A.CG	ACTT..ACC.	TT..A.T...	..G...A.CC
M. dienbienphuense (Ny01)	A17	...A...TAC	...GAC.C..	.GG-C.A.CC	.....TT.CC	-.CA..A.C.	AC.T.CA...	TT.CA.T.G.	......A.CC

Table 4 (continued)

Samples	Haplotype	Nucleotide position							
		6666666666	6666666666	7777777777	7777777777	7777777777	7777777777	7777777777	7777777788
		3336667788	8999999999	0000000111	1122222222	3333333444	4444555667	7777888888	8899999900
		0235780118	9012456789	0126789057	8901234579	0245679123	5689234391	4789012456	7924567912
M. dienbienphuense (Ny02)	A18	...A...TAC	...GAC.C..	.GG-C.A.CC	.....TT.CC	-.CA..A.C.	AC.T.CA...	TT.CA.T.G.	......A.CC
M. niphanae (Ny03)	A19	...AGC....	.A..AC.AAA	.A.-A.A.T.	CC..CT-CC.	-..C..A.C.	ACTT..A...	TT.CACT...	.G....ACC.
M. niphanae (Ny04)	A20	...AGC....	.A..AC.AAA	.A.-A.A.T.	CC..CT-CC.	-..C..A.C.	ACTT..A...	TT.CACT...	.G....ACC.
M. niphanae (Kc01)	A21	...AGC....	.A..AC.AAA	.A.-A.A.T.	CC..CT-CC.	-..C..A.C.	ACTT..A...	TT.CACT...	.G....ACC.
M. niphanae (Np01)	A22	...AGC....	.A..AC.AAA	.A.-A.A.T.	CC..CT-CC.	-.....A.C.	ACTT..A...	TT.CACT...	.G....ACC.
M. niphanae (Np02)	A23	...AGC....	.A..AC.AAA	.A.-A.A.T.	CC..CT-CC.	-.....A.C.	ACTT..A...	TT.CACT...	.G....ACC.
M. niphanae (Ny02)	A24	...AGC....	.A..AC.AAA	.A.-A.A.T.	CC..CT-CC.	-.....A.C.	ACTT..A...	TT.CACT...	.G....ACC.
M. niphanae (Ny01)	A25	...AGC....	.A..AC.AAA	.A.-A.A.C.	CC..CT-CC.	-..C..A.C.	ACTT..A...	TT.CACT...	.G....ACC.
M. sp3 (Np02)	A26	...AGC....	.A..AC.AAA	.A.-A.A.T.	CC..CT-CC.	-.....A.C.	ACTT..A...	TT.CACT...	......ACC.
M. sp3 (Np01)	A27	...AGC....	.A..AC.AAA	.A.-A.A.T.	CC..CT-CC.	-.....A.C.	ACTT..A...	TT.CACT...	.G....ACC.
M. sintangense (Cb02)	A28	...A......	.A..GA..T.	TA.-.AC..C	CTTT.C-CCC	AGT...A.A.	ACTC.CA.CA	TTCCACCT..	....G.AC..
M. sintangense (Cb01)	A28	...A......	.A..GA..T.	TA.-.AC..C	CTTT.C-CCC	AGT...A.A.	ACTC.CA.CA	TTCCACCT..	....G.AC..
M. rosenbergii (NC_006880)	A29	.....C...C	.A.GGCT...	.A.C.AC..T	C......CCC	T...A.AT..	..C.......	..CC.CT..T	...CGG...C
M. rosenbergii (Np01)	A30	.....C...C	.A.GGC....	.A.C.AC...	C......CCC	T...A.AT..	..C.......	..CC.CT..T	...CGG...C

Table 4 (continuted)

Samples	Haplotype	Nucleotide position							
		8888888888	8888888888	8888888888	8888888888	8899999999	9999999999	9999999999	9999999999
		0000011111	2222233333	4444445556	7777788899	9900000001	1111112222	2333334556	6666667777
		3456901346	1245935679	0135690189	0567823546	7913456780	3457890134	6245678791	3456892346
M. assamense (Kc01)	A01	TCGCAT-AAG	GTTTTTTAA-	-AAATAATTC	TTGGCTTTTC	AATATATCT-	ATAAACACTT	CAACTACTCA	AACTTCGTTA
M. assamense (Kc02)	A02	......-...	.........-	-.........	....	.........-	....	....	....
M. lanchesteri (Rb01)	A03	......-...	.........-	-.........	....	.........-	....	....	....
M. lanchesteri (Np01)	A04	......-...	.........-	-.........	....	.........-	....	....	....
M. lanchesteri (Rb02)	A05	......-...	.........-	-.........	....	......C..-	....	....	....
M. lanchesteri (Np02)	A06	......-...	.........-	-.........	.......A..	.........-	....	....	....
M. lanchesteri (Ay03)	A02	......-...	.........-	-.........	....	.........-	....	....	....
M. lanchesteri (Cs01)	A07	......-...	.........-	-.........	....	.........-	....	...A......	....
M. tratense (Kc01)	A08	.TA..A-.T.	ACC.A....-	-TC.AGT...	.....C..CA	T.C.CT.AC-	....GTC..A	.CT.AGT...	TCTCCTACCC
M. tratense (Ut01)	A09	.TA..A-.T.	ACC.A....-	-TC.AGT...	.....C..CA	T.C.CT.AC-	....GTC..A	.CT.AGT...	TCTCCTACCC
M. forcipatum (Tk01)	A10	.TA.GAT.CA	AC..A.A..T	-.C.CG.C.T	C..A.CC.CA	..CGATAACC	CC....GTCA	.T.AAGT..C	CG..C.A...
M. hirsutimanus (Rb01)	A11	CTA.GACGTA	AC..AA...C	-.C..G.C.T	.....C..CA	..C.GTCACC	C...-TGTCG	TT.AAG...C	T...C.A...
M. hirsutimanus (Rb02)	A12	CTA.GACGTA	AC..AA...C	-.C..G.C.T	.....C.ACA	..C.GTCACC	C...-TGTCG	TT.AAG...C	T...C.A...
M. hirsutimanus (Sb02)	A13	CTA.GACGTA	AC..AA...C	-.C..G.C.T	.....C..CA	..C.GTCACC	C...-TGTCG	TT.AAG...C	T...C.A...
M. hirsutimanus (Sb03)	A13	CTA.GACGTA	AC..AA...C	-.C..G.C.T	.....C..CA	..C.GTCACC	C...-TGTCG	TT.AAG...C	T...C.A...
M. hirsutimanus (Sb01)	A14	CTA.GACGTA	AC..AA...C	-.C..G.C.T	.....C..CA	..C.GTCACC	C...-TGTCG	TT.AAG...C	T...C.A...
M. hirsutimanus (Ny01)	A15	CTA.GACGTA	AC..AA...C	-.C..G.C.T	.....C..CA	..C.GTCACC	C...-TGTCG	TT.AAG...C	T...C.A...
M. hirsutimanus (Ny02)	A16	CTA.GACGTA	AC..AA...C	-.C..G.C.T	.....C..CA	..C.GTCACC	C...-TGTCG	TT.AAG...C	T...C.A...
M. dienbienphuense (Ny01)	A17	.TA.GAT.CA	AC..A.A..T	-.C.CG.C.T	C....CC.CA	..CGATAACC	CC....G.CA	TT.AAGT..C	C...C.A...
M. dienbienphuense (Ny02)	A18	.TA.GAT.CA	AC..A.A..T	-.C.CG.C.T	C....CC.CA	..CGATAACC	CC....G.CA	TT.AAGT..C	C...C.A...

Table 4 (continted)

Samples	Haplotype	Nucleotide position							
		8888888888	8888888888	8888888888	8888888888	8899999999	9999999999	9999999999	9999999999
		0000011111	2222233333	4444445556	7777788899	9900000001	1111112222	2333334556	6666667777
		3456901346	1245935679	0135690189	0567823546	7913456780	3457890134	6245678791	3456892346
M. niphanae (Np02)	A19	.....A-TCA	ACA.A.AG.C	C.C.CG....	.........A	..C.A..A.T	....-TGT.A	TC.AAG....	T...ATA...
M. niphanae (Ny01)	A20	...T.A-TCA	ACA.A.A..C	T.C.C....A	.........A	....A..A.T	....-TGT.A	TC.AAG.C..	T...CTA...
M. niphanae (Ny02)	A21	.....A-TCA	ACA.A.AG.C	C.C.CG....	.G..G....A	..C.A..A.T	....-TGT.A	TC.AAG...C	T...CTA...
M. niphanae (Ny03)	A22	...T.A-TCA	ACA.A.A..C	T.C.C.....	.........A	....A..A.T	....-TGT.A	TC.AAG....	T...CTA...
M. niphanae (Ny04)	A23	...T.A-TCA	ACA.A.A..C	T.C.C.....	.G..G....A	..C.A..A.T	....-GGT.A	TC.AAG....	T...CTA...
M. sintangense (Cb01)	A24	.TA..A-.T.	ACC.A...TC	ATT.A.T...	..A.....CA	T.C.CT.AC-	.AG.GTC..A	.CT.AGT..C	TCT.CTACCC
M. sintangense (Cb02)	A24	.TA..A-.T.	ACC.A...TC	ATT.A.T...	..A.....CA	T.C.CT.AC-	.AG.GTC..A	.CT.AGT..C	TCT.CTACCC
M. dienbienphuense (Ny01)	A25	.TA.GAT.CA	AC..A.A..T	-.C.CG.C.T	C....CC.CA	..CGATAACC	CC....G.CA	TT.AAGT..C	C...C.A...
M. dienbienphuense (Ny02)	A26	.TA.GAT.CA	AC..A.A..T	-.C.CG.C.T	C....CC.CA	..CGATAACC	CC....G.CA	TT.AAGT..C	C...C.A...
M. sp3 (Np01)	A27	.....A-TCA	ACA.A.AG.C	C.C.CG....	.........A	..C.A..A.T	....-TGT.A	TC.AAG....	T...CTA...
M. sp3 (Np02)	A28	.....A-TCA	ACA.A.AG.C	C.C.CG....	.........A	..C.A..A.T	....-TGT.A	TC.AAG....	T...CTA...
M. rosenbergii (Np01)	A29	C.....-...	ACCC.A...-	-..CC...C.	C.......C.	....A.A..-	...G..G..A	.C..AG..TG	......A...
M. rosenbergii (NC_006880)	A30	C.....-...	ACCC.A...-	-..CC..CC.	C.......C.	.G..A.A..-	...G..G..A	.C..AG..TG	......A...

Table 4 (continuted)

Samples	Haplotype	Nucleotide position							
			1111111	1111111111	1111111111	1111111111	1111111111	1111111111	1111111111
		9999999999	9990000000	0000000000	0000000000	0000000000	0001111111	1111111111	1111111111
		7788888888	8990011111	1333333344	5555566666	7777788889	9990001112	2222333333	4444455557
		7801245678	9013901234	5013467989	4568901479	0146823461	4590590143	4569025689	0124501374
M. assamense (Kc01)	A01	-TTTACTTAA	TGCCAACGAA	AAGTCCCACC	AAGTTCGAAT	CATACTAAAC	CATTGACCGC	TCCTATACTG	TCACTTTCTA
M. assamense (Kc02)	A02	-.........	....	....	....	....	....	....	....
M. lanchesteri (Rb01)	A03	-.........	..T.......	....	....	....	....	....	....
M. lanchesteri (Np01)	A04	-......C..	....	....	....	....	....	......T...	....
M. lanchesteri (Rb02)	A05	-.........	....	....	....	....	....	....	....
M. lanchesteri (Np02)	A06	-.........	....	....	....	....	....	....	....
M. lanchesteri (Ay03)	A02	-.........	....	....	....	....	....	....	....
M. lanchesteri (Cs01)	A07	-.........	....	....	....	....	....	....	....
M. tratense (Kc01)	A08	AA.AGT...G	.A..CGTA..	...C.A....	..AC.T-.GC	T...A..GG.	T...AT...T	CTT..A...A	ATTTAA....
M. tratense (Ut01)	A09	AA.AGT...G	.A..CGTA..	...C.A....	..AC.T-.GC	T...A..GG.	T...AT.T.T	CTT..A...A	ATTTAA....
M. forcipatum (Tk01)	A10	A.AA..CC.G	...T..TA..	GGA..A.GT.	.GA.A.T..C	.TC.AA.G.T	..CC.TT...	C....C..C.	CT.AA.A...
M. hirsutimanus (Rb01)	A11	G..A..CAGG	..TT..TA.G	G.A..A....	.GA.A.CG.C	.TCGAA.G.T	T..C.TT.AT	.T.C.C....	CT.AA.AT.G
M. hirsutimanus (Rb02)	A12	G..A..CAGG	..TT..TA.G	G.A..A....	.GA.A.CG.C	.TCGAA.G.T	T..C.TT.AT	.T.C.C....	CT.AA.AT.G
M. hirsutimanus (Sb02)	A13	G..A..CAGG	..TT..TA.G	G.A..A....	.GA.A.CG.C	.TCGAA.G.T	T..C.TT.AT	.T.C.C....	CT.AA.AT.G
M. hirsutimanus (Sb03)	A13	G..A..CAGG	..TT..TA.G	G.A..A....	.GA.A.CG.C	.TCGAA.G.T	T..C.TT.AT	.T.C.C....	CT.AA.AT.G
M. hirsutimanus (Sb01)	A14	G..A..CAGG	..TT..TA.G	G.A..A....	.GA.A.CG.C	.TCGAA.G.T	T..C.TT.AT	.T.C.C....	CT.AA.AT.G
M. hirsutimanus (Ny01)	A15	G..A..CAGG	..TT..TA.G	G.A..A....	.GA.A.CG.C	.TCGAA.G.T	T..C.TT.AT	.T.C.C....	CT.AA.AT.G
M. hirsutimanus (Ny02)	A16	G..A..CAGG	..TT..TA.G	G.A..A....	.GA.A.CG.C	.TCGAA.G.T	T..C.TT.AT	.T.C.C....	CT.AA.AT.G
M. dienbienphuense (Ny01)	A17	A.AA...C.G	...T..TA.G	GGA..A.G..	.GA.ATT..C	.TC.AA.G.T	..CC.TT...	C....CG.C.	CT.AA.A...

Table 4 (continuted)

Samples	Haplotype	Nucleotide position							
			1111111	1111111111	1111111111	1111111111	1111111111	1111111111	1111111111
		9999999999	9990000000	0000000000	0000000000	0000000000	0001111111	1111111111	1111111111
		7788888888	8990011111	1333333344	5555566666	7777788889	9990001112	2222333333	4444455557
		7801245678	9013901234	5013467989	4568901479	0146823461	4590590143	4569025689	0124501374
M. dienbienphuense (Ny02)	A18	A.AA...C.G	...T..TA.G	GGA..A.G..	.GA.ATT..C	.TC.AA.G.T	..CC.TT...	C....CG.C.	CT.AA.A...
M. niphanae (Ny03)	A19	A.AA.....G	..TT......	...C.AT..T	G.A.C.T..C	.TC.AAT..A	TG.CAT.T.T	ATT.G....A	A.TTA.A...
M. niphanae (Ny04)	A20	G.AA.....G	G.TT......	G..CGAT..T	G.A.C.T..C	.TC.AAT..A	T..CAT.T.T	ATT.G....A	A.TTA.A...
M. niphanae (Kc01)	A21	A.AA.....G	..TT......	...C.AT..T	G.A.C.T..C	.TC.AAT..A	TG.CAT.T.T	ATT......A	A.TTA.A...
M. niphanae (Np01)	A22	A.AA.....G	..TT......	...C.AT..T	G.A.C.T..C	.TC.AA...A	TG..AT.T.T	ATT....T.A	A.TTA.A...
M. niphanae (Np02)	A23	A.AA.....G	..TT......	...C.AT..T	G.A.C.T..C	.TC.AAT..A	TG..AT.T.T	ATT....T.A	A.TTA.A...
M. niphanae (Ny02)	A24	A.AA.....G	..TT......	G..C.AT..T	G.A.C.T..C	.T..AAT..A	T...AT.T.T	ATT......A	A.TTA.A.C.
M. niphanae (Ny01)	A25	A.AA.....G	..TT......	...C.AT..T	G.A.C.T..C	.TC.AAT..A	TG.CAT.T.T	ATT.G....A	A.TTA.A...
M. sp3 (Np02)	A26	A.AA.....G	..TT......	G..C.AT..T	G.A.C.T..C	.T..AAT..A	T...AT.T.T	ATT......A	A.TTA.A...
M. sp3 (Np01)	A27	AAAA.....G	..TT......	G..C.AT..T	G.A.C.T..C	.T..AAT..A	T...AT.T.T	ATT......A	A.TTA.A...
M. sintangense (Cb02)	A28	AAAAGTC..G	CAA.C.TA..	...C.A....	..AC.T-C.C	T...A..GG.	T...AT....	CT.A.C...A	A.T.A.....
M. sintangense (Cb01)	A28	AAAAGTC..G	CAA.C.TA..	...C.A....	..AC.T-C.C	T...A..GG.	T...AT....	CT.A.C...A	A.T.A.....
M. rosenbergii (NC_006880)	A29	ACC.....G.	CA......G.	..A..A....	G..CC.A...	....	..CC.T.T..	.....C.T.A	C..T..C...
M. rosenbergii (Np01)	A30	AC......G.	CA......G.	..A..A..T.	G...C.A...	....	..CC.T.T..	.....C.T.A	C..T..C...

Table 4 (continuted)

Samples	Haplotype	Nucleotide position							
		1111111111	1111111111	1111111111	1111111111	1111111111	1111111111	1111111111	1111111111
		1111111111	1111122222	2222222222	2222222222	2222222222	2222222222	2222233333	3333333333
		7777788899	9999900000	0111122333	3344444455	5566666677	7788888889	9999900000	1111111111
		5678912301	2356801278	9145605057	8901268903	8912467801	3901234673	4568915679	0123456789
M. assamense (Kc01)	A01	AATTTATATT	AATTATTATA	TTATTGTAAT	TTCAA-CTAC	AAAATCTAGC	ATTAATTATA	TCTATATATA	AACAAATTCA
M. assamense (Kc02)	A02	....	....	....	.....-....	....	....	....	....
M. lanchesteri (Rb01)	A03	....	....	....	.....-....	....	....	....	....
M. lanchesteri (Np01)	A04	....	....	....	.....-....	....	....	....	....
M. lanchesteri (Rb02)	A05	....	....	....	.....-....	....	....	....	....
M. lanchesteri (Np02)	A06	....	....	....	.....-....	....	....	....	....
M. lanchesteri (Ay03)	A02	....	....	....	.....-....	....	....	....	....
M. lanchesteri (Cs01)	A07	....	....	....	.....-....	....	....	....	....
M. tratense (Kc01)	A08	.CCCC.C..A	.CAA.ACC..	A.....C...	C..T.-A..A	TTTT....AA	..CT......	AAA.CT.T..	..TC..CAT.
M. tratense (Ut01)	A09	.CCCC.C..A	.CAA.ACC..	A.....C...	C..T.-A..A	TTTT....AA	..CT......	AAA.CT.T..	..TC..CAT.
M. forcipatum (Tk01)	A10	.T.CC.C...	CTCA...CC.	C.........	.CTCG-A..A	CT.C....TA	TC.T.C....	.AA.A.ACA.	......AC..
M. hirsutimanus (Rb01)	A11	T..CC.C.C.	CCCA...C.G	CC......T.	CCTT.-AC.A	TT.C....TA	CC.T.C....	.GA.A.ACA.	......AC..
M. hirsutimanus (Rb02)	A12	T..CC.C.C.	CCCA...C.G	CC......T.	CCTT.-AC.A	TT.C....TA	CC.T.C....	.GA.A.ACA.	......AC..
M. hirsutimanus (Sb02)	A13	T..CC.C.C.	CCCAG..C.G	CC......T.	CCTT.-AC.A	TT.C....TA	CC.T.C....	.GAGA.ACA.	......AC..
M. hirsutimanus (Sb03)	A13	T..CC.C.C.	CCCAG..C.G	CC......T.	CCTT.-AC.A	TT.C....TA	CC.T.C....	.GAGA.ACA.	......AC..
M. hirsutimanus (Sb01)	A14	T..CC.C.C.	CCCA...C.G	CC......T.	CCTT.-AC.A	TT.C....TA	CC.T.C....	.GAGA.ACA.	......AC..
M. hirsutimanus (Ny01)	A15	T..CC.C.C.	CCCAG..C.G	CC......T.	CCTT.-AC.A	TT.C....TA	CC.T.C....	.GA.A.ACA.	......AC..
M. hirsutimanus (Ny02)	A16	T..CC.C.C.	CCCAG..C.G	CC......T.	CCTT.-AC.A	TT.C....TA	CC.T.C....	.GA.A.ACA.	......AC..
M. dienbienphuense (Ny01)	A17	.T..C.C.C.	CC.A...CC.	C..C.....C	.CTC.-AC.A	TT.C....TA	CC.T.C....	.AA.A.ACA.	.C....A...

Table 4 (continuted)

Samples	Haplotype	Nucleotide position							
		1111111111	1111111111	1111111111	1111111111	1111111111	1111111111	1111111111	1111111111
		1111111111	1111122222	2222222222	2222222222	2222222222	2222222222	2222233333	3333333333
		7777788899	9999900000	0111122333	3344444455	5566666677	7788888889	9999900000	1111111111
		5678912301	2356801278	9145605057	8901268903	8912467801	3901234673	4568915679	0123456789
M. dienbienphuense (Ny02)	A18	.T..C.C.C.	CC.A...CC.	C..C.....C	.CTC.-AC.A	TT.C....TA	CC.T.CC...	.AA.A.ACA.	.C....A...
M. niphanae (Ny03)	A19	..CCACC..A	CT.A...T..	A...C.....	CAAT.AA.CA	.T.C....AA	.C.C......	.AA.A.ATA.	.C..CCAAA.
M. niphanae (Ny04)	A20	..CCACC..A	CT.A...T..	A...CA....	CA...CA.CA	.T.CG.A.AA	.C.CTC.T..	.AA.A.ACAC	.C..CCCAA.
M. niphanae (Kc01)	A21	..CCACC..A	CT.A...T..	A...C.....	CAATCAA.CA	.T.C....AA	.C.C......	.AA.A.ACA.	.C..CCAAA.
M. niphanae (Np01)	A22	..CCACC..A	CTAA...TA.	A...C.....	CA--.AA.CA	.T.C....AA	.C.C......	.AA.A.ACA.	.C..CCAAA.
M. niphanae (Np02)	A23	..CCACC..A	CT.A...T..	A...C.....	CA--.AA.CA	.T.C....AA	.C.C......	.AA.A.ACA.	.C..CCAAA.
M. niphanae (Ny02)	A24	..CCACC..A	CT.A...T..	A...C.....	CA...CA.CA	.T.C.TA.AA	.C.CTC.TAT	.AA.A.GCAC	.C..CCCCA.
M. niphanae (Ny01)	A25	..CCACC..A	CT.A...T..	A...C.....	CAAT.AA.CA	.T.C....AA	.C.C......	.AA.A.ATA.	....CCAAA.
M. sp3 (Np02)	A26	..CCAC...A	CT.A...T..	A...C.....	CA...CA.CA	.T.C....AA	.C.C......	.AA.A.ACA.	....CTAAA.
M. sp3 (Np01)	A27	..CCAC...A	CT.A...T..	A...C.....	CA...CA.CA	.T.C....AA	.C.C......	.AA.A.ACA.	....CTAAA.
M. sintangense (Cb02)	A28	...CA.C..A	.TAA.A.T..	A.G.C..G..	C.TT.-A..A	TTTC...GAA	.CCT......	AAA.CT.T..	..TC..CAT.
M. sintangense (Cb01)	A28	...CA.C..A	.TAA.A.T..	A.G.C..G..	C.TT.-A..A	TTTC...GAA	.CCT......	AAA.CT.T..	..TC..CAT.
M. rosenbergii (NC_006880)	A29	..C.C.CG.A	.CC...C.A.	C...C.C..C	C.T..-AC.A	.CG.....T.	..C.......	.G......C.	C.....CC.C
M. rosenbergii (Np01)	A30	..C.C.CG.A	.CC...C.A.	C...C.C..C	C.T..-AC.A	.CG.....T.	..C.......	.G......C.	C.....GC.C

Table 4 (continuted)

Samples	Haplotype	Nucleotide position	
		1111111111	111111
		3333333333	333333
		2222223333	333334
		0134790234	567890
M. assamense (Kc01)	A01	ATT-TATAAC	AACTAT
M. assamense (Kc02)	A02	...-......	......
M. lanchesteri (Rb01)	A03	...-......	......
M. lanchesteri (Np01)	A04	...-......	......
M. lanchesteri (Rb02)	A05	...-......	......
M. lanchesteri (Np02)	A06	...-......	......
M. lanchesteri (Ay03)	A02	...-......	......
M. lanchesteri (Cs01)	A07	...-......	.....-
M. tratense (Kc01)	A08	TC.-...CTT	..T.--
M. tratense (Ut01)	A09	TC.-...CTT	..T.T.
M. forcipatum (Tk01)	A10	.ACG..CCTT	..TCC.
M. hirsutimanus (Rb01)	A11	.A.A..CCTT	...CCC
M. hirsutimanus (Rb02)	A12	.A.A..CCTT	...CCC
M. hirsutimanus (Sb02)	A13	.A.A..CCTT	...CCC
M. hirsutimanus (Sb03)	A13	.A.A..CCTT	...CCC
M. hirsutimanus (Sb01)	A14	.A.A..CCTT	...CCC
M. hirsutimanus (Ny01)	A15	.A.A..CCTT	...CCC
M. hirsutimanus (Ny02)	A16	.A.A..CCTT	...CCC
M. dienbienphuense (Ny01)	A17	.A.G..CC.T	T..CT.

Table 4 (continuted)

Samples	Haplotype	Nucleotide position	
		1111111111	111111
		3333333333	333333
		2222223333	333334
		0134790234	567890
M. dienbienphuense (Ny02)	A18	.A.G..CC.T	T..CT.
M. niphanae (Ny03)	A19	CA.-...CTT	....T.
M. niphanae (Ny04)	A20	CA.-...CTT	.T..T.
M. niphanae (Kc01)	A21	CA.-...CTT	....T.
M. niphanae (Np01)	A22	CA.-...CT.	....T.
M. niphanae (Np02)	A23	CA.-...CTT	....T.
M. niphanae (Ny02)	A24	CAA-AT.CTT	T-----
M. niphanae (Ny01)	A25	CA.-...CTT	.T..T.
M. sp3 (Np02)	A26	TA.-...CTT	.T..T.
M. sp3 (Np01)	A27	TA.-...CTT	..T.T.
M. sintangense (Cb02)	A28	.A.-...TTT	....T.
M. sintangense (Cb01)	A28	.A.-...TTT	.-----
M. rosenbergii (NC_006880)	A29	.C.-......	.....-
M. rosenbergii (Np01)	A30	.C.-......	......

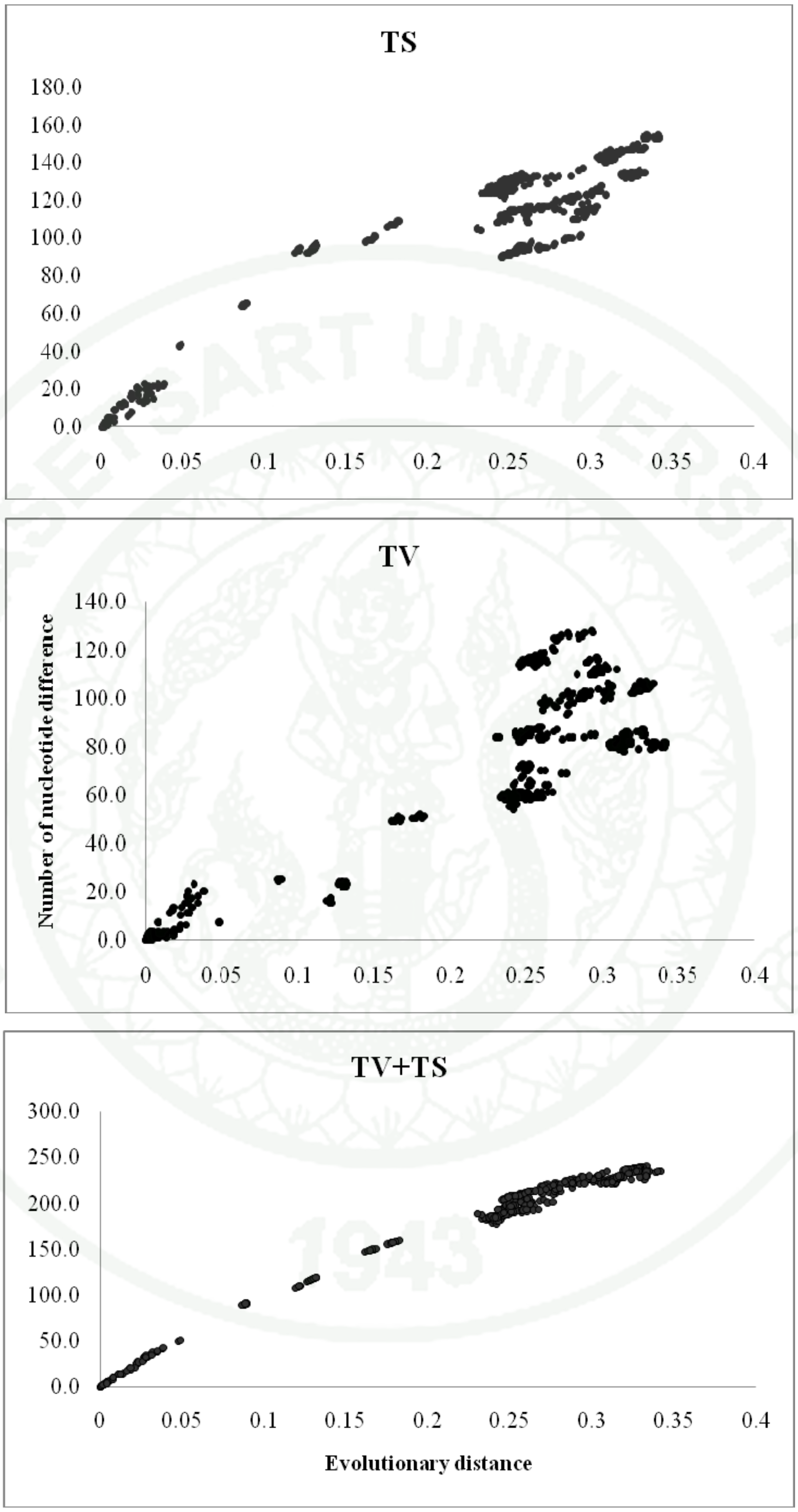

Figure 22 Transitional (TS), transveraional (TS) difference and number of all nucleotide substitution (X axis) were plotted against pairwise evolutionary distance (Y axis).

3.5 Evolutionary distance and nucleotide divergence

Evolutionary distance of all samples calculated under substitution model, TrN+I+G was showed in Table 5. Average nucleotide divergence from DnaSP was showed in Table 6. The above diagonal data showed d_{XY} between groups. The below diagonal data showed average nucleotide divergence (d_A) between groups, the information in bracket was average number of nucleotide differences between groups. The information diagonal showed nucleotide diversity within group and the number in bracket was average number of nucleotide differences within group.
Average evolutionary distance of *Macrobrachium* and outgroup was showed in Table 7, the information diagonal showed genetic distance within group. Dash stands for the sample that genetic distance within group unavailable because there was one sequence.

The intraspecific *16S rRNA* sequence divergence estimates between individual from different locality ranged 0.00-2.2% (Table 6). This shows low level of genetic difference in different provinces. On the other hand, nucleotide divergence levels among *Macrobrachium* species were high, ranging from 2-18.9% Average number of nucleotide differences ranged from 26.2- 245.2. In the same way, average evolutionary distance (Table 7) within groups showed low level, ranging from 0.00-2.6%. Average evolutionary distance among *Macrobrachium* species were high, ranging from 2.3- 33.5%.

Table 5 Evolutionary distance of *Macrobrachium* spp

.

Name	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. *M. assamense* (Kc01)																
2. *M. assamense* (Kc02)	0.001															
3. *M. lanchesteri* (Rb01)	0.002	0.002														
4. *M. lanchesteri* (Np01)	0.003	0.002	0.004													
5. *M. lanchesteri* (Rb02)	0.003	0.002	0.004	0.005												
6. *M. lanchesteri* (Np02)	0.002	0.001	0.002	0.003	0.003											
7. *M. lanchesteri* (Ay03)	0.001	0.000	0.002	0.002	0.002	0.001										
8. *M. lanchesteri* (Cs01)	0.002	0.001	0.002	0.003	0.003	0.002	0.001									
9. *M. tratense* (Kc01)	0.313	0.313	0.317	0.322	0.321	0.315	0.313	0.315								
10. *M. tratense* (Ut01)	0.309	0.309	0.312	0.318	0.316	0.311	0.309	0.312	0.008							
11. *M. forcipatum* (Tk)	0.324	0.320	0.324	0.322	0.324	0.322	0.320	0.319	0.341	0.342						
12. *M. hirsutimanus* (Rb01)	0.351	0.347	0.343	0.353	0.348	0.350	0.347	0.343	0.321	0.329	0.122					
13. *M. hirsutimanus* (Rb 02)	0.354	0.350	0.346	0.356	0.350	0.348	0.350	0.345	0.323	0.331	0.123	0.001				
14. *M. hirsutimanus* (Sb02)	0.352	0.348	0.344	0.354	0.348	0.350	0.348	0.343	0.330	0.338	0.124	0.005	0.005			
15. *M. hirsutimanus* (Sb03)	0.352	0.348	0.344	0.354	0.348	0.350	0.348	0.343	0.330	0.338	0.124	0.005	0.005	0.000		
16. *M. hirsutimanus* (Sb01)	0.350	0.346	0.342	0.351	0.346	0.348	0.346	0.341	0.327	0.335	0.122	0.004	0.005	0.001	0.001	
17. *M. hirsutimanus* (Ny01)	0.354	0.349	0.346	0.355	0.350	0.352	0.350	0.345	0.323	0.338	0.121	0.004	0.005	0.002	0.002	0.003
18. *M. hirsutimanus* (Ny02)	0.351	0.347	0.344	0.353	0.348	0.350	0.348	0.343	0.328	0.337	0.123	0.004	0.005	0.002	0.002	0.003
19. *M. dienbienphuense* (Ny01)	0.301	0.298	0.301	0.298	0.302	0.300	0.298	0.297	0.336	0.333	0.048	0.128	0.129	0.132	0.132	0.131
20. *M. dienbienphuense* (Ny02)	0.304	0.301	0.304	0.301	0.305	0.303	0.301	0.300	0.340	0.337	0.049	0.130	0.131	0.134	0.134	0.133
21. *M. niphanae* (Ny03)	0.262	0.259	0.256	0.266	0.265	0.261	0.259	0.258	0.266	0.248	0.254	0.246	0.248	0.253	0.253	0.251
22. *M. niphanae* (Ny04)	0.283	0.280	0.277	0.287	0.286	0.282	0.280	0.278	0.290	0.271	0.260	0.255	0.257	0.262	0.262	0.260
23. *M. niphanae* (Kc)	0.260	0.257	0.254	0.264	0.263	0.259	0.257	0.256	0.267	0.249	0.247	0.239	0.240	0.245	0.245	0.243
24. *M. niphanae* (Np 01)	0.267	0.264	0.261	0.271	0.270	0.262	0.264	0.263	0.274	0.255	0.249	0.246	0.245	0.257	0.257	0.255
25. *M. niphanae* (Np 02)	0.262	0.259	0.257	0.267	0.266	0.261	0.259	0.258	0.266	0.248	0.245	0.240	0.241	0.251	0.251	0.249
26. *M. niphanae* (Ny 02)	0.293	0.289	0.286	0.297	0.296	0.291	0.289	0.288	0.284	0.267	0.256	0.252	0.254	0.258	0.258	0.257
27. *M. niphanae* (Ny01)	0.267	0.264	0.261	0.271	0.270	0.266	0.264	0.263	0.271	0.253	0.253	0.251	0.253	0.258	0.258	0.256
28. *M. sp3* (Np02)	0.251	0.248	0.246	0.255	0.254	0.250	0.248	0.247	0.253	0.236	0.250	0.241	0.243	0.248	0.248	0.246
29. *M. sp3* (Np01)	0.257	0.254	0.252	0.261	0.260	0.256	0.254	0.253	0.250	0.234	0.251	0.248	0.249	0.254	0.254	0.253

Table 5 (continuted)

Name	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
30. *M. sintangense* (Cb02)	0.307	0.307	0.307	0.315	0.314	0.309	0.307	0.310	0.089	0.090	0.354	0.354	0.356	0.356	0.356	0.353
31. *M. sintangense* (Cb01)	0.307	0.307	0.307	0.316	0.315	0.309	0.307	0.309	0.088	0.089	0.344	0.346	0.348	0.348	0.348	0.345
32. *M. rosenbergii* (NC_006880)	0.179	0.181	0.186	0.187	0.184	0.183	0.181	0.183	0.321	0.317	0.304	0.344	0.346	0.352	0.352	0.349
33. *M. rosenbergii* (Np01)	0.165	0.167	0.171	0.172	0.169	0.168	0.167	0.168	0.319	0.307	0.288	0.332	0.334	0.339	0.339	0.337

Name	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
17. *M. hirsutimanus* (Ny01)																
18. *M. hirsutimanus* (Ny02)	0.002															
19. *M. dienbienphuense* (Ny01)	0.133	0.132														
20. *M. dienbienphuense* (Ny02)	0.134	0.134	0.001													
21. *M. niphanae* (Ny03)	0.254	0.252	0.266	0.269												
22. *M. niphanae* (Ny04)	0.263	0.261	0.272	0.276	0.017											
23. *M. niphanae* (Kc)	0.246	0.244	0.260	0.263	0.004	0.020										
24. *M. niphanae* (Np 01)	0.258	0.257	0.268	0.271	0.026	0.038	0.023									
25. *M. niphanae* (Np 02)	0.251	0.250	0.259	0.263	0.021	0.035	0.019	0.008								
26. *M. niphanae* (Ny 02)	0.259	0.258	0.282	0.286	0.036	0.028	0.033	0.032	0.027							
27. *M. niphanae* (Ny01)	0.259	0.257	0.268	0.271	0.004	0.020	0.008	0.031	0.026	0.039						
28. *M. sp3* (Np02)	0.249	0.247	0.265	0.269	0.018	0.023	0.016	0.029	0.022	0.027	0.019					
29. *M. sp3* (Np01)	0.255	0.254	0.273	0.277	0.019	0.026	0.017	0.029	0.023	0.028	0.022	0.004				
30. *M. sintangense* (Cb02)	0.356	0.354	0.348	0.353	0.260	0.283	0.261	0.265	0.258	0.290	0.265	0.257	0.261			
31. *M. sintangense* (Cb01)	0.348	0.346	0.348	0.352	0.262	0.284	0.263	0.267	0.260	0.290	0.266	0.258	0.260	0.000		
32. *M. rosenbergii* (NC_006880)	0.345	0.350	0.306	0.309	0.296	0.317	0.293	0.288	0.290	0.325	0.301	0.297	0.302	0.329	0.329	
33. *M. rosenbergii* (Np01)	0.339	0.338	0.289	0.292	0.279	0.301	0.276	0.272	0.273	0.309	0.284	0.280	0.284	0.326	0.326	0.012

Table 6 Average nucleotide divergence of *Macrobrachium* spp.

	1	2	3	4	5	6	7	8	9	10	11
1 *M. assamense*	0.00077 (1)	0.002 (2.2)	0.180 (233)	0.180 (232.5)	0.189 (245.2)	0.166 (214.2)	0.181 (234)	0.175 (227)	0.163 (211)	0.119 (155.5)	0.172 (329.5)
2 *M. lanchesteri*	0.002	0.008 (3)	0.181 (234.5)	0.180 (232.7)	0.189 (244)	0.167 (214.5)	0.182 (235)	0.175 (227)	0.163 (211.3)	0.121 (157.5)	0.076 (331)
3 *M. tratense*	0.180	0.181	0.008 (10)	0.176 (227)	0.178 (230.5)	0.158 (203.9)	0.071 (92)	0.177 (228.5)	0.153 (197.5)	0.182 (157.5)	0.181 (342)
4 *M. forcipatum*	0.180	0.180	0.176	-	0.091 (118.4)	0.145 (187.7)	0.179 (231)	0.042 (55.5)	0.145 (188.5)	0.175 (227)	0.261 (335)
5 *M. hirsutimanus*	0.189	0.189	0.178	0.091	0.003 (4)	0.150 (194.4)	0.184 (238)	0.096 (125.6)	0.150 (195.6)	0.189 (244.5)	0.069 (341)
6 *M. niphanae*	0.166	0.167	0.158	0.145	0.150	0.022 (26.19)	0.161 (209)	0.153 (197.4)	0.020 (26.2)	0.175 (225.8)	0.076 (310)
7 *M. sintangense*	0.181	0.182	0.071	0.179	0.184	0.161	0.0000 (0)	0.182 (234.5)	0.157 (203.5)	0.187 (241.5)	0.180 (345)
8 *M. dienbienphuense*	0.175	0.175	0.177	0.042	0.096	0.153	0.182	0.00076 (1)	0.153 (199)	0.177 (229.5)	0.178 (341.5)
9 *M. sp3*	0.163	0.163	0.153	0.145	0.150	0.020	0.157	0.153	0.004 (3)	0.175 (226.5)	0.163 (311.5)
10 *M. rosenbergii*	0.119	0.121	0.182	0.175	0.189	0.175	0.187	0.177	0.175	0.012 (14)	0.184 (345.5)
11 *E. carinicauda*	0.258	0.259	0.267	0.261	0.266	0.266	0.243	0.242	0.270	0.270	-

The above diagonal data showed d_{XY} between groups. The below diagonal data showed average nucleotide divergence (d_A) between groups. The number in bracket was average number of nucleotide differences between groups. The diagonal data showed nucleotide diversity within group and the number in bracket was average number of nucleotide differences within group.

Table 7 Average evolutionary distance of *Macrobrachium* spp.

	1	2	3	4	5	6	7	8	9	10	11
1 *M. assamense*	0.001										
2 *M. lanchesteri*	0.002	0.003									
3 *M. tratense*	0.311	0.315	0.008								
4 *M. forcipatum*	0.322	0.322	0.342	-							
5 *M. hirsutimanus*	0.350	0.348	0.330	0.123	0.003						
6 *M. dienbienphuense*	0.301	0.301	0.337	0.048	0.123	0.001					
7 *M. niphanae*	0.269	0.269	0.265	0.252	0.253	0.270	0.026				
8 *M. sp3*	0.252	0.253	0.244	0.250	0.249	0.273	0.023	0.004			
9 *M. sintangense*	0.307	0.310	0.089	0.349	0.351	0.350	0.271	0.259	0.000		
10 *M. rosenbergii*	0.173	0.177	0.316	0.296	0.343	0.299	0.294	0.291	0.327	0.012	
11 *E. carinicauda*	0.596	0.606	0.658	0.633	0.672	0.690	0.537	0.525	0.696	0.658	-

The diagonal data showed evolutionary distance within group. Dash stands for the sample that genetic distance within group unavailable because there was one sequence.

According to the genetic species concept, species is a group of genetically identical individuals. To date it had been known that not only all subspecies but also the populations within the subspecies are genetically different (Mayr, 1964). Currently, levels of sequence variation in mitochondrial genes (e.g. *Cytb*, *16S rRNA* and *ND1*) have been used as a reference point for decision species-level distinction. In decapods, nucleotide divergence of *16S rRNA* ranges from 3.5% among species, 17.6% among genera, 23.5% among families, and up to 26.2% among infraorders of Pleocyemata (Toon *et al.*, 2009). In this study, average nucleotide divergence which calculated from complete *16S rRNA* sequence of *Exoplaemon carinicauda* (NC_012566) showed 26.02% divergence from *Macrobrachium*.

Currently, crustacean species within a genus showed sequence divergence or evolutionary distance in mitochondrial marker gene ranging from 2% to 17% (Murphy and Austin, 2002; Sangthong, 2006; Schubart *et al.*, 2000a). In this study, the evolutionary distance for recognized species of *Macrobrachium*; *M. lanchesteri* and *M. rosenbergii* was 17.7%. This value is close to the range of divergence level had been reported in Australain *Macrobrachium* shrimp. The mean divergence levels amongst *Macrobrachium* species is at the higher level of that normally found between species and overlaps with levels commonly found between genera in other crustaceans (Fetzner and Crandall, 2002; Murphy and Austin, 2002). Interestingly, evolutionary distance amomg individual of *M. niphanae* and between taxa, *M. niphanae* and *M. sp3*, were 2.6 and 2.3% respectively. These values were quite high when compared with other decapods. The evolutionary distance between two cryptic specis of kuruma shrimp, *Penaeus japonicus* is about 1% in *16S rRNA* (Tsoi *et al.*, 2005) and about 1.2% in cryptic species of clawed lobster, *Metanephrops neptunus* (Chan *et al.*, 2009). Moreover, evolutionary distance in species complex of Australain *Macrobrachium* shrimp, *Macrobrachium austrliense* from 12 sites throughout eastern and central Australia showed 0.2-1.6%. While evolutionary distance among 4 additional *Macrobrachium* species ranged from 8.4-13.4%. The low divergence level between individual of *M. australiense* complex from 12 sites were typical for those obsevered among populations within species (Murphy *et al.*, 2004). Thus, evolutionary distance 2.6% within *M. niphanae* group might be indicated that the species consist of more than one species. Similarly, evolutionary distance 2.3% between *M.sp3* and *M. niphanae* might be indicated that *M. sp3* could be species. The lowest evolutionary distance among taxa was 0.2% (*M. assamense* and *M. lanchesteri*). This result showed that *M.assamense* have no genetic difference from *M. lanchesteri* although they were clear morphological different, e.g. second periopod, longitudinal groove at finger. Unfortunately, specimens of *M. assamense* were found a few in this study, thus the species are necessary to find and analys for confermation species status in further. The highest evolutionary distance among taxa in genus *Macrobrachium* and the evolutionary distance between *Macrobrachium* and *Exopalaemon* were 35.1% and 62.17% respectively. These values are in range of pairwise divergence between species of the same genus (23.0-40.2% for extreme upper) and between genus of the same family which reported by Lefébure *et al*. (2006).

3.6 Phylogenetic tree

3.6.1 Neibour joining

The Neibour joining (NJ) tree was constructed with the data from *16S rRNA* sequcences of all *Macrobrachium* and *Exopalaemon carinicauda* was used as an outgroup (Figure 23). The best-fit model was TrN+I+G and the reliability of inferred tree was tested by bootstrap test (1000 replications). Consensus tree was constructed by 50% majority rule consensus method. The NJ tree consisted 23 interior branches with 50-100% bootstrap value support. There were 12 interior branches with 100% bootstrap values. Those were interior brance which connect internal node of group of *Macrobrachium* species.

3.6.2 Maximum parsimony

The Maximum parsimony (MP) tree was contructed from the data from *16S rRNA* sequences of all *Macrobrachium* and outgroup (Figure 24). There were 438 parsimony informative sites were used in analysis and reliability of inferred tree was tested by bootstrap test (1000 replications). The MP tree consisted 21 interior branches with 50-100% bootstrap vaule support. There were 11 interior branches with 100% bootstrap values. Those were interior brance which connect internal node of group of *Macrobrachium* species.

3.6.3 Maximum likelihood

The Maximum likelihood (ML) tree was constracted from the data from *16S rRNA* sequences of all *Macrobrachium* and outgroup (Figrue 25). The best-fit model was TrN+I+G and the reliability of inferred tree was tested by bootstrap test (100 replications). The ML tree consisted 22 interior branches with 50-100% bootstrap value support. There were 9 interior branches with 100% bootstrap values. Those were interior brance which connect internal node of group of *Macrobrachium* species.

The phylogenetic trees produced by MP and ML methods were similar (Figure 24-25). Contradiction in topology was presence or absence of peripheral node. There were three major clades.

Clade I was *M. niphanae* clade, composed of *M. niphanae* and *M. sp3*. Morphological characters of two species in this clade were quite similar, rostrum straight, second periopods cylindrical and similar in form. *M. sp3* differed from *M. niphanae* in had on fine velvety pubescence on second periopods.

Clade II was *M. pilimanus* clade. This clade was composed of three species, *M. forcipatum*, *M. dienbienphuenses* and *M. hirsutimanus* which belong to *Macrobrachium pilimanus* species group. The species in this clade has morphological chracters clearly different from other *Macrobrachium*. Rostrum was short and slightly convex. Second periopod was robust, chela covered with dense and long pubescence.

Clade III, other *Macrobrachium* spp., was composed of the rest species, *M. assamense*, *M. lanchesteri*, *M. rosenbergii, M. tratense* and *M. sintangense.* This clade was quite variable in species. The major characters of this clade were; second periopod cylindrical, similar in form and major leg longer than body distinctly.

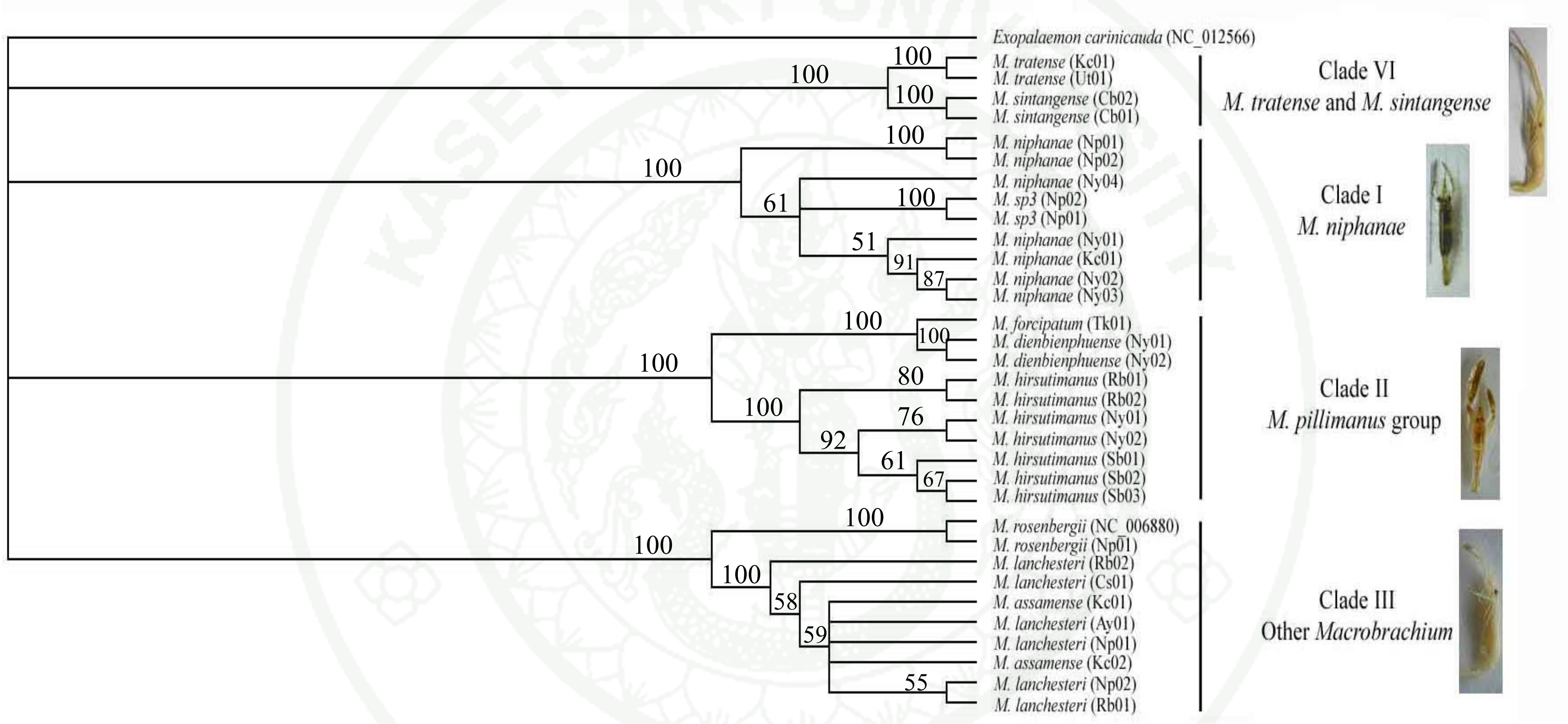

Figure 23 Topologies of phylogenetic tree from the NJ method, 50% majority. The number at the node indicated bootstrap support values.

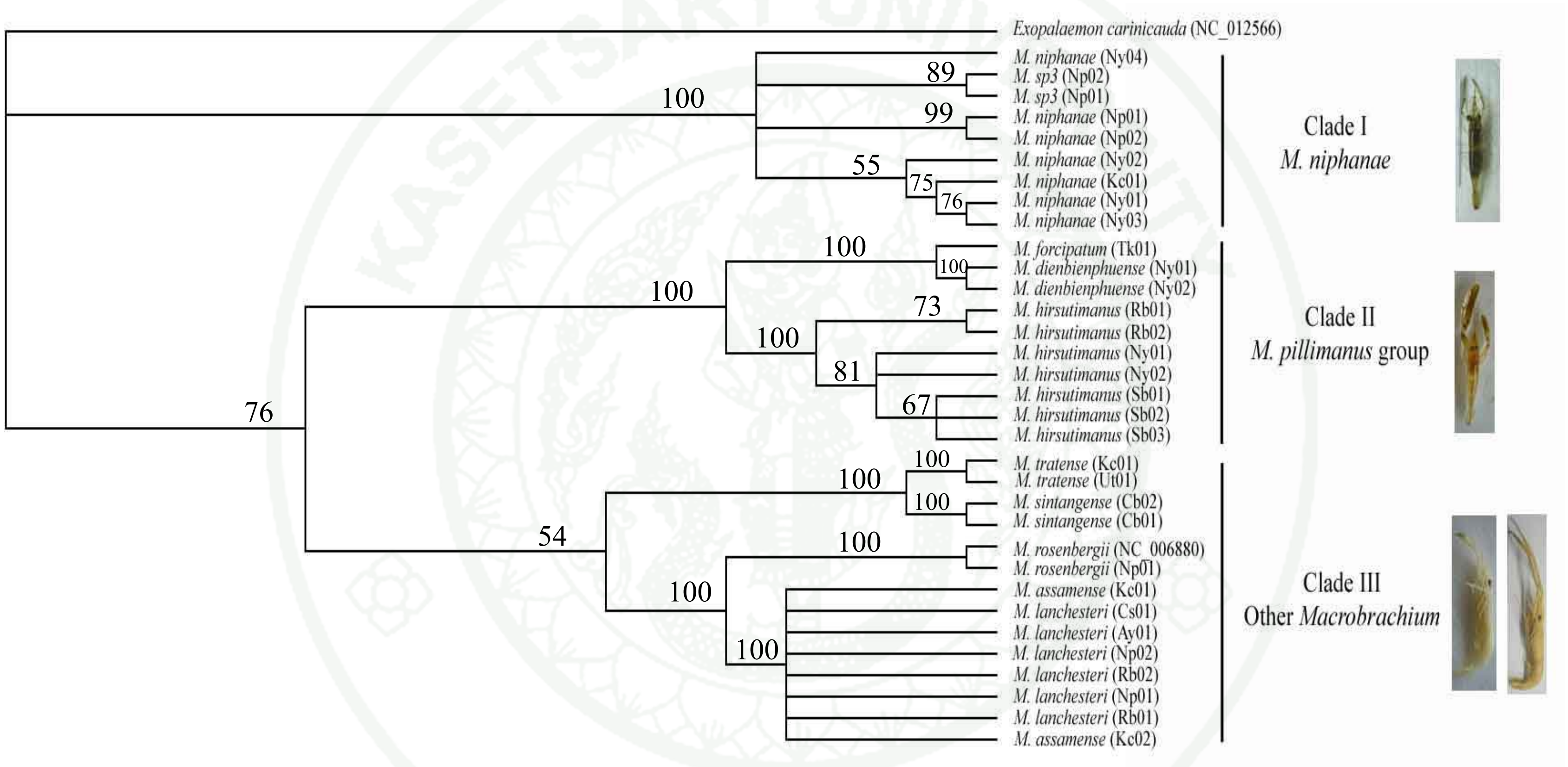

Figure 24 Topologies of phylogenetic tree from the MP method, 50% majority. The number at the node indicated bootstrap support values.

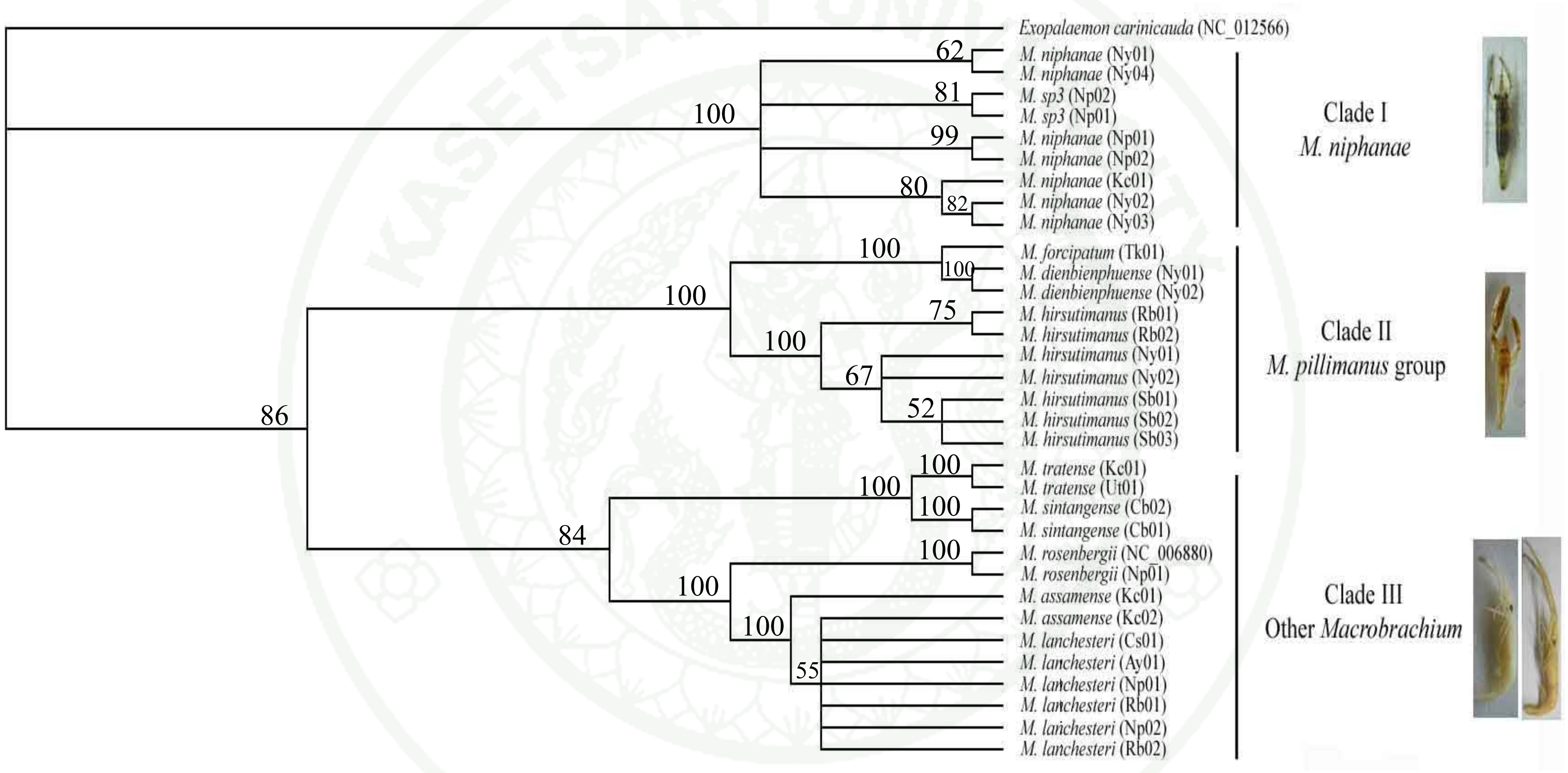

Figure 25 Topologies of phylogenetic tree from the ML method, 50% majority. The number at the node indicated bootstrap support values.

From the MP and ML trees suggested that clade II (*M. pillimanus* group) was closer to clade III (Other *Macrobrachium*) than clade I (*M. niphanae*). These two clades from sister taxon with high bootstrap values support (76/86). This result did not agree with the phylogeneic tree of genus *Macrobrachium* which obtained from analysis of partial sequences of two mitochondrial and nuclear genes (*Cox1*, *16S*, *18S* and *28S*) (Wowor *et al.*, 2009). Phylogenetic tree obtained from partial sequence suggested that *M. niphanae* was closer to *M. pillimanus* group than *M. lanchesteri* and *M. rosenbergii*. However, the species within each clade were compatible. Those were clade II consisted of species in *M. pillimanus* group and another clade (clade III) consisted of two actually species; *M. lancherteri* and *M. rosenbergii*. Clade III suggested that *M.lanchesteri* was closer to *M.rosenbergii* with the highest bootsrap value. Interestingly, *M. lanchesteri* and *M. rosenbergii* has morphological divergence, especially in size, second pereiopod and rostum but it always was observed that *M. lanchesteri* and *M. rosenbergii* were genetically closer to each other and *M. lanchesteri* always was one of the main species occurred in *M. rosenbergii* farming (Archanachai, 2005).

The NJ method gave a different topology of tree (Figure 23). That was NJ tree composed of 4 clades. Those were three major clades; I, II and III as same as the MP and ML tree. The additional clade was clade VI which composed of *M. tratense* and *M. sintangense*. The topology of NJ tree that did not agree with MP and ML tree might be caused by number of specimens which were small.

The genus *Macrobrachium* has been proven to be taxonomically difficult. This genus is quite equivocal. Sometimes, morphologically similar species are often quite genetically distinct (Liu *et al.*, 2007) and sometimes large amount of morphological divergence might be shown that there is very little variation in genetically (Murphy and Austin, 2002). These results suggests that using traditional morphological characters alone is insufficient to accurately diagnose species group of *Macrobrachium* (Holthis, 1950).

However, the *Macrobrachium* species were included in this study did not from monophyletic clade. Despite the species diversity in this genus are quite hard to classify so taxonomic mistake are easily happened. Moreover, multiple sampling within each taxonomic group is still show considerable value and possible error can be detected (Peters *et al.*, 2005). Thus a single example of an individual used as represent a species in overall analysis is not justified and should be treated with carefulness.

5. Development of species specific primer

Primer specific for three species (*M. hirsutimanus, M. rosenbergii and M. lanchesteri*) were designed by Oligos. The sequences and the position of the set of primers were shown in Table 8 and Figure 26. The expected size of PCR product of *M. hirsutimanus*, *M. rosenbergii* and *M. lanchesteri* were approximately 439, 508 and 569 bp respectively.

Table 8 Sequences of nucleotide primers

Primer's name	Nucleotide sequence	Tm (°C)
Mh_16speF2	5'-ATTTAAACAATAGATAAAATTCCC	52.5
Mh_16speR2	5'-TTAATAAGTACAGTAATGGGAGCA	51.8
MRO_16speF1	5'- AGGAAGGATCAAATTCTAGTC	47.9
MRO_16speR1	5'-ATGCCGTTTATTGATTAAGCG	55.1
ML_16speF3	5'-TACCTAAATCGCAAGTATTA	45.8
ML_16speR1	5'-TAGTTATGGTAGGAGGAATGAGCT	53.9

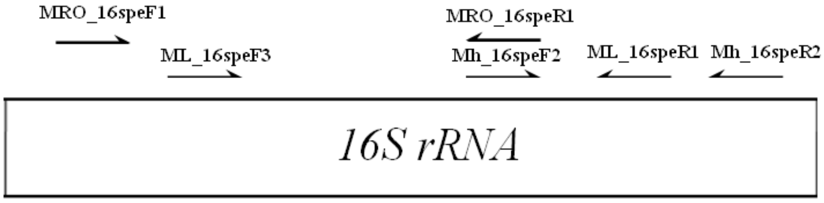

Figure 26 Diagram shows the position of the set of primers for multiplex PCR.

The total 30 *Macrobrachium* samples were amplified with the set of primer Mh_16speF2+Mh_16speR2/ MRO_16speF1+MRO_16speR1 and ML_16speF3+ML_16speR1. The reaction was performed in single reaction. The specificity of multiplex PCR was showed in Figure 27. The amplification band of three expected size were obtained 439, 508 and 569 bp for *M. hirsitimanus*, *M. rosenbergii* and *M. lanchesteri* respectively (Figure 20B). The electrophoresis pattern clearly showed the absence of cross-reaction but the band yields in *M. hirsutimanus* was poor due to the position of Mh_16speF2 was the same position of MRO_16speR1.

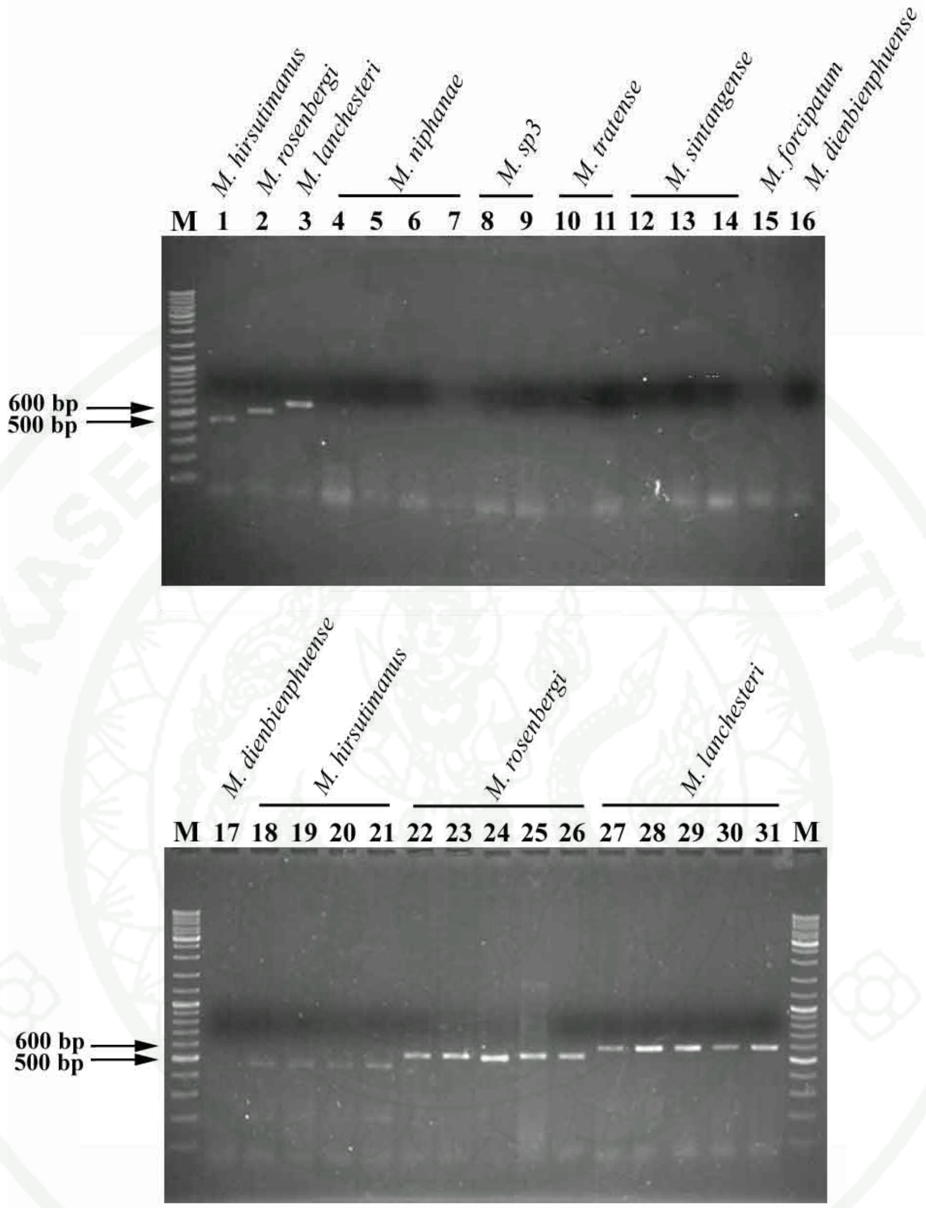

Figure 27 Multiplex PCR amplification obtained with the set of primer Mh_16speF2+Mh_16speR2/MRO_16speF1+MRO_16speR1/ ML_16speF3+ML_16speR1. M was DNA marker (GeneRuler^{TM} DNA Ladder Mix). The others were specificity of multiplex PCR of DNA template from muscle of: lane 1-3, positive control, *M. hirsutimanus, M. rosenbergii* and *M. lanchesteri*; lane 4-7, *M. niphanae*; lane 8-9, *M. sp03*; lane 10-11, *M. tratense*; lane 12-14, *M. sintangense*; lane 15, *M. forcipatum*, lane 16-17, *M. dienbienphuense*; lane 18-21, *M. hirsutimanus*; lane 22-26, *M. rosenbergii*; lane 27-31, *M. lanchesteri*.

Freshwater prawn of the genus *Macrobrachium* is a genus which notoriously difficult to identify by conservative systematic traditions. Since the dominant traits such as the rostrum and/or the second pereiopod can be change so much and so gradually during their growth (Holthis, 1950) and are influenced by environmental parameters (Dimmock *et al.*, 2004).

This study was based on only complete *16S rRNA*. Furture study, especaily using the other mitochondrial markers or nuclear sequence data and including more individuals and number of species are distinctly required to clarify the phylogenetic relationship of this genus.

CONCLUSION

Freshwater prawns of the genus *Macrobrachium* from provinces in central, western and eastern part of Thailand were classified based on morphological characters into 10 species, included *M. assamense*, *M. lanchesteri*, *M. rosenbergii*, *M. tratense*, *M. sintangense*, *M. forcipatum*, *M. dienbienphuense*, *M. hirsutimanus*, *M. niphanae* and *M. sp3* respectively.

The complete nucleotide sequences of *16S rRNA* genes in 10 species were in range 1300 to 1310 nucleotide long. The intraspecific *16S rRNA* sequence divergence estimates amongst individual from different locality ranging 0.00-2.2%, revealed low level of genetic difference in different three parts, central, western and eastern of Thailand. Average evolutionary distance within groups showed low level, ranging from 0.00-2.6%. Average evolutionary distance among *Macrobrachium* species were high, ranging from 2.3- 35.1%. *M. assmense* showed no genetic different with *M. lanchesteri* and *M. sp3* showed 2.3% genetic divergence from *M. niphanae.* Therefore, there were at least eight *Macrobrachium* species in Thailand, included *M. lanchesteri*, *M. rosenbergii*, *M. tratense*, *M. sintangense*, *M. forcipatum*, *M. dienbienphuense*, *M. hirsutimanus* and *M. niphanae* were supported species status by molecular evidence. Moreover the primer sets of multiplex PCR for identify three *Macrobrachium* species, namely *M. hirsutimanus, M. rosenbergii* and *M. lanchesteri* were successfully developed.

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APPENDIX

Key to species of *Macrobrachium*

1. a: Major second pereiopod with dense velvety pubescence ……………….…2
   b: Major second pereiopod without pubescence…………………… ……..…..7
2. a: Pubesence present on merus and carpus…………………………………….3
   b: Pubesense present only on merus or carpus……………...….….*M. niphanae*
3. a: Pubescence present on both plam and finger………………..…………...4
   b: Pubesence present only on finger……………………………….………..6
4. a: Carpus cup-shaped, less than twice as long as high, cutting edges of fingers of second pereiopod with less than 18 teeth………………….……………….…5
   b: Carpus elongated, more than twice as long as high, cutting edges of fingers of second pereiopod with more than 20 teeth….…………*M. dienbienphuense*
5. a: Propodus of third pereiopod more than 3.0 times as long as dactylus, cutting edges of fingers of pereiopod with 7-10 teeth…………………...*M. forcipatum*
   b: Propodus of third pereiopod 2.0-2.6 times as long as dactylus, female second pereiopod much smaller than that of male in form……*M. hirsutimanus*
6. a: Velvety pubescence present throughout length of fingers, finger with longitudinal grooves, rostrum straight…………………..…….*M. assamense*
   b: Velvety pubescence present only proximal half…………….*M. sintangense*
7. a: Carpus distinctly longer than chela, rostrum upturned, reaching distinctly beyond end of scaphocerite …………………………..…..….*M. lanchesteri*
   b: Carpus as long as or shorter than chela……………………………………..8
8. a: Carpus of major pereiopod longer than plam but shorter than chela……….9
   b: Carpus as long as plam and finger………………………….………*M. sp3*
9. a: Rostrum straight, no visible crest, reach to end of scaphocerite....*M. tratense*
   b: Rostrum upturned, fingers of second pereiopods with teeth on cutting edges, rostrum with more than 10 ventral teeth……………..…………*M. rosenbergii*

Reagent preparation

General reagent

2 µM dNTP from 25 µM stock solution

dATP	20	µl
dCTP	20	µl
dGTP	20	µl
dTTP	20	µl

Add each dNTP to 920 µl ultrapure water.

6X Loading buffer

Bromophenol blue	25	mg
Xylene cyanal FF	25	mg
Glycerol	3	ml

The chemical was dissolved in 5 ml distilled water, adjusted volume to 10 ml with distilled water. The 6X loading buffer was stored at 4°C.

50X Tris Acetate EDTA

Tris base	242	g
EDTA	18.6	g
Glacial acetic acid	57.1	ml

The chemical was dissolved in 900 ml distilled water, adjusted volume to 1 L with distilled water. The solution was sterilized by autoclaving for 10 minutes at 121° C, 15 lb/square inches.

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