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TSPY as a Genetic Marker for Sex Determination of Cattle Spermatozoa

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ABSTRACT

The beef industry prefers male calves as they tend to have higher growth rates and feed efficiency for meat production. The dairy industry, however, prefers female calves which produce offspring and milk for dairy products. Thus, methods are required to determine the sex of spermatozoa in order to manipulate the sex of the offspring of livestock. The ability to sex spermatozoa has great potential for commercialization in the beef and diary cattle, thus a lot of the research has been conducted to develop and refine spermatozoa sexing technology. In this study, fluorescence *in-situ* hybridization (FISH) was performed on decondensed bovine spermatozoa using testis-specific protein, Y-encoded (*TSPY*) marker, as a screening method for the detection of Y-chromosome bearing bovine spermatozoa. The PCR-produced fragments of *TSPY* were cloned, transformed, cultured, and extracted according to a standard protocol. *TSPY* fragment-bearing plasmids were labelled with SpectrumRed-dUTP by nick translation labelling. The labelled probes were hybridized onto the pre-washed slides containing decondensed bovine spermatozoa for 72 hours. After post hybridization, the washed slides were counterstained using 4,6-diamino-2-phenylindole (DAPI II) and the FISH images were captured and analysed. The results showed only certain spermatozoa cells were hybridised with red signals, indicating the presence of Y-chromosome bearing spermatozoa. It is important to note that this chromosome-specific marker can be used to verify the sex of the flow cytometrically sorted spermatozoa and open the way of elucidation for *TSPY* to be used as a marker in sex determination.

Keywords: FISH, sex determination, *TSPY*, spermatozoa

INTRODUCTION

Altering the sex ratio for a particular population may be necessary for maintaining the profitability of the dairy and beef cattle industries. Thus, a predetermination of sex prior to conception is the most cost-effective means to achieve a desired result. This particular approach is useful in cattle breeding programmes, where a specific sex of calves is required. The X- and Y-chromosome bearing spermatozoa can be used in artificial insemination (AI), in-vitro fertilization (IVF) or embryo transfer (ET) programme. However, the only proven method for sexing is cell sorting which is based on DNA differences by flow cytometry (Parati *et al*., 2006). The predetermination the sex of calves after spermatozoa sexing using flow cytometry obtained between 85% and 95% accuracy (Seidel, 1999).

The validation of the proportions of the X- and Y-chromosome bearing spermatozoa

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sorted by flow cytometry is essential. A practical approach is to use the fluorescence *in-situ* hybridization (FISH) technique. This technique is effective for validating the sorted X- and Y-chromosome bearing spermatozoa since it can be used to examine a large number of sperm cells by directly viewing sperm chromosomes without relying on flow cytometry (Kawarasaki *et al.*, 1998).

The objective of this study was to develop the FISH method using the chromosome Y-specific DNA probe which could determine the efficiency of spermatozoa sexing techniques.

MATERIALS AND METHOD

Semens were collected from using artificial vagina. Fresh semens were cryopreserved and stored in liquid nitrogen (N_2). The frozen semen were thawed and the spermatozoa was washed once with an equal volume of normal saline containing 0.9% NaCl by centrifugation for 10 minutes at 900 rpm. This was followed by smearing and fixing the spermatozoa on glass slides. The slides were then incubated with decondensation solution containing dithiotreitol (DTT) for 1.5 minutes to make the sperm nuclei accessible to the probes.

The PCR amplification was performed using primers 5'-CCC GCA CCT TCC AAG TTG TG-3' and 5'-AAC CTC CAC CTC CTC CAC GAT G-3' that is specific to testis-specific protein (*TSPY*) gene in Y-chromosome. The template DNA was prepared by PCR with the final volume of 25µl reaction mix, containing template sperm DNA, dNTP mix, 10× PCR buffer, as well as $MgCl_2$ and Taq DNA Polymerase. The amplification was performed using the following thermocycling conditions: an initial denaturation at 94°C for 4 min, followed by 35 step cycles of denaturation (94°C for 30 sec), annealing (60°C for 30 sec) and extension (72°C for 1 min), with the final extension at 72°C for 10 min. The DNA band of interest was cut and purified from agarose gel. The product was cloned into qPCR® 2.1-TOPO® plasmid using Invitrogen Cloning Kit. The extracted pasmids was labelled with SpectrumRed-dUTP by nick translation labelling.

The labelled product was mixed with blocking DNA and precipitated overnight. The DNA pellet was mixed with 10µl hybridization

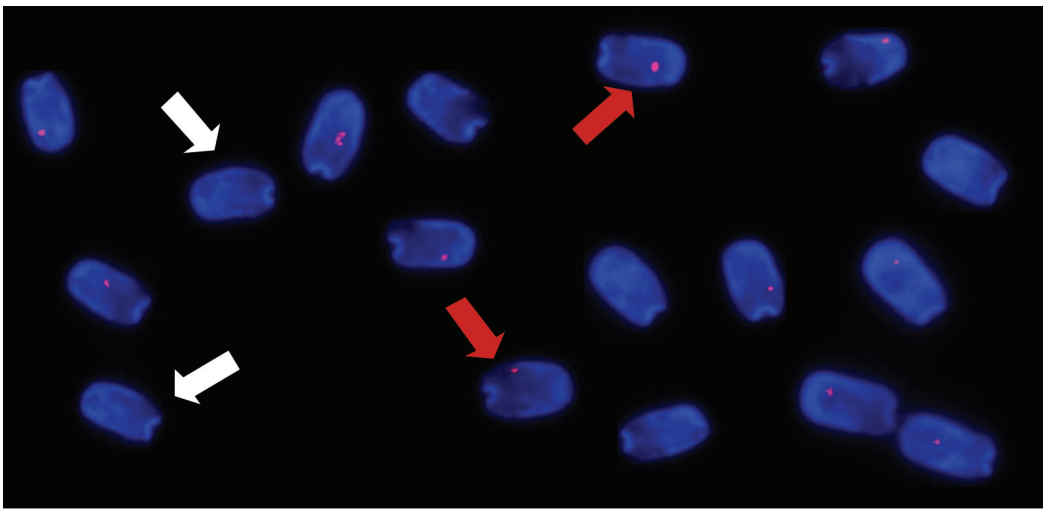

Fig. 1: Spermatozoa with red signal indicated Y-chromosome bearing spermatozoa (red arrows). Spermatozoa without red signal indicated X-chromosome bearing spermatozoa (white arrows)

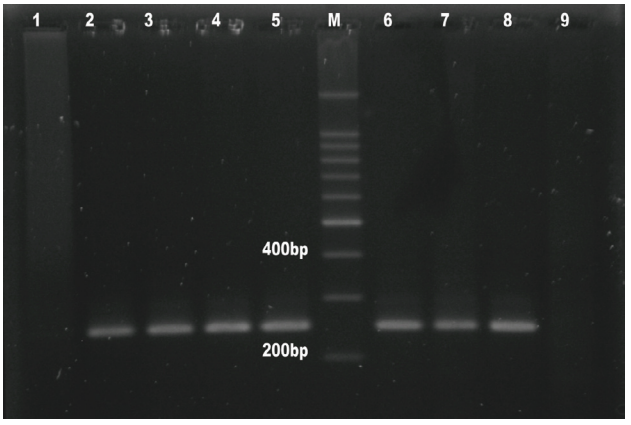

Fig. 2: DNA band from male and female bovine blood. The 260bp Y-specific bands were present in males bovine DNA (lane 2-8). M=100bp marker; lane 1=female DNA; lane 2-4=male DNA; lane 5-7=bovine's sperm DNA; lane 8=positive control, DNA from Y-chromosome bearing spermatozoa; Lane 9=negative control, without DNA template

buffer and denatured at 80ºC for two minutes. Then, the probe mixture was hybridized onto the decondensed spermatozoa slides in a moist chamber at 37ºC overnight.

The slides were washed using the washing buffer containing Nonipet-40. The slides were counterstained with 4',6-diamidino-2-phenylindole (DAPI II). After that, these slides were sealed with nail polish and the spermatozoa were observed using a phase-contrast epifluorescence microscope. The optical sections of each field of view were obtained individually and analysed.

RESULTS AND DISCUSSION

In this study, a total of 300 spermatozoa were examined. The spermatozoa with chromosome Y was indicated by the presence of red signal. The rate of Y-bearing spermatozoa was calculated by the number of sperm cells with the red signal divided by the number of the total sperm cells. The percentage of the Y-bearing spermatozoa was 50.8% and the percentage of X-bearing spermatozoa was 49.2% (*Fig. 1*). The ratio of the X- and Y-bearing spermatozoa for the whole sperm populations was found to be as expected.

The Y-encoded, testis-specific protein (*TSPY*), is a Y-specific gene (Lemos *et al.*, 2005). Meanwhile, the number of the *TSPY* copies was ranged from 20 to 60 in men and up to 200 in bull (Manz *et al*., 1998). The higher repetitive probe enhanced the signals from Y chromosome. In cattle, *TSPY* expression is apparently restricted to male germ cells and their precursors begin during foetal development. The cellular site of expression suggested a function in spermatogonial proliferation (Vogel *et al*., 1997). In this study, the specificity of *TSPY* marker was confirmed by the PCR on DNA from the blood samples of the male and female cattle. These DNA were screened for the presence of *TSPY* using the specific PCR primers amplifying a 260-bp segment of *TSPY*. All the male samples (blood and spermatozoa) are *TSPY*-positive, while all the female samples are *TSPY*-negative (*Fig. 2*).

The de-condensing of the spermatozoa head using DTT allows DNA probes to hybridize in the chromosome. One problem that normally occurs when FISH is performed on spermatozoa is the degree of condensation of the DNA. Obtaining an optimal decondensed of sperm head size for an optimum hybridization is difficult as the

time required for sperm decondensation varies considerably among species (Perreault *et al.,* 1988). In particular, a short decondensation time will cause the failure of DNA probe to access the chromatin. In contrast, if the nuclei swells more than twice of the original size, the signal from one chromosome will split and appear as two or more signals causing false score (Parrilla *et al.,* 2003).

The present study used direct DNA probe labelling by nick translation. If the reaction is optimally controlled, nick translation can give the highest sensitivity. This method does not involve extensive manipulation of the sample, and therefore the risk of contamination is low when compared to other method such as the PCR labelling (Parrilla *et al.,* 2003). Therefore, in order to increase the specificity of sex determination method using FISH, it is better to use dual colour labelling with two fluorescence colours on autosomal and Y chromosome. The time of hybridization could also be shorten to save time and improved efficiency.

CONCLUSIONS

In conclusion, FISH using *TSPY* probe can be used to determine the X- and Y-chromosome bearing spermatozoa. This technique can be used to validate the purity of the sexed spermatozoa sorted via flow cytometry.

REFERENCES

Lemos, D.C., Rios, A.F.L., Caetano, L.C., Lobo, R.B., Vila, R.A., Martelli, L., Takeuchi, P.L. and Ramos, E.S. (2005). Use of the *TSPY* gene for sexing cattle. *Genetic and Molecular Biology*, *28*(1), 117-119.

Kawarasaki, T., Welch, G.R., Long, C.R., Yoshida, M. and Johnson, L.A. (1998). Verification of flow cytometorically-sorted x- and y-bearing porcine spermatozoa and reanalysis of spermatozoa for DNA content using the fluorescence *in-situ* hybridization (FISH) technique. *Theriogenology*, *50*, 625-635.

Manz, E., Vogel, T., Glatzel, P. and Schmidtke, J. (1998). Identification of an equine Y chromosome specific gene locus (e*TSPY*) with potential in preimplantation sex diagnosis. *Theriogenology*, *49*, 364.

Parati, K., Bongioni, G., Aleandri, R. and Galli, A. (2006). Sex region determination in bovine semen: A new approach by quantitative real time PCR. *Theriogenology*, *66*, 2202-2209.

Parrilla, I., Vazquez, J.M., Oliver-Bonet, M., Navarro, J., Yelomos, J., Roca, J. and Martinez, E.A. (2003). Fluorescence *in situ* hybridization in diluted and flow cytometrically sorted boar spermatozoa using specific DNA direct probes labeled by nick translation. *Reproduction*, *126*, 317-325.

Perreault, S.D., Baebee, R.R., Elstein, K.H., Zucker, R.M. and Keefer, C.L. (1988). Interspecies differences in the stability of mammalian sperm nuclei assessed *in vivo* by sperm microinjection and *in vitro* by flow cytometry. *Biology of Reproduction*, *39*, 157-167.

Seidel, G.E.Jr. (1999). Sexing mammalian spermatozoa and embryos-state of the art. *Journal of Reproduction and Fertility Supplement*, *54*, 475–485.

Vogel, T., Dechend, F., Manz, E., Jung, C., Jakubiczka, S., Fehr, S., Schmidtke, J. and Schnieders, F. (1997). Organization and expression of bovine TSPY. *Mammalian Genome*, *8*, 491-496.

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Morphometric Variation among the Three Species of Genus *Acetes* (Decapoda: Sergestidae) in the Coastal Waters of Malacca, Peninsular Malaysia

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ABSTRACT

Small sergestid shrimps of the genus *Acetes* are locally known as 'udang garagau' which can be found along the coastal region of Malacca. A total of three species of the genus *Acetes* (namely, *A. indicus*, *A. japonicus* and *A. intermedius*) are found along the coastal waters of Malacca. The morphometric data of the three species were analyzed using one-way ANOVA and PRIMER software to examine the degree of similarity among the three species. All the morphometric characters, which include total length, carapace length, standard length, abdominal length, telson length, first antenular peduncle, second antenular peduncle, third antenular peduncle, eye length, eye wide, and eye diameter of the three species were significantly found to be different ($P < 0.05$). The dendrogram of both the male and female populations showed three major clusters indicating the three species of genus *Acetes.*

Keywords: *Acetes* shrimps, morphometric variation, Peninsular Malaysia

INTRODUCTION

Although the shrimps of the genus *Acetes*, family sergestidae, are a minor planktonic crustacean group represented by a small number of species, they are one of the economically important organisms in Asia and East African waters (Omori, 1975). It is mainly used in subsistence fisheries and is, therefore, commercially important in Peninsular Malaysia. Six species of *Acetes* from the Malay Peninsula and Singapore, namely *A. erythraeus* Nobili, *A. indicus* Milne-Edwards, *A. japonicus* Kishinouye, *A. sibogae* Hansen, *A. serrulatus* Hansen, and *A. vulgaris* Hansen are briefly reported (Pathansali, 1966). The two latter species, *A. serrulatus* and *A. vulgaris,* were recorded from Singapore waters, while the other four species of the genus *Acetes* were recorded only from the Malay Peninsula.

Morphometric characters are powerful tools for measuring discreteness and relationships among stocks (Ihssen *et al*., 1981; Melvin *et al*., 1992). In the present study, the morphometric data were used to clarify the intra-population variation in the genus *Acetes* from the coastal water of Malacca. Nonetheless, the information on the morphometric variation among the three species of *Acetes* (*A. indicus*, *A. japonicus,* and *A. intermedius*) has not been reported from the region. The population biology of *Acetes* have been reported in many studies by different

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authors (Deshmukh, 2002; Oh and Jeong, 2003; Arshad *et al*., 2007; Amin *et al*., 2008a,b). Therefore, the present study is a pioneering attempt in this direction.

MATERIALS AND METHODS

Collection of Data

The fresh samples of *Acetes* were collected monthly between February 2005 and January 2006 from the commercial push net catches landed at Klebang Besar (N $02^0$13.009/ and E 102^0 11.921/) in Malacca (*Fig. 1*). *Acetes* shrimps were caught using a push net (triangular shape) known locally as 'Sungkor' (Omori, 1975) in the coastal waters of Klebang Besar, Malacca. The dimensions of the net are 5-6 m in length, 4.0 - 4.5 m in width, and 3.0 - 3.5 m in height. The mean mesh size was 3.2 (± 0.27) cm at the anterior section, 0.75 (±0.05) cm at the middle, and 0.5 (±0.08) cm at the cod end (stretched). After collection, the samples were fixed in 10% formalin solution in the field and they were analyzed after 2-3 days of preservation. In the laboratory, these specimens were identified using a 'Nikon' dissecting microscope. Their sexes were determined by the presence or absence of petasma on the first pleopod and clasping spine on the lower antennular flagellum (Omori, 1975). The identification of the different species of *Acetes* was according to the keys developed by Omori (1975).

Data Analysis

A total of 180 specimens in the size ranging from 10 to 30 mm were used for the morphometric measurements; 60 (30 males and 30 females) specimens each from *A. japonicus*, *A. intermedius,* and *A. indicus*. Eleven selected morphometric characteristics, as shown in *Fig. 2*, were measured using the KEYENCE Digital microscope (VHX-500) for each sample. The following morphometric characters, which include the total length (TL), carapace length (CL), standard length (SL), abdominal length (AL), telson length (TLL), first antenular peduncle (P1), second antenular peduncle (P2), third antenular peduncle (P3), eye length (EL), eye wide (EW), and eye diameter (ED) were measured (*Fig. 2*). The morphometric data were analyzed using the one-way analysis of variance (ANOVA) while the PRIMER software was used for the cluster analysis of the species.

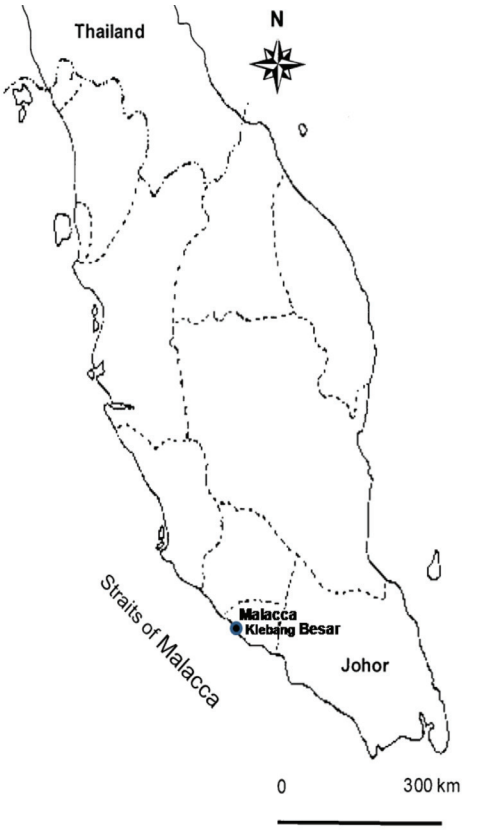

Fig.1: Sampling station (dot) and location of Klebang Besar in Malacca, Peninsular Malaysia

RESULTS AND DISCUSSION

The range and mean ± standard error values of the morphometric characters for the three species of *Acetes* (*A. japonicus, A. intermedius,* and *A. indicus*) are presented in Tables 1 and 2. The ANOVA showed that the mean differences in the total length (TL), standard length (SL), carapace length (CL), abdominal length (AL),

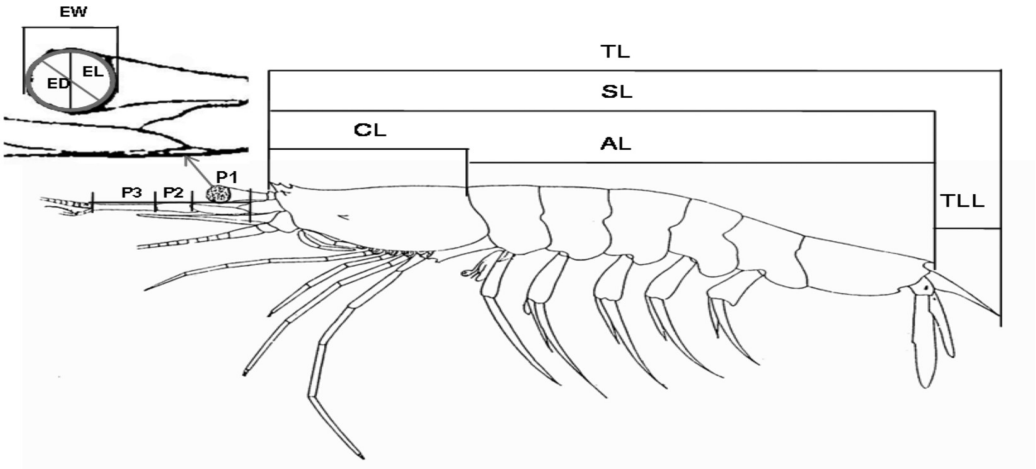

Fig. 2: Morphometric characters used for Acetes *shrimp*

telson length (TLL), first antennular peduncle (P1), second antennular peduncle (P2), third antennular peduncle (P3), eye length (EL), and eye wide (EW) among the three species were highly significant ($P < 0.05$). Nonetheless, no significant difference was observed for the male eye diameter of the different species. Meanwhile, the dendrograms (*Figs. 3* and *4*) showed three major clusters of the *Acetes* species in the coastal waters of Malacca. The similarity of matrix indicated that there were about 90 – 93% similarity between *A. japonicus* and two other species (namely, *A. indicus* and *A. intermedius*). There was about 97- 98% similarity between *A. indicus* and *A. intermedius,* based on the male and female morphometric characteristics which covered *A. japonicus* in one group and the other group that comprised two species of *A. intermedius* and *A. indicus*.

Moreover, there is no previous record on morphometric variation between the different species of *Acetes.* However, the morphometric analysis of the Malaysian Oxudercine Goby, *Boleophthalmus boddarti* was studied by Daud *et al*., 2005. The analyses of various morphometric characters showed significant differences among the three species of *Acetes.* The results shown by ANOVA had high significant differences ($P < 0.05$) of all the morphometric characters except for the eye diameter ($P > 0.05$) of the males in the three species. Meanwhile, the female population also showed highly significant differences ($P < 0.05$) for all the morphometric characters. In more specific, two major groups were observed for both the male and female of the three *Acetes* species from the study area (*Figs. 3* and *4*). The first group consists of *A. japonicus*, while the second group has *A. intermedius* and *A. indicus*.

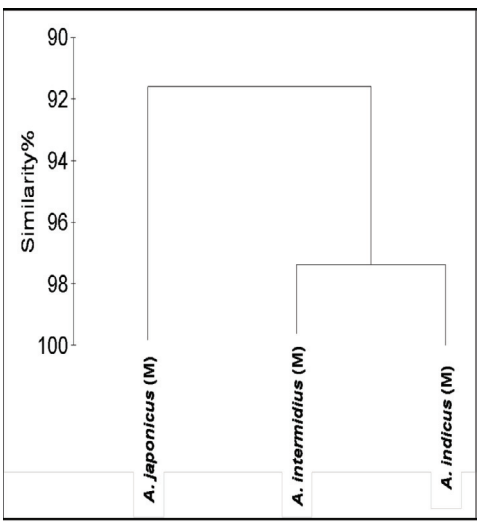

Fig. 3: Dendrogram of the three Acetes *species on the basis of morphometric characters of the male collected from Malacca coastal waters*

TABLE 1
Mean ± standard error, ranges (in parentheses) and F-values (derived from the analysis of variance) of each morphometric character (mm) in three species of genus *Acetes* (male)

MC	*A. japonicus* (M)	*A. intermedius* (M)	*A. indicus* (M)	N	F-values	P
TL	12.93^{a} ±0.16 (11.5-15)	18.90^{b} ±0.30 (17-22)	18.15^{b} ±0.59 (12.5-24)	30	69.27	0.000*
CL	3.24^{a} ±0.04 (2.98-3.61)	4.85^{b} ±0.06 (4-5.80)	4.69^{b} ±0.16 (3.41-6.45)	30	71.65	0.000*
SL	11.44^{a} ±0.13 (10-13.25)	16.75^{b} ±0.27 (15-19.50)	16.04^{b} ±0.52 (11-21)	30	78.33	0.000*
AL	8.41^{a} ±0.10 (7.5-9.75)	12.12^{b} ±0.20 (10.5-14.25)	11.61^{b} ±0.38 (8-15)	30	40.23	0.000*
TLL	1.56^{a} ±0.0.05 (1-2.25)	2.36^{b} ±0.05 (2-3.12)	2.38^{b} ±0.10 (1.66-3.54)	30	63.33	0.000*
P1	0.85^{a} ±0.02 (0.6-1.17)	1.07^{b} ±0.03 (0.75-1.50)	1.10^{b} ±0.04 (0.6-1.53)	30	12.61	0.000*
P2	0.77^{a} ±0.02 (0.58-1.17)	0.60^{b} ±0.01 (0.44-0.75)	0.97^{c} ±0.05 (0.60-1.67)	30	27.30	0.000*
P3	2.10^{a} ±0.04 (1.66-2.70)	1.05^{b} ±0.02 (0.65-1.35)	2.38^{c} ±0.10 (1.39-3.38)	30	101.72	0.000*
EL	0.60^{a} ±0.01 (0.44-0.75)	0.78^{b} ±0.01 (0.64-0.93)	0.74^{b} ±0.02 (0.5-1.11)	30	23.87	0.000*
EW	0.62^{a} ±0.01 (0.50-0.78)	0.80^{b} ±0.02 (0.58-1.07)	0.74^{b} ±0.03 (0.51-1.03)	30	22.12	0.000*
ED	0.63^{a} ±0.01 (0.50-0.73)	0.81^{a} ±0.01 (0.64-0.97)	0.75^{a} ±0.02 (0.56-1.06)	30	2.24	0.113^{NS}

For each morphometric variable, means with the same letter superscript are not significantly different.
* The mean difference is significant at 5% level; NS = not significant at 5% level

TABLE 2
Mean ± standard error, ranges (in parentheses) and F-values (derived from the analysis of variance) of each morphometric character (mm) in three species of genus *Acetes* (female)

MC	*A. japonicus* (F)	*A. intermedius* (F)	*A. indicus* (F)	N	F-values	P
TL	17.31^{a}±0.30 (14-19.75)	22.82^{b}±0.39 (19-27)	24.42^{b}±0.52 (18-30)	30	68.63	0.000*
CL	4.59^{a}±0.09 (3.5-5.38)	5.94^{b}±0.12 (4.9-7.09)	6.42^{b}±0.17 (5-8.29)	30	68.11	0.000*
SL	15.44^{a}±0.27 (12.5-17.50)	20.33^{b}±0.34 (17-24)	21.75^{b}±0.46(16-27)	30	50.34	0.000*
AL	11.06^{a}±0.19 (9-12.50)	14.50^{b}±0.25 (12-17)	15.73^{c}±0.34 (12-20)	30	23.90	0.000*
TLL	2.00^{a}±0.07 (1-2.52)	2.65^{b}±0.08 (1.97-3.25)	2.90^{b}±0.13 (1-4.1)	30	70.24	0.000*
P1	0.89^{a}±0.03 (0.55-1.29)	1.20^{b}±0.03 (0.9-1.60)	1.29^{b}±0.05 (0.80-1.81)	30	27.40	0.000*
P2	0.49^{a}±0.02 (0.33-0.72)	0.59^{b}±0.02 (0.39-0.81)	0.77^{c}±0.03 (0.44-1.13)	30	27.78	0.000*
P3	1.06^{a}±0.0 (0.61-1.92)	1.06^{a}±0.03 (0.80-1.38)	1.41^{b}±0.06 (0.94-2.39)	30	11.84	0.000*
EL	0.67^{a}±0.02 (0.47-0.87)	0.88^{b}±0.02 (0.72-1.07)	0.87^{b}±0.02 (0.66-1.17)	30	30.10	0.000*
EW	0.69^{a}±0.02 (0.50-0.93)	0.90^{b}±0.02 (0.72-1.04)	0.90^{b}±0.02 (0.67-1.18)	30	30.21	0.000*
ED	0.70^{a}±0.02(0.47-0.87)	0.91^{b}±0.02 (0.72-1.07)	0.90^{b}±0.02 (0.7-1.14)	30	30.9	0.000*

For each morphometric variable, means with the same letter superscript are not significantly different.
* The mean difference is significant at 5% level

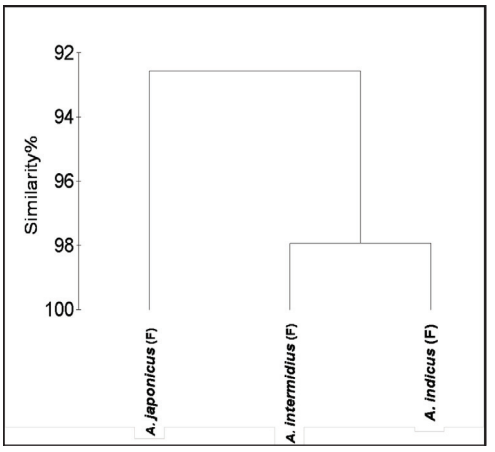

Fig. 4: Dendrogram of the three Acetes *species on the basis of morphometric characters of the female collected from Malacca coastal waters*

CONCLUSIONS

Both the similarity matrix and cluster analysis revealed that there are three different species of *Acetes* (*A. japonicus, A. intermedius,* and *A. indicus*) recorded from the coastal waters of Malacca, Malaysia. A more detailed study on systematics of the *Acetes* spp. is therefore needed from more geographical locations in Malaysia to obtain updated information on the systematic accounts and resources of *Acetes* from this country.

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REFERENCES

Amin, S.M.N., Arshad, A., Shamsudin, S.B., Japar, S.B. and Siraj, S.S. (2008a). Catch per unit effort of estuarine push net with emphasis on occurrence and abundance of *Acetes* shrimps in the coastal waters of Malacca, Peninsular Malaysia. *Pertanika Journal of Science and Technology*, *16*(2), 271-279.

Amin, S.M.N., Arshad, A., Japar, S.B. and Siraj, S.S. (2008b). Growth, mortality and yield-per-recruit of Sergestid Shrimp, *Acetes intermedius* Omori, 1975 (Decapoda: Sergestidae) from Length Frequency Analysis in the coastal waters of Malacca, Peninsular Malaysia. *Pertanika Journal of Tropical Agricultural Science*, *31*(1), 95-106.

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

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

Daud, S.K., Mohammadi, M., Siraj, S.S. and Zakaria, M.P. (2005). Morphometric analysis of Malaysian Oxudercine goby, *Boleophthalmus boddarti* (Pallas, 1977). *Pertanika Journal of Tropical Agricultural Science*, *28*(2), 121-134.

Ihssen, P.E., Booke, H.E., Casselman, J.M., McGlade, J.M., Payne, N.R. and Utter, F.M. (1981). Stock identification: Materials and methods. *Canadian Journal of Fisheries and Aquatic Sciences*, *38*, 1838-1855.

Melvin, G.D., Dadswell, M.J. and McKenzie, J.A. (1992). Usefulness of meristic and morphometric characters in discriminating populations of American shad (*Alosa sapidissima*) (Osteichthyes: Clupeidae) inhabiting a marine environment. *Canadian Journal of Fisheries and Aquatic Sciences*, *49*, 266-280.

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

Omori, M. (1975). The systematics, biogeography and fishery of epipelagic shrimp shrimps of the genus *Acetes* (Crustacea, Decapoda, Sergestidae). *Bulletin of the Ocean Research Institute*, University of Tokyo, 1-91.