J.Sci.Soc.Thailand, 24(1998) 21-36

ELECTROPHORETIC AND MORPHOMETRIC ANALYSES IN SPECIES DIFFERENTIATION OF SMALL OYSTERS, *SACCOSTREA* SPP., IN THAILAND

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(Received November 1, 1997)

ABSTRACT

Small oysters, Saccostrea *spp., were collected from different locations in Thailand, including Ko Chang-Trat ,Sri Racha-Chonburi , Samsaeb and Ko Talu-Chumporn, Ko Prab-Surat Thani, Tubtieng-Trang, and Ko Kaoyai-Satul. These oysters possessed chomata and were identified as species "A", "B" or "C" by electrophoresis. All shells were subjected to morphometric analyses except those damaged by their removal from the substrate and possible hybrids. The following dimensions were measured for each shell: dorso-ventral and antero-posterior measurements, valve thickness, overall thickness, depth of umbonal cavity, length of hinge plate , and width of adductor muscle scar. Observations of position and colour of the scar, and arrangement of chomata were also made. These parameters, in addition to 3 parameters of index of shell shape, were subjected to 2 methods of statistical analyses using the SPSS statistical computer package. The first method was the analysis of variance and Scheffe's test. Relative similarities of the populations based on gene frequencies and morphometric analysis were constructed by UPGMA program. The results of both techniques correspond with each other very well. Thus the result of morphometric analysis of oyster shells can be used in species differentiation of oysters. Another statistical method attempted was discriminant analysis. This technique was investigated to obtain the percentage of samples classified correctly and to see whether it was possible to reduce the number of morphological factors for the determination of oyster species by shell morphometric analysis. The stepwise selection procedure showed that morphological factors could be reduced to only 8 factors. The average values and standard deviations of each factor within species A, B and C are presented. The overall percentage of samples classified correctly in each species was 91.05% as compared to the electrophoretic results.*

INTRODUCTION

Oysters have been found throughout the tropics and subtropic zones . However, the phylogeny and taxonomy of tropical oysters remain confusing in those regions where speciation of oysters has not been examined thoroughly by the rather modern techniques such as electrophoretic technique or a molecular biological technique. This becomes a problem of concern in oyster cultures. The important commercial oyster species in Thailand belong to 2 genera, i.e. *Crassostrea* and *Saccostrea*. In determining the genus of oysters, the structure "chomata" is most important. Those which do not possess chomata will be grouped in the *Crassostrea*. In contrast, chomata are presence in the *Saccostrea* group (Morris, 1985). *Crassostrea* spp. are big oysters which are most important economically. They are commonly called "takrom". These oysters are further divided into takrom kram dum (*C. lugubris*) and takrom kram khao (*C. belcheri*) by the color of adductor muscle scars. If the scar is black, it will be put in the *lugubris* species. The white scar oyster belongs to the *belcheri* species (Stenzel, 1975).

The small oysters are found distributively throughout Thailand. They are less economically important than the big oysters. The habitats of these oysters are rocks, stilt roots of mangrove plants and other substrates. They can grow in a wide range of salinity, thus

their distribution is from offshore marine water to brackish water. The common names for these small oysters depend on the habitat and geographical area of the country. They are commonly referred to as "hoy pak cheeb", but in the southern part of Thailand, they can be called "hoy tieb", "hoy rug punka" or "hoy joh". The common feature of these oysters is small size. Another main feature for identification of *Saccostrea* oysters is the presence of chomata (Stenzel, 1971; Ahmed, 1975). The chomata may be regularly or irregularly spaced, partly or completely encircling the valve margin. Nevertheless, they all are assumed to be *Saccostrea cucullata* (Born, 1778) (Yusuk, 1998). In the scientific point of view it is doubtful whether these oysters really belong to the same species because the morphological characters are different. However , it has been recognized that the *"Saccostrea"* species could display ecomorphology (Tack *et al.*, 1992). Moreover, chomata is also presented in *Alectryonella* sp. but much exaggerated (Harry, 1985). Thus confusion has been persisted among the systematicists concerning the identification of small oysters of morphologically distinctive structures in Thailand.

This research was aimed to study whether the *"Saccostrea"* oysters in Thailand belong to the same species. The techniques used in this research were both electrophoretic and morphometric techniques.

MATERIALS AND METHODS

Sampling regime

Samples of small oysters, *Saccostrea* spp., were collected, during September 1995 to April 1996, from 6 provinces throughout Thailand including both the Andaman Sea and the gulf of Thailand (Fig. 1). Adductor muscles were dissected out in the field and frozen immediately in liquid nitrogen. Table 1 summarises the sample sizes at each sampling location. The frozen samples were further transported to the Plymouth Marine Laboratory where electrophoretic analysis of those samples were performed.

Electrophoresis

Electrophoresis was carried out on horizontal gels of 12 % starch (Sigma laboratory) in a Tris-Citrate-EDTA (TCE) buffer pH 7.0. Samples of adductor muscles were prepared for electrophoresis as described by Buroker *et al.* (1975). Following screening of few individuals for up to 25 enzyme systems using a variety of buffers, only 9 enzyme loci were found to give suitable resolution for investigation in all samples. These were as follows: Leucine amino peptidase (*Lap*), Isocitrate dehydrogenase (*Idh-1*), Aspartate amino transferase (*Aat-2*), Phospho-glucose isomerase (*Pgi*), Phospho—glucose mutase (*Pgm*), Esterase (*Est-2*, utilises fluorescent stain), Mannose phosphate isomerase (*Mpi-2*), Malate dehydrogenase (*Mdh-1*), and Peptidase (*Ap*, substrate *gly-leu*). *Idh-1* was monomorphic and was used solely for identification purposes. Nei's (1972) mean genetic identity (I) was calculated based on 9 enzyme loci for each pairwise comparison between populations(Table 3). A UPGMA dendrogram was constructed to illustrate the relationships between populations.

Morphometric analysis

After samples had been taken for electrophoresis, each shell was cleaned by carefully removing remaining tissues and biofouling organisms with a scapel and washing in dilute sodium hypochlorite solution. These shells were subjected to morphometric analysis. Unfortunately, some shells were destroyed at the time of collection or afterwards. The measurements of such individuals were excluded from the data analysis. Table 1 summarises

sample sizes of oyster shells examined at each sampling location. The following dimensions were measured for each shell; dorso-ventral and antero-posterior measurements of each valve (RDVM, RAPM, LDVM, LAPM), the right and left valve thicknesses (when laid on a flat surface, RT, LT), the overall thickness with the two valves fitted together (LTRT), depth of umbonal cavity (UD), length of the hinge plate (HL), position, color and width of adductor muscle scar (SP, SC, SW), and arrangement of chomata (C). The measurements were done by using a vernier caliper which could warrant the accuracy of measurement in the unit of millimeter. Because the samples consisted of a range of sizes, direct comparison of these measurements could not be used as a comparator between populations. Therefore, ratios of size-related measurements were calculated and were subjected to the statistical analysis, in addition to other factors, *ie.* index of shape of each shell (RDVM+RAPM)/RT, and (LDVM+LAPM)/LT, and index of overall shape of oyster, [(LDVM+LAPM)/LT] ÷ [(RDVM+RAPM)/RT]. The SP, SC, and C parameters were analysed as such because they are not size-dependent. Hence, there are altogether 39 morphological factors determined in this study (Table 2).

These parameters were analysed by two statistical methods. The first method was the analysis of variance (ANOVA) to test for significance differences between populations of oysters. Where the F-value was significant, Scheffe's test was employed to ascertain which populations differed significantly from which others. Based on the scores of significant different characters between pairs of populations, the relative similarities of the populations were illustrated using dendrogram derived from UPGMA computer program.

Another method of statistical analysis used in this study was discriminant analysis of the SPSS statistical computer package. This analysis was performed to classify group of species and to determine whether 39 morphometric factors used in the previous analysis could be reduced but still give similar result as determined by 39 factors. Also the percentage of corresponding between the results obtained by the electrophoretic method and the morphometric method was calculated.

RESULTS

Oysters putatively *Saccostrea cucullata* were scored for allozyme variation at 9 loci (Table 3). Variation at 5 loci, Pgi, Pgm, Mpi-2, Lap and Idh-1, was concordant within individuals and all oysters could be split into "A", "B" or "C" groups depending on their multilocus genotypes at these loci. Except for Idh-1 (fixed for one allele in group "C" and a different allele in groups "A" and "B"), the marker loci were polymorphic with groups distinquished rather by sets of alleles which varied in their diagnostic ability. Alleles found only in group "C" were $Idh\text{-}1^{1}$, $Lap^{8\text{-}10}$ and $Mpi\text{-}2^{7\text{-}10}$. Lap^{7}, and $Mpi\text{-}2^{6}$ alleles are found in "C" but rare in the other 2 groups. Alleles found only in group "B" were $Pgi^{9\text{-}12}$ and $Lap^{1\text{-}3}$ There were no alleles unique to group "A", however, Lap^{5}, Lap^{6}, Pgm^{3} and Pgm^{4} are all common in this group and rare or absent in the other 2 groups. Values of Nei's genetic identity (I) for the 8 populations based on 9 enzyme loci are given in Table 4, and this is illustrated using a UPGMA cluster analysis dendrogram (Fig 2).

The shells of *Saccostrea* oysters were grouped according to the electrophoretic results. It is surprising that the variations of oyster shell morphology existed within each group. However, all of them were then subjected to 13 morphological characteristic measurements. There were altogether 39 factors derived from ratios of size-related measurements, 3 factors of shell shape and the SP, SC, and C factors (Table 2). These factors were used in the morphometric

Table 1. Sample sizes of oyster shells examined at each sampling location.

Location	Province	Number	Species*	Abbreviation
Sri Racha	Chonburi	22	A	Chon A
Tubtieng	Trang	34	A	Trang A
Sam Saeb	Chumporn	35	B	Chum B
Ko Prab	Surat thani	26	A	Prab A
Ko Khaoyai	Satul	31	A	Satul A
Ko Talu	Chumporn	24	C	Talu C
Ko Chang	Trat	6	A	Trat A
Ko Chang	Trat	12	B	Trat B

* Species of oysters was assigned to each individual according to the electrophoretic results.

Table 2. Summary of morphological parameters used in determination of population relationship of oysters.

Factors	Factors
1. RDVM / RAPM	2. RDVM / RT
3. RDVM / LDVM	4. RDVM / LTRT
5. RDVM / UD	6. RDVM / HL
7. RAPM / RT	8. RAPM / LAPM
9. RAPM / LTRT	10. RAPM / HL
11. RAPM / SW	12. RT / LT
13. RT / LTRT	14. LDVM / LAPM
15. LDVM / LT	16. LDVM / LTRT
17. LDVM / UD	18. LDVM / HL
19. LDVM / SW	20. LDVM / C
21. LAPM / LT	22. LAPM / LTRT
23. LAPM / UD	24. LAPM / HL
25. LAPM / SW	26. LAPM / C
27. LT / LTRT	28. LT / UD
29. LT / SW	30. LTRT / UD
31. LTRT / HL	32. LTRT / SW
33. UD / HL	34. (RDVM + RAPM) / RT
35. (LDVM + LAPM) / LT	36. (LDVM+LAPM)/LT÷(RDVM+RAPM) / RT
37. SC	38. SP
39. C	

R = right valve, L = left valve, DVM = dorso-ventral measurement, APM = antero-posteroir measurement, T = thickness of valve,LTRT = overall thickness, UD = depth of umbonal cavity, HL = width of hinge plate, SP, SC and SW = position, colour, and width of adductor muscle scar, C = arrangement of chomata.

Table 3. *Saccostrea* spp. Alleles frequencies

Locus/ allele	Satul A	Trang A	Trat A	Prab A	Chon A	Chum B	Trat B	Talu C
Pgi								
1	0	0.0135	0	0	0	0	0	0
2	0	0.1216	0.0833	0.0385	0	0	0	0.3077
3	0.0192	0	0	0	0	0	0	0
4	0.8846	0.5405	0.0833	0.1346	0.1250	0	0	0.6923
5	0	0.1892	0.0833	0.2692	0.5357	0	0	0
6	0	0	0.0833	0	0	0.0135	0.0139	0
7	0.0962	0.1352	0.5835	0.5577	0.3036	0.0811	0.0417	0
8	0	0	0.0833	0	0.0357	0	0	0
9	0	0	0	0		0.3108	0.3750	0
10	0	0	0	0	0	0.4595	0.3472	0
11	0	0	0	0	0	0.0270	0.0416	0
(N)	(26)	(37)	(6)	(26)	(28)	(37)	36)	(39)
Lap								
1	0	0	0	0	0	0.0227	0.0349	0
2	0	0	0	0	0	0.1136	0.1628	0
3	0	0	0.0833	0	0	0.7500	0.6512	0
4	0	0.0250	0	0.1400	0.3030	0.0909	0.093	0.0375
5	0.1750	0.1125	0.0833	0.2800	0.1818	0.0228	0.0581	0.025
6	0.6500	0.6875	0.8333	0.5000	0.5000	0	0	0.2125
7	0.1375	0.1625	0	0.08	0.0152	0	0	0.6625
8	0.0125	0	0	0	0	0	0	0.025
9	0	0.0125	0	0	0	0	0	0.0375
10	0.025	0	0	0	0	0	0	0
(N)	(38)	(39)	(6)	(25)	(33)	(44)	(43)	(40)
Pgm								
1	0.0102	0	0	0.0600	0	0	0	0
2	0.0306	0.0156	0	0.0800	0.0333	0	0.0139	0
3	0.1122	0.0469	0.3000	0.3400	0.4000	0.0946	0.0278	0
4	0.6735	0.7031	0.7000	0.4800	0.5334	0.1081	0.0972	0.1250
5	0	0	0	0	0	0.3378		0
6	0.1633	0.2031	0	0.0400	0.0333	0.4325	0.3333	0.1500
7	0.0102	0.0313	0	0	0	0.027	0.4584	0.7000
8	0	0	0	0	0	0	0.0555	0.0250
9	0	0	0	0	0	0	0.0139	0
(N)	(49)	(32)	(5)	(25)	(30)	(37)	(36)	(40)

Table 3. (continued)

Locus/ allele	Satul A	Trang A	Trat A	Prab A	Chon A	Chum B	Trat B	Talu C
Mpi-2								
1	0.0278	0.0469	0	0.0400	0.0185	0	0	0
2	0.0417	0.0313	0.1250	0.0600	0.0185	0.0152	0.0968	0
3	0.0833	0.0938	0.2500	0.3200	0.2222	0.1364	0.1129	0
4	0.5138	0.7030	0.5000	0.5000	0.5371	0.5302	0.3710	0
5	0.3056	0.1250	0.1250	0.0800	0.1667	0.3182	0.3710	0
6	0.0278	0	0	0	0.037	0	0.0484	0.25
7	0	0	0	0	0	0	0	0.1667
8	0	0	0	0	0	0	0	0.5555
9	0	0	0	0	0	0	0	0.0278
(N)	(36)	(32)	(4)	(25)	(27)	(33)	(31)	(36)
Ap								
1	0.0476	0.0116	0	0.1136	0.1111	0.0286	0	0.0125
2	0.1429	0.0814	0	0.7273	0.7037	0.2143	0.1795	0.2125
3	0.2381	0.3372	0	0.0682	0.1667	0.0143	0.0641	0.1125
4	0.5001	0.5233	0.8000	0.0900	0.0185	0.5142	0.4231	0.6250
5	0.0476	0.0465	0.2000	0	0	0.1857	0.2564	0.0375
6	0.0239	0	0	0	0	0.0429	0.0513	0
7	0	0	0	0	0	0	0.0256	0
(N)	(21)	(43)	(8)	(22)	(27)	(35)	(39)	(40)
Est-2								
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0.0125
3	0.0208	0	0	0.0385	0.0135	0.0638	0.0395	0.0125
4	0	0	0	0	0	0	0	0
5	0.7917	0.7447	0.9167	0.8845	0.946	0.9362	0.9474	0.9125
6	0	0.1383	0	0	0	0	0	0.0125
7	0.1667	0.117	0	0.0385	0.027	0	0.0131	0.0375
8	0.0208	0	0.0833	0.0385	0.0135	0	0	0.0125
(N)	(24)	(47)	(6)	(36)	(37)	(47)	(38)	(40)
Aat-2								
1	0	0.0222	0	0	0	0.0119	0	0
2	0.0333	0.0111	0	0.0278	0	0	0	0
3	0.8334	0.9222	1	0.7778	0.9286	0.9642	0.9884	0.9750
4	0.0333	0	0	0	0	0	0	0
5	0.0667	0.0444	0	0.1944	0.0714	0.0239	0.0116	0.0250
6	0.0333	0	0	0	0	0	0	0
(N)	(30)	(45)	(6)	(18)	(35)	(42)	(43)	(40)
Mdh-2								
1	0	0	0	0.0208	0	0.0104	0	0
2	0.0300	0.0204	0	0	0.0132	0.0208	0	0.0375
3	0.9500	0.9796	1	0.9167	0.9868	0.9063	0.9886	0.9
4	0	0	0	0	0	0.0625	0	0
5	0.0200	0	0	0.0625	0	0	0.0114	0.0625
(N)	(50)	(49)	(6)	(24)	(38)	(48)	(44)	(40)
Idh-1								
1	0	0	0	0	0	0	0	1
2	0.0238	0	0	0	0	0.0526	0.0526	0
3	0.9762	1	1	1	1	0.9474	0.9474	0
(N)	(21)	(31)	(3)	(4)	(9)	(19)	(19)	(40)

Table 4. Genetic identities of 9 loci.

	Trat B	Chum B	Talu C	Trat A	Chon A	Prab A	Trang A	Satul A
Trat B	0							
Chum B	95.19	0						
Talu C	60.27	64.23	0					
Trat A	77.42	74.74	56.81	0				
Chon A	76.23	71.22	52.86	85.85	0			
Prab A	74.78	69.32	52.34	87.77	97.70	0		
Trang A	76.72	71.87	62.24	91.14	86.86	85.14	0	
Satul A	75.06	69.84	63.92	88.55	84.37	84.38	97.07	0

Table 5. Summary of the analysis of variance and Scheffe's test on 39 morphological factors derived from shell measurements and observations in 8 populations of oysters collected from different locations throughout Thailand.
* indicates $0.01 < p < 0.05$

Variables	ANOVA	Scheffe	Groups with significance differences
RDVM/ RAPM	1.6199	ns	
RDVM / RT	9.0375	*	6-2 / 6-5 / 6-8 / 1-2 / 1-5 / 1-8 / 3-5
RDVM / LDVM	0.8639	ns	
RDVM / LTRT	18.4549	*	6-3 / 6-7 / 6-8 / 1-3 / 1-7 / 1-8 / 2-3 / 2-7 / 2-8 / 4-3 / 4-7 / 4-8 / 5-7 / 5-8 / 3-8
RDVM / UD	23.3866	*	6-3 / 6-8 / 1-3 / 1-8 / 2-3 / 2-8 / 4-3 / 4-8 / 5-3 /5-8
RDVM / HL	3.5511	*	3-6
RAPM / RT	17.6481	*	6-3 / 6-7 / 6-8 / 1-3 / 1-8 / 2-3 / 2-8 / 5-3 / 5-8 / 4-3 / 4-8 / 3-8
RAPM / LAPM	4.3577	*	6-3
RAPM / LTRT	32.1676	*	1-3 / 1-8 / 1-6 / 4-8 / 4-6 / 2-3 / 2-8 / 2-6 / 7-6 / 3-6 / 8-6 / 5-3 / 5-8 / 5-6
RAPM / HL	17.9583	*	1-7 / 1-8 / 3-2 / 3-4 / 3-5 / 3-7 / 3-8 / 6-8 / 2-8 / 5-8 / 4-8 /
RAPM / SW	6.2363	*	2-1 / 2-3 / 4-3 / 5-3
RT / LT		22.8465	* 2-3 / 5-3 / 4-3 / 8-3 / 1-3 / 2-6 / 5-6 / 4-6 / 8-6 /1-6
RT / LTRT		28.8338	* 2-3 / 2-6 / 4-3 / 4-6 / 5-3 / 5-6 / 7-6 / 1-6 / 8-6 / 3-6
LDVM / LAPM	4.0036	*	6-4 / 6-2
LDVM / LT	14.0903	*	1-8 / 1-3 / 1-7 / 1-6 / 2-3 / 2-6 / 5-3 / 5-6
LDVM / LTRT	24.0952	*	1-6 / 2-6 / 5-6 / 4-6 / 3-6 / 7-6 / 8-6
LDVM / UD	4.6126	ns	
LDVM / HL	14.8330	*	3-4 / 3-5 / 3-2 / 3-7 / 3-8 / 1-2 / 1-7 / 1-8 / 6-8 / 4-8 / 5-8 / 2-8
LDVM / SW	22.2031	*	6-3 / 6-8 / 1-3 / 1-8 / 2-3 / 2-8 / 4-3 /4-8 / 5-3 / 5-8
LDVM / C		3.8992	* 6-2 / 6-3
LAPM / LT		22.8818	* 2-8 / 2-3 / 2-7 / 2-6 / 5-8 / 5-3 /5-7 / 5-6 / 1-8 / 1-3 /1-7 / 1-6 4-3 / 4-6

Table 5. (Continued)

Variables	ANOVA	Scheffe	Groups with significance differences
LAPM / LTRT	31.6757		1-8 / 1-6 / 2-8 / 2-6 / 5-8 / 5-6 / 4-6 / 7-6 / 3-6 / 8-6
LAPM / UD	5.1466	*	1-5 / 1-6
LAPM / HL	15.0508	*	3-6 / 3-4 / 3-5 / 3-2 / 3-7 / 3-8 / 1-7 / 1-8 / 6-8 / 4-8 / 5-8 / 2-8
LAPM / SW	3.2490	ns	
LAPM / C	16.6869	*	1-6 / 8-6 / 5-6 / 2-6 / 4-6 / 7-6 / 3-6
LT / LTRT	33.4305	*	1-6 / 4-6 / 5-6 / 7-6 / 2-6 / 3-6 / 8-6
LT / UD	12.0095	*	8-2 / 8-5 / 7-6 / 8-6 / 3-2 / 3-5 / 3-6 / 1-6 / 4-6
LT / SW	24.0774	*	8-3 / 8-6 / 1-6 / 2-6 / 5-6 / 7-6 / 4-6 / 3-6
LTRT / UD	15.8508	*	8-4 / 8-2 / 8-5 / 8-6 / 7-2 / 7-5 / 7-6 / 3-2 / 3-5 / 3-6 /1-6 / 4-6
LTRT / HL	9.6530	*	3-4 / 3-5 / 3-2 / 3-8 /3-6 / 1-6
LTRT / SW	60.3333	*	3-4 / 3-1 / 3-6 / 8-6 / 7-6 / 5-6 / 2-6 / 4-6 / 1-6
UD / HL	21.2108	*	3-5 / 3-1 / 3-2 / 3-4 / 3-6 /8-6 / 7-6 / 5-6 / 1-6 / 2-6 / 4-6
(RDVM+RAPM) / RT	25.6747	*	1-3 / 1-8 / 1-6 / 2-8 / 2-6 / 4-6 / 5-6 / 3-6 / 8-6
(LDVM+LAPM) / LT	4.8086	*	1-5
(LDVM+LAPM)/ LT ÷(RDVM+RAPM)/ RT	18.2026	*	3-2 / 3-5 / 3-8 / 3-7 / 6-8 / 6-7 / 2-8 / 2-7 / 1-2 / 1-5 / 1-8 / 1-7 / 4-8 / 4-7 / 5-8 / 5-7
SC	15.7905	*	2-6 / 8-6 / 5-6 / 1-6 / 3-6 / 4-6 / 7-6
SP	4.8716	*	1-8 / 2-8 / 3-8 / 4-8 / 5-8 / 6-8
C	27.9652	*	2-6 / 2-1 / 2-3 / 7-3 / 8-3 / 5-1 / 5-3 / 4-3 / 3-6 / 1-3

Table 6. Summary of dissimilarity and similarity of 39 morphological factors determined between populations of *Saccostrea* oysters.

Population Concerned	Dissimilarity	Similarity
7-8	0	39
6-8	24	15
6-7	15	24
6-5	15	24
6-4	16	23
6-3	23	16
6-2	18	21
6-1	18	21
5-8	14	25
5-7	4	35
5-4	0	39
5-3	19	20
5-2	0	39
4-8	11	28
4-7	2	37
4-3	15	24
4-2	0	39
4-1	0	39
3-8	11	28
3-7	5	34
3-2	19	20
3-1	12	27
2-8	15	24
2-7	4	35
2-1	4	35
1-8	15	24
1-7	7	32

* 1 = Chonburi A, 2 = Trang A, 3 = Chumporn A, 4 = Prab A, 5 = Satul A, 6 = Talu C, 7 = Trat A, 8 = Trat B

Table 7. The avearge values and standard deviation of 8 morphological factors determined in the discriminant analysis.

Sp.	RDVM/UD	SC	C	LDVM/C	LTRT/SW	LDVM/HL	UD/HL	RT/ LTRT
A	5.77 (2.87)*	1.94 (0.22)	1.26 (0.63)	2.07 (0.68)	2.04 (0.55)	2.51 (0.61)	0.75 (0.36)	0.33 (0.11)
B	15.33 (9.09)	1.98 (0.15)	2.23 (0.98)	2.04 (1.27)	1.51 (0.49)	2.87 (0.59)	0.31 (0.13)	0.47 (0.13)
C	3.12 (1.03)	1.38 (0.49)	1.67 (0.74)	2.13 (0.48)	2.64 (0.57)	2.15 (0.54)	0.98 (0.26)	0.38 (0.11)

* standard deviation

Table 8. Summary of the percentage of correct classification by the morphometric identification.

Actual group		No. of cases	Predicted group membership		
Group A	1	119	108 (90.8%)	6 (5.0%)	5 (4.2%)
Group B	2	47	2 (4.3%)	44 (93.6%)	1 (2.1%)
Group C	3	24	3 (12.5%)	0 (0%)	21 (87.5%)

Percent of "grouped" cases correctly classified: 91.05%

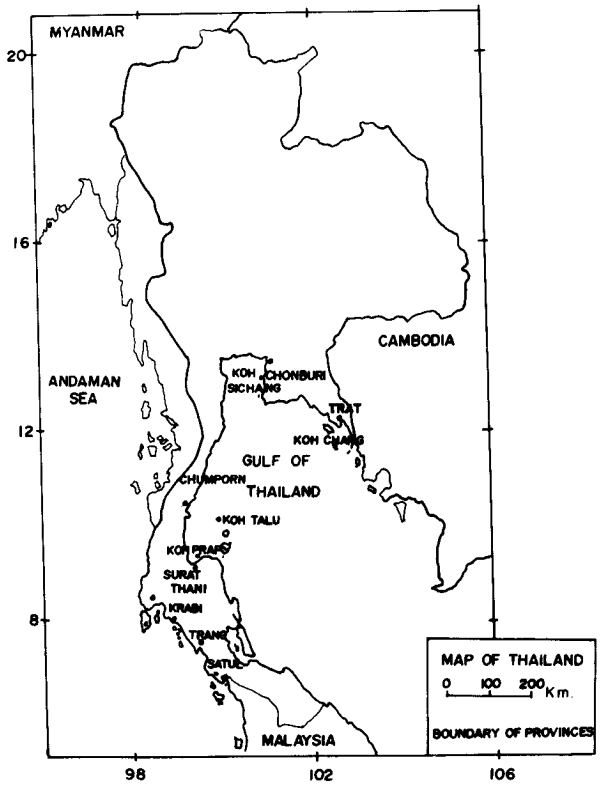


Fig. 1. Sampling locations of *Saccostrea* oysters throughout Thailand.

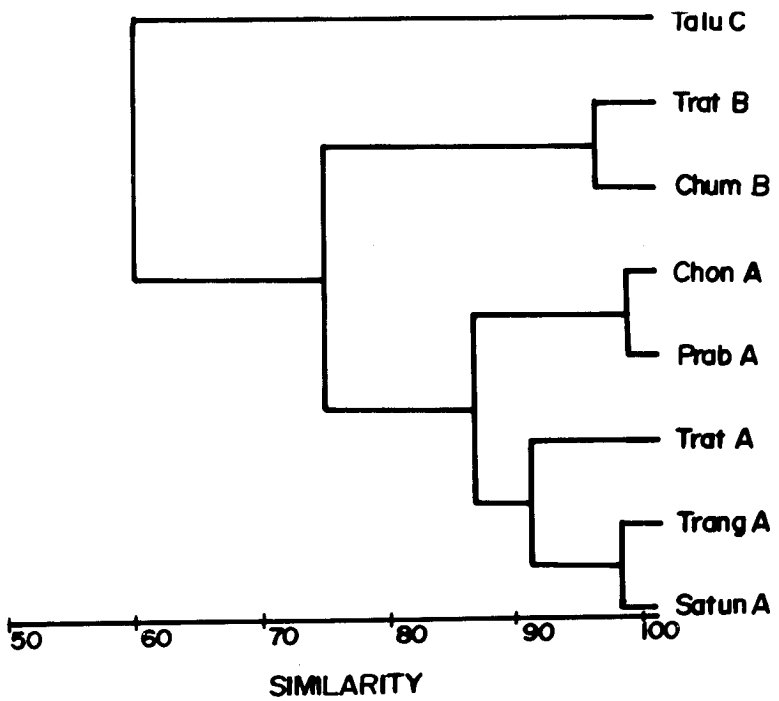
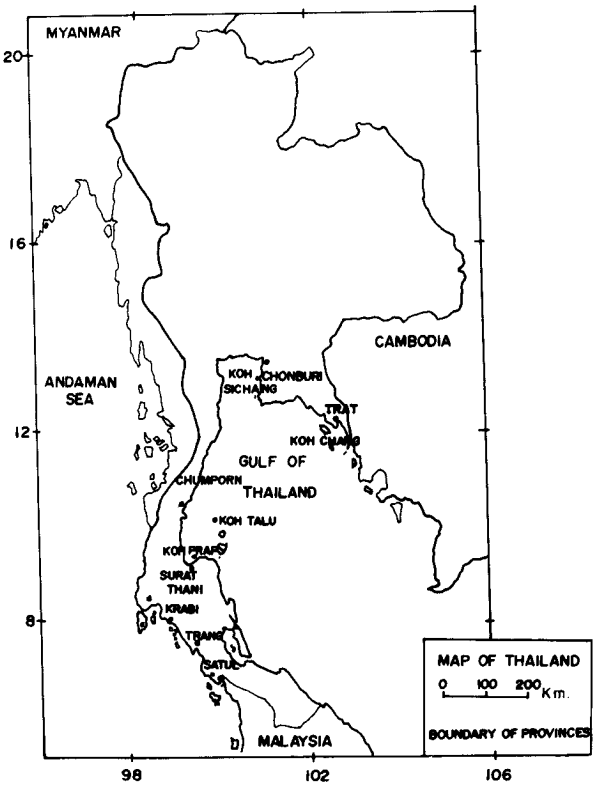


Fig. 2. A constructed dendrogram of population relationship based on genetic identities of 9 enzyme loci.



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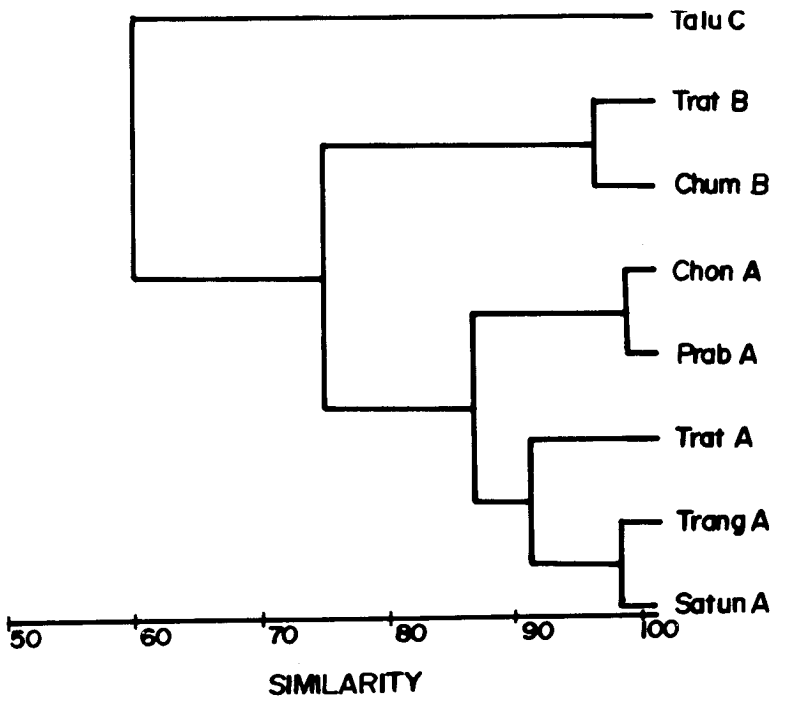

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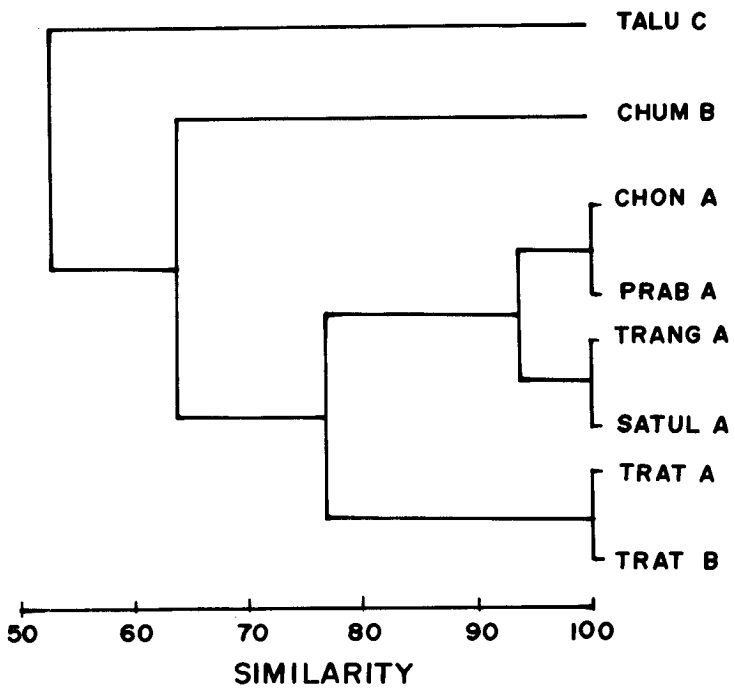

Fig. 3. A constructed dendrogram of population relationship based on relative morphological similarities of populations.

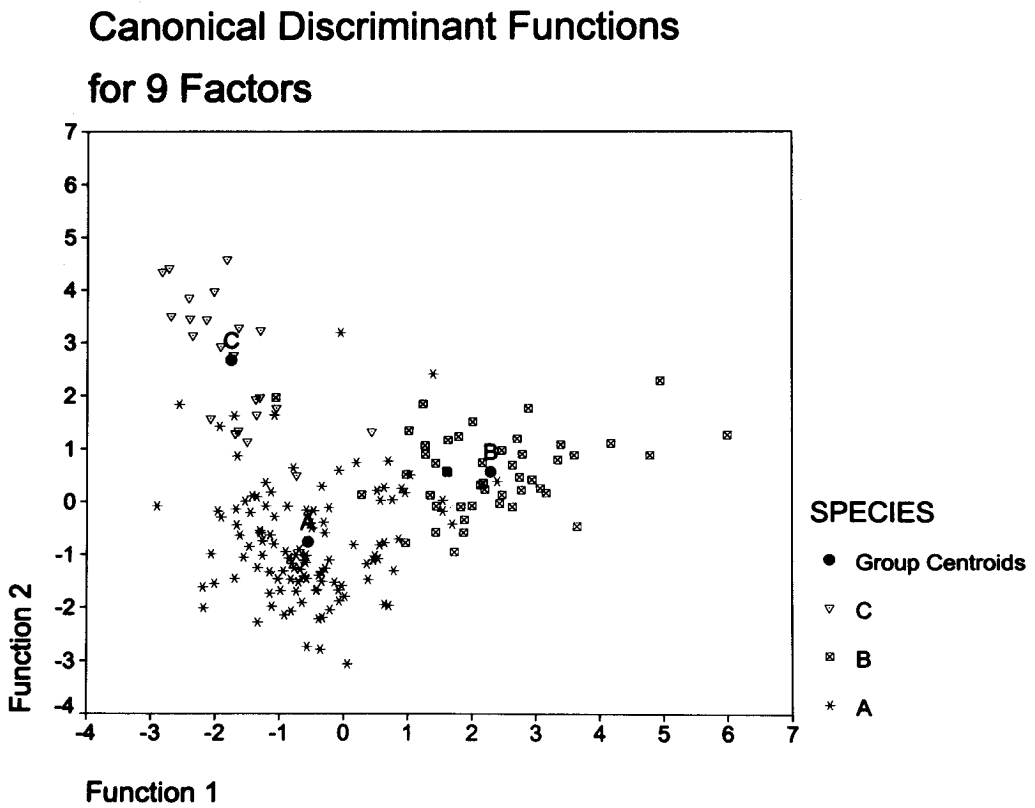

Fig. 4. A plot of distribution of species A, B, and C as determined by the morphometric technique.

analysis of similarity between populations of *Saccostrea* oysters collected along the coastline of Thailand. Individuals which were scored as hybrids by the electrophoretic technique, were removed from the morphometric analysis. Table 5 gives the results of analysis of variance of 39 morphological factors determined for the relationship between populations. Where F -value was significant, the results of Scheffe's test were given to indicate which population differed significantly from which other. A summary of similarity and dissimilarity between populations is shown in Table 6. Simple matching coefficients were calculated, and they were used for construction of dendrogram by the UPGMA program. The result, as shown in figure 3, shows that the morphometric method cannot separate the "A" and " B" species collected from the same location, *ie.*, Ko Chang-Trat.

Stepwise selection algorithm in discriminant analysis was used to determine which potentially useful morphometric factors should be included in species identification. With the probabilities of F-to-enter 0.01 and F-to-remove 0.014, the stepwise selection procedure showed that only 8 factors were included. These factors are RDVM/UD, SC, C, LDVM/C, LTRT/SW, LDVM/HL, UD/HL, and RT/LTRT. Table 7 shows the average values and standard deviations of each morphological factors determined in the discriminant analysis of species A, B, and C. The percentage of samples correctly classified by the morphometric analysis was 91.05% as determined by the canonical discriminant function (Table 8). The plot of distribution of species A, B, and C is shown in Figure 4.

DISCUSSION

Among oysters *Crassostrea cucullata* (von Born, 1778) has been considered as a superspecies by Stenzel (1971) who, however, assigned this tuberculated or denticulated species and others like it to the genus *Saccostrea*. According to him the Sydney rock oyster *Crassostrea commercialis* was a subspecies of *Saccostrea cucullata*. This introduced a controversy in the field of oyster taxonomy. Iredale and Roughley (1933) had identified the Sydney rock oyster as a species of Saxostrea, but Thomson (1954) placed it in the genus *Crassostrea* and ever since then the Sydney rock oyster had been known to oystermen and to biologists as *Crassostrea commercialis*. Thomson (1954) identified small oysters of Australia as *C. tuberculata* (Lamarck). The name *C. tuberculata* was also accepted for the cucullata-like oysters of the Philippines (Carreon, 1968) and the cucullata-like oysters of Pakistan (Ahmed, 1975).

Saccostrea cucullata is widely distributed in the Indo-Pacific tropics and subtropics. It will not be surprising to find variants or subspecies of this species in different part of the world (Ahmed, 1975). For instance, there were two ecomorphs of this species occurring in East Africa (Stenzel, 1971) and one subspecies *commercialis* in the Australian waters (as believed by Stenzel). Ahmed (1975) discussed that to call *S. cucullata* a complex superspecies, as did Stenzel (1971), was not justified unless genetic differences and partial or complete reproduction isolation could be demonstrated from different stocks of this species from different parts of the Indo-Pacific. Ahmed (1975) went on to state that this species may simply be polymorphic instead of being polytypic. Morris (1985) mentioned that specimens of *S. cucullata* collected in the mangrove area of Hong Kong were thin shelled and stunted, but developed into typical morphology when placed in the exposed area. Moreover, in sheltered areas the oysters may be covered by a variable number of thin, black, vertically projecting hyote spines. These spines had been considered as diagnostic of *S. echinata* by Quoy and Gaimard (1835). Thus this species, *S. cucullata*, had been recorded under the name *S. echinata* from a number of localities throughout the tropical Indo-Pacific (Morris, 1985). Later on, it was confirmed by Morton and Morton (1983) that *S. echinata* represented the juvenile stage of *S. cucullata*.

In Thailand Brohmanonda *et al.* (1988) had classified all small oysters collected from many provinces *ie.*, Chonburi, Trat, Chanthaburi, Prachaub Kirikhan, Chumporn, Satul, Trang, and Krabi as *Saccostrea commercialis* (Iredale & Roughley). This was contrary to other authors (Amornjaruchit, 1988; Yusuk, 1988) who referred to as *S. cucullata* (Born, 1778). Small rock oysters were also recognized on the rocky shores of the Sichang Island, Chonburi, but they were classified as *Saccostrea mordax* (Gould) (Tsuchiya & Liardwitayapasit, 1986). In addition with the fact that the small oysters in this species could display ecomorphology, thus it has become a problem of concern for the taxonomists in naming these small oysters.

The classifications of oysters using solely their shell characteristics has been of great value since the time of Linnaeus (1758). However, these features are often insufficient to reach the proper taxonomic identification and to discuss evolutive tendencies in a well defined group. Moreover, identification is very important in shellfish aquaculture. Thus other methods have been applied for classification, in addition to the descriptive morphology. Among these, electrophoretic technique was found to be an extremely valuable tool for systematic studies (Avise, 1975). Therefore many papers related to taxonomy published later on had applied genetic analysis by electrophoretic technique to morphological characteristics. However, many authors concluded that morphological and genetic variation were not related. For example, Groue and Lester (1981) performed morphological and genetic analyses of oysters in the gulf of Mexico. They concluded that morphological variation was probably a response to differences in environmental factors such as salinity. While other authors including Beaumont and Khamdan (1991) who studied population differentiation in pearl oyster, *Pinctada radiata* (leach), considered that estimates of population relationship was more reliable by electrophoretic data than shell shape. The effect of environment upon shell morphology was particular pronounced in oysters, as shown by Galtsoff (1964), in which the principal axes of shell growth were not as permanent as they were in clams, scallops, and other bivalves.

This research applied the value of electrophoresis to morphological characters in identification of small oysters in Thailand. The morphological characters used in this study were among those important in classification of oysters (Stenzel, 1971), namely depth of umbonal cavity, arrangement of chomata, index of shell shape, shell structure, width of hinge plate, etc. The morphometric analyses were done on the SPSS statistical computer package. The dendrograms based on gene frequencies and morphometric analyses were constructed by UPGMA program. It can be shown that the identification of oysters by the electrophoretic and morphometric analyses gave similar results. Thus the technique is proved to be of high value for field work. However, entering 39 morphometric factors in the computer for oyster grouping is such a lot of work. So another statistical technique, discriminant analysis, was performed to see whether some of these factors could be reduced without changing the outcome of the result. It can be shown that the factors could be reduced to only 8. Therefore, it is certain that this technique, morphometric analysis, is of great value in identification of oyster species particularly in the field. The technique has other advantages over the electrophoretic technique, i.e., simple, easy, rapid, low cost, and high percentage of correct classification.

In this study the impact of environment on shell morphology was less than that of genetics. This is because polymorphism can be observed in Trat and Chumporn. The differentiation in environment could maintain polymorphism within the species with different genotypes inhabiting geographically adjacent environment. The results of morphometric analysis correspond quite well with the results of electrophoresis. This occurs from the fact that the shells were primarily grouped according to the electrophoretic results. This could eliminated the hybrids from the analysis , thus it can be certain about the results occurred. In addition

this study supports Stenzel (1971) and Ahmed (1975) in the morphological characters which are important in classification of *Saccostrea* oysters, i.e., depth of umbonal cavity, adductor muscle scar, arrangement of chomata, length of the hinge plate, thickness of right valve and overall thickness. Index of shell shape was found to be less important in this morphometric analysis .

The idea of placing the tuberculated oyster species in the genus *Saccostrea* has been accepted by many taxonomists. Based on the results of electrophoretic studies it can be concluded that there are at least 3 species of the *Saccostrea* oysters in Thailand. However, to be certain in assigning the name to each group, comparison with type specimen should also be made by both electrophoretic technique and molecular biological technique. Needless to say the morphology is still quite useful in grouping of oysters before further analyses could be done.

The knowledge from this study can be applied to use in oyster cultures. Taxonomy should receive more careful consideration in oyster culture. Ideally, for commercial purposes, an oyster shell should be well-cupped and low ability to accumulate pollutants from water. These characteristics are genetic dependent. Failing to recognize the taxa would confuse, rather than aid, the understanding of their biology and could lead to failure or success of breeding practices. Moreover, in order to study the impact of human to the environment, the biodiversity should be studied first for background knowledge of the extant species at a particular time. This technique, morphometric analysis, is proved to be of great value in identification of oysters by any layman.

ACKNOWLEDGEMENT

The authors greatfully acknowledge the efforts of many people who helped in this research: Prof. Dr. Visut Baimai, Mahidol University; Assoc. Prof. W. Yusuk, Kasetsart University; The Coastal Aquaculture Division, Fishery Department, Ministry of Agriculture and Economics; Miss A Sungpetch; Mr. C Dilokratanatrakul and Dr. Kitt Visoottiviseth. This research was funded by the Darwin Initiative for the Survival of Species.

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