Cytogenetic Studies on Metaphase Chromosomes of Six Bivalve Species of Families Mytilidae and Veneridae (Nucinelloidea, Mollusca)

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Summary Chromosomes of 6 bivalve species were studied from mitotic metaphases using cell suspension techniques. Among Family Mytilidae, *Modiolus barbatus* (Linnaeus 1758) has a diploid chromosome number of $2n=32$ with 5 metacentric, 2 submetacentric, 5 subtelocentric and 4 telocentric chromosome pairs, *Septifer excisus* (Wiegmann 1837) has $2n=26$ with 9 metacentric, 2 submetacentric, and 2 telocentric chromosome pairs and *Septifer bilocularis* (Linnaeus 1758) also has $2n=26$ with 8 metacentric, 2 submetacentric and 3 subtelocentric chromosome pairs. Among Family Veneridae, *Ruditapes decussatus* (Linnaeus 1758) has $2n=38$ with 6 metacentric, 5 submetacentric, 3 subtelocentric and 5 telocentric chromosome pairs, *Circe scripta* (Linnaeus 1758) has $2n=38$ with 6 metacentric, 6 submetacentric, 4 subtelocentric and 3 telocentric chromosome pairs and *Venus verrucosa* (Linnaeus 1758) has also $2n=38$ with 7 metacentric, 4 submetacentric, 4 subtelocentric and 4 telocentric chromosome pairs. These results are reported for the first time in Egypt.

Key words Chromosomes, Karyotypes, Ideograms, Mytilidae, Veneridae, Bivalvia.

The bases of cytotaxonomy were laid down when the principle of chromosome individuality was accepted and the chromosome theory of heredity became established. It was noticed that the karyotype characterized by the chromosome number, size and morphology, is a definite and constant feature of each species. Since 1992, new karyological data have been added for 65 bivalve species among which the first karyological data are given for 49 species. Within the bivalve class, the most frequent chromosome number of $2n=38$ was reported by Nakamura (1985) and Thiriott-Quievreux (1994).

Chromosome studies have been reported on 14 species of Family Veneridae: the chromosome number $2n=38$ is most frequently (Menzel 1968a, Gerard 1978, Rasotto *et al.* 1981, Wang *et al.* 1998), while chromosome number and morphology (Koulman and Wolff 1977, Corni and Trentini 1990), karyotype (Ieyama 1990) and karyometric analysis (Ieyama 1985, Borsa and Thiriott-Quievreux 1990) are described.

The diploid chromosome number ($2n$) of 30 bivalve species (Family: Mytilidae) range from 22 to 32. This diploid chromosome complements have all types of chromosome, and the total diploid chromosome length has been reported mostly from the Mytilidae and shows an increase as $2n$ increase. The karyotype variety is very apparent in the Mytilidae (Ahmed 1974, Diupotex-Chong *et al.* 1978, Ieyama 1984, Thiriott-Quievreux 1984, Cornet 1993, Pasantes *et al.* 1996, Holland *et al.* 1999, Vitturi *et al.* 2000, Thiriott-Quievreux 2002). Previous data are compiled in Table 1.

This report presents the chromosome numbers, karyotypes and morphometric measurements of chromosomes in 3 species of Family Mytilidae, *Modiolus barbatus*, *Septifer excisus* and *Septifer bilocularis* and 3 species of Family Veneridae, *Ruditapes decussatus*, *Circe scripta* and *Venus verrucosa*, specially in Egypt. These outcomes are compiled in Table 2.

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Table 1. Interspecific comparisons of karyological data

Species/Family	Origin	2n	Karyotype	Reference
Mytilidae				
<i>Septifer bilocularis</i>	Japan	26	3m, 2m/sm, 7st, 1t	Ieyama 1983b
<i>Septifer excisus</i>	Japan	26	2m, 1m/sm, 2sm, 7st, 1t	Ieyama 1983b
<i>Septifer keenae</i>	Japan	28	3m, 2m/sm, 2sm, 4st, 2t/st, 1t	Ieyama 1983b
<i>Septifer virgatus</i>	Japan	28	4m, 1m/sm, 2sm, 4st, 1t/st, 2t	Ieyama 1983b
<i>Modiolus barbatus</i>	Italy	32	6m, 8sm, 2st	Libertini <i>et al.</i> 1996
<i>Modiolus barbatus</i>	Italy	32		Rasotto <i>et al.</i> 1981
<i>Modiolus arcuatulus</i>	Egypt	24	5m, 7sm	Ghobashy <i>et al.</i> 1995
<i>Modiolus arcuatulus</i>	Japan	32	6m, 6sm, 3st, 1t	Ieyama 1984c
<i>Mytilus edulis</i>	France	28	6m, 8sm/st	Martinez-Lage <i>et al.</i> 1996
<i>Mytilus edulis</i>	France	28	2m, 6sm, 6st	Thiriout-Quievreux and Ayraud 1982
<i>Mytilus galloprovincialis</i>	Spain	28	6m, 8sm/st	Martinez-Lage <i>et al.</i> 1994
<i>Mytilus galloprovincialis</i>	France	28	1m, 6sm, 7st	Thiriout-Quievreux and Ayraud 1982
<i>Mytilus trossulus</i>	Canada	28		Gonzalez-Tizon <i>et al.</i> 2000b
<i>Mytilus trossulus</i>	Canada	28	7m, 7sm	Martinez-Lage <i>et al.</i> 1997
<i>Mytilus californianus</i>	Canada	28	7m, 7sm	Martinez-Lage <i>et al.</i> 1997
<i>Mytilus coruscus</i>	Japan	28	5m, 3sm, 6st	Ieyama 1984c
<i>Mytilus desolations</i>	Indean	28	5m, 2m/sm, 4sm/st, 3st	Thiriout-Quievreux 1984
<i>Brachidontes minimus</i>	W Medit erranean	28	5m, 9st	Thiriout-Quievreux, unpublished
<i>Brachidontes pharaonis</i>	S Medit erranean	28	6m/sm, 8st	Vitturi <i>et al.</i> 2000
<i>Brachidontes rodriguezi</i>	Argentina	28	2m, 12st, 2t	Torreiro <i>et al.</i> 1999
Veneridae				
<i>Circe scripta</i>	Japan	38	19m	Ieyama 1980
<i>Venus verrucosa</i>	Italy	38		Rasotto <i>et al.</i> 1981
<i>Ruditopes decussatus</i>	Spain	38		Rodriguez-Juiz <i>et al.</i> 1996
<i>Ruditopes decussatus</i>	France	38	9m/sm, 10st/t	Borsa and Thiriout 1990
<i>Ruditopes philippinarum</i>	Canada	38		Gonzalez-Tizon <i>et al.</i> 2000b
<i>Ruditopes aureus</i>	France	38	17m/sm, 2st/t	Borsa and Thiriout 1990
<i>Venerupis pullastra</i>	Spain	38	3m, 8sm, 8st	Insua and Thiriout-Quievreux 1992
<i>Venerupis rhomboides</i>	Spain	38	4m, 8sm, 4st, 3t	Insua and Thiriout-Quievreux 1992
<i>Chione cancellata</i>	USA	38		Menzel 1968a
<i>Irus mitis</i>	Japan	38	15m, 4sm	Ieyama 1980

m: metacentric, sm: submetacentric, st: subtelocentric, t: telocentric.

Table 2. Summary of karyological data of the present study

Family/Species	2n	Karyotype						Reference
		m	m/sm	sm	st	st/t	t	
Mytilidae								
<i>Septifer bilocularis</i>	26	3	2		7		1	Ieyama 1983b
<i>Septifer bilocularis</i>	26	8		2	3			Present study
<i>Septifer excisus</i>	26	2	1	2	7		1	Ieyama 1983b
<i>Septifer excisus</i>	26	9		2			2	Present study
<i>Modiolus barbatus</i>	32	6		8	2			Libertini <i>et al.</i> 1996
<i>Modiolus barbatus</i>	32	5		2	5		4	Present study
Veneridae								
<i>Circe scripta</i>	38	19						Ieyama 1980
<i>Circe scripta</i>	38	6		6	4		3	Present study
<i>Ruditopes decussatus</i>	38		9			10		Borsa and Thiriout 1990
<i>Ruditopes decussatus</i>	38	6		5	3		5	Present study
<i>Venus verrucosa</i>	38							Rasotto <i>et al.</i> 1981
<i>Venus verrucosa</i>	38	7		4	4		4	Present study

m: metacentric, sm: submetacentric, st: subtelocentric, t: telocentric.

Materials and methods

Samples of the different species investigated were collected from different localities along the coast of the Red Sea from Hurghada to Quseir City. The collected specimens were transferred directly to the laboratory and kept alive in tanks of aerated seawater.

The whole animals were prepared by the warm-dry method of Kligerman and Bloom (1977) with some modification made by Nakamura (1986) as follows: The bivalves were kept in 0.005% colchicine solution for 2–6 h before being sacrificed, and then the gonads and gills were removed, cut into small pieces and soaked in 0.075 M KCl hypotonic solution. These pieces were fixed in freshly mixed Carnoy's fixative (3 : 1 methyl alcohol : acetic acid) with 3 changes of 20 min duration. Tissues were then minced gently in 50% acetic acid to prepare a cell suspension. A drop of the cell suspension was pipetted out and placed on heated clean glass slides. For conventional karyotypes, slides were stained directly with Giemsa (4% in phosphate buffer, pH 6.8) for 20 min.

The best spread chromosome configurations were cut out from the photographs and chromosomes were paired on the basis of centromeric position and size for karyotyping. The following morphological features were used to compare the karyotypes: the relative length (RL), the arm ratio (Ar) and the centromeric index (Ci). Fundamental number (FN) was calculated on the basis that metacentric, submetacentric and subtelocentric having a value of four and acrocentric has a value of two. Nomenclature of chromosome type adopted by Levan *et al.* (1964) were used in the present study.

Results and discussion

Modiolus barbatus

The chromosome number from the species *Modiolus barbatus* was determined from gills,

Fig. 1. Mitotic metaphase spread (a) and karyotype (b) of *Modiolus barbatus* $2n=32$.

Fig. 2. Ideograms of the chromosomes of *Modiolus barbatus* constructed with respect to the relative length (a) and the centromeric index (b).

oogonial and spermatogonial germ line cells; gills offered better results for chromosomal analysis than gonads. The photograph of the cell spread and the karyotype of this species showed that it has a diploid chromosome number of $2n=32$ with fundamental number (FN) of 56 (Fig. 1a). An ideogram (Fig. 2a, b) was constructed from the relative length and centromeric indices with the centromere drawn at the same level to make the visual comparison easier.

The karyotype of *Modiolus barbatus* show 4 different groups of chromosomes (Fig. 1b). Group A is composed of 5 metacentric chromosome pairs with an arm ratio ranging from 1.09 to 1.64, relative length from 6.02% to 10.42% and centromeric index varying from 37.93 to 47.78. Group B has 2 submetacentric chromosome pairs with an arm ratio ranging from 1.88 to 2.00, relative length from 5.21% to 6.65% and centromeric index varying from 33.33 to 34.78. Group C consists of 5 subtelocentric chromosome pairs with an arm ratio ranging from 3.12 to 4.17, relative length from 4.05% to 6.95% and centromeric index varying from 19.35 to 24.27 and group D contains 4 telocentric chromosome pairs with arm ratio ranging from 7.67 to 9.60, relative length from 4.51% to 6.13% and centromeric index varying from 9.43 to 11.54 (Table 3). The diploid chromosome number of *Modiolus barbatus* $2n=32$ is in agreement with that reported by Libertini *et al.* (1996). While the karyotype reported by them is composed of 6 metacentric, 8 submetacentric and 2 subtelocentric chromosome pairs, that of our result composed of 5 metacentric, 2 submetacentric, 5 subtelocentric and 4 telocentric chromosome pairs.

Septifer excisus

The cell spread, karyotype and ideogram of a model sample of this species (Fig. 3, 4) demon-

(a)

(b)

Fig. 3. Mitotic metaphase spread (a) and karyotype (b) of *Septifera excisus* $2n=26$.

Fig. 4. Ideograms of the chromosomes of *Septifer excisus* which were constructed in respect to the relative length (a) and the centromeric index (b).

Table 3. Average of ten cell spreads of chromosomes measurements and classification of *Modiolus barbatus*

Chromosome No.	Chromosome length			Relative length %			Arm ratio	Centromeric index %	Classification
	Long arm	Short arm	Total	Long arm	Short arm	Total			
	Mean $\pm$ S.D.	Mean $\pm$ S.D.	Mean $\pm$ S.D.	Mean $\pm$ S.D.	Mean $\pm$ S.D.	Mean $\pm$ S.D.	Mean $\pm$ S.D.	Mean $\pm$ S.D.	
1	0.94 $\pm$ 0.14	0.86 $\pm$ 0.13	1.80 $\pm$ 0.19	5.44 $\pm$ 0.46	4.98 $\pm$ 0.44	10.42 $\pm$ 0.89	1.09 $\pm$ 0.16	47.78 $\pm$ 1.34	M
2	0.95 $\pm$ 0.14	0.65 $\pm$ 0.10	1.60 $\pm$ 0.18	5.50 $\pm$ 0.53	3.76 $\pm$ 0.35	9.26 $\pm$ 0.86	1.46 $\pm$ 0.17	40.63 $\pm$ 1.12	M
3	0.90 $\pm$ 0.13	0.55 $\pm$ 0.09	1.45 $\pm$ 0.16	5.21 $\pm$ 0.48	3.18 $\pm$ 0.30	8.39 $\pm$ 0.87	1.64 $\pm$ 0.17	37.93 $\pm$ 1.23	M
4	0.75 $\pm$ 0.12	0.51 $\pm$ 0.08	1.26 $\pm$ 0.17	4.34 $\pm$ 0.41	2.95 $\pm$ 0.23	7.29 $\pm$ 0.65	1.47 $\pm$ 0.16	40.48 $\pm$ 1.12	M
5	0.60 $\pm$ 0.09	0.44 $\pm$ 0.06	1.04 $\pm$ 0.15	3.47 $\pm$ 0.34	2.55 $\pm$ 0.23	6.02 $\pm$ 0.55	1.36 $\pm$ 0.16	42.31 $\pm$ 1.38	M
6	0.75 $\pm$ 0.12	0.40 $\pm$ 0.06	1.15 $\pm$ 0.16	4.34 $\pm$ 0.37	2.31 $\pm$ 0.21	6.65 $\pm$ 0.61	1.88 $\pm$ 0.18	34.78 $\pm$ 1.24	SM
7	0.60 $\pm$ 0.10	0.30 $\pm$ 0.04	0.90 $\pm$ 0.14	3.47 $\pm$ 0.32	1.74 $\pm$ 0.18	5.21 $\pm$ 0.46	2.00 $\pm$ 0.19	33.33 $\pm$ 1.12	SM
8	0.91 $\pm$ 0.14	0.29 $\pm$ 0.03	1.20 $\pm$ 0.16	5.27 $\pm$ 0.44	1.68 $\pm$ 0.16	6.95 $\pm$ 0.59	3.14 $\pm$ 0.23	24.17 $\pm$ 1.09	ST
9	0.78 $\pm$ 0.13	0.25 $\pm$ 0.03	1.03 $\pm$ 0.15	4.51 $\pm$ 0.42	1.45 $\pm$ 0.15	5.96 $\pm$ 0.51	3.12 $\pm$ 0.25	24.27 $\pm$ 1.10	ST
10	0.58 $\pm$ 0.09	0.16 $\pm$ 0.02	0.74 $\pm$ 0.12	3.36 $\pm$ 0.31	0.93 $\pm$ 0.13	4.29 $\pm$ 0.42	3.63 $\pm$ 0.32	21.62 $\pm$ 1.08	ST
11	0.55 $\pm$ 0.08	0.15 $\pm$ 0.02	0.70 $\pm$ 0.11	3.18 $\pm$ 0.30	0.87 $\pm$ 0.12	4.05 $\pm$ 0.38	3.67 $\pm$ 0.34	21.43 $\pm$ 1.08	ST
12	0.50 $\pm$ 0.07	0.12 $\pm$ 0.02	0.62 $\pm$ 0.10	2.89 $\pm$ 0.27	0.69 $\pm$ 0.10	3.58 $\pm$ 0.32	4.17 $\pm$ 0.39	19.35 $\pm$ 1.07	ST
13	0.96 $\pm$ 0.14	0.10 $\pm$ 0.01	1.06 $\pm$ 0.15	5.55 $\pm$ 0.53	0.58 $\pm$ 0.09	6.13 $\pm$ 0.58	9.60 $\pm$ 0.78	09.43 $\pm$ 1.00	T
14	0.93 $\pm$ 0.13	0.12 $\pm$ 0.02	1.05 $\pm$ 0.15	5.38 $\pm$ 0.51	0.69 $\pm$ 0.10	6.07 $\pm$ 0.58	7.75 $\pm$ 0.71	11.43 $\pm$ 1.03	T
15	0.80 $\pm$ 0.12	0.10 $\pm$ 0.02	0.90 $\pm$ 0.13	4.63 $\pm$ 0.43	0.58 $\pm$ 0.09	5.21 $\pm$ 0.50	8.00 $\pm$ 0.77	11.11 $\pm$ 1.03	T
16	0.69 $\pm$ 0.10	0.09 $\pm$ 0.01	0.78 $\pm$ 0.12	3.99 $\pm$ 0.35	0.52 $\pm$ 0.09	4.51 $\pm$ 0.42	7.67 $\pm$ 0.73	11.54 $\pm$ 1.05	T
Sum			17.28 $\pm$ 1.13						

Table 4. Average of ten cell spreads of chromosomes measurements and classification of *Septifer excisus*

Chromosome No.	Chromosome length			Relative length %			Arm ratio	Centromeric index %	Classification
	Long arm	Short arm	Total	Long arm	Short arm	Total			
	Mean $\pm$ S.D.	Mean $\pm$ S.D.	Mean $\pm$ S.D.	Mean $\pm$ S.D.	Mean $\pm$ S.D.	Mean $\pm$ S.D.			
1	0.90 $\pm$ 0.14	0.80 $\pm$ 0.13	1.70 $\pm$ 0.19	6.76 $\pm$ 0.58	6.01 $\pm$ 0.55	12.77 $\pm$ 1.08	1.13 $\pm$ 0.16	47.06 $\pm$ 1.32	M
2	0.80 $\pm$ 0.12	0.75 $\pm$ 0.12	1.55 $\pm$ 0.17	6.01 $\pm$ 0.55	5.63 $\pm$ 0.54	11.64 $\pm$ 1.00	1.07 $\pm$ 0.13	48.39 $\pm$ 1.43	M
3	0.84 $\pm$ 0.12	0.71 $\pm$ 0.12	1.55 $\pm$ 0.17	6.31 $\pm$ 0.60	5.33 $\pm$ 0.51	11.64 $\pm$ 1.01	1.18 $\pm$ 0.17	45.81 $\pm$ 1.23	M
4	0.90 $\pm$ 0.13	0.65 $\pm$ 0.10	1.55 $\pm$ 0.18	6.76 $\pm$ 0.63	4.88 $\pm$ 0.44	11.64 $\pm$ 1.00	1.38 $\pm$ 0.18	41.94 $\pm$ 1.11	M
5	0.53 $\pm$ 0.09	0.47 $\pm$ 0.07	1.00 $\pm$ 0.15	3.98 $\pm$ 0.34	3.53 $\pm$ 0.31	7.51 $\pm$ 0.64	1.13 $\pm$ 0.14	47.00 $\pm$ 1.35	M
6	0.50 $\pm$ 0.08	0.36 $\pm$ 0.05	0.86 $\pm$ 0.13	3.76 $\pm$ 0.33	2.70 $\pm$ 0.24	6.46 $\pm$ 0.65	1.39 $\pm$ 0.16	41.86 $\pm$ 1.20	M
7	0.48 $\pm$ 0.07	0.38 $\pm$ 0.05	0.86 $\pm$ 0.13	3.61 $\pm$ 0.34	2.85 $\pm$ 0.24	6.46 $\pm$ 0.61	1.26 $\pm$ 0.15	44.19 $\pm$ 1.26	M
8	0.45 $\pm$ 0.07	0.35 $\pm$ 0.04	0.80 $\pm$ 0.12	3.38 $\pm$ 0.31	2.63 $\pm$ 0.26	6.01 $\pm$ 0.55	1.29 $\pm$ 0.15	43.75 $\pm$ 1.23	M
9	0.30 $\pm$ 0.05	0.25 $\pm$ 0.04	0.55 $\pm$ 0.09	2.25 $\pm$ 0.22	1.88 $\pm$ 0.19	4.13 $\pm$ 0.35	1.20 $\pm$ 0.15	45.45 $\pm$ 1.41	M
10	0.57 $\pm$ 0.09	0.31 $\pm$ 0.05	0.88 $\pm$ 0.14	4.28 $\pm$ 0.42	2.33 $\pm$ 0.20	6.61 $\pm$ 0.59	1.84 $\pm$ 0.18	35.23 $\pm$ 1.09	SM
11	0.55 $\pm$ 0.09	0.29 $\pm$ 0.03	0.84 $\pm$ 0.13	4.13 $\pm$ 0.39	2.18 $\pm$ 0.21	6.31 $\pm$ 0.57	1.90 $\pm$ 0.18	34.52 $\pm$ 1.11	SM
12	0.75 $\pm$ 0.12	Zero	0.75 $\pm$ 0.12	5.63 $\pm$ 0.54	Zero	5.63 $\pm$ 0.54	∞	Zero	T
13	0.42 $\pm$ 0.07	Zero	0.42 $\pm$ 0.07	3.16 $\pm$ 0.29	Zero	3.16 $\pm$ 0.29	∞	Zero	T
Sum			13.31 $\pm$ 1.04						

strate that the diploid chromosome number is $2n=26$ and the fundamental number is $FN=48$. These chromosomes consist of three groups: Group A which contains 9 metacentric chromosome pairs with arm ratio ranging from 1.07 to 1.39, relative length from 4.13% to 12.77% and centromeric index from 41.86 to 48.39. Group B is composed of 2 submetacentric chromosome pairs with arm ratio ranging from 1.84 to 1.90, relative length from 6.31% to 6.61% and centromeric index from 34.52 to 35.23. Group C consists of 2 telocentric chromosome pairs with arm ratio being ∞ , relative length from 3.16% to 5.63% and centromeric index being zero (Table 4).

The diploid chromosome number of *Septifer excisus* (Wiegmann 1837) $2n=26$ is in agreement with that reported by Ieyama (1983). While the previous karyotype is composed of 2 metacentric, one metacentric/submetacentric, 2 submetacentric, 7 subtelocentric and one telocentric chromosome pairs, while that of our result composed of 9 metacentric, 2 submetacentric and 2 telocentric chromosome pairs.

Septifer bilocularis

The chromosomes of 52 mitotic metaphases taken from 17 animals were counted. 43 cells had $2n=26$ and fundamental number (FN) of 52 (Fig. 5a). The karyotype consists of 3 groups: Group A consisting of 8 metacentric chromosome pairs with an arm ratio ranging from 1.00 to 1.61, relative length from 5.56% to 11.12%, and centromeric index from 44.12 to 50.00. Group B composed of 2 submetacentric chromosome pairs with an arm ratio ranging from 1.73 to 1.92, relative length from 6.48% to 11.12%, and centromeric index from 34.29 to 36.67. Group C contains 3 subtelocentric chromosome pairs with an arm ratio ranging from 3.63 to 4.38, relative length from 6.86% to

(a)

(b)

Fig. 5. Mitotic metaphase spread (a) and karyotype (b) of *Septifera bilocularis* $2n=26$.

A B C
Chromosome Group
(a)

A B C
Chromosome Group
(b)

Fig. 6. Ideograms of the chromosomes of *Septifer bilocularis* which were constructed in respect to relative length (a) and the centromeric index (b).

Table 5. Average of ten cell spreads of chromosomes measurements and classification of *Septifer bilocularis*

Chromosome No.	Chromosome length			Relative length %			Centromeric index %	Classification
	Long arm	Short arm	Total	Long arm	Short arm	Total		
	Mean $\pm$ S.D.	Mean $\pm$ S.D.	Mean $\pm$ S.D.	Mean $\pm$ S.D.	Mean $\pm$ S.D.	Mean $\pm$ S.D.		
1	0.65 $\pm$ 0.10	0.55 $\pm$ 0.09	1.20 $\pm$ 0.17	6.02 $\pm$ 0.54	5.10 $\pm$ 0.48	11.12 $\pm$ 0.92	45.83 $\pm$ 1.24	M
2	0.52 $\pm$ 0.09	0.45 $\pm$ 0.08	0.97 $\pm$ 0.14	4.82 $\pm$ 0.43	4.17 $\pm$ 0.39	8.99 $\pm$ 0.74	46.39 $\pm$ 1.22	M
3	0.50 $\pm$ 0.09	0.40 $\pm$ 0.06	0.90 $\pm$ 0.14	4.63 $\pm$ 0.44	3.71 $\pm$ 0.33	8.34 $\pm$ 0.77	44.44 $\pm$ 1.11	M
4	0.48 $\pm$ 0.08	0.42 $\pm$ 0.06	0.90 $\pm$ 0.14	4.45 $\pm$ 0.42	3.89 $\pm$ 0.35	8.34 $\pm$ 0.71	46.67 $\pm$ 1.27	M
5	0.37 $\pm$ 0.06	0.35 $\pm$ 0.05	0.72 $\pm$ 0.13	3.43 $\pm$ 0.31	3.24 $\pm$ 0.28	6.67 $\pm$ 0.54	48.61 $\pm$ 1.35	M
6	0.38 $\pm$ 0.04	0.30 $\pm$ 0.04	0.68 $\pm$ 0.11	3.52 $\pm$ 0.32	2.78 $\pm$ 0.22	6.30 $\pm$ 0.57	44.12 $\pm$ 1.24	M
7	0.30 $\pm$ 0.04	0.30 $\pm$ 0.04	0.60 $\pm$ 0.10	2.78 $\pm$ 0.24	2.78 $\pm$ 0.24	5.56 $\pm$ 0.49	50.00 $\pm$ 1.54	M
8	0.37 $\pm$ 0.04	0.23 $\pm$ 0.03	0.60 $\pm$ 0.10	3.43 $\pm$ 0.31	2.13 $\pm$ 0.20	5.56 $\pm$ 0.49	38.33 $\pm$ 1.20	M
9	0.76 $\pm$ 0.12	0.44 $\pm$ 0.06	1.20 $\pm$ 0.16	7.04 $\pm$ 0.65	4.08 $\pm$ 0.33	11.12 $\pm$ 1.06	1.73 $\pm$ 0.18	SM
10	0.46 $\pm$ 0.07	0.24 $\pm$ 0.03	0.70 $\pm$ 0.11	4.26 $\pm$ 0.39	2.22 $\pm$ 0.21	6.48 $\pm$ 0.56	36.67 $\pm$ 1.15	SM
11	0.71 $\pm$ 0.11	0.19 $\pm$ 0.02	0.90 $\pm$ 0.13	6.58 $\pm$ 0.55	1.76 $\pm$ 0.17	8.34 $\pm$ 0.73	34.29 $\pm$ 1.11	ST
12	0.70 $\pm$ 0.11	0.16 $\pm$ 0.02	0.86 $\pm$ 0.12	6.48 $\pm$ 0.57	1.48 $\pm$ 0.15	7.96 $\pm$ 0.69	21.11 $\pm$ 1.09	ST
13	0.58 $\pm$ 0.09	0.16 $\pm$ 0.02	0.74 $\pm$ 0.11	5.38 $\pm$ 0.51	1.48 $\pm$ 0.15	6.86 $\pm$ 0.66	18.60 $\pm$ 1.06	ST
Sum			10.97 $\pm$ 1.05				21.62 $\pm$ 1.09	

Table 6. Average of ten cell spreads of chromosomes measurements and classification of *Ruditapes decussatus*

Chromosome No.	Chromosome length			Relative length %			Centromeric index %	Classification	
	Long arm	Short arm		Long arm	Short arm				Total
		Mean± S.D.	Mean± S.D.		Mean± S.D.	Mean± S.D.			
1	0.60±0.09	0.50±0.08	1.10±0.16	4.19±0.34	3.49±0.31	7.68±0.63	45.45±1.56	M	
2	0.60±0.09	0.40±0.07	1.00±0.14	4.19±0.33	2.79±0.23	6.98±0.61	40.00±1.32	M	
3	0.48±0.07	0.32±0.05	0.80±0.12	3.35±0.31	2.23±0.20	5.58±0.52	40.00±1.19	M	
4	0.44±0.05	0.36±0.05	0.80±0.12	3.07±0.27	2.51±0.21	5.58±0.52	45.00±1.28	M	
5	0.45±0.05	0.33±0.04	0.78±0.11	3.14±0.30	2.30±0.20	5.44±0.50	42.31±1.33	M	
6	0.40±0.06	0.32±0.06	0.72±0.12	2.79±0.24	2.23±0.20	5.02±0.47	44.44±1.42	M	
7	0.46±0.05	0.26±0.03	0.72±0.11	3.21±0.31	1.81±0.19	5.02±0.47	36.11±1.11	SM	
8	0.45±0.06	0.25±0.03	0.70±0.11	3.14±0.29	1.74±0.18	4.88±0.43	35.71±1.12	SM	
9	0.40±0.05	0.22±0.03	0.62±0.10	2.79±0.23	1.54±0.19	4.33±0.41	35.48±1.13	SM	
10	0.38±0.04	0.22±0.03	0.60±0.10	2.65±0.22	1.54±0.17	4.19±0.41	36.67±1.11	SM	
11	0.38±0.04	0.22±0.03	0.60±0.11	2.65±0.22	1.54±0.17	4.19±0.41	36.67±1.11	SM	
12	0.62±0.08	0.18±0.02	0.80±0.13	4.32±0.34	1.26±0.15	5.58±0.53	22.50±1.07	ST	
13	0.56±0.08	0.18±0.02	0.74±0.12	3.91±0.31	1.26±0.15	5.17±0.49	24.32±1.07	ST	
14	0.38±0.05	0.12±0.02	0.50±0.08	2.65±0.22	0.84±0.13	3.49±0.32	24.00±1.08	ST	
15	0.74±0.11	0.10±0.01	0.84±0.13	5.17±0.45	0.70±0.10	5.87±0.51	11.90±0.89	T	
16	0.74±0.12	0.10±0.01	0.84±0.13	5.17±0.45	0.70±0.10	5.87±0.51	11.90±0.89	T	
17	0.80±0.11	Zero	0.80±0.11	5.87±0.51	Zero	5.87±0.51	Zero	T	
18	0.68±0.09	Zero	0.68±0.09	4.75±0.36	Zero	4.75±0.36	Zero	T	
19	0.68±0.09	Zero	0.68±0.09	4.75±0.41	Zero	4.75±0.41	Zero	T	
Sum			14.32±1.09						

8.34%, and centromeric index from 18.60 to 21.62 (Fig. 5b, Table 5). The ideograms were constructed (Fig. 6a, b).

The diploid chromosome number of *Septifer bilocularis* (Linnaeus 1758) $2n=26$ is in agreement with that reported by Ieyama (1983). While the karyotype that he showed is composed of 3 metacentric, 2 metacentric/submetacentric, 7 subtelocentric and one telocentric chromosome pairs, our karyotype was composed of 8 metacentric, 2 submetacentric and 3 subtelocentric chromosome pairs.

The cytogenetic findings of the present investigation of three species *Modiolus barbatus*, *Septifer excisus* and *Septifer bilocularis*, are in complete agreement with those reported using the classical morphological and anatomical methods for taxonomy. The results of these three species are in agreement with those reported by Ahmed 1974, Diupotex-Chong *et al.* 1978, Ieyama 1984, Thiriou-Quievreux 1984, Cornet 1993, Pasantes *et al.* 1996, Holland *et al.* 1999, Vitturi *et al.* 2000, Thiriou-Quievreux 2002 in which they reported that the diploid chromosome number of Family Mytilidae to which *Modiolus barbatus*, *Septifer excisus* and *Septifer bilocularis* belong ranges from $2n=22$ to $2n=32$.

Ruditapes decussatus

A diploid composition of $2n=38$ was found in 46 mitotic metaphase from 18 animals (Fig. 7a). For karyotyping, chromosomes of 10 well-spread metaphase plates were cut out from photomicrographs and paired on the basis of size and centromeric position. Chromosome measurements were taken from the 10 best spreads and the means and SD of length of short arm and long arm,

(a)

(b)

Fig. 7. Mitotic metaphase spread (a) and karyotype (b) of *Ruditapes decussatus* $2n=38$.

Fig. 8. Ideograms of the chromosomes of *Ruditapes decussatus* which were constructed in respect to relative length (a) and the centromeric index (b).

chromosome length, relative length ($100 \times \text{absolute chromosome pair length} / \text{total length of haploid complement}$), arm ratio (length of short arm/length of long arm) and centromeric index ($100 \times \text{length of short arm} / \text{total length of chromosome}$) together with the chromosome classification are given in Table 6.

The karyotype consists of four groups (Fig. 7b): Group A consists of 6 metacentric chromosome pairs with relative length ranging from 5.02% to 7.68%, arm ratio from 1.20 to 1.50 and centromeric index varies from 40.00 to 45.45. Group B which composed of 5 submetacentric chromosome pairs with relative length ranging from 4.19% to 5.02%, arm ratio from 1.73 to 1.82 and centromeric index varies from 35.48 to 36.67. Group C has 3 subtelocentric chromosome pairs with relative length ranging from 3.49% to 5.58%, arm ratio from 3.11 to 3.44 and centromeric index varies from 22.50 to 24.32. Group D which contains 5 telocentric chromosome pairs with relative length ranging from 4.75% to 5.87%, arm ratio from 7.40 to ∞ and centromeric index varies from zero to 11.90 (Table 6). An ideogram (Fig. 8a, b) was constructed from relative length and centromeric indices with the centromeres drawn at the same level to make visual comparison easier.

The diploid chromosome number of *Ruditapes decussatus* (Linnaeus 1758) $2n=38$ is in agreement with that reported by Borsa and Thiriou-Quievreux (1990). While the karyotype that they reported is composed of 9 metacentric/submetacentric and 10 subtelocentric/telocentric chromosome pairs, that of our result composed of 6 metacentric, 5 submetacentric, 3 subtelocentric and 5 telocentric chromosome pairs.

(a)

(b)

Fig. 9. Mitotic metaphase spread (a) and karyotype (b) of *Circe scripta* $2n=38$.

(a)

(b)

Fig. 10. Ideograms of the chromosomes of *Circe scripta* which were constructed in respect to relative length (a) and the centromeric index (b).

Table 7. Average of ten cell spreads of chromosomes measurements and classification of *Circe scripta*

Chromosome No.	Chromosome length			Relative length %			Arm ratio	Centromeric index %	Classification		
	Long arm	Short arm		Total	Long arm	Short arm				Total	
		Mean±S.D.	Mean±S.D.			Mean±S.D.					Mean±S.D.
1	1.05±0.15	1.00±0.15	2.05±0.19	4.95±0.31	4.71±0.38	9.66±0.83	1.05±0.16	48.78±1.36	M		
2	0.85±0.13	0.70±0.10	1.55±0.17	4.01±0.32	3.30±0.23	7.31±0.66	1.21±0.18	45.16±1.23	M		
3	0.65±0.09	0.45±0.06	1.10±0.14	3.06±0.29	2.13±0.21	5.19±0.43	1.44±0.19	40.91±1.13	M		
4	0.48±0.07	0.32±0.04	0.80±0.10	2.26±0.23	1.50±0.19	3.76±0.29	1.50±0.19	40.00±1.11	M		
5	0.35±0.05	0.25±0.04	0.60±0.08	1.65±0.21	1.18±0.15	2.83±0.23	1.40±0.19	41.67±1.18	M		
6	0.29±0.03	0.26±0.03	0.55±0.08	1.37±0.19	1.22±0.16	2.59±0.23	1.12±0.15	47.27±1.24	M		
7	1.15±0.17	0.65±0.09	1.80±0.18	5.42±0.41	3.06±0.25	8.48±0.67	1.77±0.19	36.11±1.11	SM		
8	0.90±0.14	0.50±0.09	1.40±0.16	4.24±0.34	2.36±0.22	6.60±0.53	1.80±0.20	35.71±1.09	SM		
9	0.70±0.12	0.40±0.07	1.10±0.16	3.30±0.31	1.88±0.18	5.18±0.46	1.75±0.19	36.36±1.08	SM		
10	0.72±0.12	0.38±0.05	1.10±0.16	3.39±0.33	1.79±0.17	5.18±0.44	1.89±0.18	34.55±1.08	SM		
11	0.78±0.13	0.32±0.05	1.10±0.16	3.68±0.36	1.50±0.17	5.18±0.44	2.44±0.21	29.09±1.05	SM		
12	0.76±0.13	0.34±0.05	1.10±0.16	3.58±0.31	1.60±0.15	5.18±0.45	2.24±0.21	30.91±1.11	SM		
13	1.15±0.17	0.35±0.04	1.50±0.19	5.42±0.45	1.65±0.19	7.07±0.53	3.29±0.23	23.33±1.07	ST		
14	1.05±0.17	0.30±0.03	1.35±0.18	4.95±0.44	1.41±0.165	6.36±0.51	3.50±0.29	22.22±1.07	ST		
15	0.83±0.12	0.27±0.02	1.10±0.15	3.91±0.32	1.27±0.14	5.18±0.44	3.07±0.26	24.55±1.08	ST		
16	0.47±0.08	0.13±0.02	0.60±0.08	2.21±0.21	0.61±0.09	2.82±0.23	3.62±0.31	21.67±1.04	ST		
17	0.86±0.14	0.10±0.02	0.96±0.13	4.05±0.39	0.47±0.06	4.52±0.39	8.60±0.65	10.42±0.89	T		
18	0.90±0.16	Zero	0.90±0.16	4.24±0.39	Zero	4.24±0.39	∞	Zero	T		
19	0.65±0.09	Zero	0.65±0.09	3.06±0.25	Zero	3.06±0.25	∞	Zero	T		
Sum			21.21±1.03								

Table 8. Average of ten cell spreads of chromosomes measurements and classification of *Venus verrucosa*

Chromosome No.	Chromosome length			Relative length %			Arm ratio	Centromeric index %	Classification		
	Long arm	Short arm		Total	Long arm	Short arm				Total	
		Mean±S.D.	Mean±S.D.			Mean±S.D.					Mean±S.D.
1	1.10±0.17	0.80±0.13	1.90±0.19	5.41±0.49	3.94±0.27	9.35±0.73	1.38±0.17	42.11±1.23	M		
2	0.96±0.15	0.76±0.12	1.72±0.19	4.72±0.23	3.74±0.25	8.46±0.67	1.26±0.19	44.19±1.23	M		
3	0.61±0.11	0.55±0.10	1.16±0.16	3.00±0.21	2.70±0.21	5.70±0.53	1.11±0.16	47.41±1.45	M		
4	0.70±0.11	0.46±0.09	1.16±0.17	3.44±0.24	2.26±0.20	5.70±0.51	1.52±0.20	39.66±1.11	M		
5	0.72±0.12	0.44±0.07	1.16±0.16	3.54±0.21	2.16±0.20	5.70±0.49	1.64±0.19	37.93±1.13	M		
6	0.55±0.09	0.47±0.07	1.02±0.15	2.71±0.22	2.31±0.23	5.02±0.44	1.17±0.17	46.08±1.37	M		
7	0.55±0.09	0.35±0.05	0.90±0.14	2.71±0.22	1.72±0.19	4.43±0.34	1.57±0.20	38.89±1.09	M		
8	1.10±0.16	0.50±0.08	1.60±0.18	5.41±0.39	2.46±0.22	7.87±0.65	2.00±0.21	31.25±1.10	SM		
9	0.52±0.08	0.30±0.04	0.82±0.12	2.56±0.23	1.48±0.18	4.04±0.33	1.73±0.19	36.59±1.06	SM		
10	0.45±0.06	0.25±0.04	0.70±0.13	2.21±0.21	1.23±0.18	3.44±0.31	1.80±0.19	35.71±1.12	SM		
11	0.50±0.09	0.20±0.03	0.70±0.12	2.46±0.20	0.98±0.15	3.44±0.29	2.50±0.21	28.57±1.03	SM		
12	1.06±0.15	0.30±0.04	1.36±0.19	5.21±0.41	1.48±0.20	6.69±0.59	3.53±0.27	22.06±1.00	ST		
13	1.03±0.17	0.33±0.03	1.36±0.18	5.07±0.42	1.62±0.18	6.69±0.55	3.12±0.22	24.26±1.01	ST		
14	0.96±0.12	0.24±0.03	1.20±0.16	4.72±0.39	1.18±0.17	5.90±0.53	3.69±0.31	20.00±0.99	ST		
15	0.73±0.11	0.23±0.03	0.96±0.13	3.59±0.33	1.13±0.16	4.72±0.42	3.17±0.26	23.96±0.97	ST		
16	0.80±0.10	0.10±0.02	0.90±0.13	3.94±0.33	0.49±0.06	4.43±0.41	8.00±0.57	11.11±0.83	T		
17	0.58±0.09	0.07±0.02	0.65±0.10	2.85±0.24	0.35±0.04	3.20±0.25	8.00±0.58	10.77±0.81	T		
18	0.54±0.09	0.06±0.01	0.60±0.10	2.66±0.20	0.29±0.02	2.95±0.22	9.00±0.61	10.00±0.88	T		
19	0.46±0.07	Zero	0.46±0.07	2.26±0.19	Zero	2.26±0.19	∞	Zero	T		
Sum			20.33±1.10								

Circe scripta

The chromosomes of 37 mitotic metaphases taken from 15 animals were counted. 49 cells had $2n=38$ and fundamental number (FN) of 72 (Fig. 9a). The karyotype consists of four groups: Group A consisting of 6 metacentric chromosome pairs with arm ratio ranging from 1.05 to 1.56, relative length varies from 2.59% to 9.66% and centromeric index from 40.00 to 48.78. Group B which composed of 6 submetacentric chromosome pairs with arm ratio ranging from 1.75 to 2.44, relative length from 5.18% to 8.46%, and centromeric index varies from 29.09 to 36.36. Group C contains 4 subtelocentric chromosome pairs with arm ratio ranging from 3.29 to 3.62, relative length from 2.82% to 7.07%, and centromeric index varies from 21.67 to 24.55. Group D has 3 telocentric chromosome pairs with arm ratio ranging from 8.60 to ∞ , relative length from 3.06% to 4.52%, and centromeric index varies from (Fig. 9b, Table 7). An ideogram (Fig. 10a, b) was constructed.

The diploid chromosome number of *Circe scripta* (Linnaeus 1758) $2n=38$ is in agreement with that reported by Ieyama (1980). While the karyotype of which is composed of 19 metacentric, that of our result composed of 6 metacentric, 6 submetacentric, 4 subtelocentric and 3 telocentric chromosome pairs.

Venus verrucosa

A diploid complement of $2n=38$ was found in 32 mitotic metaphase from 16 animals (Fig. 11a). For karyotyping, the chromosomes of 10 well-spread metaphase plates were cut out from pho-

(a)

(b)

Fig. 11. Mitotic metaphase spread (a) and karyotype (b) of *Venus verrucosa* $2n=38$.

(a)

(b)

Fig. 12. Ideograms of the chromosomes of *Venus verrucosa* which were constructed in respect to relative length (a) and the centromeric index (b).

tomicographs and paired on the basis of size and centromeric position.

The karyotype (Fig. 11b) consists of four groups: Group A consists of 7 metacentric chromosome pairs with relative length ranging from 4.43% to 9.35%, arm ratio from 1.11 to 1.64 and centromeric index varies from 37.93 to 47.41. Group B which composed of 4 submetacentric chromosome pairs with relative length ranging from 3.44% to 7.87%, arm ratio from 1.73 to 2.50 and centromeric index varies from 28.57 to 36.59. Group C has 4 subtelocentric chromosome pairs with relative length ranging from 4.72% to 6.69%, arm ratio from 3.12 to 3.69 and centromeric index varies from 20.00 to 23.96. Group D which contains 4 telocentric chromosome pairs with relative length ranging from 2.26% to 3.20%, arm ratio from 8.00 to ∞ and centromeric index varies from zero to 10.77 (Table 8). An ideogram (Fig. 12a, b) was constructed. The diploid chromosome number of *Venus verrucosa* (Linnaeus 1758) $2n=38$ is in agreement with that reported by Rasotto *et al.* (1981).

The cytogenetic findings of the present investigation on the 3 species, *Ruditopes decussatus*, *Circe scripta* and *Venus verrucosa*, are in complete agreement with those recorded using the classical morphological and anatomical methods for taxonomy. The results of these three species are in agreement with that reported by (Menzel 1968, Koulman and Wolff 1977, Gerard 1978, Rasotto *et al.* 1981, Ieyama 1990, Corni and Trentini 1990, Borsa and Thiriout-Quievreux 1990, Wang *et al.* 1998) in which they reported that the most frequently chromosome number of Family Veneridae—to which *Ruditopes decussatus*, *Circe scripta* and *Venus verrucosa* belonged—is $2n=38$.

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