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CHROMOSOME STUDIES ON THE MARINE SHRIMPS *PENAEUS VANNAMEI* AND *P. CALIFORNIENSIS* (DECAPODA)

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ABSTRACT

Chromosome numbers were obtained from eggs of *Penaeus vannamei* and *Penaeus californiensis*. They had 2n = 88 chromosomes. This was confirmed by the haploid chromosome number of n = 44 in testes. No evident karyological difference was observed between these species, in which 4 metacentric, 10 submetacentric, 56 subtelocentric, and 18 acrocentric chromosomes were observed. To obtain mitotic metaphase chromosomes from marine shrimp eggs, 2 methods are described. The effect of colchicine incubation for chromosome condensation was investigated.

Karyological information is useful for breeding research, chromosome manipulation experiments, taxonomy, and evolutionary studies of crustaceans.

There are only two reports on the karyology of *P. vannamei* Boone and one on *P. californiensis* Holmes. Because of the different chromosome numbers reported in *P. vannamei,* a further investigation was initiated to provide an alternative cytogenetic technique for better karyotype analysis.

Until now, studies on decapod crustacean cytogenetics have been concerned with data mostly on the number of chromosomes (see Nakamura *et al.*, 1988; Murofushi and Deguchi, 1990, for review). In the genus *Penaeus,* previous reports on 10 species have not provided enough information on chromosome morphology.

In bivalves, banding techniques have been useful for the identification of individual chromosomes and have become an essential part of cytogenetic analysis (Insua and Thiriot-Quiévreux, 1991).

This paper describes and compares the chromosome number and the tentative karyotype of the marine shrimps *P. vannamei* and *P. californiensis.* In addition, I propose two modifications of the air-drying technique to obtain mitotic chromosome metaphases that will improve karyotype analysis and banding techniques in the genus *Penaeus.*

MATERIALS AND METHODS

Mitotic Chromosomes (diploid number).—Samples of spawn from 30 *P. vannamei* from Nayarit, México, and 28 *P. californiensis* from Bahía Magdalena, Baja California Sur, México, were collected about 3 h after spawning. The water temperature was 27.5°C and salinity 35 ppt. The number of cleaving eggs was counted in 200 eggs to confirm the fertilization rate to be at least 60%. Eggs were washed with fresh sea water using a 40-μm mesh, then placed in 30-ml assay tubes to get about 1-cm sample depth.

A modified procedure of Rodríguez-Romero *et al.* (1991) was used. Five concentrations of colchicine (Sigma Chemical Co.) in sea water were tested with eggs of *P. vannamei*: 0.006, 0.012, 0.025, 0.05, and 0.1%; each incubated for 30, 60, and 90 min. Eggs of *P. californiensis* were incubated for 45 min with 0.05% colchicine in sea water. Thus, 16 treatments for colchicine concentration level and incubation period were investigated. The eggs were incubated in 0.9% sodium citrate in distilled water for 30 min. The sample was then fixed in a freshly prepared mixture of methanol : acetic acid (3:1) with 5 changes, each lasting 10 min, resuspending the eggs at each change. The fixed eggs were stored in a refrigerator at 4°C for 24 h. Two quick changes of the freshly prepared fixer were made followed by 5 changes of 50% acetic acid in distilled water at 40°C, each lasting 10 min. The tubes were centrifuged at 1,000 rpm for 3 min after each change. The eggs turned from white to a translucent and crystalline appearance.

First Method for Obtaining Chromosome Metaphases.—Ten ml of the resuspended eggs were placed in a glass petri dish. The petri dish was placed on a hot plate for 5 s to reach a temperature about 50–60°C. Using an 8-cm plastic rod with 1 end flattened to a 2-cm diameter, the eggs were crushed randomly and quickly for about 1 min in the petri dish. The eggs were then put into a 12-ml glass centrifuge tube and observed against the light. A clear suspension with small fragments like crystalline splinters was seen in the centrifuge tube. The remaining uncrushed eggs stayed at the bottom of the tube and were discarded. The remaining suspension was centrifuged at 500 rpm for 5 min. The supernatant was decanted, 10 ml of 50% acetic acid was added, and the cells were resuspended. Slide preparation was made using the technique of Thiriot-Quiévreux and Ayraud (1982). One-half ml of the suspension was dropped from a height of 8 cm onto a hot (50°C) glass slide, slightly inclined. After the impact, the liquid was immediately removed using a 2-ml plastic pipet.

Second Method for Obtaining Chromosome Metaphases.—One egg in 0.1 ml of 50% acetic acid was taken into a pasteur pipet and dropped from a height of 5 cm on a hot (50°C) glass slide. After the impact, the egg was crushed and squashed on the slide, using the flattened

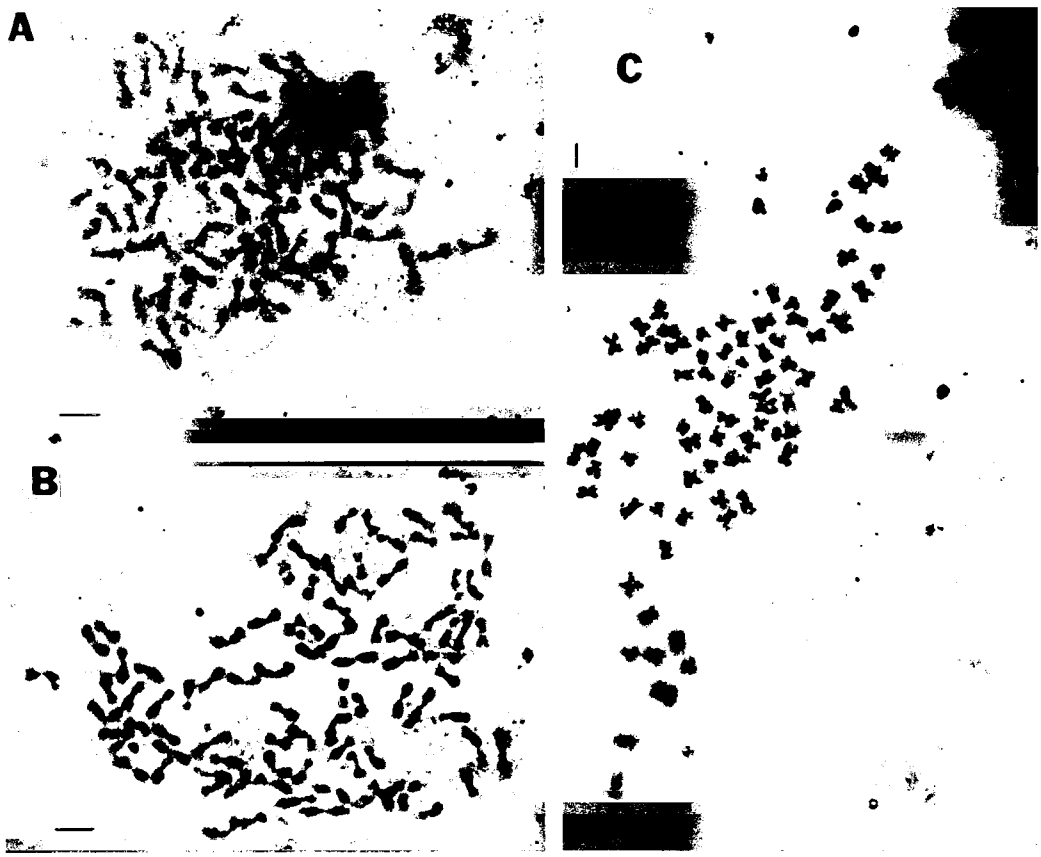

Fig. 1. Different degrees of condensation on mitotic metaphase chromosomes obtained from eggs. A, 0.006% colchicine, 30 min, *Penaeus vannamei*. B, C, 0.05% colchicine, 45 min, *Penaeus californiensis*. Bars = 5 µm.

plastic tube previously described. Although this technique is for individual analysis of eggs, it can be done using more eggs (5–10).

For each treatment, 4 slides were made using the first method and 2 using the second.

Meiotic Chromosomes (Haploid Number).—Six males of *P. vannamei* (first generation) and 6 wild males of *P. californiensis*, from 20–25 g in body weight, were injected intramuscularly with 2 µg of colchicine per gram of body weight. The testes were dissected 4–6 h after the injection. The testes were cut into small pieces and immersed in 0.9% sodium citrate in distilled water at 25°C for 20 min. The samples were then fixed in a freshly prepared mixture of methanol : acetic acid (3:1) with 5 changes, each lasting 10 min. The small pieces were broken up in 50% acetic acid, using small tweezers, until a suspension was obtained. Slide preparation was made using the same technique as in the first method for obtaining chromosomes.

Two hours after the slide preparation, chromosomes were stained for 30 min with 5% Giemsa (Spectrum Chemical Co.) diluted with phosphate buffer, pH 6.8. Slides were then rinsed in distilled water and allowed to air-dry for 2 days.

Mitotic and meiotic chromosomes were observed at 100× magnification and counted using a camera lucida attached to a Zeiss microscope. From mitotic chromosomes, the best 30 metaphases of each species were photographed at a magnification of 100× using a Zeiss photomicroscope. Three metaphases of each species were chosen for karyotyping. Chromosomes were arranged in homologous pairs, classified by their relative arm-length ratio (long arm/short arm), according to Levan *et al.* (1964), and then arranged by decreasing size.

Results

No evident difference was seen in the degree of the chromosome condensation using different concentrations of colchicine. However, longer incubation led to a greater chromosome condensation. The longest chromosomes ranging from 2.7–7.9 µm were obtained from a 30-min treatment. In this study, these chromosomes were considered a low degree of condensation (Fig. 1A). The most effective treatment was for 45 min, where the range of the chromosome lengths was from 1.6–6.5 µm. These chromosomes were considered a medium degree of condensation

Fig. 2. Different degrees of condensation on mitotic metaphase chromosomes obtained from eggs. D, E, 0.006% colchicine, 60 min, *P. vannamei*. F, 0.025% colchicine, 90 min, *P. vannamei*. Bars = 5 μm.

(Fig. 1B, C). Chromosomes ranging from 1.3–4.0 μm were observed from a 60-min treatment. These chromosomes were considered a medium to high degree of condensation (Fig. 2D, E). The most condensed chromosomes were obtained in the treatment lasting 90 min. The range of the chromosome lengths was from 0.8–2.0 μm (Fig. 2F). Clear haploid chromosome spreads were obtained from testicular tissue from three of six *P. vannamei* (Fig. 3A), and four of six *P. californiensis* (Fig. 3B). The length of the meiotic chromosomes was 1.5–3 μm.

From the metaphase chromosome counts obtained from eggs of *P. vannamei* and *P. californiensis*, the diploid chromosome number was considered to be $2N = 88$ for both species. The percentage of the modal diploid cells for *P. vannamei* was 54.2% of 168 cells, and for *P. californiensis* was 57.5% of 120 cells. The chromosome number ranged from 79–91 for *P. vannamei* and 80–90 for *P. californiensis* (Table 1). The diploid chromosome number was confirmed by obtaining the haploid chromosome number of $N = 44$ from testes. The chromosome number varied from 38–47 in *P. vannamei* and from 38–46 in *P. californiensis* (Table 2).

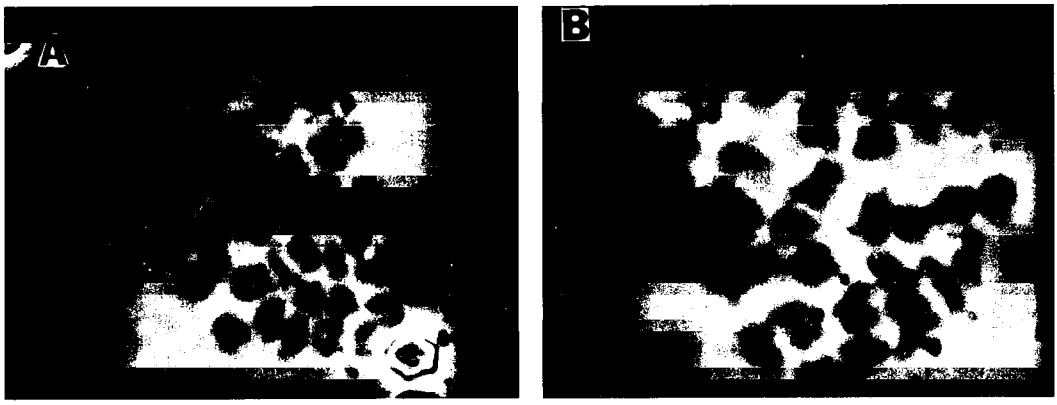

Fig. 3. Meiotic metaphase chromosomes obtained from testes. A, *Penaeus vannamei.* B, *Penaeus californiensis.* Bars = 5 μm.

From the best three mitotic metaphases analyzed from the eggs of *P. vannamei,* the following distribution of the 44 pairs is proposed (Table 3): two pairs were metacentric "m" (numbers 10 and 20), five pairs were submetacentric "sm" (numbers 9, 27, 37, 38, and 41), three pairs were acrocentric "t" (numbers 2, 40, and 43), and the remaining 34 pairs were subtelocentric "st." The karyotype with this analysis was: 4m + 10sm + 68st + 6t (Fig. 4). If the standard deviation of the arm-ratio values of pair numbers 8, 11, 13, 25, 29, 32–36, and 44 are taken into account, these 11 pairs, originally "st," also fall as "t." The karyotype then could be: 4m + 10sm + 46st + 28t, and all possible combinations. An intermediate karyotype value was 4m + 10sm + 56st + 18t. From the best three mitotic metaphases from the eggs of *P. californiensis* (Table 3): two pairs were "m" (numbers 11 and 16), five pairs were "sm" (numbers 9, 25, 31, 33, and 42), nine pairs were telocentric "T," but they were considered as acrocentric "t," caused by the possibility that the short arms were not distinguishable on the prints (numbers 14, 23, 30, 38–41, 43, and 44), and the remaining 28 pairs were "st." The karyotype with this analysis was: 4m + 10sm + 56sm + 18t (Fig. 5). Again, considering the standard deviation of the arm-ratio values of pair numbers 4, 6, 7, and 8, these four pairs, originally "st," also fall as "t." The karyotype could then be: 4m + 10sm + 48st + 26t, and all possible combinations. An intermediate karyotype value was: 4m + 10sm + 52st + 22t.

Table 1. Number of diploid metaphases counted (MC), their chromosome number (CN), and percentage (%) obtained from eggs of *Penaeus vannamei* and *Penaeus californiensis.*

	P. vannamei			*P. californiensis*	
	MC	%	CN	MC	%
	5	2.98	79	0	0
	6	3.57	80	7	5.83
	3	1.78	81	0	0
	7	4.17	82	0	0
	2	1.19	83	0	0
	12	7.15	84	12	10.01
	4	2.38	85	4	3.33
	17	10.12	86	13	10.84
	9	5.35	87	5	4.16
	91	54.17	88*	69	57.50
	8	4.76	89	8	6.67
	3	1.79	90	2	1.66
	1	0.59	91	0	0
Total	168	100.00		120	100.00

* Modal diploid chromosome number (2*N*).

Discussion

Most of the chromosomes in penaeid karyotypes are subtelocentric or acrocentric: *P. aztecus* Ives: 18M + 18SM + 52A (Goswami, 1985), *P. vannamei, P. stylirostris* (Stimpson), *P. californiensis,* and *P. occidentalis* Streets: 14M + 78A (Mayorga, 1982), and *P. monodon* Fabricius: 18M + 70A (Fanjun and Dong, 1993); 16m + 20sm + 10st + 42t (Kumar and Lakra, 1996). However, Jixun *et al.* (1989) observed 54m + 20(m,sm) + 10sm + 4(sm,st) in *P. orientalis* Kishinouye.

The large number and small size, with progressively decreasing length, make it difficult to identify them individually. It is even more difficult to distinguish between subtelocentric and acrocentric chromosomes.

Table 2. Number of haploid metaphases counted (MC), their chromosome number (CN), and percentage (%) obtained from testes of three individuals (IND) of *Penaeus vannamei* and four of *Penaeus californiensis*.

	P. vannamei										
	38	39	40	41	42	43	44*	45	46	47	
IND											Total MC
1	1	0	2	2	2	4	8	3	1	0	23
2	0	0	2	2	4	7	15	8	3	1	42
3	0	1	2	3	2	1	6	3	0	0	18
Total	1	1	6	7	8	12	29	14	4	1	83
%	1.2	1.2	7.23	8.44	9.64	14.46	35	16.9	4.82	1.2	100
	P. californiensis										
	38	39	40	41	42	43	44*	45	46		
IND										Total MC	
1	0	4	1	2	3	0	8	4	0	22	
2	1	1	3	1	0	0	7	2	1	16	
3	1	0	0	0	4	4	9	2	0	20	
4	5	2	6	4	1	5	10	5	1	39	
Total	7	7	10	7	8	9	34	13	2	97	
%	7.2	7.2	10.31	7.2	8.25	9.28	35.1	13.41	2	100	

* Modal haploid chromosome number (N = 44).

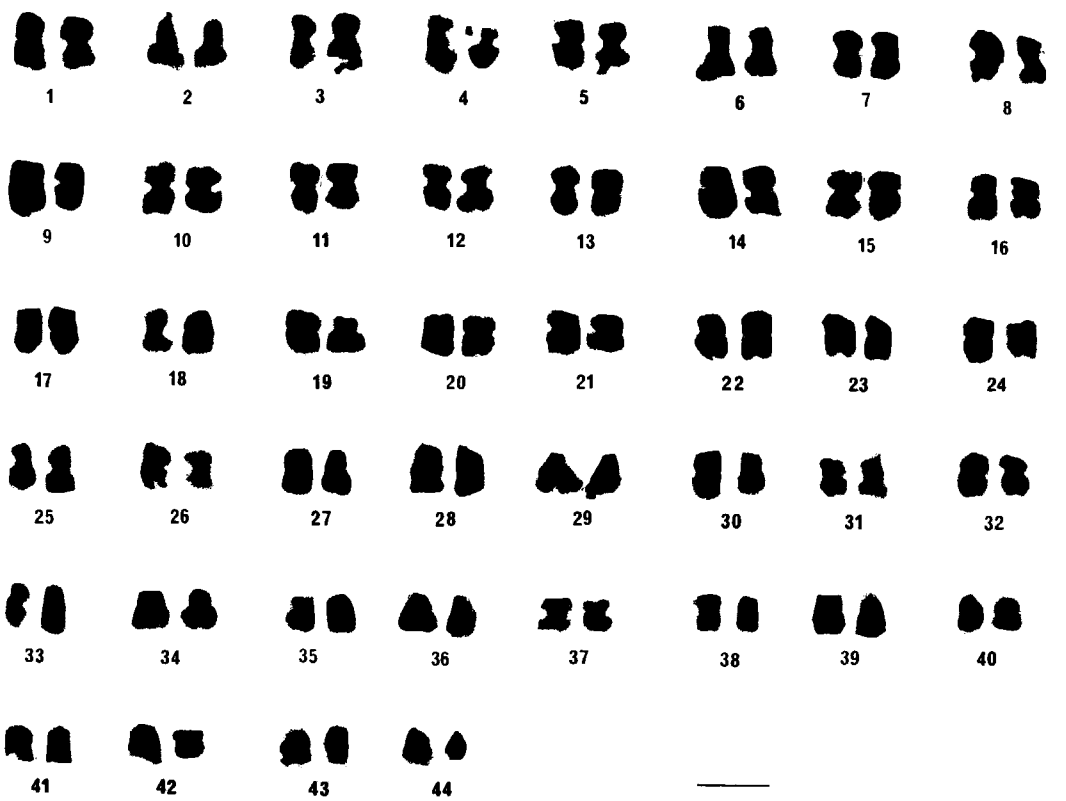

Fig. 4. Tentative karyotype of *Penaeus vannamei*. Two pairs, numbers 10 and 20, are metacentric; five pairs, numbers 9, 27, 37, 38, and 41, are submetacentric; the remaining 37 pairs are considered as 56 subtelocentric and 18 telocentric. Bar = 5 μm.

Table 3. Mean relative length (L) ± *s*, arm ratio (R) ± *s*, and classification (C) of metaphase chromosomes in three cells of *Penaeus vannamei*, and three cells of *Penaeus californiensis*, from embryos.

Chromosome pairs	*P. vannamei*			*P. californiensis*		
	L ± *s*	R ± *s*	C*	L ± *s*	R ± *s*	C*
1	5.95 ± 2.480	3.87 ± 0.562	st	4.68 ± 0.939	5.13 ± 1.635	st
2	5.55 ± 2.099	11.0 ± 6.340	t	4.62 ± 1.024	6.17 ± 0.873	st
3	5.55 ± 2.099	4.31 ± 0.699	st	4.49 ± 1.099	5.99 ± 0.903	st
4	5.40 ± 2.036	4.45 ± 0.505	st	4.35 ± 0.868	6.09 ± 1.071	st-t
5	5.40 ± 2.036	4.45 ± 0.505	st	4.22 ± 1.017	5.55 ± 0.969	st
6	5.40 ± 2.036	4.45 ± 0.505	st	4.09 ± 0.996	5.92 ± 1.685	st-t
7	5.28 ± 1.995	4.34 ± 0.659	st	4.01 ± 1.017	5.77 ± 1.732	st-t
8	5.28 ± 1.995	6.96 ± 2.230	st-t	3.88 ± 1.124	6.38 ± 1.335	st-t
9	5.28 ± 1.995	2.66 ± 0.242	sm	3.82 ± 1.017	2.36 ± 0.664	sm
10	5.15 ± 1.822	1.47 ± 0.173	m	3.76 ± 0.904	4.96 ± 1.485	st
11	5.02 ± 2.041	6.53 ± 2.490	st-t	3.69 ± 0.996	1.52 ± 0.174	m
12	5.02 ± 2.041	4.76 ± 1.120	st	3.69 ± 0.996	4.15 ± 0.734	st
13	4.88 ± 2.003	4.84 ± 2.355	st-t	3.63 ± 1.016	5.80 ± 1.242	st
14	4.88 ± 2.003	4.64 ± 0.949	st	3.63 ± 1.016	∞	T
15	4.88 ± 2.003	4.64 ± 0.949	st	3.48 ± 0.939	4.90 ± 1.588	st
16	4.88 ± 2.003	4.64 ± 0.949	st	3.48 ± 0.939	1.49 ± 0.323	m
17	4.48 ± 2.003	4.13 ± 1.046	st	3.48 ± 0.939	5.65 ± 1.194	st
18	4.48 ± 2.003	4.85 ± 0.439	st	3.42 ± 0.979	5.48 ± 1.464	st
19	4.48 ± 2.003	4.76 ± 2.026	st	3.29 ± 0.750	5.23 ± 1.186	st
20	4.48 ± 2.003	1.44 ± 0.200	m	3.23 ± 0.822	5.92 ± 0.500	st
21	4.35 ± 1.822	4.67 ± 2.070	st	3.23 ± 0.822	5.92 ± 0.500	st
22	4.35 ± 1.822	4.74 ± 0.635	st	3.16 ± 0.715	5.85 ± 0.514	st
23	4.35 ± 1.822	4.74 ± 0.635	st	3.02 ± 0.832	∞	T
24	4.35 ± 1.822	4.74 ± 0.635	st	3.02 ± 0.832	4.86 ± 1.063	st
25	4.35 ± 1.822	6.43 ± 2.268	st-t	2.96 ± 0.715	2.64 ± 0.164	sm
26	4.35 ± 1.822	4.74 ± 0.635	st	2.96 ± 0.715	4.72 ± 0.919	st
27	4.35 ± 1.822	2.48 ± 0.500	sm	2.89 ± 0.800	4.45 ± 0.795	st
28	4.22 ± 1.654	5.42 ± 0.549	st	2.83 ± 0.822	5.03 ± 0.537	st
29	4.16 ± 1.761	5.68 ± 5.640	st-t	2.83 ± 0.822	5.03 ± 0.537	st
30	4.03 ± 1.712	4.98 ± 0.928	st	2.76 ± 0.904	∞	T
31	3.96 ± 1.814	4.82 ± 1.096	st	2.64 ± 0.825	2.61 ± 0.380	sm
32	3.96 ± 1.814	6.20 ± 2.206	st-t	2.56 ± 0.854	4.49 ± 0.588	st
33	3.82 ± 1.787	5.94 ± 5.109	st-t	2.56 ± 0.854	2.52 ± 0.181	sm
34	3.76 ± 1.894	5.11 ± 4.711	st-t	2.51 ± 0.753	4.29 ± 0.304	st
35	3.76 ± 1.894	6.26 ± 2.920	st-t	2.30 ± 0.609	4.75 ± 1.527	st
36	3.76 ± 1.894	5.93 ± 3.423	st-t	2.30 ± 0.609	4.75 ± 1.527	st
37	3.55 ± 1.696	2.51 ± 0.438	sm	2.30 ± 0.609	4.75 ± 1.527	st
38	3.55 ± 1.696	2.51 ± 0.438	sm	2.17 ± 0.590	∞	T
39	3.48 ± 1.686	4.15 ± 1.222	st	2.09 ± 0.697	∞	T
40	3.29 ± 1.598	7.12 ± 3.930	t	2.03 ± 0.609	∞	T
41	3.29 ± 0.598	2.52 ± 0.468	sm	2.03 ± 0.609	∞	T
42	3.29 ± 0.598	3.36 ± 2.900	st	1.77 ± 0.350	2.07 ± 0.288	sm
43	2.83 ± 1.196	7.53 ± 6.800	t	1.63 ± 0.230	∞	T
44	2.03 ± 0.694	4.89 ± 4.399	st-t	1.50 ± 0.118	∞	T

* m = metacentric, sm = submetacentric, st = subtelocentric, t = acrocentric, and T = telocentric.

There were no evident morphological differences between the karyotypes of *P. vannamei* and *P. californiensis*. For this reason, from a preliminary analysis, the karyotype of both species obtained in this study is proposed to be 4m + 10sm + 56st + 18t. Mayorga (1982) also found no morphological differences when describing the karyotypes as 14M + 78A of four species of shrimps from Perú. The 14M correspond to the more diversified description of this study (from México) of 4m + 10sm, and the 78A correspond to 56st + 18t. The karyotype from México has two pairs of chromosomes fewer than those from Perú.

Two previous reports of *P. vannamei* gave different chromosome numbers: Mayorga (1982) gave $2N = 92$ and $N = 46$. Chow *et al.* (1990), gave $N = 44$. My study agreed with the haploid number. I assume the haploid chromosome number of $N = 44$ was from shrimps of México.

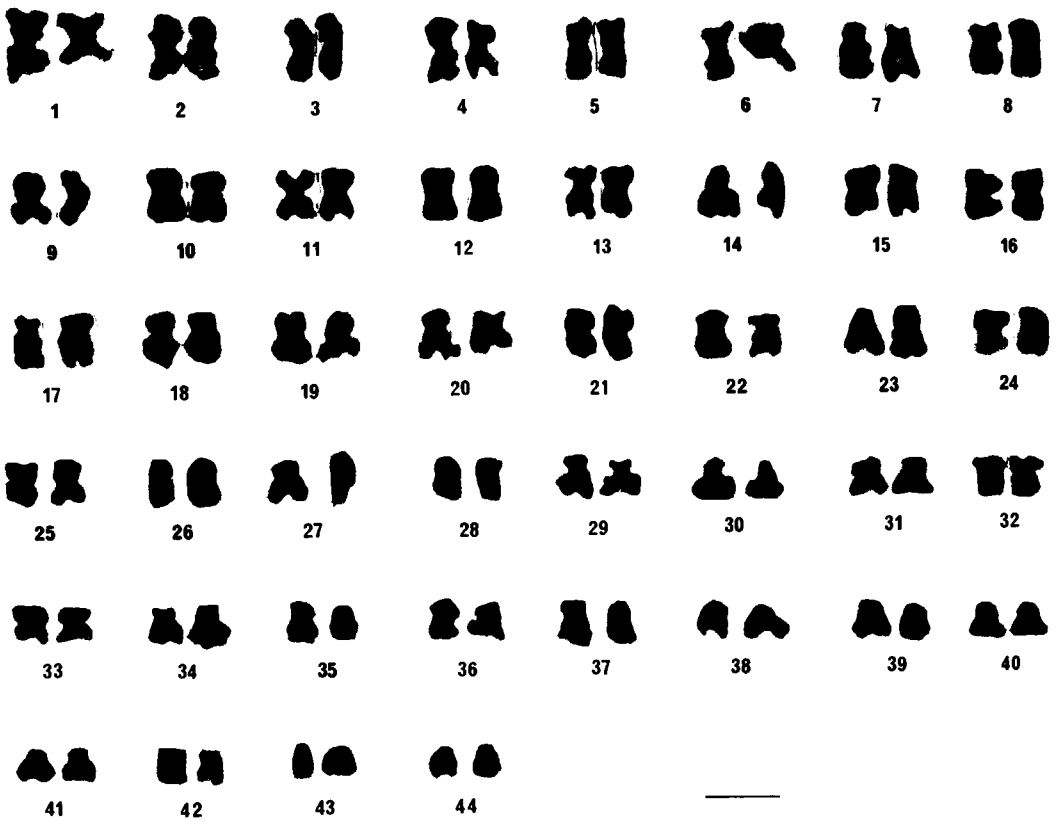

Fig. 5. Tentative karyotype of *Penaeus californiensis*. Two pairs, numbers 11 and 16, are metacentric; five pairs, numbers 9, 25, 31, 33, and 42, are submetacentric; the remaining 37 pairs are considered as 55 subtelocentric and 19 telocentric. Bar = 5 μm.

Chow *et al.* (1990) suggested that chromosome rearrangements have accompanied the speciation process in *Penaeus* without polyploidy. The diploid number in *P. japonicus* Bate has been reported to be $2N = 92$ by Niiyama (1948, cited in Murofushi and Deguchi, 1990; 1959, cited in Nakamura *et al.*, 1988) and $2N = 86$ by Hayashi and Fujiwara (1988). The work of Mayorga (1982) and my study give other evidence of these rearrangements.

The chromosome fusion from 92 to 88 leaves an intermediate value of 90. In the Gulf of México, *P. setiferus* (L.) has $2N = 90$ (Milligan, 1976), and $N = 45$ (Chow *et al.*, 1990). Further research will be necessary to verify this geographic difference in chromosome number among species of *Penaeus*.

According to Rodríguez-Romero *et al.* (1991), chromosomes with a medium degree of condensation are the most suitable for traditional C, G, Nor, etc., banding studies. My two modifications of the air-drying technique in eggs from marine shrimp will allow specified chromosome condensation by controlling the time of colchicine-treatment incubation. In addition, it will allow future chromosome-banding techniques in penaeids that will achieve a better description of karyotypes.

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