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The Chromosome Number of the Persian Gulf Shrimp Penaeus *semisulcatus*

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Abstract

The chromosome number of Penaeus *semisulcatus*, which is commonly known as the green tiger prawn and which lives in the Persian Gulf, has been determined by three approaches and the efficacies of these approaches have been compared. In one, various tissues after intramuscular injection of colchicine, in another embryonic cells and in the third blastema tissue regenerated after amputation of a pair pereopods were used. All three approaches when optimized were effective and their results suggest that the diploid chromosome number of P. *semisulcatus* is 90. This figure falls within the range of diploid chromosome numbers previously reported for other species of shrimp (86 to 92). Most of the chromosomes of P. *semisulcatus* are metacentric.

Keywords: *Decapods, Penaeidae, Penaeus semisulcatus, chromosome number, karyotype, Persian Gulf.*

1. Introduction

Penaeus *semisulcatus* belongs to the genus Penaeus and to the family penaeidae. This shrimp, commonly known as the green tiger prawn, is endemic to Iran and constitutes more than 90 percent of the harvest of the Persian Gulf. It is considered to be amongst the best shrimps in terms of nutritional value and thus has vast economic importance.

Twenty seven species of the genus Penaeus have been identified and the chromosome number and less often the karyotype of some of these have been reported. P. aztecus (Milligan 1976; Goswami 1985), P. setiferus, P. duorarum (Milligan 1976), P. japonicus (Niiama 1948), P. monodon (Xiang et al. 1990), P. occidentalis, P. californiensis, P.

stylirostris, P. vanammei (Zambrano-Mayorgam, 1982), P. penicillatus (Xiang, 1991) and P. orientalis (Jixun et al., 1989; Xiang 1988) are amongst the species whose chromosomes have been studied. The reported 2n chromosome numbers of these species range from 86 to 92. The 2n chromosome numbers of P. *semisulcatus* has been variously reported as 90 and 88 by two research groups (Nayak and Ahmed, 1989; Xiang et al., 1991). Given that this species is endemic to Iran, we set forth to ascertain its actual chromosome number.

The study of the chromosomes of decapod crustaceans is notoriously difficult, mainly because of the large number and small size of their chromosomes (Chow et al., 1990; Hayashi and Fujiwara 1988; Xiang, 1988). Furthermore, a tissue with a high percentage of dividing cells is not always readily available in decapods.

Most studies on the chromosomes of shrimps of the genus Penaeus have been done on tissues extracted after injection of colchicine (Zambrano-Mayorga, 1982; Xiang, 1988, Xiang et al., 1990; Xiang et al., 1991). In some cases in which the testes was used, the haploid chromosome number was determined without colchicine injection (Chow et al., 1990). Chromosomes of embryos and blastema tissue regenerated after amputation of a leg have also been investigated (Jixum et al., 1989; Hayashi and Fujiwara 1988). An advantage of embryonic and blastema tissues is that they contain a high percentage of dividing cells. In the present study, all these procedures were used to determine the chromosome number of P. *semisulcatus* and their relative usefulness was assessed.

2. Materials and Methods

Young or fully adult shrimps of the species P. *semisulcatus* were either harvested from the Persian Gulf offshore the port of Booshehr, or obtained from the prawn hatchery of the Booshehr Research Station for Reproduction and Culture of the Prawn of the Persian Gulf, P. *semisulcatus*. The embryos were all obtained from the hatchery. For optimization of each method of chromosomal preparation used, a range of conditions was tested.

To study the chromosomes of adults, a previously described colchicine intramuscular injection protocol was used except that KCl

hypotonic treatments with the concentration range 0-0.4% (0%, 0.05%, 0.15%, 0.25%, 0.4%) for five different time intervals ranging between 5 and 45 minutes were tested (Xiang, 1988; Xiang et al., 1990; Xiang, 1991). Gill, hepatopancreas, antennal gland and/or testes tissues were used. In many cases where testes tissue was used, colchicine was not injected.

Chromosomal spreads were prepared by three methods (A, B and C. In method A, a tissue section was minced in a few drops of cold 50% acetic acid, two drops of Carnoy solution (3 methanol: 1 acetic acid) were added, the suspension was dropped onto a prewarmed (50) slide and flame dried. In method B, a tissue section was directly placed on a slide in a few drops of Carnoy solution, minced and flame dried (Xiang et al., 1988; Xiang et al., 1990; Xiang et al., 1991). In method C, a tissue fragment was placed in 6 ml Carnoy solution and a cell suspension was obtained by repeated pipetting. The suspension was quickly centrifuged, most of the supernatant was removed and the cell pellet was resuspended in the few remaining drops. The suspension was dropped onto slides and dried at 45 ^{o}C.

Slides prepared by either of the above methods were kept at room temperature for 3 hours and finally stained with a 5% Giemsa solution prepared in phosphate buffered saline (pH=6.8) for 5,10,15 or 20 minutes.

Preparation of chromosomes from embryos of P. *semisulcatus* was accomplished according to procedures reported for P. orientalis, except that colchicine treatment was at 31^{o}C and incubation times of 45, 60 and 80 minutes were applied (Jixun et al. 1989). For staining, embryos were covered with a few drops of 1% orceine prepared in 45% acetic acid and kept at 25^{o}C for 5 to 24 hours, 30^{o}C for 2 to 4 hours, 35^{o}C for 2 hours or 40^{o}C for 30 to 60 minutes. Each single embryo was then transferred onto a clean slide, carefully washed with 45% acetic acid and gently squashed under a coverslip.

For the study of chromosomes of regenerated blastema, all equipment were sterilized and sea water was autoclaved. Younger shrimps weighing 3 to 7 grams were preferably used. Prior to amputation, they were kept for 15 minutes in sea water containing 4 μl/l formalin or 4 μg/l malachite green and then for one hour in sea

water without additives to relieve the stress of the disinfection treatment. After amputation of the pereopods from the base, the shrimps were maintained at 32°C. Regenerated buds began to appear three days after amputation and their length by day 6, the time at which chromosomes were studied, were 1 to 3 mm. The blastema tissue was removed and placed in a 10 mg/100 ml or 15 mg/100 ml colchicine solution for 45 minutes, then in a 0.55% KCl solution for 5, 10, 15, 20, 25, 30 or 35 minutes. The colchicine and hypotonic treatments were carried out at 30°C. Fixation, slide preparation and staining were as with the intramuscular injection protocol (method A).

Microscopic observations were done with a Zeiss Photomicroscope III. Grouping of the chromosomes on the basis of the location of the centromeres and length of chromosomal arms was done only with chromosomal spreads of embryonic cells (Levan et al., 1964).

3. Results

Countable chromosomal spreads were obtained from all three protocols. In contrast to previously reports, the hepatopancreas and antennal glands were not suitable tissues for chromosome preparation in our hands. Diploid counts were achieved in a few cells of the gill using the intramuscular injection protocol (Fig.1). Optimum treatment for gill tissue included a 5.5 to 6 hour incubation in colchicine, a 25 minute incubation at 25°C in pure water and preparation of concentrated cell suspension by pipetting in Carnoy solution (method C).

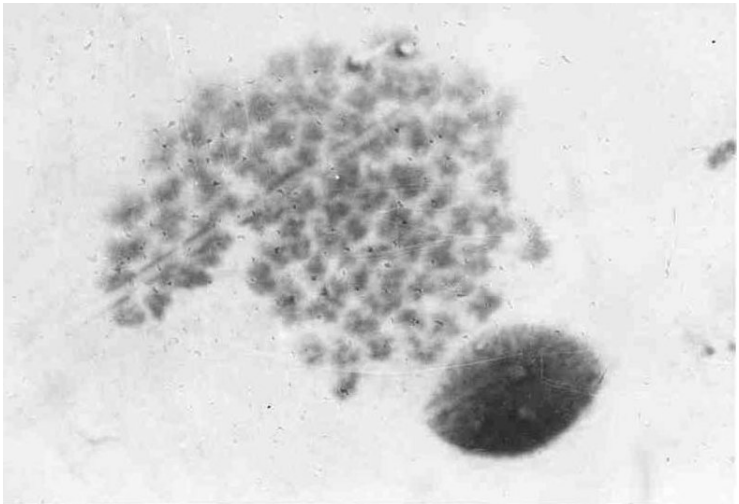

Figure 1. Chromosomal spread of gill tissue obtained by the injection protocol (x 1000). The slide was prepared by method C.

The intramuscular injection protocol proved particularly suitable for testes tissue (Fig. 2). The optimum treatments for this tissue were a 5.5 to 6 hour colchicine incubation and a 15 minute hypotonic treatment in pure water. Slides prepared by method A and method B were better than those obtained by method C. Generally either no or many chromosomal spreads were seen in the vicinity of any single testicular lobe, consistent with previous reports that cells of different lobes are intermittently either nondividing on actively dividing (Chow *et al.,* 1990).

Embryos proved to be good sources of chromosomal spreads of acceptable quality. A typical spread is shown in Figure 3a. Amongst the variables tested, optimal treatments included incubation in colchicine for 60 minutes, staining with orceine for 12 to 24 hours at 25^{o}C, and a wash with 45% acetic acid. Shorter and longer incubations with colchicine resulted, respectively, in thin chromosomes which did not stain well or condensed chromosomes wherein the centromere was not easily located. Elimination of the acetic acid wash caused a high level of background staining (Fig. 3b).

Cells of regenerated blastema tissue also produced good chromosomal spreads (Fig. 4). A 15mg/100ml colchicine incubation and a 10 minute hypotonic treatment were best. The modal diploid number of chromosomes counted in each of the three protocols is 90 (Table 1). Male and female shrimps were equally used as sources of regenerated blastema chromosomal spreads and the modal number for both sexes was 90. The chromosomes of eighty three diploid cells were counted in the three protocols combined, and 47 of these corresponding to 56.6% of the cells had 90 chromosomes (Table 1). Seventy eight chromosomal spreads of testes cells, prepared by the intramuscular injection protocol were also counted. Forty three of these corresponding to 55.1% contained 45 chromosomes (Table 2). It, therefore, appears that the haploid and diploid chromosome numbers of P. *semisulcatus* are, respectively, 45 and 90.

The chromosomal spreads obtained allow us to propose a karyotype for P. *semisulcatus*: 24 chromosome pairs are metacentric, 11 pairs are submetacentric, 1 pair is subtelocentric and 9 pairs are telocentric or acrocentric. It was not possible to distinguish between telocentric and

acrocentric chromosomes. Because of the small size of the chromosomes of P. *semisulcatus* and the small number of chromosomal spreads suitable for karyotyping, the karyotype suggested may not be absolutely accurate. Nevertheless, the preponderance of metacentric and submetacentric chromosomes is evident in the chromosomes of Figures 3a and 4b.

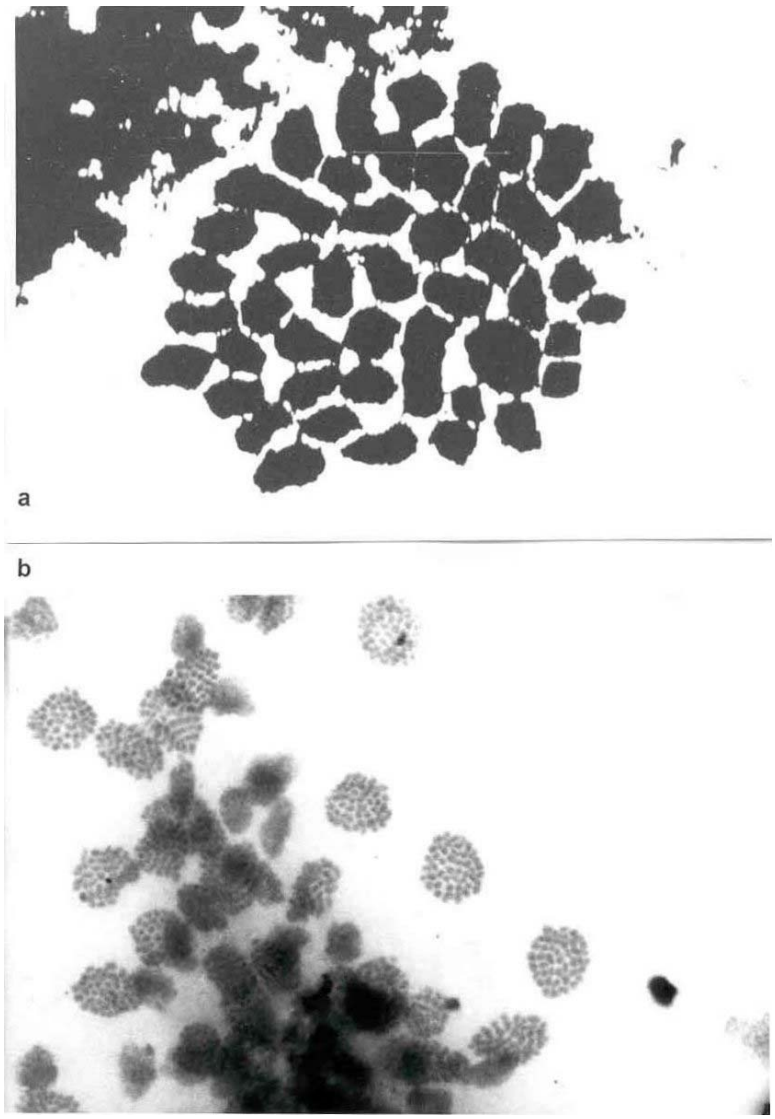

Figure 2. Chromosomal spreads of testes tissue. There was no colchicine treatment and slides were prepared by method A. a-the chromosomes of a single cell (x 1000) b-the chromosomes of several cells clustered in a testicular lobe (x 450).

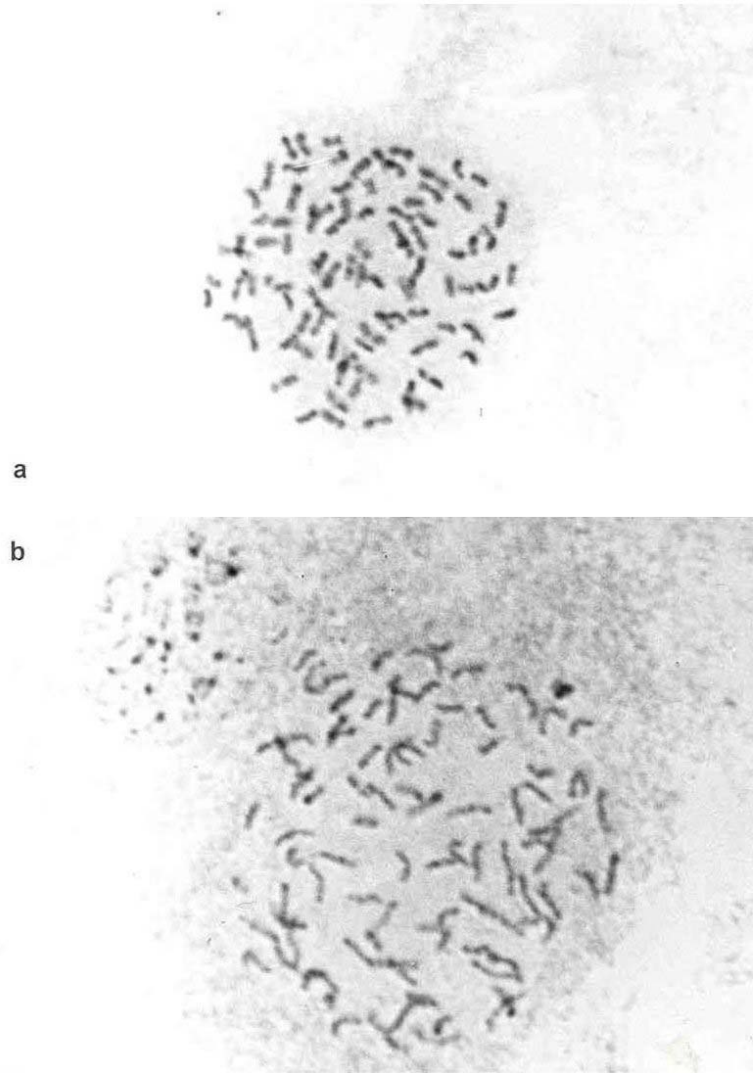

Figure 3. Chromosomal spreads of embryonic cells (x 1250). Colchicine treatment was for 60 minutes and staining with orceine was for 24 hours. a-with 45% acetic acid wash b-without acetic acid wash.

4. Discussion

Three protocols for preparation of chromosomal spreads of P. *semisulcatus* cells were tested. The intramuscular injection protocol was most useful for cells of the testes. Although haploid chromosomal counts could readily be obtained, the quality of the chromosomes was not appropriate for karyotyping. Chromosomal spreads of a quality which allow karyotyping was obtained from embryonic and regenerated blastema tissues. Preliminary results indicate that inclusion of a hypotonic treatment on embryonic cells may produce

premetaphase chromosomes of a quality which would allow banding studies (not shown).

The chromosomal counts obtained with all three protocols suggest that the diploid chromosome number of P. *semisulcatus* is 90. This is consistent with a previous report of Chinese scientists and inconsistent with one presented by Indian scientists (Xiang et al. 1991; Nayak and Ahmed 1989). The only other species in the genus Penaeus for which a 2n chromosome number of 90 has been reported is P. setiferus (Milligan 1976).

A karyotype of the chromosomes of P. *semisulcatus* is also suggested wherein 35 of the shrimp's 45 chromosomal pairs are classified as either metacentric or submetacentric. This also contradicts results presented by the Indian scientists who reported 40 acrocentric and only 4 metacentric pairs (Nayak and Ahmed 1989). The discrepancy may be due to a misidentification of the species used by the Indian scientists. Most of the chromosomes of other species of shrimps whose karyotypes have been reported are also metacentric (Chow et al. 1990; Goswami 1985; Jixun et al. 1989).

Crustacea show a wide variety of interesting chromosome related features (Lecher et al. 1995). Their haploid chromosome numbers show a wide range from 3 (Acanthoeyclops) to 188 (Astacus). The latter is one of the highest numbers of chromosomes known for animals. In some orders, primitive groups tend to have lower chromosome numbers while in others, the reverse is true. Crustacea also have a wide range of DNA content, from 0.37 pg (Daphnia) to 22.6pg (Decapoda) per haploid genome. The modal value is two to three picogragms. A strict correlation between chromosome number and DNA content does not exist. Much repetitive DNA exists in the genome of some species. Amplification and polyploidy have been suggested as possible mechanisms for change in DNA content during evolution. Endoploidy and B chromosomes have been reported in many species. A great variety of sex-determination mechanisms exist in crustacea, including male heterogamety, female heterogamety and more complex sex chromosome mechanisms.

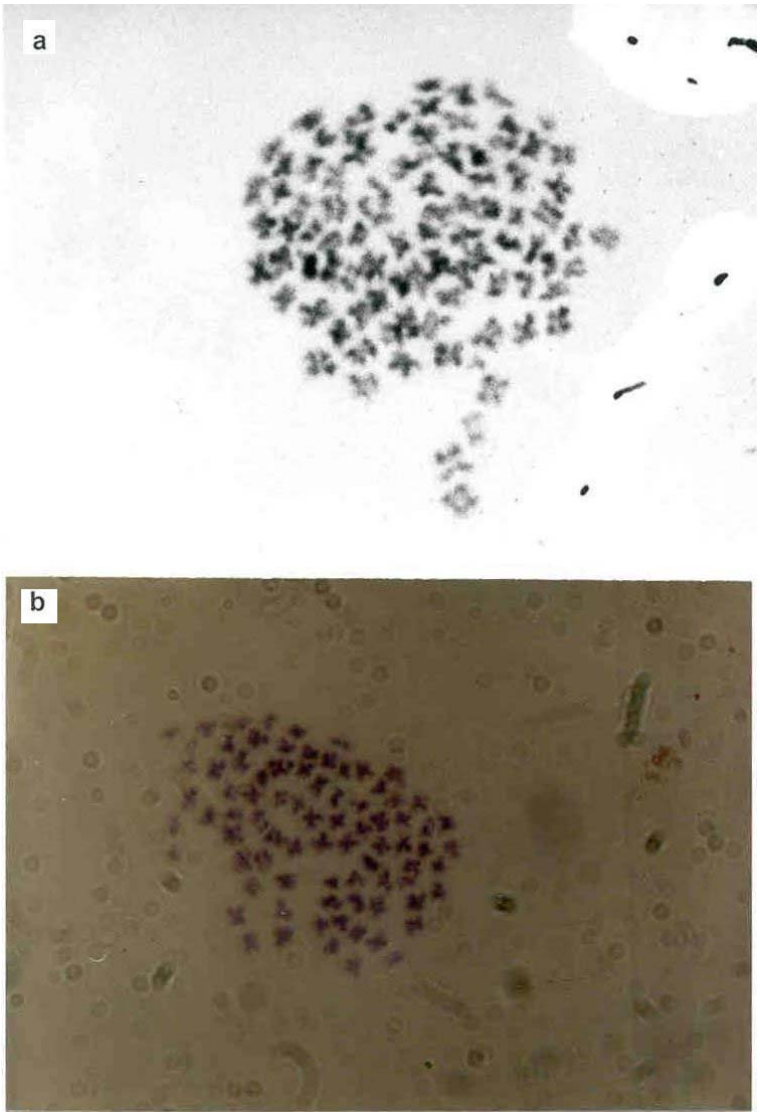

Figure 4. Chromosomal spreads of regenerated blastema tissue (1000X).

Decapoda is one of the largest groups of crustacea and many of the features mentioned above apply to this group of organisms. The range of their haploid chromosome numbers is large, from 27 in Liocarcinus vernalis to 188 in Astacus trowbridgi (Lecher et al. 1995). Their DNA content per haploid genome ranges from 1.3 pg to 22.6 pg.. Polyploidy has been suggested to be a relevant factor in Astacidea evolution. Analysis of repeated sequences has proved to be useful in assessing Decapod phylogeny.

Penaeidae are considered "primitive" amongst the Decapoda (Lecher et al. 1995). Most studies have focused on members of the genus Penaeus and their chromosome numbers are lower than that of

most other Decapoda. The evolutionary relationship between some species has been suggested on the basis of karyotype studies and nuclear content of DNA (Chow et al. 1990; Xiang et al. 1990). It has been speculated that P. duorarum and P. aztecus have evolved from P. setiferus. It is hoped that the application of improved karyotype techniques as well as molecular biology approaches will enhance our understanding of the genetics and evolution of the Decapoda in general and the penaeidae in particular.

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References

Chow S., Dougherty W.J., and Sandifer, P.A. (1990) Meiotic chromosome complements and nuclear DNA contents of four species of shrimps of the genus Penaeus. Journal of Crustacean Biology 10:29-36.

Goswami U (1985). Chromosomal studies in Penaeus aztecus Ives larvae. Mahasagar-bull. Nation, Inst. Oceano. 18:75-77.

Hayashi K-I and Fujiwara Y (1988). A new method for obtaining metaphase chromosomes form the regeneration blastema of Penaeus (Marsupenaeus) japonicus. Nippon Suisan Gakkaishi 54: 1563-1565.

Hayashi K-I, Fujiwara Y and Tatara A (1990) Chromosomes of whiskered velvet shrimp Metapenaeopsis barbata (DeHaan) (Decapoda, Penaeidae). Researches on Crustacea 18: 49-51.

Jixun D, Zhang Q and Boa Z (1989). Karyotype studies on Penaeus orientalis. Journal of Ocean University of Qingdao 19: 97-103.

Lecher P, Defaye D and Noel P (1995). Chromosomes and nuclear DNA of crustacea. Invertebrate Reproduction and Development 27: 85-114.

Levan A, Fredga K and Sandberg AA (1964). Nomenculature for centromeric position on chromosomes. Hereditas 52: 201-220.

Milligan DJ (1976). A method for obtaining metaphase chromosome spreads from marine shrimp with notes on the karyotype of Penaeus aztecus, P. setiferus and P. duorarum. Proc VII. World Maricul. Soc. San Diego, Calif. pp 327-332.

Nayak B and Ahmed MJ (1989). Studies on the chromosomes of four species of prawns. Cytobios. 60:189-196.

Niiyama H (1948). Cytogenetics, Oguma conference. Oguma commemoration volume on cytology and genetics, In: Biology Data Book, Vol 1, 2nd ed., Federation of American Societies for Experimental Biology, Bethesda, Maryland, pp 19-20.

Van Zalinge NP (1984). The shrimp fisheries in the gulf between Iran and the Arabian Peninsula. In: Gulland JA, Rothschild BJ (Eds.). in Panaeid Shrimp-Their Biology and Management, Farnham, Fishing News Books Ltd, p 71 .

Xiang JH (1988). The chromosome study on chinese shrimp, Penaeus orientalis kishinouye. Oceanol. Limnol. Sin. Haiyang-Yu-Huzhao 19:205-209.

Xiang JH, Liu RY, Zhou LH, Wang HF (1990). A study on the chromosome in a marine shrimp, Penaeus monodon. Annual Research Reports, Experimental Marine Biology Laboratory, Academia Sinica 1:115-121 .

Xiang JH, Zhou LH, Liu RY and Yu D (1991). The chromosomes of three shrimp Penaeus penicillatus, P. *semisulcatus* and P. japonicus. Mar. Sci. Haiyang Kexue 4: 72-73.

Zambrano-Mayorga M (1982). Genetic de Crustaceos. Documenta 10: 3-6.