

## KARYOTYPE ANALYSIS AND CHROMOSOMAL LOCALIZATION BY FISH OF RIBOSOMAL DNA, TELOMERIC (TTAGGG)<sub>N</sub> AND (GATA)<sub>N</sub> REPEATS IN *HALIOTIS FULGENS* AND *H. CORRUGATA* (ARCHEOGASTROPODA: HALIOTIDAE)

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**ABSTRACT** The chromosomes of the blue abalone *Haliotis fulgens* and the yellow abalone *Haliotis corrugata* were analyzed by means of DAPI staining, and fluorescence *in situ* hybridization with 18S-5.8S-28S rDNA, telomeric (TTAGGG)<sub>n</sub> and (GATA)<sub>n</sub> repeats DNA probes. The diploid chromosome number found was 36 for both California abalone species. However, the karyologic comparison between *H. fulgens* and *H. corrugata* indicated that the blue abalone has 8M + 8SM + 2ST, whereas the yellow abalone has 10M + 7SM + 1ST. The physical location of 18S-5.8S-28S rDNA clusters was found in the terminal region of the long arms of two pairs of submetacentric chromosomes (4, 11 and 2, 4 respectively). Localization of heteromorphisms of FISH-rDNA between homologue chromosomes and between sister chromatids, and the presence of interstitial hybridization signals was found in metacentric and onto submetacentric chromosomes. The presence of microsatellites (TTAGGG)<sub>n</sub> and (GATA)<sub>n</sub> was evidenced after FISH treatment by DNA probes. The telomeric sequence occurred at the ends of all mitotic chromosomes in both abalone species, whereas the (GATA)<sub>n</sub> repetitive sequence was found on chromosomal interstitial zones and at the ends of some chromosomes, this was manifested after FISH on interphase nucleus. In addition, this study contributes with new karyologic information of haliotids from California and gives support to the Tethys model about biogeographical origin, from the Mediterranean Sea to the East Pacific Ocean.

**KEY WORDS:** abalone chromosomes, *Haliotis fulgens*, *Haliotis corrugata*, FISH, rDNA, (TTAGGG)<sub>n</sub>, (GATA)<sub>n</sub>

### INTRODUCTION

The abalones (*Haliotis* spp.) constitute a remarkable group of marine gastropods molluscs that are found throughout most of the world, chiefly in temperate and tropical seas (Leighton 2000). In Mexico they are found along the Pacific coast of the Baja California Peninsula where they are commercially exploited because of the high price reached worldwide (Oakes & Ponte 1996). Because of their economical importance several studies on Haliotidae have been carried out, including population genetic analysis, seed production and ecology (Lindberg 1992, González & Scoresby 1996, Hamm & Burton 2000, Zúñiga et al. 2000, Del Río-Portilla & González-Avilés 2001). However, over the past 3 decades, several abalone species have experienced precipitous declines in abundance (Tegner et al. 1989). Overexploitation, pollution, habitat degradation and diseases have probably maximized the decline process (Burton & Tegner 2000).

The native haliotids to the northeastern Pacific Ocean are assigned to eight species, but taxonomic analysis has united two of these as subspecies of *Haliotis kamtschatkana* (Geiger & Poppe 2000). Attention has been focused on cytogenetics, because this field furnishes useful information on taxonomy of the Haliotidae family. According to Geiger & Groves (1999) based on chromosomal data, a model of progressive increase in chromosome number can be postulated, from a diploid number (2n) of 28 chromosomes in Mediterranean species like *H. tuberculata* (Arai & Wilkins 1986), to 2n = 32 in Indo-Pacific species (Jarayabhand et

al. 1998), to the highest number 2n = 36 in North Pacific species (Okumura et al. 1999). This would suggest that *H. tuberculata* is a relict species from the ancient Tethys Sea, and that the abalones dispersed eastwards, which is in agreement with the eastward dispersal pattern in the Pacific. In this scenario, the eight species of California abalones would be early representatives of the family. However, karyologic studies on abalones from California have only been performed on the black abalone *H. cracherodii* (Minkler 1977) and the red abalone *H. rufescens* (Gallardo-Escárate et al. 2004). The karyologic comparison among California abalones species showed that all species comprised equal chromosome number (2n = 36), although the relationships among chromosome types have not yet been studied.

Fluorescence *in situ* hybridization (FISH) is a powerful tool for understanding the genomic organization. By visualizing hybridization sites of a specific DNA probe, FISH permits the direct mapping of genes or DNA fragments on specific chromosomes and subchromosomal regions. This method has been used only in few studies to examine the chromosomes in the phylum Mollusca. In fact, available karyologic data on the class Gastropoda indicate that more than 300 species have been analyzed cytogenetically, whereas <20 of them have been examined using this kind of techniques (Vitturi et al. 2002). In Haliotidae, no karyologic studies by FISH have been carried out, therefore the chromosomal gene localization in abalones has not been analyzed.

The present paper was organized with the followings objectives: (a) to describe the karyotype of two California abalone species, *Haliotis fulgens* (Philippi 1845) and *Haliotis corrugata* (Wood 1828), (b) to examine the localization of 18S-5.8S-28S

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rDNA by means of FISH, (c) to ascertain the presence of telomeric (TTAGGG)<sub>n</sub> and (GATA)<sub>n</sub> sequences.

## MATERIALS AND METHODS

### Abalone Collection and Chromosome Preparation

Specimens of blue abalone, *Haliotis fulgens* and yellow abalone, *H. corrugata* were collected by diving from a subtidal population in Cedros Island, Baja California, Mexico (28°03'N; 115°8'W). The metaphases were obtained from trochophore larvae and the chromosome preparation was performed according to Gallardo-Escárate et al. (2004). The slides were washed in three changes of Phosphate Buffer Saline (1 × PBS) for 5 min each time, and incubated with DAPI solution in the dark for 25 min at room temperature (25°C) using a Coplin jar. DAPI solution stain was prepared with 4,6-diamidino-2-phenylindole (DAPI) (Sigma-Aldrich, USA) in 1 × PBS at 0.5 µg/mL.

### Karyotyping by Image Analysis

Chromosome spreads were karyotyped from fluorescent digital images. The best metaphases were captured using a motorized epifluorescent microscope (Leica model DMRXA2) equipped with a RGB color digital camera of 36-bits and 3.3 mega pixels (Leica model DC300). For identification of homologue chromosomes, arm lengths (short and long) were measured with an Image ProPlus software version 4.0 (Copyright 1993–1998 Media Cybernetics). Measurements were used to determine relative lengths of chromosomes (length of the chromosome pair / total complement length × 100) and centromeric index (length of short arm / total length of the chromosome × 100) of each chromosome. To perform a quantitative analysis between *H. fulgens* and *H. corrugata* a karyo-ideogram was plotted according to Spotorno (1985).

### Fluorescence In situ Hybridization

To decrease unspecific hybridizations, slides were pretreated with 100 µg/mL RNase in 2xSSC (pH 7.0) buffer at 37°C for 1 h and then washed twice for 5 min each in 2 × SSC at RT and one time at 37°C for 5 min. Then the slides were incubated in 5 µg/mL pepsin in 0.01 M HCl for 10 min at 37°C. After the treatment the slides were washed two times for 5 min each in 1 × PBS, fixed in 1% formaldehyde for 10 min at RT, washed twice for 5 min each in 1 × PBS, rinsed in 0.85% NaCl and dehydrated in a series of ethanol concentrations (30%, 50%, 70%, 90% and 100%). Finally, slides were air dried and stored at -80°C.

The chromosomal localization of the major rDNA gene cluster was performed using a probe containing 18S-5.8S-28S genes plus intergenic spacer of *D. melanogaster* (pDm238) (Roiha et al. 1981) and cloned in pBR322 plasmid of *E. coli* (strain JM109). The plasmid purification was performed with a NucleoSpin Plasmid kit (Macherey-Nagel) and it was labeled with Dig-Nick Translation Mix kit (Roche Molecular Biochemicals) according to manufacturer's instructions. After obtaining the labeled probe, the hybridization mixture was added onto pretreated chromosome preparations and heated to 72°C for 7 min to denature DNA. *In situ* hybridization was allowed to proceed at 37°C overnight, followed

by posthybridization washes, three times for 5 min each in 50% formamide in 2 × SSC at 44°C, three times for 5 min each in 0.1 × SSC at 60°C, 5 min in 2 × SSC at RT, 5 min in TNT at RT and 30 min in TNB at RT.

FISH with telomeric (TTAGGG)<sub>n</sub> and (GATA)<sub>n</sub> DNA probes were amplified by PCR in volumes of 50 µL of the reaction mixture in absence of template using (TTAGGG)<sub>5</sub> and (CCCTAA)<sub>5</sub> and (GATA)<sub>7</sub> and (TATC)<sub>7</sub> as primers, respectively (Ijdo et al. 1991). The PCR conditions were performed by 10 cycles at 94°C for 1 min, 55°C for 30 sec, 72°C for 1 min and 30 standard PCR amplification cycles at an annealing temperature of 60°C. Nick translation labeling with digoxigenine was performed and *in situ* hybridization was carried out as mentioned earlier and followed by posthybridization washes at 42°C (telomeric probe) or 37°C (GATA probe). For detection of hybridization, chromosomes were incubated in 0.5 µg/mL mouse antidigoxigenine and detected in 0.2 µg/mL Rabbit antiMouse FITC-conjugated, and finally in FITC-conjugated Goat antiRabbit at a concentration of 0.1 µg/mL. Chromosome slides were counterstained and mounted with 30 µL of IP-V 500 ng/mL (propidium iodide plus Vectashield antifading medium). Fluorescence images of FISH were obtained by using an epifluorescence microscope (Axioskop 2 plus, Zeiss) and recorded by a cooled CCD camera (CoolSnap, Photometrics Inc.).

## RESULTS

### Karyotype of *H. fulgens*

Metaphase chromosomes of the blue abalone *H. fulgens*, showed a diploid number equal to 36 chromosomes. The mean values and standard deviations of the total lengths, relative lengths and centromeric index were estimated from chromosome arm lengths using image analysis (Table 1). The maximum length of chromosomes was 6.07 ± 0.12 µm and the minimum was 3.13 ± 0.21 µm. The ratio of the chromosome arms plotted in the karyo-ideogram indicated that this species possess 8 metacentric pairs (1, 3, 6, 9, 10, 16, 17 and 18), 8 submetacentric pairs (2, 4, 5, 7, 8, 11, 12 and 13), and 2 pairs of subtelocentric chromosomes (14 and 15). The distribution of relative chromosome lengths was derived to perform a karyotypic arrangement (8M + 8SM + 2ST) (Fig. 1).

### In situ Hybridization in *H. fulgens*

FISH using a probe derived from 18S-5.8S-28S rDNA genes produced strong signals on the telomere of the long arms of 2 pairs of submetacentric chromosomes (pairs 4 and 11). Metaphase chromosomes from larvae showed heteromorphisms of NORs between sister chromatids and low interstitial signals were mainly found in submetacentric chromosomes. However, the specific localization of regions with low signal intensity showed a high cytogenetic variability in all metaphases analyzed (Fig. 2a and 2b).

After FISH treatment with the telomeric sequence (TTAGGG)<sub>n</sub>, abalone's chromosomes were found positive to telomeric probe. Strong signals were found at the ends of all chromosome types analyzed (Fig. 2c). The visualization of the interphase nucleus labeled with the telomeric probe allowed to evidence the distribution of telomeres in clusters at the nuclear periphery (Fig. 2d). The presence of the (GATA)<sub>n</sub> microsatellite

TABLE 1.  
Total chromosome lengths, relative length and centromeric index for *Haliotis fulgens* ( $2n = 36$ ).

Chromosome Pair No.	Total Length ( $\mu\text{m}$ ) (Mean $\pm$ std)	Relative Length (%) (Mean $\pm$ std)	Centromeric Index (Mean $\pm$ std)	Chromosome Type*
1	6.07 $\pm$ 0.12	8.00 $\pm$ 0.27	47.45 $\pm$ 0.16	M
2	5.06 $\pm$ 0.81	6.67 $\pm$ 0.12	34.09 $\pm$ 0.15	SM
3	4.84 $\pm$ 0.11	6.37 $\pm$ 0.38	48.37 $\pm$ 0.17	M
4	4.72 $\pm$ 0.68	6.23 $\pm$ 0.49	39.81 $\pm$ 0.18	SM
5	4.16 $\pm$ 0.93	5.48 $\pm$ 0.22	34.16 $\pm$ 0.14	SM
6	4.62 $\pm$ 0.35	6.08 $\pm$ 0.12	44.69 $\pm$ 0.13	M
7	4.56 $\pm$ 0.68	6.01 $\pm$ 0.07	39.41 $\pm$ 0.10	SM
8	4.41 $\pm$ 0.79	5.82 $\pm$ 0.40	37.27 $\pm$ 0.17	SM
9	4.24 $\pm$ 0.49	5.59 $\pm$ 0.20	42.74 $\pm$ 0.15	M
10	4.14 $\pm$ 0.36	5.45 $\pm$ 0.10	43.77 $\pm$ 0.12	M
11	4.04 $\pm$ 0.69	5.32 $\pm$ 0.20	37.98 $\pm$ 0.15	SM
12	3.98 $\pm$ 0.87	5.24 $\pm$ 0.26	34.51 $\pm$ 0.09	SM
13	3.81 $\pm$ 0.51	5.02 $\pm$ 0.31	40.59 $\pm$ 0.15	SM
14	3.86 $\pm$ 1.38	5.09 $\pm$ 0.21	24.73 $\pm$ 0.14	ST
15	3.63 $\pm$ 1.29	4.78 $\pm$ 0.25	24.82 $\pm$ 0.17	ST
16	3.50 $\pm$ 0.25	4.61 $\pm$ 0.19	44.97 $\pm$ 0.12	M
17	3.13 $\pm$ 0.08	4.13 $\pm$ 0.23	48.29 $\pm$ 0.20	M
18	3.13 $\pm$ 0.21	4.13 $\pm$ 0.22	45.20 $\pm$ 0.09	M

\* M, metacentric; SM, submetacentric; ST, subtelocentric.

on chromosomes of *H. fulgens* was found positive for the signal of hybridization (Fig. 2e). The localization of these regions was found in chromosomal interstitial zones and at the ends of some chromosomes, this was manifested after FISH treatment on interphase nuclei (Fig. 2f).

#### Karyotype of *H. corrugata*

Metaphases examined in the yellow abalone *H. corrugata*, showed a chromosome number of  $2n = 36$ . By arranging into chromosome pairs, the karyotype consisted of 10 metacentric pairs (1, 3, 6, 9, 10, 11, 14, 15, 17 and 18), 7 submetacentric pairs (2, 4, 5, 7, 8, 12 and 13), and 1 pair of subtelocentric chromosomes (16). Chromosome pair 10 showed overlapping between metacentric and submetacentric types (Fig. 3). According to chromosome total lengths, relative lengths and centromeric index, the maximum chromosome length was  $5.82 \pm 0.12 \mu\text{m}$  and the minimum was  $3.46 \pm 0.05 \mu\text{m}$  (Table 2).

#### In situ hybridization in *H. corrugata*

Strong signals on the telomere of the long arms of 2 pairs of submetacentric chromosome (2 and 4) were produced by NORs with FISH treatment. Heteromorphisms of NORs were found between sister chromatids. Presence of interstitial regions was found in metacentric and in submetacentric chromosomes. The localization of low hybridization signals showed high variability (Fig. 4a, 4b).

Telomeric (TTAGGG)<sub>n</sub> DNA probe showed positive hybridization in chromosomes of yellow abalone and strong signals were found (Fig. 4c). FISH onto interphase nucleus showed a peripheral distribution of the telomeres at the nuclear periphery (Fig. 4d). The

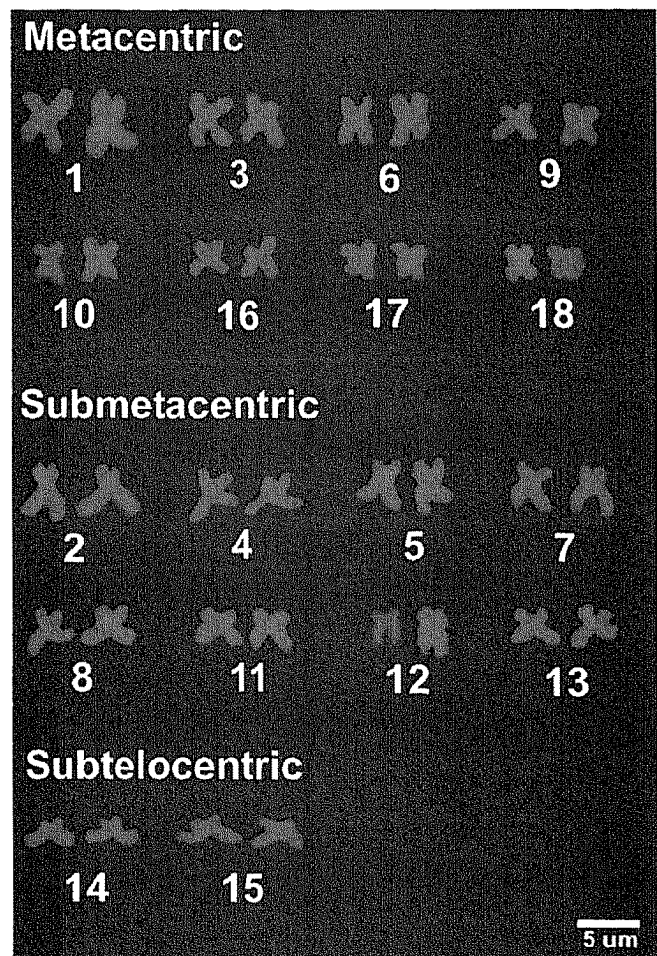
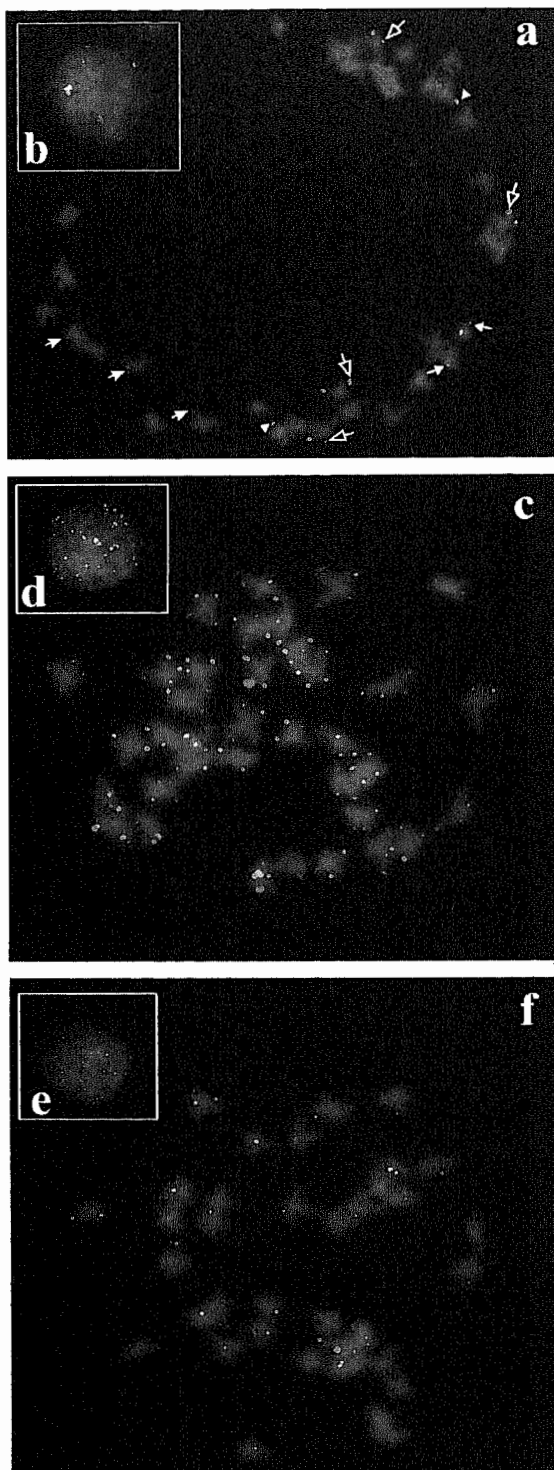
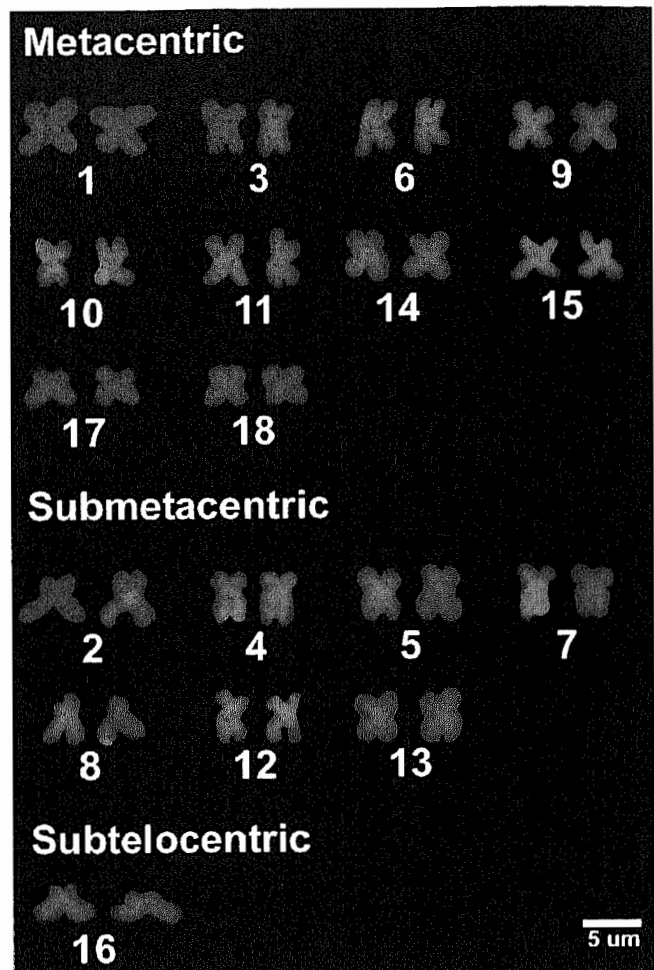


Figure 1. Karyotype of *Haliotis fulgens* ( $2n = 36$ ).



**Figure 2.** a) Fluorescence *in situ* hybridization of *Haliotis fulgens*. a) Mitotic metaphase chromosomes from trochophore larvae after FISH with 18S-5.8S-28S rDNA probe. b) Interphase nucleus with rDNA probe. c) Metaphase chromosomes after FISH treatment with the telomeric sequence (TTAGGG)<sub>n</sub>. d) Interphase nucleus with telomeric probe. e) Metaphase chromosomes after being labelled with the (GATA)<sub>n</sub> probe. f) Interphase nucleus labelled with (GATA)<sub>n</sub> sequence. Empty white arrows indicate identification of the NORs in homologue chromosome. Arrows head show NOR heteromorphism between sister chromatids. White arrows indicate interstitial signals of 18S-5.8S-28S region.



**Figure 3.** Karyotype of *Haliotis corrugata* ( $2n = 36$ ).

presence of (GATA)<sub>n</sub> sequence was corroborated in *H. corrugata* chromosomes (Fig. 4e). The localization of these regions was also variable (Fig. 4f).

#### DISCUSSION

The genus *Haliotis* is a complex and diverse group of archaeogastropods related by an economical importance and inconclusive systematics. In this sense, several studies have been carried out to approach the genetic characteristics of abalones throughout the world (Lee & Vacquier 1995, Elliott 2000). Cytogenetic analyses such as chromosome number and morphology are useful to trace phylogenetic patterns and evolutionary relationships. Karyologic data in abalone species show a variation of diploid number from 28–36 chromosomes (Jarayabhand et al. 1998). The review of karyotypes reported in abalones and their geographic distribution (Geiger & Poppe 2000), showed that abalones from the European Mediterranean region have a  $2n = 28$ . Representative abalones from the Indo-Pacific region have a characteristic  $2n = 32$ , with the exception of *H. aquatilis* with a diploid number equal to 34 chromosomes. The abalones found in the South Japan region show a  $2n = 32$ . Finally, abalones from the North Pacific or Japanese abalones have a diploid chromosome number of 36. In reference to karyologic data from species belonging to the North Oriental Pacific and specially from California, only the karyotypes of the

TABLE 2.  
Total chromosome lengths, relative length and centromeric index for *Haliotis corrugata* ( $2n = 36$ ).

Chromosome Pair No.	Total Length ( $\mu\text{m}$ ) (Mean $\pm$ std)	Relative Length (%) (Mean $\pm$ std)	Centromeric Index (Mean $\pm$ std)	Chromosome Type*
1	5.82 $\pm$ 0.12	7.28 $\pm$ 0.27	48.02 $\pm$ 0.06	M
2	5.00 $\pm$ 0.08	6.26 $\pm$ 0.12	35.90 $\pm$ 0.11	SM
3	4.82 $\pm$ 0.03	6.03 $\pm$ 0.38	46.70 $\pm$ 0.05	M
4	4.80 $\pm$ 0.06	6.00 $\pm$ 0.49	39.95 $\pm$ 0.08	SM
5	4.59 $\pm$ 0.02	5.74 $\pm$ 0.22	36.93 $\pm$ 0.06	SM
6	4.53 $\pm$ 0.06	5.66 $\pm$ 0.12	43.81 $\pm$ 0.09	M
7	4.52 $\pm$ 0.05	5.66 $\pm$ 0.07	34.51 $\pm$ 0.09	SM
8	4.45 $\pm$ 0.02	5.57 $\pm$ 0.40	36.91 $\pm$ 0.08	SM
9	4.44 $\pm$ 0.04	5.55 $\pm$ 0.20	49.16 $\pm$ 0.07	M
10	4.34 $\pm$ 0.01	5.43 $\pm$ 0.10	41.09 $\pm$ 0.07	M
11	4.32 $\pm$ 0.08	5.40 $\pm$ 0.20	47.98 $\pm$ 0.16	M
12	4.29 $\pm$ 0.02	5.36 $\pm$ 0.26	37.38 $\pm$ 0.04	SM
13	4.24 $\pm$ 0.02	5.30 $\pm$ 0.31	34.82 $\pm$ 0.08	SM
14	4.22 $\pm$ 0.09	5.28 $\pm$ 0.21	44.74 $\pm$ 0.10	M
15	4.07 $\pm$ 0.03	5.09 $\pm$ 0.25	47.04 $\pm$ 0.03	M
16	4.03 $\pm$ 0.02	5.04 $\pm$ 0.19	22.44 $\pm$ 0.04	ST
17	4.01 $\pm$ 0.01	5.02 $\pm$ 0.23	48.59 $\pm$ 0.02	M
18	3.46 $\pm$ 0.05	4.32 $\pm$ 0.22	45.50 $\pm$ 0.07	M

\* M, metacentric; SM, submetacentric; ST, subtelocentric.

black abalone *H. cracherodii* (Minkler 1977) and recently of the red abalone *H. rufescens* (Gallardo-Escárate et al. 2004) have been reported. Both abalone species showed a chromosome number of  $2n = 36$ . The increase in diploid chromosome number suggests that abalones from the European Mediterranean region are relict species from the ancient Tethys Sea, and those abalones were dispersed eastwards, which is in agreement with the eastward dispersal pattern in the Pacific. In this scenario, the California abalone species would be more recent haliotids.

This study is the first karyologic report of the blue abalone *Haliotis fulgens* and the yellow abalone *H. corrugata*. Our results showed that a  $2n = 36$  chromosomes was observed in both abalone species. This result confirms a characteristic diploid number for abalone species from California. However, the karyologic comparison between *H. fulgens* and *H. corrugata* indicate that the blue abalone has  $8M + 8SM + 2ST$ , whereas the yellow abalone has  $10M + 7SM + 1ST$  (Fig. 5). In addition, *H. rufescens* and *H. cracherodii* described for this region showed that these species are similar in the metacentric chromosome number equal to eight pairs (8M). The differences are that the red abalone has  $9SM + 1ST$ , whereas the black abalone has  $8SM + 2ST$ . According to Patterson (1969) basal gastropods have a low diploid number of chromosomes, whereas recent gastropods species frequently exhibit higher chromosome numbers. Parallel to the increase of chromosome number, in certain aquatic organisms often an enlargement of submetacentric and telocentric chromosomes is observed, probably by chromosomal fission (Thiriou-Quévieux 1994).

The karyologic conformation of *H. fulgens* and *H. corrugata* suggests a deletion process of the short arms in some submetacentric chromosome pairs, originating subtelocentric pairs. In this scenario, it is possible to suggest that *H. fulgens* is an abalone species with considerable chromosomal loss and rearrangements in comparison with *H. corrugata*. Furthermore, the highest number of metacentric chromosomes of the yellow abalone suggests that this species is the most ancestral among the California abalones karyo-

logically reported. Phylogenetic relationships studied by Lee & Vacquier (1995), and Coleman & Vacquier (2002) among California species, showed that *H. corrugata* was phylogenetically closer to Japanese abalone species in comparison with other species like *H. rufescens*, whereas *H. fulgens* appears as the genetically most distant species or the most recent, compared with the other California abalones.

Fluorescence *in situ* hybridization is a powerful technique used to characterize and to detect DNA segments in chromosomes. This method of molecular cytogenetics allows the physical location of genes independently of their transcription activity. Although the FISH technique has been extensively used in many animal and plant species, in molluscs it has mainly been applied in bivalves (Zhang et al. 1999, Vitturi et al. 2000b, Insua et al. 2001, Martínez et al. 2002, Cross et al. 2003). In reference to gastropods, less than 20 of 300 species analyzed cytogenetically have been examined by FISH (Vitturi et al. 2002).

The ribosomal RNA genes are a group of DNA sequences that produce structural RNA to support protein synthesis. The nucleotide sequences of these genes are often polymorphic, which has enabled taxonomic investigation of organisms with ambiguous phylogenetic relationships (Littlewood 1994). Furthermore, rDNA sequences are useful for the initiation of physical genome mapping, because the gene exists in multiple copies, which improves its detection. According to our results, the 18S-5.8S-28S rDNA of *H. fulgens* and *H. corrugata* was localized by FISH at the ends of two pairs of submetacentric chromosomes (4, 11 and 2, 4 respectively). To our knowledge, in abalone only one study has been reported in reference to location of the nucleolar organizing region (NOR). According to Okumura et al. (1999), variations in NOR-bearing chromosomes were observed in *H. discus hannai* and located terminally on the long arms of two chromosome pairs. However, these NORs were visualized by silver nitrate staining (Ag-NOR staining) and therefore any NORs in the nonactive state remained undetected. Our results showed localization of hetero-

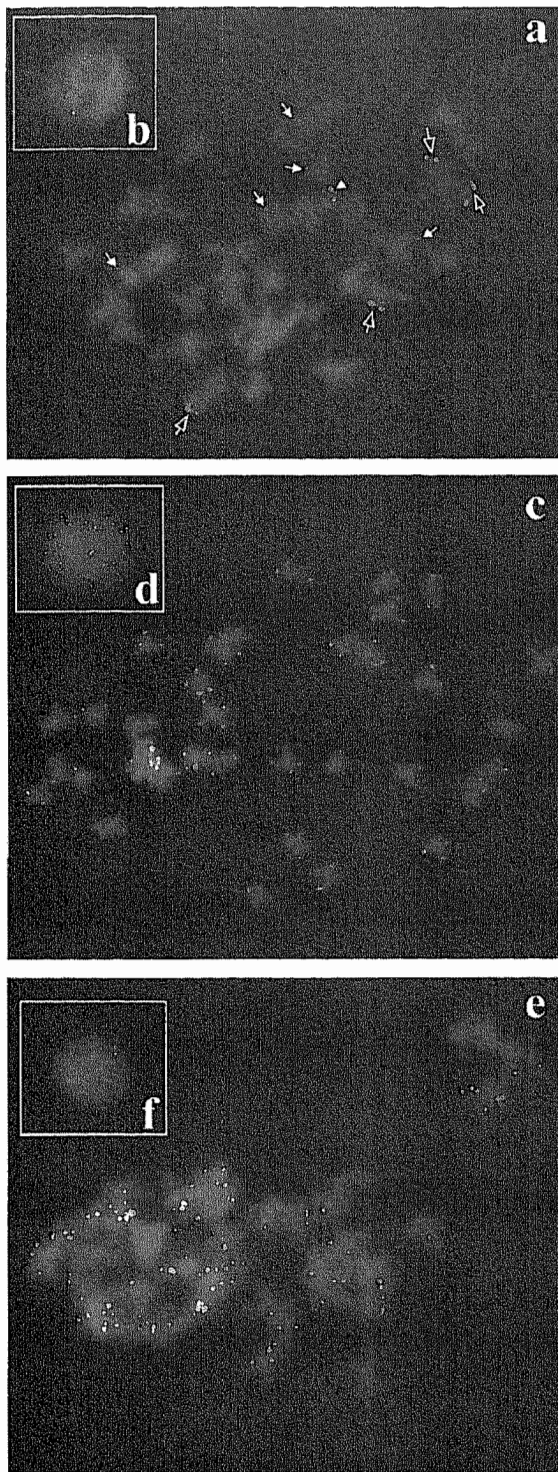


Figure 4. a) Fluorescence *in situ* hybridization of *Haliotis corrugata*. a) Mitotic metaphase chromosomes from trochophore larvae after FISH with 18S-5.8S-28S rDNA probe. b) Interphase nucleus with rDNA probe. c) Metaphase chromosomes after FISH treatment with the telomeric sequence (TTAGGG)<sub>n</sub>. d) Interphase nucleus with telomeric probe. e) Metaphase chromosomes after being labeled with the (GATA)<sub>n</sub> probe. f) Interphase nucleus labelled with (GATA)<sub>n</sub> sequence. Empty white arrows indicate identification of the NORs in homologue chromosome. Arrows head show NOR heteromorphism between sister chromatids. White arrows indicate interstitial signals of 18S-5.8S-28S region.

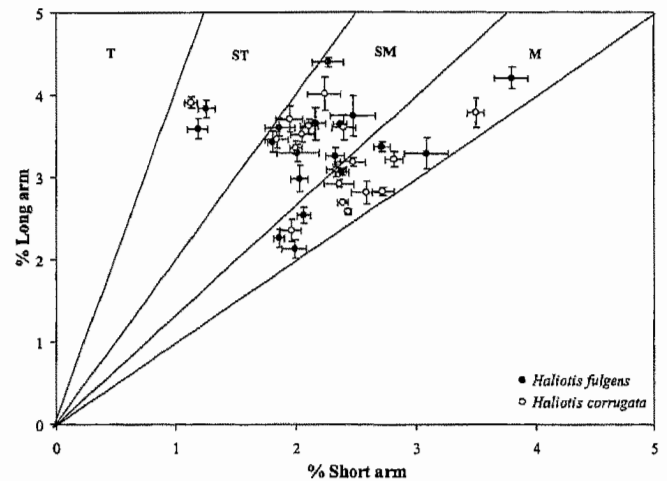


Figure 5. Karyo-ideogram of *Haliotis fulgens* (black circles) and *H. corrugata* (white circles). Relative lengths and confidence intervals were potted for each species.

morphisms of FISH-rDNA between homologues and between sister chromatids, and presence of interstitial hybridization signals were found in metacentric as well as onto submetacentric chromosomes. These results may indicate that the copy number variability of rDNA and mistakes are caused by either deleting or duplicating rDNA clusters during the DNA replication. This FISH-rDNA variability is in agreement with other studies reported in molluscs (Martínez-Expósito et al. 1997, Torreiro et al. 1999, Insua et al. 2001, Martínez et al. 2002, Cross et al. 2003).

In this study, we used in addition to the rDNA FISH, *in situ* hybridization with (TTAGGG)<sub>n</sub> and (GATA)<sub>n</sub> probes to test for the presence of these sequences in *H. fulgens* and *H. corrugata* chromosomes. The results demonstrated that the telomeric sequence occurred at the ends of all mitotic chromosomes in abalones. In molluscs, conservation of the telomeric region has been reported in bivalves (Guo & Allen 1997) and gastropods (Vitturi et al. 2002), and therefore the telomere repeat is widespread within the Phylum.

The genome of blue and yellow abalones, revealed evident hybridization sites by the (GATA)<sub>n</sub> FISH. In fact, this result was according to that reported in other gastropods species (Vitturi et al. 2000a, Colomba et al. 2002), in reference to the (GATA)<sub>n</sub> microsatellite occurring among Mollusk taxa. Other studies have shown a heterogeneous distribution of the sequence that was found to be absent or, if present, occurred in a low amount (Vitturi et al. 2002). In conclusion, studies by FISH performed in molluscs are useful to examine the genomic constitution at the molecular level and for the reconstruction of chromosomal changes during evolution. In this context, rDNA probes have been used to examine the Robertsonian process in *Nucella lapillus* (Pascoe et al. 1996). In addition, the use of the microsatellite (GATA)<sub>n</sub> revealed that this sequence does not occur in the Y chromosome of the neogastropod *Fasciolaria lignaria* (Vitturi et al. 2000a). Therefore this sequence could be used to test sex-linked arrangements in abalone species.

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