Rev. Brasil. Genet. 13, 4, 675-685 (1990)
(Brazil. J. Genetics)

CHROMOSOMES OF *Pomacea sp.* (PERRY, 1811) (MESOGASTROPODA, MOLLUSCA)

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ABSTRACT

A search was made for differences in *Pomacea sp* through karyotype analysis. The genus *Pomacea* was studied as a biological control of *Biomphalaria glabrata*, an intermediate host of *Schistosoma mansoni*.

A standard chromosome analysis was performed with the air dried technique, tested in embryos at the young veliger stage. The meiotic preparations were obtained through the same technique with the male gonad.

According to our results the present species has a diploid chromosome number of 2n = 28, represented by 9 metacentric pairs, 4 submetacentric pairs and 1 subtelocentric pair. The short arm of the subtelocentric pair showed a positive reaction to silver stained preparations, v.g.; the site of the secondary constriction (NOR).

The meiotic analysis confirmed the presence of n = 14 chromosomes.

INTRODUCTION

The species belonging to the genus *Pomacea* (Ampullariidae) are large mollusks measuring more or less 21 cm (Malek, 1985) which can be found in swamps, bogs, canals, lakes, etc.

The classification of this species has been very complex because of the morphoanatomical characteristics of the animals. Sowerby (1909), Pain (1949, 1950a,b,

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1956a,b,c, 1960) and Blume and Pain (1951) reported the occurrence of *Pomacea* in South America. Lopes (1955) studied the genitalia of *P. haustrum* and *P. sordida* as well as several biomorphological features of *P. canaliculata* (Lopes, 1956a) and *P. lineata* (Lopes, 1956b) and Hylton Scott (1957) of Ampullariidae. More recently, Thiengo (1987, 1989) described in detail, the morphology of *P. lineata* and *P. sordida*, respectively and Fausto Filho (1962, 1965) reported on the biology of *P. haustrum*. The biological control of *Biomphalaria glabrata* (intermediate host of *Schistosoma mansoni*) using *P. haustrum* and *P. lineata* as natural competitors was investigated (Milward de Andrade, 1972, 1974; Milward de Andrade and Guimarães, 1971, 1975, 1977, 1978; Matthiensen, 1976; Guimarães, 1978, among others). These experiments were conducted at different sites under natural conditions and a perceptible reduction in the *B. glabrata* population was observed. Some biological features of *Pomacea sp.* were investigated by Moraes and Veiga (1981a,b) in order to select the species best adapted to preying conditions for the control of *Biomphalaria*.

The karyotypic study of *Pomacea sp* may contribute to the elucidation of the numerous systematic problems concerning this genus and could be useful for the determination of the species with the highest potential for the biological control of *Biomphalaria*.

No cytogenetic studies have been published in the literature on the genus *Pomacea* (Mesogastropoda). According to Patterson and Burch (1978), the variation in chromosome number ranges from n = 7 in *Viviparus contectus* to n = 20 in several members of the family Pleuroceridae. However, three species of the family Ampullariidae whose chromosome number was determined by Lufty and Demian (1964), *Pila ovata, Lamistes bolteni* and *Marisa cornuarietis*, all had a diploid number of n = 14 chromosomes.

The species of *Pomacea sp*, although dioic, do not present sexual dimorphism, a fact that impairs the selection of sex for meiotic preparations, which are obtained from male specimens. During copulation, however, the males can usually be identified by their smaller diameter (Guimarães, 1978; Thiengo, personal communication).

By obtaining the karyotype of this species we could then study others of the same genus to determine possible morphological differences in chromosomes between species and to investigate possible chromosomal mechanisms of sex determination.

MATERIAL AND METHOD

Adult *Pomacea sp* specimens were collected from a small artificial lake located on the grounds of the Biosciences Institute, University of São Paulo, maintained in the laboratory in an aquarium containing *Salvinea sp* and fed lettuce leaves. Eggs were collected from specimens maintained in the laboratory. Embryos in the trochophore and young veliger stage were used to obtain mitotic metaphases. The embryos were removed from the egg capsule (calcarea) with tweezers. They develop inside a pink albumen of the same colour as observed in their digestive gland.

Mitotic metaphases were obtained by an air dried technique as used by Kawano *et al*. (1984) for *B. glabrata*:

1) Embryos are decapsulated, washed in distilled water and placed in 0.05% colchicine for 60 minutes at ± 28°C, with illumination.

2) Hypotonization is performed using 0.4% sodium citrate for 30 minutes at 37°C.

3) For pre-fixation, the embryos are transferred to 5% acetic acid for 5 minutes.

4) The material is fixed with 3:1 ethanol:acetic acid, three immersions of 20 minutes each.

5) Embryos are dissociated in 50% acetic acid with the aid of a pipette.

6) The material is dribbled onto a thoroughly clean slide heated to 40°C on a hot plate.

7) The slides are allowed to dry naturally overnight.

8) The material is stained with 2% Giemsa for 20 minutes, using a phosphate buffer solution, pH 6.8.

This same technique was used to obtain mitotic metaphases from individual embryos. Gonadal preparations were made by the same air dried technique in order to obtain the different meiotic phases. $AgNO_3$ staining was used to visualize the nucleolar organizer region by the method of Howell and Black (1980).

The mitotic metaphases and the different meiotic phases were analyzed and photographed with a Zeiss microscope. The chromosomes were classified morphologically according to Levan *et al*. (1964). Ten of the best metaphases were measured to construct the idiogram presented in Figure 4.

RESULTS

Figure 1 presents a mitotic metaphase preparation from a *Pomacea sp* embryo. The karyotype is presented in Figure 2 and showed a diploid number of 2n = 28 chromosomes. Paired chromosomes were classified by size. Chromosomes 1, 2 and 3 are larger metacentric pairs. Chromosome 4 is a subtelocentric pair which was positive for $AgNO_3$ staining in the secondary constriction, a fact indicating that this is the nucleolar organizer region. Mitotic metaphase preparations from individual embryos were obtained in order to determine the occurrence of sexual dimorphism in the karyotype. Figure 3 shows two mitotic metaphases from the same embryo, with evidence of variation in the short arm of the subtelocentric pair. Chromosomes 5, 9, 12 and 14 are submetacentric pairs, while chromosomes 6, 7, 8, 10, 11 and 13 are metacentric pairs. On the basis of centromere position, the chromosomes can be classified into 9 metacentrics, 4 submetacentrics and 1 subtelocentric.

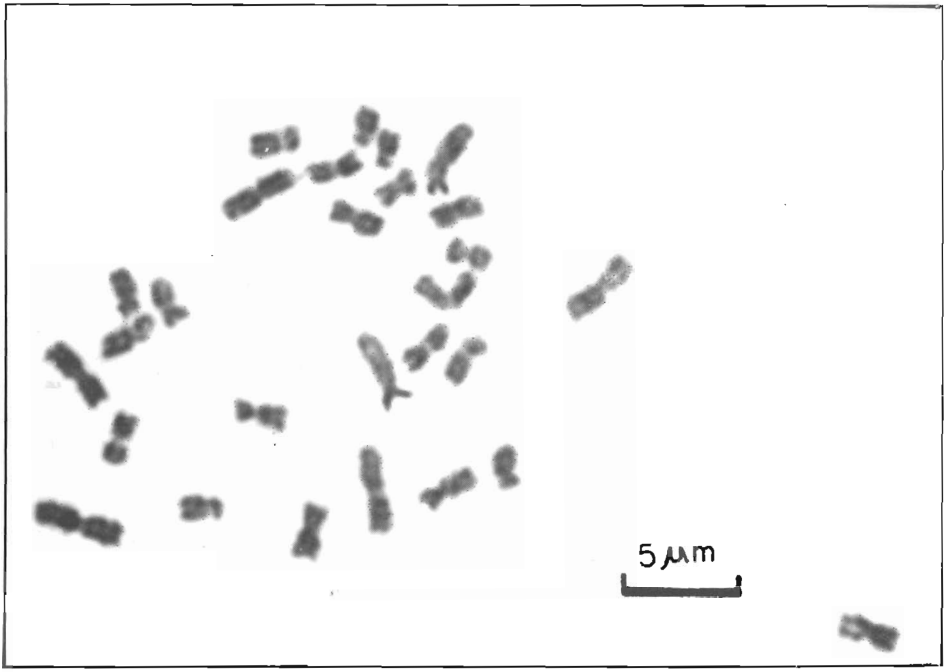

Figure 1 - Mitotic metaphase from *Pomacea sp* embryo.

The data in Table I were used to determine the relative arm length for the calculation of centromere position in the chromosomes and the idiogram constructed from these data is illustrated in figure 4. Figure 5A illustrates a meiotic pachytene phase (the nucleus stained by $AgNO_3$), Figure 5B illustrates a diakinesis and Figure 5C metaphase I. Figure 5D presents a polyploid metaphase cell from male gonadal preparation and Figure 5E a polyploid nucleus with five nucleoli.

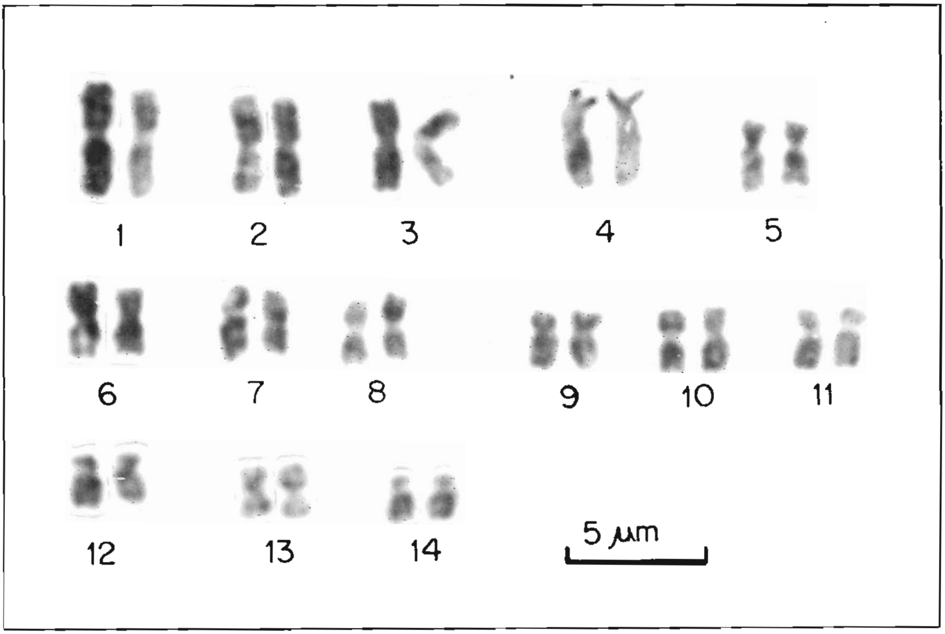

Figure 2 - Karyotype of *Pomacea sp.*

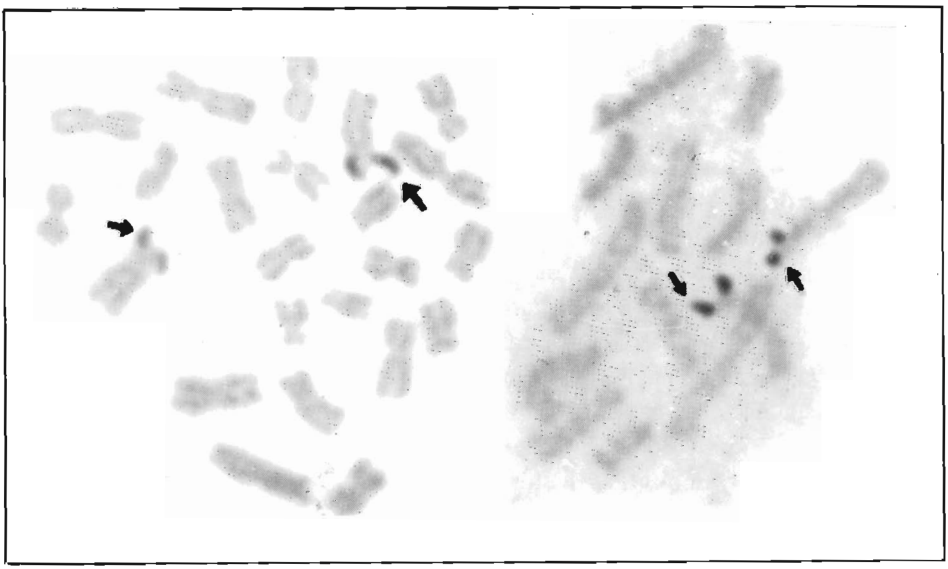

Figure 3 - Mitotic metaphase from the same embryo, the arrows indicate NOR silver staining, chromosome pair no. 4.

Table I - Relative chromosomes lengths and arm ratios of *Pomacea sp.* M = Metacentric, SM = submetacentric, ST = subtelocentric.

Chromosome pair no.	Long arm	Short arm	Total length	Centromeric index	Relative length	Arm ratio	Centromere position
1	18.35	15.62	33.97	45.98	10.11	1.17	M
2	16.77	15.05	31.82	47.29	9.47	1.11	M
3	15.84	14.12	29.96	47.12	8.92	1.12	M
4	22.85	7.02	29.87	23.50	8.89	3.25	ST
5	15.85	9.72	25.57	38.00	7.61	1.71	SM
6	13.85	11.12	24.97	44.53	7.43	1.24	M
7	12.35	10.85	23.20	46.76	6.90	1.13	M
8	11.90	9.72	21.62	44.95	6.43	1.22	M
9	13.85	7.42	21.27	34.88	6.33	1.86	SM
10	11.07	9.75	20.82	46.82	6.20	1.13	M
11	10.45	9.15	19.60	46.68	5.83	1.14	M
12	12.55	6.27	18.82	33.31	5.60	2.00	SM
13	9.52	8.12	17.64	46.03	5.25	1.17	M
14	10.87	5.80	16.67	34.79	4.96	1.87	SM

DISCUSSION

The order Mesogastropoda, which belongs to the subclass Prosobranchia presents a variation in chromosome number from n = 7 detected in *Viviparus contectus* to n = 18 in a member of the family Pittonidae and to n = 20 in several Pleuroceridae (Patterson, 1969; Patterson and Burch, 1978). Thiaridae species presenting n = 45 to 60 were considered to be polyploid (Patterson, 1969).

Two contrasting hypotheses have been proposed to explain evolution within the Prosobranchia. One of them was proposed by Patterson (1969) who suggested that the more primitive mollusks may have a low chromosome number, whereas Hinegardner (1974) thinks that the low DNA content may be correlated with more evolved species. However, Vitturi *et al.* (1982) proposed a new evolutionary viewpoint for Prosobranchia on the basis of the chromosomal data obtained by Patterson (1969) and of the DNA content of 38 species determined by Hinegardner (1974). Thus, according to Vitturi *et al.* (1982), in the Prosobranchia subclass, evolution may have

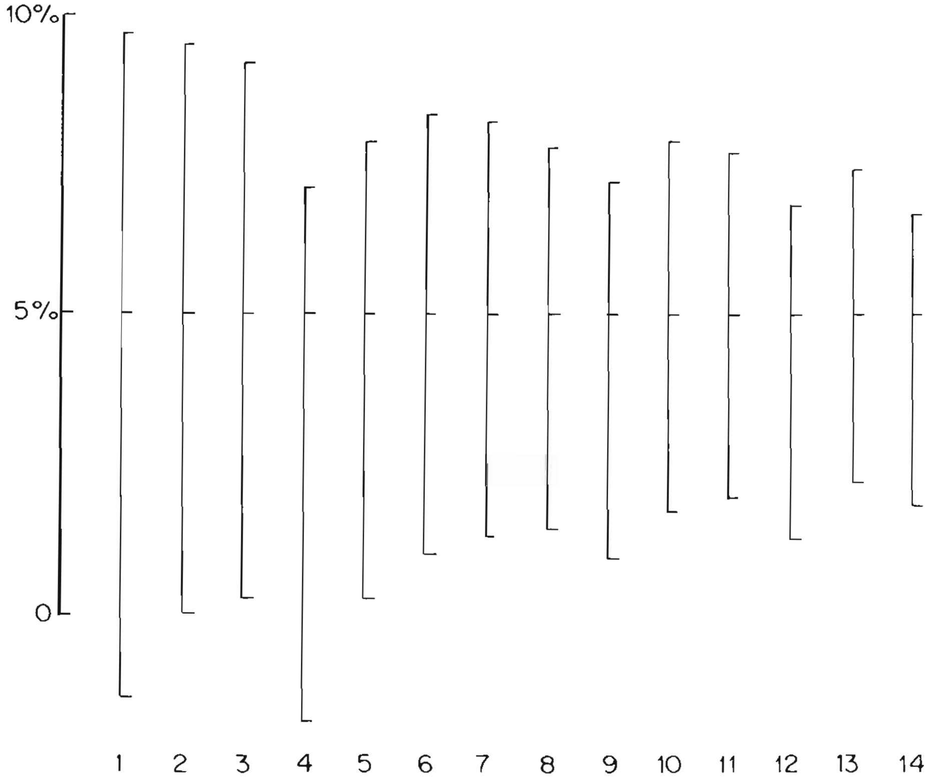

Figure 4 - Idiogram of *Pomacea sp*.

been accompanied by an increase in chromosome number as indicated by the n = 9 - 18 values for Archaeogastropoda, n = 16 - 18 for Mesogastropoda and n = 28 - 36 for Neogastropoda, according to the species studied by them.

On the other hand, in the single cytogenetic study carried out on the family Ampullariidae, Lufty and Demian (1964) found that *Pila ovata, Lanistes bolteni* and *Marisa cornuarietis* all had n = 14 chromosomes, as also was found in the *Pomacea sp* studied in the present investigation.

The species studied here was sent to Thiengo for determination and she reached the conclusion that it probably is a type of *Pomacea lineata*, since crossing tests were not performed. Thus, all the genera studied thus far within the family Pilidae have a stable haploid number equal to 14 chromosomes. The family Ampullariidae which belongs to the superfamily Viviparacea (n = 8 to 14) has the largest chromosome number (n = 14).

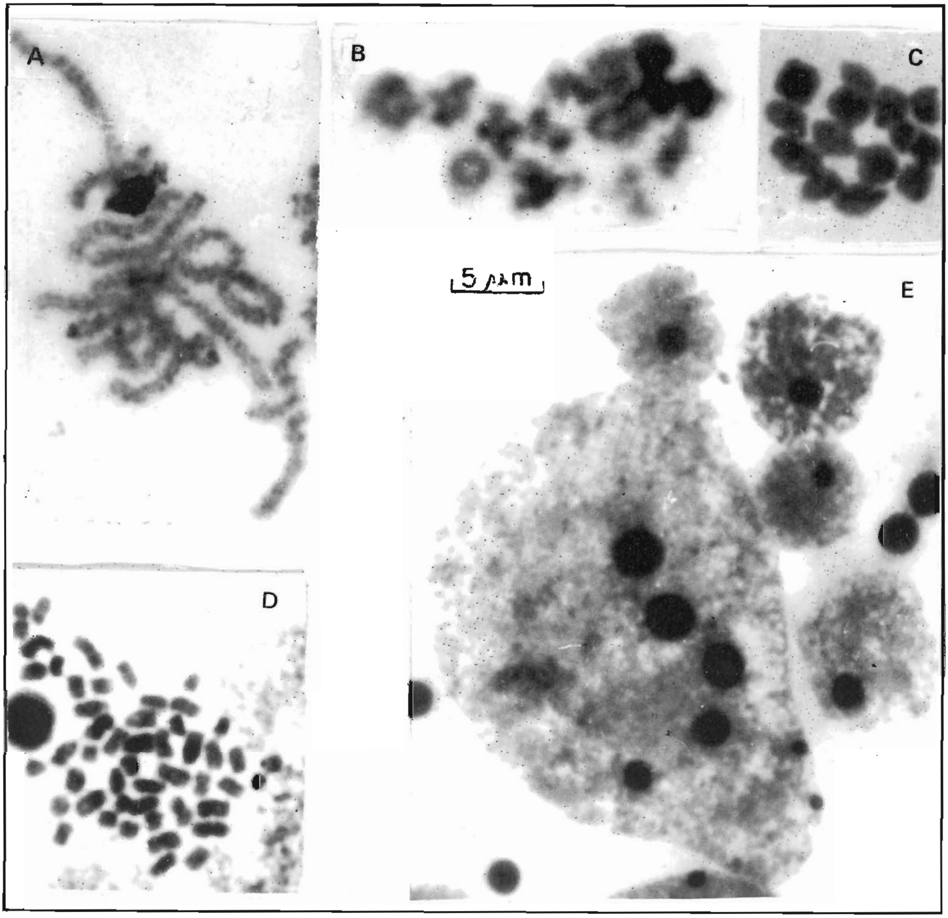

Figure 5 - (A) Meiotic pachytene phase, with nucleolus stained by $AgNO_3$; (B) Meiotic diakinesis phase; (C) Meiotic metaphase I; (D) Polyploid metaphase cell; (E) Polyploid nucleus with five nucleoli.

The chromosome morphology data obtained in the present study cannot be discussed in a comparative manner owing to the lack of data for other species within the *Pomacea*.

No sex dimorphism was detected at the cytogenetic level. It was not possible to detect sex chromosomes by the method utilized. Vitturi *et al*. (1982) also failed to show sexual dimorphism in other species of the same order.

In gonodal preparations, polyploid nuclei are frequent as also seen in other molluscs. These nuclei divide mitotically as shown in Figure 5D.

ACKNOWLEDGMENTS

This research was partially supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq. Grant no. 301469/85-7 (T. Kawano) and FINEP no. 4.3.86.091.00.

The authors also thank Ms. Vera H. Monezi for typing.

Publication supported by FAPESP.

RESUMO

O estudo cariotípico em *Pomacea sp* é importante em virtude das grandes dificuldades taxonômicas apresentadas por este gênero e também por ser utilizado em experiências como controlador biológico da *Biomphalaria glabrata*, hospedeiro intermediário do *Schistosoma mansoni*.

A técnica utilizada para a obtenção de metáfases foi a de suspensão celular, elaborada em nosso laboratório. O cariótipo foi obtido em preparações realizadas com embriões, no estágio de veliger jovem. Esta espécie possui o número diplóide de 2n = 28 cromossomos constituído de 9 pares metacêntricos, 4 pares submetacêntricos e 1 par subtelocêntrico. O braço curto do par subtelocêntrico se mostrou positivo ao $AgNO_3$, local da constrição secundária (RON). A análise meiótica confirmou a presença de n = 14 cromossomos.

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(Received December 15, 1989)