

Chromosomal studies on two Egyptian freshwater snails, *Cleopatra* and *Bithynia* (Mollusca-Prosobranchiata)

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Amany A. Tohamy* and Shaimaa M. Mohamed*

*Zoology Department, Faculty of Science, Helwan University, Cairo, Egypt
Corresponding Author: E-mail: amanytohamy2005@yahoo.com

ABSTRACT

This study deals with the karyotype of *Cleopatra bulimoides* and a *Bithynia* spp., both belonging to class Gastropoda and subclass Prosobranchia but to two different families, namely Thiariidae and Bithyniidae. The observed diploid chromosome number for *Cleopatra* was $2n=28$, and for *Bithynia* $2n=32$. The mitotic chromosomes of *Cleopatra* consisted of four metacentric pairs, eight submetacentric pairs and two telocentric pairs of chromosomes. The mitotic chromosome number of *Bithynia* snails was six metacentric pairs, four submetacentric pairs, three subtelocentric pairs and three telocentric pairs of chromosomes. Results of the present study have scientific and practical significance complementary to biochemical and molecular studies in animal taxonomy.

Key words: chromosomes, karyotype, *Cleopatra*, *Bithynia*.

INTRODUCTION

The freshwater snail fauna has been studied long time ago with a wide variety of interests. In recent times, the interest of zoologists has been focused on the group of molluscs which play an important role in transmitting helminthic diseases of man and his livestock.

Most studies on *Cleopatra* and *Bithynia* were carried out for parastiological purposes, *Cleopatra* as the intermediate host of *Prohemistomum vivax* infecting dog, cat and kite in Egypt and occasionally man and *Bithynia* snails as the first intermediate host of *Opisthorchis viverrini* (Wykoff *et al*, 1965).

There are relatively few studies on the karyotype and sex determination in prosobranchs. karyological studies of some species of subclass *Prosobranchia* were described by several authors (Jacob, 1959,

Patterson, 1973, Vitturi and Catalano, 1984, Yasseen, 1994 and Chaudhury and Pandit, 1997). It has been concluded that numerous species of the subclass *Prosobranchia* exhibit a wide range of haploid chromosome numbers ranging from $n = 7$ to $n = 36$. Vitturi *et al* (1982 and 1988) Nakamura (1986) and Yasseen (1994) examined the chromosome numbers of a diversity of prosobranchs. They found that the Patellogastropoda has 9, the Pleurotomarioidea, Fissurelloidea and Neritoidea has 11-21 and the Trochoidea has 18 chromosomes. Mesogastropoda has 7-20 and neogastropoda 28-36 chromosomes. Nakamura (1985) found in *Haliototis diversicolor aquatilis* that the number of chromosomes was $n = 16$. Dasilva and Brown (1982) found that three species of genus *Bullia* have a diploid chromosome number of 64 ($n = 32$) although they may be polyploid in the gill

and digestive gland. There are some evidence that males are heterogametic while females are homogametic, but more species need to be studied (Vitturi *et al.*, 1988 and Thiriot-Quievreux, 1990). Karyological information on the prosobranch family Thiariidae, in which the genus *Melanopsis* is included, until recently, is comparatively scarce. Chromosome numbers of only seven species were listed in the review of Patterson (1969). Haploid numbers of 16, 18 and 19 chromosomes in diploid species and also polyploid species with haploid chromosome complements of 35-60 elements were reported. Yaseen (1996) reported a diploid number of $2n = 22$ in *Melanoides tuberculata*. (Müller). This species has also been found to be polyploid (Jacob, 1959 and Barsiene *et al* 1996).

Numerous species are known to be polymorphic in chromosome numbers (Suzuki *et al*, 1981), for example, the intertidal gastropod, *Nucella lapillus*, displays a chromosomal dimorphism with population monomorphic chromosome numbers $2n = 26$ or $2n = 36$ (Janson, 1983).

Cytogenetic parameters such as chromosome number and morphology have long been used to characterize species and can give valuable clues to phylogeny evolution and taxonomic relationships. The present investigation is concerned with the karyological studies of two genera of gastropod snails, namely *Cleopatra* and *Bithynia*, which belong to the same subclass *Prosobranchia* and to two different families, Thiariidae and Bithyniidae in a trial to clarify their taxonomic status.

MATERIALS AND METHODS

The snails were collected from Giza and Fayoum governates all year round from July 2002 to August 2003, from slow running fresh water streams. Snails were taken alive to the laboratory, then maintained in tanks of aerated

water and fed continuously to promote growth. Chromosome preparations were obtained from the gills and gonads. Preparation of chromosomes and karyotypes of snails were made according to the method described by Barsiene *et al.* (2000).

After taxonomic verification of each snail, pooled snails (about 10 snails for each species) were placed directly in 0.1% colchicine at room temperature, for one day. Snails were dissected and their gills and gonads separated and treated with 0.48% KCl as hypotonic solution, at room temperature. Tissues were then carefully minced in the hypotonic solution and transferred to centrifuge tubes where they were left for 1-1.5 hours, after which they were centrifuged for 10 minutes at 2500 rpm. The cell pellet was fixed in freshly prepared mixture of absolute methanol and glacial acetic acid (3:1) for 15 minutes, and then centrifuged at 2500 rpm with three changes of 15 minutes duration. Finally, 1-2 ml of freshly prepared fixative were added to cell pellet and 3-5 drops of cell suspension were dropped on clean wet glass slides (previously kept at 4° C in 70% ethanol) which is flame-dried.

The prepared microscopic slides were stained in 4% Giemsa for 30-45 minutes and examined under a high power microscope with an oil immersion and photographs were taken with a high contrast Kodak film. For karyotyping, chromosomes were cut out of the photographs and paired on the basis of size and centromere position. Within each population, measurements of the chromosomes of ten karyotypes from different specimens were made using digitizer table. Relative chromosome length was expressed as a percentage of the total length (in μm) of the haploid complement. The centromeric index was calculated by dividing 100 times the length of the short arm by the total chromosome length. The centromere position

was also expressed in terms of the arm ratio (length of long arm divided by the length of short arm). Terminology relating to centromere position followed that of Levan *et al.* (1964).

RESULTS

Cleopatra and *Bithynia* snails showed a diploid number of chromosomes $2n = 28$ and $2n = 32$, respectively, as indicated from the

metaphase spread preparations and the corresponding karyotypes (Figs. 1a, b and Figs. 2 a,b). Chromosomes of each species were arranged in karyotypes in a descending manner, according to the total lengths and centromere positions. Tables 1 and 2 show the means and standard deviations of short arms, long arms, total and relative lengths, centromeric indices and arm ratios, with the classification of the two populations studied.

Table (1): Measurements and classification of the chromosomes of *Cleopatra* snails.

Pair Ch. No.	Short arm M ± SD	Long arm M ± SD	Total length M ± SD	Relative length M ± SD	Centromeric index M ± SD	Arm ratio M ± SD	Classification (shape)
1	3.49 ± 0.42	4.00 ± 0.32	7.49 ± 0.66	9.79 ± 0.32	46.53 ± 2.61	1.43 ± 0.11	m
2	2.78 ± 0.64	3.97 ± 0.56	6.75 ± 0.45	8.82 ± 0.20	41.03 ± 8.50	1.54 ± 0.69	m
3	1.98 ± 1.16	3.94 ± 0.82	5.12 ± 0.61	7.72 ± 0.31	37.93 ± 18.42	1.70 ± 0.22	m
4	1.90 ± 1.17	3.83 ± 1.41	5.02 ± 0.67	7.46 ± 0.46	37.80 ± 19.43	1.60 ± 0.29	m
5	2.45 ± 1.38	4.61 ± 1.47	7.06 ± 0.57	9.22 ± 0.34	34.86 ± 19.54	1.88 ± 0.08	Sm
6	2.23 ± 0.67	3.90 ± 1.07	6.12 ± 0.65	7.98 ± 0.32	36.96 ± 11.56	2.07 ± 1.49	Sm
7	2.09 ± 0.53	3.40 ± 0.48	5.48 ± 0.54	7.15 ± 0.32	34.94 ± 8.08	1.90 ± 0.58	Sm
8	1.69 ± 1.06	3.40 ± 0.81	5.09 ± 0.45	6.65 ± 0.22	32.46 ± 19.60	1.57 ± 0.67	Sm
9	1.46 ± 0.64	3.21 ± 0.51	4.67 ± 0.44	6.09 ± 0.23	30.89 ± 12.13	2.80 ± 1.86	Sm
10	1.22 ± 0.78	3.25 ± 0.75	4.48 ± 0.28	5.84 ± 0.13	27.14 ± 17.79	2.18 ± 0.99	Sm
11	1.11 ± 0.11	3.40 ± 0.94	4.31 ± 0.36	6.29 ± 0.17	25.75 ± 22.09	2.10 ± 1.07	Sm
12	0.49 ± 0.73	3.15 ± 0.33	3.69 ± 0.56	4.93 ± 0.44	26.02 ± 16.09	2.60 ± 2.26	Sm
13	0.60 ± 0.88	3.94 ± 0.86	4.97 ± 0.41	6.48 ± 0.18	12.07 ± 18.27	4.67 ± 3.72	t
14	0.43 ± 0.61	3.83 ± 0.53	4.25 ± 0.25	5.56 ± 0.19	9.72 ± 14.16	3.47 ± 1.76	t

m = metacentric, Sm = Submetacentric, t = telocentric, M = mean, SD = standard deviation

Table 2: Measurements and classification of the chromosomes of *Bithynia* snails.

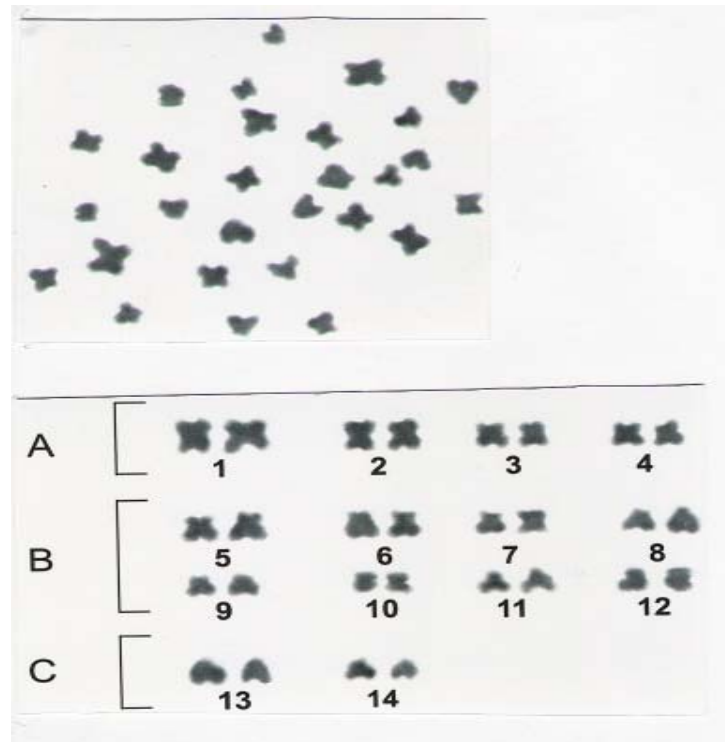
Pair Ch. No.	Short arm M ± SD	Long arm M ± SD	Total length M ± SD	Relative length M ± SD	Centromeric index M ± SD	Arm ratio M ± SD	Classification (shape)
1	3.96 ± 0.77	6.34 ± 0.56	10.31 ± 0.72	9.33 ± 0.80	38.33 ± 5.79	1.66 ± 0.45	m
2	3.78 ± 0.28	5.39 ± 0.50	9.17 ± 0.50	8.29 ± 0.39	41.28 ± 3.22	1.38 ± 0.11	m
3	3.53 ± 0.30	5.06 ± 0.20	8.59 ± 0.49	7.76 ± 0.40	41.04 ± 1.38	1.43 ± 0.97	m
4	3.37 ± 0.05	4.88 ± 0.42	8.22 ± 0.44	7.46 ± 0.13	40.97 ± 2.06	1.44 ± 0.12	m
5	3.24 ± 0.11	4.09 ± 0.60	7.93 ± 0.56	7.16 ± 0.12	41.05 ± 3.66	1.45 ± 0.22	m
6	2.63 ± 0.30	4.04 ± 0.32	6.67 ± 0.44	6.03 ± 0.15	39.38 ± 3.51	1.55 ± 0.24	m
7	2.64 ± 0.86	4.90 ± 0.97	7.54 ± 0.54	6.80 ± 0.06	35.51 ± 3.01	2.35 ± 1.97	Sm
8	2.43 ± 1.37	4.71 ± 1.21	7.14 ± 0.60	6.44 ± 0.24	33.58 ± 19.45	1.92 ± 0.35	Sm
9	2.14 ± 1.20	4.83 ± 1.46	6.99 ± 0.54	6.31 ± 0.23	31.19 ± 17.97	1.59 ± 0.34	Sm
10	1.70 ± 0.90	4.46 ± 0.55	6.16 ± 0.51	5.56 ± 0.25	26.96 ± 12.83	2.60 ± 3.82	Sm
11	0.77 ± 1.07	5.04 ± 1.09	5.81 ± 0.43	5.25 ± 0.32	13.1 ± 18.55	6.18 ± 0.92	St
12	1.01 ± 1.13	4.37 ± 1.67	5.38 ± 0.60	5.75 ± 0.30	18.77 ± 18.64	4.33 ± 0.23	St
13	0.79 ± 1.05	3.99 ± 0.98	4.78 ± 0.91	4.24 ± 0.59	20.48 ± 19.28	5.05 ± 0.90	St
14	0.62 ± 0.65	4.73 ± 1.02	5.55 ± 0.34	4.82 ± 0.15	12.5 ± 13.27	5.44 ± 4.55	t
15	0.61 ± 1.15	4.67 ± 1.48	5.21 ± 0.44	4.97 ± 0.13	11.71 ± 22.22	5.40 ± 0.12	t
16	0.38 ± 0.85	3.8 ± 0.81	4.18 ± 1.04	3.74 ± 0.72	7.03 ± 15.74	10.84 ± -	t

m = metacentric, sm = Submetacentric, st = subtelocentric, t = telocentric, M = mean, SD = standard deviation.

The Karyotype of *Cleopatra* snails (Fig.1b and table 1) consists of three groups : Group A was composed of four metacentric pairs of chromosomes with arm ratio of 1.43-1.70, relative lengths of 7.46 % to 9.79% and centromeric indices of 37.80 to 46.53, group B contained eight submetacentric pairs of chromosomes with arm ratio ranging from 1.57 to 2.80, relative lengths of 4.93% to 9.22% and centromeric indices of 25.75 to 36.96, group C included two telocentric pairs of chromosome with arm ratio 3.47 to 4.67, relative lengths of 5.56% to 6.48% and centromeric indices from 9.72 to 12.07. Fig. 2b shows the karyotype of *Bithynia* snails which consists of four groups : Group A was composed of six metacentric pairs of

chromosomes with arm ratio ranging from 1.38 to 1.66, relative lengths of 6.03% to 9.33% and centromeric indices of 38.33 to 41.28, group B contained four submetacentric pairs of chromosomes with arm ratio ranging from 1.59 to 2.60, relative lengths of 5.56% to 6.80% and centromeric indices of 26.96 to 35.51, group C included three subtelocentric pairs of chromosomes with arm ratio ranging from 4.33 to 6.18, relative lengths of 4.24% to 5.75% and centromeric indices of 13.1 to 20.48 and group D consisted of three telocentric pairs of chromosomes with arm ratio ranging from 5.44 to 10.48, relative lengths of 3.47% to 4.97% and centromeric indices of 7.03 to 12.5.

Fig. (1): *Photomicrographs showing the cell spread (a) and karyotype (b) of Cleopatra snail.*



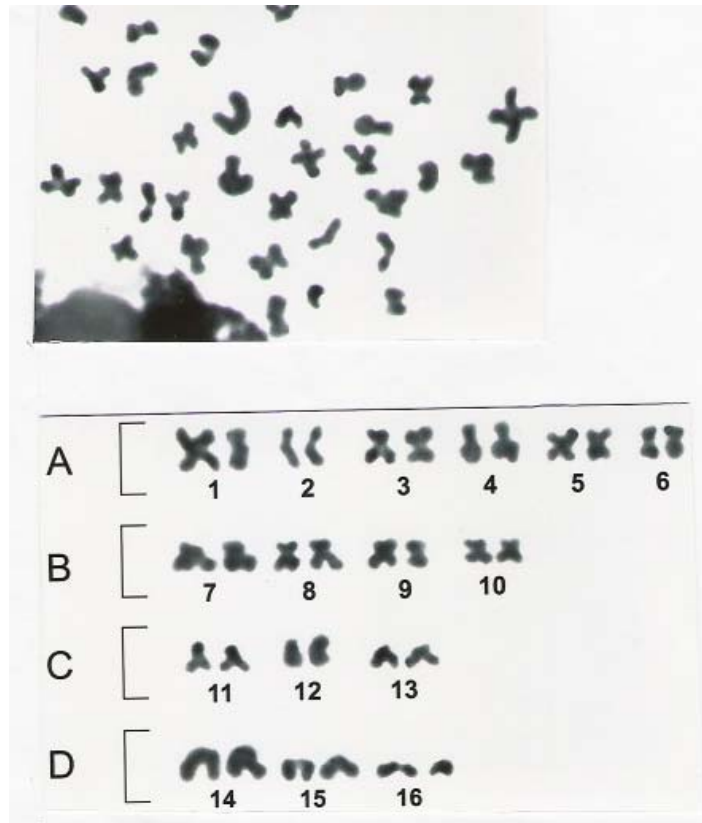
The absolute lengths of chromosomes of *Cleopatra* snails range from 3.69 to 7.49 μm and the relative lengths ranged from 4.93% to

9.79% (Table 1). In *Bithynia* snails, the absolute lengths of chromosomes ranged from 4.18 to 10.31 μm , while the relative lengths

ranged from 3.74% to 9.33% (Table 2). Comparison of the karyotypes of the two snail populations showed the existence of not only numerical differences but also differences in the relative lengths of their chromosomes and

their type. Idiograms (Figs. 3 a,b) are constructed from the total lengths of chromosomes, with the centromeres drawn at the same level to make the visual comparison easier.

Fig. (2): Photomicrographs showing the cell spread (a) and karyotype (b) of *Bithynia* snail.



Figs. 4 and 5 show the polyploidy in *Cleopatra* and *Bithynia* snails, respectively. There were insignificant incidences of polyploid nuclei. Fig. 6 and 7 show different stages of meiotic division in *Cleopatra* and *Bithynia* respectively. The first meiotic division begins with a long prophase (Figs. 6, 7 a-e), which is subdivided into four stages, leptotene, zygotene, pachytene, and diplotene. During the leptotene, the chromatin of the chromatids was stretched out very thinly, and it was not possible to identify individual

chromosomes i.e. the chromosomes were entangled, slender thread-like structures with densely stained granules (Figs. 6b and 7b). At the zygotene, the chromosomes were much shorter, more contracted and quite clearly visible (Figs. 6c and 7c). At the pachytene stage, chromosomes were more condensed and showed the haploid number of bivalent and still arranged in bouquet (Figs. 6d and 7d). During the diplotene, the chromosomes were much shorter, more contracted and quite clearly visible (Figs. 6e and 7e).

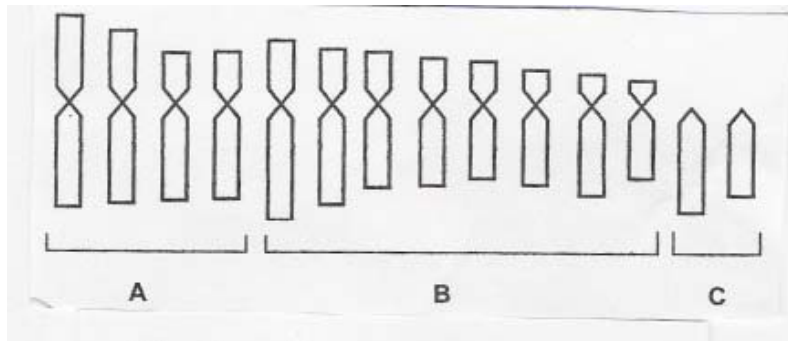


Fig. (3a): Idiogram of Cleopatra snail.

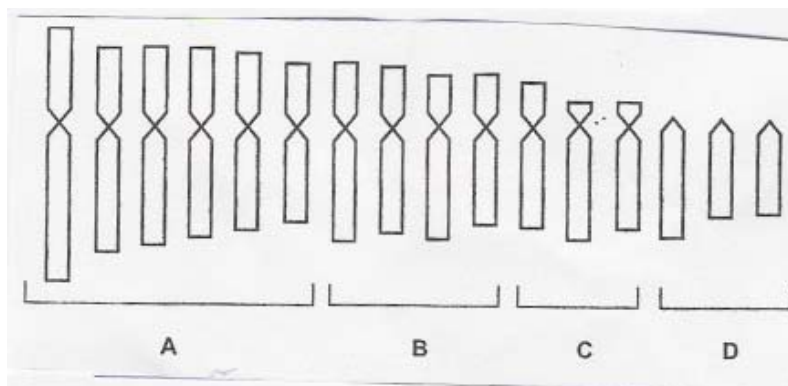


Fig. (3b): Idiogram of Bithynia snail.

Fig. (4): Photomicrographs showing the polyploidy in Cleopatra.

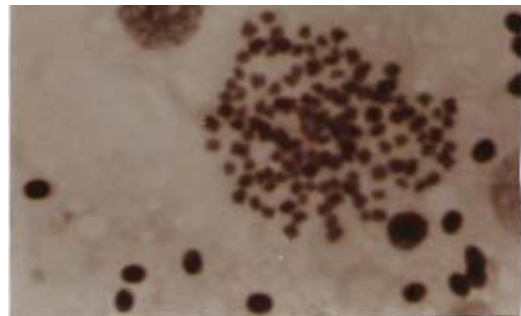


Fig. (5): Photomicrographs showing the polyploidy in Bithynia.



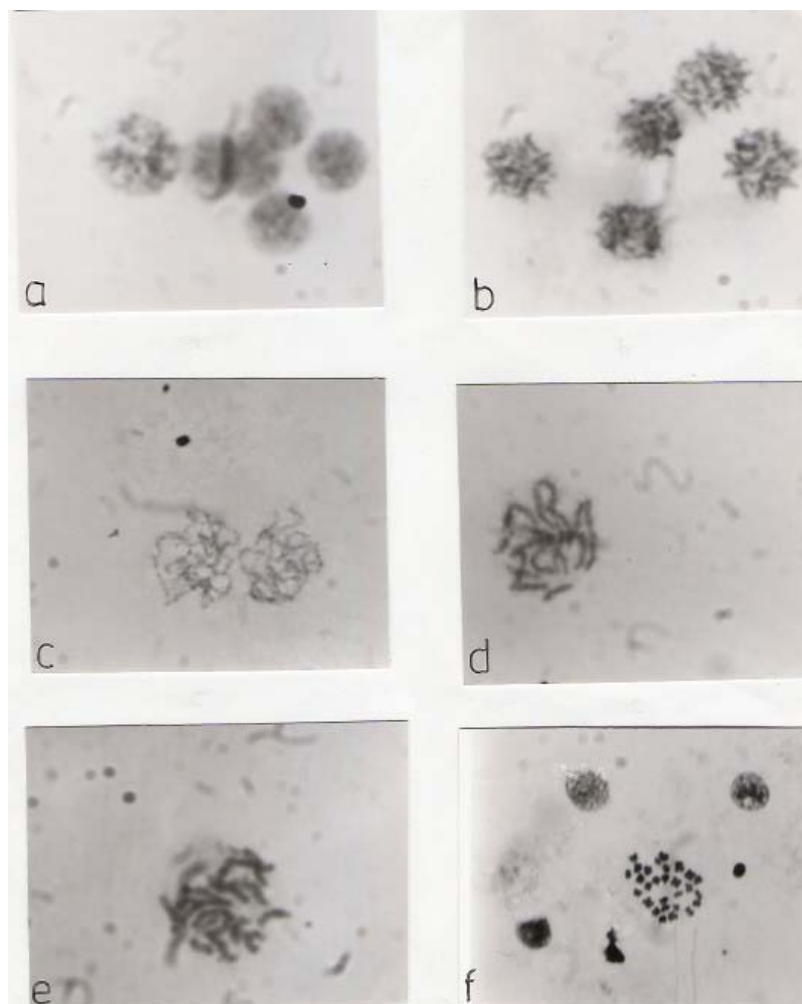


Fig. (6): Giemsa - stained chromosomes from gonads of *Cleopatra bulimoides*. (a) A group of cell in the early prophase stage. (b) A group of in the early leptotene stage. (c) Zygotene stage. (d) Pachytene stage. (e) diplotene stage .

DISCUSSION

The karyotypes of the freshwater *Cleopatra* and *Bithynia* snails indicated that the diploid chromosome number of *C. bulimoides* is $2n = 28$ while that of *Bithynia* spp is $2n = 32$.

These results are in partial agreement with those of Patterson and Burch (1978) who reported that the haploid chromosome numbers of order

Mesogastropoda ranged from $n = 7$ to $n = 18$, with the exception of species of families Thiaridae and Cypraeidae. These families have higher haploid numbers ranging from $n = 20$ to $n = 60$, which is attributed to the presence of polyploidy phenomena. Park (1994), also, reported that the haploid number of *Parafossarulus manchouricus* (Family Bithyniidae) is $n = 17$.

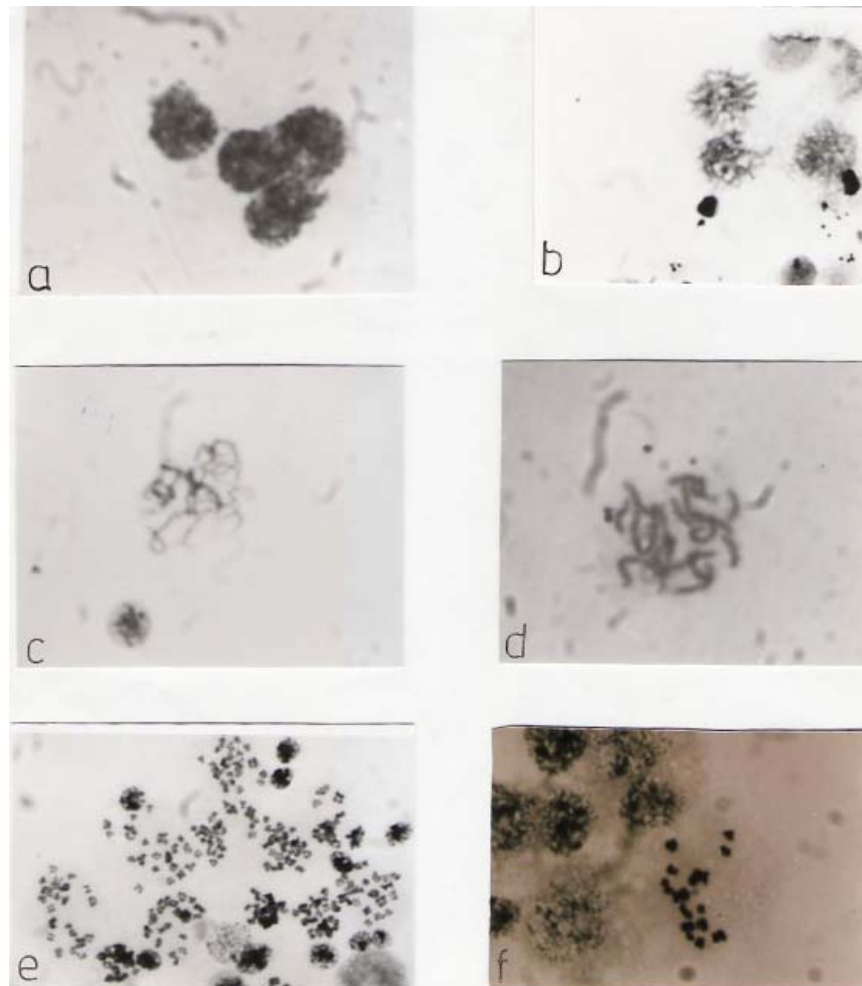


Fig. (7): Giemsa - stained chromosomes from gonads of *Bithynia Sp.* (a) Agroup of cell in the early prophase stage. (b) Agroup of in the early leptotene stage. (c) Zygotene stage. (d) Pachytene stage. (e) Agroup of in the early metaphase. (f) Metaphase stage.

Yaseen (1994) has, also, reported a diploid chromosome number of $2n = 28$, in *Cleopatra* as well as different levels of polyploidy for certain phenotypes. While, no reports are available on the number of chromosomes of *Bithynia* snails and the present study is the first report on this species in Egypt. A wide range of chromosome numbers (14 to 36) has been reported in other prosobranch species (Nakamura and Ojima 1990). The difference in the chromosome

numbers has occurred without large genome alterations or Robertsonian translocations (Nakamura and Ojima, 1990). According to these authors, the tandem fusion of smaller chromosomes produced large elements in karyotypes of the genus *Semisulcospira* (Mesogastropoda), Pleuroceridae (Melaniidae) relative to Thiariidae. Comparison between the two snail species under investigation revealed some significant differences. Polyploidy was observed in the two snail populations in the

digestive gland. Similarly, Dasilva and Brown (1982) found polyploidy in the gill and digestive gland of *Bullia* snails. The chromosome numbers of the two snail species are similar in their ranges with those of mesogastropod snails to which they belong.

In the present study, diakinesis stage could not be observed. The stages of diakinesis and bivalent formation were seldom observed by Yassen (1995), in his study on the chromosomes of the Egyptian freshwater snail, *Melanoides tuberculata*.

The literatures on karyotype analysis of freshwater snails are not abundant, due to difficulties in obtaining mitotic fields with enough quality to carry out chromosome studies (Park *et al*, 1999).

In conclusion, cytogenetic studies contribute useful information supplementary to the morphological, biochemical and other characters used for systematic analysis of freshwater snails.

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الملخص العربي

دراسات كروموسومية على اثنين من قواقع المياه العذبة المصرية ، كليوباترا وبيثينيا (أمامية الخياشيم - الرخويات) .

. 32 = 2

. 28 = 2