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KARYOTYPE ANALYSES OF SIX SPECIES OF NORTH AMERICAN FRESHWATER MUSSELS (BIVALVIA, UNIONIDAE)

Gab-Man Park and J. B. Burch

ABSTRACT

The mitotic chromosomes from gonadal tissue of six species of freshwater bivalves of the family Unionidae were analyzed using air-drying techniques. The diploid chromosome number of all six species is 38. The karyotypes of the six species are as follows: *Elliptio complanata* – five pairs with median centromeres (*m*), 11 pairs with submedian centromeres (*sm*), two pairs with subterminal centromeres (*st*), and one pair with terminal centromeres (*t*); *Anodonta grandis* – five *m*, 12 *sm* and two *st*; *Anodonta "grandis"* – three *m*, eight *sm* and eight *st*; *Anodontoides ferussacianus* – two *m*, 15 *sm* and two *st*; *Epioblasma triquetra* – four *m*, 13 *sm* and two *st* in males, and apparently five *m*, 12 *sm* and two *st* in females; *Ptychobranchus fasciolaris* – three *m*, 15 *sm* and one *st*.

Key words: *Elliptio complanata, Anodonta grandis, Anodontoides ferussacianus, Epioblasma triquetra, Ptychobranchus fasciolaris*, chromosome numbers, karyotypes, freshwater Unionidae, North America.

INTRODUCTION

Cytotaxonomic studies can contribute important information supplementary to the morphological, biochemical and other characters used for systematic analyses. Chromosomal studies on marine bivalves have been noticeably useful in systematics (*e.g.*, Menzel & Menzel, 1965; Longwell *et al.*, 1967; Menzel, 1968; Ahmed & Sparks, 1970; Nadamitsu & Shinkawa, 1973; Ieyama & Inaba, 1974: Ieyama, 1975, 1977, 1990, 1992; Dixon & Flavell, 1986; Pasantes *et al.*, 1990; Insua *et al.*, 1994), but until rather recently there have been relatively few studies of the chromosomes of freshwater bivalves. Keyl (1956) stated that high chromosome numbers and relatively small nuclei made a satisfactory cytotaxonomic analysis of the Unionidae impossible.

Nakamura (1985) reviewed chromosome numbers and karyological data for 125 species in 22 of the Recent bivalve families. Chromosomal information known until now about the Unionoidean clams, refer to 29 species (Table 1). Lillie (1901) reported n = 16 for *Unio complanata* based on paraffin embedding and microtome sectioning techniques, which does not give an accurate analysis (see Burch, 1960). Griethuysen *et al.* (1969) reported 2n = 38 of *Anodonta anatina* and *Unio pictorum*. Jenkinson (1976, 1984) published chromosome numbers of 15 species of North American naiads. Karyological investigations in the freshwater Bivalvia have been previously carried out on one species of Margaritiferidae and nine Unionidae (Nadamitsu & Kanai, 1975, 1978; Park *et al.*, 1988; Park & Kwon, 1991).

The present report gives the chromosome numbers and compares the karyotypes of six species of North American Unionidae.

MATERIALS AND METHODS

The specimens used in this study, all members of the family Unionidae, were collected in Michigan and Colorado, U.S.A., in 1993 and 1994. The species are listed below.

Elliptio complanata (Lightfoot 1786), Ocqueoc River, Presque Isle County, Michigan.
Anodonta grandis Say 1829, Dexter, Four Mile Lake, Washtenaw County, Michigan; Douglas Lake, Cheboygan County, Michigan; Burt Lake, Cheboygan County, Michigan.
Anodonta "grandis," Colorado, supplied by Dr. Hsiu-Ping Liu, University of Colorado.

TABLE 1. Chromosome numbers reported previously in unionoidean bivalves.

Species	Method	2n	Karyotype	Country	Reference
Margaritiferidae					
Margaritifera leavis	[AP[1]]	38	19 *sm*[1]	Japan	Nadamitsu & Kanai (1975)
Margaritifera margaritifera	[AF]	38		U.S.A.	Jenkinson (1976)
Unionidae					
Ambleminae					
Lamprotula gottschei	[AF]	38	7 *m* + 12 *sm*	Korea	Park & Kwon (1991)
Pseudodon obovalis omiensis	[SQ]	38	9 *m* + 10 *sm*	Japan	Nadamitsu & Kanai (1978)
Unioninae					
Elliptio ["*Unio*"] *complanata*	[SE]	[32] n= 16		USA	Lillie (1901)
Gonidea angulata	[AF]	38		USA	Jenkinson (1976)
Inversidens japonensis	[SQ]	38	6 *m* + 13 *sm*	Japan	Nadamitsu & Kanai (1978)
Lasmigona costata	[AF]	38		USA	Jenkinson (1976)
Lanceolaria acrorhyncha	[AF]	38	5 *m* + 14 *sm*	Korea	Park & Kwon (1991)
Quadrula quadrula	[AF]	38		USA	Jenkinson (1976)
Tritogonia verrucosa	[AF]	38		USA	Jenkinson (1976)
Unio pictorum	[SQ]	38		Netherl.	Griethuysen *et al.* (1969)
Unio elongatus	[SQ]	[38] n = 19		Italy	Vitturi *et al.* (1982)
Unio douglasiae	[AF]	38	6 *m* + 13 *sm*	Korea	Park & Kwon (1991)
Unio douglasiae sinuolatus	[AF]	38	4 *m* + 15 *sm*	Korea	Park & Kwon (1991)
Sorenia triangularis	[AF]	38	5 *m* + 13 *sm* + 1 *t*	Korea	Park & Kwon (1991)
Anodontinae					
Alasmidonta arcula	[AF]	38		USA	Jenkinson (1976)
Alasmidonta marginata	[AF]	38		USA	Jenkinson (1976)
Anodonta anatina	[SQ]	38		Netherl.	Griethuysen *et al.*, (1969)
Anodonta grandis	[AF]	38		USA	Jenkinson (1976)
Anodonta woodiana	[AF]	38	7 *m* + 12 *sm*	Korea	Park *et al.*, (1988)
Anodonta arcaeformis flavotincta	[AF]	38	7 *m* + 12 *sm*	Korea	Park & Kwon (1991)
Anodonta woodiana	[AF]	38	7 *m* + 12 *sm*	Korea	Park & Kwon (1991)
Anodontoides ferussacianus	[AF]	38		USA	Jenkinson (1976)
Lampsilinae					
Lampsilis radiata luteola	[AF]	38		USA	Jenkinson (1976)
Potamilus alatus	[AF]	38		USA	Jenkinson (1976)
Ptychobranchus fasciolaris	[AF]	38		USA	Jenkinson (1976)
Toxolasma lividus glans	[AF]	38		USA	Jenkinson (1976)
Villosa iris	[AF]	38		USA	Jenkinson (1976)
Villosa lienosa	[AF]	38		USA	Jenkinson (1976)

[1]Abbreviations: AF = air-dried or flame-dried; SE = sectioned; SQ = squash preparation; *m* = medianly constricted chromosome; *sm* = submedianly constricted; *st* = subterminally constricted; *t* = centromere in the terminal region of the chromosome.

Anodontoides ferussacianus (Lea 1834), Maple Bay, Burt Lake, Cheboygan County, Michigan.
Epioblasma triquetra (Rafinesque 1820), Clinton River at Elizabeth Lake Road Bridge, Oakland County, Michigan.
Ptychobranchus fasciolaris (Rafinesque 1820), Clinton River at Elizabeth Lake Road Bridge, Oakland County, Michigan.

Mitotic chromosome spreads of gonadal tissues were obtained by a modification of the standard air-dry technique. The prepared slides were observed under a Nippon Kokaku microscope with a 100x oil immersion objective and a 10x ocular.

The nomenclature of centromeric position for the classification of chromosomes as proposed by Levan *et al.* (1964) has been followed in our study.

Voucher specimens of the species used in this study have been placed in the Museum of Zoology, the University of Michigan, Ann Arbor.

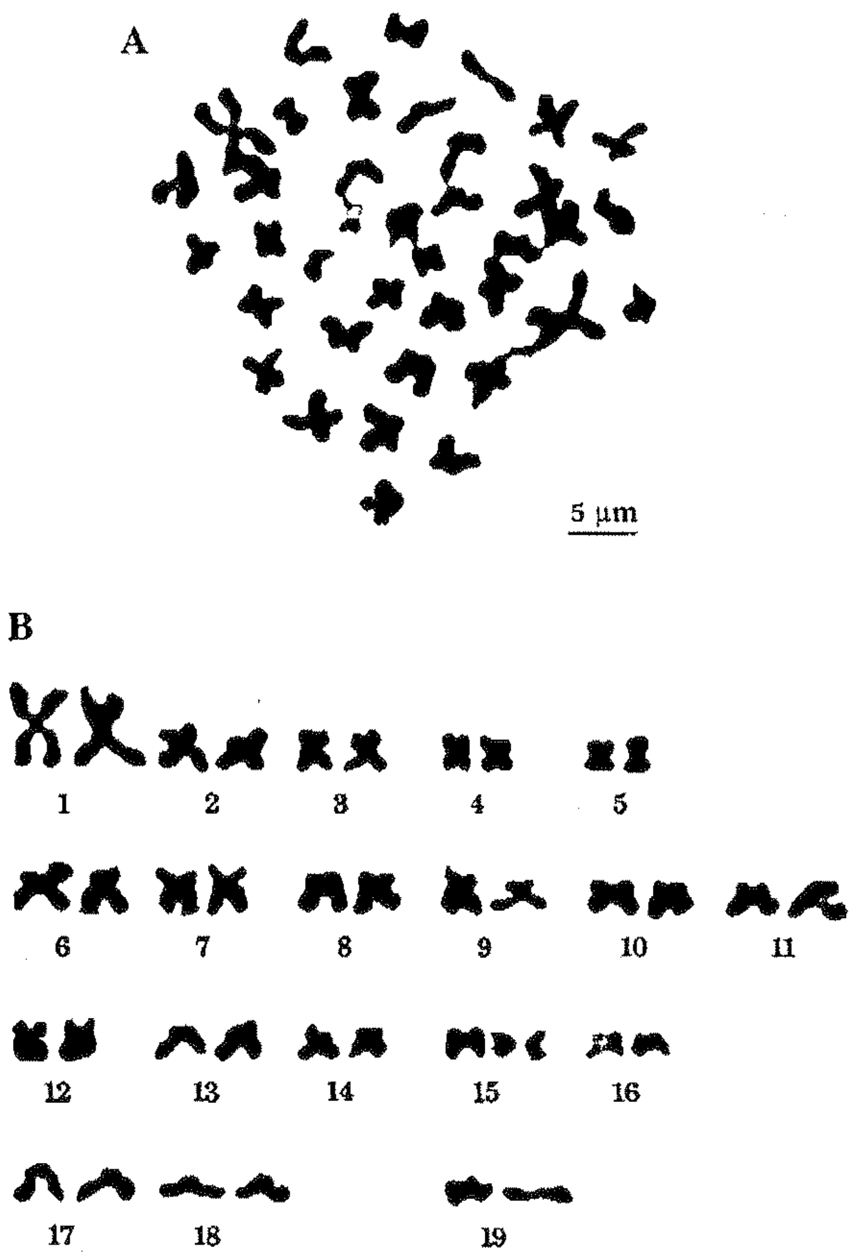

FIG. 1. *Elliptio complanata*, female. A, Metaphase chromosomes of a gonadal cell; B, arrangement of the chromosomes in A into sets with medianly (*m*), submedianly (*sm*) and subterminally (*st*) placed centromeres, and then arranged in each set in order of decreasing size.

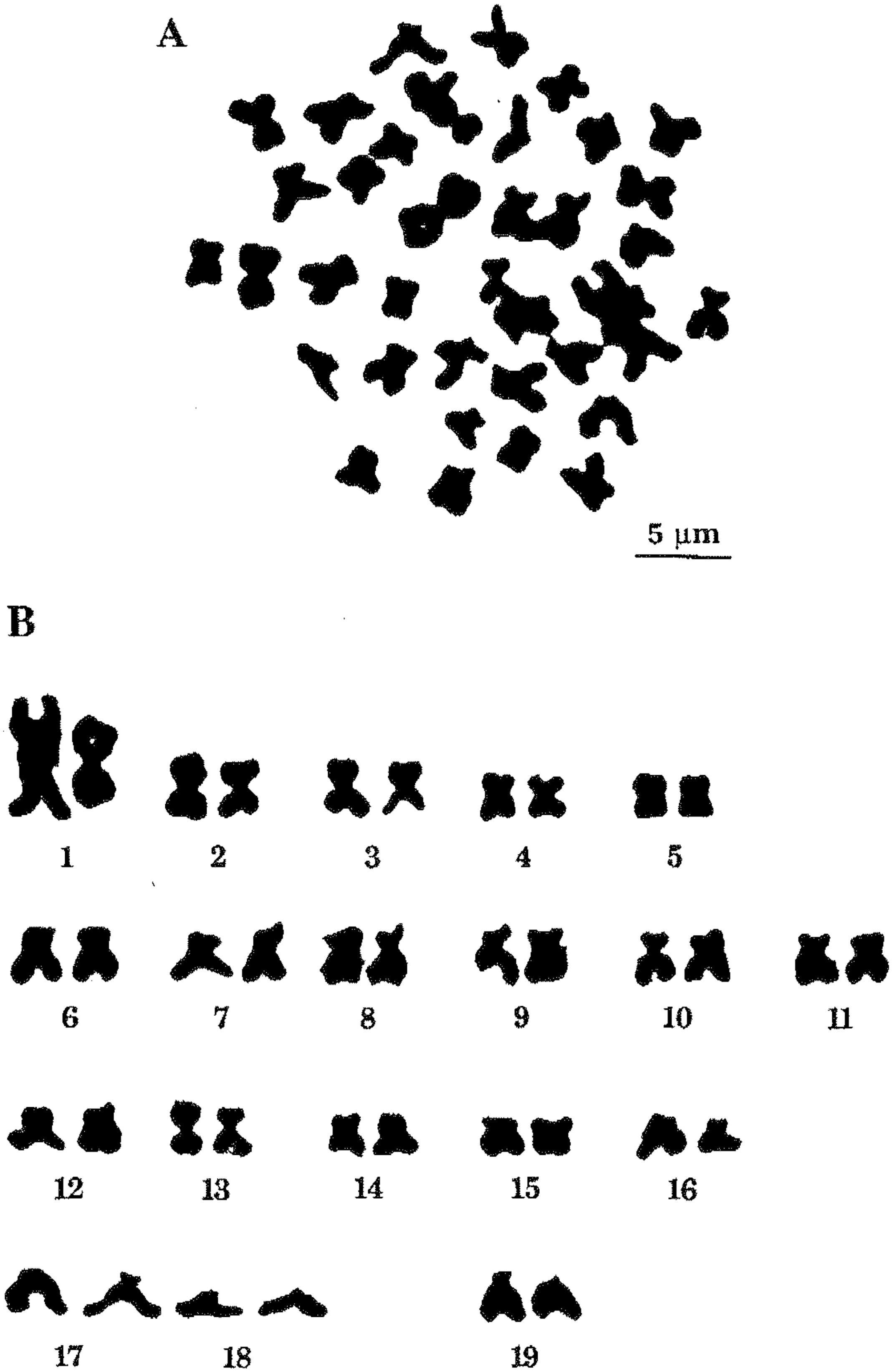

FIG. 2. *Elliptio complanata*, male. A, Metaphase chromosomes of a gonadal cell; B, arrangement of the chromosomes in A into karyotypic sets. [There is an overlapping of one of the chromosomes in pair 1 by a chromosome of pair 9.]

TABLE 2. Total mean lengths (µm) and relative mean lengths (%) of mitotic metaphase chromosome pairs in the male and female *Elliptio complanata*.

Chromosome pair	Male[1]		Female[1]		
	Total length	Relative length	Total length	Relative length	Type
1	5.88 ± 0.15	10.69 ± 0.11	5.81 ± 0.21	10.01 ± 0.29	*m*
2	3.46 ± 0.26	6.29 ± 0.26	3.61 ± 0.34	6.28 ± 0.26	*m*
3	3.08 ± 0.30	5.60 ± 0.37	3.49 ± 0.18	6.07 ± 0.17	*m*
4	2.69 ± 0.29	4.89 ± 0.26	2.97 ± 0.43	5.17 ± 0.39	*m*
5	2.31 ± 0.08	4.20 ± 0.15	2.66 ± 0.36	4.63 ± 0.86	*m*
6	3.46 ± 0.44	6.29 ± 0.31	3.61 ± 0.51	6.28 ± 0.57	*sm*
7	3.27 ± 0.23	5.95 ± 0.17	3.43 ± 0.12	5.97 ± 0.65	*sm*
8	3.10 ± 0.47	5.64 ± 0.41	3.20 ± 0.10	5.57 ± 0.30	*sm*
9	2.72 ± 0.15	4.95 ± 0.35	3.11 ± 0.23	5.41 ± 0.08	*sm*
10	2.70 ± 0.23	4.91 ± 0.28	2.80 ± 0.07	4.87 ± 0.27	*sm*
11	2.69 ± 0.19	4.89 ± 0.25	2.80 ± 0.28	4.87 ± 0.52	*sm*
12	2.55 ± 0.20	4.64 ± 0.05	2.71 ± 0.45	4.71 ± 0.43	*sm*
13	2.41 ± 0.28	4.38 ± 0.34	2.48 ± 0.22	4.31 ± 0.30	*sm*
14	2.35 ± 0.14	4.27 ± 0.17	2.39 ± 0.08	4.16 ± 0.03	*sm*
15	2.19 ± 0.11	3.98 ± 0.32	2.28 ± 0.61	3.97 ± 0.21	*sm*
16	2.10 ± 0.23	3.82 ± 0.30	1.98 ± 0.05	3.44 ± 0.34	*sm*
17	2.81 ± 0.55	5.11 ± 0.09	3.11 ± 0.44	5.41 ± 0.86	*st*
18	2.58 ± 0.12	4.69 ± 0.26	2.62 ± 0.28	4.56 ± 0.52	*st*
19	2.63 ± 0.08	4.78 ± 0.50	2.44 ± 0.21	4.24 ± 0.34	*t*

[1]Based on measurements from three sets in female and two sets in male of karyotyped cells.

TABLE 3. Total mean lengths (µm) and relative mean lengths (%) of mitotic metaphase chromosome pairs from three localities of *Anodonta grandis* from three localities.[1]

Chromosome pair	Burt Lake*		Douglas Lake**		Dexter***		Type
	TL[2]	RL[2]	TL	RL	TL	RL	
1	4.92 ± 0.52	8.27 ± 0.30	5.56 ± 0.33	8.78 ± 0.66	5.25 ± 0.19	8.42 ± 0.66	*m*
2	3.86 ± 0.27	6.49 ± 0.06	5.00 ± 0.21	7.89 ± 0.39	4.41 ± 0.38	7.07 ± 0.24	*m*
3	3.30 ± 0.07	5.55 ± 0.11	3.33 ± 0.24	5.26 ± 0.13	3.94 ± 0.32	6.32 ± 0.26	*m*
4	2.80 ± 0.35	4.71 ± 0.25	2.78 ± 0.32	4.39 ± 0.28	3.20 ± 0.21	5.13 ± 0.14	*m*
5	2.72 ± 0.13	4.57 ± 0.34	2.78 ± 0.28	4.39 ± 0.11	2.61 ± 0.09	4.19 ± 0.37	*m*
6	4.15 ± 0.33	6.99 ± 0.19	5.00 ± 0.11	7.89 ± 0.09	4.17 ± 0.19	6.69 ± 0.33	*sm*
7	3.86 ± 0.24	6.49 ± 0.46	3.89 ± 0.15	6.14 ± 0.14	3.58 ± 0.20	5.74 ± 0.52	*sm*
8	3.30 ± 0.16	5.55 ± 0.52	3.61 ± 0.21	5.70 ± 0.40	3.34 ± 0.33	5.36 ± 0.43	*sm*
9	3.24 ± 0.01	5.45 ± 0.12	3.33 ± 0.30	5.26 ± 0.37	3.30 ± 0.18	5.29 ± 0.37	*sm*
10	2.96 ± 0.20	4.98 ± 0.29	3.33 ± 0.30	5.26 ± 0.51	3.07 ± 0.10	4.92 ± 0.30	*sm*
11	2.79 ± 0.12	4.69 ± 0.14	3.06 ± 0.14	4.83 ± 0.07	2.91 ± 0.11	4.67 ± 0.41	*sm*
12	2.76 ± 0.35	4.64 ± 0.09	3.06 ± 0.20	4.83 ± 0.14	2.89 ± 0.08	4.63 ± 0.10	*sm*
13	2.76 ± 0.23	4.64 ± 0.20	2.78 ± 0.37	4.39 ± 0.10	2.85 ± 0.16	4.57 ± 0.23	*sm*
14	2.65 ± 0.04	4.46 ± 0.28	2.50 ± 0.09	3.95 ± 0.52	2.85 ± 0.15	4.57 ± 0.37	*sm*
15	2.61 ± 0.26	4.39 ± 0.44	2.22 ± 0.22	3.50 ± 0.22	2.82 ± 0.07	4.52 ± 0.21	*sm*
16	2.61 ± 0.08	4.39 ± 0.38	2.22 ± 0.15	3.50 ± 0.16	2.62 ± 0.10	4.20 ± 0.11	*sm*
17	2.43 ± 0.14	4.09 ± 0.09	2.22 ± 0.15	3.50 ± 0.24	2.38 ± 0.19	3.82 ± 0.07	*sm*
18	2.96 ± 0.21	4.98 ± 0.25	3.89 ± 0.22	6.14 ± 0.45	3.32 ± 0.20	5.32 ± 0.25	*st*
19	2.79 ± 0.26	4.69 ± 0.10	2.78 ± 0.21	4.39 ± 0.50	2.85 ± 0.34	4.57 ± 0.32	*st*

[1]Based on measurements from two sets *, four sets ** and three sets *** of karyotyped cells.
[2]TL = Total length; RL = relative length.

RESULTS

Elliptio complanata (Lightfoot 1786)

In five female and three male specimens studied, the chromosome number 2n = 38 was observed. The karyotype was the same in both sexes. It consisted of five pairs of medianly

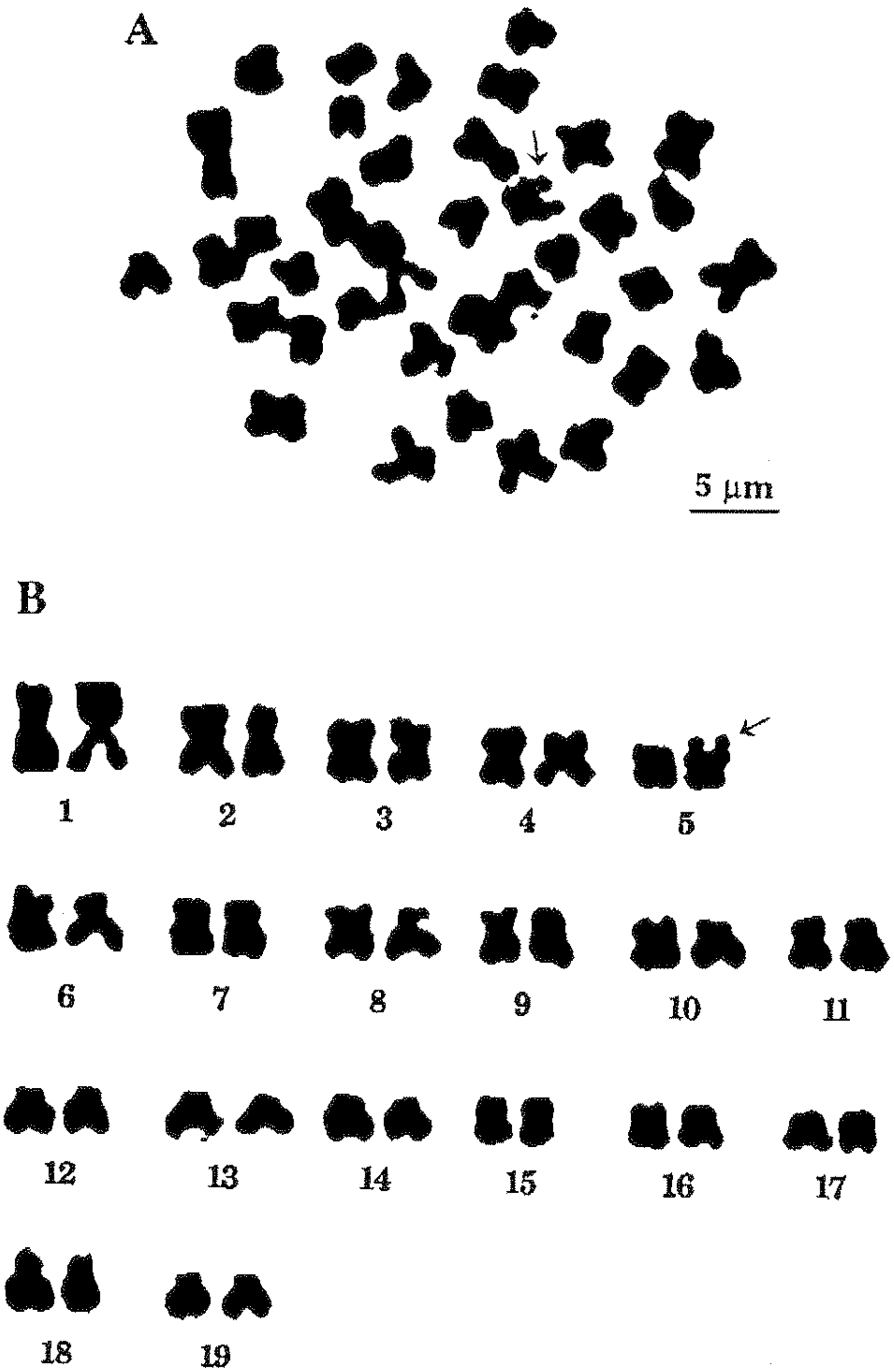

FIG. 3. *Anodonta grandis*, female, Burt Lake. A, Metaphase chromosomes of a gonadal cell; B, arrangement of the chromosomes in A into karyotypic sets.

constricted chromosomes (*m*), 11 pairs of submedianly constricted chromosomes (*sm*), two pairs with subterminally placed centromeres (*st*), and one pair with terminal centromeres (*t*) (Figs. 1, 2). The largest pair of chromosomes in both sexes were noticeably larger than the chromosome next in size in decreasing length. The nombre fondamental (NF) [fundamental number] was 72. Table 2 gives measurements of the oogonial metaphase chromosomes. The mean total

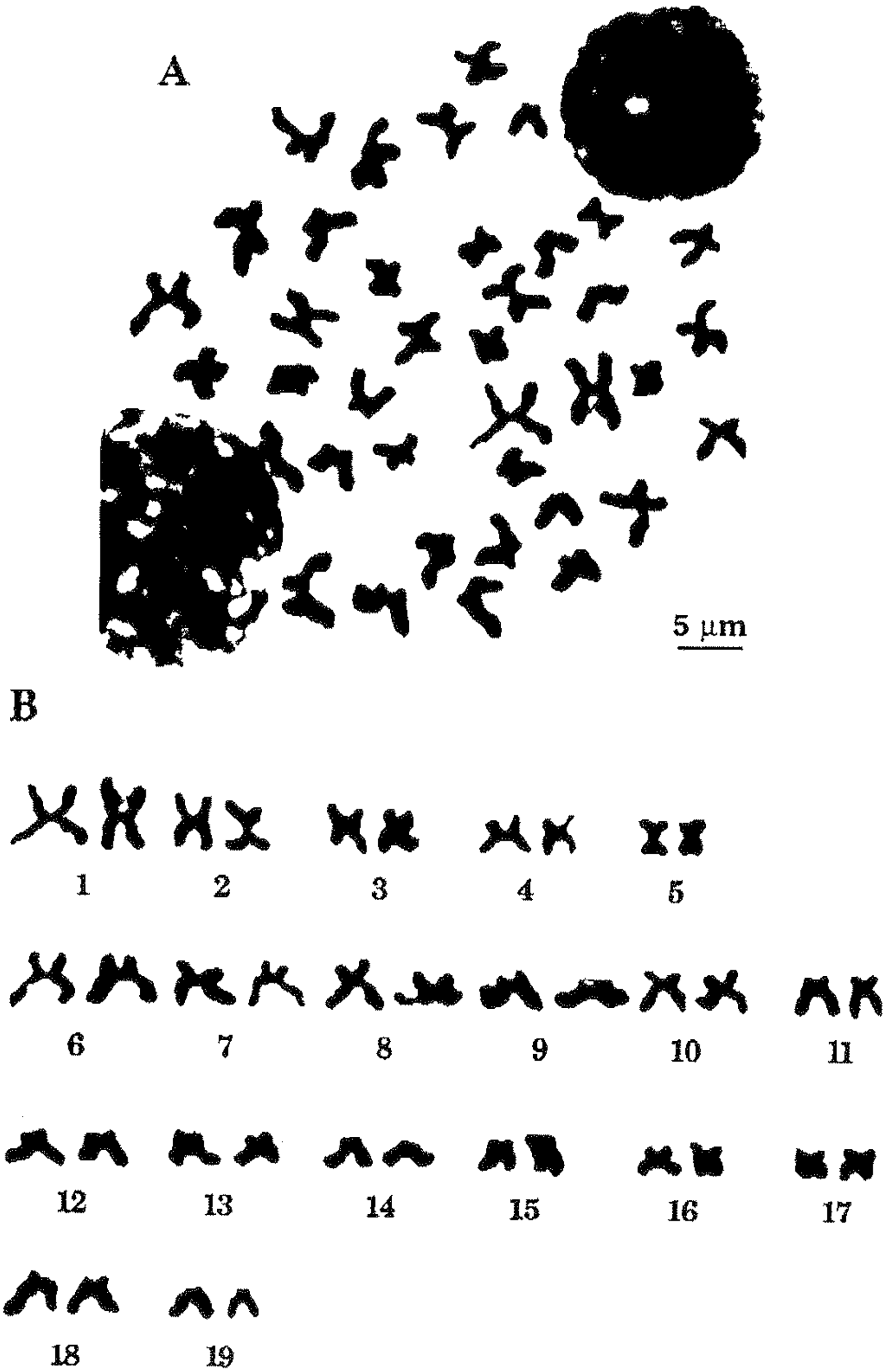

FIG. 4. *Anodonta grandis*, female, Douglas Lake. A, Metaphase chromosomes of a gonadal cell; B, arrangement of the chromosomes in A into karyotypic sets.

length of mitotic metaphase chromosomes in the females and males was 57.5 ± 5.17 and 54.98 ± 4.50 µm, respectively.

Anodonta grandis Say 1829

Five females from each collection site were examined. The diploid chromosome number was 38 in each cell studied was 38. The karyotype consisted of five *m* pairs, 12 *sm* pairs and

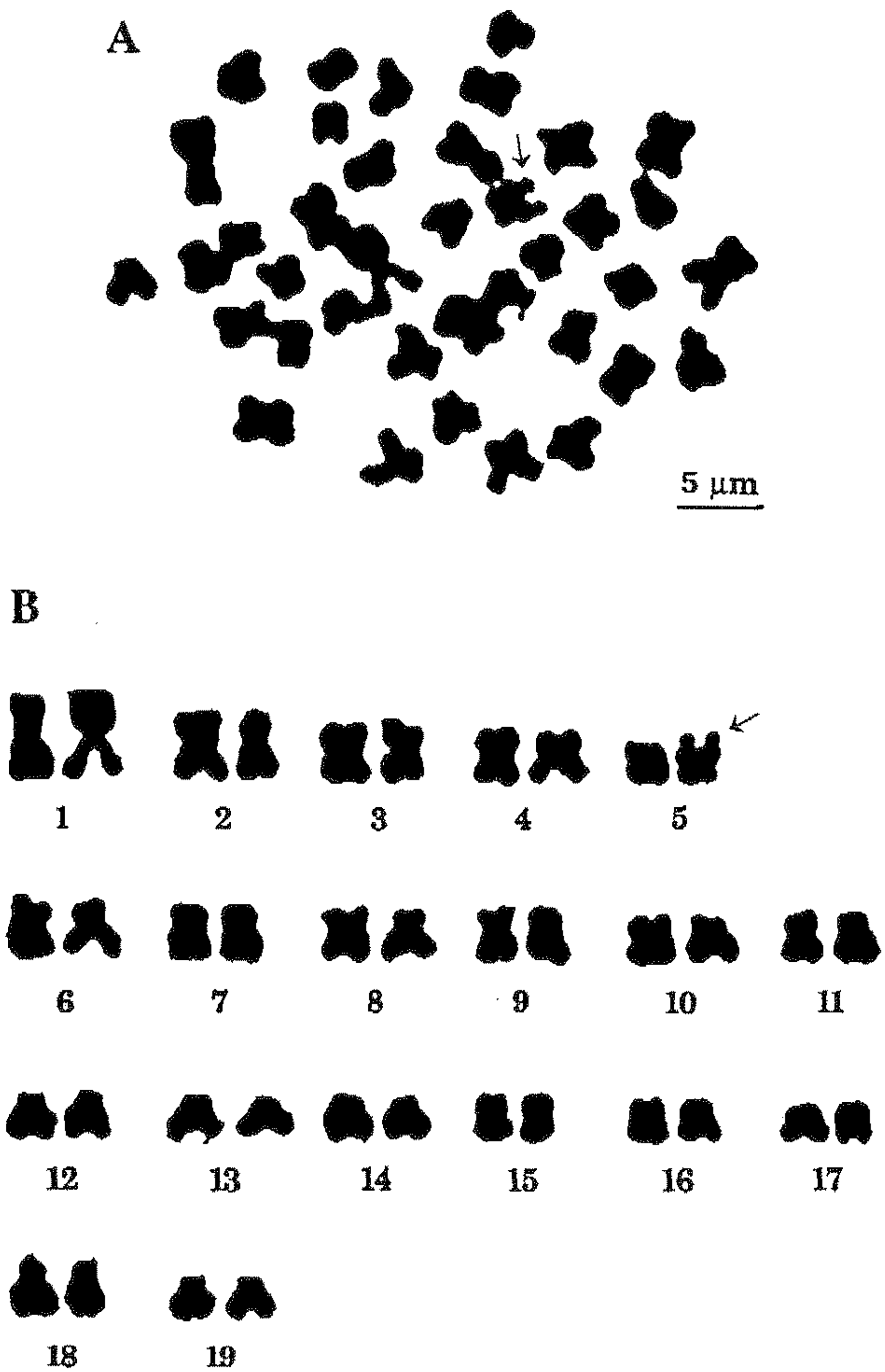

FIG. 5. *Anodonta grandis*, female, Dexter. A, Metaphase chromosomes of a gonadal cell; B, arrangement of the chromosomes in A into karyotypic sets. Arrow indicates secondary constriction.

TABLE 4. Total mean lengths (μm) and relative mean lengths (%) of mitotic metaphase chromosome pairs of Anodonta sp. (*"grandis,"* Colorado).[1]

Chromosome pair	Total length	Relative length	Type
1	5.01 ± 0.49	7.62 ± 0.13	*m*
2	3.70 ± 0.66	5.62 ± 0.39	*m*
3	2.73 ± 0.67	4.15 ± 0.56	*m*
4	4.34 ± 0.33	6.60 ± 0.22	*sm*
5	4.06 ± 0.31	6.17 ± 0.31	*sm*
6	3.70 ± 0.29	5.62 ± 0.06	*sm*
7	3.69 ± 0.27	5.61 ± 0.13	*sm*
8	3.53 ± 0.14	5.37 ± 0.06	*sm*
9	3.30 ± 0.25	5.02 ± 0.27	*sm*
10	3.26 ± 0.20	5.00 ± 0.35	*sm*
11	3.01 ± 0.36	4.58 ± 0.16	*sm*
12	4.33 ± 0.41	6.58 ± 0.48	*st*
13	3.57 ± 0.26	5.43 ± 0.35	*st*
14	3.32 ± 0.22	5.05 ± 0.24	*st*
15	3.01 ± 0.34	4.58 ± 0.16	*st*
16	3.00 ± 0.28	4.56 ± 0.35	*st*
17	2.84 ± 0.20	4.32 ± 0.54	*st*
18	2.70 ± 0.16	4.10 ± 0.17	*st*
19	2.59 ± 0.22	3.94 ± 0.06	*st*

[1]Based on measurements from two sets of karyotyped cells.

TABLE 5. Total mean lengths (μm) and relative mean lengths (%) of mitotic metaphase chromosome pairs in female *Anodontoides ferussacianus*.[1]

Chromosome pair	Total length	Relative length	Type
1	3.00 ± 0.19	7.42 ± 0.20	*m*
2	2.83 ± 0.25	7.00 ± 0.14	*m*
3	3.33 ± 0.11	8.28 ± 0.34	*sm*
4	2.67 ± 0.34	6.61 ± 0.61	*sm*
5	2.67 ± 0.27	6.61 ± 0.72	*sm*
6	2.50 ± 0.09	6.19 ± 0.05	*sm*
7	2.33 ± 0.10	5.76 ± 0.44	*sm*
8	2.17 ± 0.26	5.37 ± 0.13	*sm*
9	2.06 ± 0.11	5.10 ± 0.25	*sm*
10	2.02 ± 0.20	5.00 ± 0.51	*sm*
11	2.00 ± 0.17	4.95 ± 0.22	*sm*
12	2.00 ± 0.14	4.95 ± 0.16	*sm*
13	1.83 ± 0.08	4.53 ± 0.61	*sm*
14	1.67 ± 0.09	4.13 ± 0.11	*sm*
15	1.67 ± 0.05	4.13 ± 0.10	*sm*
16	1.50 ± 0.06	3.71 ± 0.14	*sm*
17	1.33 ± 0.15	3.29 ± 0.45	*sm*
18	1.50 ± 0.16	3.71 ± 0.34	*st*
19	1.34 ± 0.11	3.32 ± 0.19	*st*

[1]Based on measurements from five sets of karyotyped cells.

two *st* pairs (Figs. 3, 4, 5). Table 3 shows measurements of the chromosomes. The mean total lengths of the mitotic metaphase chromosomes per cell was 59.47 ± 3.97 μm for the Burt Lake specimens, 63.34 ± 4.20 μm for the Douglas Lake specimens, and 62.36 ± 3.59 μm for the Dexter specimens. Chromosome pair 5 in the five individuals from the Dexter site had secondary

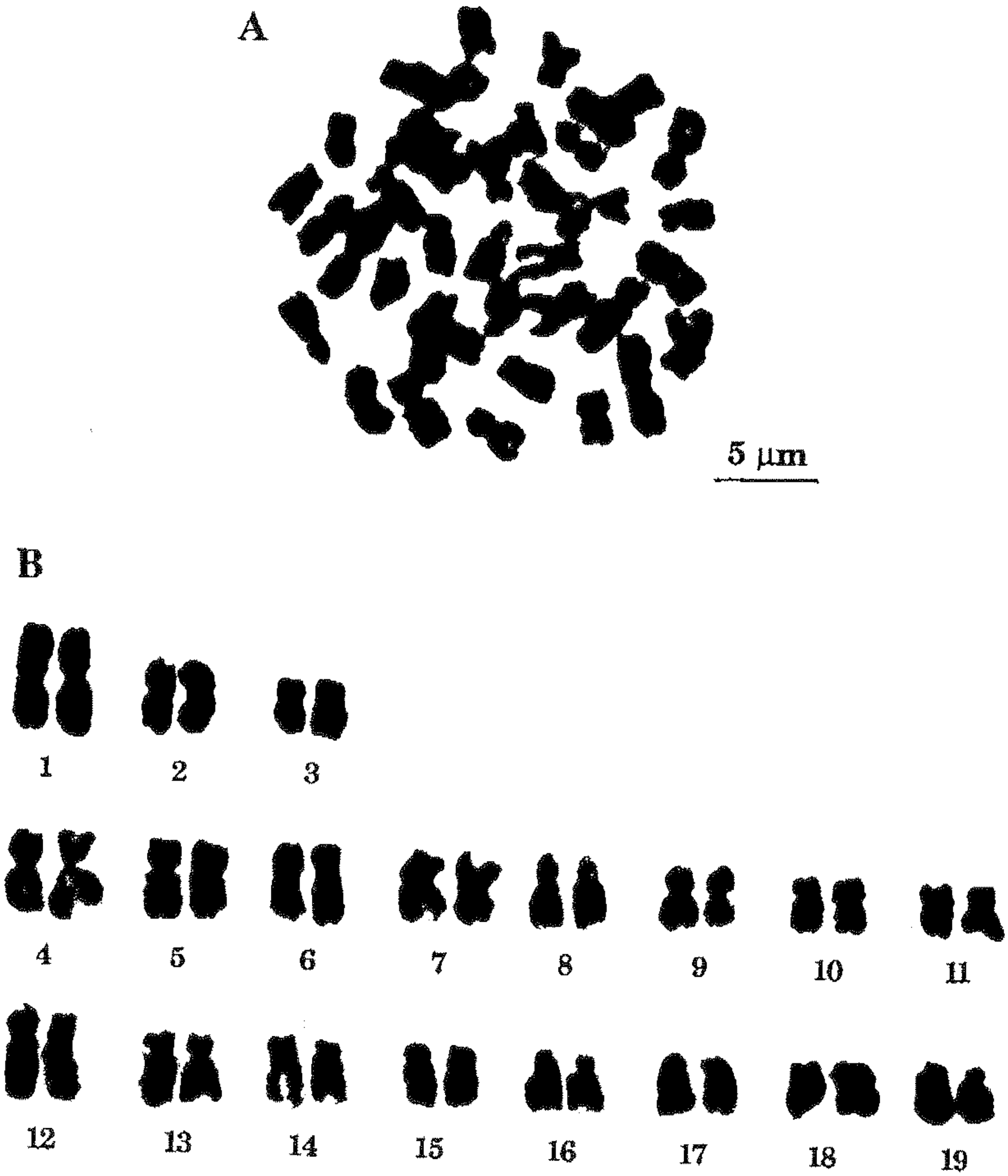

FIG. 6. *Anodonta "grandis,"* female, (Colorado). A, Metaphase chromosomes of a gonadal cell; B, arrangement of the chromosomes in A into karyotypic sets.

constrictions in the long arms.

Since all of the chromosomes had two arms, the NF was 76.

Anodonta sp. (*"grandis,"* Colorado)

Mitotic chromosomes were observed in 20 cells of four female specimens. Each cell had 38 chromosomes (2n = 38) (Fig. 6). Table 4 shows measurements of mitotic metaphase chromosomes in two sets of karyotypes cells. The karyotypes consisted of three *m* pairs of chromosomes, eight *sm* pairs and eight *st* pairs. The NF is 76. The mean lengths of the mitotic metaphase

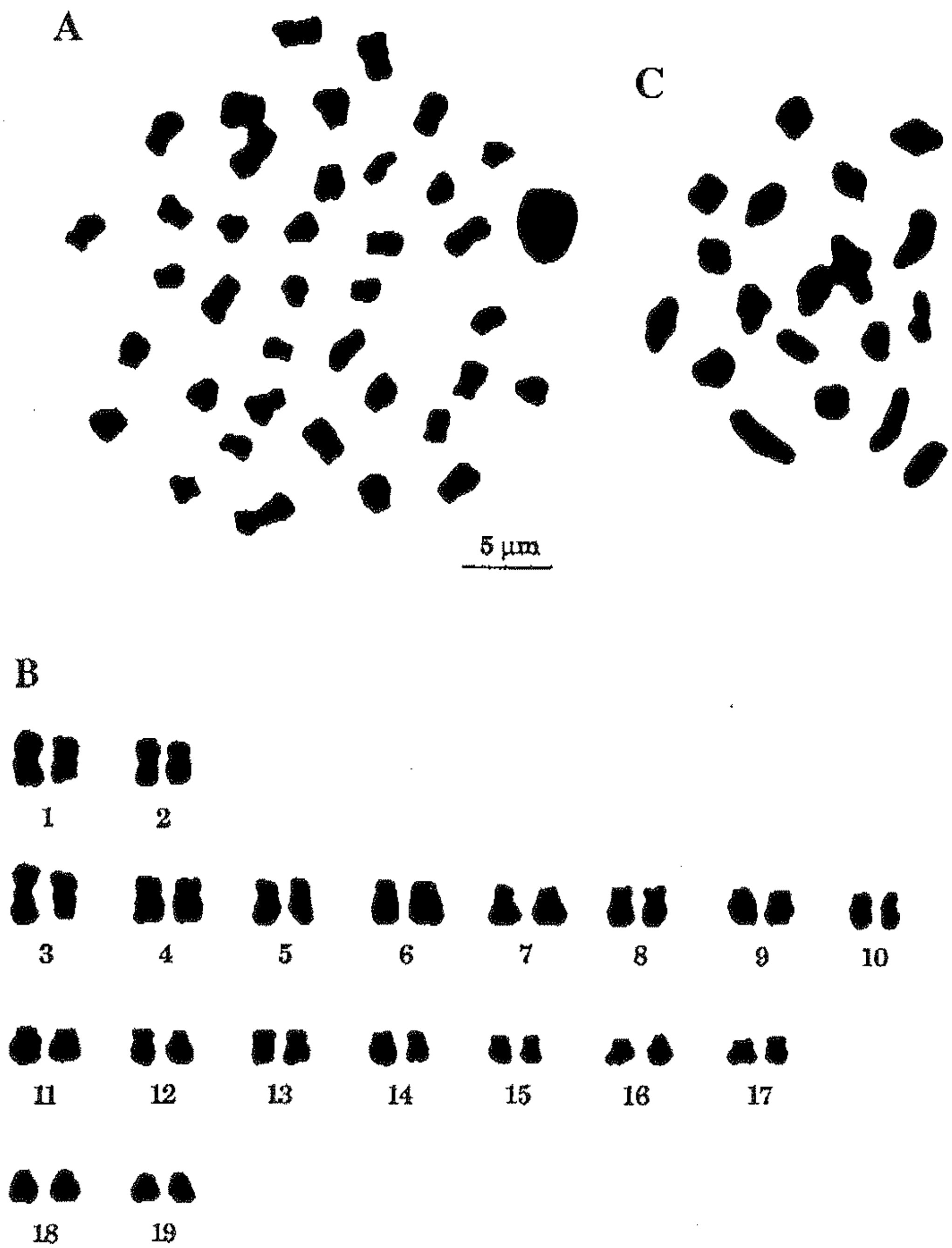

FIG. 7. *Anodontoides ferussacianus*. A, Metaphase chromosomes of a gonadal cell; B, arrangement of the chromosomes in A into karyotypic sets; C, meiotic metaphase I chromosomes.

chromosomes ranged from 5.01 ± 0.49 to 2.59 ± 0.22 μm, and the relative mean lengths of the chromosomes ranged from 7.62 ± 0.13 to 3.94 ± 0.06 μm (Table 4).

The karyotype of this Colorado population appears to be different from that of *Anodonta grandis* from Michigan.

Anodontoides ferussacianus (Lea 1834)

Mitotic and meiotic chromosomes were counted in 32 cells of five specimens. The haploid chromosome number of *Anodontoides ferussacianus* is n = 19 (Fig. 7C) and the diploid number is 38 (Fig. 7A,B). The karyotype is shown in Fig. 7C. It consists of two *m* pairs, 15 *sm* pairs and

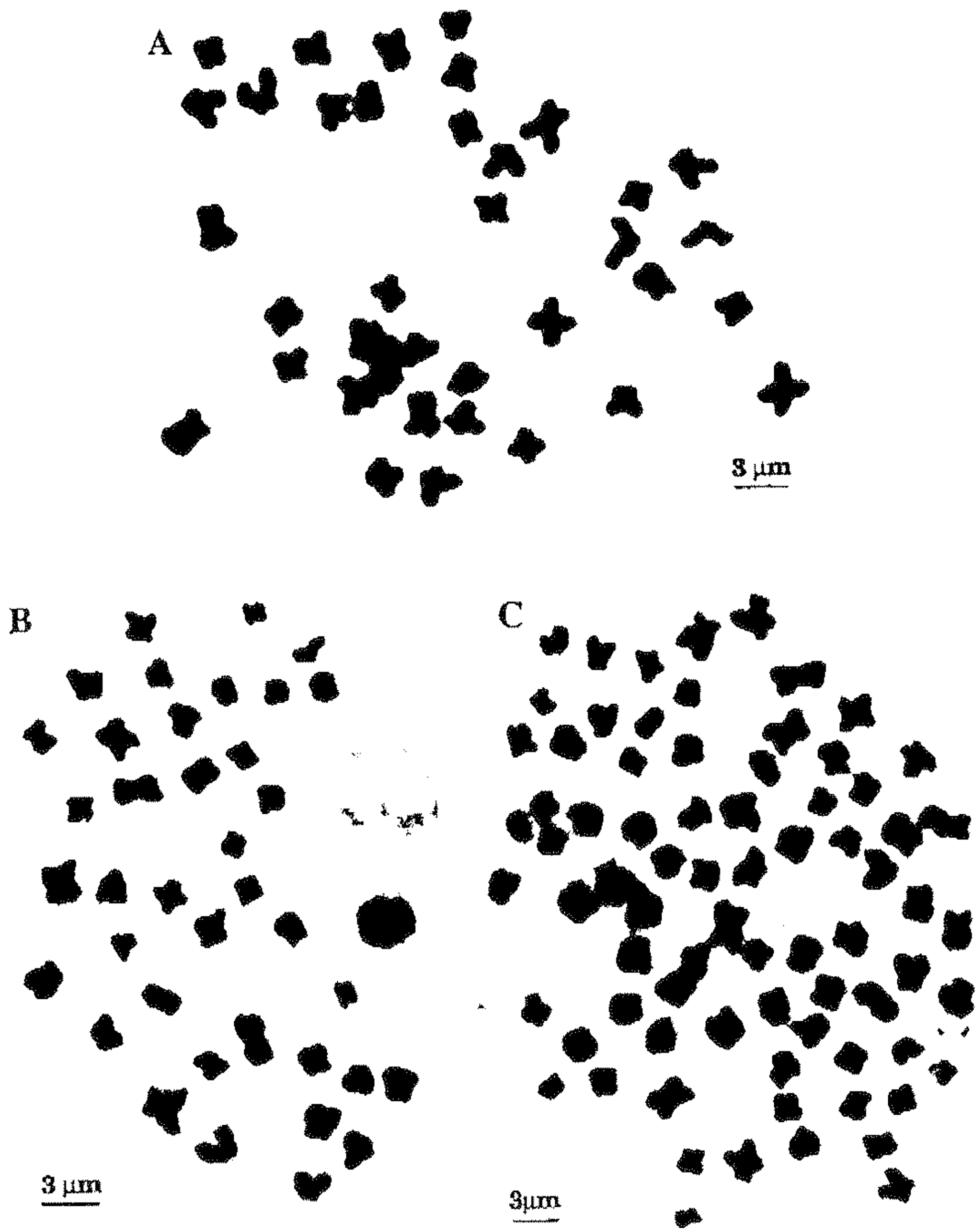

FIG. 8. *Epioblasma triquetra*. Mitotic metaphase chromosomes of gonadal cells. A, Female; B, male; C, tetraploid cell, male.

TABLE 6. Total mean lengths (µm) and relative mean lengths (%) of mitotic metaphase chromosomes in female and male *Epioblasma triquetra*.[1]

Chromosome pair	Female			Male		
	Total length	Relative length	Type	Total length	Relative length	Type
1	2.70 ± 0.31	6.62 ± 0.47	M	3.00 ± 0.28	8.90 ± 0.20	*m*
2	2.55 ± 0.45	6.25 ± 0.01	M	2.25 ± 0.40	6.68 ± 0.55	*m*
3	2.10 ± 0.16	5.15 ± 0.58	M	1.95 ± 0.16	5.79 ± 0.35	*m*
4	1.95 ± 0.11	4.78 ± 0.41	M	1.50 ± 0.14	4.45 ± 0.10	*m*
5	1.80 ± 0.09	4.41 ± 0.17	M	2.55 ± 0.35	7.57 ± 0.09	*sm*
6	2.70 ± 0.36	6.62 ± 0.05	SM	2.25 ± 0.37	6.68 ± 0.67	*sm*
7	2.55 ± 0.33	6.25 ± 0.11	SM	2.10 ± 0.18	6.23 ± 0.14	*sm*
8	2.40 ± 0.28	5.88 ± 0.23	SM	1.80 ± 0.10	5.34 ± 0.12	*sm*
9	2.40 ± 0.25	5.88 ± 0.41	SM	1.80 ± 0.08	5.34 ± 0.29	*sm*
10	2.25 ± 0.17	5.51 ± 0.47	SM	1.65 ± 0.26	4.90 ± 0.43	*sm*
11	2.10 ± 0.18	5.15 ± 0.16	SM	1.50 ± 0.17	4.45 ± 0.08	*sm*
12	1.95 ± 0.08	4.78 ± 0.59	SM	1.50 ± 0.13	4.45 ± 0.22	*sm*
13	1.80 ± 0.06	4.41 ± 0.30	SM	1.35 ± 0.10	4.01 ± 0.34	*sm*
14	1.80 ± 0.04	4.41 ± 0.30	SM	1.35 ± 0.08	4.01 ± 0.18	*sm*
15	1.65 ± 0.10	4.04 ± 0.41	SM	1.20 ± 0.06	3.56 ± 0.08	*sm*
16	1.65 ± 0.09	4.04 ± 0.11	SM	1.20 ± 0.06	3.56 ± 0.12	*sm*
17	1.50 ± 0.14	3.68 ± 0.05	SM	1.00 ± 0.05	2.97 ± 0.35	*sm*
18	2.55 ± 0.23	6.25 ± 0.70	ST	1.95 ± 0.14	5.79 ± 0.05	*st*
19	2.40 ± 0.20	5.88 ± 0.23	ST	1.80 ± 0.10	5.34 ± 0.37	*st*

[1]Based on measurements from two sets of karyotyped cells.

TABLE 7. Total mean lengths (µm) and relative mean lengths (%) of mitotic metaphase chromosomes in female *Ptychobranchus fasciolaris*.[1]

Chromosome pair	Total length	Relative length	Type
1	5.00 ± 0.27	8.76 ± 0.37	*m*
2	3.59 ± 0.33	6.29 ± 0.29	*m*
3	3.13 ± 0.15	5.48 ± 0.58	*m*
4	3.91 ± 0.25	6.85 ± 0.25	*sm*
5	3.75 ± 0.10	6.57 ± 0.27	*sm*
6	3.59 ± 0.33	6.29 ± 0.29	*sm*
7	3.28 ± 0.12	5.75 ± 0.41	*sm*
8	3.13 ± 0.25	5.48 ± 0.35	*sm*
9	2.97 ± 0.54	5.20 ± 0.23	*sm*
10	2.88 ± 0.38	5.04 ± 0.36	*sm*
11	2.81 ± 0.25	4.92 ± 0.52	*sm*
12	2.80 ± 0.30	4.90 ± 0.46	*sm*
13	2.59 ± 0.37	4.54 ± 0.42	*sm*
14	2.50 ± 0.11	4.38 ± 0.18	*sm*
15	2.34 ± 0.36	4.10 ± 0.23	*sm*
16	2.25 ± 0.19	3.94 ± 0.11	*sm*
17	2.19 ± 0.21	3.84 ± 0.47	*sm*
18	1.88 ± 0.25	3.29 ± 0.30	*sm*
19	2.50 ± 0.24	4.38 ± 0.50	*st*

[1]Based on measurements from three sets of karyotyped cells.

two *st* pairs of chromosomes. Mean measurements of the mitotic metaphase chromosomes of five cells are given in Table 5. The mean lengths of the chromosomes ranged from 3.00 ± 0.19 to 1.34 ± 0.11 μm. The NF is 76.

Epioblasma triquetra (Rafinesque 1820)

The chromosome number of this sexually dimorphic species is 2n = 38 (Fig. 8). The relative lengths and total lengths of the male and female mitotic metaphase chromosomes analyzed are given in Table 6. The karyotype of this species was found to be slightly different in the two

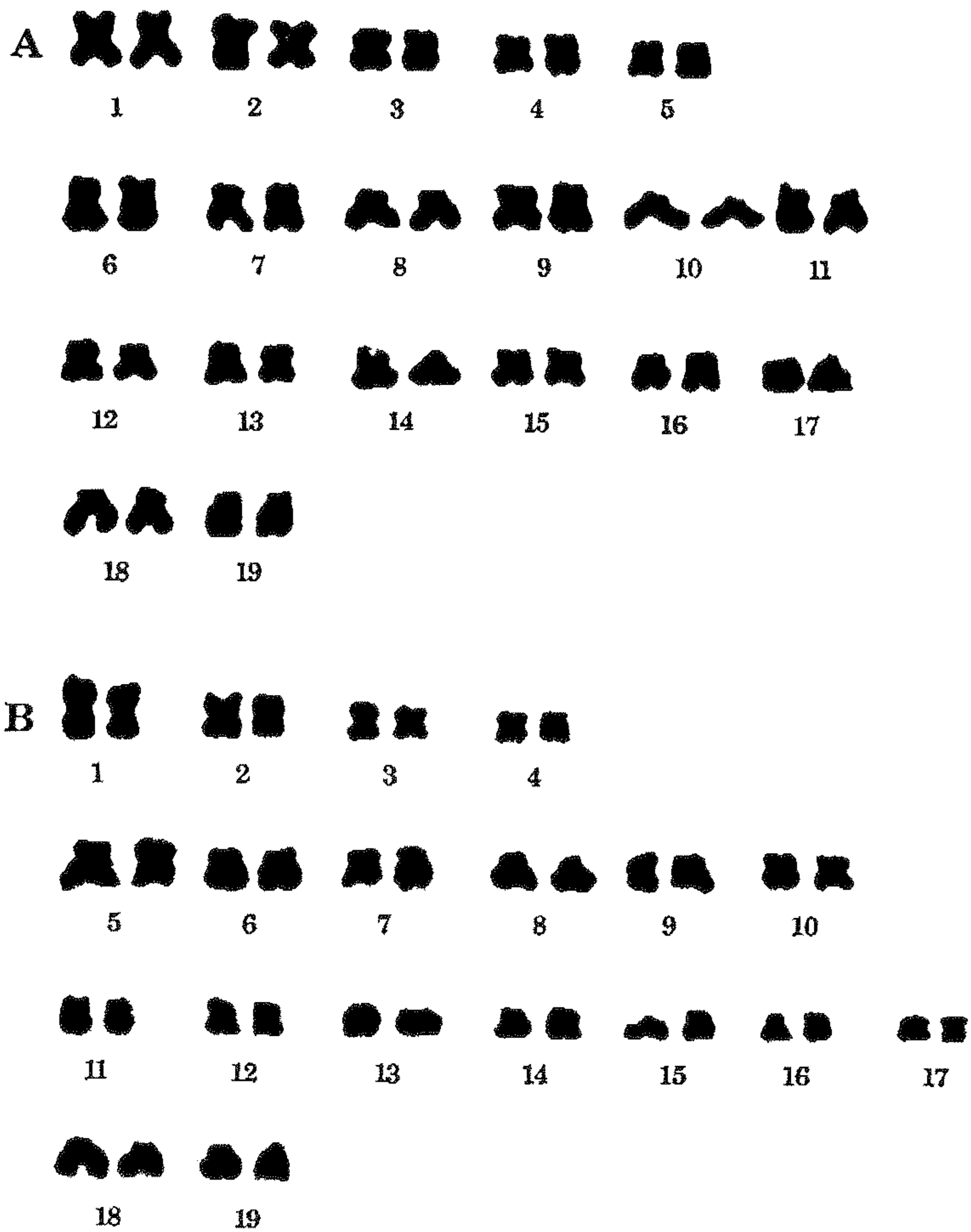

FIG. 9. *Epioblasma triquetra*. A, Karyotype of a female (from the chromosomes in Fig. 8A); B, karyotype of a male (from Fig. 8B).

sexes. In females it is five *m*, 12 *sm*, and two *st* (Fig. 9A). In males it is four *m*, 13 *sm* and 2 *st* (Fig. 9B). Chromosome pair no. 5 was medianly constricted in the females but submedianly constricted in males. If this difference is not due to an artifact of our technique, then the chromosomes of pair no. 5 may have some specific relationship to sex determination.

The chromosome complements of the males and the females studied showed some other differences. In females, the mean total length of the mitotic metaphase chromosomes was 40.80 ± 3.63 μm while in males it was 33.70 ± 3.20 μm.

The NF of *Epioblasma triquetra* is 76. In males, tetraploid sets of chromosomes (Fig. 8C) were observed in 40 testicular cells.

Ptychobranchus fasciolaris (Rafinesque 1820)

Three female specimens were examined. Thirty-eight chromosomes were counted in 20 cells (Fig. 10A). In addition, 30 cells had tetraploid sets of chromosomes (Fig. 10B). The normal

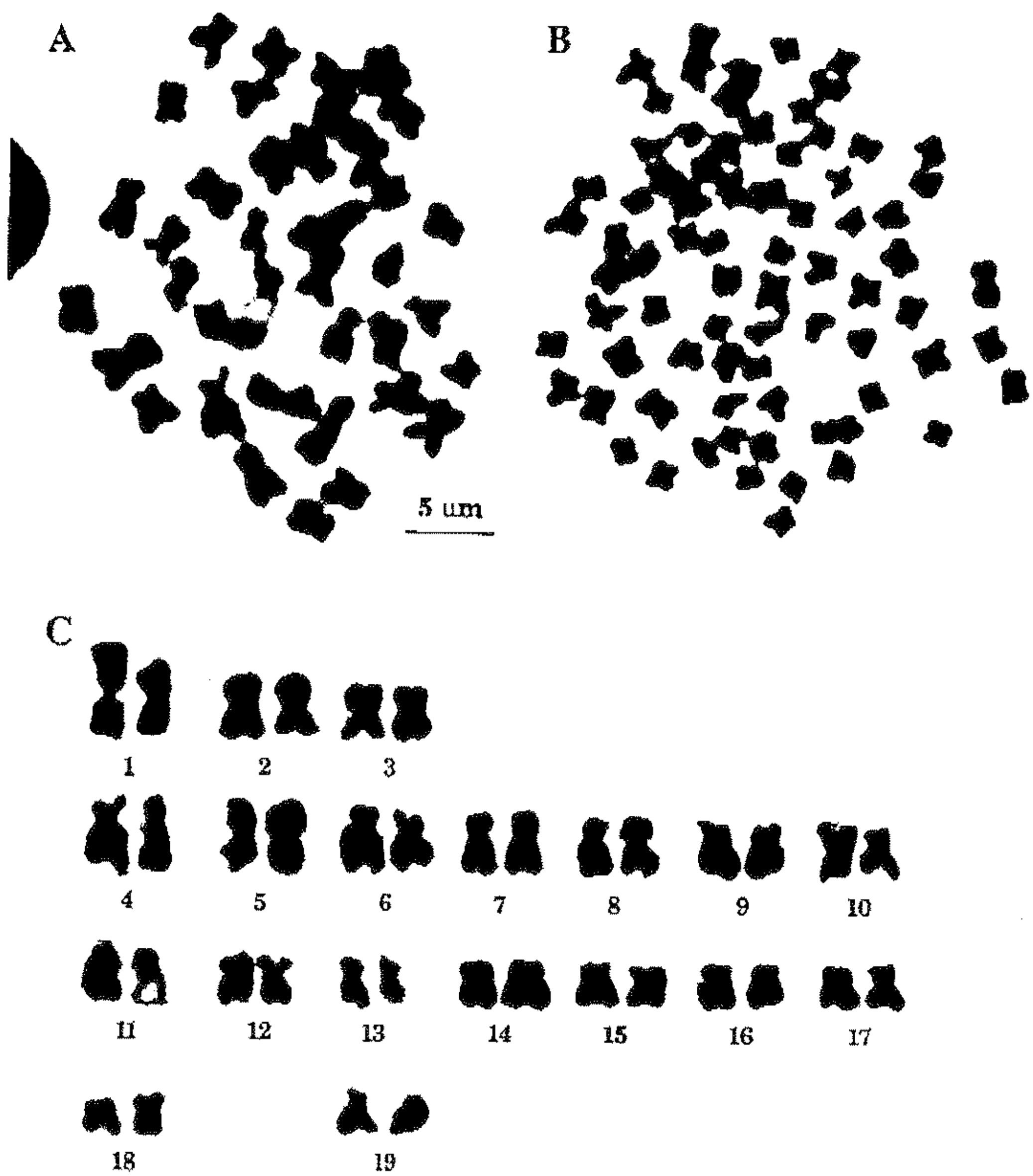

FIG. 10. *Ptychobranchus fasciolaris*, female. Mitotic metaphase chromosomes of gonadal cells. A, Diploid cell; B, karyotype of the chromosomes in A; C, tetraploid cell.

diploid chromosome complements consisted of three pairs of *m* chromosomes, 15 *sm* pairs and one *st* pair (Fig. 10C, Table 7). The NF of this species is 76. The size range of the mitotic metaphase chromosomes was from 5.00 ± 0.27 µm in a largest chromosome set to 1.88 ± 0.25 µm in a small one (Table 7).

Results of the above karyological analyses are summarized in Table 8.

DISCUSSION

The chromosome data provided by our study add to that previously reported for North American freshwater bivalves. The chromosome numbers (each with 2n = 38) we found for *Anodonta grandis, Anodontoides ferussacianus* and *Ptychobranchus fasciolaris* confirm the reports of Jenkinson (1976). We now provide also the karyotype of these three species. The number of chromosomes (2n = 38) we observed in *Elliptio complanata* differs from that reported (n = 16) by Lillie (1901) in oogenesis of *"Unio"* [*Elliptio*] *complanata*. Griethuysen *et al.* (1969) and Jenkinson (1976) previously doubted the accuracy of Lillie's report, and here we correct Lillie's error. Lillie's observations were based on tissues prepared by the paraffin sectioning method. Such early observations were often inaccurate because of limitations of the paraffin technique (Burch, 1960). In addition to the above four species, we found the chromosome number of *Epioblasma triquetra* and a western North American *Anodonta* population [*A. "grandis"* from Colorado] also to be 2n = 38, and we give the karyotype for these two species, and for *Elliptio complanata*.

The diploid chromosome numbers of the six species of North American Unionidae reported

TABLE 8. Karyotypes of six species of freshwater North American Unionidae reported here.

Species	Karyotype formula	No. chromosome pairs
Unionidae		
Unioninae		
Elliptio complanata	5 *m* + 11 *sm* + 2 *st* + 1 *t*	19
Anodontinae		
Anodonta grandis	5 *m* + 12 *sm* + 2 *st*	19
Anodonta sp.	3 *m* + 8 *sm* + 8 *st*	19
Anodontoides ferussacianus	2 *m* + 15 *sm* + 2 *st*	19
Lampsilinae		
Epioblasma triquetra		
(Male)	4 *m* + 13 *sm* + 2 *st*	19
(Female)	5 *m* + 12 *sm* + 2 *st*	19
Ptychobranchus fasciolaris	3 *m* + 15 *sm* + 1 *st*	19

here are all 2n = 38 (Table 8), which is the same number reported for 23 other unionid species on three continents (Table 1). An additional 26 unionid species of North America were reported (without being named specifically) to have the same chromosome number (Jenkinson, 1984). It is remarkable that the evolution of unionid mussels has proceeded without any change in chromosome number. Such a conservatism has been pointed out for various gastropod and other molluscan taxa (Burch, 1965; Patterson, 1969), has been suggested for families of marine bivalves (Menzel, 1968), and is noticeable in the chromosome numbers in the bivalve and polyplacophoran taxa listed by Nakamura (1985).

For the species studied so far in the Margaritiferidae (one species from North America and one from East Asia) and the Unionidae (42 species from North America, three from Western Europe, and nine species from East Asia), all have 38 chromosomes. These two freshwater mussel families are replaced in Australia by the family Hyriidae. It is perhaps significant to note that the only three hyriid species for which there is chromosomal information available, the chromosome number is 2n = 34 (McMichael & Hiscock, 1958), four less chromosomes than occurring in the freshwater mussel families found in the Northern Hemisphere. It will be interesting to determine the chromosome numbers of the other Southern Hemisphere freshwater unioidean family, the Mycetopodidae of South America and southern Africa.

In addition to the normal diploid complement of chromosomes, tetraploid sets of chromosomes were observed in both male and female *Epioblasma triquetra*, and in *Ptychobranchus fasciolaris*. Tetraploid sets of chromosomes in normally diploid specimens of *Crassostrea ariakensis* were reported by Ieyama (1975), and in *C. rhizophorae* by Rodriguez-Romero *et al.* (1979). Ieyama indicated that the tetraploid cells may have been due to the colchicine treatment. Ahmed (1974) noted that the numerous polyploid-looking metaphases (4n or higher) were observed in testicular squashes. These observations may have been due to artifacts, *i.e.*, to the close proximity and confluence of several synchronously dividing spermatogonia.

In comparing the karyotypes of the six North American species we investigated, each are distinct (Table 8), except possibly for *Anodonta grandis* and female *Epioblasma triquetra*. Unfortunately, limited material of the latter species was available. If the male and female chromosomes are measured accurately, and evaluated and aligned properly, then the occurrence of one more set of *m* chromosomes and one less set of *sm* chromosomes in females compared with males needs to be studied further [J.B.B.], especially in regard to a possible role in sex determination.

The karyotype of Michigan *Anodonta grandis* from three localities was analyzed to be 5 *m*, 12 *sm*, and 2 *st*, and that of *A. grandis* from Colorado to be 3 *m*, 8 *sm*, and 8 *st* (Table 8). Those differences would clearly separate the Michigan and Colorado populations. However, the distinction between *sm* and *st* chromosomes is sometimes rather slight and may be due more to possible artifacts of slide preparation than to actual karyotypic differences [J.B.B.]. Populations of the *A. grandis* complex throughout its geographic distribution obviously need more study.

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Gab-Man PARK*

Museum of Zoology
Department of Biology
University of Michigan
Ann Arbor, Michigan 48109
U.S.A.

J. B. BURCH

Museum of Zoology and Department of Biology, College of Literature, Science & Arts, and
School of Natural Resources & Environment
University of Michigan
Ann Arbor, Michigan 48109, U.S.A.

*Present address: Department of Biology, Kangweon National University, Chuncheon, Kangwon-Do, 200-701, Korea.