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Chromosome Study of *Anodonta anatina* (L., 1758) (Bivalvia, Unionidae)*

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The chromosome complement of the freshwater mussel *Anodonta anatina* was studied using Giemsa, Ag-NOR and chromomycin A_3 staining. The diploid chromosome number of this species is 2n=38 and the arm number (NF) = 76. The nucleolar organizer region (NOR) was found on one chromosome pair and it was connected to GC rich chromatin as visualized by CMA_3 staining.

Key words: *Anodonta anatina,* chromosomes, freshwater bivalve, karyotype, NOR.

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Freshwater clams (*Unionacea*) belong to the most common and widespread species among freshwater animals. Most studies on the distribution and role of these organisms have been done in lakes and small streams (STRAYER *et al.* 1994). The species of the genus *Anodonta* live in waters of moderate or high productivity at high temperatures occurring during the summer months (BAUER 2001). *Anodonta anatina* is a paleoarctic freshwater unionid mollusk which is common both in lotic or in lentic habitats. It is a dominant species among *Unionidae* in Poland (PIECHOCKI & DYDUCH-FALNIOWSKA 1993) and also in Great Britain (ALDRIDGE 2000). This may be due to the relatively low sensitivity of *A. anatina* to environmental pollution as compared to other unionid species (PIECHOCKI & DYDUCH-FALNIOWSKA 1993).

The chromosome number within *Unionidae* is known for 27 species, with most having 38 chromosomes (for a review see NAKAMURA 1985; THIRIOT-QUIÉVREUX 2002). Six species of *Anodonta*, including *A. anatina*, have been studied cytogenetically, but only the diploid chromosome number (2n=38) and fundamental arm number (NF=76) have been established (NAKAMURA 1985; BARSIENE 1994). The locations of the nucleolar organizer regions (NORs) have only been studied in Chinese mussel (*Anodonta woodiana*) (WOZNICKI 2004).

The present report describes the karyotype and the NORs of *Anodonta anatina* from Poland.

Material and Methods

The chromosome complements of twenty eight specimens of *Anodonta anatina* from Wulpinskie Lake (North-Eastern Poland) were studied. The shell length of the mussels ranged from ca 8 to ca 12 centimeters.

Because of a very low level of the mitotic index, a stimulation of cell divisions was performed. For that purpose, a 0.4% solution of cobalt chloride was injected *in vivo* (0.05 ml per specimen). Cobalt chloride blocks two major steps of cellular respiration, inducing tissue hypoxia, which leads to cell proliferation (WEBB 1962 cited by CUCCHI & BARUFFALDI 1989).

After 60 hours, a 0.1% colchicine solution was injected into the mussel's foot *in vivo* for 6 hours. From 0.5 to 1.0 ml of colchicine solution was used (depending on the size of the mussel). Gills were dissected and homogenized in distilled water and then hypotonized for 60 min in distilled water. Cell suspensions were fixed in 3:1 methanol/acetic acid and centrifuged three times at 1000 rpm. Each slide preparation was made using the air-drying technique (THIRIOT-QUIÉVREUX & AYRAUD 1982).

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For conventional karyotypes, chromosome preparations were stained with 5% Giemsa in distilled water for 20 min. CMA_3 staining was done according to SOLA *et al.* (1992) and Ag-NOR staining as described by HOWELL & BLACK (1980).

Chromosome spreads were analyzed under a Nikon Optiphot 2 fluorescent microscope equipped with UV filters for identification of fluorescent signals and photographed by a Coolpix 995 camera.

Five metaphase plates were karyotyped. Morphometric measurements of chromosomes were made using the freeware computer application MicroMeasure version 3.3 available on the Internet at: http://www.colostate.edu/Depts/Biology/MicroMeasure. The relative length (RL) (100 x chromosome length/total haploid length) and the centromeric index (CI) (100x length of the short arm/total chromosome length) were calculated. Chromosomes were classified according to LEVAN *et al.* (1964). In the case of five animals, sequential staining CMA_3/Ag-NOR was done and at least five metaphase plates from each specimen were analyzed. About 50 interphase nuclei were observed from the same five individuals after silver staining.

Results

From 17 individuals of *Anodonta anatina*, 170 Giemsa-stained metaphase plates were analyzed, showing that the diploid chromosome number was 2n=38 (Fig. 1). Their relative length ranged from 4.37 to 2.31 (Table 1), and the karyotype consisted of six pairs of metacentric, twelve pairs of submetacentric and one pair of subtelocentric chromosomes (NF=74) (Fig. 1) (Table 1).

Staining with fluorochrome CMA_3 revealed bright positive bands at a terminal position on the short arm of one chromosome pair of *Anodonta anatina* (Fig. 2). The same results were obtained using silver staining (Ag-NOR). The sequential CMA_3/Ag-NOR staining procedure of the same metaphases showed that the CMA_3 and silver positive signals appeared at the same chromosome site of the metacentric chromosome (Fig. 3). The number of silver-stained interphase nucleoli in *A. anatina* cells never exceeded 2 nucleoli per cell.

Discussion

The karyotype of *A. anatina* described as 2n=38 (Fig. 1) is coincident with that reported for this species and other *Anodonta sp.* (*A. grandis*, *A. piscinalis*, *A. cygnea*, *A. subcircularis*, *A. woodiana*) (NAKAMURA1985; BARSIENE 1994; WOZNICKI 2004). Within the bivalve class this is the most frequent chromosome number (THIRIOT-QUIÉVREUX 1994).

The fundamental chromosome arm number (NF) reported for four *Unionidae* species equaled 76 (because only bi-armed chromosomes were found) (NAKAMURA 1985). The *Anodonta anatina* karyotype described in the present paper had one uniarmed chromosome pair, therefore NF equaled 74 (Fig. 1) (Table 1). The relative length of chromosomes seems to be similar in *A. anatina* (Table 1) and *A. woodiana* (WOZNICKI 2004), but the morphology of chromosomes was different (Fig. 1). The main differences between these two karyotypes were the number of submetacentric chromosomes in *Anodonta anatina* (12 pairs),

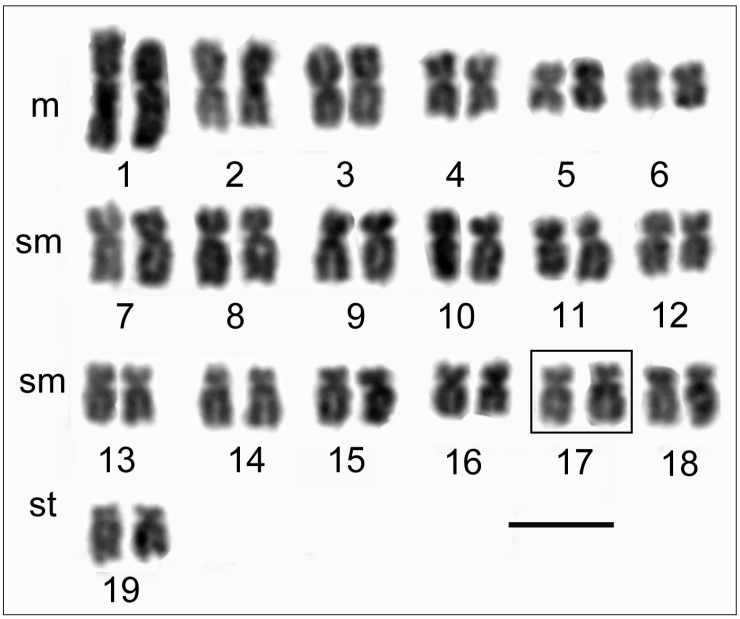

Fig. 1. Karyotype of *Anodonta anatina*. m – metacentric chromosomes, sm – submetacentric and st – subtelocentric chromosomes. NOR bearing chromosome pair is framed. Bar = 5 μm.

Table 1

Relative lengths (RL) and centromeric indices (CI) of *Anodonta anatina* chromosomes

Chromosome pair no.	RL	SD	CI	SD	Classification
1	4.37	±0.02	40.23	±0.08	m
2	3.38	±0.02	46.56	±0.15	m
3	3.07	±0.01	45.62	±3.54	m
4	2.55	±0.01	41.96	±1.53	m
5	1.93	±0.01	41.26	±0.58	m
6	1.90	±0.01	41.30	±0.22	m
7	3.17	±0.01	36.55	±0.63	sm
8	2.98	±0.01	34.82	±1.32	sm
9	2.88	±0.01	35.61	±2.57	sm
10	2.78	±0.02	34.51	±3.52	sm
11	2.40	±0.03	31.68	±2.94	sm
12	2.52	±0.03	35.67	±0.25	sm
13	2.37	±0.01	33.71	±2.79	sm
14	2.29	±0.04	26.19	±2.10	sm
15	2.42	±0.02	31.16	±3.80	sm
16	2.12	±0.02	33.82	±1.41	sm
17	2.41	±0.03	29.84	±2.49	sm
18	2.15	±0.04	27.94	±1.88	sm
19	2.31	±0.03	20.83	±1.45	st

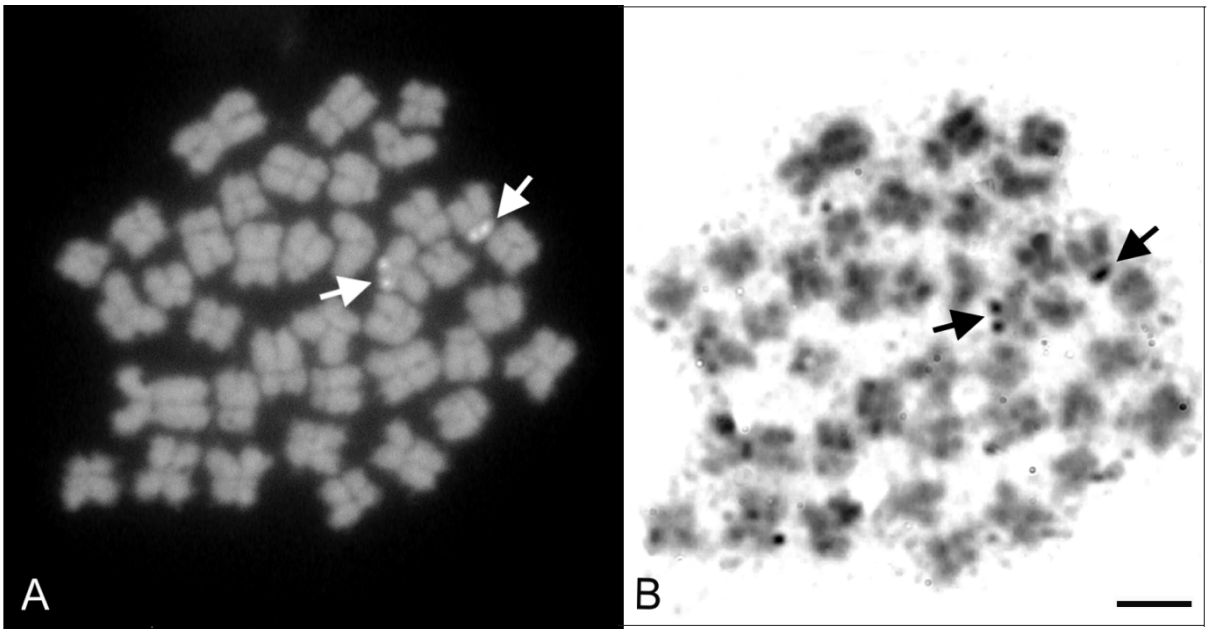

Fig. 2. Metaphase chromosomes of *Anodonta anatina* after A – CMA_3-staining, B – Ag-staining. Arrows indicate NOR chromosomes. Bar = 5 μm.

which was higher than in *A. woodiana* (5 pairs), and the presence of one pair of subtelocentric chromosomes in *Anodonta anatina*.

One NOR locus situated telomerically at the short arm of a submetacentric chromosome is also common for both studied *Anodonta* species (Fig. 2) (WOZNICKI 2004). The single NOR locus in *A. anatine* represents one of the NOR patterns observed in bivalves. The number of NOR bearing chromosome pairs in these mollusks varies from one in *Mya arenaria* (THIRIOT-QUIÉVREUX *et al.* 1998), *Donax trunculus* (MARTINEZ *et al.* 2002) and *Brachidontes pharaonis* (VITTURI *et al.* 2000) to three in *Mytilus californianus* (MARTINEZ-LAGE *et al.* 1997; GONZALEZ-TIZON *et al.* 2000) and *Mytilus trossulus* (MARTINEZ-LAGE *et al.* 1997). A single pair of chromosomal NORs located terminally is

supposed to be an ancestral character (AMEMIYA & GOLD 1990; THIRIOT-QUIÉVREUX 1994).

To date chromosomes of five species of *Mytilidae* (MARTINEZ-LAGE *et. al*. 1994, 1995; TORREIRO *et al*. 1999; VITTURI *et al.* 2000), one species of *Donacidae* (MARTINEZ *et al*. 2002), one species of *Dreissenidae* (WOZNICKI & BOROŃ 2003), one species of *Solenidae* (FERNANDEZ-TAJES *et al*. 2003) and one species of *Unionidae* (WOZNICKI 2004) have been stained using fluorochrome Chromomycin A_3 (CMA_3), which binds to GC rich chromatin (AMEMIYA & GOLD 1986).

GC rich CMA_3 positive heterochromatin connected to NORs is typical in fish and amphibians (AMEMIYA & GOLD 1986), although it has also been observed in bivalve mollusks (MARTINEZ--EXPOSITO *et. al*. 1997; MARTINEZ-LAGE *et. al.* 1994). Staining with fluorochrome CMA_3 has revealed the existence of GC bands on one chromosome pair, at the same location as Ag-NOR in *Anodonta anatina* (Fig. 2) The same pattern of NOR/GC-rich region location was described in *Anodonta woodiana* from Poland (WOZNICKI 2004). Other bivalve species show CMA_3 positive bands on two or more chromosome pairs. In different groups these are or are not connected with NORs (MARTINEZ-LAGE *et al.* 1995; VITTURI *et al.* 2000; MARTINEZ *et al.* 2002; WOZNICKI & BOROŃ 2003).

The present findings provide a cytogenetic characterization of *Anodonta anatina* and the second case of NOR description in a species from the genus *Anodonta*.

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