

CHROMOSOME BANDING PATTERNS OF THE GUDGEON, *GOBIO GOBIO* (ACTINOPTERYGII, CYPRINIDAE)

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Background. The gudgeon is one of the most divergent fish species in Europe. Morphological variability related to sex, size, ecological characters, and geographical distribution are well documented in the literature. Apart from the only few data on the karyotype, chromosome banding patterns of this species have not been studied. Cytogenetic features are very useful tools in taxonomic descriptions of cyprinid species. The aim of this study was to describe and characterise the banding patterns of the gudgeon karyotype.

Material and methods. Cytogenetic examinations of 15 specimens of gudgeon from the upper part of the Odra River, Poland, were carried out. Different chromosome banding techniques: Giemsa staining, C-banding, silver nitrate, chromomycin A₃ and DAPI staining were used.

Results. The karyotype of gudgeon consisted of $2n = 50$ chromosomes, and $NF = 98$ chromosome arms. Ag-NORs were located on one submeta-subtelocentric chromosome pair and the size polymorphism of NORs was detected. The chromosome sites with G-C rich DNA on one submeta-subtelocentric chromosome pair and heterochromatin block in the centromeric regions were found. Low accumulation of A-T pair rich regions were indicated by DAPI staining.

Conclusion. The presently described new chromosomal features of *Gobio gobio* substantially enhance our knowledge on the taxonomy of this species at cytogenetic level. Jointly with data on morphological- and genetic variability they could be used to determine the phylogeny of the genus *Gobio* and related species.

Key words: chromosome banding patterns, gudgeon, *Gobio gobio*, fish, karyotype, NOR polymorphism

INTRODUCTION

The Gobioninae, along with other seven subfamilies, belongs to the family Cyprinidae (cf. Howes 1991). Hosoya (1986) considered them to be monophyletic, based on sensory canal patterns, morphology of supraoccipital and frontal, and modification of the anterior vertebrae. Three species; *G. gobio*, *G. kessleri*, and *G. albipinnatus*, occurring in Poland, have been traditionally assigned to the genus *Gobio*. According to Bănărescu (1992) *G. gobio* from Europe, Siberia, and Central Asia represents *Gobio* sensu stricto. Naseka (1966) divided the genus *Gobio* sensu Bănărescu into two genera *Gobio* and *Romanogobio*, and the latter genus containing two other species: *R. kessleri* and *R. albipinnatus*. Among Gobioninae only *Gobio* has a wide distribution range, extending throughout most of Europe, the Black Sea watershed in northern Anatolia, several landlocked lakes in Central Anatolia, some rivers in Central Asia, and most of Siberia in the watershed of the Arctic Ocean as far east as the Lena and Kolyma rivers (Bănărescu 1999). Many geographical populations of this fish, classified as subspecies or “nations” have been described within its geo-

graphical range. According to Bănărescu (1999) five European subspecies are now recognised.

The majority of the morphological characters of the *Gobio* species and specifically the gudgeon, *G. gobio*, such as sexual dimorphism, age, growth, feeding habits, longevity, and some aspects of reproduction biology have been already described (see a review of Bănărescu 1999). *G. gobio*, considered one of the most divergent fish species in Europe, with high level of interpopulational variability, has not been studied in relation to its banding chromosomal patterns. However, some cytogenetic features as C-banding, nucleolar organizer regions (NORs) and location of GC-rich DNA sites on chromosomes have been useful in cyprinid taxonomy (Buth et al. 1991, Boroń 2001).

Some papers (Raicu et al. 1973, Sofradžija and Berberović 1975, Hafez et al. 1978, Vujošević et al. 1983, Vasil'ev 1985, Klinkhard et al. 1995) described this species as possessing, diploid number of $2n = 50$ chromosomes, which is the most frequent among all other cyprinid lineages (Buth et al. 1991, Ráb and Collares-Pereira 1995).

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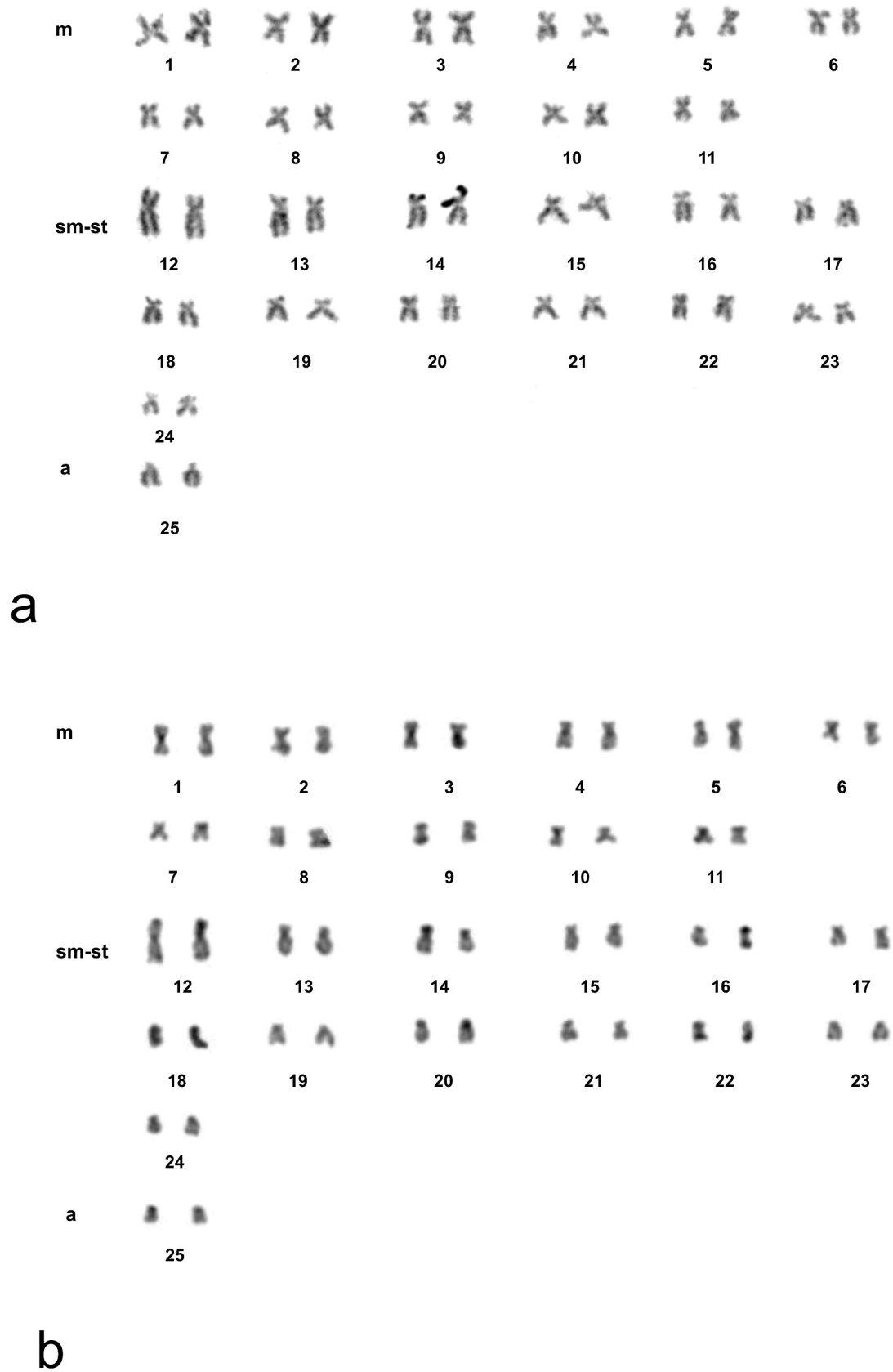


Fig. 1. Karyotype of the gudgeon, *Gobio gobo*: (a) after Giemsa plus Ag-NOR staining; (b) after C-banding

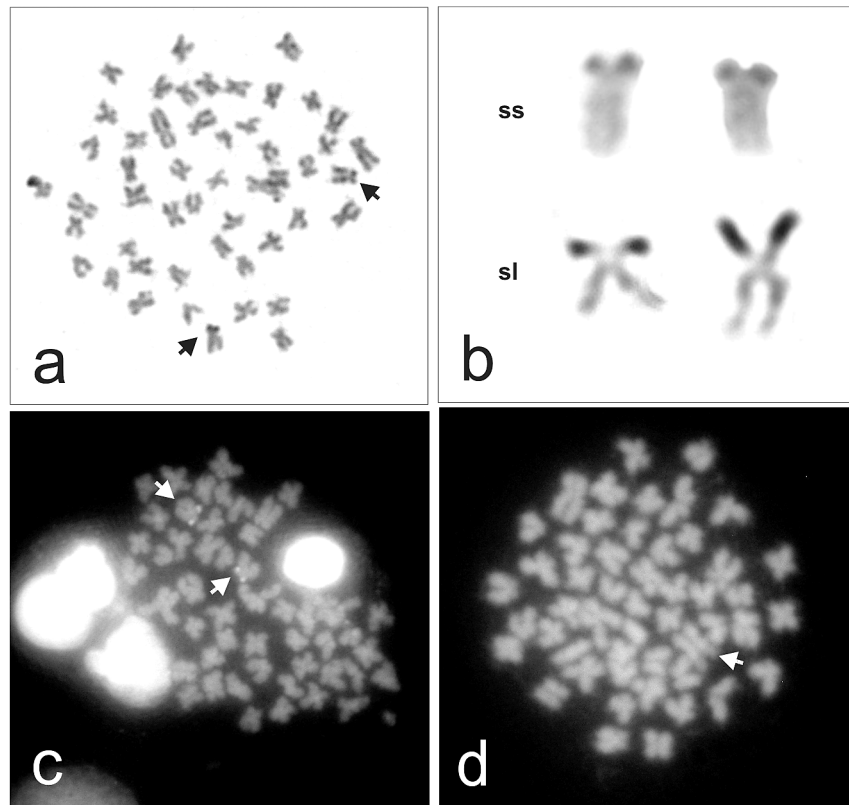


Fig. 2. Chromosomes of the gudgeon, *Gobio gobio*, after different staining: (a) Ag-NOR; (b) “ss” and “sl” cytotypes of NOR-bearing chromosomes; (c) chromomycine A₃ (arrows indicate the NOR sites); (d) DAPI (arrow indicates sm-st chromosome).

In the presently reported study we provide, for the first time, a new data on the location of Ag-stained NORs, AT-rich DNA regions (stained with DAPI), and GC-rich DNA regions on the chromosomes of the gudgeon *G. gobio* from the upper part of the Odra River in Poland.

MATERIALS AND METHODS

Fifteen specimens (six males and nine females) of gudgeon from the Odra River, near Legnica, (Poland) were studied. Mitotic chromosome preparations were obtained from the head kidney by standard air-drying technique (Ráb and Roth 1988). Conventional 5% Giemsa staining, C-banding (Haff and Schmid 1984), NOR sites by silver nitrate staining (Howell and Black 1980) and chromomycine A₃ staining (Sola et al. 1992) were applied. The distribution of A-T pairs on chromosomes was revealed by DAPI staining (Sola et al. 1992). The chromosomes were classified according to Levan et al. (1964).

RESULTS

All individuals of *G. gobio* were characterized by the same $2n = 50$ number of chromosomes. The karyotypes consisted of 11 pairs of metacentric chromosomes, 13 pairs of submeta-subtelocentric, and one pair of acrocentric chromosomes, $NF = 98$ (Fig. 1). Ag-NOR sites on one submeta-subtelocentric chromosome pair No. 14 were found (Fig. 2a). Two cytotypes with size polymorphism in the NOR-bearing pair were observed (Fig. 2b). Chromosome sites with G-C-rich regions were shown on one submeta-subtelocentric pair

(Fig. 2c). C-positive blocks of heterochromatin mainly in the centromeric regions in some chromosomes pairs were detected (Fig. 1b). DAPI staining revealed A-T pair rich regions on “q” arm of one submeta-subtelocentric chromosome (Fig. 2d).

DISCUSSION

The chromosome diploid number of *G. gobio* $2n = 50$ and diploid arm number (NF) ranging from 88 to 98 have been reported by other authors (Raicu et al. 1973, Sofradžija and Berberović 1975, Hafez et al. 1978, Vujošević et al. 1983). The same number of chromosomes was obtained in the presently reported study, and this feature is stable for the species of the genus *Gobio*, except *Gobio uranoscopus* from Slovakia with $2n = 52$ chromosomes (Ráb and Collares-Pereira 1995). Diploid arm number $NF = 98$, determined in the present paper, and chromosome formulae of the gudgeon $2n = 50; (11 m + 13 sm-st + 1a)$ (Fig. 1) is similar with the results of the specimens from other populations previously described.

The majority of the European species, as well as most of other cyprinid fish species of North America, possessed single NOR-bearing chromosome pair (only about 30% species had multiple NOR sites) (Buth et al. 1991, Klinkhard et al. 1995). In cyprinids, NORs are located on all type of the chromosome (from metacentric to acrocentric) (Foresti et al. 1981, Galetti et al. 1985, Galetti jr. et al. 1984, Moreira-Filho et al. 1984, Takai and Ojima 1986), but in European species NORs are mainly located on sm/a chromosomes (Ráb and Collares-Pereira 1995). Classical rearrangements (inversion and translocation) are the most causes of interspecific NOR chro-

mosome differences (Gold and Amemiya 1986, Amemiya and Gold 1988). Silver nitrate staining, which was done for the first time in this species, shown one sm-st NOR bearing chromosome pair (Fig. 2a). The size polymorphism of NOR-bearing chromosomes (pair No. 14) was observed in two individuals, and they have been described as two cytotypes “ss” and “sl” (Fig. 2b). The NORs enlargement that is an effect of amplification is common among different fish species including cyprinids. Size differences between homologous NORs have been found in other fish species (Sanchez et al. 1990, Jankun et al. 2003) and amphibian (Schmid 1982). Amplification or deletion of NOR sites could be an effect of the crossing-over disorders caused by wrong meiotic conjugation between repetitive nucleotide sequences of homologous chromosomes (Schmid and de Almeida 1988). These kinds of chromatin rearrangements lead to inactivation of NOR site (Foresti et al. 1981, Moreira-Filho et al. 1984, Takai and Ojima 1986).

Distribution of heterochromatin in cyprinids generally is limited to centromeric regions and NOR sites (for review see: Klinkhardt et al. 1995, Ráb and Collares-Pereira 1995), but also some species with different type of heterochromatin location were found (Boroń 2001). C-positive blocks of heterochromatin in the centromeric regions of some investigated chromosomes in gudgeon are presented in this study (Fig. 1b).

In several cyprinid species there is non specific DAPI heterochromatin (Mayer et al. 1986, Schmid and Guttenbach 1988). In all investigated specimens, one small signal on a “q” arm of sm-st large chromosome after DAPI staining were found (Fig. 2d) that could be an effect of low accumulation of A-T pairs in gudgeon genome.

Using classical cytogenetic methods presented in this some species specific chromosome markers were found. However, they seem to be not sufficient for more detailed differentiation and identification of the gudgeon chromosomes. So, the next step using the molecular cytogenetic techniques (Fluorescence In Situ Hybridization—FISH) to enable identifying and description of some specific sequences on chromosomes are required.

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