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Chromosome Banding Study of the Golden Loach, *Sabanejewia aurata balcanica* from Slovakia (Cobitidae)

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Abstract The karyotype of a subspecies of the golden loach, *Sabanejewia aurata balcanica* from eastern Slovakia was studied by conventional Giemsa staining, Ag-NOR staining, and C-banding. The diploid chromosome number was 2n=50. The karyotype comprised 2 pairs of metacentric, 6 pairs of submetacentric and 17 pairs of subtelocentric to acrocentric chromosomes. Both metacentric pairs and 2 large subtelocentric pairs had massive pericentromeric blocks, while all other elements had only weak blocks of heterochromatin. The NORs were localized on the short arms of one middle-sized subtelocentric pair. The karyotype of *S. a. balcanica* differs from that of *S. aurata kubanica*, suggesting chromosomal polymorphism of this widely distributed, polytypic cobitid species. The polymorphic karyotypes of the golden loach may thus demonstrate transient stages, linking primitive and advanced cobitid karyotypes.

Karyotype studies have become an invaluable tool contributing to the solution of many systematic and evolutionary problems in fishes. Generally, their importance can be increased by utilizing the results of chromosome banding methods which present a greater set of detailed characters. However, because of the structural and size differences between chromosomes of higher groups of vertebrates and those of fishes (Medrano et al., 1988), there has been only limited success in the application of banding methods to fish chromosomes.

Cobitid fishes belong to the group to which several chromosome banding methods have been successfully applied (Saitoh et al., 1984; Saitoh, 1986, 1989; Lee et al., 1987). However, the number of species thus investigated remains rather limited.

The golden loach, *Sabanejewia aurata*, is a highly polytypic species and is widely distributed in the extensive Ponto-Caspian-Aral zoogeographic region. This species comprises several definite subspecies and also some forms of unknown and/or uncertain systematic position (Vasiljeva and Vasiljev, 1988). Among these subspecies and forms, *S. aurata kubanica* is the only subspecies that has been studied karyologically (Vasiljeva and Vasiljev, 1988). The other *Sabanejewia* species investigated karyologically are *S. caspia* (see Vasiljev, 1985) and *S. larvata* (see Lodi and Marchioni, 1980). The diploid chromosome numbers of these three *Sabanejewia* loaches are 2n=50 but their karyotypes differ: those of *S. caspia* and *S. larvata* are nearly identical but differ slightly from that of *S. aurata cubanica*.

The present report describes the karyotype, distribution of C-positive heterochromatin and localization of nucleolus organizer regions (NORs) in a golden loach which was collected from eastern Slovakia and referred to the subspecies *S. aurata balcanica* (Karaman, 1922) by Balon (1966).

Material and methods

The 24 individuals examined (5 males, 6 females and 13 juveniles) were collected from the Laborec River (tributary of Tisa River, Danube River basin) near Hankovce Village in eastern Slovakia. The intraspecific interrelationships of karyotyped material will be analyzed in detail elsewhere (Vasiljeva and Ráb, in press). Standard procedures for chromosome preparation followed Ráb and Roth (1988). At least 10 metaphase plates from each individual were karyotyped (265 in total). Chromosomes were classified according to the system of Levan et al.

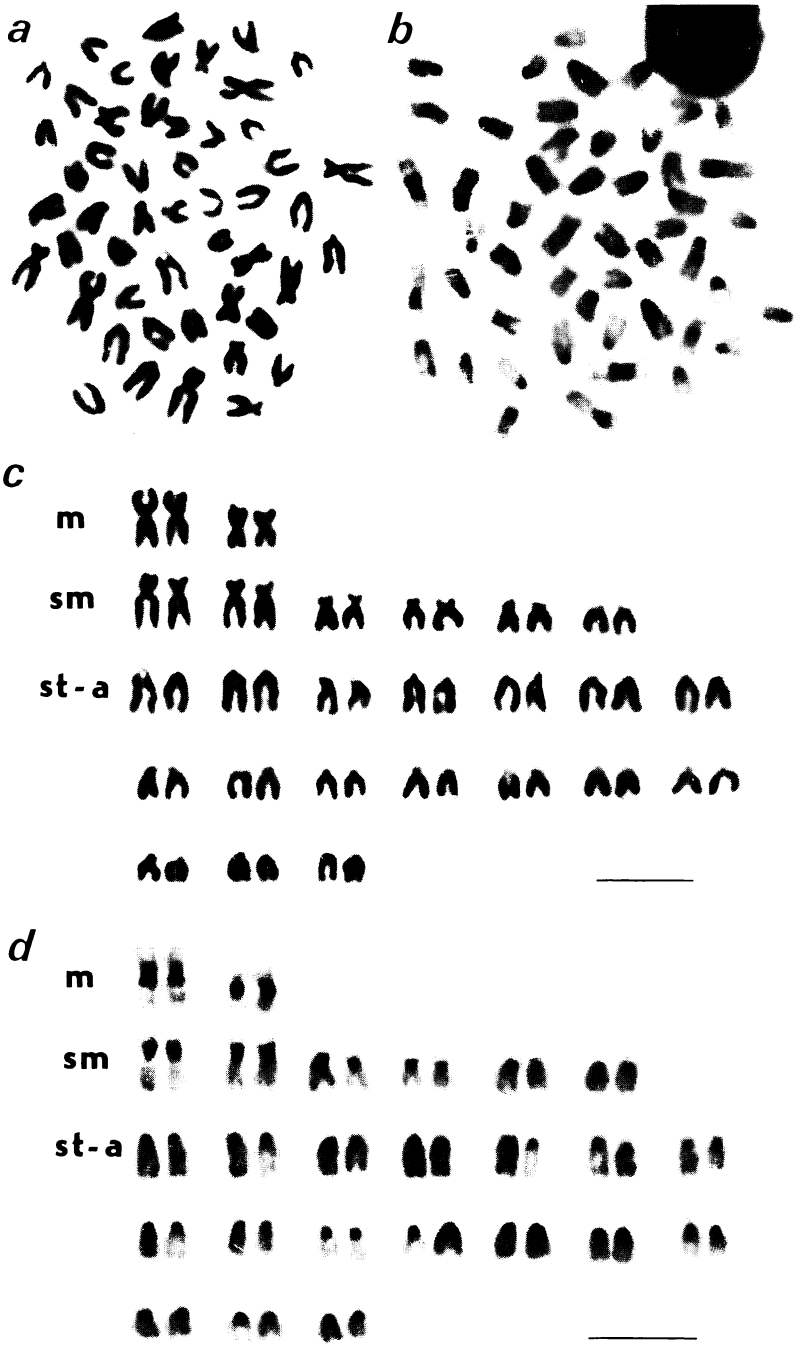

Fig. 1. Metaphase cells (a, b) and karyograms (c, d) of female *Sabanejewia aurata balcanica* arranged from conventionally Giemsa-stained (c) and C-banded (d) chromosomes. Bars indicate 5 μm.

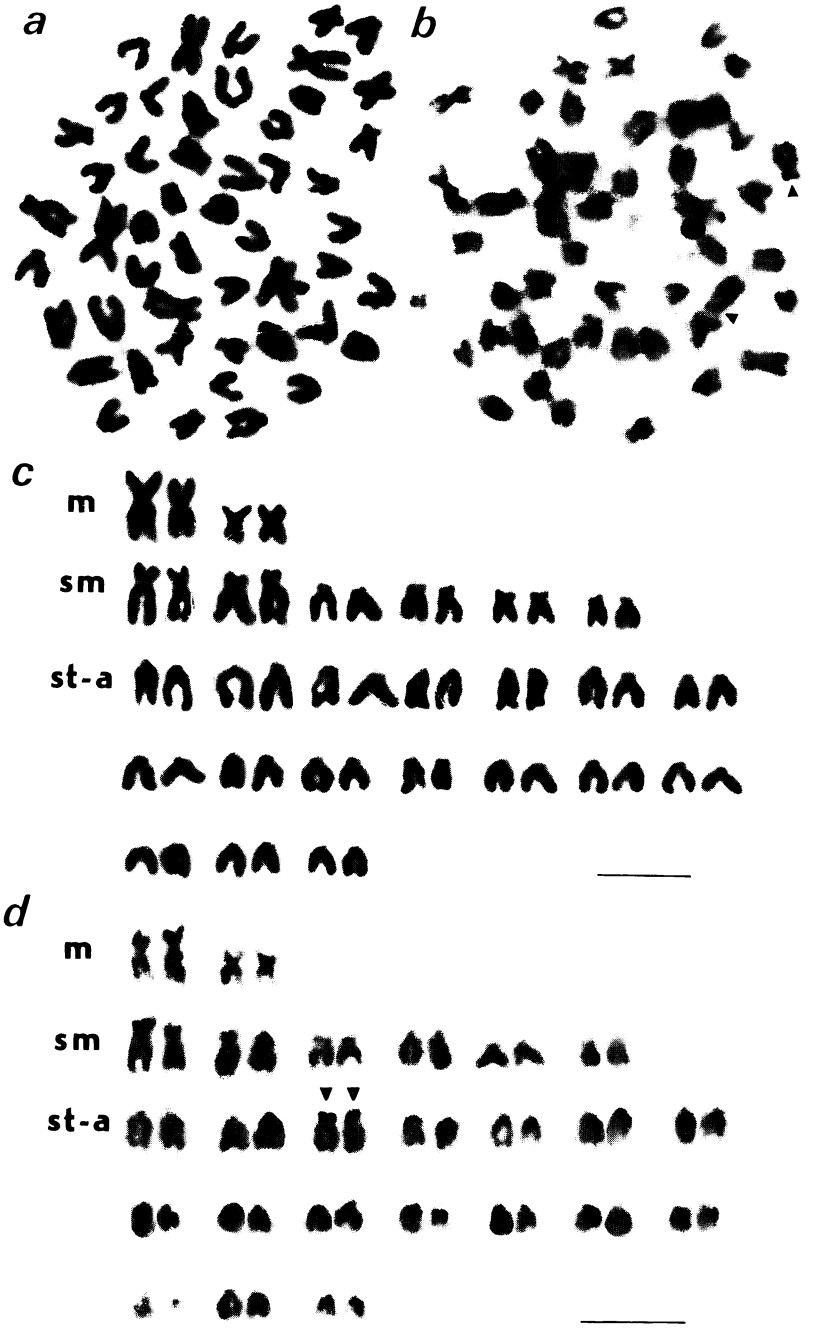

Fig. 2. Metaphase cells (a, b) and karyograms (c, d) of male *Sabanejewia aurata balcanca* arranged from conventionally Giemsa-stained (c) and silver-stained (d) chromosomes. Positions of NORs are shown by arrows. Bars indicate 5 μm.

(1964). Silver staining for the detection of NORs followed Howell and Black (1980), and the C-banding technique followed Haaf and Schmid (1984).

Results

The diploid chromosome number of all individuals was 2n=50. The karyotype comprised 2 pairs of metacentric (m), 6 pairs of submetacentric (sm) and 13 pairs of subtelocentric (st) to acrocentric (a) chromosomes (Figs. 1, 2). Easily identifiable marker chromosomes were both larger and smaller pairs of m, the largest 2 pairs of sm, and 1 pair of middle-sized st carrying terminal NORs on the short arms (Fig. 2b, d). Except for the NOR-bearing pair, all other marker chromosomes had specific distribution patterns of heterochromatin: the massive blocks were localized in the pericentromeric regions. Other chromosomes of the complement had only weak heterochromatic blocks in their centromeric regions (Fig. 1b, d). There were found neither heteromorphic pairs nor sex related heterochromatin blocks indicating sex chromosomes in karyotypes of either females (Fig. 1) or males (Fig. 2).

Discussion

Karyotypes of *Sabanejewia* species and/or subspecies analyzed so far are similar to each other, though some characteristics are species and/or subspecies specific (Table 1). The karyotype of *S. aurata kubanica* from the Nevinka River (Kuban River basin) possesses 1 large and 2 smaller pairs of metacentrics and 7 pairs of submetacentrics, while that of *S. aurata balcanica* from the Laborec River (Danube River basin) contains 1 large and 1 smaller pair of metacentrics and 6 pairs of submetacentrics. Two large sm pairs are shared by both subspecies. The other st and a chromosomes decrease gradually in size and differ in number in both subspecies. This comparison suggests that *S. aurata* is a karyotypically polymorphic species throughout its range.

A comparison of polymorphic karyotypes of *S. aurata* with the karyotype of *S. larvata* from Italy (vicinity of Torino) is difficult, because the chromosomes shown in the published pictures are more spiralized. However, 2 smaller pairs of metacentrics in *S. larvata* (see Lodi and Marchioni, 1980) could be safely identified, and these elements are probably homologous with those in *S. aurata kubanica*. On the other hand, 2 large submetacentric marker pairs are missing in *S. larvata*, although they have been found not only in the karyotype of the golden loach but also in those of several *Cobitis* species (e.g. Cataudella et al., 1977; Ueno et al., 1980, 1985; Vasiljev, 1985, etc.). The other remarkable character of the karyotype of *S. larvata* and *S. caspia*, which Vasiljev (1985) stated to be the same (although the latter has never been described in detail), is the presence of a higher proportion of uni-armed chromosomes. This fact may indicate the ancestral character state of the karyotypes of *S. larvata* and *S. caspia* (see Ueno et al., 1985). From the above characteristics, the polymorphic karyotypes of the golden loach may thus represent a link between the karyotypes of *S. larvata* and *S. caspia* and the more advanced karyotypes of some other cobitids, e.g., European *Cobitis taenia*, Eurasian *C. granoei*, Korean *C. koreensis* and *C. longicorpus*, and Japanese *C. biwae*, *C. takatsuensis*, and *C. "taenia striata"*.

It is necessary to test this hypothesis using chromosome banding data. Our findings concerning the distribution of C-heterochromatin along the metacentric marker chromosomes showed large heterochromatic blocks in the pericentromeric regions, i.e., on both sides of the centromere. Centric fusion sometimes results in such heterochromatin distribution on metacentric chromosomes (Gropp and Winking, 1981). Other available data on distribution of C-heterochromatin in cobitids (Saitoh et al., 1984;

Table 1. Karyotype of *Sabanejewia* species so far analyzed. m, metacentrics; sm, submetacentrics; st, subtelocentrics; a, acrocentrics.

Species	2n	Haploid karyotype structure				References
		m	sm	st	a	
S. larvata	50	2	3	11	9	Lodi and Marchioni, 1970
S. caspia	50	2	3	11	9	Vasiljev, 1985
S. aurata kubanica	50	3	7	—15—		Vasiljeva and Vasiljev, 1988
S. aurata balcanica	50	2	6	—17—		This study

Saitoh, 1986, 1989) showed nearly the same localization of large heterochromatic blocks along the metacentrics in the karyotypes of so-called *Cobitis "taenia striata"* (see Saitoh and Aizawa, 1987). Therefore, a similar pattern of heterochromatin distribution is probably found among some other cobitid species, and one may expect that fusion events played a role in the origin of these m chromosomes in loaches. However, the number of m chromosomes in the karyotypes of cobitids varies, whereas the diploid chromosome number of non-polyploid species is $2n=50$ (rarely 48). This fact may point against the origin of m chromosomes in cobitid karyotypes by fusion and suggests instead some type of intrachromosomal rearrangements, e.g. pericentric inversion or addition of heterochromatin blocks. The results of Ag-NOR staining in the karyotype of *S. aurata balcanica* cannot be discussed in a comparative sense, because so far as we know this kind of chromosome banding has been applied to two *Misgurnus* species only (Lee et al., 1987) and not to any other *Sabanejewia* or closely-related *Cobitis* species.

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ヨーロッパ産シマドジョウの1亜種, *Sabanejewia aurata balcanica* の染色体分染

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ヨーロッパ産シマドジョウの1種の東部スロバキア産亜種, *Sabanejewia aurata balcanica* の染色体を，通常のギムザ染色，銀染色，およびC-バンド染色によって調べた．染色体数は2n＝50で，核型は中部着糸型2対，次中部着糸型6対，および次端部または端部着糸型17対よりなることがわかった．中部着糸型対全部と大型の次中部着糸型2対の動原体近傍には多量のヘテロクロマチンが認められたが，他の染色体には小さなヘテロクロマチンしかなかった．仁形成部位は中型の次端部着糸型対の単腕部にあった．本亜種の核型は *S. aurata kubanica* のものとはことなる．このことから，広い分布域を持ち大きな変異を示す本種が核型でも多型的であることがわかった．核型のこのような多型的状態は，ドジョウ科の原始的核型と派生的核型の中間的な段階を表しているのであろう．