Karyotypes of Two Species of Arowana, *Osteoglossum bicirrhosum* and *O. ferreirai*

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The South American osteoglossid genus *Osteoglossum* is represented by two species, i.e., *O. bicirrhosum* Vandelli and *O. ferreirai* Kanazawa. The former species, well known among aquarists as arowana (or silver arowana), is reported to have 2n=56 chromosomes (Uyeno, 1973). The latter, which became known to science rather recently from the Rio Negro system (Kanazawa, 1966) and often called the black arowana in allusion to a blackish tint over the body, has not been studied cytologically. Our recent investigations with several specimens of these two species have revealed that the chromosomes of *O. ferreirai* are considerably different from those of *O. bicirrhosum* in shape and number. Some emendatory findings were also obtained for *O. bicirrhosum*.

Material and methods

Four specimens of *Osteoglossum bicirrhosum*, 43.5~118.0 mm in standard length, and three specimens of *O. ferreirai*, 57.5~72.0 mm in standard length, were secured from aquarium fish dealers. Each fish was given an intraperitoneal injection of 5 μM colchcine at a dose of 0.025 ml/g body weight. After three hours, gill and kidney tissues were removed and treated with 0.25% tripsin solution for 10~15 minutes. Dulbecco buffer solution (NaCl 8.0 g, Na_2HPO_4

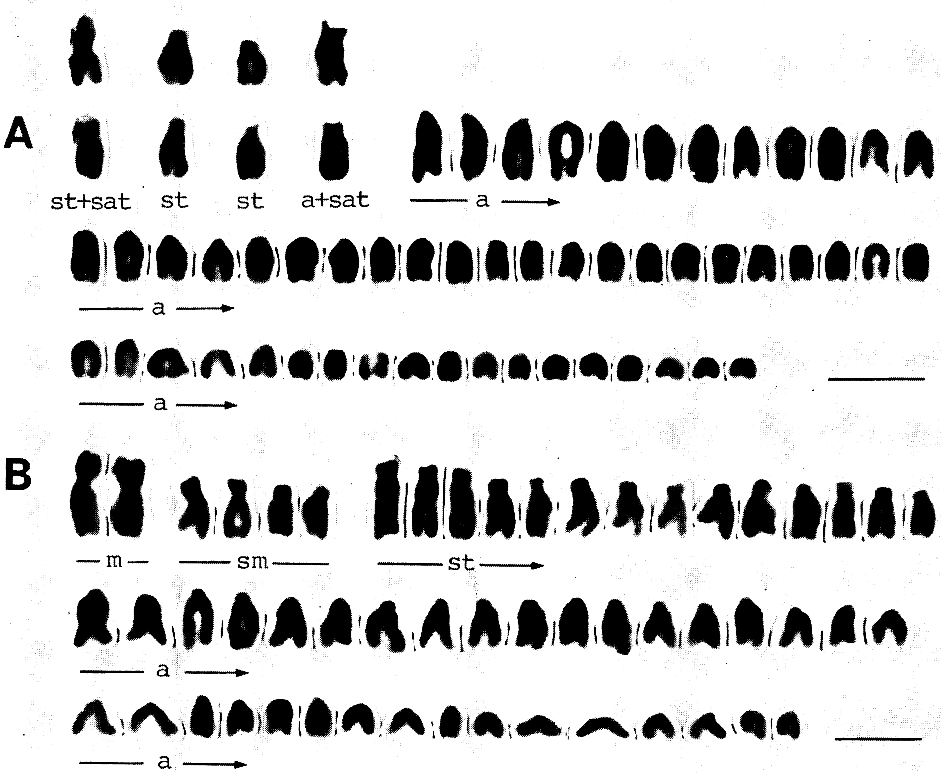

Fig. 1. Karyotypes of two species of *Osteoglossum*. A: *O. bicirrhosum*, 2n=56. SAT-chromosomes and other subtelocentric chromosomes from a nuclear plate other than that karyotypes in A are shown above A, to show the presence of satellites on only two chromosomes. B: *O. ferreirai*, 2n=54. a, acrocentric; m, metacentric; sat, satellite; sm, submetacentric; st, subtelocentric. Scale bars indicate 5 μm.

1.15 g and KH_2PO_4 0.2 g in 1 *l* of distiled water) was used to dissolve tripsin. Then the tissues were treated with 0.075 M KCl hypotonic solution for 30～40 minutes and fixed in 3: 1 methanol-acetic acid. The tissues thus fixed were cut into pieces, and tissue pieces were tapped onto slide glasses. Further preparation procedures were made in accordance with the routine flame-drying and Giemsa-staining methods. For detecting nucleolus organizer regions (NORs), the silver-staining techniques devised by Kodama et al. (1980) were employed. The classification of chromosomes followed Levan et al. (1964).

Results

Osteoglossum bicirrhosum. Chromosome counts for well-spread metaphase plates showed a modal diploid number of 56 in 119 of the 160 cells scored (Table 1). The chromosome number of *O. bicirrhosum* is determined as 2n=56. The karyotype from 24 excellent metaphase plates consisted of three subtelocentric chromosomes and 53 acrocentric chromosomes (Fig. 1A). One of the subtelocentric chromosomes and one of the acrocentric chromosomes had a satellite on each of their short arms (st+sat and a+sat in Fig. 1A). The existence of satellites on these chromosomes was confirmed in Ag-stained plates; intensely stained NORs were observed on two chromosomes, one appearing to be subtelocentric and the other acrocentric (Fig. 2). One of the remaining two subtelocentric chromosomes in the nuclear plate karyotyped in Fig. 1A appeared to have satellites. However, the presence of satellites on that chromosome was not indicated in other plates (one example is shown in Fig. 1), nor were there intensely stained NORs on chromosomes other than the above-mentioned two SAT-chromosomes (Fig. 2).

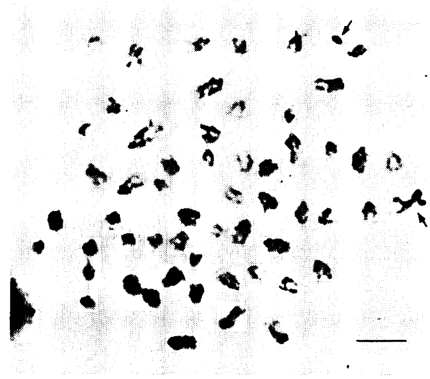

Fig. 2. Ag-stained chromosomes of *Osteoglossum bicirrhosum* with a subtelocentric chromosome bearing NORs (arrow, upper right) and an acrocentric chromosome bearing NORs (arrow, middle right). Scale bar indicates 5 μm.

Osteoglossum ferreirai. The chromosome number of this species is determined as 2n=54 on the basis of chromosome count frequencies for 30 well-spread plates (Table 1). The karyotype from eight excellent metaphase plates was composed of two large metacentric, four submetacentric, 14 subtelocentric and 34 acrocentric chromosomes (Fig. 1B). The metacentic chromosomes may appear to be subtelocentric, but their arm ratios based on precise measurements fell within the limit of metacentrics. Satellites were not observed.

Discussion

Uyeno (1973) described the karyotype of *Osteoglossum bicirrhosum* as consisting of one submetacentric, one subtelocentric and 54 acrocentric chromosomes. Our finding agrees with Uyeno's (1973) description in chromosome number, but not in karyotype. The subtelocentric chromosome with satellites observed in our study seems to correspond to Uyeno's (1973) submetacentric chromosome. The karyotype of *O. bicirrhosum* is characterized by the con-

Table 1. Frequency distribution of diploid chromosome counts in *Osteoglossum bicirrhosum* and *O. ferreirai*.

Species	No. of cells scored	2n						
		50	51	52	53	54	55	56
O. bicirrhosum	160	1	3	4	8	11	14	119
O. ferreirai	30	1	2	2	3	20	2	

stitution made up solely of subtelo- and acrocentric chromosomes, if our observations are correct, and the chromosome number (56) which is highest among the 14 osteoglossiform species thus far studied cytologically (Uyeno, 1973; Urushido et al., 1975; Beamish and Uyeno, 1978; present study).

The karyotype of *Osteoglossum ferreirai* differs from that of *O. bicirrhosum* among others in the lower number of chromosomes by two and the presence of two large-sized metacentric chromosomes. This suggests the occurrence of either fusion of the Robertsonian type or fission of chromosomes in the course of chromosomal differentiation of the two species. However, this alone cannot explain satisfactorily the differences in the karyotypes of the two species, such as the heteromorphism in *O. bicirrhosum* and the presence of submetacentrics in *O. ferreirai*. Some mechanisms of chromosomal rearramgement other than fusion and fission have certainly taken part in their chromosomal differentiation.

Acknowledgment

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オステオグロッスム属2種の染色体

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オステオグロッスム科の2種，*Osteoglossum bicirrhosum* と *O. ferreirai* の染色体を調査した．*O. bicirrhosum* は 2n=56 の染色体をもち，核型は 3 st +53 a，次端部着糸型と端部着糸型染色体のそれぞれ1個には付随体が観察された．*O. ferreirai* の核型は 2n=2 m+4 sm+14 st+34 a=54 であり，前種とはかなりの相違を示した．*O. ferreirai* には大型の二腕染色体があり，しかも染色体が2個少ないところから，両者の染色体の分化には fusion あるいは fission の機構が働いたものと考えられる．しかし，*O. bicirrhosum* には heteromorphism がみられ，また *O. ferreirai* には多数の次中部着糸型染色体があるところから，両種間の染色体分化には上述の機構以外の染色体再編成が関与しているものと考えられる．

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