Chromosome description and localization of Nucleolus Organizing Regions (NORs) by Ag-staining Technique in *Alburnus filippii* (Cyprinidae, Cypriniformes) in Anzali Lagoon, North Iran

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Received: March 2009 Accepted: April 2010

Keywords: *Alburnus filippii*, Nucleolus Organizer Region (NOR), Karyotype

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The silver staining procedure is commonly used to visualize the activity of ribosomal RNA genes, since it has been established that only those NORs of metaphase chromosomes that are functionally active during the preceding interphase are capable of staining (Galetti *et al.,* 1984; Schmid *et al.,* 2006). Nucleolus organizer regions (NORs) are used as markers to indicate intra- and inter-species chromosomal polymorphism in many groups of fish (Ra´b *et al.,* 2008). There are no available reports specifying the activity of nucleolus organizer regions location in Kura bleak (*A. filippii*) chromosomes by silver staining. The aim of this paper is to present the chromosome complement, number and location of the nucleolar organizer regions (NORs) of *A. filippii* (Kessler, 1877), commonly known as Kura bleak from northern Iran, a survey initiated by Gul et al. (2006).

The samples (six females and four males) were collected in 2006 from Anzali Wetland (37º28'N, 49º27'E) Guilan, Iran. The fish were alive transported to the laboratory and were kept for 72 h prior to processing (Pourkazemi et al., 2010). The preparation of chromosomes was performed according to Collares- Pereira *et al*. (1998) but reducing the colchicine incubation time. Detection of the nucleolus organizer regions (NORs) was done following the silver staining method of Howell and Black (1980) with slight modifications to reveal the presence of active rDNA clusters.

In the present study the number of diploid chromosomes were 2n=50, of which six pairs were metacentric (M), 9 pairs were submetacentric (Sm), 4 pairs were subtelocentric (St) and 6 pairs were acrocentric (a). The chromosomes are arranged in decreasing size. In this metaphase the fundamental number (number of chromosome arms) was therefore 88 and no heteromorphic sex chromosomes were observed.

Nucleolar organizer regions (NORs) were observed at the end of the short arms of one pairs of medium-sized subtelocentric chromosomes. NOR locations in *A. filippii* are presented in Figure1.

[DOR: 20.1001.1.15622916.2011.10.2.17.8]

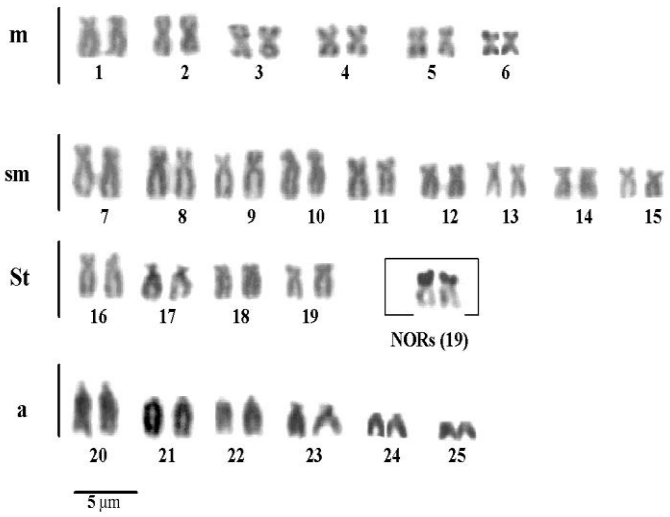

Figure1: Giemsa-stained karyotype of *Alburnus filippii*. M, Metacentric; Sm, Submetacentric; St subtelocentric and A, acrocentric chromosomes. The NOR-bearing chromosome pair is shown after silver impregnation in the box

The chromosomes of this leuciscine cyprinid fish have not been studied very. Gul et al. (2006) reported a diploid number 2*n* = 50 for *A. filippii* specimens of the Aras river basin in eastern Turkey, and a karyotype composed of 8 pairs of metacentric (m), 8 pairs of submetacentric (Sm) and 9 pairs of acrocentric (a) chromosomes. The presence of different populations, races and/or sub species arising from mutation, race improvement and hybridization with other indigenous species could be the possible explanation for differences in number and type of chromosomes reported in a species found distributed in different aquatic ecosystems (Ráb, and Collares-Pereira, 1995).

Our results are also consistent with the diploid chromosome number reported for *A. filippii* of the Aras river basin in eastern Turkey (Gul *et al.*, 2006) but with different chromosome arm number (NF) and karyotypic formula. The chromosome numbers and karyotypes of *A. filippii* in Anzali lagoon are similar to other European leuciscinae cyprinids, although some small differences are apparent, due to the difficulty of recognition of the centrometric position of small chromosomes. Cytogenetic studies conducted by Arkhipchuk (1999) in *Alburnus* showed similar karyotypes in *A. akili*, *A. doriae* and *A. alburnus* from Poland (2n = 50). The cytogenetic analysis of *A. alburnus* from Germany (Table 1) showed the same diploid number as reported by Schmid et al. (2006) but with different fundamental numbers (Germany: 14M+ 14SM + 8ST+ 14A; Poland: 32M/SM + 18ST/A). In addition, the nucleolus organizer regions were detected in one pair of submetacentric (Sm) chromosomes in the same study. This discrepancy could be explained by the lower technical resolution of the karyotype by Arkhipchuk (1999) although the hypotheses that some degree of chromosome divergence occurred cannot be excluded. The remaining members of this subfamily analyzed, showed evidence of low variation in diploid number and NF (from NF = 78 in *A. heckeli* to NF = 88 in *A. filippii*), like other families of the order Cypriniformes (Table 1).

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Table 1: Summary of the chromosome features of species of *Alburnus*

Species	Karyotype							References
	2n	NF	m	sm	st	a	NORs	
A. akili	50	18	82	32	-	-	-	Arkhipchuk, 1999
A. alburnus	50	18	82	32	-	-	-	Arkhipchuk, 1999
A. alburnus	50	86	14	14	8	14	sm	Schmid *et al.*, 2006
A. arborella	52	87	-	-	-	-	-	Vasil'ev, 1980
A. atropatenae	50							Klinkhardt *et al.*, 1995
A. caeruleus	50							Klinkhardt *et al.*, 1995
A. doriae	50	86	18	14	14	14	-	Arkhipchuk, 1999
A. filippii	50	88	12	18	8	12	st	**Present study**
A. hebes (1)	50							Klinkhardt *et al.*, 1995
A. heckeli	50	78	12	12	16	10	-	Simovic et al., 1994
A. hohenackeri	50							Klinkhardt *et al.*, 1995
A. kosswigi (2)	50							Klinkhardt *et al.*, 1995
A. microlepis(1)	50							Klinkhardt *et al.*, 1995
A. nasreddini	50							Vasil'ev, 1980
A. orontis	50							Vasil'ev, 1980
A. qalilus	50							Vasil'ev, 1980
A. scoranzoides	50							Vasil'ev, 1980
A. tarichi	50							Vasil'ev, 1980

diploid numbers (2n), fundamental number (NF), metacentric (m), submetacentric (sm), subtelocentric (st) and acrocentric (a). (1) = *Alburnus sellal*, (2) = *Alburnus escherichii*

However, Knowledge of chromosome morphology becomes more important in cases of closely related taxa with the same chromosome number and also may be a very useful tool to identify the species of *Alburnus* in different aquatic ecosystems. More sensitive techniques may be useful in identifying individual chromosomes and may reveal karyotype variations and possible phylogenetic relationships between species of *Alburnus*.

Acknowledgments

The study was supported by the University of Gilan. We thank Gh. Khalili, K. Bakhshalizadeh and Sh. Bakhshalizadeh for kindly providing fish for the study and for the technical support. We thank the Faculty of Natural Resources and Dr. Dadman International Sturgeon Research Institute. We are also grateful to the anonymous reviewers for their valuable comments and advice.

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[DOR: 20.1001.1.15622916.2011.10.2.17.8]

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