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RESEARCH PAPER

Cytogenetic Analysis of *Seminemacheilus Lendlii* (Hanko, 1925) (Teleostei: Nemacheilidae)

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Abstract

The aim of this study is to reveal chromosomal properties of endemic *Seminemacheilus lendlii* fish species. Metaphase plates were obtained from kidney cells. The diploid chromosome number was observed 2n=50; consisting of eight pairs of metacentric, 10 pairs of submetacentric and seven pairs of subtelocentric chromosomes. The fundamental number of chromosomal arms (FN) was found as 86. Positive C-bands were seen on the centromeric region for several chromosomes. Also, heterochromatic blocks were determined on the short and/or long arms of some chromosome pairs. Four nucleolus organizer regions (NORs) were observed on the silver stained metaphase plates. This study may contribute to cytogenetic data of Anatolian loaches.

Keywords: Anatolian loach, chromosome, karyotype, C-banding, nucleolus organizer region (NOR).

***Seminemacheilus lendlii* (Hanko, 1925) (Teleostei: Nemacheilidae)'nin Sitogenetik Analizi**

Özet

Bu çalışmanın amacı; *Seminemacheilus lendlii*'nin kromozomal özelliklerini ortaya çıkarmaktır. Metafaz plakları böbrek hücrelerinden elde edildi. Diploit kromozom sayısı 2n=50 olmak üzere karyotipinin sekiz çift metasentrik, 10 çift submetasentrik ve yedi çift subtelosentrik kromozomdan oluştuğu belirlendi. Kol sayısı (FN) 86 olarak hesaplandı. C-bantlar çok sayıda kromozomun sentromerinde belirlendi. Ayrıca bazı kromozomların kısa ve/veya uzun kolları üzerinde heterokromatik bloklar gözlendi. Gümüş boyalı metafazlarda dört nükleolus organizatör bölge (NOR) tespit edildi. Bu çalışmanın Anadolu çöpçü balıklarının sitogenetiğine katkı sağlayacağı düşünülmektedir.

Anahtar Kelimeler: Anadolu çöpçü balığı, kromozom, karyotip, C-bantlama, çekirdekçik organize edici bölge (NOR).

Introduction

The family Nemacheilidae has 40 species in the inland waters of Turkey. One of these species belongs to *Barbatula* genera; 35 of them *Oxynoemacheilus* genera; one *Paracobitis* genera; two *Seminemacheilus* genera, both endemic for Turkey; and one *Turcinoemacheilus* genera (Kuru *et al*., 2014). Anatolian loach *Seminemacheilus lendlii* (Hanko, 1925) was recorded as *Nemacheilus lendli* by Geldiay and Balık (1988) and as *Noemacheilus lendli* by Kuru (2004). *S. lendlii* was considered to be a member of the family Cobitidae in both studies. Fricke *et al*. (2007) were reported that this species belongs to Balitoridae family. However, recently it has been shown that *S. lendlii* belongs to Nemacheilidae family (Freyhof *et al*., 2011). As mentioned above taxonomy of the nemacheilid loaches is very complex and problems still exist. *S. lendlii*, which is an endemic species to our inland waters, distributed in Black Sea basins and Middle Anatolia's lake basins (Fricke *et al*., 2007).

Chromosomal studies have been carried out for many years in Anatolian fish species. These studies contribute to fish systematics and taxonomy. Despite the rich species number of the Anatolian loaches the chromosomal studies are inadequate (Değer, 2011; Gaffaroğlu *et al*., 2012). The aim of this study is to reveal C-banding and AgNOR staining properties of *S. lendlii* in addition to basic karyotypic analysis.

Materials and Methods

Nineteen specimens (12 females, 7 males) of *S. lendlii* were collected from Sultansazlığı, Kayseri, Turkey (38°22'N, 35°21'E). These specimens were

carried live to the Ahi Evran University Genetics Laboratory. Chromosome preparations were prepared according to Collares-Pereira (1992)'s "Air Drying Technique". Sumner (1972)'s C-banding technique was performed for detection constitutive heterochromatin regions while Howell and Black (1980)'s silver ($AgNO_3$) staining method was used for identify NORs. Metaphase slides were photographed in Leica DM 3000 microscope. Chromosomes were measured by digital caliper and karyotype was arranged manually. Chromosomes were classified according to Levan *et al*. (1964).

Results

Totally 127 Giemsa stained metaphase plates were counted for determining the diploid chromosome number. The diploid chromosome number of *S. lendlii* was 2n=50; consisting of eight pairs of metacentric (m), 10 pairs of submetacentric (sm) and seven pairs of subtelocentric (st) chromosomes (Figure 1a, b). FN was found as 86. Sex chromosomes were not determined. C-bands were observed on the centromeres of numerous chromosomes (Figure 1c). Additional heterochromatic blocks were determined on the short and/or long arms of some chromosome pairs (Figure 1c). The NORs were observed on the short arms of two submetacentric chromosomes pairs. Also on the some metaphases NORs were observed on the short arms of one, two or three chromosomes (Figure 1d).

Discussion

The number of diploid chromosomes of *S. lendlii* is the same with the result of Sember *et al*.'s research (2015) but chromosome morphologies and FN's are different (Table 1). The chromosome morphologies and FN's of same families's species pose differences from each other (except *O. tigris*) (Table 1). In addition, it was observed that *S. lendlii* has the same number of diploid chromosomes with other loach species spreading in Anatolia but their chromosome morphologies pose a slight difference (Table 1). Also, *S. lendlii* has the same number of diploid chromosomes as many species of *Barbatula* and *Nemacheilus* too (Arai, 2011). Furthermore, it was also observed that *S. lendlii* is similar to many species from *Cobitis*, *Misgurnus* and *Sabanejewia* genera spreading in different countries in terms of the number of diploid chromosomes (Arai, 2011). On the other hand, as reported on *T. kosswigi* (Gaffaroğlu *et al*., 2012), *O. angorae* (Kaya *et al*., 2005), *O. persa* and *O. tongiorgii* (Esmaeili *et al*., 2015) sex chromosomes were not observed in *S. lendlii* too. The similar result was reported for the other loach species by Değer (2011) as well.

It was reported that C-banding was frequently being used in karyo-systematic studies and was important in specifying the phylogenetic relationships between the species. Constitutive heterochromatin regions can be detected with this banding method. These regions are mostly localized in the centromere of the chromosomes. The settlements and the sizes of these sections can differ by species (Arslan and Arslan, 2007). *S. lendlii* shows high similarity with Sember *et al*. (2015)'s study about C-banding phenotype. This study indicates that C-bandings pose similarities with *Cobitis elazigensis*, *Oxynoemacheilus argyrogramma*, *O. frenatus* and *Oxynoemacheilus* sp. in terms of the settlement of the centromeres of the chromosomes (Değer, 2011). It was observed that many chromosomes in the centromere of *T. kosswigi* (Gaffaroğlu *et al*., 2012) had less density compared to *S. lendlii*. In addition, the heterochromatic sections monitored as a block in some chromosomes of *T. kosswigi* (Gaffaroğlu *et al*., 2012), was observed in this study too (Fig. 1c). These heterochromatic blocks were may be formed after pericentric inversions and/or centric fusions (Boron, 1995).

The NOR sections where the rRNA genes in the chromosomes are mostly repeated can be observed with silver staining. The number and settlement of NOR can be unique to species, even to the populations. NOR can generally be located at the end of the short arms of the chromosomes, sometimes at the end of the long ones (Mayr *et al*., 1986; Amemiya and Gold, 1990; Rab *et al*., 1990; Gaffaroğlu, 2003). In terms of NOR phenotype *S. lendlii* shows similarity with Sember *et al*. (2015)'s study. While *S. lendlii* indicates similarities with *O. frenatus* and *Oxynoemacheilus* sp. which have NOR in two pairs chromosomes in terms of the number of NOR's, it indicates differences with *C. elazigensis* and *O. argyrogramma* which have NOR in a pair chromosome (Değer, 2011). Furthermore, the NOR's detected in two pairs of chromosome in *S. lendlii* was also different from some species of *Cobitis*, *Nemacheilus* and *Sabanejewia* (Arai, 2011). In terms of NOR settlement, it indicates similarities with *C. elazigensis* which has NOR at the end of the short arms of the chromosomes; however, it indicates differences with *O. argyrogramma*, *O. frenatus* and *Oxynoemacheilus* sp. having NOR at the end of the long arms of the chromosomes (Değer, 2011). In addition, Değer (2011) had been reported that NOR's were observed in submetacentric chromosomes of *C. elazigensis* and *O. argyrogramma* while in the acrocentric chromosome of *O. frenatus* and *Oxynoemacheilus* sp. NOR localities of *S. lendlii* has been similar to *C. elazigensis* and *O. argyrogramma*. On the other hand, NOR number polymorphism that observed in *S. lendlii* has been reported in many fish species. The reason for this situation could based on the differentiation of cistron numbers and transcriptional activity (Miller *et al*., 1976; Warburton and Henderson, 1979; Gaffaroğlu, 2003).

In conclusion, chromosomal properties of

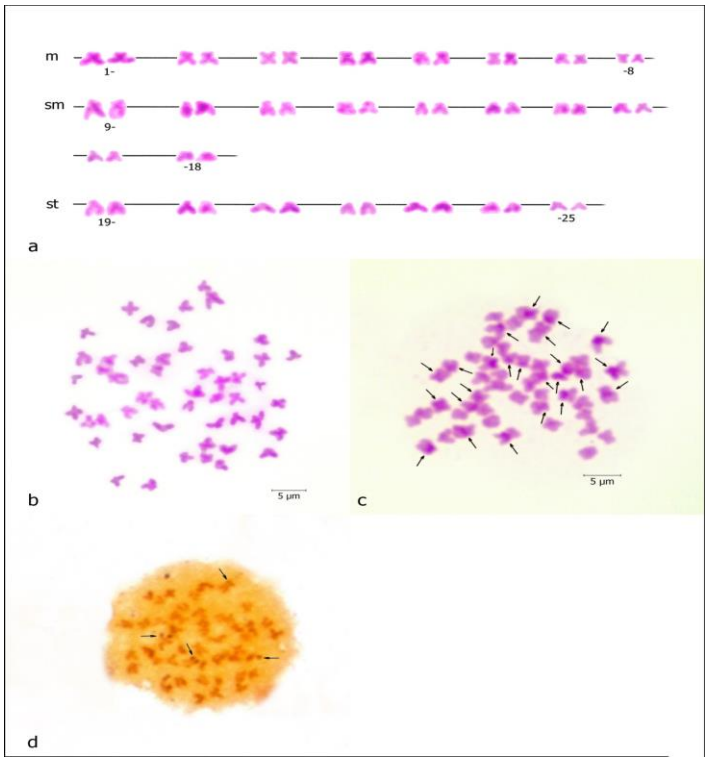

Figure 1. (a and b) Karyotype and Giemsa stained metaphase plate (c) C-banded metaphase plate (d) $AgNO_3$-stained metaphase plate of *S. lendlii* species.

Table 1. Chromosomal studies carried out in some representatives of the superfamily Cobitoidea distributed in the Anatolia

Species	2n	Chromosome morphology	FN	References
Oxynoemacheilus angorae	50	14m+14sm+22a	78	Kaya *et al*., 2005
Oxynoemacheilus tigris	50	18m+18sm+14a	86	Kılıç, 2006
Oxynoemacheilus panthera	50	14m+18sm+18a	82	Tanrıkulu, 2008
Cobitis elazigensis	50	18m-sm+32a	68	Değer, 2011
Oxynoemacheilus argyrogramma (Tigris River population)	50	44m-sm+6a	94	Değer, 2011
Oxynoemacheilus argyrogramma (Euphrates River population)	50	42m-sm+8a	92	Değer, 2011
Oxynoemacheilus frenatus	50	32m-sm+18a	82	Değer, 2011
Oxynoemacheilus sp.	50	30m-sm+20a	80	Değer, 2011
Turcinoemacheilus kosswigi	50	8m+14sm-st+28a	72	Gaffaroğlu *et al*., 2012
Seminemacheilus lendlii	50	16m+24sm+10st-a	90	Sember *et al*., 2015
Seminemacheilus lendlii	50	16m+20sm+14st	86	In this study

endemic *S. lendlii* have been determined for the first time. This study will be contributed to loach cytogenetic and taxonomy.

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References

Amemiya, C.T. and Gold, J.R. 1990. Cytogenetic studies in the North American minnows (Cyprinidae). Hereditas, 112: 231-247.

Arai, R. 2011. Fish karyotypes, A Check List. Springer, Japan, pp.

Arslan, A. and Arslan, E. 2007. Karyosistematik de C-bantlama (Konstitutif heterokromatin)'nın önemi. Selçuk Üniversitesi Fen Edebiyat Fakültesi Fen Dergisi, 29: 121-126.

Boron, A. 1995. Chromosome banding studies of *Noemacheilus barbatulus* (Linnaeus, 1758) from Poland. Caryologia, 48(3-4): 239-246.

Collares-Pereira, M.J. 1992. *In vivo* direct chromosome preparation (Air Drying Technique). In: 1st Int. Workshop on Fish Cytogenetic Techniques,

Concarneau-France, pp. 15-19.

Değer, D. 2011. Dicle ve Fırat su sistemlerinde yaşayan bazı Cobitoidea türleri üzerine karyolojik araştırmalar. Doktora Tezi. Diyarbakır: Dicle Üniversitesi.

Esmaeili, H.R., Pirvar, Z., Ebrahimi, M. and Geiger, M.F. 2015. Karyological and molecular analysis of three endemic loaches (Actinopterygii: Cobitoidea) from Kor River basin. Iran Molecular Biology Research Communications, 4(1): 1-13.

Freyhof, J., Erk'akan, F., Özeren, C. and Perdices, A. 2011. An overview of the western Palaearctic loach genus *Oxynoemacheilus* (Teleostei: Nemacheilidae). Ichthyological Exploration of Freshwaters, 22(4): 301-312.

Fricke, R., Bilecenoglu, M. and Sari, H.M. 2007. Annotated checklist of fish and lamprey species (Gnathostoma and Petromyzontomorphi) of Turkey, including a red list of threatened and declining Species. Stuttgarter Beitrage zur Naturkunde Serie A (Biologie), 706: 1-172.

Gaffaroğlu, M. 2003. Karakaya Baraj Gölü'nde yaşayan Cyprinidae familyasına ait bazı türlerin karyolojik analizleri. Doktora Tezi. Malatya: İnönü Üniversitesi.

Gaffaroğlu, M., Karasu, M. and Unal, S. 2012. Karyotype of river loach *Turcinoemacheilus kosswigi* Bănărescu and Nalbant, 1964 (Cypriniformes, Balitoridae) from the Euphrates River, Turkey. Journal of Agricultural Science and Technology, 14: 821-826.

Geldiay, R. and Balık, S. 1988. Türkiye tatlısu balıkları. Ege Üniversitesi Basımevi, İzmir.

Howell, W.M. and Black, D.A. 1980. Controlled silver staining of nucleolus organizer regions with a protective colloidal developer: a 1 step method. Experientia, 36: 1014-1015.

Kaya, T.Ö., Gül, S. and Nur, G. 2005. Karyotype analysis in *Orthrias angorae* (Steinctachner, 1897). Kafkas Universitesi Veteriner Fakültesi Dergisi, 11(2): 137-140.

Kılıç, B. 2006. Kura-Aras Havzasından *Orthrias tigris* (Heckel, 1843)'de kromozomal çalışmalar. Yüksek Lisans Tezi. Kars: Kafkas Üniversitesi.

Kuru, M. 2004. Türkiye içsu balıklarının son sistematik durumu. Gazi Üniversitesi Gazi Eğitim Fakültesi Dergisi, 24(3): 1-21.

Kuru, M., Yerli, S.V., Mangıt, F., Ünlü, E. and Alp, P. 2014. Fish biodiversity in inland waters of Turkey. Journal of Academic Documents for Fisheries and Aquaculture, 3: 93-120.

Levan, A., Fredga, K. and Sandberg, A.A. 1964. Nomenclature for centromeric position on chromosomes. Hereditas, 52: 201-220.

Mayr, B., Rab, P. and Kalat, M. 1986. NORs and counterstain-enhanced fluorescence studies in Cyprinidae of different ploidy level. Genetica, 69: 111-118.

Miller, O.J., Miller, D.A., Dev, V.G., Tantravahi, R. and Crocef, C.M. 1976. 184 Expression of human and suppression of mouse nucleolus organizer activity in mouse-human 185 somatic cell hybrids. Proceedings of the National Academy of Sciences of the United States 186 of America, 73(12): 4531-4535.

Rab, P., Roth, P. and Arefjev, V.A. 1990. Chromosome studies of European leuciscine fishes (Pisces, Cyprinidae) karyotype of *Aspius aspius*. Caryologia, 43(3-4): 249-255.

Sember, A., Bohlen, J., Šlechtová, V., Altmanová, M., Symonová, R. and Ráb, P. 2015. Karyotype differentiation in 19 species of river loach fishes (Nemacheilidae, Teleostei): extensive variability associated with rDNA and heterochromatin distribution and its phylogenetic and ecological interpretation. BMC Evolutionary Biology, 15: 251. DOI: 10.1186/s12862-015-0532-9.

Sumner, A.T. 1972. A simple technique for demonstrating centromeric heterochromatin. Experimental Cell Research, 75: 304-306.

Tanrıkulu, D. 2008. Kura Aras Havzası'nda bulunan Çöpçü Balığı'nda (*Orthrias panthera*, Heckel 1843) karyotip analizi. Yüksek Lisans Tezi. Kars: Kafkas Üniversitesi.

Warburton, D. and Henderson, A.S. 1979. Sequential silver staining and hybridization in situ on nucleolus organizing regions in human cells. Cytogenetics and Cell Genetics, 24: 168-175.