

The karyotype of *Hyla savignyi* Audouin, 1827 (Amphibia: Anura) from Southern Armenia

A. Martirosyan, I. Stepanyan

Institute of Zoology, Armenian Academy of Sciences, P. Sevak 7, Yerevan 0014, Armenia
E-mail : ilona_e@pochta.ru

Abstract. Karyotypes, meiosis and spermatogenesis of *Hyla savignyi* Audouin, 1827 from Southern Armenia were studied for the first time by conventional Giemsa staining, C- and Ag-banding methods. A karyotype with $2n=24$ was revealed. All chromosomes are macrochromosomes (8M+12sM+4sT, NF=48) and form bivalents in meiosis I. A secondary constriction was found in both sexes on the 9-th autosome pair which was NOR-positive. Blocks of C-heterochromatin were revealed on the short arms of pairs 3 and 8 and on the long arms of pairs 7 and 9. Only one specimen (female) had an additional NOR on one of homologues of pair 5. A comparison of the karyotypes of *H. savignyi* and *H. arborea schelkownikovi* Chernov, 1926 reveals their close relationship.

Key words: Amphibia, *Hyla*, karyotypes, C-heterochromatin, NOR, chromosome polymorphism.

INTRODUCTION

Savigny's tree frog, *Hyla savignyi* Audouin, 1827, is widespread in Southern Armenia, while Shelkownikov's tree frog, *Hyla arborea schelkownikovi* Chernov, 1926, is often met in Northern Armenia. According to earlier literature data, Savigny's tree frog was considered as a subspecies of the European tree frog *Hyla arborea savignyi* (Nikolsky, 1918; Chernov, 1939; Dal, 1954). However, recent studies have shown that Savigny's tree frog differs from the European tree frog in ecology, morphology (absence of anterodorsally-oriented loop), mating calls, and biochemical peculiarities (Schneider, Nevo, 1972; Egiastian, Schneider, 1990). In the light of recent data, the Savigny's tree frog is separated as a distinct species (Bannikov et al., 1977; Ananjeva et al., 1998). Nevertheless, the karyotype of tree frogs from Armenia still is not studied.

In present study, comparison of data on chro-

mosomal sets of Savigny's tree frog and Shelkownikov's tree frog from Armenia with regards to their systematic position is presented for the first time.

MATERIAL AND METHODS

The *Hyla savignyi* Audouin, 1827 were collected in 2003 and 2004 from the Ararat valley in two southern regions of Armenia. The karyotypes of two females and two males from the vicinity of the village Urtsadzor (Ararat region; 39°55'N/44°50'E; 1110 m) and four males and four females from the vicinity of the town Ejmiatsin (Armavyr region; 40°09'N/44°17'E; 870 m) were studied.

Chromosome smears were prepared from bone marrow, spleen and testes as described in Haertel et al. (1974) and Macgregor, Varley (1986). C-banding was done according to Sumner (1972) with some modifications. Silver staining was performed following the Howell, Black (1978) technique. Chromosome slides were examined



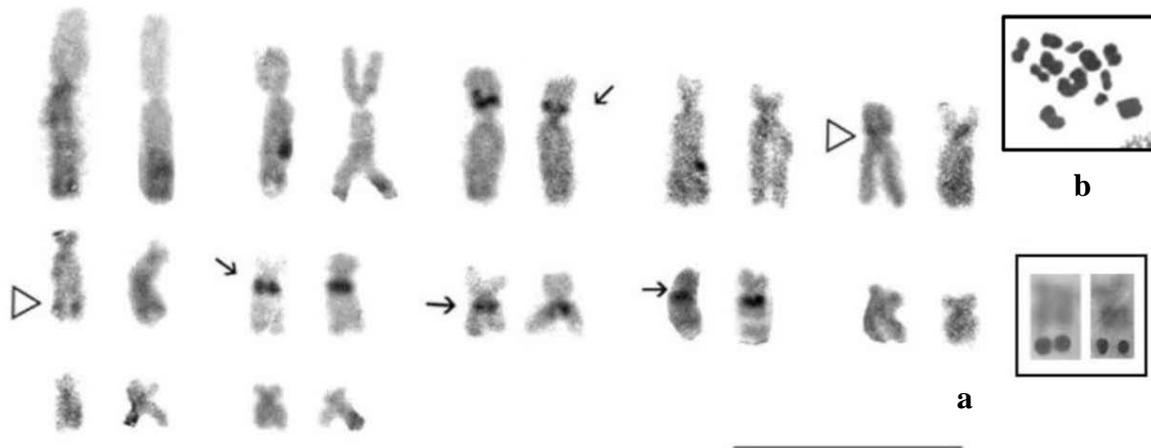


Fig. 1. Male chromosomes of *Hyla savignyi* from Armenia. **a** - C-banded karyogram. Arrows indicate C-positive regions. White triangles indicate weakly marked C-banded regions. NOR-bearing chromosomes are framed. **b** - diakinesis. Bar = 10 μ m.

under "NU-2E" (K. Zeiss, Germany) microscope, with a 1125 magnification (90 x 12.5). Homologous chromosome pairs were identified according to the classification of Levan et al. (1964).

RESULTS

The chromosome complements of *Hyla savignyi* from both regions of Southern Armenia are similar and comprise 24 chromosomes (NF=48). The karyotypes consist of 4 pairs of metacentric, 6 pairs of submetacentric and 2 pairs of subtelocentric chromosomes. A secondary constriction was observed in the long arm of the 9-th pair in both sexes (Figs 1, a; 2, a, c).

The karyotype of one female collected near Urtsadzor displayed dimorphism in the 5-th pair of chromosomes, in which the large submetacentric homologue possesses a secondary constriction in the short arm, while a middle-sized submetacentric homologue has no secondary constriction (Fig. 2, b, c).

At male diakinesis 12 ring-shaped, stick-shaped, and half-round-shaped bivalents were revealed (Fig. 1, b).

Blocks of C-heterochromatin were observed in the interstitial regions of the short arms of the 3-rd (submetacentric) and 8-th (metacentric) pairs as well as in the long arms of the 7-th and 9-th (submetacentric) pairs in the karyotypes of all males (Fig. 1, a). Weakly stained C-banded regions also appeared on the pericentromeric part of the short arm of the 5-th pair and the terminal part of the long arm of the 6-th pair of chromosomes (Fig. 1, a). Ag-banding revealed an argentophilous body placed on the 9-th pairs of chromosomes of all males and five females (Figs 1, a; 2, a, b). In addition, a NOR-bearing region appeared on the satellite of a large submetacentric chromosome pair 5 in the karyotype of one female from Urtsadzor (Fig. 2, b, c).

DISCUSSION

Hylidae are a large family of tree frogs widespread in the American, Eurasian and Australian continents, and consist of approximately 870 known species, combined into four subfamilies (Faivovich et al., 2005).

The diploid numbers of chromosomes in most



Fig. 2. Female chromosomes of *Hyla savignyi* from Armenia. **a** - mitotic metaphase of normal female. **b**- Mitotic metaphase of a deviant female. **c** - karyogram of a deviant female. Arrows indicate NOR-positive regions. NOR-bearing chromosomes of female are shown in the frame. Bar = 10 μ m.

of the Hylidae range from 22 to 34 (Morescalchi, 1965, 1973, 1977; Stephenson, Stephenson, 1970; Schmid, 1978; Wiley, Little, 2000; Wiley, 2003; Schmid, Steinlein, 2003). The majority of species within the genus *Hyla* have $2n=24$ (NF=48) (Morescalchi, 1973, 1977; Miura, 1995; Mable, Bogart, 1995; Schmid, Steinlein, 2003; Wiley, 2003; Al-Shehri, Al-Salech, 2005) and include metacentric and submetacentric chromosomes. The karyotype of *H. savignyi* from Armenia also includes $2n=24$, however it consists of metacentric, submetacentric and subtelocentric chromosomes.

The morphometric analysis carried out by conventional Giemsa staining and Ag-banding has shown that two homologues of the 9-th pair of chromosomes in all males and five females of *H. savignyi* from Armenia have secondary constrictions in the long arms. However, the chromosomal complement of one female had an additional secondary constriction on the short arm of one of homologues of autosomal pair 5. Heterozygosity had previously been documented for a male *Xenopus laevis* (Daudin, 1802) (Pipidae), which had lost one of the two NORs in the 12-th autosomal pair. This individual was viable, and showed a

Table. The karyotype features of *Hyla savignyi* and *H. arborea schelkownikovi*. 2n - diploid number of chromosomes, NF- fundamental number of chromosome arms, Sec. cns. - second constriction, Sex - sex chromosomes, C-bd - C-positive heterochromatic blocks, NOR – localization of nucleolar organizer regions. m - metacentric, sm - submetacentric, st - subtelocentric chromosomes; p - short arm, q - long arm.

Species	Population	2n	NF	Sec. cns	Sex	C-bd	NOR	Karyotype formula	Sources
<i>Hyla savignyi</i> (males, females)	Armenia (Ejmiatsin Urtsadzor)	24	48	9-th	-	2, 4, 7, 8,9	9q	8m+12sm+4st	Authors' data
	Urtsadzor	24	48	5-th 9-th	-	-	5p 9q		
	Saudi Arabia	24	48	9-th	9 XX/ XY	-	9q	10m+10sm+4a	Al-Shehri, Al-Salech, 2005
<i>H. arborea schelkownikovi</i> (males)	Armenia (Alaverdi)	24	48	9-th	-	-	9q	12m+8sm+4st	Authors' data

single nucleolar constriction and a single nucleolus (Kahn, 1962; Birnstiel et al., 1966). The present study documents an additional case of NOR heterozygosity in amphibians.

According to Al-Shehri and Al-Salech (2005), the 9-th chromosome pair of *H. savignyi* is represented by submetacentric sex chromosomes of the XY type in males and XX in females. The X chromosome is longer than Y and had a secondary constriction in the long arms. We revealed a secondary constriction in the long arm of submetacentric chromosomes of chromosome pair 9 in all studied specimens (both, males and females), that is evidence that this chromosome pair does not consist of sex chromosomes. The heteromorphic pair 5 also can not be considered as a pair of sex chromosomes as was observed in one female specimen only. The absence of C- and Ag-banding data of chromosomal sets of *H. savignyi* from Saudi Arabia points to that its karyotype was insufficiently investigated. As a result, the additional karyological data obtained by different staining methods of hylas from various populations are needed for reliable comparison of our new data.

Chromosomal sets of *H. savignyi* from Southern Armenia and Saudi Arabia (Al-Shehri, Al-Salech, 2005) are similar in diploid chromosomal number, fundamental number of chromosomal arms, presence of a chromosomal pair with secondary constriction, 4 pairs of metacentric, 5 pairs of submetacentric and 2 pairs of subtelocentric chromosomes, but differ in 2 pairs of metacentric, 2 pairs of submetacentric chromosomes and the sex chromosomes (Table). However, these differences can be the result of different methods of chromosome measurement. Thus, Al-Shehri and Al-Salech (2005) considered chromosome pairs 11 and 12 as acrocentric. According to our measurements, these pairs are subtelocentric. It is quite possible, that differences in measurement methods also affect the other chromosomal pairs.

The comparison of the karyotypes of *Hyla savignyi* and *H. arborea schelkownikovi* from Armenia shows similarities in their diploid number of chromosomes, fundamental number of chromosomal arms, second constriction bearing chromosomes (9-th pair) and morphology of 8 metacentric, 8 submetacentric, 4 subtelocentric chromo-



Fig. 3. Male chromosomes of *H. arborea schelkownikovi* from Armenia. **a** - karyogram, the pair of argemontophylous chromosomes is framed. Bar = 10 μ m.

somes (Fig. 3; Table). These species differ by 4 metacentric and 4 submetacentric chromosomes (Table). Unfortunately, C-banding patterns were not obtained in *H. arborea schelkownikovi* from Northern Armenia and our results were based on conventional Giemsa staining data.

Phylogenetic analyses, based on approximately 5100 base pairs from four mitochondrial (12S, tRNA, valine, 16S, and cytochrome b), and five nuclear genes, have shown close similarity between these two species. As a result, *H. savignyi* and *H. arborea* fall into one cluster in "*H. arborea*" species group (Faivovich et al., 2005). Our study of the karyotypes of *H. savignyi* and *H. arborea* also reveals high affinity in their chromosome sets, although we have found some differences.

ACKNOWLEDGEMENTS

We would like to thank S. Pipoyan, T. Tadevosyan and M. Arakelyan for their help in preparing of the manuscript.

REFERENCES

- Ananjeva N.B., Borkin L.J., Darevsky I.S., Orlov N.L. 1998.** The encyclopedia of nature of Russia. Amphibians and Reptiles. Moscow. 576 p. (In Russian).
- Al-Shehri A.H., Al-Salech A.A. 2005.** Karyotype of amphibians in Saudi Arabia 2: The karyotype of *Hyla savignyi* // *J. Biol. Sci.* 5(6): 768-770.
- Bannikov A.G., Darevsky I.S., Ishenko W.G. 1977.** Identification key of amphibians and reptiles from fauna USSR. Moscow. 415 p. (In Russian).
- Birnstiel M.L., Wallace H., Sirlin J.L., Fischberg M. 1966.** Localization of the ribosomal DNA complements in the nucleolar organizer region of *Xenopus laevis* (pp. 431-448) // Vincent W., Miller O. (Eds.). *International Symposium on the Nucleolus. Its Structure and function.* Bethesda.
- Chernov S.A. 1939.** Herpetofauna of Armenian SSR and Nakhichevan ASSR // *Zool. Sb. Armyanskogo Filiala Acad. Nauk USSR.* 1: 79-194. (In Russian).
- Dal S.K. 1954.** Animals of Armenian SSR. 1. 415 p. (In Russian).
- Egiasaryan E.M., Schneider H. 1990.** The mating call of tree frogs in Armenia (Anura, Hylydae) // *Zool. Anzeig.* 225: 113-122.
- Faivovich J.N., Haddad C.F.B., Garcia P.C.A., Frost D.R., Campbell J.A., Wheeler W.C. 2005.** Systematic

review of the frog family Hylidae, with special reference to Hylinae: phylogenetic analysis and taxonomic revision // *Bull. American Mus. Natur. Hist.* 294: 1-240.

- Haertel T.D., Owezarszak A., Storm R.M. 1974.** A comparative study of the chromosomes from five species of the genus *Rana* (Amphibia, Salientia) // *Copeia*. 1: 109-114.
- Howell W.M., Black D.A. 1978.** A rapid technique for producing silver-stained nucleolus organizer regions and trypsin – Giemsa bands on human chromosomes // *Hum. Genet.* 43: 53-56.
- Kahn J. 1962.** The nucleolar organizer in the mitotic chromosome complement of *Xenopus laevis* // *Quart. J. Microbiol. Sci.* 103: 407-409.
- Levan A., Fredga K., Sandberg A.A. 1964.** Nomenclature for centromeric position on chromosomes // *Hereditas*. 52: 201-220.
- Macgregor H.C., Varley J.M. 1986.** Working with animal chromosomes. Moscow. 272 p. (Russian translation).
- Mable B.K., Bogart J.P. 1995.** Hybridization between tetraploid and diploid species of tree frogs (Genus *Hyla*) // *J. Hered.* 86: 432-440.
- Miura I. 1995.** The late replication banding patterns of chromosomes are highly conserved in the genera *Rana*, *Hyla* and *Bufo* (Amphibia: Anura) // *Chromosoma*. 103: 567-574.
- Morescalchi A. 1965.** Il corredo cromosomico di *Hyla arborea arborea* (L.) e di *Hyla regilla baird* and *girand*: Il problema del cromosomi sessuali e l'evoluzione del cariotipo di hyla // *Caryologia*. 18: 193-206.
- Morescalchi F. 1973.** Cytotaxonomy of the Amphibia, (pp. 240-244) // Chiarelli A., Capanna E. (Eds). *Cytotaxonomy and Vertebrate Evolution*. London-New York. 348 pp.
- Morescalchi A. 1977.** New developments in vertebrate cytotaxonomy. I. Cytotaxonomy of the amphibians // *Genetica*. 50: 179-193.
- Nikolsky A.M. 1918.** Fauna of Russia and Adjacent countries. Amphibians (Amphibia). Petrograd. 55p. (In Russian).
- Schmid M. 1978.** Chromosome banding in amphibia I. Constitutive heterochromatin and nucleolus organizer regions in *Bufo* and *Hyla* // *Chromosoma*. 66: 361-388.
- Schmid M., Steinlein C. 2003.** Chromosome banding in amphibia. XXIX. The primitive XY/XX sex chromosomes of *Hyla femoralis* (Anura, Hylidae) // *Cytogenet. Genome Res.* 101: 74-79.
- Schneider H., Nevo E. 1972.** Bio-acoustic study of the yellow-lemon tree frog, *Hyla arborea savignyi* Audouin // *Zool. Jb. Physiol.* 76: 497-506.
- Stephenson E.M., Stephenson N.G. 1970.** Karyotypes of two hylids // *Chromosoma*. 30: 38-50.
- Sumner A.T. 1972.** A simple technique for demonstrating centromeric heterochromatin // *Exp. Cell Res.* 75: 304-306.
- Wiley G.E., Little M.L. 2000.** Replication banding patterns of the diploid-tetraploid tree frogs *Hyla chrysoscelis* and *Hyla versicolor* // *Cytogenet. Cell Genet.* 88: 11-14.
- Wiley J.E. 2003.** Replication banding and FISH analysis reveal the origin of the *Hyla femoralis* karyotype and XY/XX sex chromosomes // *Cytogenet. Genome Res.* 101: 80-83.

Received August 14, 2007.

Accepted by N.V. Golub, October 10, 2007.

Published December 28, 2007.