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Chromosomal Analyses of *Cobitis phrygica* Battalgazi, 1944 and *C. simplicispina* Hanko, 1925 (Teleostei, Cobitidae)

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Summary The purpose of this study is to reveal diploid chromosome number, karyotype and chromosomal banding properties with C-banding and Ag-staining in *Cobitis phrygica* Battalgazi, 1944 and *C. simplicispina* Hanko, 1925 from Turkey. Metaphase chromosomes were obtained from kidney cells. Both species had a same diploid chromosome number of $2n=50$. Karyotypes were composed of four pairs of metacentric, four pairs of submetacentric and 17 pairs of subtelo-acrocentric chromosomes in *C. phrygica*, and eight pairs of metacentric, eight pairs of submetacentric and nine pairs of subtelo-acrocentric chromosomes in *C. simplicispina*. Fundamental arm numbers were calculated as 66 in *C. phrygica* and as 82 in *C. simplicispina*. C-bands were observed on the pericentromeric regions of most chromosomes in both species. Nucleolus organizer regions (NORs) were determined on one pair of chromosomes in both species.

Key words *Cobitis phrygica*, *Cobitis simplicispina*, Karyotype, C-band, Nucleolus organizer region, Turkey.

At least 18 species of the genus *Cobitis* belonging to Cobitidae are known in Turkey. It was reported that an endemic *C. phrygica* distributes in Acıgöl, Salda and Söğüt lakes watersheds of Turkey (Fricke *et al.* 2007, Kuru *et al.* 2014, Çiçek *et al.* 2015) and an endemic *C. simplicispina* distributes in the Sakarya and Kızılırmak basins and around the Tuz lake (Erk'akan *et al.* 2003). Also, some localities of *C. simplicispina* were recorded from Denizli and Muğla provinces (Yılmaz *et al.* 2006, Güçlü *et al.* 2013). Recently, the *Cobitis* that distributes in Turkey have been revised because of having complex and interesting problems about their systematics and phylogeny (Erk'akan *et al.* 1999). Erk'akan *et al.* (1999) pointed out that *C. phrygica* Battalgazi, 1944 should be a synonym of *C. simplicispina*. However, it was mentioned as *C. phyrigica* Battalgil, 1944 by Kuru (2004) and as *C. phrygica* Battalgazi, 1944 by Fricke *et al.* (2007) and Kuru *et al.* (2014). The problems in taxonomy are still continuing.

Chromosomal investigation is one of the research topics in fish for solving the systematic and taxonomic problems. However, difficulties in obtaining chromosomes from fishes and their chromosomes are to be small in length, being outnumbered and the lack of a standard method reduces success in this area (Ulupınar and Alaş 2002). Chromosomal study has been reported in only one species, *C. elazigensis* from Anatolia (Değer 2011). The aim of the present work is to describe chromosomal properties with conventional, Ag-staining and C-banding in endemic loaches of *C. phrygica* and *C. simplicispina* from Anatolia.

Materials and methods

Five female and one male specimens of *C. phrygica* were collected from Salda Lake, Burdur, Turkey (37°31′N, 29°43′E) and four female and two male specimens of *C. simplicispina* were collected from Küfi Creek, Denizli, Turkey (38°21′N, 29°50′E). The specimens were carried alive to the laboratory. Metaphase chromosome preparations were obtained by the air drying technique (Collares-Pereira 1992).

C-banding technique of Sumner (1972) and Ag-staining technique of Howell and Black (1980) were applied. Preparations were screened in a Leica DM 3000 microscope and photographs were taken with a CCD camera and an AKAS software. Chromosome shapes were classified according to Levan *et al.* (1964). For calculating of the fundamental arm number (FN) meta-submetacentric (m-sm) chromosomes were taken as biarmed whereas subtelo-acrocentric (st-a) chromosomes were taken as uniarmed.

Results

The diploid chromosome numbers of *C. phrygica* and *C. simplicispina* were $2n=50$ (Figs. 1A, 2A). Chromo-

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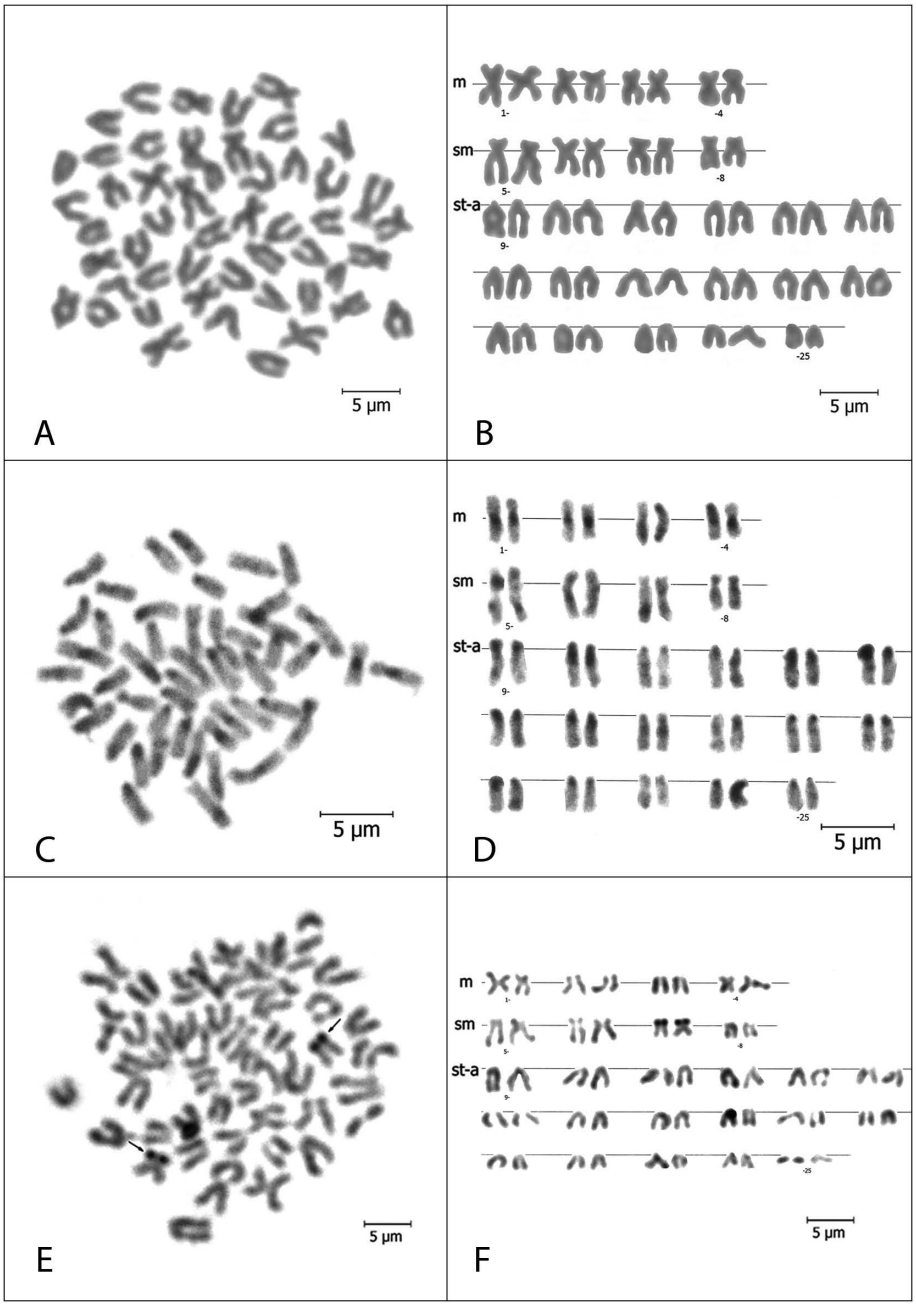

Fig. 1. Giemsa stained metaphase spread (A), arranged karyotype (B), C-banded metaphase spread (C) arranged karyotype (D) Ag-stained metaphase spread (E) and arranged karyotype (F) of *C. phrygica*. The arrows indicate the Ag-NORs.

somes were arranged as: four pairs of m, four pairs of sm and 17 pairs of st-a chromosomes in *C. phrygica* (Fig. 1B), and eight pairs of m, eight pairs of sm and nine pairs of st-a chromosomes in *C. simplicispina* (Fig. 2B). FN's were calculated as 66 in *C. phrygica* and as 82 in *C. simplicispina*. Sex chromosome was not detected in both species. C-bands were determined on the pericentromeric regions of most chromosome pairs in both species (Figs. 1C, 2C). Almost chromosomes have C-bands in both species, although the long arms of Nos. 5, 13, and 33–38 chromosomes show the interstitial C-bands in *C. phrygica* and 17 and 18 chromosomes show the interstitial C-bands in *C. simplicispina* (Figs. 1D, 2D). Furthermore, Ag-NORs were observed on the terminal regions of the short arms of sm chromosomes (chromosome No. 7) in *C. phrygica* (Fig. 1E, F) and on the terminal regions of the long arms of the chromosomes of the largest sm pair in *C. simplicispina* by Ag-staining (Fig. 2E). Additional Ag-NORs were observed on the terminal regions of the short arms of one pair of sm chromosomes in *C. simplicispina* on some Ag-stained metaphases of male and female specimens (Fig. 2F).

Discussion

Karyotypes of *Cobitis* are highly diversified in terms of chromosome shape but they share almost the same chromosome number $2n=50$ (Rab *et al.* 2007). *C. phry-*

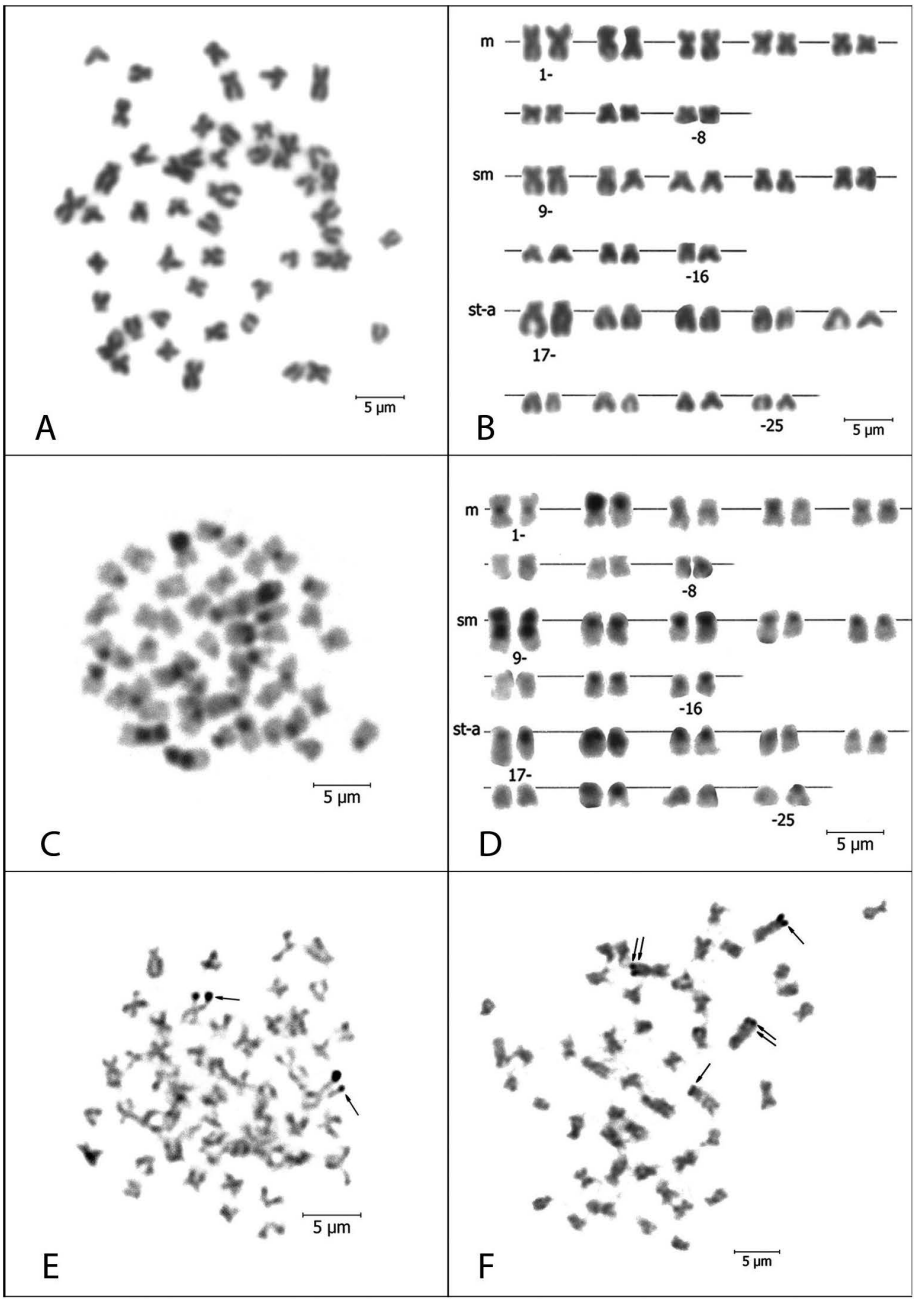

Fig. 2. Giemsa stained metaphase spread (A), arranged karyotype (B), C-banded metaphase spread (C), arranged karyotype (D), Ag-stained (E, F) metaphase spreads of C. simplicispina. The arrows indicate the Ag-NORs.

Table 1. Chromosomal data of Anatolian *Cobitis* species.

Species	2*n*	Karyotype	FN	References
C. elazigensis	50	18m-sm+32a	68	Değer (2011)
C. phrygica	50	8m+8sm+34st-a	66	This study
C. simplicispina	50	16m+16sm+18st-a	82	This study

gica and *C. simplicispina* showed similar morphology with other loaches. Also, their chromosome numbers are the same with the Anatolian endemic species *C. elazigensis* (Değer 2011) but their karyotypes are different (Table 1). Numbers of biarmed and uniarmed chromosomes of *C. phrygica* are similar with *C. elazigensis*. Otherwise, number of biarmed chromosomes of *C. simplicispina* is more than *C. elazigensis* whereas uniarmed chromosomes of *C. simplicispina* is less than *C. elazigensis*. Consequently, FN of *C. phrygica* is lower than *C. elazigensis* while FN of *C. simplicispina* is higher than *C. elazigensis*. *C. phrygica* and *C. simplicispina* have the same chromosome number with *C. calderoni*, *C. elongatoides*, *C. linea*, *C. maroccana*, *C. taurica* and *C. vardarensis* (Madeira *et al.* 1992, Rab *et al.* 2000, Rabova *et al.* 2001, Janko *et al.* 2005, Esmaeili *et al.* 2015). Nevertheless, st-a chromosomes of *C. elongatoides*, *C. linea*, *C. taurica* and *C. vardarensis* are lower than *C. phrygica and C. simplicispina*. Uniarmed chromosome numbers of *C. calderoni* and *C. maroccana* (Madeira *et al.* 1992) almost are the same with *C. phrygica*. Also,

Sabanejewia aurata that is in the same family with *C. phrygica* and *C. simplicispina* was 2*n*=50. However, there are some differences in the chromosome morphology (Boron 2000). As reported in other *Cobitis* species (Rabova *et al.* 2001, Değer 2011, Esmaeili *et al.* 2015) sex chromosome was not determined in *C. phrygica* and *C. simplicispina*.

C-bands contain transcriptionally inactive highly repetitive DNA sequences (Sumner 1972, Boron 2000). *C. phrygica* and *C. simplicispina* are resemble to *C. elazigensis* in terms of the localization of C-bands on the centromeric regions of almost all chromosomes (Değer 2011) and to *C. vardarensis* which has pericentromeric C-bands on all chromosomes (Rabova *et al.* 2001) and to *C. taenia* which has pericentromeric C-bands on several m-sm chromosomes (Boron 1999). *C. phrygica* and *C. simplicispina* also show similarity with *S. aurata* that centromeric C-bands on some chromosomes have been reported before (Boron 2000). However, telomeric C-bands additionally to pericentromeric C-bands that were reported in this species (Boron 2000) are not observed in *C. phrygica* and *C. simplicispina*. Moreover, *C. phrygica* and *C. simplicispina* are similar to *Misgurnus fossilis* in the same family which has pericentromeric C-bands on some chromosomes (Boron 2000).

The number and locations of Ag-NORs have been used as a systematic and taxonomic character and these characters benefit to fish cytotaxonomy (Boron 1999). The karyotype and Ag-NOR phenotypes appears to be highly variable in the *Cobitis* (Rab *et al.* 2007). It was reported that two Ag-NORs were detected on some *Cobitis* species after Ag-staining (Arai 2011). About Ag-NOR number of *C. phrygica* and *C. simplicispina* show similarity to *C. maroccana* and *C. vardarensis* but localization of Ag-NORs seems different from these species (Madeira *et al.* 1992, Rabova *et al.* 2001). In addition, *C. phrygica* and *C. simplicispina* share similar property with *C. elazigensis* and *S. aurata* which has one pair of Ag-NOR bearing sm chromosomes (Boron 2000, Değer 2011). Also, *C. phrygica* and *C. simplicispina* share the same Ag-NOR number with *C. taenia* but there are differences in Ag-NOR localization (Boron 1999). Otherwise, Ag-NOR number polymorphisms that were reported in *C. elongatoides* (Rab *et al.* 2000) and *C. vardarensis* (Rabova *et al.* 2001) was observed in *C. simplicispina*.

Detailed chromosomal studies should be carried out on the other *Cobitis* species that are distributed in Anatolia shall be better to understand the systematics and taxonomy of this genus.

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