



Chromosomal Characteristics of *Oxynoemacheilus axylos* Yoğurtçuoğlu, Kaya & Freyhof, 2022 and *O. nasreddini* Yoğurtçuoğlu, Kaya & Freyhof, 2021 (Actinopterygii: Nemacheilidae) in Türkiye

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Abstract: Two endemic Anatolian nemacheilids were studied cytogenetically for the first time. Diploid chromosome numbers, chromosome morphologies in the karyotypes and also C-band and Ag-NOR banding properties were recorded for the two species of *Oxynoemacheilus*. The diploid chromosome number is 50 and karyotypes contained 34 biarmed and 16 monoarmed chromosomes in *Oxynoemacheilus axylos* Yoğurtçuoğlu et al. 2022. In *O. nasreddini* Yoğurtçuoğlu et al. 2021, there are 30 biarmed and 20 monoarmed chromosomes. No heteromorphic sex chromosomes were determined. C-band patterns and the number of Ag-NORs were different between the two studied species.

Key words: Anatolian loach, karyotype, C-banding, nucleolus organizer region

Introduction

The family Nemacheilidae Regan, 1911 includes five genera: *Oxynoemacheilus*, *Seminemacheilus*, *Turcinoemacheilus*, *Paracobitis* and *Schistura*; they are distributed in the inland waters of Türkiye (ÇIÇEK et al. 2020). *Oxynoemacheilus* is the most species-rich genus in the family, with 61 valid species of freshwater fishes in the Middle East (YOĞURTÇUOĞLU et al. 2022). More than half of these species occur in Türkiye. Taxonomy of Anatolian species of *Oxynoemacheilus* has been revised in details in the last years and several new species have been described (YOĞURTÇUOĞLU et al. 2021, 2022). *Oxynoemacheilus axylos* was described from Lake Tuz Basin (Cihanbeyli, Gölyazı, Melendiz

and Samsam) (YOĞURTÇUOĞLU et al. 2022). *Oxy-noemacheilus nasreddini* is distributed in tributaries within the basins of Akşehir, Eber, Eğirdir and Ilgın Lakes in Central Anatolia (YOĞURTÇUOĞLU et al. 2021, 2022).

The studies on the karyotypes help to determine the genetic structure of fish species (PHIMPHAN et al. 2020). These studies are useful in cytotaxonomy, phylogeny and fish fauna management (PHIMPHAN et al. 2020). Application of these studies has received considerable attention in Anatolian fishes in recent years (ÜNAL-KARAKUŞ 2021). However, few studies are available on the Anatolian species of *Oxynoemacheilus*, likely due to their small sizes (Ünal-KARAKUŞ 2021). The chromosomal properties of *O. panthera* (TANRIKULU 2008), *O. tigris*

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(Kılıç et al. 2011), *O. frenatus* (DEĞER 2011), *O. argyrogramma* (DEĞER 2011), *Oxy-noemacheilus* sp. from Euphrates River (DEĞER 2011), *O. angorae* (GAFFAROĞLU et al. 2014), *O. atili* (KARASU-AYATA et al. 2018) and *O. simavicus* (KARASU-AYATA et al. 2021) were reported from Türkiye. Most of these reports were based on conventional cytogenetic technique to determine the diploid chromosome numbers and chromosome morphology. Only a few (*O. angorae*, *O. argyrogramma*, *O. atili*, *O. frenatus* and *O. simavicus*) included chromosomal banding (C-banding and Ag-NORs) analyses (DEĞER 2011, GAFFAROĞLU et al. 2014, KARASU-AYATA et al. 2018, Ünal-KARAKUŞ 2021).

Cytogenetic studies may provide a useful tool in the cytotaxonomy of loaches of the genus *Oxy-noemacheilus* (Rábová et al. 2001). Most fish species differ by their karyotype structure, especially in number, size and morphology of chromosomes. Differences originating during their karyotype evolution should play an important role in speciation of fish species (MAJTÁNOVÁ et al. 2016). These differences should be revealed with modern cytogenetic approaches. There are no data about the cytogenetics of *O. axylos* and *O. nasreddini*. We aimed to reveal chromosomal properties with conventional cytogenetic methods such as Giemsa staining, C-banding and silver-staining.

Materials and Methods

The individuals (two females and five males) of *O. axylos* were collected from Melendiz Creek, Selimiye Village, Aksaray Province (38° 18' N; 34° 15' E), whereas the individuals (two females and two males) of *O. nasreddini* were collected from Bulasın Stream, Ilgın, Konya Province (38° 19' N; 31° 51' E). The species identification followed GELDIAY & BALIK (2007) and YOĞURTÇUOĞLU et al. (2022). The individuals were transported alive to the laboratory and kept in a well-aerated aquarium. The research procedure was approved by the Local Animal Ethics Committee of Türkiye (Protocol Number: 68429034/05). For karyological studies, air-drying technique of BERTOLLO et al. (2015) was followed. Each fish was injected intraperitoneally with a colchicine solution (0.1%; 1 ml/ 100 g body weight). The fishes were kept in a well-aerated aquarium and, after two hours, head kidney tissue was extracted and placed in hypotonic solution 0.56% of KCl. After this step, the cellular suspension was centrifuged at 1200 rpm for 10 min. The hypotonic solution was discarded and the pellet was suspended and washed three times

in methanol : glacial acetic acid (3 : 1). After centrifugation at 1200 rpm for 10 minutes, the drops of cellular suspension were put on a clean slide. The slides were allowed to air dry. Some of them were stained with 10% Giemsa for 20 minutes. At least 10 slides were prepared from each specimen. C-banding was done by using the method given by SUMNER (1972) with slight modifications. For silver staining of the chromosomes, the method given by HOWELL & BLACK (1980) was followed. All chromosome preparations were stored in the laboratory. All prepared slides were observed under a Leica DM3000 microscope. Mitotic metaphases (with Giemsa stained, C-banded and silver stained) were photographed using AKAS software. Karyotypes were prepared by arranging chromosomes in pairs by size. For each chromosome, the average lengths of the short and long arms and arm ratio (the ratio of the long arm length to the short arm) were measured by digital calliper and then the chromosomes were classified according to the criteria given by LEVAN et al. (1964). For calculating fundamental arm number (Fn), meta- and submetacentrics were taken as biarmed, whereas subtelo-acrocentrics were taken as uniarmed.

Results

Oxy-noemacheilus axylos and *O. nasreddini* shared the same diploid chromosome set ($2n$) 50 (Figs. 1a, 2a). Karyotype structure contained eight pairs of metacentric, nine pairs of submetacentric and eight pairs of subtelo-acrocentric chromosomes in *O. axylos* (Fig. 1b), whereas seven pairs of metacentric, eight pairs of submetacentric and ten pairs of subtelo-acrocentric chromosomes in *O. nasreddini* (Fig. 2b). Fn was calculated as 84 in *O. axylos* and as 80 in *O. nasreddini*. Heteromorphic sex chromosomes were not observed in the two species.

Dark C-bands were detected in the pericentromeric regions of most of the chromosomes (except Nos. 4 and 6 metacentrics, 8 submetacentric, 2 and 4 subtelo-acrocentrics) in *O. axylos* (Fig. 1c, d). Light C-bands were detected in the pericentromeric regions of only a few chromosomes in *O. nasreddini* (Fig. 2c, d).

Single Ag-NORs were detected in *O. axylos* (Fig. 1e) and *O. nasreddini* (Fig. 2e). Ag-NOR was located in the terminal regions of the short arm of one homologue of the largest submetacentric chromosome in *O. axylos* (Fig. 1f). Ag-NORs were located in the terminal regions of the short arms of the largest submetacentric chromosomes in *O. nasreddini* (Fig. 2f).

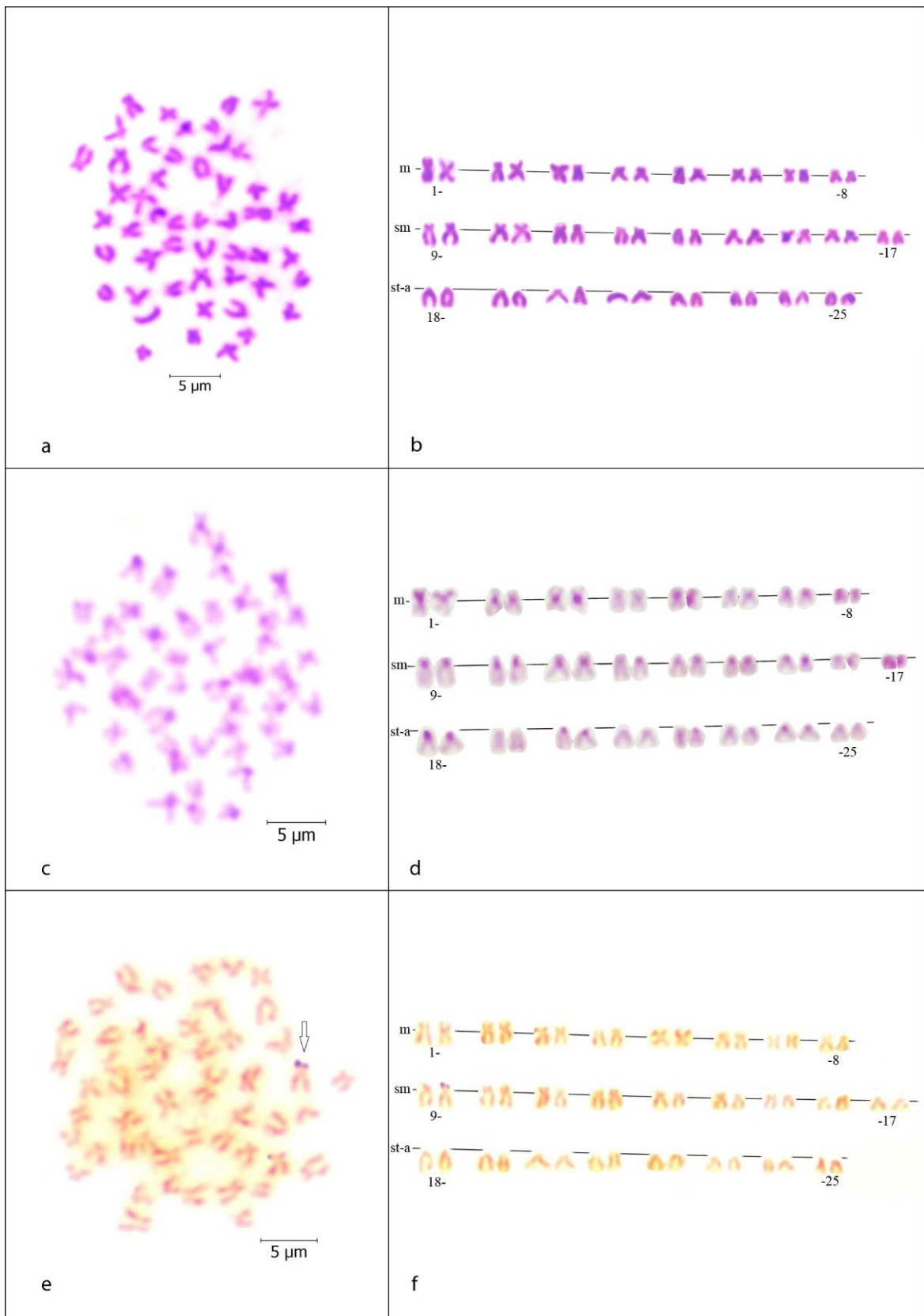


Fig. 1. Metaphases and karyotypes of *Oxyoemacheilus axylos*: a. Giemsa stained metaphase; b. the arranged karyotype of Giemsa stained metaphase; c. C-banded metaphase; d. the arranged karyotype of C-banded metaphase; e. silver-stained metaphase (arrow indicates the Ag-NOR); f. the arranged karyotype of silver-stained metaphase. Scale bar = 5 µm. m: metacentric, sm: submetacentric, st-a: subtelo-acrocentric.

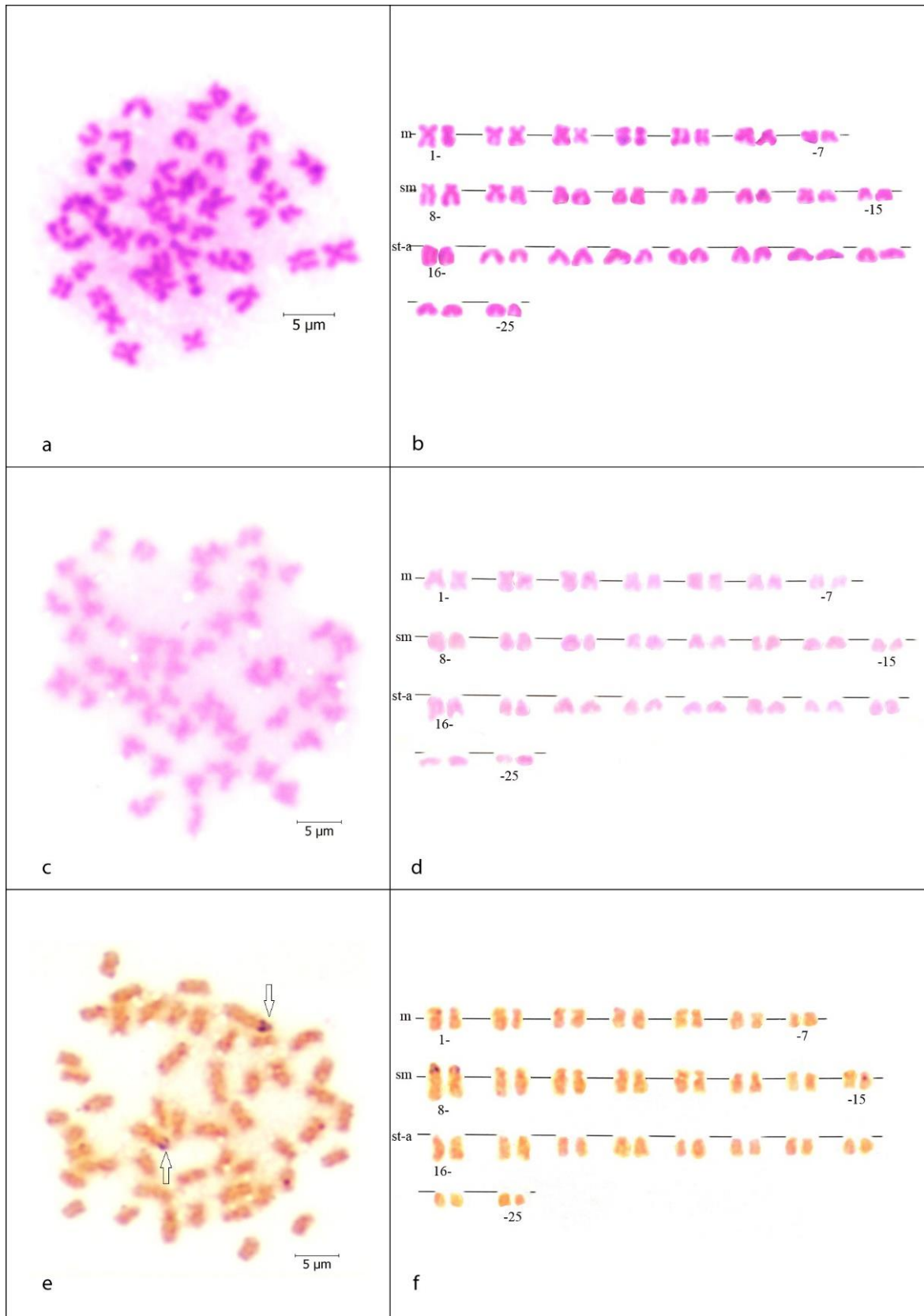


Fig. 2. Metaphases and karyotypes of *Oxynoemacheilus nasreddini*: a. Giemsa stained metaphase; b. the arranged karyotype of Giemsa stained metaphase; c. C-banded metaphase; d. the arranged karyotype of C-banded metaphase; e. silver-stained metaphase (arrows indicate the Ag-NORs); f. the arranged karyotype of silver-stained metaphase. Scale bar = 5 µm. m: metacentric, sm: submetacentric, st-a: subtelo-acrocentric.

Discussion

Nemacheilid loaches exhibit mostly $2n = 50$, with chromosomal morphologies ranging from prevalence of biarmed to unarmed chromosomes in the karyotype and quite variable NOR phenotypes (Ráb et al. 2021). Cytogenetic studies on *O. axylos* and *O. nasreddini* showed similarity in $2n$; however, chromosome morphologies, C-band patterns and Ag-NOR signal numbers differed. The karyotype of *O. axylos* has more biarmed chromosomes and an additional pair of metacentric and a pair of submetacentric chromosomes as compared to *O. nasreddini*. *Oxynoemacheilus nasreddini* has two additional pairs of subtelo-acrocentric chromosomes in the karyotype compared to *O. axylos*. The diploid chromosome number $2n = 50$ is uniform, suggesting a slow rate of karyotype change, mostly by intrachromosomal rearrangements like pericentric inversions and (or) translocations (MAJTÁNOVÁ et al. 2016). These chromosomal rearrangements have likely occurred in the karyotype evolution of *O. axylos* and *O. nasreddini*. On the other hand, dark C-bands were observed in almost all of the chromosomes of *O. axylos*, whereas light C-bands were observed in a few chromosomes of *O. nasreddini*. Only one Ag-NOR signal was detected in *O. axylos*, while two Ag-NOR signals were detected in *O. nasreddini*.

The $2n$ chromosome set, previously reported in eight Anatolian species of *Oxynoemacheilus* (Table 1), was also confirmed by our study on *O. axylos* and *O. nasreddini*. However, karyotypic differences are observed among the Anatolian species of *Oxynoemacheilus* (Table 1). The changes on the chromosome morphologies with the same $2n$ numbers are probably mainly due to pericentric inversions and/or translocations in the karyotype evolution in the Anatolian species of *Oxynoemacheilus* (KARASU-AYATA et al. 2021). To compare with the previous studies, the Fn's of *O. axylos* and *O. nasreddini* are between the reported values (76–94) (Table 1). The differences of Fn's correspond to difference of karyotypes among the species of *Oxynoemacheilus* (Table 1). Having more biarmed chromosomes, Fn of *O. axylos* is higher than *O. nasreddini*. We found that the two species have mainly biarmed chromosomes in their karyotypes. Similarly, such biarmed chromosomes prevail also in other Anatolian species of *Oxynoemacheilus* (Table 1) and should be a cytotaxonomic character in this group. The number of biarmed chromosomes of *O. axylos* and *O. nasreddini* are higher than *O. panthera* (TANRIKULU 2008), *Oxynoemacheilus* sp., *O. frenatus* (DEĞER 2011), *O. atili* (KARASU-AYATA

et al. 2018) and *O. simavicus* (KARASU-AYATA et al. 2021) from Anatolia.

Chromosomal studies in species of *Oxynoemacheilus* in different countries are also limited (ESMAEILI et al. 2015). In this context, *O. axylos* and *O. nasreddini* are similar to Iranian loach species, *O. persa* and *O. tongiorgii* (ESMAEILI et al. 2015), having more biarmed chromosomes than monoarmed chromosomes in $2n = 50$. The number of biarmed chromosomes was reported as 42 in *O. persa* and *O. tongiorgii* (ESMAEILI et al. 2015), thus, Fn's of these species are much higher than those of *O. axylos* and *O. nasreddini*.

From the other members of the family Nemacheilidae in Anatolia, only data on cytogenetics of *Turcinoemacheilus kosswigi* (GAFFAROĞLU et al. 2012) and *Seminemacheilus lendlii* (ÜNAL et al. 2016) have been reported. *Seminemacheilus lendlii* (ÜNAL et al. 2016) has more biarmed chromosomes, similarly to *O. axylos* and *O. nasreddini*, whereas *T. kosswigi* (GAFFAROĞLU et al. 2012) had more unarmed chromosomes in the karyotype.

Heteromorphic sex chromosomes were not observed in the two studied species. This phenomenon has also been observed in many species of genus *Oxynoemacheilus* (DEĞER 2011, GAFFAROĞLU et al. 2014, ESMAEILI et al. 2015, KARASU-AYATA et al. 2018, KARASU-AYATA et al. 2021) as well as in other genera of the family Nemacheilidae (GAFFAROĞLU et al. 2012, ÜNAL et al. 2016).

Ag-NORs are chromosomal markers that consist of repeated sequences of ribosomal genes. The number and location of Ag-NORs have been widely used in cytotaxonomy and phylogenetic studies because chromosomal characters are often species-specific (PHIMPHAN et al. 2020). Structure, number and morphology of a NOR may be specific to even fish populations and species. Normally, most fishes have only a single Ag-NOR (PHIMPHAN et al. 2020). A single Ag-NOR bearing submetacentric chromosome pair in *O. nasreddini* is consistent with results for *O. atili* (KARASU-AYATA et al. 2018), *O. simavicus* (ÜNAL-KARAKUŞ 2021) and *O. argyrogramma* (DEĞER 2011). However, *O. nasreddini* differs from *O. frenatus* and *Oxynoemacheilus* sp. (DEĞER 2011) as they have multiple Ag-NOR numbers. *Oxynoemacheilus axylos* has only one Ag-NOR signal differing it from other Anatolian *Oxynoemacheilus* species (DEĞER 2011, KARASU-AYATA et al. 2018, ÜNAL-KARAKUŞ 2021). Larger submetacentric chromosomes having Ag-NORs could be a cytogenetic marker for *O. axylos* and *O. nasreddini*. The Ag-NOR number detected in the present study differs from *S. lendlii*, which has four Ag-NOR signals

Table 1. Karyological data of the genus *Oxynoemacheilus* from Türkiye.

Species	2n	Chromosome morphology	Fn	References
<i>O. panthera</i>	50	14m+18sm+18a	82	TANRIKULU (2008)
<i>O. argyrogramma</i>	50	44m-sm+6a	94	DEĞER (2011)
<i>O. frenatus</i>	50	32m-sm+18a	82	DEĞER (2011)
<i>O. sp.</i>	50	30m-sm+20a	80	DEĞER (2011)
<i>O. tigris</i>	50	18m+18sm+14a	86	Kılıç et al. (2011)
<i>O. angorae</i>	50	8m+28sm+14st-a	86	GAFFAROĞLU et al. (2014)
<i>O. atili</i>	50	10m+18sm+22st-a	78	KARASU-AYATA et al. (2018)
<i>O. simavicus</i>	50	12m+14sm+24st-a	76	KARASU-AYATA et al. (2021)
<i>O. axylos</i>	50	16m+18sm+16at-a	84	This study
<i>O. nasreddini</i>	50	14m+16sm+20st-a	80	This study

2n: diploid chromosome number, Fn: fundamental number, m: metacentric, sm: submetacentric, st-a: subtelo-acrocentric

(Ünal et al. 2016). However, the location of these regions on the submetacentric chromosomes are similar with that in *S. lendlii* (ÜNAL et al. 2016).

Constitutive heterochromatin regions are revealed by the application of a standard C-banding procedure (SUMNER 1972). These regions are an important tool in terms of chromosomal identification of the species and determination of sex chromosomes. The differences in constitutive heterochromatin localisation could be used as a cytogenetic marker and reveal the chromosome evolution of the species (ARSLAN & ARSLAN 2007). The C-band data (darkness, located on the pericentromeres of most of the chromosomes) in *O. axylos* show similarity to those reported from Anatolia: *O. argyrogramma*, *O. frenatus*, *Oxynoemacheilus* sp. (DEĞER 2011), *O. angorae* (GAFFAROĞLU et al. 2014), *O. atili* (KARASU-AYATA et al. 2018) and *O. simavicus* (Ünal-KARAKUŞ 2021). This character is a common characteristic tool in many fish species (PHIMPHAN et al. 2020). *Oxy-noemacheilus nasreddini* differs from other Anatolian species of *Oxynoemacheilus* in the pattern of C-bands: it has light C-bands in a few chromosomes. Moreover, *O. axylos* shows similarities to *S. lendlii* (ÜNAL et al. 2016) and *T. kosswigi* (GAFFAROĞLU et al. 2012) in the pattern of C-band distribution.

In conclusion, the present study is the first report on the chromosomal characteristics of *O. axylos* and *O. nasreddini*. Although the studied species have the same diploid chromosome number ($2n = 50$), there are differences in the Fns, numbers of mono- and banded chromosomes and chromosome having Ag-NORs as well as in the patterns of C-banded chromosomes. This result indicated that karyological data as a useful distinctive tool for nemacheilids. Moreover, molecular cytogenetic

studies in the Anatolian members of *Oxynoemacheilus* are essential for better understanding of their karyotype evolution.

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