

**Research Article**

## **Karyological analysis of two species of mudskippers (Teleostei: Gobioidae: Oxudersidae) in the Musa estuary, Persian Gulf, Iran**

**Saberi F.<sup>1</sup>; Gharzi A.<sup>1\*</sup>; Jazayeri A.<sup>2</sup>; Akmali V.<sup>1</sup>; Chehri K.<sup>1</sup>**

Received: November 2022

Accepted: February 2023

### **Abstract**

Cytological parameters including whole sets of chromosomes, known as karyology, has become increasingly prevalent in resolving taxonomic issues for uncertain species. This study focuses on the karyological analysis of haploid and diploid chromosomes in two amphibious fish species, *Periophthalmus waltoni* and *Boleophthalmus dussumieri* which inhabit muddy beaches of the Persian Gulf. This survey is the first to report on the chromosome number of *P. waltoni*. To achieve this task, 10 male and 10 female specimens were collected from the beaches of Musa estuary, Persian Gulf, Iran. Mitotic chromosomes and haploid chromosomes were obtained from branchial and testicular tissue samples in males, respectively. The chromosomes were stained using the traditional Giemsa staining technique. The diploid chromosome number for *P. waltoni* was determined to be  $2n=44$  in both males and females, with a fundamental number (NF) of 82, whereas the numbers for *B. dussumieri* were 46 and 82. The karyotype of *P. waltoni* consisted of 14 large metacentrics, four large sub-metacentric, eight large sub-telocentric, four medium metacentric, six medium sub-metacentric, two medium telocentric, two small sub-telocentric, and four small telocentric chromosomes. In contrast, the karyotype of *B. dussumieri* comprised 18 large metacentrics, eight large sub-metacentric, two large telocentric, eight medium metacentric, two medium sub-metacentric, two medium telocentric chromosomes, and four microchromosomes. No sex chromosomes were identified in either species. Notably, the results revealed that the chromosome count and morphology differed among the species within the same genus, and the use of the Ag-NOR banding technique accentuated these differences.

**Keywords:** *Periophthalmus waltoni*, *Boleophthalmus dussumieri*, Chromosomes, Cytology, Persian Gulf

1-Department of Biology, Faculty of Science, Razi University, Kermanshah, Iran

2-Department of Biology, Faculty of Sciences, Shahid Chamran University of Ahvaz, Ahvaz, Iran

\* Corresponding author's Email: ahgharzi@yahoo.com

## Introduction

Since 1960s, Karyological studies in fish have made significant contributions to fields such as genetics, taxonomy, phylogeny, and environmental toxicology. Through karyological analysis, researchers can obtain fundamental data on the number, size, and appearance of chromosomes, which can aid in understanding evolutionary mechanisms and clarifying the natural relationships and probable phylogeny of species (Dai and Han, 2018).

The common term 'mudskippers' refers to a group of fish that have adapted to an amphibious lifestyle, living in both aquatic and terrestrial environments. Four genera of Mudskippers, namely *Periophthalmodon*, *Periophthalmus*, *Boleophthalmus*, and *Scartelaos*, were previously classified as members of the oxudercinae gobies within the Gobiidae family (Ishimatsu and Gonzales, 2011; Ghanbarifardi *et al.*, 2016). However, they are now considered part of the Oxudercidae family: Oxudercinae (Nelson *et al.*, 2016; Yang *et al.*, 2022). The Gobiidae family is known for being one of the largest vertebrate families (Gill and Mooi, 2012; Kovalchuk *et al.*, 2018), and the most diverse family of bony fish (Fricke *et al.*, 2021). The majority of Genus-level species diversity in Gobiidae and Oxudercidae mainly occurred from the Late Miocene through the Early Pleistocene (Jeon *et al.*, 2021).

Ghanbarifardi *et al.* (2016) conducted a study to examine the morphological and molecular

phylogenetics of the goby fish and seven species of mudskippers along the coasts of the Sea of Oman. The results based on the maximum likelihood and Bayesian inference revealed that the monophyly of the Oxudercinae and Amblyopinae subfamilies was not supported, and the use of these subfamilies as classification units is not recommended. Additionally, the results of the molecular phylogeny of two mitochondrial genes (Cyt b, COI) and one nuclear gene (Rag1) indicated that mudskippers belong to the *Periophthalmus* lineage (Agorreta *et al.*, 2013; Polgar *et al.*, 2017) which challenges their previous classification as members of the Gobiidae family. Mudskippers are uniquely adapted to living in muddy fields and can survive on land for an extended period (Murdy, 2011; Lorente-Martínez *et al.*, 2018; Yang *et al.*, 2022).

These mudskippers have been evaluated as a model of the water-to-land evolutionary transition of Devonian proto-amphibians, which are believed to be the ancestors of all present tetrapods (Ishimatsu and Gonzales, 2011; Yang *et al.*, 2022). According to Murdy (1989) and Esmaeili *et al.* (2018), three mudskipper species are distributed along the coasts of the Persian Gulf and the Sea of Oman, including *Periophthalmus waltoni* Koumans, 1941, *Boleophthalmus dussumieri* (Valenciennes, 1837), and *Scartelaos tenuis* (Day, 1876). These species have evolved since the Indian subcontinent collided with Asia, and the populations

of Southeast Asia were separated from this region (Esmaeili *et al.*, 2018). Today, they can be found on muddy beaches and in mangrove forests in southern Iran (Ghanbarifardi and Damadi, 2021). The genus *Periophthalmus* is less dependent on water than *Boleophthalmus*, and spends most of its time out of the water (Murdy, 2011; Jaafar *et al.*, 2016; Lorente-Martínez *et al.*, 2018). Of the three species in the Persian Gulf, *P. waltoni* has been reported as native to the Persian Gulf and the Sea of Oman (Abdoli *et al.*, 2009), although it has also been observed in the Narmada River in Gujarat, India (Murdy, 1989). This species can be found in a variety of sediments, ranging from soft and fine-grained to hard and coarse-grained ones. On the Iranian coasts, *B. dussumieri* species lives in soft and fine-grained sediments (Ghanbarifardi *et al.*, 2014).

In India, this species is considered a semiterrestrial, and edible fish are found along the coasts of Mumbai (Krishnaja and Rege, 1982; Giglia-Mari and Berneburg, 2012). Its chromosome number is 46 relatively large acrocentric chromosomes (Krishnaja, 1980). This species has been introduced as a suitable cytogenetic model for the induction of chromosomal abnormalities (Krishnaja and Rege, 1980; Gadhia *et al.*, 2008).

Although the karyotypic characteristics of some families of marine fish have been already known, so far, there is no significant information on the Perciformes group

(Galvão *et al.*, 2011). Moreover, despite the diversity of fish species in Iranian waters, karyological studies on them are relatively scarce and require further exploration. As mudskippers have evolved in parallel with terrestrial vertebrates (Hickman *et al.*, 2020), determining the number and the type of chromosomes of the present species, as models for illustrating the water-to-land transition, could facilitate investigations into other cytogenetic characteristics, such as nucleolar organizer regions (NORs), using the Ag-NOR banding technique.

## Materials and methods

### Sample collection

Twenty specimens, including six male and four female *P. waltoni*, as well as five male and five female *B. dussumieri*, were collected from the Musa estuary (48° 59' 8" E and 30° 5' 20" N) in the northwest of the Persian Gulf (Figs. 1 and 2).

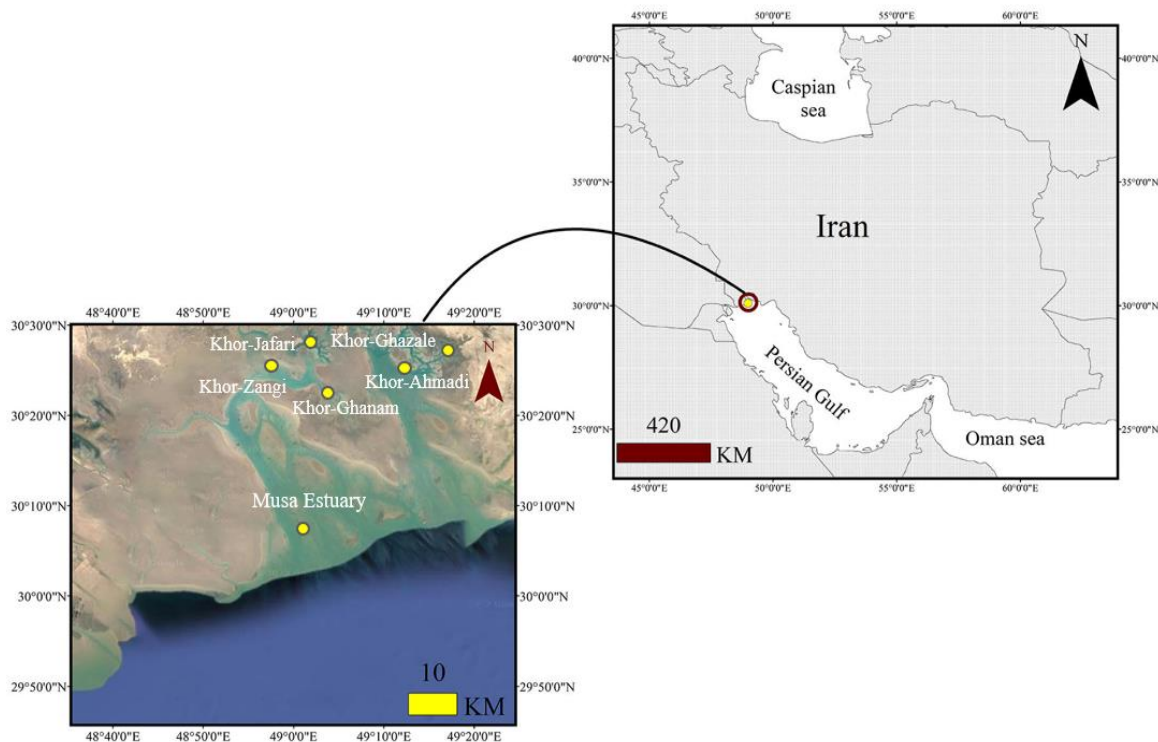


**Figure 1: A: *Periophthalmus waltoni*. B: *Boleophthalmus dussumieri* from Khor Jafari, Musa estuary, Persian Gulf, Iran. Scale bars=1 cm.**

The specimens were captured alive by hand and transferred to the laboratory.

Then, morphological characteristics were examined and species

identification was performed using a valid identification key (Murdy, 1989).



**Figure 2:** A map showing Musa estuary, with the sampling area

#### *Tissue extraction process and optimization of karyotype preparation*

To conduct karyological studies, alive fish were first weighed accurately before intraperitoneally injecting a colchicine solution into the abdominal wall at a concentration of 0.01 mg/mL (0.01 %) and a dose of 0.1 mL per gram of body weight. After 5 to 5.4 hours, the samples were killed in an anaerobic jar with chloroform-soaked cotton wool.

Afterward, the specimens were dissected and the spleen, kidneys, and gill tissues were removed. To separate the gills, a linear cut was made on the ventral surface from the end of the mouth to the beginning of the pectoral fins and the gills were gently separated

and then the testicles were removed. The remaining organs were isolated, and the kidneys which located under the peritoneum and attached to the spine, were carefully separated. The isolated tissues were later kept in a hypotonic potassium chloride solution of 0.09 M for 10 min at room temperature. Then, a few drops of cold and fresh fixative were added, and the mixture was left to rest for ten hours. After homogenization and centrifugation with a g-force (1500  $\times$ g for 10 minutes), the supernatant was removed and a fresh and cold fixative solution (a mixture of acetic acid and methanol at a 3:1 ratio) was added to the pellet. This process was repeated twice and the resulting pellet was

thoroughly washed. Finally, one ml of cold fixative solution was added dropwise to the pellet and homogenized with a Pasteur pipette. The resulting solution was then dripped onto cold and clean slides from a distance of 60-80 cm on a sloped surface. The slides were allowed to dry at room temperature and coded before being stained with 5% Giemsa for 10 min.

#### *Photographing and counting chromosomes*

The stained slides were labeled and the best metaphase plaques were photographed using an optical microscope (Olympus-cx31) with a 100x objective and a BEL microscope equipped with a camera. The total length of each chromosome and the length of its long and short arms were measured from the photos of metaphase plaques. Homologous chromosomes were classified by the previous method reported by Levan *et al.* (1964), and the final karyotype was arranged. Standard methods were employed to separate chromosomes, identify homologs, and arrange karyotypes. Subsequently, the chromosome analysis software Karyovision, Image J, Photoshop V8, and Excel were utilized to examine and analyze the chromosomes on the slides.

To determine the size and classification of chromosomes, various measurements were taken. These included the measurement of the short arm length (Ls) and long arm length (Ll), with the total chromosome length (LT) being calculated as the sum of these two values. Additionally, the

relative length (RL) and centromeric index (CI) were also measured, alongside LT, according to established protocols (Turpin and Lejeune, 1965; Nascimento *et al.*, 2022).

Chromosomes were classified according to size by applying the symbols "L, M, and S" as representatives of the large, medium, and small chromosomes, respectively. The symbols "m, sm, t, st", were used to classify chromosomes according to type as metacentric, submetacentric, telocentric, and subtelocentric, respectively. The fundamental number (FN) was two for metacentric, submetacentric, subtelocentric chromosomes, and microchromosomes, and one for telocentric chromosomes. With this information, the karyotype formula was determined.

#### **Results**

This study utilized tissue samples from the gills, kidneys, and testes to prepare the karyotype and determine the number of chromosomes. The diploid chromosome number obtained in the present study was confirmed by the observed metaphase step for more than 100 cells in each species. The most optimal metaphase plaques were obtained from gill and testis tissues in males of both species.

In the present study, based on the counting process, more than 30 haploid plaques in *P. waltoni* (n=22) were observed in the meiotic nuclei of the testis (Fig. 3C). Also, the diploid chromosome number (2n=44, NF=82)

was obtained from the mitotic plaques of gill tissue, containing 19 pairs of di-

brachial chromosomes and 3 pairs of monobrachial chromosomes (Table 1).

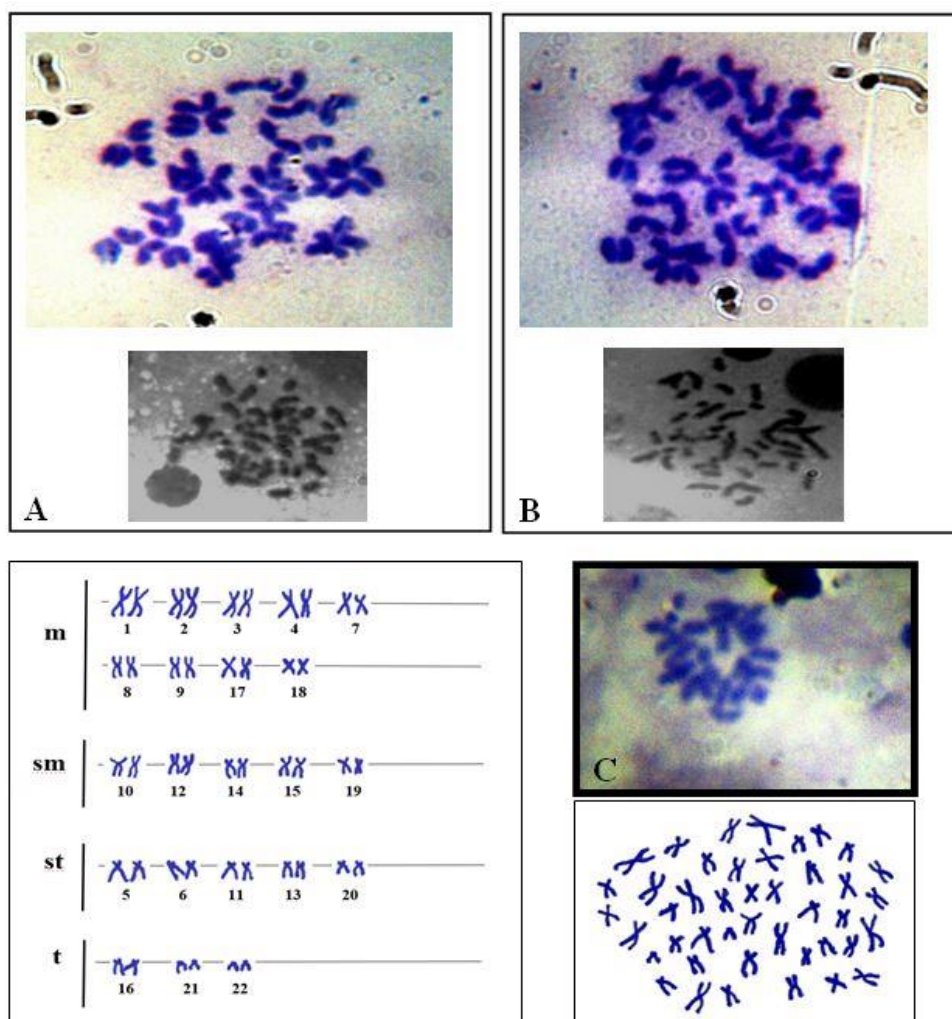


Figure 3: Karyotype prepared from chromosomes of metaphase stage of mitosis in male (A) and female (B) *P.waltoni*, C: Haploid chromosome in a male,  $2n=44$  by conventional staining.

Table 1: Karyotype data of two mudskippers.

| Species              | Plural | Constitute a model of chromosome               | arm number |
|----------------------|--------|--|------------|
| <i>P. waltoni</i>    | 44     | $2n = 9m + 5sm + 5st + 3t$                     | 82         |
| <i>B. dussumieri</i> | 46     | $2n = 13m + 5sm + 3t + 2\mu\text{-chromosome}$ | 82         |

Chromosomes were classified into four groups based on the location of their centromeres (Levan *et al.*, 1964), including 9 pairs of metacentric chromosomes (pairs of chromosomes 1, 2, 3, 4, 7, 8, 9, 17, 18), 5 pairs of submetacentric chromosomes (pairs of chromosomes 12, 10, 15, 14 and 19), 3 pairs of telocentric chromosomes (pairs

of chromosomes 16, 21 and 22) and 5 pairs of subtelocentric chromosome (pairs of chromosomes 6, 11, 13, 16, 20). The variations of chromosome length ranged from 1.73  $\mu\text{m}$  to 8.63  $\mu\text{m}$  (Fig. 4 and Table 2). NO sex chromosomes were observed, in this species, indicating a lack of -hetero chromosomal status.

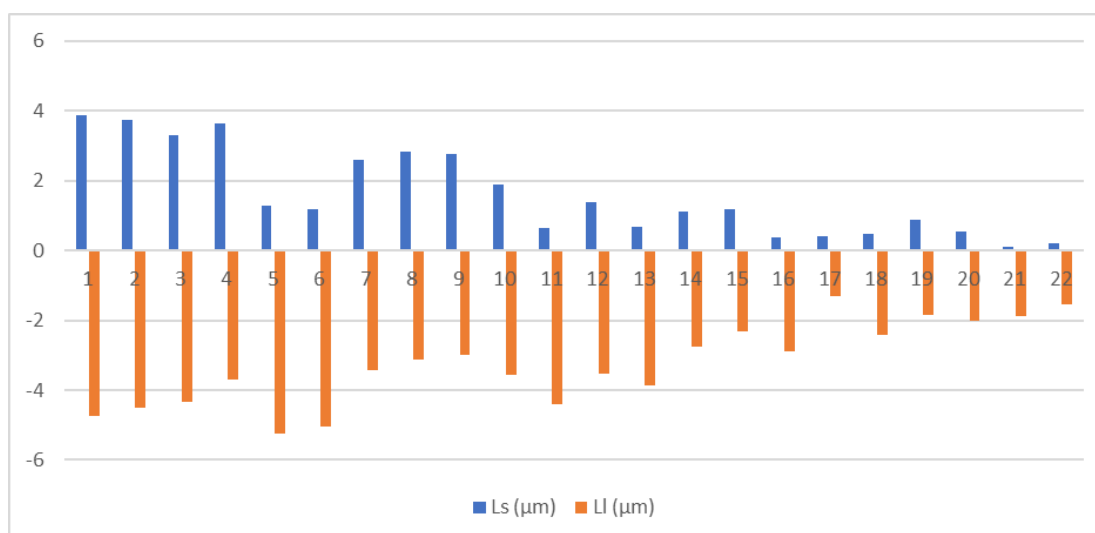


Figure 4: Idiogram of chromosomes of metaphase stage of mitosis in *P. waltoni*.

Table 2: Mean length of the short arm chromosome (Ls), Longarm chromosome (LI), Total arm chromosome (LT), The ratio of the arms (LI/Ls), Relative length (RL), Centromeric index (CI), and standard deviation (SD) of RL, CI from 10 karyotypes of male and female species (*Periophthalmus waltoni*), 2n=44.

| Chro, pair   | Ls (µm)      | LI (µm)      | LT (µm)       | Ratio LI/Ls | RL±SD       | CI±SD       | Chro, size | Chro, type       | Number of arms |
|--------------|--------------|--------------|---------------|-------------|-------------|-------------|------------|------------------|----------------|
| 1            | 3.88         | 4.75         | 8.63          | 1.22        | 0.080±0.001 | 0.449±0.015 | Large      | Metacentric      | 4              |
| 2            | 3.75         | 4.50         | 8.25          | 1.20        | 0.077±0.003 | 0.454±0.032 | Large      | Metacentric      | 4              |
| 3            | 3.31         | 4.35         | 7.66          | 1.31        | 0.071±0.001 | 0.432±0.012 | Large      | Metacentric      | 4              |
| 4            | 3.63         | 3.70         | 7.33          | 1.01        | 0.068±0.001 | 0.495±0.038 | Large      | Metacentric      | 4              |
| 5            | 1.30         | 5.25         | 6.55          | 4.03        | 0.061±0.002 | 0.198±0.036 | Large      | Sub- telocentric | 4              |
| 6            | 1.19         | 5.05         | 6.24          | 4.24        | 0.058±0.000 | 0.190±0.010 | Large      | Sub- telocentric | 4              |
| 7            | 2.61         | 3.44         | 6.05          | 1.31        | 0.056±0.001 | 0.431±0.019 | Large      | Metacentric      | 4              |
| 8            | 2.84         | 3.11         | 5.95          | 1.09        | 0.055±0.003 | 0.477±0.024 | Large      | Metacentric      | 4              |
| 9            | 2.77         | 3.00         | 5.77          | 1.08        | 0.054±0.002 | 0.480±0.022 | Large      | Metacentric      | 4              |
| 10           | 1.88         | 3.55         | 5.43          | 1.88        | 0.050±0.002 | 0.346±0.014 | Large      | Sub- metacentric | 4              |
| 11           | 0.64         | 4.39         | 5.03          | 6.85        | 0.047±0.000 | 0.127±0.036 | Large      | Sub- telocentric | 4              |
| 12           | 1.37         | 3.52         | 4.89          | 2.56        | 0.045±0.001 | 0.280±0.000 | Large      | Sub- metacentric | 4              |
| 13           | 0.67         | 3.85         | 4.52          | 5.74        | 0.042±0.002 | 0.148±0.000 | Large      | Sub- telocentric | 4              |
| 14           | 1.13         | 2.77         | 3.90          | 2.45        | 0.036±0.002 | 0.289±0.025 | Medium     | Sub- metacentric | 4              |
| 15           | 1.18         | 2.32         | 3.50          | 1.96        | 0.032±0.001 | 0.337±0.018 | Medium     | Sub- metacentric | 4              |
| 16           | 0.38         | 2.90         | 3.28          | 7.63        | 0.030±0.001 | 0.115±0.022 | Medium     | Telocentric      | 2              |
| 17           | 0.42         | 1.31         | 1.73          | 3.11        | 0.016±0.001 | 0.242±0.011 | Medium     | Metacentric      | 4              |
| 18           | 0.47         | 2.43         | 2.90          | 5.11        | 0.027±0.001 | 0.162±0.034 | Medium     | Metacentric      | 4              |
| 19           | 0.88         | 1.84         | 2.72          | 2.09        | 0.025±0.000 | 0.323±0.011 | Medium     | Sub- metacentric | 4              |
| 20           | 0.56         | 2.00         | 2.56          | 3.57        | 0.024±0.000 | 0.218±0.036 | Small      | Sub- telocentric | 4              |
| 21           | 0.12         | 1.88         | 2.00          | 15.66       | 0.018±0.000 | 0.060±0.000 | Small      | Telocentric      | 2              |
| 22           | 0.20         | 1.55         | 1.75          | 7.75        | 0.016±0.003 | 0.114±0.000 | Small      | Telocentric      | 2              |
| <b>Total</b> | <b>35.18</b> | <b>71.49</b> | <b>106.64</b> |             |             |             |            |                  | <b>82</b>      |

The chromosomal expansion of *B. dussumieri* from the mitotic plaques of gill exhibited chromosome number 2n=46 and NF=82, containing 20 pairs of brachial and 3 pairs of monobrachial chromosomes. In addition, the haploid

chromosome number in testis tissue was N=23 (Fig. 5C). The range of chromosome length variations was from a minimum of 1.30 to a maximum of 9.86. Similar to *P. waltoni* species, sex chromosomes were not observed in this

species. Also, the chromosomal assemblage of *Boleophthalmus dussumieri* included 13 pairs of metacentric chromosomes (pairs of chromosomes 1, 2, 3, 4, 5, 7, 10, 14, 15, 16, 17, 18, 19), 5 pairs of submetacentric chromosomes (pairs of chromosomes 6, 8, 9, 11 and 20) and 3

pairs of telocentric chromosomes (pairs of chromosomes 12, 13 and 21). In addition, chromosomes pairs 22 and 23 were classified into the microchromosome group (Fig. 6 and Table 3). Cytogenetic data for the species in this study and other studies have been compiled in Table 4.

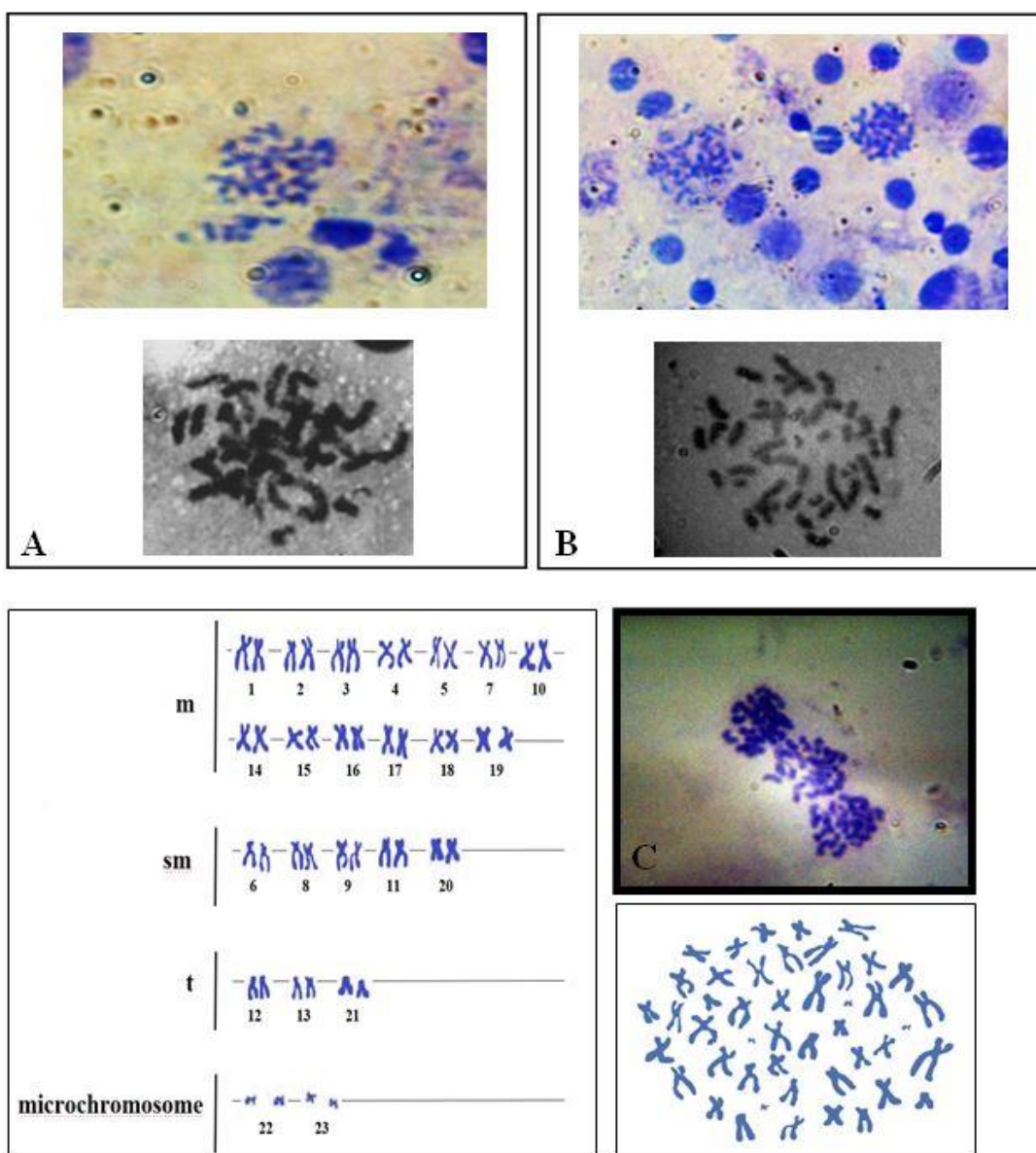


Figure 5: Karyotype prepared from chromosomes of metaphase stage of mitosis in male (A) and female (B) *B.dussumieri*, C: Haploid chromosome in a male,  $2n=46$  by conventional staining.



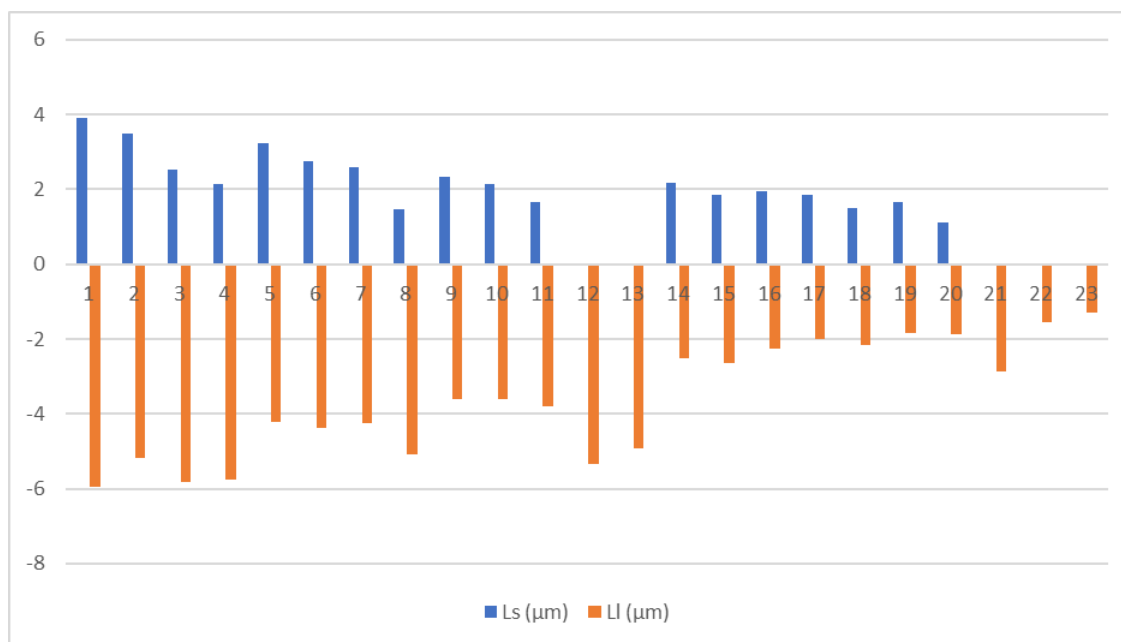


Figure 6: Idiogram of chromosomes of metaphase stage of mitosis in *B. dussumieri*.

Table 3: Mean length of the short arm chromosome (Ls), Longarm chromosome (LI), Total arm chromosome (LT), The ratio of the arms (LI/Ls), Relative length (RL), Centromeric index (CI), and standard deviation (SD) of RL, CI from 10 karyotypes of male and female species, (*Boleophthalmus dussumieri*), 2n=46.

| Chro, pair   | Ls (μm)      | LI (μm)      | LT (μm)       | Ratio LI/Ls | RL±SD       | CI±SD       | Chro, size | Chro, type      | Number of arms |
|--------------|--------------|--------------|---------------|-------------|-------------|-------------|------------|-----------------|----------------|
| 1            | 3.92         | 5.94         | 9.86          | 1.51        | 0.079±0.001 | 0.602±0.005 | Large      | Metacentric     | 4              |
| 2            | 3.48         | 5.17         | 8.65          | 1.48        | 0.070±0.003 | 0.597±0.032 | Large      | Metacentric     | 4              |
| 3            | 2.52         | 5.81         | 8.33          | 2.30        | 0.067±0.001 | 0.697±0.012 | Large      | Metacentric     | 4              |
| 4            | 2.15         | 5.77         | 7.92          | 2.68        | 0.064±0.001 | 0.728±0.038 | Large      | Metacentric     | 4              |
| 5            | 3.23         | 4.22         | 7.45          | 1.30        | 0.060±0.002 | 0.566±0.036 | Large      | Metacentric     | 4              |
| 6            | 2.76         | 4.39         | 7.15          | 1.59        | 0.058±0.000 | 0.613±0.010 | Large      | Sub-metacentric | 4              |
| 7            | 2.59         | 4.25         | 6.84          | 1.64        | 0.055±0.001 | 0.621±0.019 | Large      | Metacentric     | 4              |
| 8            | 1.47         | 5.08         | 6.55          | 3.45        | 0.053±0.003 | 0.775±0.024 | Large      | Sub-metacentric | 4              |
| 9            | 2.33         | 3.6          | 5.93          | 1.54        | 0.048±0.002 | 0.607±0.022 | Large      | Sub-metacentric | 4              |
| 10           | 2.15         | 3.61         | 5.76          | 1.67        | 0.046±0.002 | 0.626±0.014 | Large      | Metacentric     | 4              |
| 11           | 1.66         | 3.79         | 5.45          | 2.28        | 0.044±0.000 | 0.695±0.036 | Large      | Sub-metacentric | 4              |
| 12           | 0            | 5.33         | 5.33          | ∞           | 0.043±0.001 | 1±0.000     | Large      | Telocentric     | 2              |
| 13           | 0            | 4.92         | 4.92          | ∞           | 0.039±0.002 | 1±0.000     | Large      | Telocentric     | 2              |
| 14           | 2.16         | 2.50         | 4.66          | 1.15        | 0.037±0.002 | 0.536±0.025 | Large      | Metacentric     | 4              |
| 15           | 1.87         | 2.63         | 4.50          | 1.40        | 0.036±0.001 | 0.584±0.018 | Large      | Metacentric     | 4              |
| 16           | 1.94         | 2.27         | 4.21          | 1.17        | 0.034±0.001 | 0.539±0.022 | Medium     | Metacentric     | 4              |
| 17           | 1.87         | 2            | 3.87          | 1.06        | 0.031±0.001 | 0.516±0.011 | Medium     | Metacentric     | 4              |
| 18           | 1.50         | 2.16         | 3.66          | 1.44        | 0.029±0.001 | 0.590±0.034 | Medium     | Metacentric     | 4              |
| 19           | 1.66         | 1.84         | 3.50          | 1.10        | 0.028±0.000 | 0.525±0.011 | Medium     | Metacentric     | 4              |
| 20           | 1.12         | 1.88         | 3.00          | 1.67        | 0.024±0.000 | 0.626±0.036 | Medium     | Sub-metacentric | 4              |
| 21           | 0            | 2.88         | 2.88          | ∞           | 0.023±0.000 | 1±0.000     | Medium     | Telocentric     | 2              |
| 22           | 0            | 1.55         | 1.55          | ∞           | 0.012±0.003 | 1±0.000     | Small      | μ-Chromosome    | 2              |
| 23           | 0            | 1.30         | 1.30          | ∞           | 0.010±0.000 | 1±0.000     | Small      | μ-Chromosome    | 2              |
| <b>Total</b> | <b>40.38</b> | <b>82.89</b> | <b>123.27</b> |             |             |             |            |                 | <b>82</b>      |

**Table 4: Review of cytogenetic data from mudskippers, by (De Lima Filho, 2015), with additions.**

| Species   | 2n | Chro morphology    | NF | Reference                      | Local               |
|---|----|--------------------|----|--------------------------------|---------------------|
| <i>Boleophthalmus boddarti</i>  | 46 |                    |    | Verma (1968)                   | India               |
|   | 46 | 46m/sm             | 92 | Subrahmanyam (1969)            | Indo-Pacific        |
|   | 46 |                    |    | Verma (1968)                   | India               |
|   | 46 | 46a                |    | Nishikawa <i>et al.</i> (1974) | Japan               |
|   | 46 | 14m+18sm+14a       | 46 | Krishnaja and Regan (1980)     | India               |
|   | 46 |                    | 78 | Khuda-Bukush and Barat (1987)  | Indo-Pacific        |
| <i>Boleophthalmus dussumieri</i>  | 46 | 13m+5sm+3t+2μ-chro | 82 | Gadhia <i>et al.</i> (2008)    | India               |
|   |    |                    |    | <b>Present report</b>          | <b>Persian Gulf</b> |
| <i>Boleophthalmus glaucus</i><br>( <i>Scartelaos cantoris</i> )                 | 46 | 12m+20sm+2st+12a   | 80 | Manna and Prasad (1974)        | Indo-Pacific        |
| <i>Boleophthalmus pectinirostris</i>  | 46 | 46st/a             | 46 | Aria and Sawada (1975)         | Indo-Pacific        |
| <i>Periophthalmus borneensis</i>  | 48 |                    |    | Verma (1968)                   | Indo-Pacific        |
| <i>Periophthalmus cantonensis</i><br>( <i>Periophthalmus novaeaguineensis</i> ) | 46 | 46t/a              |    | Nogusa (1957, 1960)            | Japan               |
|   | 46 |                    |    | Arai and Kobayasi (1973)       | Japan               |
|   | 46 | 34m/sm+12t/a       | 80 | Nishikawa <i>et al.</i> (1974) | Japan               |
|   | 46 | 18m+12sm+16st/a    | 76 | Aria and Sawada (1975)         | Indo-Pacific        |
| <i>Periophthalmus chrysopilus</i>   | 56 |                    |    | Matsuo (1998)                  | Indo-Pacific        |
| <i>Periophthalmus modestus</i>  | 46 | 16m/sm+30a         | 62 | Lee (1968a)                    | Indo-Pacific        |
|   | 46 | 46a/t              | 46 | Nishikawa <i>et al.</i> (1974) | Japan               |
| <i>Periophthalmus schollosseri</i>  | 46 | 46a                | 46 | Matsuo (1998)                  | Indo-Pacific        |
| <i>Periophthalmus serperaster</i>   | 46 | 8m+16sm+4st+18a    | 74 | Khuda-Bukhsh and Nayak (1987)  | Indo-Pacific        |
| <i>Periophthalmus waltoni</i>   | 44 | 9m+5sm+5st+3t      | 82 | <b>Present report</b>          | <b>Persian Gulf</b> |
| <i>Scartelaos histophorus</i>   | 48 | 8m/sm+40st/a       | 56 | Matsuo (1998)                  | Indo-Pacifico       |

Remark: chro=chromosome, 2n=diploid number, NF=fundamental number, m=metacentric, sm=submetacentric, st=subtelocentric, t=telocentric, a=acrocentric.

## Discussion

Karyotypic studies are efficient for addressing the systematic and evolutionary problems in fish, as well as assessing the impact of genetically active substances on fish genomes (Sharma *et al.*, 2002; Gadhia *et al.*, 2008). However, due to the small size and large number of chromosomes in fish, obtaining high-quality chromosome preparations can be challenging (Nelson, 2006; Nasri *et al.*, 2010; Cherednichenko *et al.*, 2022). Despite the challenges of obtaining high-quality chromosome preparations, karyotypic studies in fish can provide valuable information on their genetics and evolution. This study represents the

first report on chromosome analysis in both mitotic and meiotic divisions in *Periophthalmus waltoni* and *Boleophthalmus dussumieri*, two fish species found along the coast of the Persian Gulf. We obtained *P. waltoni* chromosome number 2n=44, NF=82, with a mixture of metacentric, telocentric, subtelocentric, and submetacentric chromosomes by counting diploid chromosomes. In comparison, the related species *Periophthalmus modestus* belonging to the same genus has a chromosome number of 2n=46, consisting of acrocentric and telocentric chromosomes (Nishikawa *et al.*, 1974).

The chromosome number of *Periophthalmus cantonensis* was also  $2n=46$ , consisting of 17 pairs of di-brachial chromosomes and 6 pairs of monobrachial chromosomes. The karyotype of this species is characterized by two pairs of relatively longer arms and one pair of smaller monobrachial chromosomes (Nogusa, 1957, 1960; Arai, 2011). In previous studies, (Nogusa, 1957, 1960) reported three pairs of small chromosomes for *P. cantonensis*, while other research only identified a relatively small pair of chromosomes (Arai, 2011). In the present study, chromosomes were classified into 13 pairs of large chromosomes, six pairs of medium chromosomes, and three pairs of small chromosomes. Furthermore, three pairs of chromosomes were bi-armed, while the remainder were quadri-armed.

In our study, the diploid chromosome number of *B. dussumieri* was found to be  $2n=46$ ,  $NF=82$ , and we observed that five pairs of chromosomes were bi-armed while the remaining pairs were quadri-armed. This chromosome number is consistent with previous reports (Verma, 1968; Nishikawa *et al.*, 1974; Gadhia *et al.*, 2008). While the study conducted by (Krishnaja and Regan, 1980) reported that the karyotype of *B. dussumieri* comprised of 46 acrocentric chromosomes that were relatively large in size, but in this study chromosomes were metacentric, submetacentric, and telocentric, along with two pairs of microchromosomes. In addition, the chromosomes were categorized into 15

large pairs, 6 medium pairs, and 2 small pairs. Chromosome aberrations in this species were rare and occurred at a rate close to zero. This fish can be a useful biological model for studying the teratogenic, and carcinogenic effects of environmental chemicals (Krishnaja and Rege, 1982; Giglia-Mari and Berneburg, 2012). These findings support the idea that *B. dussumieri* can serve as a model for in vivo detection of potential mutagens (Krishnaja and Rege, 1980; Gadhia *et al.*, 2008).

The diploid chromosomes number in *B. dussumieri* is the same as that of two other species, *Boleophthalmus pectinirostris*, and *Boleophthalmus boddaerti*, which are also  $2n=46$ . *B. pectinirostris* has monobrachial chromosomes and is characterized by one to two pairs of small chromosomes (Nogusa, 1957,1960; Nishikawa *et al.*, 1974). It is reported that the karyotype of *B. boddaerti* is completely metacentric, with a large pair of heteromorphic chromosomes that may be sex chromosomes (Subrahmanyam, 1969). The absence of heterochrony or sex chromosome status in *B. boddaerti*, is similar to the findings in *P. waltoni* and *B. dussumieri*. Additionally, *B. boddaerti*, has only six pairs of metacentric chromosomes, while the rest are sub-metacentric, acrocentric, and telocentric (Manna and Prasad, 1974).

In a recent study by (da Silva *et al.*, 2021), the cytogenetic analysis of approximately 139 species of Gobiiformes revealed a diploid diversity of  $2n=30-56$  chromosomes.

The most common chromosome numbers were  $2n=46$  and  $2n=44$ . Within the Oxudercidae family, the diploid chromosome number ranges from  $2n=40$  in *Aboma lactipes* (Arai, 2011) to  $2n=50$  in *Gobius niger* (Vitturi and Catalano, 1989). Also, a meta-analysis of cytogenetic data from 169 species of the Oxudercidae family showed that it is the most diverse in chromosome number. Among these species, 37% (25 spp.) had  $2n=44$  chromosomes, 31% (21 spp.) had  $2n=46$  chromosomes, and the remaining 32% (23 spp.) had  $2n=38-56$  chromosomes. The Gobiidae family has the second-largest number of cytogenetic data for 50 species, with 42% (21 spp.) having  $2n=46$  chromosomes, 24% (12 spp.) having  $2n=44$  chromosomes, and the remaining 34% (17 spp.) having  $2n=30-50$  chromosomes (da Silva *et al.*, 2021).

Finally, the available chromosomal data for the families Gobiidae, Blenniidae, and Labrisomidae show greater karyotypic diversity within and between species compared to the other groups of Perciformes, which are more homogeneous in different chromosome characteristics (Galvão *et al.*, 2011). The Eloteridae and Buttidae families, typically have an acrocentric chromosome number of  $2n=46$  with  $NF=46$ , while the Oxudercidae family tends to have a higher frequency of  $2n=46$  chromosome number with  $NF$  greater than 46. Other families of Gobiiformes with either old or recent divergence, also have species with a

chromosome number of  $2n=46$  (da Silva *et al.*, 2021).

## References

- Abdoli, L., Kamrani, E., Abdoli, A. and Kiabi, B., 2009.** Length-weight relationships for three species of mudskippers (Gobiidae: Oxudercinae) in the coastal areas of the Persian Gulf, Iran. *Journal of Applied Ichthyology*, 25(2), 236-237. DOI: 10.30906/1026-2296-2019-25-1-1-5.
- Agorreta, A., San Mauro, D., Schlieven, U., Van Tassell, J.L., Kovačić, M., Zardoya, R. and Rüber, L., 2013.** Molecular phylogenetics of Gobioidi and phylogenetic placement of European gobies. *Molecular Phylogenetics and Evolution*, 69(3), 619-633. DOI: 10.1016/j.ympev.2013.07.017.
- Arai, R., 2011.** Fish karyotypes: A checklist. *Springer Science Publisher*, London, United Kingdom. 235 P.
- Cherednichenko, O., Pilyugina, A. and Nuraliev, S., 2022.** Cytogenetical bioindication of pesticidal contamination. *Pesticides in the Natural Environment*, 4(10), 227-260. DOI: 10.1016/B978-0-323-90489-6.00010-0.
- Da Silva, S.A.S., de Lima-Filho, P.A., da Motta-Neto, C.C., da Costa, G.W.W.F., Cioffi, M.D.B., Bertollo, L.A.C. and Molina, W.F., 2021.** High chromosomal evolutionary dynamics in sleeper gobies (Eleotridae) and notes on disruptive biological factors in

- Gobiiformes karyotypes (Osteichthyes, Teleostei). *Marine Life Science & Technology*, 3(3), 293-302. DOI: 10.1007/s42995-020-00084-6.
- Dai, Y. and Han, H., 2018.** Karyological Analysis of Two Species in the Subfamily Schizothoracinae (Cypriniformes: Cyprinidae) from China, with Notes on Karyotype Evolution in Schizothoracinae. *Turkish Journal of Fisheries and Aquatic Sciences*, 18 (1), 175-186. DOI: 10.4194/-2712-v18-1-20.
- De Lima Filho, P.A.D., 2015.** Diversidade cromossômica e padrões ecomorfológicos em Gobiidae (Perciformes) no litoral e ilhas oceânicas do Brasil. *Rio Grande do Norte publisher*, Brasilia, Brazil. 141 P.
- Esmaeili, H.R., Sayyadzadeh, G., Eagderi, S. and Abbasi, K., 2018.** Checklist of freshwater fishes of Iran. *FishTaxa*, 3(3), 1-95.
- Fricke, R., Eschmeyer, W.N. and Fong, J.D., 2021.** Species by family/subfamily in Eschmeyer's Catalog of Fishes. *Online Version*. Available at: <http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp>.
- Gadhia, P.K., Gadhia, M., Georje, S., Vinod, K.R. and Pithawala, M., 2008.** Induction of chromosomal aberrations in mitotic chromosomes of fish *Boleophthalmus dussumieri* after exposure in vivo to antineoplastics Bleomycin, Mitomycin-C, and Doxorubicin. *Indian Journal of Science and Technology*, 1(1), 1-6. DOI: 10.1016/0165-1218(82)90147-1.
- Galvão, T.B., Bertollo, L.A.C. and Molina, W.F., 2011.** Chromosomal complements of some Atlantic Blennioidei and Gobioidae species (Perciformes). *Comparative cytogenetics*, 5(4), 247- 259. DOI: 10.3897/CompCytogen5i4.1834.
- Ghanbarifardi, M. and Damadi, E., 2021.** Molecular phylogeny of Gobiidae from Iran based on mitochondrial and nuclear markers. *Journal of Animal Research (Iranian Journal of biology)*, 34(4), 362-374. DOI: 10.1134/S1063074019050055.
- Ghanbarifardi, M., Aliabadian, M., Esmaeili, H.R. and Polgar, G., 2014.** Morphological divergence in the Walton's mudskipper, *Periophthalmus waltoni* Koumans, 1941, from the Persian Gulf and Gulf of Oman (Gobioidae: Gobiidae). *Zoology in the Middle East*, 60(2), 133-143. DOI: 10.1080/09397140.2014.914717.
- Ghanbarifardi, M., Esmaeili, H.R., Gholami, Z., Aliabadian, M. and Reichenbacher, B., 2016.** Molecular phylogeny of three mudskippers (Gobiidae) from the Persian Gulf and Gulf of Oman (Makran). *Journal of Applied Ichthyology*, 32(3), 416-420. DOI: 10.1111/jai.12999.
- Giglia-Mari, G. and Berneburg, M., 2012.** DNA in 3R: Repair, Replication, and Recombination. *Molecular Biology International*,

- 2012, 1 page. DOI: 10.1155/2012/658579.
- Gill, A.C. and Mooi, R.D., 2012.** Thalasseleotrididae, new family of marine gobioid fishes from New Zealand and temperate Australia, with a revised definition of its sister taxon, the Gobiidae (Teleostei: Acanthomorpha). *Zootaxa*, 3266(1), 41-52. DOI: 10.11646/zootaxa.3266.1.3.
- Hickman, C.P., Roberts. K. and Larson, I., 2020.** Integrate principles of zoology. sixteenth edition. 11<sup>th</sup> ed., *Published by McGraw-Hill*, New York, United States. 98 P.
- Ishimatsu, A. and Gonzales, T.T., 2011.** Mudskippers: front runners in the modern invasion of land. *The biology of gobies, Enfield: Science Publishers*, Boca Raton, United States. 38 P.
- Jaafar, Z., Polgar, G. and Zamroni, Y., 2016.** Description of a new species of *Periophthalmus* (Teleostei: Gobiidae) from the Lesser Sunda Islands. *Raffles Bulletin of Zoology*, 64, 278-283.
- Jeon, H.B., Jun, J., Choi, S.H. and Suk, H.Y., 2021.** Diversification and colonization processes in Gobioidei predicted based on mitochondrial 12S rRNA with focusing on Oxudercidae. *Mitochondrial DNA Part B*, 6(3), 1166-1172. DOI: 10.1080/23802359.2021.1901620.
- Kovalchuk, O., Świdnicka, E. and Stefaniak, K., 2018.** A new record of *Gobius jarosi* (Teleostei, Gobiidae) from the Early Miocene of Poland with inference to paleogeography and palaeoecology of the Carpathian Basin. *Historical Biology*, 31(10), 1394-1401. DOI: 10.1080/08912963.2018.1457032.
- Krishnaja, A.P. and Rege, M.S., 1980.** Some observation on the chromosome of certain teleosts using a simple method. *Indian Journal of Experimental Biology*, 18(3), 268-270.
- Krishnaja, A.P. and Rege, M.S., 1982.** Induction of chromosomal aberrations in fish *Boleophthalmus dussumieri* after exposure in vivo to Mitomycin-C and heavy metals. *Genetic Toxicology*, 102(1), 71-82. DOI: 10.1016/0165-1218(82)90147-1.
- Krishnaja, A.P., 1980.** Fish as an in vivo cytogenetic model in the detection of potential mutagens, Ph.D. Thesis, *Bombay University*, Bombay, India. 91 P.
- Levan, A., Fredga, K. and Sandberg, A.A., 1964.** Nomenclature for centromeric position on chromosomes. *Hereditas*, 52, 201-220. DOI: 10.1111/j.1601-5223.1964.tb01953.x.
- Lorente-Martínez, H., Agorreta, A., Torres-Sánchez, M. and San Mauro, D., 2018.** Evidence of positive selection suggests possible role of aquaporins in the water-to-land transition of mudskippers. *Organisms Diversity & Evolution*, 18(4): 499-514. DOI: 10.1007/s13127-018-0382-6.
- Manna, G.K. and Prasad, R., 1974.** Chromosome analysis in three species of fishes belonging to the

- family Gobiidae. *Cytologia*, 39(3), 609–618. DOI: 10.1508/cytologia.39.609.
- Murdy, E., 2011.** Systematics of Oxudercinae. *The biology of gobies*, Arlington, United States. pp. 99-106.
- Murdy, E.O., 1989.** A taxonomic revision and cladistic analysis of the *oxudercine gobies* (Gobiidae: Oxudercinae). Records of the Australian Museum, *Supplements*, 11, 1–93. DOI: 10.3853/ j.0812-7387.11.1989.93.
- Nascimento, T., Gongalves, S.R., Bàez, M., Seijo, G. and Guerra, M., 2022.** Molecular cytogenetics reveals an uncommon structural and numerical chromosomal heteromorphism in *Zephyranthes brachyandra* (Amaryllidaceae). *Boletín de la Sociedad Argentina de Botánica*, 57(1), 31-40. DOI: 10.3897/CompCytogen.v11i3.13418.
- Nasri, M., Keivany, Y. and Dorafshan, S., 2010.** First karyological analysis of smallmouth lotak, *Cyprinion kais* Heckel, 1843, an endemic Cyprinid fish from the Tigris-Euphrates Basin. *Italian Journal of Zoology*, 77(3), 272–276. DOI: 10.1080/11250000903140717.
- Nelson, J.S., 2006.** Fishes of the world. 4<sup>th</sup> ed., *Published simultaneously in Canada*, New Jersey, United States. 127 P.
- Nelson, J.S., Grande, T.C. and Wilson, M.V., 2016.** Fishes of the World. 5<sup>th</sup> ed., *Published by John Wiley & Sons, Inc., Hoboken*, New Jersey, United States. 452 P.
- Nishikawa, S., Amaoka, K. and Nakanishi, K., 1974.** A comparative study of chromosomes of twelve species of gobioid fishes in Japan. *Japanese Journal of Ichthyology*, 21(2), 61-71. DOI: 10.11369/jji1950.21.61.
- Nogusa, S., 1957.** Chromosome Studies in Pisces VII. A Comparative Study of the Chromosomes in Six Species of the Gobiidae. *Japanese Journal of Ichthyology*, 6(4-6), 141-146. DOI: 10.11369/jji1950.6.141.
- Nogusa, S., 1960.** A comparative study of the chromosomes in fishes with particular considerations on taxonomy and evolution. *Members Hyogo University Agriculture*, 3(1), 61-71.
- Polgar, G., Ghanbarifardi, M., Milli, S., Agorreta, A., Aliabadian, M., Esmaeili, H.R. and Khang, T.F., 2017.** Ecomorphological adaptation in three mudskippers (Teleostei: Gobioidae: Gobiidae) from the Persian Gulf and the Gulf of Oman. *Hydrobiologia*, 795(1), 91-111. DOI: 10.1007/s10750-017-3120-8.
- Sharma, O.P., Tripathi, N.K. and Sharma, K.K., 2002.** A Review of Chromosome Banding in Fishes. Some Aspects of Chromosome Structure and Function: Chromosome Structure and Function. *Online ISBN*, 109-122. DOI: 10.1007/978-94-010-0334-6\_11.
- Subrahmanyam, K., 1969.** A karyotypic study of the estuarine fish *Boleophthalmus boddarti* (Pallas)

with calcium treatment. *Current Science*, 38: 437–439.

**Turpin, R. and Lejeune, J., 1965.** Les Chromosomes Humains. Paris: Gautier-Villars. French. *University of Sistan and Baluchestan*, 94-95.

**Verma, G.K., 1968.** Studies on the structure and behaviour of chromosomes of certain fresh water and marine gobiid fishes. *Annals of the National Academy of Medical Science*, 1968, 166-178.

**Vitturi, R. and Catalano, E., 1989.** Multiple chromosome polymorphism in the gobiid fish *Gobius niger* jazo L. 1758 (Pisces,

Gobiidae). *Cytologia*, 54(2), 231-235. DOI:

10.1508/cytologia.54.231.

**Yang, Y., Yoo, J.Y., Baek, S.H., Song, H.Y.J.o.S., Jung, S.H. and Choi, J.H., 2022.** Chromosome-level genome assembly of the shuttles hopfish, *Periophthalmus modestus*. *GigaScience*, 11(1), 77-89. DOI:

10.1093/gigascience/giab089.