Iranian Journal of Fisheries Sciences 22(2) 368-383 2023
DOI: 10.22092/ijfs.2023.129085

Research Article

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

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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: *Periphthalmus waltoni*, *Boleophthalmus dussumieri*, Chromosomes, Cytology, Persian Gulf

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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 (48º 59' 8" E and 30º 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).

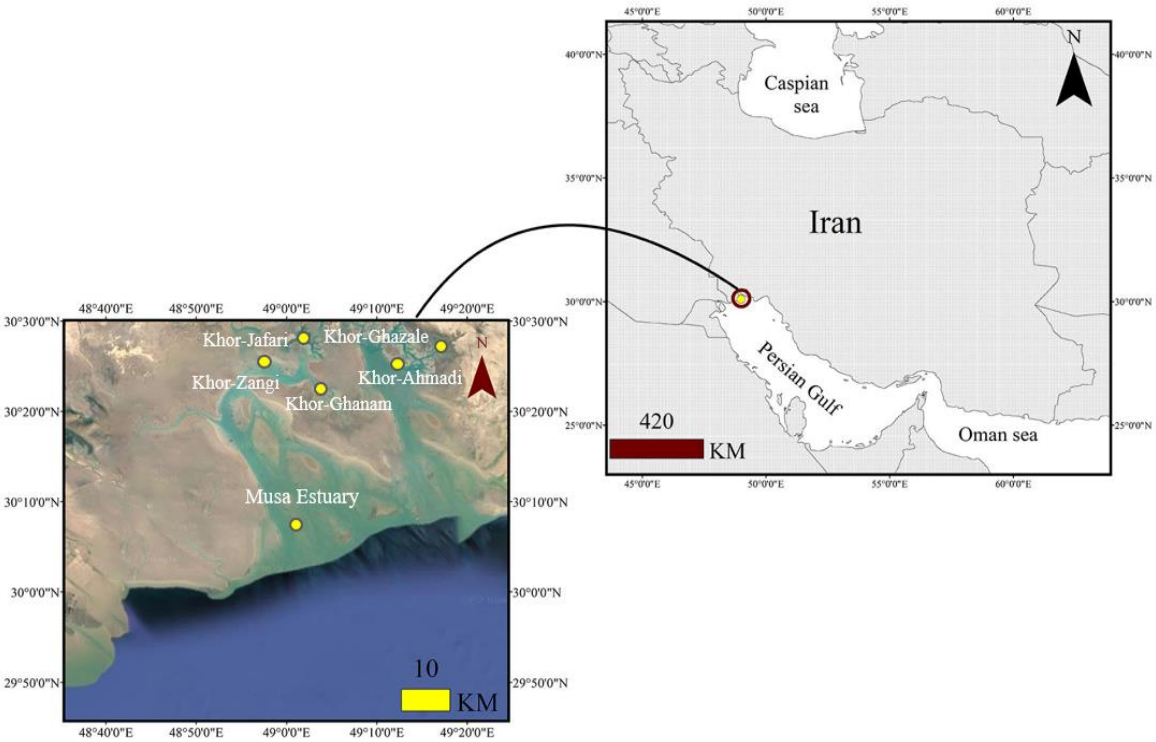

Figure 2: A map showing Musa estuary, with the sampling area

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 ×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).

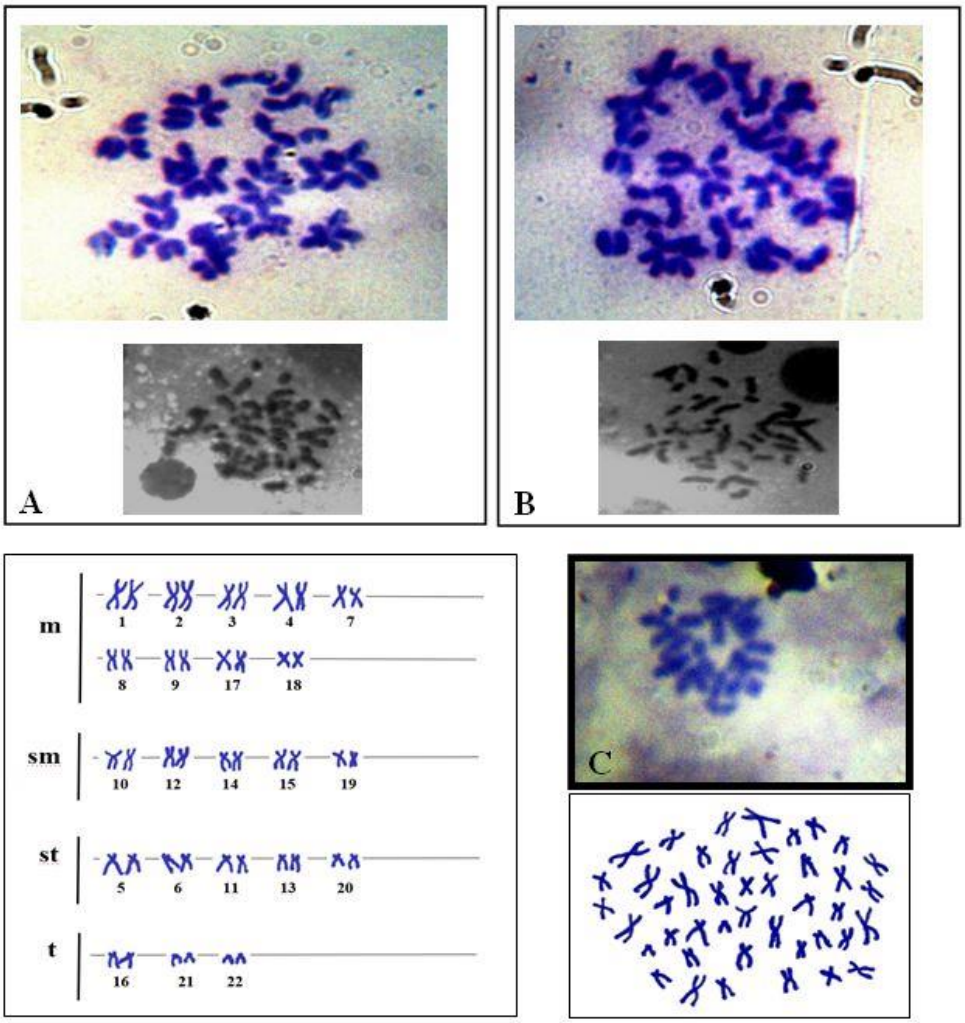

Figure 3: Karyotype prepared from chromosomes of metaphase stage of mitosis in male (A) and female (B) *P.waltoni*, C: Haploid chromosome in a male, 2n=44 by conventional staining.

Table 1: Karyotype data of two mudskippers.

Species	Plural	Constitute a model of chromosome	arm number
P. waltoni	44	2n = 9m + 5sm + 5st + 3t	82
B. dussumieri	46	2n = 13m + 5sm + 3t + 2μ-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 μm to 8.63 μm (Fig. 4 and Table 2). NO sex chromosomes were observed, in this species, indicating a lack of -hetero chromosomal status.

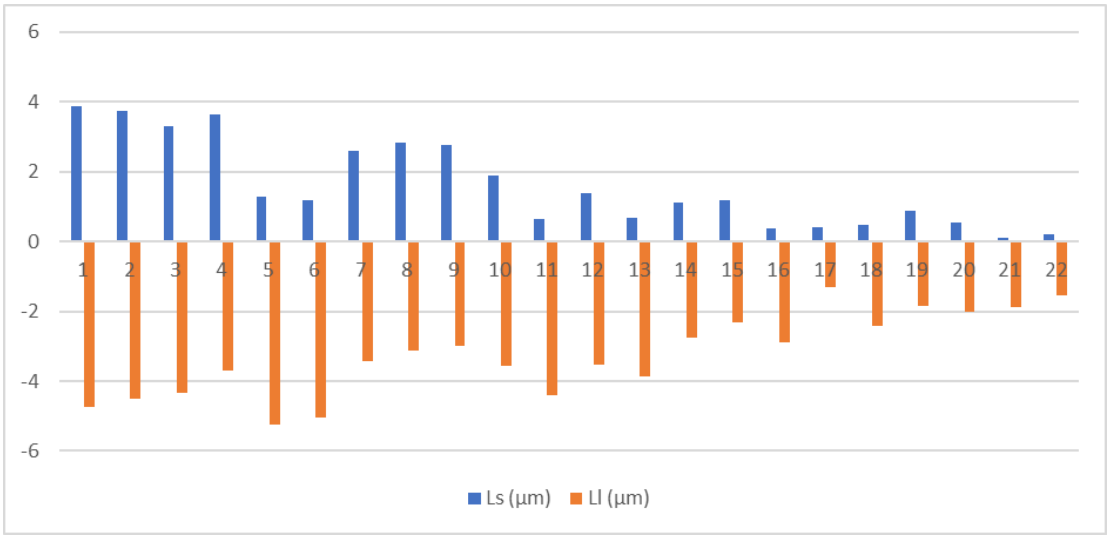

Figure 4: Idiogram of chromosomes of metaphase stage of mitosis in *P. waltoni*.

Table 2: Mean length of the short arm chromosome (Ls), Longarm chromosome (Ll), Total arm chromosome (LT), The ratio of the arms (Ll/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)	Ll (µm)	LT (µm)	Ratio Ll/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
Total	**35.18**	**71.49**	**106.64**						**82**

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.

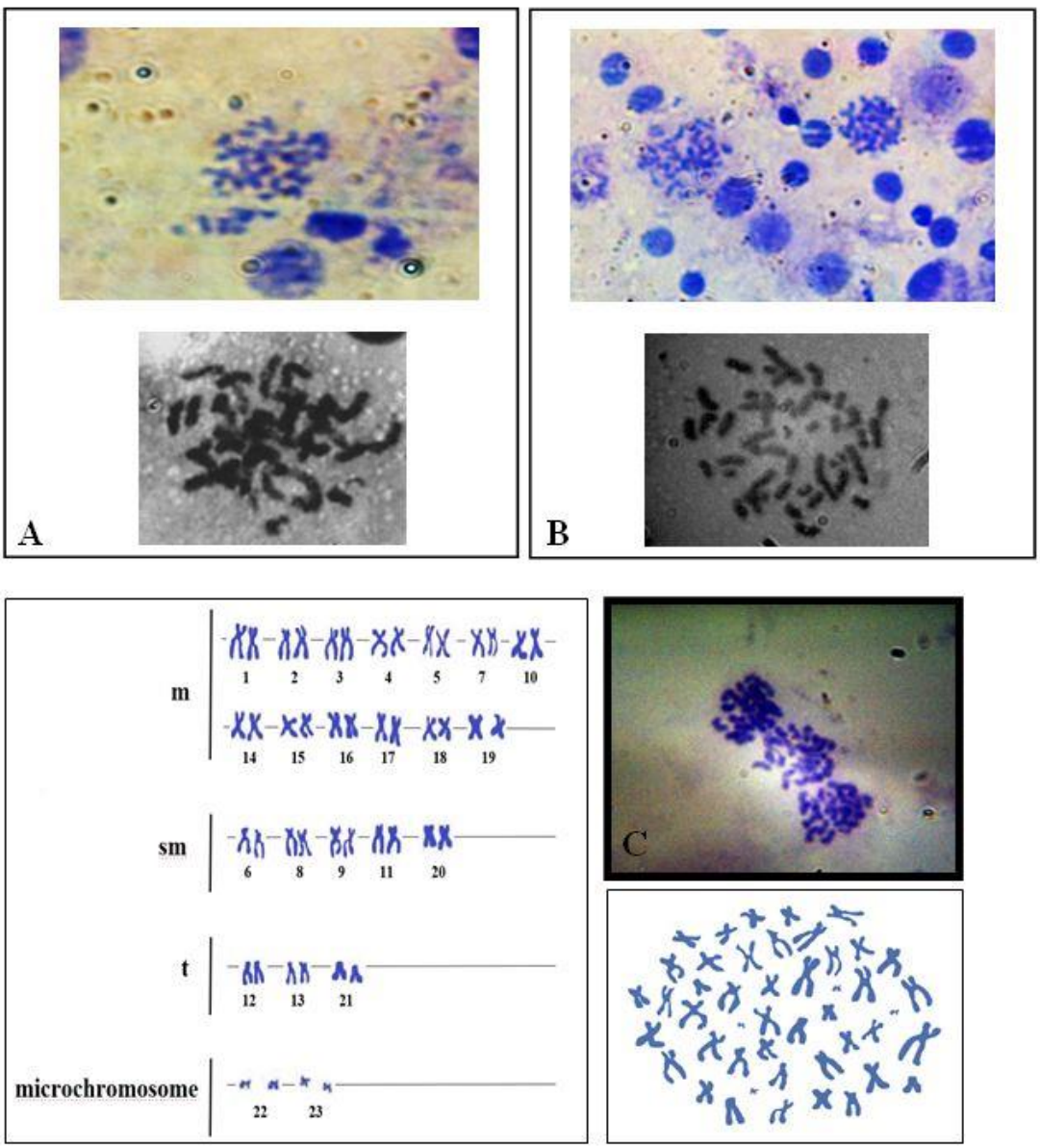

Figure 5: Karyotype prepared from chromosomes of metaphase stage of mitosis in male (A) and female (B) *B.dussumieri*, C: Haploid chromosome in a male, 2n=46 by conventional staining.

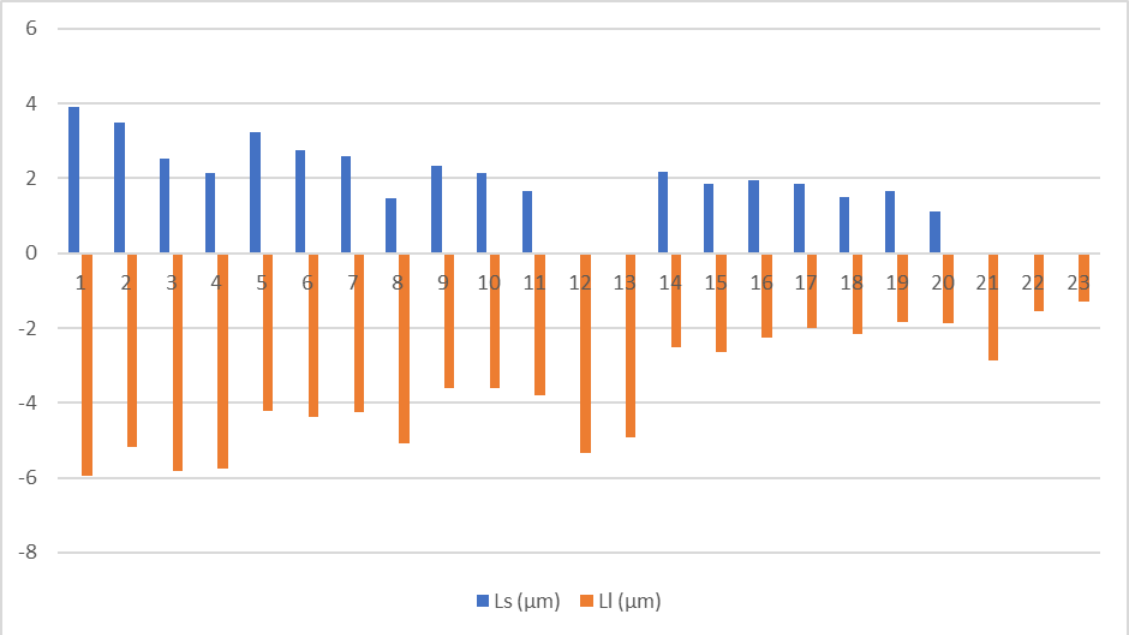

Figure 6: Idiogram of chromosomes of metaphase stage of mitosis in *B. dussumieri*.

Table 3: Mean length of the short arm chromosome (Ls), Longarm chromosome (Ll), Total arm chromosome (LT), The ratio of the arms (Ll/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)	Ll (µm)	LT (µm)	Ratio Ll/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
Total	**40.38**	**82.89**	**123.27**						**82**

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

Species	2n	Chro morphology	NF	Reference	Local
Boleophthalmus boddarti	46			Verma (1968)	India
	46	46m/sm	92	Subrahmanyam (1969)	Indo-Pacific
	46			Verma (1968)	India
	46	46a		Nishikawa *et al.* (1974)	Japan
	46	14m+18sm+14a	46	Krishnaja and Regan (1980)	India
	46		78	Khuda-Bukush and Barat (1987)	Indo-Pacific
	46			Gadhia *et al.* (2008)	India
Boleophthalmus dussumieri	**46**	**13m+5sm+3t+2µ-chro**	**82**	**Present report**	**Persian Gulf**
Boleophthalmus glaucus (*Scartelaos cantoris*)	46	12m+20sm+2st+12a	80	Manna and Prasad (1974)	Indo-Pacific
Boleophthalmus pectinirostris	46	46st/a	46	Aria and Sawada (1975)	Indo-Pacific
Periophthalmus borneensis	48			Verma (1968)	Indo-Pacific
Periophthalmus cantonensis (*Periophthalmus novaeguineaensis*)	46	46t/a		Nogusa (1957, 1960)	Japan
	46			Arai and Kobayasi (1973)	Japan
	46	34m/sm+12t/a	80	Nishikawa *et al*. (1974)	Japan
	46	18m+12sm+16st/a	76	Aria and Sawada (1975)	Indo-Pacific
Periophthalmus chrysospilos	56			Matsuo (1998)	Indo-Pacific
Periophthalmus modestus	46	16m/sm+30a	62	Lee (1968a)	Indo-Pacific
	46	46a/t	46	Nishikawa *et al*. (1974)	Japan
Periophthalmus scholosseri	46	46a	46	Matsuo (1998)	Indo-Pacific
Periophthalmus serperaster	46	8m+16sm+4st+18a	74	Khuda-Bukhsh and Nayak (1987)	Indo-Pacific
Periophthalmus waltoni	**44**	**9m+5sm+5st+3t**	**82**	**Present report**	**Persian Gulf**
Scartelaos histophorus	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% (12spp.) having 2n=44 chromosomes, and the remaining 34% (17spp.) having 2n=30-50 chromosomes (da Silva *et al.*, 2021).

Finally, the available chromosomal data for the families Gobiidae, Bleniidae, 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).

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