Marine Biology (2004) 146: 161–165
DOI 10.1007/s00227-004-1422-7

RESEARCH ARTICLE

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Karyology of the toadfish *Porichthys plectrodon* (Jordan and Gilbert, 1882) (Batrachoididae) from Margarita Island, Venezuela

Received: 29 October 2002 / Accepted: 8 June 2004 / Published online: 14 July 2004

Abstract This paper reports the results of cytogenetic analyses carried out on *Porichthys plectrodon* using conventional Giemsa staining, C-banding and silver staining techniques. A diploid chromosome count of $2n=44$ was observed, consisting of 8 metacentric, 10 submetacentric, 6 subtelocentric and 20 acrocentric chromosomes. Differences in length made it possible to identify homologous chromosomes within the metacentric group. Constitutive heterochromatin was distributed as large pericentromeric blocks in pairs 1 and 2, while the rest of the chromosomes were marked in centromeric regions, some more conspicuously than others. One pair of small-sized acrocentric NOR-bearing chromosomes (21) was identified by the nucleolar regions located terminally on their short arms.

Introduction

The family Batrachoididae is a small group of fish, commonly known as toadfish, containing around 70 species divided into 21 genera and three subfamilies (Nelson 1994; Greenfield et al. 1994; Collette 1995, 2001; Greenfield 1997). Although no fishing statistics are available, larger species of toadfishes are commonly found in local markets and may fetch high prices in Venezuela and French Guiana (http://www.fishbase.org/Summary/FamilySummary.cfm?ID=189).

The Batrachoididae family includes four species (*Opsanus tau*, *O. beta*, *Porichthys notatus* and *Halobatrachus didactylus*) which are considered important experimental organisms for biomedical and physiological studies (see Freshwater et al. 2000 and Palazón-Fernández et al. 2001 for references).

Cytogenetic data on batrachoidid species are restricted to number and chromosome formulae for *Amphychthys cryptocentrus*, *Batrachoides manglae* and *Thalassophryne maculosa* from Venezuela (Nirchio et al. 2002b, 2004), *B. pacifici* from Panama (Nirchio et al. 2002a), *Porichthys porosissimus* from Brazil (Brum et al. 2001) and *H. didactylus* from Spain (Palazón et al. 2003).

In this study we describe the diploid number, chromosome formulae, C-band pattern, and NOR locations in *Porichthys plectrodon* from Margarita Island, Venezuela. This toadfish is found in the western Atlantic Ocean from Virginia to Brazil (Hoese and Moore 1998) and is one of the five species of toadfish recognized in Venezuela (Cervigón 1993). This species is found on sand or mud beds, mostly at depths between 10 and 250 m, in a wide variety of salinities and temperatures (Lane 1967; Moore 1970).

Materials and methods

Eleven specimens (eight males, three females) of *P. plectrodon* from Margarita Island, Venezuela were analyzed. Voucher specimens were deposited at the Ichthyology Collection of the Escuela de Ciencias Aplicadas del Mar, Universidad de Oriente (Venezuela).

The preparation of chromosomes was performed according to Nirchio et al. (2002b) but reducing the

Communicated by P.W. Sammarco, Chauvin

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colchicine incubation time. Each specimen was injected intraperitoneally with a colchicine solution (0.5%; 1 ml/100 g fish weight). The fish were maintained in a well-aerated aquarium and after 3 h they were sacrificed, and the kidneys were extracted and placed in a hypotonic solution of 0.4% KCl. Every single kidney was minced with fine forceps and then with a glass syringe by repeated aspiration and forced release until a fine cellular suspension was obtained. After 30 min in the hypotonic solution, the cellular suspension was centrifuged at 1,000 rpm for 3 min. The hypotonic solution was discarded and the cellular button was suspended and washed three times in a methanol-acetic acid mixture 3:1 (V:V).

One droplet of the cellular suspension was dropped on a clean microscope slide, previously chilled in a freezer, from a height of 45 cm. The slides were briefly put over a flame and then allowed to air-dry.

For the conventional karyotype, the preparations were stained for 20 min with 5% Giemsa in phosphate buffer pH 6.88. Detection of the nucleolus organizer regions (NORs) was done following the silver staining method of Howell and Black (1980). C-bands were obtained according to the methods described by Sumner (1972).

A total of 134 mitotic spreads (10–12 plates per individual) were scanned to determine the modal chromosome number, and 55 spreads (five plates per individual) were used to determine the number of NORs.

The mitotic figures were photographed using a green filter and 50 ASA film. The resulting photographs were then scanned and stored as *.tif images. Long arm (L), short arm (S) and whole chromosome length were measured for each chromosome to the nearest 0.01 mm, using the measuring tool in Adobe Photoshop software v.7.0. The length of each chromosome pair in relation to the total chromosome length (RL%) was obtained from these values. Chromosomes were identified by the arm ratio criteria proposed by Levan et al. (1964).

Results

All specimens of *P. plectrodon* under study were characterized by a diploid chromosome count of $2n=44$, obtained in 97% of cells examined (130 cells). The hypomodal and hypermodal counts, on the whole, barely reached 3% of all the cells recorded, and probably resulted from preparation-caused defects such as chromosome loss, overlap, miscounting, and additional chromosomes from another spread.

The standard karyotype of the species, prepared by arrangement of chromosomes into groups based on L/S ratio, is shown in Fig. 1. No differences were observed between sexes. This karyotype was composed of 8 metacentric (M), 10 submetacentric (SM), 6 subtelocentric (ST), and 20 acrocentric (A) elements, with an arm number (NF = *Nombre Fondamentale*) of 62. The length of the M chromosomes compared to the total length of the diploid chromosome length (RL%) was 29.5%, ranging from 3.1% to 13.5% per chromosome. For the SM chromosomes, RL% was 19.7%, ranging from 3.2% to 4.9%. For the ST chromosomes, RL% was 12.5%, ranging from 3.9% to 4.4%. For the A chromosomes, RL% was 38.3%, ranging from 2.3% to 4.5%. The sizes of the metacentric chromosomes were very distinct: each pair, from the smallest to the largest, was approximately half the size of the following pair, making the identification of homologous pairs a simple task. However, minimal differences in chromosome size and arm ratio in the submetacentric, subtelocentric and acrocentric chromosomes made it difficult to classify homologous pairs with such certainty. Table 1 summarizes chromosome measurements of the different pairs.

Silver NOR staining of the selected metaphase spreads revealed one pair of small-sized acrocentric NOR-bearing chromosomes with black dots located terminally on the short arms of these chromosomes. They could be identified as pair 21 (Fig. 1A). No NOR polymorphisms were observed, but in several cases an intimate association was seen between the NOR-bearing arms of the two homologous chromosomes (Fig. 2C).

The visualization of C-banding revealed large positive pericentromeric bands at pairs 1 and 2. The heterochromatic blocks occupy approximately 38% of the chromosomes in pair 1 and around 27% in pair 2. In the rest of the chromosomes, heterochromatin was found at centromeric positions, some more conspicuous than others (Fig. 1B).

Discussion

Available data on the karyotype of batrachoidid species report a diploid chromosome number of $2n=46$, a condition repeated in several species, with the exception of *P. porosissimus* (Brum et al. 2001) and *P. plectrodon*, which each possess a complement of $2n=44$ (Table 2). Assuming that the karyotype for present teleosteans derives from an ancestral diploid number of $2n=48$ uniarmed chromosomes (Ohno 1974; Gold 1979; Vitturi et al. 1995), a chromosome number under $2n=48$ (Table 2) and the presence of a high number of biarmed elements could indicate that the karyotype of *P. plectrodon* is a derivative character (apomorphic) among Batrachoididae.

C-banding revealed that the heterochromatin in *P. plectrodon* occupies a wide pericentromeric region of the pairs 1 and 2. Other less conspicuous pericentromeric bands were observed in most of the chromosomes (Fig. 1B). This pattern of heterochromatin distribution is similar to the one reported for *P. porosissimus* (Brum et al. 2001) and can be attributed to the Robertsonian fusion of two large pairs of uniarmed chromosomes, reinforcing the hypothesis of Brum et al. (2001) regarding the origin of these metacentric chromosome pairs. On the other hand, C-banding in *T. maculosa* has shown the presence of heterochromatic regions restricted

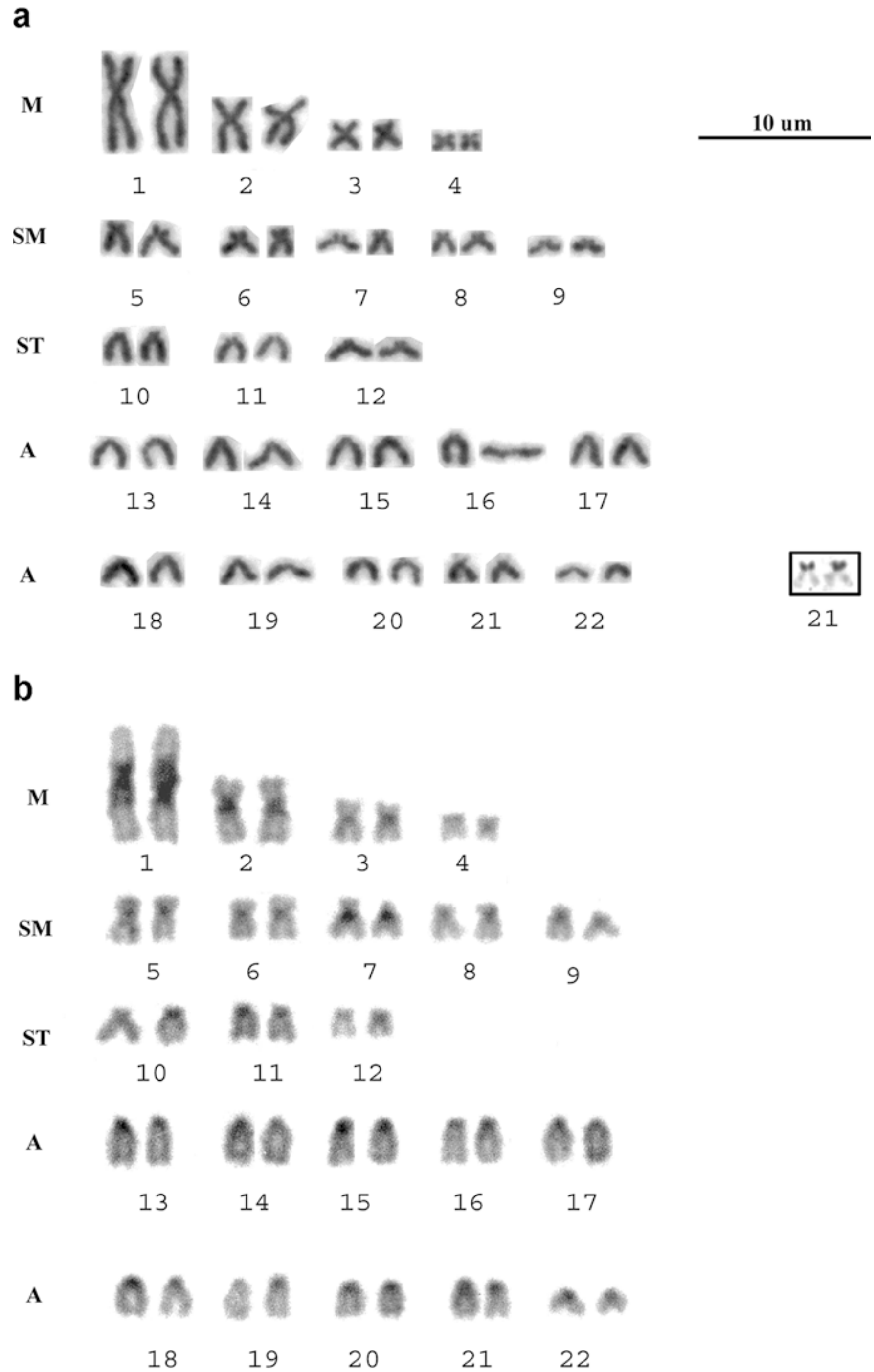

Fig. 1 a Karyotype of *Porichthys plectrodon* stained conventionally with Giemsa. NOR-bearing chromosomes appear in the *square inset*. **b** C-banded karyotype. *Bar* 10μm

Table 1 Chromosome number and formulae in six species of toadfish. *NF* Arm number (*Nombre Fondamentale*), *M* metacentric, *SM* submetacentric, *ST* subtelocentric, *A* acrocentric

Species	2*n*	Karyotype formula	NF	Reference
Amphychthys cryptocentrus	46	4M:2SM:40A	52	Nirchio et al. 2002a
Batrachoides manglae	46	6M:6SM:34A	58	Nirchio et al. 2002a
B. pacifici	46	6M:6SM:34A	58	Nirchio et al. 2002b
Halobatrachus didactylus	46	8M:12SM:26A	66	Palazón et al. 2003
Porichthys porosissimus	44	14M/SM:30ST/A	58	Brum et al. 2001
P. plectrodon	44	8M:10SM:6ST:20A	62	Present study
Thalassophryne maculosa	46	12M:6SM:20ST:8A	64	Nirchio et al. 2004

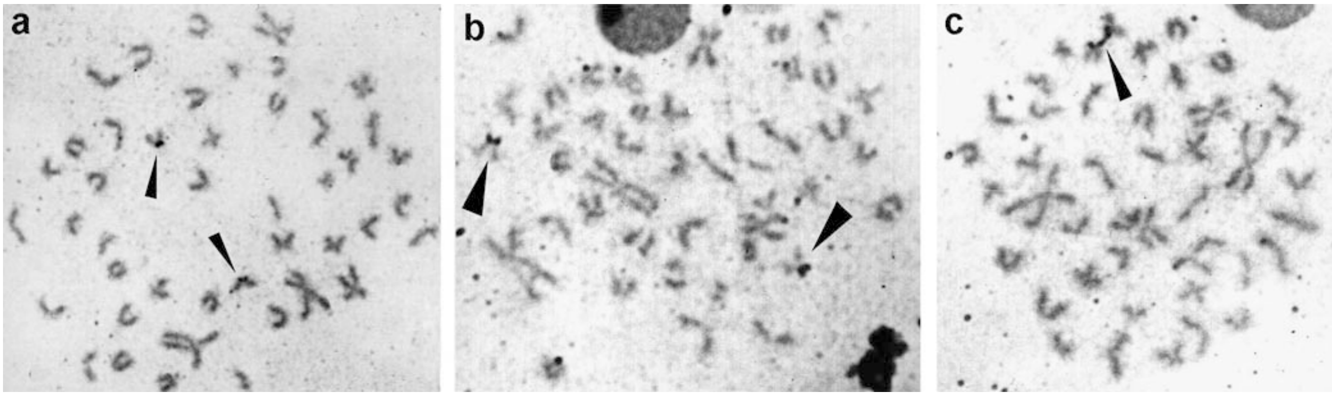

Fig. 2a–c Metaphase plates of *Porichthys plectrodon* stained for NOR. Observe constancy of NOR (**a**, **b**) and NOR-bearing chromosome association (**c**)

to centromeres (Nirchio et al. 2004). This dissimilar feature between *Talasophryne* and *Porichthys* suggests that C-bands could be a good cytotaxonomic marker in the family Batrachoididae.

As far as the available information allows generalization, NORs in the Batrachoididae seem to be a non-conservative feature. Accordingly, *P. plectrodon* exhibited positive signals on the telomeric regions of the short arm of pair 21 (Fig. 1A); *Halobatrachus didactylus* possesses a single pair of NOR-bearing chromosomes but submetacentric instead of subtelocentric (Palazón et al. 2003). Unsatisfactory results were obtained by Brum et al. (2001) when attempting to detect NORs by silver impregnation in *P. porosissimus*, but they inferred the occurrence of more than a single NOR-bearing pair on the basis of the presence of one to three nucleoli per nucleus, whereas Nirchio et al. (2004) established that *T. maculosa* does not possess additional NORs since fluorescence in situ hybridization revealed fluorescent signals on the telomeric region of the short arm of only one medium-sized subtelocentric chromosome pair (pair 16), the same that was identified using silver salts.

Although more information is required for inferring phylogenetic relationships between batrachoidid species, available data suggest that chromosome translocation events involving the active NOR sites detectable by silver impregnation seem to be associated with the diversification in the group; hence NOR sites have a potentially high cytotaxonomical value within this fish group.

An interesting feature observed in this study was an intimate association between the NOR-bearing arms of two homologous acrocentric chromosomes (Fig. 2C). Similar behaviour has occasionally been observed in *Gobius fallax* (Thode et al. 1983) and *Cyprinus carpio* (Anjum and Jankun 1998). A study using several staining techniques in *Oedalechilus labeo* (Rossi et al. 2000) has also presented two active NOR-bearing chromo-

Table 2 Mean ± SD of the absolute length of each chromosome pair [*AL*(μm)], length of each chromosome as percentage of total length of the haploid complement (*RL%*), value of the arm ratio (*C*) and type of chromosome morphology according to arm ratio (*T*)

Chromosome number	AL (μm)	RL%	C	T
1	5.480 ± 0.140	13.550 ± 0.350	1.450 ± 0.060	M
2	3.330 ± 0.180	8.240 ± 0.420	1.360 ± 0.110	M
3	1.860 ± 0.100	4.600 ± 0.250	1.040 ± 0.050	M
4	1.250 ± 0.030	3.100 ± 0.080	1.320 ± 0.100	M
5	2.000 ± 0.130	4.930 ± 0.340	2.390 ± 0.210	SM
6	1.750 ± 0.030	4.320 ± 0.080	1.750 ± 0.110	SM
7	1.530 ± 0.020	3.780 ± 0.060	2.440 ± 0.210	SM
8	1.430 ± 0.020	3.520 ± 0.070	2.140 ± 0.270	SM
9	1.230 ± 0.020	3.030 ± 0.060	2.000 ± 0.120	SM
10	1.780 ± 0.060	4.390 ± 0.140	5.730 ± 0.600	ST
11	1.700 ± 0.020	4.200 ± 0.050	3.080 ± 0.230	ST
12	1.580 ± 0.040	3.900 ± 0.090	3.780 ± 0.470	ST
13	1.840 ± 0.040	4.560 ± 0.100	47.500 ± 0.870	A
14	1.810 ± 0.020	4.490 ± 0.050	46.750 ± 0.430	A
15	1.810 ± 0.020	4.490 ± 0.040	46.750 ± 0.430	A
16	1.800 ± 0.050	4.440 ± 0.120	46.250 ± 1.090	A
17	1.770 ± 0.040	4.370 ± 0.100	45.500 ± 0.870	A
18	1.620 ± 0.020	3.990 ± 0.060	41.500 ± 0.500	A
19	1.400 ± 0.080	3.450 ± 0.180	35.750 ± 1.790	A
20	1.380 ± 0.090	3.410 ± 0.230	35.250 ± 2.170	A
21	1.140 ± 0.000	2.820 ± 0.010	29.000 ± 0.000	A
22	0.970 ± 0.040	2.400 ± 0.090	24.500 ± 0.870	A

somes associated at the tips of their NOR-bearing arms, but their association pattern was not described. According to Anjum and Jankun (1998), such associations between chromosomes may result from a tendency of the NOR-bearing chromosomes to be found and most likely reflect their joint participation in the formation of the common nucleolus during the preceding interphase.

For comparative purpose, we calculate the arm number (NF) of all toadfishes so far karyotyped by assigning a value of 2 to biarmed chromosomes (metacentric and submetacentric) and a value of 1 to uniarmed chromosomes (subtelocentric and acrocentric). Thus, NF is 52 for *A. cryptocentrus*, 58 for *B. manglae*, *B. pacifici* and *P. porosissimus*, 64 for *T. maculosa*, 66 for *H. didactylus* and 62 for *P. plectrodon* (see Table 2 for references). According to LeGrande (1981), differences in the NF among closely related species can be the result of pericentric inversions, whereas the differences in the diploid number ($2n$) presumably represent Robertsonian changes (fusions, fissions). Thus, the karyotype of *P. plectrodon* can be interpreted as the result of structural chromosomic rearrangements involving at least the central fusion of four pairs of uniarmed chromosomes to form two large pairs of biarmed elements (metacentric or submetacentric) resulting in the reduction of the diploid chromosome number from the ancestral fish karyotype ($2n=48$) to $2n=44$, as well as a series of pericentric inversions, generating biarmed chromosomes and so increasing the NF.

In conclusion, the chromosome analysis of species within the Batrachoididae family, using conventional staining procedures, reveals a great variability in the size, shape and number of chromosomes, as well as in NOR sites and constitutive heterochromatin distribution, suggesting that the diversification in the family is closely related to numerical and structural changes in the chromosomes. Therefore, cytological information may be a powerful tool in further taxonomical research work on Batrachoididae.

References

Anjum R, Jankun M (1998) NOR-bearing chromosomal associations revealed through silver and sequential chromomycin A3 staining in the mirror carp, *Cyprinus carpio*. Caryologia 51:167–171

Brum MJI, Affonso PRAM, Mota LCG, Pauls E, Netto MRCB (2001) Cytogenetic characterization of *Porichthys porosissimus* (Valenciennes, 1857) (Batrachoididae, Batrachoidiformes) from the Rio de Janeiro Coast, Brazil. Chromosome Sci 5:15–18

Cervigón F (1993) Los peces marinos de Venezuela, 2nd edn, vol II. Fundación Científica Los Roques, Caracas, Venezuela

Collette BB (1995) *Potamobatrachus trispinosus*, a new freshwater toadfish (Batrachoididae) from the Rio Tocantins, Brazil. Ichthyol Explor Freshw 6:333–336

Collette BB (2001) *Opsanus dichrostomus*, a new toadfish (Teleostei: Batrachoididae) from the western Caribbean Sea and southern Gulf of Mexico. Occasional Papers of the Museum of Zoology the University of Michigan, no. 731

Freshwater DW, Khyn-Hansen C, Sarver SK, Walsh PJ (2000) Phylogeny of *Opsanus* spp (Batrachoididae) inferred from multiple mitochondrial-DNA sequences. Mar Biol 136:961–968

Gold J (1979) Cytogenetics. In: Hoar WS, Randall DJ (eds) Fish physiology, vol 8. Academic Press, New York, pp 353–405

Greenfield DW (1997) *Allenbatrachus*, a new genus of Indo-Pacific toadfish (Batrachoididae). Pac Sci 51:306–313

Greenfield DW, Mee JKL, Randall JE (1994) *Bifax lacinaia*, a new genus and species of toadfish (Batrachoididae) from the south coast of Oman. Fauna Saudia Arabia 14:276–281

Hoese HD, Moore RH (1998) Fishes of the Gulf of Mexico: Texas, Louisiana and adjacent waters. Texas A&M University Press, College Station

Howell WM, Black DA (1980) Controlled silver staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method. Experientia 3:1014–1015

Lane ED (1967) A study of the Atlantic midshipman, *Porichthys porosissimus*, in the vicinity of Port Aransas, Texas. Contrib Mar Sci Univ Tex 12:1–53

LeGrande WH (1981) Chromosomal evolution in North American catfishes (Siluriformes: Ictaluridae) with particular emphasis on the m adtoms, Noturus. Copeia 1:33–52

Levan A, Fredga K, Sandberg AA (1964) Nomenclature for centromeric position on chromosomes. Hereditas 52:201–220

Moore RH (1970) Diurnal variations in the activity and metabolism of the Atlantic midshipman, *Porichthys porosissimus*. Contrib Mar Sci Univ Texas 15:33–43

Nelson JS (1994) Fishes of the world, 3rd edn. Wiley, New York

Nirchio M, Gómez J, Villalaz J (2002a) Cariotipo del pez sapo *Batrachoides pacifici* (Batrachoididae: Teleostei) de la costa del Pacífico de Panamá. Saber 13:82–84

Nirchio M, Turner BJ, Pérez JE, Gaviria JI, Cequea H (2002b) Karyotypes of three species of toadfish (Batrachoididae: Teleostei) from Margarita island, Venezuela. Sci Mar 66:3–4

Nirchio M, Fenocchio AS, Swarça AC, Dias AL, Giuliano-Caetano L, Ron E, Gaviria JI, Pérez JE (2004) Cytogenetic characterization of *Thalassophryne maculosa* Günther, 1861 (Pisces: Batrachoididae) from Margarita Island, Venezuela. Caribb J Sci (in press)

Ohno S (1974) Protochordata, Cyclostomata and Pisces. In: John B (ed) Animal cytogenetics, vol 4. Bornträger, Berlin

Palazón-Fernández JL, Arias AM, Sarasquete C (2001) Aspects of the reproductive biology of the toadfish, *Halobatrachus didactylus* (Schneider, 1801) (Pisces: Batrachoididae). Sci Mar 65:131–138

Palazón JL, Nirchio M, Sarasquete C (2003) Conventional karyotype and nucleolar organizer regions of the toadfish *Halobatrachus didactylus* (Schneider, 1801) (Pisces: Batrachoididae). Sci Mar 67:445–449

Rossi AR, Gornung E, Crosetti D, De Innocentiis S, Sola L (2000) Cytogenetic analysis of *Oedalechilus labeo* (Pisces: Mugilidae), with a report of NOR variability. Mar Biol 136:159–162

Sumner AT (1972) A simple technique for demonstrating centromeric heterocromatin. Exp Cell Res 75:304–306

Thode G, Cano J, Alvarez MC (1983) Associations of nucleolus organizer chromosomes shown by silver staining in *Gobius fallax*. J Hered 74:480–482

Vitturi R, Catalano E, Colomba M, Montagnino L, Pellerito L (1995) Karyotypes analysis of *Aphanius fasciatus* (Pisces, Cyprinodontiformes) Ag-NORs and C-band polymorphism in four populations from Sicily. Biol Zentralbl 114:392–402