Bol. Inst. Oceanogr. Venezuela, 46 (2): 129-135 (2007); 1 Fig., 1 Tab.

FIRST DESCRIPTION OF THE KARYOTYPE AND Ag-NORs LOCALIZATION OF *STEPHANOLEPIS SETIFER* (BENNETT, 1831) (TETRAODONTIFORMES: MONACANTHIDAE) WITH REMARKS ON SEX CHROMOSOME DIFFERENTIATION

MAURO NIRCHIO[1]. & CLAUDIO OLIVEIRA[2].

[1]*Escuela de Ciencias Aplicadas del Mar. Universidad de Oriente, Isla de Margarita, Venezuela. nirchio@cantv.net*

[2]*Instituto de Biociências, Universidade Estadual Paulista, Sao Paulo, Brasil.*

ABSTRACT: A cytogenetic study of *Stephanolepis setifer* by conventional Giemsa stain and localization of Nucleolus Organizer Regions (NORs) by silver staining is reported. Females possess a diploid chromosome number of 2n=34, all of which are acrocentric, Males exhibit a karyotype with 2n=33 chromosomes, consisting of 32 acrocentric chromosomes including two unpaired acrocentric chromosomes, and one medium-size metacentric chromosome. The results suggest the presence of a multiple sex chromosome system of X1X1X2X2/X1X2Y type. The X1 and X2 chromosome sizes suggest that the metacentric chromosome is derived from Robertsonian fusion between the original Y chromosome and an autosome which probably corresponded to one element of the pair 16 and another of the pair 17, resulting in a neo-Y sex chromosome. Silver staining of metaphase spreads revealed a single pair of Ag-NORs interstitially located on the chromosome pair numbered 7. The comparison of our results with previous reports in Tetraodontiformes species suggest that its evolutionary divergence has been accompanied by macrostructural karyotype changes as far as microstructural alteration in NORs cluster.

Key words: Cytogenetic, multiple sex chromosomes, Ag-NORs

RESUMEN: A cytogenetic study of *Stephanolepis setifer* by conventional Giemsa stain and localization of the Nucleolus Organizer Regions (NORs) by silver staining is reported. Females feature a diploid chromosome number of 2n=34 chromosomes, all of which are acrocentric. Males exhibit a karyotype with 2n=33 chromosomes, consisting of 32 acrocentric chromosomes, including two unpaired ones and one medium-size metacentric chromosome. The results suggest the presence of a multiple sex chromosome system of the X1X1X2X2/X1X2Y type. The size of chromosomes X1 and X2 suggests that the metacentric chromosome is derived from a Robertsonian fusion between the original Y chromosome and an autosome, probably corresponding to one element of pair 16 and to another of pair 17, resulting in a neo-Y sex chromosome. Silver staining of metaphase spreads revealed a single pair of Ag-NORs interstitially located on the seventh pair. The comparison of our results with previous reports in other Tetraodontiforme species suggests that their evolutionary divergence has been accompanied by both macrostructural karyotype changes and microstructural NOR alterations.

Key words: cytogenetics, multiple sex chromosomes, Ag-NORs

INTRODUCTION

The order Tetraodontiformes is represented by 101 genera and 357 species; most are marine, although 14 of them occur only in freshwater and another eight may be found in freshwater (NELSON 2006). Within the Tetraodontiformes, the Monacanthidae includes about 32 genera and 102 species (NELSON 2006; ACERO & POLANCO 2006). The pygmy filefish, *Stephanolepis setifer*, is a monacanthid which occurs in the Western Atlantic from North Carolina to Brazil, including Bermuda, the Gulf of Mexico, and the Caribbean Sea (MATSUURA, 2002).

Cytogenetic studies of Tetraodontiformes have, thus far, covered only about 17% of the species (ca. 60 species) but have characterized the group as karyotypically very diverse (Table 1). With the aim of contributing to the cytogenetic knowledge of Tetraodontiformes, in the present study we report the karyotype and the localization of Ag-NORs for *Stephanolepis setifer,* with remarks on sex chromosome differentiation in the species.

MATERIALS AND METHODS

Four males and six females of *Stephanolepis setifer* collected with inshore fish traps in Cubagua Island, Venezuela, were analyzed. Their sex was established by external sexual dimorphism (second soft dorsal-fin ray greatly prolonged in mature males Fig. 1) and confirmed by the presence of well differentiated testes or ovaries during dissection of the fishes. Voucher specimens are deposited in the Fish Collection of the Escuela de Ciencias Aplicadas del Mar at Universidad de Oriente.

Chromosome preparations were obtained from cephalic kidney cells by conventional air-drying techniques (BERTOLLO *et al.* 1983) using stimulation of mitotic activity (LEE & ELDER 1980). Silver-stained Nucleolus Organizer Regions (Ag-NORs) were obtained as described by HOWELL & BLACK (1980). The best metaphases were photographed using a Motic camera and images were digitally processed using the Adobe Photoshop CS2 software. The chromosomes were classified according to the arm ratio (LEVAN *et al.* 1964). Fundamental number (NF) was determined considering subtelocentrics (ST) and acrocentrics (A) as having one chromosome arm and metacentrics (M) and submetacentric (SM) as having two chromosome arms.

RESULTS AND DISCUSSION

Tetraodontiform fishes display diploid numbers and karyotype formulae that include exclusively acrocentric chromosomes as well as very differentiated and distinct karyotypes with chromosome numbers ranging from 2n=28 to 2n=48, and NF values from 33 to 78 (Table 1).

In female *S. setifer* the karyotype has 2n=34 chromosomes, all of which are acrocentric. Males exhibit a karyotype with 2n=33 chromosomes, consisting of 32 acrocentric chromosomes including two unpaired acrocentrics, and one medium-size metacentric chromosome. This result suggests the presence of a multiple sex chromosome system of X1X1X2X2/ X1X2Y type in *S. setifer* (Fig. 1). This multiple sex chromosome system requires the attachment of the Y chromosome to an autosome by translocation or Robertsonian fusion, which results in males and females having different diploid chromosome numbers and different numbers of metacentric and acrocentric chromosomes (e.g. UYENO & MILLER 1971; THORGAARD 1978). The X1 and X2 chromosome sizes in *S. setifer* suggest that the metacentric chromosome is derived from Robertsonian fusion between the original Y chromosome and an autosome which probably corresponded to one element of the pair 16 and another of the pair 17, resulting in a neo-Y sex chromosome.

Identifying which sex is heterogametic and which is homogametic provides information about the sex-determination mechanism present in the species (DEVLIN & NAGAHAMA 2002). Five different systems of sexual chromosome determination (ZZ/ZW, XX/XY, XX/X0, X1X1X2X2/X1X2Y, XX/XY1Y2 and ZZ/ZW1W2) have been identified in neotropical fish (NIRCHIO & OLIVEIRA, 2006; ALVES *et al.* 2006). The main evolutionary events that determine sexual chromosome systems include additions or deletions of heterochromatic blocks (e.g. PHILLIPS & IHSSEN, 1985; ANDREATA *et al.* 1992; CANO *et al.* 1996; STEIN *et al.* 2001), reductions in chromosome size (e.g. PARK & KANG 1979), increments in chromosome size (e.g. GALETTI *et al.* 1981), and chromosome rearrangements (e.g. UYENO & MILLER 1971; THORGAARD 1978; BERTOLLO *et al.* 1983; de ALMEIDA TOLEDO *et al.* 1984; PEZOLD 1984; VITTURI *et al.* 1991).

Among Monacanthidae, the genera *Cantherhines*, *Navodon*, *Paramonachantus*, *Rudarius* and *Oxymonacanthus* have been reported as possessing exclusively subtelocentric or acrocentric chromosome complements with the same diploid number in both genders, but for the species so far cytogenetically analyzed of the genus *Stephanolepis*, except for *S. japonicus*, a multiple sex chromosomes of the X1X1X2X2/ X1X2Y type as the one here reported for *S. setifer* have been reported also in *S. cirrhifer*, and *S. hispidus* (Table 1). Since the occurrence of cytologically differentiated sex chromosomes in marine fishes appears to be rare (GALETTI *et al.* 2000), the presence of differentiated sex chromosomes only in *Stephanolephis* suggest that this mechanism might be a derived feature of the genus. Additional studies in different tetraodontiform species will be important to know if those sex chromosomes originated only once in the order or, alternatively, this was a common feature for the group.

NOR-silver staining is one of the methods used to locate 18S and 28S rRNA cistrons in chromosomes (HOWELL & BLACK 1980) and requires transcriptional activity of the ribosomal genes during the preceding interphase (see HUBBEL 1985, SÁNCHEZ-PINA *et al.* 1984, JIMÉNEZ *et al.* 1988).

Table 1. Table 1. Chromosome number in tetraodontiform species. a: acrocentric, st: subtelocentric, sm= submetacentric, m=metacentric.

Family	Species	2N	Karyotype formula	NF	Reference
TRIACANTHIDAE	*Triacanthus brevironsis*	48	48 a	48	CHOUDHURY *et al.* 1982
	T. striglifer	48	48 a	48	RISHI *et al.* 1973
	Arothron hispidus	42	36 sm+ 6 st/a	78	NAIARAJAN & SUBRAHAMANIAN, 1974
	A. immaculatus	42	14 m+ 16sm+ 12 st/a	72	ARAI & NAGAIWA 1976
	A. immaculatus	42	12 m+ 16sm+ 16 st/a	68	CHOUDURY *et al.* 1982
	A. leopardus	40	14 m + 14 sm+ 12 st/a	68	ARAI & NAGAIWA 1976
	A. nigropunctatus	38	14 m+20 sm+ 4 st/a	72	ARAI & NAGAIWA 1976
	A. reticularis	42	12 m+ 14 sm+16 st/a	68	CHOUDURY *et al.* 1982
	Canthigaster coronate	28	8 m/sm + 20 st/a	36	ARAI, 1983
	C. rivulata	34	4m + 6sm+ 10st/a +14a	54	ARAI & NAGAIWA 1976
	Chelonodon patoca	40	14m+ 16sm+ 10st/a	70	ARAI & NAGAIWA 1976
	Fugu chrysops	44	6m+ 14sm+ 24 st/a	64	ARAI & NAGAIWA 1976
	F. niphobles	44	20m/sm + 24st/a	64	ARAI & KAISUYAMA 1973
	F. pardalis	44	-	-	ARAI 1983
	F. poecilonotus	44	-	-	ARAI 1983
	Lagocephalus laevigatus	46	-	-	ARAI 1983
TETRAODONTIDAE	*L. lunaris*	44	10m+ 14sm+ 20st/a	68	CHOUDURY *et al.* 1982
	Monotetra palambagensis	42	-	-	HINEGARDNER & ROSEN 1972
	Sphoeroides greeleyi	46	24sm/sm + 22st/a	70	BRUM & MOIA, 2002
	S. tyleri	46	12 m/sm + 34st/a	58	BRUM *et al*, 1996
	S. spengleri	46	20m/sm + 26st/a	66	BRUM *et al.* 1996
	S. testudineus	46	20m/sm + 26st/a	66	NOLETO *et al.* 2006
	Takifugu niphobeles	44	4m/sm + 16sm + 24st/a	64	MIYAKI *et al.* 1995
	T. pardalis	44	6m/sm + 16sm + 22st/a	66	MIYAKI *et al.* 1995
	T. poecilonotus	44	12m + 10st + 22st/a	66	MIYAKI *et al.* 1995
	T. radiatus	44	8m + 14st + 22st/a	66	MIYAKI *et al.* 1995
	T. rubripes	44	10m + 12st + 22st/a	66	MIYAKI *et al.* 1995
	T. xanthopterus	44	8m + 14st + 22st/a	66	MIYAKI *et al.* 1995
	Tetraodon cutcutia	42	16m+ 12st+ 10a	70	KHUDA-BUKHSH & BARAI 1987
	Tetraodon fluviatilis	42	2m + 4sm + 2st + 34a	50	MANDRIOLI *et al.* 2000
	Tetraodon nigroviridis	42	20m/sm + 22st	62	FISCHER *et al.* 2000
	Diodon blekeeri	46	-	58	ARAI & NAGAIWA 1976
	D. holocanthus	46	20m/sm + 26st/a	66	SA-GABRIEL & MOLINA 2001
DIODONTIDAE	*Chilomycterus spinosus*	52	16m/sm+36st/a	68	BRUM *et al.* 1996
	Chilomicterus antennatus	52	6m + 46st/a	58	SA-GABRIEL & MOLINA 2005
	Cicliothys spinosus	50	4m+ 18sm + 12st+ 16a	76	NOLETO *et al.* 2006

Continuación Tabla 1

Family	Species	2N	Karyotype	NF	Reference
OSTRACIDAE	*Lactoria diaphana*	36	-	48	Arai 1983
	Ostracion cubicus	50	4sm + 46st/a	54	Arai & Nagaiwa 1976
	O. immaculatus	50	4sm + 46st/a	54	Arai 1983
BALISTIDAE	*Balistapus undulatus*	42	42st/a	42	TAKAI & OJIMA 1987
	Balistes vetula	44		44	SA-GABRIEL & MOLINA 2005
	Balistoides conspicullum	44	44st/a	44	TAKAI & OJIMA 1987
	B. viridescens	44	2m + 2sm + 40st/a	48	TAKAI & OJIMA 1987
	Melichthys niger	40	-	40	SA-GABRIEL & MOLINA 2005
	Melichthys vidua	40	40st/a	40	KITAYAMA & OJIMA 1984
	Odonus niger	42	42st/a	42	KITAYAMA & OJIMA 1984
	Parika scaber	40	40st/a	40	MUKOFUSHI *et al.* 1989
	Pseudobalistes flavimarginatus	44	2sm + 42st/a	46	ARAI & NAGAIWA 1976
	Rhineacanthus aculeatus	44	44st/a	44	ARAI & NAGAIWA 1976
	R. echarpe	44	44st/a	44	KITAYAMA & OJIMA 1984
	R. verrucosus	44	44st/a	44	ARAI & NAGAIWA 1976
	Rucanus arcodas	36	36st/a	36	ARAI & NAGAIWA 1976
	Suflamen chysopterus	46	46st/a	46	ARAI & NAGAIWA 1976
	S. traenatus	46	46st/a	46	TAKAI & OJIMA 1987
	Cantherhines macroceus	40	40st/a	40	ARAI & NAGAWAIA 1976
	Navodon modestus	40	40st/a	40	MUKOFUSHI & YOSIDA 1979
MONACANTHIDAE	*Stephanolepis cirrifher (M)*	33	33st/a		MUKOFUSHI *et al.* 1980
	Stephanolepis cirrifher (F)	34	34 st/a		MUKOFUSHI *et al.* 1980
	Stephanolepis hispidus (M)	33	33a + 1sm		SA-GABRIEL & MOLINA 2005
	Stephanolepis hispidus (F)	34	34 a		SA-GABRIEL & MOLINA 2005
	Stephanolepis setifer (M)	33	32a + 1m	34	THIS PAPER.
	Stephanolepis setifer (F)	34	34 a	34	THIS PAPER.
	Stephanolepis japonicus	34	34st/a	34	MUKOFUSHI & YOSIDA 1979
	Paramonachantus japonicus	34	34st/a	34	MUKOFUSHI & YOSIDA 1979
	Rudarius ercodes	36	36st/a	36	ARAI & NAGAIWA 1976
	Oxymonacanthus longirostris	36	36st/a	36	ARAI & NAGAIWA 1976

Silver staining of metaphase spreads revealed a single pair of Ag-NORs interstitially located on the chromosome pair number 7 (Figure 1). As far as ribosomic cistrons are concerned, silver impregnation performed on some Tetraodontiformes species has revealed that NOR localization in this group is also a cytogenetically variable feature. Indeed, a survey of Brazilian tetraodontiforms (BEZERRA 2007) showed a single NOR located interstitially on chromosome pair number 2 in *Melichthys niger* (Balistidae), pericentromerically located on pair 7 in *Cantherhines macrocerus* and on pair 5 in *C. pullus* (Monacanthidae), terminally located on the short arm of the metacentric chromosome pair number 2 in *Sphoeroides testudineus* (Tetraodontidae), and on the tips of the short arms of the subtelocetric pair number 7 in *Chilomycterus antennatus* (Diodontidae).

The high diversity of karyotypes indicates that the evolutionary divergence in tetraodontiforms has been frequently accompanied by macrostructural rearrangements that originated different diploid number. On the other hand, the occurrence of Ag-NORs in different

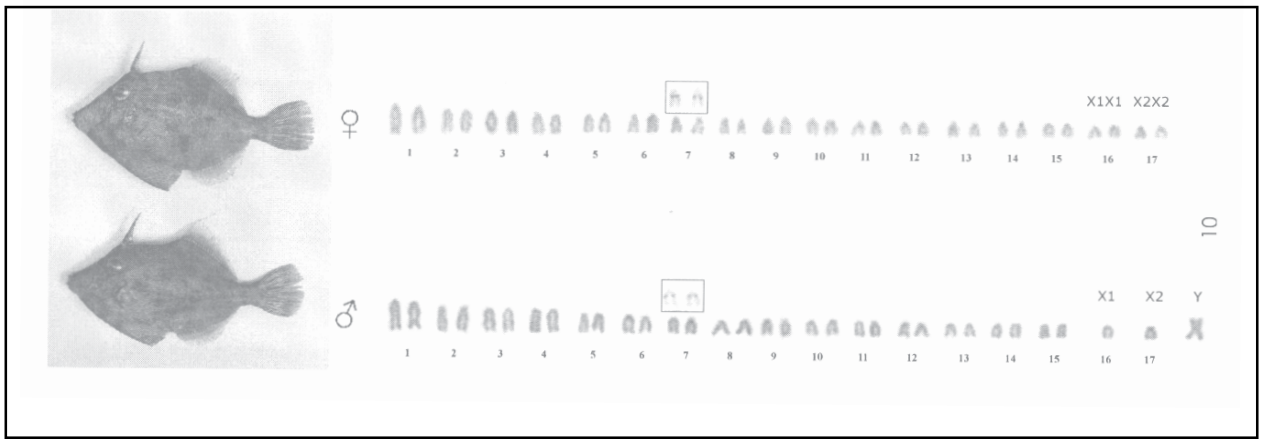


Fig. 1. Karyotype of *Stephanolepis setifer.* NOR-bearing chromosomes appear in the box.

chromosomes and positions also shows that microstructural changes were fixed in karyotype evolution in tetraodontiforms.

ACKNOWLEDGMENTS

Consejo de Investigación of Universidad de Oriente (Venezuela), provided funds to support this study. We are grateful to Ian Harrison for contribute to the improvement of the final version of the manuscript.

REFERENCES

ACERO, A.P & A.F. POLANCO. 2006. Peces del Orden Tetraodontiformes de Colombia. *Biota Colombiana* 7(1): 155-164.

ALVES, A.L., C. OLIVEIRA, M. NIRCHIO, A. GRANADO & F. FORESTI. 2006. Karyotypic relationships among the tribes of Hypostominae (Siluriformes: Loricariidae) with description of XO sex chromosome system in a Neotropical fish species. *Genetica* 128:1–9.

ANDREATA, A.A., L. F. ALMEIDA-TOLEDO, C. OLIVEIRA & S. D. A. TOLEDO Filho. 1992. Chromosome studies in Hypoptopomatinae (Pisces, Siluriformes, Loricariidae): 1. XX/XY sex chromosome heteromorphism in *Pseudotocinclus tietensis*. *Cytologia* 57, 369–372.

Arai, R. & I. KATSUYAMA. 1973. Notes on the chromosomes of three species of shore-fishes. *Bull. Nat. Sci. Mus. Tokyo Ser* A16(3): 405-409.

ARAI, R. & K. NAGAIWA. 1976. Chromosomes of Tetraodontiform Fishes from Japan. *Bull. Nat. Sci. Mus. Tokio Ser* A 2(1):59-72.

_____. 1983. Karyological and osteological approach to phylogenetic systematic of tetraodontiform fishes. *Bull. Nat. Sci. Mus.* Tokyo Ser. A9(4):175-210.

BERTOLLO, L. A. C., C. S. TAKAHASHI & O. M. FILHO. 1983. Multiple sex chromosomes in the genus *Hoplias* (Pisces: Erythrinidae). *Cytologia*, 48, 1 –12.

BEZERRA, DE I. I. C. 2007. *Padrões cariotípicos em Tetraodontiformes. (Osteychthyes): Redução genómica e mecanismos de diversificação*. Trab.Grad. Universidade Federal do Rio Grande do Norte, 68 pp.

BRUM, M. J. I. & L. C. G. MOTA. 2002. C-banding and nucleolar organizer regions of *Sphoeroides greelegy* (Tetraodontidae, Tetraodontiformes). *Caryologia* 55 (2): 171-174.

_____, M. M. O. CORREA, C. A. PURCELL, V. P. RIBEIRO & R. S. MURATORI. 1996. Contribuição ao conhecimento citogenético da ordem Tetraodontiformes II . Os cariótipos de *Sphoeroides tyleri* (Tetraodontidae) e de *Chilomycterus spinosus* (Diodontidae). VI Simp. Citog. Evol. Aplic. Pixes Neotropicais, Sao Carlos, Sao Paulo.

CANO, J., A. PRETEL, S, MELENDEZ., F. GARCIA, V. CAPUTO, A. S. FENOCCHIO & L. A. C. BERTOLLO. 1996. Determination of early stages of sex chromosome differentiation in the sea bass *Dicentrarchus labrax* L. (Pisces: Perciformes). *Cytobios* 87: 45–59.

CHOUDHURY, R. C., R. PRASAD & C. C. DAS. 1982. Karyologycal studies in five tetraodontiform fishes from Indian Ocean. *Copeia* 1982: 728-732.

DE ALMEIDA TOLEDO, L., F. FORESTI & DE ALMEIDA TOLEDO FILHO, S. H. 1984. Complex sex chromosome system in *Eigenmannia* sp. (Pisces, Gymnotiformes). *Genetica* 64, 165– 169.

DEVLIN R.H. & Y. NAGAHAMA. 2002. Sex determination and sex differentiation in fish: an overview of genetic, physiological, and environmental influences. *Aquaculture*, 208: 191–364.

FISCHER, C., C. OZOUF-COSTAZ, H. ROEST CROLLIUS, C. DASILVA, O. JAILLON, L. BOUNEAU, C. BONILLO, J. WEISSENBACH, A. BERNOT. 2000. Karyotype and chromosome location of characteristic tandem repeats in the pufferfish *Tetraodon nigroviridis*. *Cytogenet*. *Cell Genet*. 88:50-55.

GALETTI J R., P. M., F. FORESTI, L. A. C. BERTOLLO, O. MOREIRA FILHO. 1981. Heteromorphic sex chromosomes in three species of the genus *Leporinus* (Pisces, Anostomidae). *Cytogenet*. *Cell Genet*. 29: 138– 142.

_____., C. T. AGUILAR & W. F. MOLINA. 2000. An overview of marine fish cytogenetics. *Hydrobiologia* 420: 55–62.

HINEGARDNER, R. & D. E. ROSEN. 1972. Cellular DNA content and evolution of teleostean fishes, *Amer*. *Nat*. 106 (951):621-624.

Howell, W. M & D. A. BLACK. 1980. Controlled silver staining of Nucleolus Organizer Regions with a protective colloidal developer: a 1-step method. *Experientia* 36: 1014-1015.

HUBBEL, H. R. 1985. Silver staining as an indicator of active ribosomal genes. *Stain Technol*. 60: 285-294.

JIMÉNEZ, R., M. BURGOS & R. DÍAZ DE LA GUARDIA. 1988. A study of the silver staining significance in mitotic NORs. *Heredity* 60: 125-127.

KHUDA-BUKHSH, A. R. & A. BARAT. 1987. Chromosomes in fifteen species of Indian teleosts (Pisces). *Caryologia* 40 (1-2): 131-144.

KITAYAMA, E & Y. OJIMA. 1984. A preliminary report on the phylogeny of five balistid fish in terms of several chromosome banding techniques in combination with a statistical analysis. *Proc*. *Japan*. *Acad*. *Ser*. *B* 60 (3): 58-61.

LEE, M. R. & F. F. BELDER. 1980. Yeast stimulation of bone marrow mitosis for cytogenetic investigations. *Cytogenet*. *Cell Genet*. 26:36-40.

LEVAN, A., A. FREDGA & A. SANDBURG. 1964. Nomenclature for centromeric position on chromosomes. *Hereditas* 52: 201-220.

MANDRIOLI, M., B. CUOGHI, M. MARINI, & G. C. MANICARDI. 2000. Cytogenetic Analysis of the Pufferfish *Tetraodon fluviatilis* (Osteichthyes). *J*. *Chrom*. *Res*. 8 (3): 237-242.

MATSUURA, K. 2002. *Monacanthidae. In: Carpenter, K.E. (ed.) The living marine resources of the Western Central Atlantic. Volume 3: Bony fishes part 2 (Opistognathidae to Molidae), sea turtles and marine mammals*. FAO Species Identification Guide for Fishery Purposes and American Society of Ichthyologists and Herpetologists Special Publication No. 5.Rome FAO. 1375-2127.

MIYAKI, K., O. TABETA & H. KAYANO. 1995. Karyotypes in six species of puffer fishes genus *Takifugu* (Tetraodontidae, Tetraodontiformes). *Fish*. *Sci* 61: 594-598.

MUROFUSHI, M. & T. H. YOSIDA. 1979. Cytogenetical studies on fishes. I. Karyotypes of four filefishes. *Jpn*. *J*. *Genet*. 54: 191-195.

_____., S. OIKAWA, S. NISHIKAWA & T. H. YOSIDA. 1980. Cytogenetical studies on fishes. III. Multiple sex chromosome mechanism in the fish, *Stephanolepis cirrhifer*. *Jpn*. *J*. *Genet*., 55 (2): 127-132.

_____., T. KAKATSUBO & P. J. SMITH. 1989. Karyological study on the New Zealand leather jacket, *Perika scaber*, fish of the order Tetraodontiformes. *Bull. Biogeogra. Soc. Jpn.*, 44: 35-58.

NATARAJAN, R. & K. SUBRAHANJAM. 1974. A karyotype study of some teleosts from Portonovo waters. *Proc. Ind. Acad. Sci.* (B) 70:173-196.

NELSON, J. S. 2006. Fishes of the World. 4th eds. New York: John Wiley and Sons, Inc. New Yoerk. USA 60 pp.

NIRCHIO M, & C. OLIVEIRA. 2006. Citogenética de Peces Ed. Universidad de Oriente, Cumaná, Venezuela, 216 pp.

NOLETO R. B., M. R. VICARI, R. R.CIPRIANO, R. R. ARTONI & M. CESTARI. 2006 Physical mapping of 5S and 45S rDNA loci in pufferfishes (Tetraodontiformes). *Genetica* 130:133–138.

PARK, E. H. & Y. S. KANG. 1979. Karyological confirmation of conspicuous ZW sex chromosomes in two species of Pacific anguilloid fishes (Anguilliformes: Teleostomi). *Cytogenet. Cell Genet*. 23, 33– 38.

PEZOLD, F. 1984. Evidence for multiple sex chromosomes in the freshwater goby, *Gobionellus shufeldti* (Pisces: Gobiidae). *Copeia* 1984: 235–238.

PHILLIPS, R. B. & P. E. IHSSEN, 1985. Identification of sex chromosomes in lake trout (*Salvelinus namaycush*). *Cytogenet. Cell Genet*. 39, 14–18.

RHISHI, K. K. 1973. A preliminary report on the karyotypes of eighteen marine fishes. *Res. Bull. (N.S.) Panjab Univ.*, 24, Parts III-IV:161-162.

SÁ-Gabriel, L. G. & W. F. MOLINA. 2001 Proc. 47o. Congr. Nacional Genetica, GA214.

_____. & W. F. MOLINA. 2005. Sex Chromosomes in *Stephanolepis hispidus* (Monacanthidae, Tetraodontiformes). *Cytologia* 69 (4): 447-452.

SÁNCHEZ-PINA, M. A,, F. J. MEDINA, M. M. FERNÁNDEZ-GÓMEZ & M. C. RISUENO. 1984. Ag-NOR proteins are present when transcription is impaired. *Biol. Cell* 5: 199-202.

STEIN, J. D., R. B. PHILLIPS & R. H. DEVLIN, 2001. Identification of sex chromosomes in chinook salmon (*Oncorhynnchus tshawytscha*). *Cytogenet. Cell Genet*. 98, 108– 110.

TAKAI, A. & Y. OJIMA. 1987. Comparative chromosomal studies in three balistid. *Kromosomo* (Tokio) 47-48: 1545-1550.

THORGAARD, G. H., 1978. Sex chromosomes in the sockeye salmon: a Y-autosome fusion. *Can. J. Genet. Cytol.* 20: 349–354.

UYENO, T. & R. R. MILLER. 1971. Multiple sex chromosomes in a Mexican cyprinodontid fish. *Nature* 231, 452– 453.

VITTURI, R., CATALANO, E. & F. LAFARGUE. 1991. Evidence for heteromorphic sex chromosomes in *Zeus faber* Pisces Zeiformes nucleolus organizer regions and C-banding pattern. *Cytobios* 68, 37– 44.

RECIBIDO: Septiembre 2007
ACEPTADO: Noviembre 2007