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Chromosome differentiation in the subantarctic Bovichtidae species *Cottoperca gobio* (Günther, 1861) and *Pseudaphritis urvillii* (Valenciennes, 1832) (Pisces, Perciformes)

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Abstract: A cytogenetic study on the bovichtid species *Cottoperca gobio* from the Magellan Strait and *Pseudaphritis urvillii* from Tasmania showed both species have a plesiomorphic number of chromosomes (2*n*=48). However, *C. gobio* has a more conservative karyotype composed entirely of acrocentric chromosomes (Fundamental Number=48); the presence of two metacentric pairs in *P. urvillii* (FN=52) makes this species karyologically more derived. The differences in the number of chromosomal arms, and the chromosomal location of the nucleolar organizer regions indicate karyological divergence in the two separating stocks from which *C.gobio* and *P.urvillii* originated. During the diversification of this notothenioid family, probably coincident with the fragmentation of Gondwana, the stock that split off with the Australian Plate gave rise to the Tasmanian species and experienced more chromosomal modifications than the stock from which *C. gobio* is derived. The pattern of constitutive heterochromatin suggests a possible homology between a pair of chromosomes in bovichtids and other notothenioids.

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Introduction

The perciform suborder Notothenioidei comprises the majority of the recent Antarctic fish fauna with over 120 species belonging to the families Bovichtidae, Nototheniidae, Artedidraconidae, Harpagiferidae, Bathydraconidae and Channichthyidae (Hureau 1986, Gon & Heemstra 1990, Eastman 1993, Miller 1993). The classification of Balushkin (1992) separates the new family Pseudaphritidae (comprising only the monotypic genus *Pseudaphritis*) from the family Bovichtidae (including the genera *Cottoperca* and *Bovichtus*) using as the main diagnostic character for the family Bovichtidae the *scaleless body*. However, this is not correct since the head, trunk and tail of *Cottoperca gobio* (Günther, 1861) are covered with scales (Nakamura *et al.* 1986). It seems likely that *Pseudaphritis* and *Bovichtus* belong to two separate families, but the phylogenetic position of *Cottoperca* has also to be clarified. The Bovichtidae are regarded as the most primitive family (Regan 1913, Hureau 1986, Andriashev 1987, Eastman 1993) with species showing many morphological characters considered plesiomorphic for the suborder. They do not possess any of the morpho-physiological and biochemical adaptations to the extreme Antarctic environmental conditions shared by most other notothenioids (Eastman 1993). According to phylogenetic reconstructions of notothenioids based on both morphological and molecular characters, the Bovichtidae is the basal clade in the suborder (Iwami 1985, Eastman 1993, Bargelloni *et al.* 1994). They have therefore been used as a "functional outgroup" in cladistic analysis since an unequivocal sister group for notothenioids has not yet been identified (Eakin 1981, Eastman 1993). The geographic distribution also distinguishes this family from other notothenioids: most of the species live outside the High Antarctic region having been recorded from subantarctic and cold temperate waters of the Southern Ocean. Among the eleven species known at the present (belonging to genera *Bovichtus, Cottoperca, Pseudaphritis*) *Cottoperca gobio* is present in southern South America, *Pseudaphritis urvillii* (Cuvier, 1831) occurs in Tasmania and southeastern mainland Australia. The genus *Bovichtus* is the most widespread in the Southern Ocean inhabiting shallow waters of Australia, New Zealand, South America, and subantarctic islands. Only one species, *B. elongatus*, is found south of the Antarctic Convergence (Hureau & Tomo 1977, Tomo 1981, Hardy 1988, Gon & Heemstra 1990). Eastman (1993) considered the hypothesis that the genus *Bovichtus* populated the peri-Antarctic islands from South America and the Scotia arc after the formation of the Antarctic Circumpolar Current (25 Ma) and that *B. elongatus* could be an immigrant to the Antarctic region from South America or the Scotia arc.

Preliminary information on chromosome number and morphology of *Cottoperca gobio* (Prirodina 1986) supported the notion of the primitive phylogenetic position of bovichtids among notothenioid fishes. However, no further cytogenetic

studies were made on bovichtids despite the recent increase of research in this field (Ozouf-Costaz 1987, Ozouf-Costaz & Doussau de Bazignan 1987, Ozouf-Costaz *et al.* 1991, Morescalchi *et al.* 1992 a,b, Capriglione *et al.* 1994). This lack was mainly due to the logistic difficulties in collecting species of the family, which are scarce and scattered around the Southern Ocean.

International cooperative programmes for Antarctic research between Italy and Australia and Italy and France allowed us to begin a cytogenetic study on the bovichtid species *Cottoperca gobio* and *Pseudaphritis urvillii*. *C. gobio* inhabits shallow coastal waters in the South America subantarctic regions. *P. urvillii* is a small euryaline fish native to lowland streams throughout coastal Tasmania and the Bass Strait islands; it is also found in Victoria, New South Wales and southern South Australia (Hortle & White 1980, Fulton 1990).

In this paper we have analysed the karyotypes of the two species after conventional Giemsa staining, C-banding and AgNOR staining and the data compared with information from other notothenioid families. Our hope was that knowledge of chromosomal differentiation could be integrated with information from other biochemical, physiological and molecular traits to provide a more complete phylogenetic hypothesis for these fishes.

Material and methods

Specimens of *Cottoperca gobio* (two females) were captured by fishing in the Magellan Strait, 53°02'S, 70°50'W, at 2 m depth, in January 1993. They are deposited in the MNHN collections (MNHN 1994-0117 and MNHN 1994-0115). Samples of *Pseudaphritis urvillii* (three females, two undetermined juveniles) were collected by electrofishing from the North West Bay River at Margate, Tasmania, *c.* 43°02'S, 147°13'E, in October 1993. These specimens are deposited in the Museum of the Institute of Comparative Anatomy, University of Genova. After capture, fish were kept in well oxygenated sea-water tanks. Mitotic cells were obtained from the kidney according to the method of Doussau de Bazignan & Ozouf-Costaz (1985) with the following modifications: colchicine 0.5% was injected at 0.3 ml/100 g body weight for periods ranging from 2h30–6 h. Fish were anaesthetized in 0.5% phenoxy-2-ethanol in sea-water. Hypotonic treatment ranged between 15 and 50 min (*P. urvillii*) and between 50 and 75 min (*C. gobio*).

Chromosome spreads were made from fixed cell suspensions kept at -20°C. Conventional staining of chromosomes followed current methods (Hartley & Horne 1985): C-banding according to BSG method (Sumner 1972), with slight modifications basically related to a reduction of temperature during the barium treatment; AgNOR staining according to the protocol of Howell & Black (1980). Characterization of chromosome morphology followed Levan *et al.* (1964). Conventionally the bi-armed metacentric (M) have been placed before the uni-armed acrocentric chromosomes (A) in the karyotypes.

Results

Cottoperca gobio

Chromosome preparation from this species presented difficulties, due to a rather low mitotic index and problems with hypotonic treatment. Both specimens had 48 acrocentric chromosomes (2n=48, FN=48) of slightly decreasing size. One chromosomal pair was heteromorphic (pair 1 in the karyotype) with an unequal secondary constriction near the centromere (Fig. 1). AgNOR staining showed a single pair of active nucleolar organizer regions (NORs) which were located in this chromosome pair (Fig. 3a). In nearly all the metaphase plates from both specimens, there was a clear heteromorphism of the Ag-NORs with one NOR twice the size of the other. The AgNOR bearing chromosome pair also revealed a heteromorphic positive C-band in addition to centromeric heterochromatin (Fig. 1b). Besides the centromeric heterochromatin, one of the larger chromosomes had a strongly positive pericentric C-band. In the homolog of this chromosome the intercalate C-band was much smaller and very close to the centromere. A slight telomeric C-band was also visible in one of the larger chromosomes, but not in all metaphases. All other chromosomes showed only C-bands at the centromere. However, no telomeric or centromeric associations between chromosomes were observed in this species.

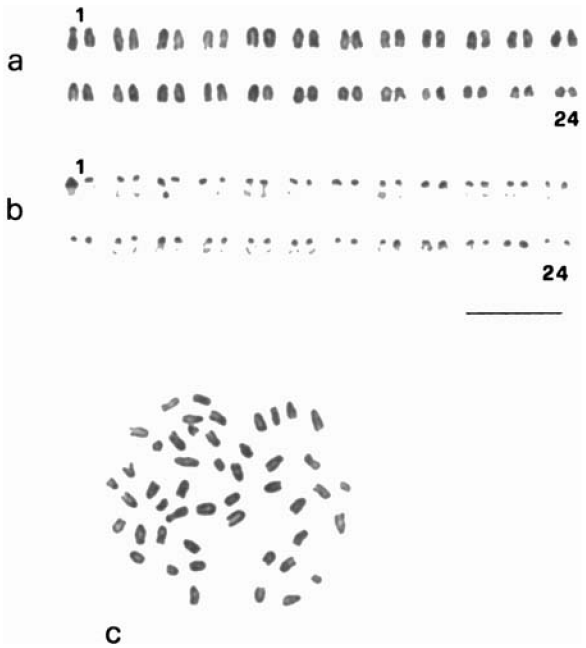

Fig. 1. *Cottoperca gobio*. **a.** Conventional Giemsa-stained karyotype. **b.** C-banded karyotype. **c.** metaphase plate. Bar represents 10 μm.

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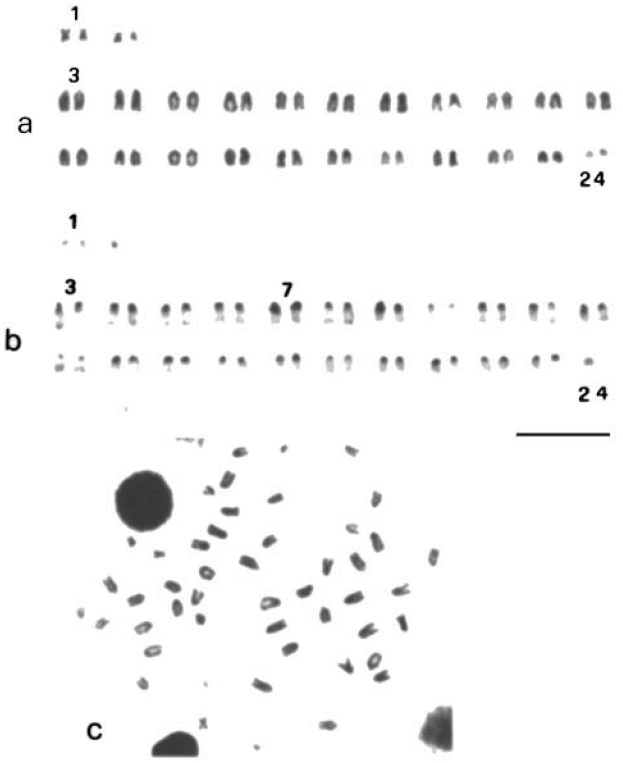

Fig. 2. *Pseudaphritis urvillii*. **a**. Conventional Giemsa-stained karyotype. **b**. C-banded karyotype. **c**. metaphase plate. Bar represents 10 μm.

Pseudaphritis urvillii

Well spread metaphase plates were obtained after the longest hypotonic treatment. Chromosome counts from all the individuals showed a diploid number of 48 chromosomes that could be grouped into 24 pairs. Two pairs were formed by small metacentric elements, while the remaining chromosomes were acrocentric, giving a FN of 52 (Fig. 2). The metacentric chromosomes were among the smallest elements; the acrocentrics formed a gradually decreasing series.

A pair of A, was easily identified in approximately 90% of Giemsa stained karyotypes (pair 3). The chromosomes of this pair were among the largest in the complement and usually showed an extended, achromatic region, at the centromeric and pericentromeric level. This achromatic region appeared heteromorphic in length and corresponded well to a major active NOR. In all the individuals examined AgNOR staining showed a polymorphic NOR in these two homologous chromosomes; in most metaphases two or four additional chromosomes showed centromeric silver staining. These chromosomes can be considered to have minor NORs at centromeric position (Fig. 3b). C-banding indicated centromeric constitutive heterochromatin in almost all acrocentric chromosomes, but nearly lacking in the metacentrics and in the two smallest acrocentrics (Fig. 2b). Some telomeres appeared lightly C-positive. Silver positive

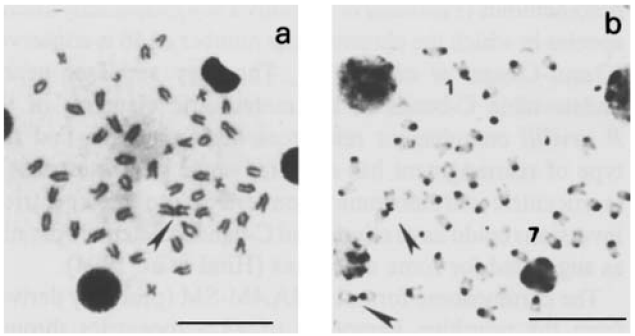

Fig. 3. **a.** Silver stained chromosomes of *Cottoperca gobio*: a single pair of chromosomes (arrows) is carrying heteromorph NORs. **b.** Silver stained chromosomes of *Pseudaphritis urvillii* : at least three pairs of chromosomes are NORs bearing, the major NORs are located near the centromeric region of the two largest acrocentrics (arrows). Bar represents 10 μm.

regions of NOR bearing chromosomes, correspond to dark C-bands after barium treatment. Moreover in C-banded metaphases (Fig. 1b, 4b) a pair of chromosomes (corresponding in size to chromosome pairs five to ten, here chromosome no. 7) showed a large heterochromatic band in a pericentric position, which appeared well separated from the centromere band when chromosomes were less contracted. This pattern is very similar to the C-band pattern in the heteromorphic chromosome pair no. 1 in the karyotype of *Cottoperca gobio* (Fig. 2b). The pair of chromosomes with intercalated C-band in *Pseudaphritis urvillii* do not show pericentric NORs detectable by silver staining.

In some well spread metaphases, centromeric associations between two pairs of medium-size acrocentric chromosomes were found. Heterochromatic chromosomal regions involved in these associations probably correspond to minor Ag-NORs (Fig. 4a, b).

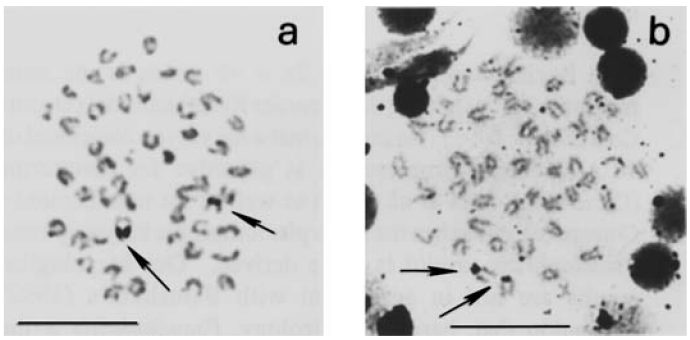

Fig. 4. Centromeric association between acrocentric NORs bearing chromosomes (arrows) of *Pseudaphritis urvillii:* **a.** Giemsa-stained chromosomes. **b.** C-banded chromosomes. Note the constitutive heterocromatin associated to the major NOR region in the chromosome of the pair 1 and the large interstitial C-band in the chromosome of the pair 7. Bar represents 10 μm.

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Discussion

Both bovichtid species have 2n = 48 which is the most frequent number among the suborder Notothenioidei (Ozouf-Costaz *et al.* 1991). Assuming that a karyotype composed of 48 acrocentric chromosomes is primitive for perciforms (Ohno 1974, Sola *et al.* 1981) as well as for notothenioids, *Cottoperca gobio* has the more plesiomorphic karyotype and *Pseudaphritis urvillii* is more derived. Our karyological results are not in agreement with Balushkin's (1992) contention that, based on osteology, *Pseudaphritis* is the most plesiomorphic notothenioid. Karyological and morphological data do not provide completely congruent information about notothenioid relationships, probably because the two systems diverged at different rates. During the diversification of bovichtids that was associated with the fragmentation of Gondwana, the two separating stocks which gave rise to the modern *Cottoperca* and *Pseudaphritis*, followed different pathways of karyological diversification. The stock that split off with the Australian Plate incorporated morphological chromosomal modifications leading to a chromosomal formula 2n=44A,4M in the Tasmanian *P.urvillii*. The stock that remained in the area of southern South America and Falkland Islands, from which *C. gobio* is derived, was conservative in chromosome morphology, maintaining the plesiomorphic formula 2n=48A. Among the notothenioids that have been cytogenetically characterized only one Bathydraconidae (*Psilodraco breviceps*) has a karyotype of all acrocentric chromosomes (Prirodina 1994). Prirodina (1986) obtained the karyotype of *C. gobio* from gill epithelium cells and found a diploid chromosome number of 48–50, all acrocentrics of decreasing size. This variation in chromosome number was probably due to technical difficulties, such as the hypotonic treatment. Starting from an ancestral karyotype of acrocentric elements, pericentric inversions could have given rise to the two metacentric pairs in *P. urvillii*. Pericentric inversions seem to be common in other notothenioids (Prirodina & Neyelov 1984), especially among species in which the chromosome number of 48 is conserved (Ozouf-Costaz *et al.* 1991). The very small or nearly undetectable C-bands in the metacentric elements of the *P. urvillii* complement reinforces the hypothesis that this type of rearrangment has occurred since processes of AM (acrocentric chromosomes converted into metacentrics) inversions could have eliminated C-banded heterochromatin, as suggested for some other taxa (Hirai *et al.* 1994).

The chromosome formula 44A,4M-SM (probably derived from the primitive karyotype of 48 acrocentrics through pericentric inversions) is shared by several notothenioid species including bovichtids, nototheniids and channichthyids (most of the chromosome numbers and formulas currently known in notothenioids are listed in Ozouf-Costaz *et al.* 1991, table I). It is noteworthy that at least one species in each notothenid subfamily conserves this formula, but not the more derived Pleuragramminae. Among the subfamily Nototheniinae this same formula is present in genera phyletically very distant from each other *(Patagonotothen, Lepidonotothen*). Among the family Channichthyidae, *Chaenocephalus aceratus* and *Chionodraco rastrospinosus* have 44A+4M. Moreover in most of the notothenioid species with a rearranged chromosomal formula, two pairs of meta-submetacentrics are present. This pattern indicates that pericentric inversions could have been an initial step in karyological diversification among Notothenioidei. An alternative hypothesis could be that pericentric inversions are a frequent mutation which became fixed in various phylogenetic lines.

The two bovichtid species, as revealed by AgNOR staining, have different chromosomal NORs locations. In *C. gobio* active heteromorphic NORs are located on a single pair of chromosomes: NOR regions on the two homologous strands appear to be different in size after different staining techniques (Ag-NOR, Giemsa, C-banding) suggesting that their heteromorphism may be structural, and probably derived by duplication of rDNA. Differences in size in homologous NORs were sometimes explained as different transcriptional activity due to a functional conditions in some fishes (Garcia *et al.* 1987, Lopez *et al.* 1988), or as simply structural heteromorphism (Vitturi *et al.* 1990). Because of the low number of specimens examined we cannot conclude if the heterozygosity for single/doubled NORs founded in *C.gobio* is restricted to our individuals or represents intraspecific variability as often reported in fishes (Foresti *et al.* 1981, 1989, Vitturi *et al.* 1990, Sola *et al.* 1990) and other organisms (Long & David 1980). The strong relationship of the NOR region to C-banding indicates an association between rDNA and constitutive heterochromatin as in other teleosts (Schmid & Guttenbach 1988, Pendas *et al.* 1994) and also in other vertebrates (Babu & Verma 1985, Hadjiolov 1985). In the Tasmanian species at least three pairs of chromosomes have active NORs. The present data cannot answer the question if such differences between the two bovichtids are due to a differential expression of nuclear genes for rRNA or to an amplification and/or dispersion of rDNA in the genome of *P. urvillii*, the more karyologically derived species. The AgNOR positive material is actually composed of nucleolar proteins (Jordan 1987, Roussel *et al.* 1994) providing information only on the active NORs. In order to detect the true amount and the chromosomal locations of rDNA clusters in bovichtids as well in other notothenioids we have planned further investigations using such cytomolecular tools e.g. *in situ* hybridization with rDNA probes (Pendas *et al.* 1993, 1994). If we compare the NORs pattern in other notothenioids previously studied, a different location of at least the major active NORs seems clear: in most of the species of nototheniids and channichthyids major NORs are located on a pair of small submetacentric chromosomes (Ozouf-Costaz *et al.* 1991, Morescalchi *et al.* 1992 a,b).

Our present data on C-banding pattern and the association between NORs and C-positive heterochromatin allow us to

propose some working hypotheses to be tested during future work. We suggest a possible homology between the chromosome of the pair 1 in the karyotype of *C. gobio* and the chromosome no. 7 in the complement of *P. urvillii*. The two proposed homologues share the same C-banding pattern showing, besides the constitutive centromeric heterochromatin, an additional characteristic interstitial pericentromeric C-band. Ribosomal genes, linked to this C-positive heterochromatin, are heteromorphic and active in *C. gobio* and they could be inactivated (undetectable by Ag-NOR staining) in *P. urvillii*. Moreover, if we consider the pattern of C-banding in the channichthyid *Chionodraco hamatus* previously studied, we can easily recognize a pair of chromosomes that are very similar to the pair described above. These chromosomes could also be homologous: they are acrocentric with a dark interstitial C-band rich in GC heterochromatin (revealed by Cromomycin A3) and associated with a minor NOR (Morescalchi *et al.* 1992a, figs. 10 & 11). A highly repetitive DNA family was also described as preferentially localized in C-positive heterochromatic regions in *C. hamatus* and is supposed to be more widely distributed also in the Nototheniidae (Capriglione *et al.* 1994).

If this hypothesis is corroborated, it would indicate a very strong conservation of the heterochromatic regions corresponding to the interstitial C-band in the supposed homologous chromosomes of bovichtids and channichthyids. Bovichtids, in fact, separated very early from the ancestral notothenioid stock: this event must have occurred long before the major environmental changes associated with the isolation of Antarctica that created conditions suitable for the diversification of the other notothenioid taxa. Moreover, channichthyids are the most phyletically derived taxon among Notothenioidei (Eastman 1993). This in turn could possibly signify a basic functional role linked to the heterochromatic chromosomal region which is conserved in phyletically very distinct families.

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References

ANDRIASHEV, A.P. 1987. A general review of the Antarctic bottom fish fauna. *In* KULLANDER, S.O. & FERNHOLM, B., *eds. Fifth Congress of European Ichthyologists, Proceedings, Stockholm, 1985*. Stockholm: Swedish Museum of Natural History, 357-372.

BABU, K.A. & VERMA, R.S. 1985. Structural and functional aspects of nucleolar organizer regions (NORs) of human chromosomes. *International Review of Cytology*, **94**, 151-176.

BALUSHKIN, A.V. 1992. Classification, phylogenetic relationships, and origins of the families of the suborder Notothenioidei (Perciformes). *Journal of Ichthyology*, **30**, 132-147.

BARGELLONI, L., RITCHIE, P.A., PATARNELLO, T., BATTAGLIA, B., LAMBERT, D.M. & MEYER, A. 1994. Molecular evolution at subzero temperatures: mitochondrial and nuclear phylogenies of fishes from Antarctica (Suborder Notothenioidei), and the evolution of antifreeze glycopeptides. *Molecular Biology and Evolution*, **11**, 854-863.

CAPRIGLIONE, T., MORESCALCHI, A., OLMO, E., ROCCO, L., STINGO, V. & MANZO, S. 1994. Satellite DNAs, heterochromatin and sex chromosomes in *Chionodraco hamatus* (Channichthyidae, Perciformes). *Polar Biology*, **14**, 285-290.

DOUSSAU DE BAZIGNAN, M. & OZOUF-COSTAZ, C. 1985. Une technique rapide d'analyse chromosomique appliquée à sept espèces de poissons antarctiques. *Cybium*, 3 series, **9**, 57-74.

EAKIN, R.R. 1981. Osteology and relationship of the fishes of the Antarctic family Harpagiferidae (Pisces, Notothenioidei). *Antarctic Research Series*, **31**, 81-147.

EASTMAN, J.T. 1993. *Antarctic fish biology: evolution in a unique environment*. San Diego: Academic Press, 322 pp.

FORESTI, F., ALMEIDA TOLEDO, L.F. & TOLEDO, S.A. 1981. Polymorphic nature of nucleolus organizer regions in fishes. *Cytogenetics and Cell Genetics*, **31**, 137-144.

FORESTI, F., ALMEIDA TOLEDO, L.F. & TOLEDO, S.A. 1989. Supernumerary chromosome system, C-banding pattern characterization and multiple nucleolus organizer regions in *Moenkhausia sanctaefilomenae* (Pisces, Characidae). *Genetica*, **79**, 107-114.

FULTON, W. 1990. *Tasmanian freshwater fishes*. Fauna of Tasmania handbook no. 7. Hobart: University of Tasmania, 80 pp.

GARCIA, E., ALVAREZ, M.C. & THODE, G. 1987. Chromosome relationship in the genus *Blennius* (Blennidae, Perciformes): C-banding pattern suggests two karyo-evolutional pathways. *Genetica*, **72**, 27-36.

GON, O. & HEEMSTRA, P.C. 1990. *Fishes of the Southern Ocean*. Grahamstown: J.L.B. Smith Institute of Ichthyology, 462 pp.

HADJIOLOV, A.A. 1985. *The nucleolus and ribosome biogenesis*. Wien: Springer, 258 pp.

HARDY, G.S. 1988. A revision of *Bovichtus* Cuvier, 1831 (Pisces: Bovichthyidae) from Australasia, with description of a new deepwater species from the New Zealand Subantarctic. *Journal of Natural History*, **22**, 1639-1655.

HARTLEY, S.E. & HORNE, M.T. 1985. Cytogenetic techniques in fish genetics. *Journal of Fish Biology*, **26**, 575-582.

HIRAI, H., YAMAMOTO, M.T., OGURA, K., SATTA, Y., YAMADA, M., TAYLOR, R.W. & IMAI, I.T. 1994. Multiplication of 28S rDNA and NOR activity in chromosome evolution among ants of the *Myrmecia pilosula* species complex. *Chromosoma*, **103**, 171-178.

HORTLE, M.E. & WHITE, W.G. 1980. Diet of *Pseudaphritis urvillii* (Cuvier & Valencienne) (Pisces: Bovichthyidae) from South-Eastern Tasmania. *Australian Journal of Marine and Freshwater Research*, **31**, 533-39.

HOWELL, W.M. & BLACK, D.A. 1980. Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method. *Experientia*, **36**, 1014-1015.

HUREAU, J.-C. 1986. Relations phylogénetiques au sein des Notothenioidei. *Océanis* **12**(5), 367-376.

HUREAU, J.-C. & TOMO, A.P. 1977. *Bovichthys elongatus* n. sp., poisson Bovichthyidae, famille nouvelle pour l'Antarctique. *Cybium*, **3**, 67-74.

IWAMI, T. 1985. Osteology and relationships of the family Channichthyidae. *Memoirs of National Institute of Polar Research, Tokyo*, Ser E, **36**, 1-69.

JORDAN, G. 1987. At the heart of the nucleolus. *Nature*, **329**, 489-490.

LEVAN, A., FREDGA, K. & SANDBERG, A.A. 1964. Nomenclature for centromeric position of chromosomes. *Hereditas*, **52**, 201-220.

LONG, E.O. & DAVID, I.D. 1980. Repeat genes in eukaryotes. *Annual Review of Biochemistry*, **49**, 727-764.

LOPEZ, J.R., ALVAREZ, M.C., THODE, G. & MARTINEZ, G. 1988. Karyotype divergence in *Symphodus melops* and *Symphodus roissaly* (Labridae, Perciformes): C-banded and Ag-NOR karyotypes. *Genoma*, **32**, 35-39.

MILLER, R.G. 1993. *A history and atlas of the fishes of the Antarctic Oceans.* Nevada: Foresta Institute, 792 pp.

MORESCALCHI, A., HUREAU, J.-C., OLMO, E., OZOUF-COSTAZ, C., PISANO, E. & STANYON, R. 1992a. A multiple sex-chromosome system in Antarctic ice-fishes. *Polar Biology*, **11**, 655-661.

MORESCALCHI, A., PISANO, E., STANYON, R. & MORESCALCHI, M.A. 1992b. Cytotaxonomy of Antarctic teleosts of the *Pagothenia/Trematomus* complex (Nototheniidae, Perciformes). *Polar Biology*, **12**, 553-558.

NAKAMURA, I., INADA, T., TAKEDA, M. & HATANAKA, H. 1986. *Important fishes trawled off Patagonia.* Tokyo: Japan Marine Fishery Resource Research Center, 369 pp.

OHNO, S. 1974. Protochordata, Cyclostomata and Pisces. *In* JOHN, B., *ed. Animal Cytogenetics, Vol. 4, Chordata 1.* Berlin: Gebruder Borntraeger, 92 pp.

OZOUF-COSTAZ, C. 1987. Apport de la caryologie aux études taxinomiques et phylogènetiques des poissons. *In Actes du Colloque sur la Recherche Francaise dans les Terres Australes*, Strasbourg, 155-163.

OZOUF-COSTAZ, C. & DOUSSAU DE BAZIGNAN, M. 1987. Chromosome relationships among 15 species of Notothenіidae. *In* KULLANDER, S.O. & FERNHOLM, B., *eds. Fifth Congress of European Ichthyologists, Proceedings, Stockholm, 1985.* Stockholm: Swedish Museum of Natural History, 413-419.

OZOUF-COSTAZ, C., HUREAU, J.-C. & BEAUNIER, M. 1991. Chromosome studies on fish of the suborder Notothenioidei collected in the Weddell Sea during EPOS 3 cruise. *Cybium*, **15**, 271-289.

PENDAS, A.M. MORAN P., FREIJE, J.P. & GARCIA-VAZQUEZ, E. 1994. Chromosomal mapping and nucleotide sequence of two tandem repeats of Atlantic salmon 5S rDNA. *Cytogenetics and Cell Genetics*, **67**, 31-36.

PENDAS, A.M., MORAN, P. & GARCIA-VAZQUEZ E. 1993. Multi-chromosomal location of ribosomal RNA genes and heterochromatin association in brown trout. *Chromosome Research*, **1**, 63-67.

PRIRODINA, V.P. 1986. Karyotypes of *Cottoperca gobio* (Bovichthyidae, Notothenioidei) as compared to karyotypes of other Notothenioidei. Morphology and distribution of fishes of the Southern Ocean. *Proceeding of the Zoological Institute, Leningrad*, **153**, 67-71. [In Russian.]

PRIRODINA, V.P. 1994. Review of karyotypic and taxonomic diversity in the suborder Notothenioidei. *Journal of Ichthyology*, **34**(6), 1-13.

PRIRODINA, V.P. & NEYELOV, A.-V. 1984. Les formules chromosomiques de deux espèces de poissons du genre *Notothenia* (Famille Nototheniidae) provenant de l'Ouest Antarctique. *Transactions of the Zoological Institute, Leningrad*, **127**, 32-37.

REGAN, C.T. 1913. The Antarctic fishes of the Scottish National Antarctic Expedition. *Transactions of the Royal Society of Edinburgh*, **49**, 229-292.

ROUSSEL, P., SIRRI, V. & HERNANDEZ-VERDUN, D. 1994. Quantification of Ag-NOR protein using Ag-NOR staining on Western blots. *Journal of Histochemistry and Cytochemistry*, **42**, 1513-1517.

SCHMID, M. & GUTTENBACH, M. 1988. Evolutionary diversity of reverse (R) fluorescent chromosome bands in vertebrates. *Chromosoma*, **97**, 101-114.

SOLA, L., CATAUDELLA, S. & CAPANNA, E. 1981. New developments in vertebrate cytotaxonomy. III Karyology of bony fishes: a review. *Genetica*, **54**, 285-328.

SOLA, L., MONACO, P.J. & RASCH, E.M. 1990. Cytogenetics of bisexual/unisexual species of *Poecilia*. I. C-bands, Ag-NOR polymorphisms, and sex chromosomes in three populations of *Poecilia latipinna*. *Cytogenetics and Cell Genetics*, **53**, 148-154.

SUMNER, A.T. 1972. A simple technique for demonstrating centromeric heterochromatin. *Experimental Cell Research*, **75**, 304-306.

TOMO, A.P. 1981. Contribution al conocimiento de la fauna ictiologica del sector Antartico Argentino. *Publicationes de la Direccion Nacional Instituto Antartico Argentino*, **14**, 1-242.

VITTURI, R., MAZZOLA, A., CATALANO, E. & LO CONTE, M.R. 1990. Karyotype analysis, nucleolar organizer regions (NORs), and C-banding pattern of *Dicentrarchus labrax* (L.) and *Dicentrarchus punctatus* (Block, 1792) (Pisces, Perciformes) with evidence of chromosomal structural polymorphism. *Cytologia*, **55**, 425-430.