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The chromosomal complement of the artedidraconid fish *Histiodraco velifer* (Perciformes: Notothenioidei) from Terra Nova Bay, Ross Sea

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Abstract. The karyotype of *Histiodraco velifer* from the Antartic Ocean was analyzed using various banding methods and in situ hybridization with a telomeric probe. A male and a female had a diploid set of 46 chromosomes (6 submetacentric + 40 acrocentric, FN = 52); the nucleolar organizer was CMA_3-positive and was located on the short arm of a medium-sized submetacentric pair. All chromosomes stained uniformly with DAPI, whereas C-banding revealed heterochromatic blocks that were mostly located centromerically and telomerically and were resistant to *Alu*I digestion. The substantial identity of the karyotype of *H. velifer* with that of the other artedidraconids investigated so far suggests that chromosome changes must have played a less than significant role in the speciation among the lineages of this fish family endemic to Antarctica.

The perciform suborder of Notothenioidei is a highly diversified group of fish living in the waters surrounding Antarctica. In terms of number of both species and biomass they are important constituents of the Southern Ocean environment. The high rate of endemism of these fishes is the consequence of the long-standing isolation (25 Myr) of this continent due to its geographical distance from other lands, representing a physical barrier, and to a circum-Antarctic current, the Antartic Convergence, representing a hydrological barrier. The vicariance events produced by this separation caused the subdivision of Notothenioidei into 120 species grouped in six families (Bovichtidae, Nototheniidae, Artedidraconidae, Harpagiferidae, Bathydraconidae and Channichthydae) (Eastman, 1993).

The Artedidraconids comprise four genera and 24 species easily distinguished from all the other notothenioids by the presence of a mental barbel (Eakin, 1990; Eastman, 1993) (Fig. 1). The family is largely confined to the Antarctic shelf and most species live in water less than 800 m deep. As a group, they are probably the most sedentary of notothenioids, spending almost all their time motionless on the substrate (Hubold, 1991).

Fig. 1. *Histiodraco velifer* from Terra Nova Bay, Ross Sea. Arrow indicates the mental barbel.

Data on the karyology of Artedidraconidae – rather scarce and confined to the chromosome morphology of eight species –

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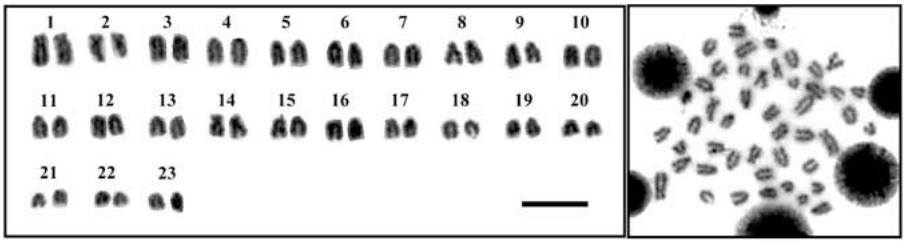

Fig. 2. Karyotype (left) and Giemsa-stained metaphase plate (right) of *H. velifer*. Scale bar = 5 μm.

indicate pronounced gross-karyotype stability (Ozouf-Costaz et al., 1991). Indeed, a diploid number of 46 mostly acrocentric chromosomes is present in all the species analyzed. There are slight differences with regard to a pair of chromosomes, acrocentric in the species of the genus *Artedidraco* and submetacentric in those of *Pogonophryne*.

A cytogenetic study of the sole species making up the genus *Histiodraco, H. velifer* (Fig. 1), was conducted to verify the role, if any, played by chromosome rearrangements in the speciation of Artedidraconidae. The description of this karyotype is reported for the first time, complete with banding and FISH data.

Materials and methods

For this study we used one male and one female of *H. velifer* collected in the vicinity of Terra Nova Bay Station during the Italian Antarctic Expedition 2001–2002 and identified according to Eakin (1990). Chromosome preparations were obtained directly from cephalic kidney and testis cells after in vivo colchicine treatment. Cell suspensions, hypotonic treatment and cell fixation were performed as described previously (Caputo et al., 1996). Chromosome number and standard morphology were determined by conventional Giemsa staining at pH 7.0. Chromosome nomenclature was in accordance with Levan et al. (1964).

Characterization of nucleolar organizer regions (NORs) was carried out by the one-step silver nitrate method (Howell and Black, 1980). C-banding was performed according to Sumner (1972) using $Ba(OH)_2$ at 45 °C. Fluorescent staining with GC-specific chromomycin A_3 (CMA_3) and AT-specific DAPI was done according to Schmid et al. (1983) and Schweizer (1976), respectively. *Alu*I enzyme digestion was carried out according to Mezzanotte et al. (1983).

In situ hybridization was performed using the digoxigenin-labeled all human telomere probe (Oncor). Hybridization, detection and counterstaining procedures were carried out according to the manufacturer's protocol.

Results

The diploid complement of both male and female consisted of 2n = 46 chromosomes (Fig. 2). The karyotype showed 20 pairs of acrocentric and/or subtelocentric chromosomes and three pairs of submetacentrics (pairs 1, 2 and 14) (FN = 52). In both specimens the chromosomes gradually decreased in size. Heteromorphic sex chromosomes were not observed.

After C-banding, constitutive heterochromatin was detected in the centromeric and telomeric regions of almost all chromosomes. Heterochromatin appeared as thin bands in all chromosomes except for the NOR-bearing element where it stained more strongly (Fig. 3A). Moreover, distinct interstitial bands were noted in the long arms of chromosomes 3. The C-positive material appeared resistant to digestion with the restriction enzyme *Alu*I except for nucleolus-associated heterochromatin which appeared sensitive to this endonuclease (Fig. 3C). As revealed by $AgNO_3$ and CMA_3, the NOR phenotype was of terminal-centromeric type (see Takai and Ojima, 1986) and was located on the short arm of the submetacentric chromosomes 14 (Fig. 3D, E). In situ hybridization with a telomeric probe revealed fluorescent signals at the ends of all chromosomes, without interstitial spots (Fig. 3F). Finally, the chromosomes were uniformly stained with DAPI (Fig. 3B).

Discussion

Despite their usefulness in interpreting phyletic relationships among other vertebrates like mammals and reptiles, chromosome data are relatively less informative in the case of bony fishes. In fact, the modal complement is made up of around 48 acrocentric elements which is considered the plesiomorphic complement for teleosts by several authors (e.g. Ohno, 1970). The NOR location, usually considered to be especially relevant to taxonomic studies, is rather "repetitious" among most bony fishes (usually terminal-centromeric; see Takai and Ojima, 1986). The present data on *Histiodraco velifer* confirm the pronounced gross-karyotype stability of these vertebrates. Indeed, like other artedidraconids karyotyped to date, this taxon showed 46 chromosomes, with 20 acrocentric and three submetacentric pairs (see Ozouf-Costaz et al., 1991; Pisano et al., 1998). According to Ozouf-Costaz et al. (1991) this seems to indicate that the karyotype is not a powerful criterion for taxonomic studies of this group, being at most useful to distinguish the artedidraconid family within Notothenioidei. Also the NOR location appeared plesiomorphic (terminal-centromeric) and uninformative at specific level.

The chromosome complement of *H. velifer* also appeared to be scarcely differentiated from the one of the notothenioid fishes that are closest morphologically and genetically, the Harpagiferidae family (Hastings, 1993; Bargelloni et al., 1997). Indeed, though very little cytogenetic information is available on Harpagiferidae, the two sole species karyotyped to date show a very similar chromosome formula with a single additional pair of acrocentrics (2n = 48; Pisano et al., 1998) compared with Artedidraconidae. Considering 2n = 48 as the basic complement for recent teleosts, this suggests that it can also be considered a plesiomorphic character for Harpagiferidae. Compared with this family, the karyotype of Artedidraconidae (2n = 46) might represent an apomorphic karyotype state origi-

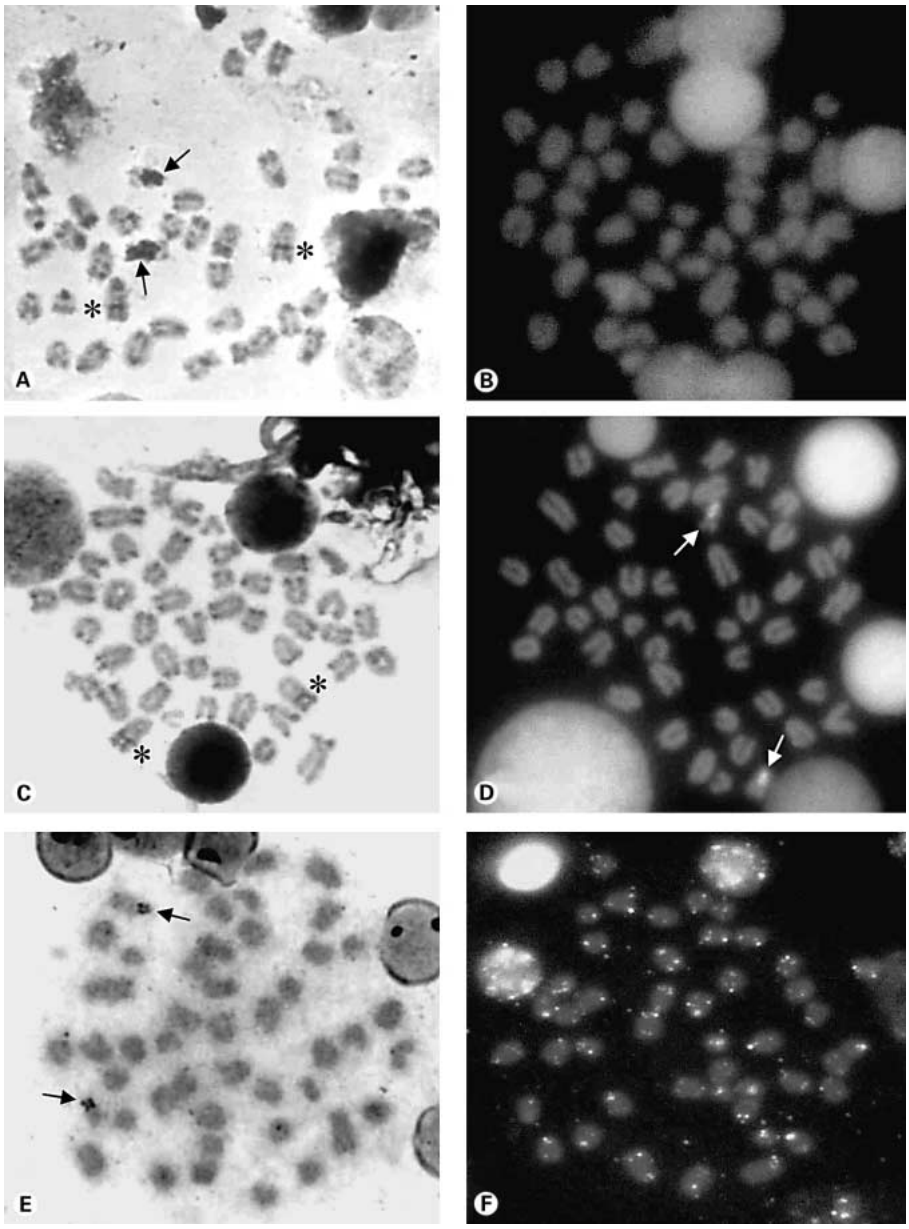

Fig. 3. Metaphases of *H. velifer* after (**A**) CBG-banding (arrows indicate nucleolus-associated heterochromatin; asterisks indicate an interstitial heterochromatic block), (**B**) DAPI staining, (**C**) *Alu*I digestion (asterisks indicate an interstitial heterochromatic block), (**D**) CMA_3 staining (arrows indicate the NORs), (**E**) silver staining (arrows indicate the NORs), and (**F**) in situ hybridization with a telomeric probe.

nating from a whole-arm translocation, probably a tandem fusion which seems to be a common rearrangement event in the chromosome evolution of teleosts (Caputo et al., 1997). The interstitial band evidenced in the larger acrocentric pair by CBG-banding may actually be a sign of just such rearrangements. However, in situ hybridization with a telomeric probe indicated that these sequences are probably lost during fusion events. Alternatively, short regions of $(TTAGGG)_n$ repeats may be retained but may be so small as to escape detection.

Interestingly, the sister taxon of artedidraconids, the Harpagiferidae family, is an exception among the Antarctic clades of notothenioids in that two species are Subantarctic and the remaining four are associated with peri-Antarctic islands. The formation of the Antarctic Circumpolar Current 25 Myr ago might thus be the vicariant event responsible for the speciation and distribution of this clade (Eastman, 1993). This hydrological change thus allows us to date the sole karyotypical difference distinguishing the two families to the beginning of the Miocene. Further karyological study of the other harpagiferid and artedidraconid species would be helpful to confirm their 2n = 48 and 2n = 46 karyotypes, respectively.

In conclusion, the present data show once again that despite their marked differences in morphological, ecological and taxonomic features, Antarctic fish have not undergone profound chromosomal rearrangements during their evolutionary diversification. This finding further highlights the marginal nature of chromosomal speciation mechanisms in marine teleosts, whose populations lack the demographic preconditions required for the chromosomal variants to reach homozygosis (e.g. Caputo et al., 1997). Indeed, there appears to exist a close relationship

between the absence of geographical barriers throughout the marine environment and the high vagility of these vertebrates (eggs, larvae or adults) on one side and the rare occurrence of macrostructural chromosome rearrangements on the other. Finally, cellular homeostasis might be important for karyotype maintenance among these fishes, restricting changes in the complement to cryptic chromosome rearrangements.

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