Intraspecific Karyotypical Diversity in Brackish Water Fishes of the Eleotridae Family (Pisces, Perciformes)

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Summary The Eleotridae family consists of 35 genera and 150 species distributed in freshwaters/brackish waters, estuaries or shores of tropical and subtropical regions. A few species are found in the Central America and South America. Cytogenetic analyses were performed on sympatric species *Dormitator maculatus* (12 individuals; 9 males and 3 females) and *Eleotris pisonis* (5 individuals; 2 males and 3 females) collected in the lower course of the Pium River, Northeastern Brazil. Both the species have $2n=46$ with striking differences for the chromosome formulas (*D. maculatus*, $40m-sm+6st-a$ and *E. pisonis*, $46a$). The two species revealed preferentially centromeric or telomeric heterochromatic regions and absence of structural chromosome heteromorphisms between the sexes. The NORs are located on the short arm of a submetacentric pair (pair 4) in *D. maculatus* and in interstitial position on a medium acrocentric (pair 21) in the *E. pisonis*. Meiotic analyses carried out on *D. maculatus* and *E. pisonis* showed a modal value of 23 in both MI and in MII. Comparison with cytogenetic data of Mexican populations (*D. maculatus*, $34m-sm+12st-a$; *E. pisonis*, $2m-sm+42st-a$) indicated that the karyotypical changes among the populations from Northeastern Brazil and the Mexican coast are mainly due to pericentric inversion, although translocations, fission and fusion are involved in the karyotypic diversification of the group. The differences observed among the karyotype macrostructure of South and Central American forms suggest the existence of distinct species in these two regions.

Key words Intraspecific karyotypical diversity, Eleotridae.

The Eleotridae family consists of 35 genera and 150 species distributed in freshwaters/brackish waters, estuaries or shores of tropical and subtropical regions (Nelson 1994). It is represented by a few species in the east of South America that inhabit the lower courses of coastal rivers. Two of these species, *Dormitator maculatus* and *Eleotris pisonis*, are widely distributed geographically, stretching from the Gulf of Mexico and the Bahamas to the south of Brazil (Menezes and Figueiredo 1985).

Cytogenetical data for Eleotridae family are scarce (Arai and Sawada 1974, Krysanov and Elovenko 1981). The chromosome analyses already performed seem that a diploid value of 46 chromosomes is a basal characteristic for the group. However, some exceptions have been observed regarding the chromosome formulas in the different genera.

The *Dormitator* genus has representatives on both the Atlantic (*D. maculatus*) and Pacific (*D. latifrons*) sides of Central America. In spite of the barrier that separates them, cytogenetics analyses have revealed that their karyotypic macrostructures are still quite similar ($2n=46$, $34m/sm+12st/a$) (Uribe-Alcocer 1985, Uribe-Alcocer and Ramirez-Scamilla 1989). However, the karyotypes of the species *D. maculatus* and *E. pisonis* of the Mexican coast (Uribe-Alcocer and Ramirez-Scamilla 1989, Montes-Perez 1981) present considerable divergences for the diploid number and chromosome formulas.

In the present study, chromosome banding (C banding and Ag-NORs) and also meiotic analy-

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ses was used in *D. maculatus* in *E. pisonis* to establish phylogenetic relationships among these genera and to ascertain possible chromosomal diversification among geographically isolated populations of America.

Material and methods

Dormitator maculatus (9 males and 3 females) and *Eleotris pisonis* specimens (2 males and 3 females) were collected, in sympatry and syntopy at 5 km from the mouth of the Pium River, Rio Grande do Norte state, Northeastern Brazil.

Mitotic stimulation (Lee and Elder 1980) was used for to increase the metaphase figures, obtained according to Gold *et al.* (1990). Gonadal tissue samples from males were utilized for meiotic analyses (Kligerman and Bloom 1977). C banding (Sumner 1972) and nucleolar organizing regions (Ag-NORs) analyses (Howell and Black 1980) were established to both species. Karyotypic formula was determinate according with the Levan *et al.* (1964) chromosome classification.

Results

Both species presented $2n=46$ with striking differences for the chromosome formulas. *D. maculatus* species presented a karyotype with many two-armed chromosomes, $40m/sm+6st$, $NF=92$. The NORs are located on a single submetacentric pair (pair 4) showing telomeric ribosomal sites on the short arm (Fig. 1a). C-banding performed on *D. maculatus* showed heterochromatic segments, mainly distributed in centromeric position (Fig. 1b), without numerical or structural chromosome heteromorphisms sex related.

E. pisonis showed a karyotype presenting 46 acrocentric chromosomes. NORs sites were located on pericentromeric position on the long arms of the pair 21 (Fig. 2a). In this species, C-banding revealed heterochromatic blocks located on the centromeric and pericentromeric regions (Fig. 2b).

The meiotic analyses in the two species presented spermatogonials metaphases with $2n=46$ chromosomes, 23 bivalents in MI (metaphase I) and 23 chromosomes in MII (Fig. 3a–f).

Discussion

Many cytogenetical analyses have shown that fish populations, although grouped taxonomically within the same nominal species, have reached such a degree of chromosome differentiation that they could be considered as distinct species (Moreira-Filho and Bertollo 1991).

Factors such as inversions, translocations and the heterochromatin process, involving mainly the sex chromosomes, are important agents of post-zygotic barriers that impede introgression and consequently reinforce growing stages of differentiation up to the species level.

The sample of *E. pisonis* of the Pium river presented a karyotype with $2n=46$ acrocentric chromosomes ($NF=46$). Similar karyotypes were also observed for others species of this genus, such as *E. acanthopomus* (Arai and Sawada 1974) and *E. muralis* (Khuda-Bukhsh and Nayak 1990).

The modal diploid number ($2n=46$) found in 13 Eleotridae species previously studied, is also common in the Gobiidae group (Caputo *et al.* 1996, Caputo *et al.* 1997), with close phylogenetic relationship with the Eleotridae. This diploid number shows it to be a symplesiomorphic trait in Eleotridae, indicating a numerical reduction in the chromosomes compared to the basal karyotype of the Perciformes ($2n=48$). However, the results for *E. pisonis* from the northeastern region of Brazil showed that they were significantly distant from those of the Mexican populations of the same species that presented a numerically and structurally differentiated karyotypic formula showing $2m-sm+42st-a$ and $NF=46$ (Uribe-Alcocer and Diaz-Jaimes 1996).

Fig. 1. a) *Eleotris pisonis* karyotype stained with Giemsa, $2n=46a$. b) C-banding. c) somatic metaphase. d) NOR-bearing chromosomes (pair 21).

Fig. 2. Karyotype of *Dormitator maculatus* by a) conventional staining, 2n=46. b) C-banding. c) somatic metaphase. d) Arrows indicate NOR-bearing chromosomes (pair 4).

Fig. 3. Meiotic metaphases. a–c) *Eleotris pisonis*. d–f) *Dormitator maculatus*. From right to left, spermatogonial metaphase ($2n=46$), metaphase I (23 bivalents) and metaphase II (23 chromosomes), respectively.

The vast distribution area (north of the United States to the south of Brazil) of the *Eleotris pisonis* species and the natural geographic barriers provided by the estuary regions that flow into the Atlantic, including the mouths of the Orinoco and Amazon Rivers, make maintenance of gene flow unlikely between the extremes of distribution of this species, similarly to others Atlantic species (Briggs 1974, Floeter and Gasparini 2000). Thus it appears that causer events for population stratification, in this case, bring about the establishment of biologically distinct species that are still grouped as a single nominal species. Apparently the karyotype of the Mexican populations is more derived than that the South American forms, because of the presence of a biarmed pair, derived from centric fusion. In this genus, other events, such as chromosome fission, act in the chromosome evolution of the genus, bearing in mind that the *E. picta* karyotype, $2n=52$ and $NF=52$ (Uribe-Alcocer and Diaz-Jaimes 1996), may have originated by this process.

The *D. maculatus* population of Pium river presented a karyotype with $2n=46$, $40m-sm+6st-a$ and $NF=86$, and no indication was observed of sex chromosome heteromorphism. In contrast, the population from the Itinga River in Mongaguá, in Southeastern Brazil, showed $2n=46$, $40m/sm+6st/a$ in the females and $41m/sm+5st/a$ in the males, indicating the possible presence of a XX/XY sex chromosomes (Oliveira and Almeida-Toledo 1985), where the males presented one biarmed chromosome (Y) not found in the females. Cytogenetical studies carried out on this species in Mexico showed a karyotype consisting of $34m/sm+12st/a$ and $NF=80$ (Maldonado-Monroy *et al.* 1985). These results showed numeric conservatism among the Brazilian populations and the Mexican population, differing with regards to the karyotypic macrostructure.

Meiotic analysis ($MI=23$ and $MII=46$) in these species corroborated with the diploid numbers, no showing evidence of sex chromosomes.

The Eleotrid sleeper *Gobiomorphus breviceps*, from New Zealand, shows variations in the colors of different populations seemed to indicate population stratifications and reduced genetic flow (Maldonado-Monroy *et al.* 1985). These striking karyotypic modifications can be attributed to peri-

centric inversion, with evident modification in the fundamental number between the 2 forms observed. In addition, such inversion process seems to play an active role in the karyotypic evolution of *Oxyeleotris marmorata*, on the islands of Java, Sumatra and Kalimantan/Borneo (considered as the Indo-Malaysian region). *O. marmorata* presented a karyotype of $2n=46$ ($42m+4sm$, $NF=92$), with an extra metacentric chromosomes in polymorphic condition in the Java population (Masagca 2000). Also in this case, geographic isolation seems to have been decisive in the karyotypic differentiation of the populations. Thus the cytogenetic analyses, as for *E. pisonis*, indicated the need for taxonomic revision in *D. maculatus* since it seems to harbor biologically different forms.

The results of C banding in the *D. maculatus* and *E. pisonis*, did not show differences among karyotypes of males and females. Heterochromatic blocks could be observed located in centromeric and/telomeric position, with some pairs, in the first species, showing smaller totally heterochromatic arms. Thus it is possible that heterochromatin processes may play a part in the karyotypic differentiation observed among the geographically distinct populations from the Pium river (Northeastern Brazil) (present paper), Itinga river (Southeastern Brazil) (Oliveira and Almeida-Toledo 1985) and from the Gulf of Mexico (Maldonado-Monroy *et al.* 1985).

NORs sites are localized on a unique pair in the two species, similarly to others Perciformes groups (Molina 2000). The lack of data on other species of this family does not permit inference on the constancy of this pattern for the group.

Thus it seems evident that several kinds of rearrangements such as pericentric inversion, fusions and fission added to heterochromatin processes play an active role in the chromosome diversity observed among Eleotridae samples from South and Central America, strongly suggesting that they seem biologically cryptic forms and not more populations of a same species.

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