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RESEARCH PAPER

High chromosomal evolutionary dynamics in sleeper gobies (Eleotridae) and notes on disruptive biological factors in Gobiiformes karyotypes (Osteichthyes, Teleostei)

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Abstract

The order Gobiiformes is made up of more than 2200 species, representing one of the most diverse groups among teleost fishes. The biological causes for the tachytelic karyotype evolution of the gobies have not yet been fully studied. Here we expanded cytogenetic data for the Eleotridae family, analyzing the neotropical species *Dormitator maculatus*, *Eleotris pisonis*, *Erotelis smaragdus,* and *Guavina guavina*. In addition, a meta-analytical approach was followed for elucidating the karyotype diversification versus biological aspects (habitat and egg type) of the Gobiiformes. The species *E. smaragdus* and *E. pisonis* present $2n = 46$ acrocentric chromosomes (NF = 46), *D. maculatus* $2n = 46$ (36sm + 4st + 6a; NF = 86), and *G. guavina,* the most divergent karyotype, with $2n = 52$ acrocentric chromosomes (NF = 52). Besides numeric and structural diversification in the karyotypes, the mapping of rDNAs and microsatellites also showed noticeable numerical and positional variation, supporting the high chromosomal evolutionary dynamism of these species. In Gobiiformes, karyotype patterns which are more divergent from the basal karyotype ($2n = 46$a) are associated with characteristics less effective to dispersion, such as the benthic habit. These adaptive characteristics, connected with the organization of the repetitive DNA content in the chromosomes, likely play a synergistic role in the remarkable karyotype diversification of this group.

Keywords Chromosome evolution · Dispersive potential · Goby · Karyotype diversification · Microsatellites · rDNA

Introduction

Gobiiformes (Osteichthyes, Teleostei) constitute one of the most diverse groups among vertebrates, encompassing nine families, 268 genera, and notably 2211 fish species (Betancur-R et al. 2017; Eschmeyer and Fong 2020; Nelson et al. 2016). Its wide geographic distribution covers the areas of Oceania, Asia, Europe, North America, and Latin America, inhabiting marine, brackish and freshwater environments. Some species live in hypersaline waters or even great oceanic depths (Muus and Nielsen 1999; Oto et al. 2017; Suzuki et al. 2015), however they generally occur in estuaries, rocky marine coasts, or are associated with coral reefs (Baensch and Riehl 1991; Kottelat and Freyhof 2007; Patzner et al. 2012). Their reproductive strategies include (1) parental care for eggs and larvae, (2) internal or external fertilization and (3) males' sex change under certain environmental conditions (Nakashima et al. 1996; Skóra et al. 1999).

The percomorph fish clade Gobiiformes, despite its great diversity, is a monophyletic group (Thacker 2003). Its origin dates from the Paleocene (~ 65 Ma), and now has representatives in marine, estuarine, and continental waters of vast areas of tropical and subtropical regions (Fanta 1997; Rocha et al. 2005; Ruber et al. 2003). In general, they represent a fish model of rapid and intense karyoevolutionary

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divergences (Lima-Filho et al. 2012; Molina 2005). A wide range of chromosomal rearrangements is associated with the karyotype diversification of this group, in which pericentric inversions and Robertsonian fusions stand out, in addition to others such as tandem fusions and fission events on a smaller scale (Amores et al. 1990; Caputo et al. 1997; Prazdnikov et al. 2013). Moreover, chromosome polymorphisms are also frequent in Gobiiformes populations (Caputo et al. 1997; Ene 2003; Nishikawa et al. 1974; Webb 1986), indicating continuous processes of karyotypic changes. In this scenario, meta-analysis can provide patterns under a phylogenetic perspective, or correlate the rapid and intense chromosomal changes with the biological characteristics of the species.

Cytogenetic data for Gobiiformes are restricted to five out of nine families, representing less than 10% of the valid species present in this order. Nevertheless, even though most of the chromosomal data are restricted to Giemsa-stained karyotypes (Arai 2011), they point to a high numerical and structural chromosome diversity within this group (Fanta 1997; Lima-Filho et al. 2012; Rocha et al. 2005; Ruber et al. 2003). Such high karyotype diversity in some marine fishes are punctually attributed to the diversity of habitats, limited dispersive capacity, and rich behavioral repertoire of the species (Lima-Filho et al. 2016; Molina et al. 2014a; Rocha et al. 2005). On the basis of its greatest frequency, it has been suggested that the karyotype composed by 46 acrocentric chromosomes corresponds to the basal one for Gobiiformes (Vasil'ev and Gregorian 1994). However, this suggestion needs to be confirmed, since it is based on a small set of cytogenetic data available for some families, without considering the phylogenetic extent within the order.

The cytogenetic diversification in Gobiiformes shows a very extensive panel of chromosome changes (Amores et al. 1990; Caputo et al. 1997; Lima-Filho et al. 2014a, b; Prazdnikov et al. 2013). In Eleotridae, popularly known as "sleepers" (the name given due to their mode of life, hiding in dens in the substrate and low vagility), karyotype divergences among biogeographic regions (Molina 2005), chromosome polymorphisms (Uribe-Alcocer and Ramirez-Escamilla 1989), and differentiated sex chromosomes (Oliveira and Almeida-Toledo 2006) have already been reported despite the limited cytogenetic data available for this group.

Extensive cytogenomic analyses, associated with phylogenetic meta-analyses of biological traits, have led to increased understanding of the macro and microstructural reorganization levels of the chromosomes. Therefore, to correlate the biological characteristics of the Gobiiformes groups with the possible basal karyotype for the order, we performed a detailed analysis of the karyotypes by applying standard and advanced molecular cytogenetic techniques in four Gobiiformes species belonging to different genera. In addition to conventional chromosomal methods, base-specific fluorochromes Mithramycin A (MM), DAPI (4′,6-diamidino-2-phenylindole) and fluorescence in situ hybridization (FISH) using the repetitive sequences of 18S rDNA, 5S rDNA and microsatellite sequences [$(CA)_{15}$ and $(CAA)_{10}$] as probes were performed.

Results

Karyotypes, C-, Ag- and DAPI/MM banding

The species *E. smaragdus* and *E. pisonis* have karyotypes with $2n = 46$ acrocentric chromosomes (NF = 46), *D. maculatus* has $2n = 46$ composed of 36sm + 4st + 6a (NF = 86), and *G. guavina* has $2n = 52$ acrocentric chromosomes (NF = 52) (Fig. 1).

The C-positive heterochromatin shows a diversified distribution and content among the species. In *E. smaragdus,* it occurs as conspicuous centromeric and terminal blocks in the chromosomes, in *E. pisonis* as small centromeric segments and in *D. maculatus* and *G. guavina* with an irregular distribution in centromeric, interstitial, and terminal blocks. In all species, some heterochromatic blocks occupy the interstitial regions or the entire arms of two-armed chromosomes (Fig. 1).

Ag-NORs sites are located on a single pair of chromosomes and are the only regions in the karyotypes exhibiting a $MM^+/DAPI^-$ pattern (Fig. 1; in the boxes). These sites are localized in the terminal position on the long arms of pair 9 in *E. smaragdus*, in the interstitial position of the long arms of pair 21 in *E. pisonis*, in the terminal position of the short arms of pair 4 in *D. maculatus*, and in the interstitial region of the long arms of pair 19 in *G. guavina* (Fig. 1; in the boxes).

FISH mapping

The 18S rDNA sites are congruent with the Ag-NORs signals in all species but located in non-homologous chromosomes. The 5S rDNA sites, in addition to numerical variation, also show large interspecific divergences in their chromosomal location (Fig. 1). In *E. smaragdus,* they have a proximal location on the chromosome pairs 7 and 14; in *E. pisonis,* they are interstitially co-located with the 18S rDNA site in pair 21; in *G. guavina*, they occupy an interstitial position on pair 4. In addition, *D. maculatus* exhibits a structural polymorphism. In this case, some individuals have 18S and 5S rDNA sites on the short arms of pairs 4 and 5, respectively, while others have only one homologue of pair 4 carrying an 18S rDNA site, the other homologue of this same pair carrying co-located 18S rDNA/5S rDNA sites, and a single homologue of pair 5 carrying a 5S rDNA site (Fig. 1).

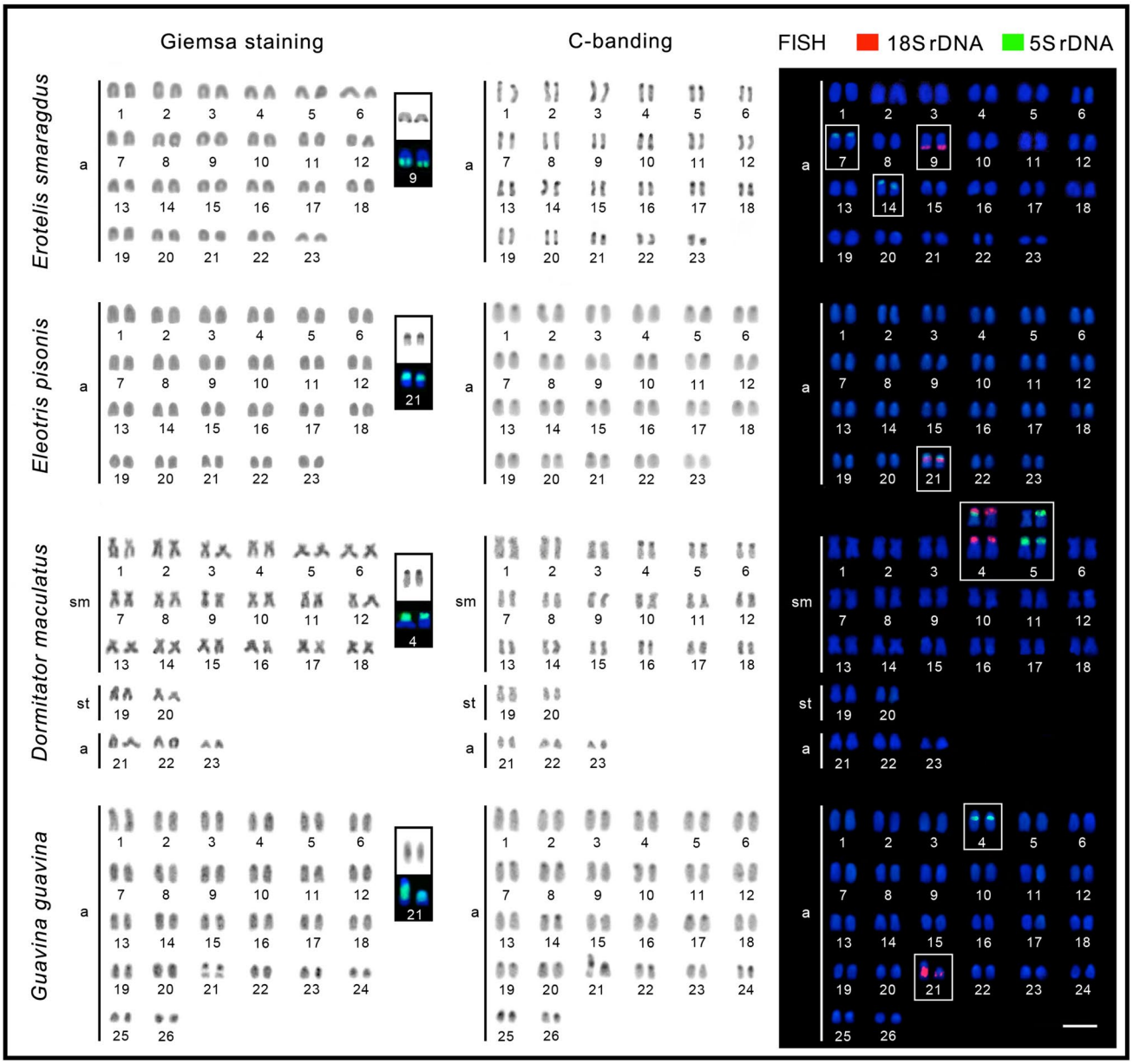

Fig. 1 Karyotypes of *E. smaragdus*, *E. pisonis*, *D. maculatus* and *G. guavina*, after Giemsa staining, C-banding, and fluorescence in situ hybridization with 18S rDNA (red) and 5S rDNA (green) probes. The Ag-NORs and $MM^{+}/DAPI^{-}$ regions (green) are showed in the boxes of the first column. The two rDNA arrays in the chromosomes of *D. maculatus* are highlighted in the larger box. Scale bar = 5 μm

The mapping of microsatellites $(CA)_{15}$ and $(CAA)_{10}$ was performed for *E. pisonis, D. maculatus* and *G. guavina*. The results show the distribution of these motifs in both heterochromatic and euchromatic regions (Fig. 2). The $(CA)_{15}$ repeats occur in all chromosomes of the three species, mainly in the terminal region of their long arms. In *E. pisonis*, this motif additionally occurs in both arms of pairs 15 and 21. In *D. maculatus*, these sequences occupy the terminal regions of both arms of most chromosomes. On the other hand, in *G. guavina*, they have a very variable distribution, occurring exclusively in the terminal position of the short or long arms, in both arms of the chromosomes, or in the interstitial regions of a few chromosomal pairs (Fig. 2). In contrast, microsatellite sequences $(CAA)_{10}$, do not occur in all chromosomes of any given species. In *E. pisonis,* they are mainly located in the terminal regions of the long arms in most of the chromosomes. However, in *D. maculatus* and in *G. guavina*, they occur in the terminal region of only one or both chromosome arms, but with a different distribution pattern along the chromosomes in each species (Fig. 2).

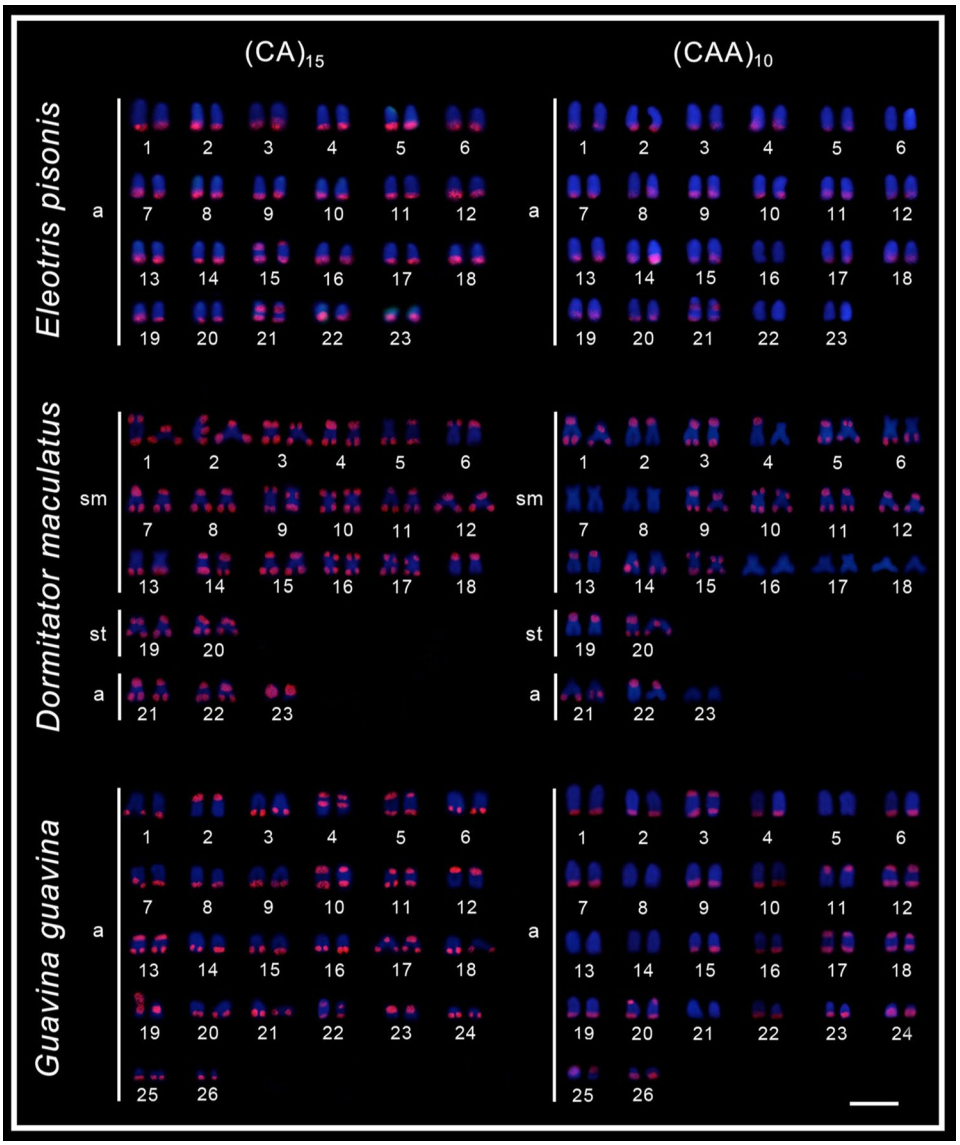

Fig. 2 Hybridization patterns from the repetitive microsatellite motifs $(CA)_{15}$ and $(CAA)_{10}$ in the chromosomes of *E. pisonis*, *D. maculatus*, and *G. guavina*. Scale bar = 5 µm

Meta-analysis

The cytogenetic survey on Gobiiformes covered 139 species, and showed a diploid variation from $2n = 30$ to 56 chromosomes, where $2n = 46$ represents the most frequent condition, followed by $2n = 44$ chromosomes, also present in high frequency and prevalent in some clades. Oxudercidae is the most representative family, with cytogenetic data available for 69 spp. Of these, 37% (25 spp.) have $2n = 44$ chromosomes, 31% (21 spp.) have $2n = 46$ chromosomes, and the remaining 32% (23 spp.) have diploid numbers varying from $2n = 38$ to $2n = 56$ chromosomes. The NF in this group ranges from 40 to 92. Gobiidae is the second group with the largest number of accessible cytogenetic data (50 spp.), among which, 42% (21 spp.) have $2n = 46$ chromosomes, 24% (12 spp.) have $2n = 44$ chromosomes, and the remaining 34% (17 spp.) have $2n = 30$ to $2n = 50$ chromosomes. The NF variation was shown to be extensive in this family, ranging from 38 to 98. Cytogenetic data for Eleotridae encompassed 11 species: 64% (7 spp.) with $2n = 46$ chromosomes, and the others with $2n = 48$ or 52 chromosomes. The NF ranges from 46 to 90, with NF = 46 being the most frequent. The Butiidae and Odontobutidae families are the least investigated: the former with five species analyzed exhibiting $2n = 46$ or $2n = 48$, NF from 48 to 58, and the only four species of the second having $2n = 44$ acrocentric chromosomes.

The association between karyotype diversification and biological factors affecting the dispersive potential in Gobiiformes species revealed a greater divergence in $2n$ and NF in the benthic species (66% and 73%, respectively, of the karyotypes different from the basal pattern), while species with pelagic and bentho-pelagic habits share more conservative karyotype patterns. Similar trends occur in

the Gobiidae and Oxudercidae families with the greatest samples (Table 1).

In another comparison, the 2*n* and NF data of the karyotypes of 124 Gobiiformes species were analyzed with respect to their preferential environments. Species with more than one habitat were divided into grouped categories. The results show that the chromosome variation is not precisely related to environmental categories (Table 2). However, the 2*n* of species from freshwater and freshwater/estuarine habitats are mainly equal to the considered basal karyotype (2*n* = 46) for the order. Similar results were obtained for the families Gobiidae and Oxudercidae.

Discussion

The four Eleotridae species exhibited a conspicuous karyotype diversification regarding their fundamental number, chromosome morphology (Table 3), and organization of repetitive sequences on the chromosomes. These data are consistent with a wider evaluation of the karyotype evolution among Gobiiformes (Molina et al. 2014b).

The evolutionary history of some Eleotridae groups, such as *Dormitator* in the Atlantic, is recent (0.19–0.35 Ma) and linked with population fragmentation derived from some major geological and ecological events, such as the uplift of

Table 1 Frequency of diploid number (2*n*) and chromosome arms number (NF) in Gobiiformes species and families Gobiidae and Oxudercidae grouped according to benthic (*B*), pelagic (*P*) and bentho-pelagic (*B*/*P*) habitat categories

2*n*	*B* (%)	*P* (%)	*B*/*P* (%)	NF	*B* (%)	*P* (%)	*B*/*P* (%)
Gobiiformes							
< 46	43 (41.7)	2 (50.0)	11 (52.4)	< 46	13 (13.0)	–	4 (23.5)
46	35 (34.0)	2 (50.0)	10 (47.3)	46	22 (22.0)	1 (33.3)	8 (47.0)
> 46	25 (24.3)	–	–	> 46	65 (65.0)	2 (66.7)	5 (29.5)
Total	103	4	21		100	3	17
Gobiidae							
< 46	22 (52.4)	1 (33.3)	4 (50.0)	< 46	4 (10.8)	1 (25.0)	1 (7.1)
46	12 (28.6)	2 (66.7)	4 (50.0)	46	13 (35.2)	1 (25.0)	6 (42.9)
> 46	8 (19.0)	–	–	> 46	20 (54.0)	2 (50.0)	7 (50.0)
Total	42	3	8		37	4	14
Oxudercidae							
< 46	22 (50.0)	1 (100.0)	6 (54.0)	< 46	6 (14.0)	–	2 (28.6)
46	16 (32.6)	–	5 (46.0)	46	5 (11.6)	–	1 (14.2)
> 46	11 (22.4)	–	–	> 46	32 (74.4)	1 (100)	4 (57.2)
Total	49	1	11		43	1	7

Table 2 Frequency of the diploid number (2*n*) and chromosome arms number (NF) in Gobiiformes species and in the families Gobiidae and Oxudercidae, grouped according to the type of aquatic environment

2*n*	Sets of aquatic environments						
	M–M/E (%)	F–F/E (%)	E–F/M/E (%)	NF	M–M/E (%)	F–F/E (%)	E–F/M/E (%)
Gobiiformes							
< 46	20 (54.1)	17 (37.0)	15 (36.7)	< 46	6 (16.6)	9 (21.0)	3 (7.3)
46	7 (18.9)	21 (45.7)	19 (46.3)	46	7 (19.4)	15 (34.8)	9 (22.0)
> 46	10 (27.0)	8 (17.3)	7 (17.0)	> 46	23 (64.0)	19 (44.2)	29 (70.7)
Total	37	46	41		36	43	41
Gobiidae							
< 46	10 (45.4)	4 (23.5)	6 (66.7)	< 46	4 (17.3)	1 (6.2)	1 (9.0)
46	6 (27.3)	10 (58.8)	3 (33.3)	46	7 (30.4)	10 (62.5)	5 (45.5)
> 46	6 (27.3)	3 (17.7)	–	> 46	12 (52.3)	5 (31.3)	5 (45.5)
Total	22	17	9		22	16	11
Oxudercidae							
< 46	11 (78.6)	5 (34.0)	13 (40.6)	< 46	2 (15.3)	4 (33.3)	2 (7.7)
46	1 (7.1)	7 (46.0)	13 (40.6)	46	1 (8.7)	2 (16.7)	2 (7.7)
> 46	2 (14.3)	3 (20.0)	6 (18.8)	> 46	10 (76.0)	6 (50.0)	22 (84.6)
Total	14	15	32		13	12	26

M marine, *F* freshwater, *E* estuarine and presence in more than one aquatic environment

Table 3 Cytogenetic data for Eleotridae species

Species	2*n*	Karyotype	NF	Distribution	References
Dormitator latifrons	46	12 m + 22sm + 10st + 2a	90	Eastern Pacific	Uribe-Alcócer et al. (1983), Uribe-Alcocer and Ramirez-Escamilla (1989)
D. maculatus	46	36sm + 4st + 6a	86	Western Atlantic	Molina (2005), Present study
D. maculatus	46	14 m + 28sm + 2st + 2a ♀ 13 m + 28sm + 3st + 2a ♂	90	Western Atlantic	Oliveira and Almeida-Toledo (2006)
D. maculatus	46	34 m/sm + 12st/a	80	Caribbean	Maldonado-Monroy et al. (1985)
Eleotris acanthopoma	46	46a	46	Indo-Pacific	Arai and Sawada (1974)
E. oxycephala	46	46a	46	Indo-Pacific	Yu et al. (1987)
E. picta	52	52a	52	Western Atlantic	Uribe-Alcocer and Diaz-Jaimes (1996)
E. pisonis	46	46a	46	Western Atlantic	Molina (2005), Present study
E. pisonis	44	2 m/sm + 42st/a	46	Caribbean	Uribe-Alcocer and Diaz-Jaimes (1996)
Erotelis smaragdus	46	46a	46	Western Atlantic	Present study
Gobiomorus dormitor	48	2 m + 4sm + 42a	54	Western Atlantic	Maldonado-Monroy et al. (1985)
Guavina guavina	52	52a	52	Western Atlantic	Present study
Hypseleotris cyprinoides	48	48a	48	Indo-Pacific	Suzuki (1996)
Mogurnda mogurnda	46	6sm + 40st/a	52	Western Pacific	Arai (2011)

m metacentric, *sm* submetacentric, *st* subtelocentric, *a* acrocentric chromosome, *NF* number of chromosome arms

Central American Isthmus and regional isolation by climate and oceanographic changes (Galván-Quesada et al. 2016). These processes, on macro- or micro-scales, apparently had direct evolutionary effects on genomic diversification and on the fixation of chromosome rearrangements alongside their distribution limits (Molina 2005). *D. maculatus* has different regional karyotypes, such as in the Brazilian northeast (2*n* = 46; NF = 86) (Molina 2005, present data), and southeastern (2*n* = 46; NF = 90) (Oliveira and Almeida-Toledo 2006) coasts, in Western Atlantic and Caribbean (2*n* = 46; NF = 80) (Maldonado-Monroy et al. 1985). These karyotype divergences highlight a cryptic macroevolution pattern and support an under perceived scenario of profuse allopatric speciation in the *Dormitator maculatus* complex.

Similarly, karyotype divergences also occur among *E. pisonis* populations from the Brazilian (2*n* = 46; NF = 46) (Molina 2005, present data) and Caribbean coasts (2*n* = 44; NF = 46) (Uribe-Alcocer and Diaz-Jaimes 1996). As a whole, such karyotype variations also suggest the occurrence of cryptic species within the Eleotridae family (Molina 2005). However, despite exhibiting 2*n* variations (2*n* = 44–52), Eleotridae species most often have 2*n* = 46 chromosomes, a condition also found in *E. pisonis*, *E. smaragdus* and *D. maculatus*, suggesting that it may represent a basal trait for this family. Karyotypes with 2*n* > 46, as in *G. guavina* (2*n* = 52; the highest diploid value in the group), and NF > 46 (Table 3), indicate the importance of fission events, as well as pericentric inversions in the karyotype evolution of this fish group. Such rearrangements are also frequent in large marine groups as Percomorpha (Galetti et al. 2000).

Besides karyotype variations, marked intra- and interspecific heterogeneities in the amount and location of heterochromatin occur among the Eleotridae species. While a reduced and centromeric heterochromatic pattern occurs in *G. guavina* and *E. pisonis*, the C-positive heterochromatin is present in the interstitial and terminal regions of chromosomes of *E. smaragdus* and *D. maculatus*. This diversified heterochromatic organization is phylogenetically wide and has been recognized in several gobiiform groups (Caputo et al. 1997; Lima-Filho et al. 2012, 2014a), indicating an intense inner chromosomal reorganization of repetitive DNAs, probably associated with changes in the macrostructure of the Eleotridae chromosomes.

The mapping of rDNA sequences has shown a wide variation at both population and interspecific levels in Gobiiformes (Lima-Filho et al. 2012, 2014a, b; Ocalewicz and Sapota 2011). In Eleotridae, although only two Ag-NORs/18S rDNA sites occur, they show distinct size and location in conspicuously different chromosomal pairs among the species, thus suggesting the occurrence of disruptive events of the syntenic order in these chromosomes.

Evidence of significant internal reorganizations in the Eleotridae chromosomes is also provided by the differentiated distribution that the 5S rDNA sites have in this group. Location of the 18S and 5S rDNA sites in different chromosomes, like in *G. guavina* and *E. smaragdus*, is a common condition in several fish groups (Gornung 2013). However, syntenic arrays such as in *E. pisonis*, hitherto uncommon in Gobiiformes, constitute a derived condition. Indeed, collectively the rDNA sites create very exclusive species-specific patterns. The set of diversifications related to rDNA

sequences and the bearing chromosome indicates that microstructural changes are frequent in Eleotridae and probably extend to other chromosomes of the species. Interestingly, *D. maculatus* exhibits a rDNA polymorphism related to the 18S and 5S sequences on pairs 4 and 5 of the karyotype comprising different arrangements which include a syntenic 18S/5S state in only one homologue of pair 4. This polymorphism reinforces the dynamic condition of the ribosomal DNAs among Eleotridae species and suggests a transient stage toward the colocalization of the 18S/5S sequences in the same chromosome pair.

Like the rDNA, microsatellite sequences are also evolutionarily dynamic, susceptible to high mutational rates in the genome (Oliveira et al. 2006), and can present independent evolutionary paths in chromosomes (Xu et al. 2017). In *E. pisonis*, *D. maculatus* and *G. guavina*, the $(CA)_{15}$ and $(CAA)_{10}$ microsatellites are clustered on different regions of the chromosomes, presenting an incomplete overlap with the C-banding regions. In these species, the heterogeneity of heterochromatin is identified by the heterochromatic and euchromatic regions harboring both, one or neither $(CA)_{15}$ and $(CAA)_{10}$ repeats. This level of heterogeneity suggests that these regions are evolutionarily less stable and potentially associated with the high karyotype changes in Eleotridae.

As a whole, the inter- and intraspecific diversification of the karyotypes, and the great potential for population fragmentation, make Eleotridae a target group for deeper taxonomic approaches in the search for the real meaning of its biodiversity.

Additional remarks on karyoevolution, biological features and geographic dispersion of Gobiiformes

The significant diversification of chromosomal numbers and karyotypic formulas (Arai 2011), distinguishes Gobiiformes from other large groups of marine fish with a clear $2n = 48$ conservatism (Motta-Neto et al. 2019). Phylogenetic relationships (Betancur-R et al. 2013; Thacker 2009) indicate a higher frequency of karyotypes with $2n = 46$ acrocentric chromosomes distributed from basal clades to recent lineages of this order. While in families Eleotridae and Butiidae $2n = 46$ acrocentric chromosomes (NF = 46) is a prevalent condition, Oxudercidae shows a greater frequency of $2n = 46$ chromosomes, but with NF > 46. Apart from the Odontobutidae, which possess $2n = 44$ chromosomes, other families of Gobiiformes, with ancient or recent divergence, have some species with $2n = 46$ chromosomes. The presence of a high incidence of karyotypes with $2n = 46$ chromosomes in Apogonidae (Araújo et al. 2010), a family closely related to Gobiiformes (Betancur-R et al. 2017), suggests that $2n = 44$ chromosomes is a homoplasic and recurrent trait in some groups of Gobiiformes. In addition, Gobiiformes also include variations in intraspecific diploid number (Caputo et al. 1999; Prazdnikov et al. 2013), in 5S rDNA sites (Lima-Filho et al. 2012; present data), in karyotypes of congeneric species (Caputo et al. 1997; Grigoryan and Vasiliev 1993; Thode et al. 1988), and in the emergence of sex chromosomes (Lima-Filho et al. 2014b; Pezold 1984).

This diversified scenario is also supported by the high evolutionary variation of the ribosomal sequences, indicating a massive internal reorganization in the chromosomes. Although generally present on a single pair of chromosomes, the present study shows that 18S rDNAs can be found in different positions and on different chromosomes among gobiiform species, which is consistent with the findings of Lima-Filho et al. (2012) and Ocalewicz and Sapota (2011). Similar reorganizations are also found for 5S sites in parallel to large numerical variations. In addition, syntenic arrangements such as those in *E. smaragdus*, or complex polymorphic arrangements showed in *D. maculatus*, along with their location on the sex chromosomes (Lima-Filho et al. 2014b), complement the evolutionary dynamism of these sequences.

Some biological characteristics of Gobiiformes, such as particular habitats and reproductive strategies, seem to act on the dispersive potential of the species, thus supporting population stratifications and the fixation of chromosomal rearrangements. Some divergent cytogenetic patterns are found in marine species, contrasting with the more obvious biogeographic stratification of freshwater species. This is in accordance with to the patterns of genetic variability in Gobiiformes, whose pelagic species have a more homogeneous genetic structure than the benthic ones (Giovannotti et al. 2009).

The extensive variation in NF values among the Eleotridae species (NF = 46–90; Table 4), and in Gobiiformes generally (NF = 40–96; Arai 2011) indicates a significant participation of pericentric inversions in the karyotype evolution of these groups. Genomic-based studies revealed that large inversions are common in fishes and keep favorable allelic combination

Table 4 Collection sites and the sample sizes (*N*) of the Eleotridae genera

Species	Sampling Site	*N*
Dormitator maculatus	Pium River (5° 56′ 51.2″ S, 35° 14′ 09.2″ W)	10 (8♂, 2♀)
Eleotris pisonis	Pium River (5° 56′ 51.2″ S, 35° 14′ 09.2″ W)	25 (15♂, 10♀)
Erotelis smaragdus	Curimataú River (6° 19′ 15.50″ S, 35° 2′ 29.31″ W)	15 (10♂, 5♀)
Guavina guavina	Potengi River (5° 41′ 07.2″ S, 35°14′ 28.1″ W)	4 (1♂, 3♀)

involved in local environmental adaptations (Kess et al. 2020; Kirubakaran et al. 2016; Pearse et al. 2014). Inversions are central to the evolution of many species (Faria et al. 2019), which the eco-evolutionary effects are extensive, encompassing morphological, physiological, behavioral adaptations and phyletic diversification (Ayala et al. 2017; Berg et al. 2016, 2017; Wellenreuther and Bernatchez 2018). In the order Gobiiformes, the reorganization of genomic architecture promoted by inversions possibly favored fine-scale adaptation to the several environments and salinity gradients occupied, and it is likely that such mechanisms have played an equally important role in the evolution of the lineages within this group. Despite offering an apparent chance for greater gene flow among populations, marine environments are large and subdivided by extensive ecosystems that become progressively occupied during species colonization. The available data illustrate the unusual chromosomal diversity found in Eleotridae and other Gobiiformes fishes, offering a new example of congruence of phyletic and karyotype diversification within the marine ichthyofauna.

Materials and methods

Sampling

The collection sites, numbers, and sex of the individuals investigated are presented in Fig. 1 and Table 4. All the specimens were collected under the appropriate authorization of the Brazilian environmental agency ICMBIO/SISBIO (License number 19135-4).

Chromosome preparations, C-, Ag- and DAPI/MM banding

The specimens were subjected to in vivo mitotic stimulation with bacterial and fungal antigen complexes (Molina et al. 2010). Mitotic chromosomes were obtained from cell suspensions of fragments of the anterior kidney (Gold et al. 1990) and stained with Giemsa 5% diluted in phosphate buffer (pH 6.8).

The nucleolus organizing regions (NORs) and the C-positive heterochromatic regions were identified following the method described by Howell and Black (1980) and Sumner (1972), respectively. Additionally, the chromosomes were also stained with the base-specific fluorochromes DAPI and MM (Schweizer 1976).

Fluorescence in situ hybridization (FISH) for repetitive DNA mapping

The location of the rDNA sites on chromosomes were determined using fluorescence in situ hybridization with 5S and 18S rDNA probes, containing approximately 200 bp and 1400 bp, respectively. The probes were amplified by PCR from the nuclear DNA of *Rachycentron canadum* (Teleostei, Rachycentridae), using primers NS1 5′-GTA GTC ATA TGC TTG TCT C-3′ and NS8 5′-TCC GGT GCA TCA CCT ACG GA-3′ (White et al. 1990) and A 5′ (5′-TAC GCC CGA TCT CGT CCG ATC-3′ and B 5′-CAG GCT GGT ATG GCC GTA AGC-3′ (Pendás et al. 1994), respectively. The 18S rDNA probe was labeled with digoxigenin-dUTP-11, and the 5S rDNA probe with biotin-14-dATP using nick translation according to the manufacturer's specifications (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA). The hybridization signals were detected using anti-digoxigenin rhodamine (Roche, Mannheim, Germany) for the 18S rDNA probe, and streptavidin-FITC (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) for the 5S rDNA probe.

Simple sequence repeats (SSRs) were mapped by in situ hybridization (Kubat et al. 2008) using the oligonucleotides $(CA)_{15}$ and $(CAA)_{10}$ labeled with AlexaFluor 555, at the 5′ terminal position during the synthesis process (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA). The chromosomes were counterstained with Vectashield/DAPI (1.5 µg/ml).

Image analysis and processing

At least 30 metaphase spreads per individual were analyzed to confirm the $2n$, karyotype structure, and FISH results. Images were captured using an Olympus BX51 microscope (Olympus Corporation, Ishikawa, Japan) with CoolSNAP and the images were processed using the Image Pro Plus 4.1 software (Media Cybernetics, Silver Spring, MD, USA). Chromosomes were classified as metacentric (m), submetacentric (sm), subtelocentric (st), or acrocentric (a), according to their arm ratios (Levan et al. 1964).

Meta-analysis

Searches for associations among karyotype, biological and ecological features were performed using several scientific web portals. Diploid numbers ($2n < 46$; $2n = 46$, $2n > 46$) and chromosome arm numbers (NF < 46; NF = 46, NF > 46) comprising 139 species, 54 genera and five families of Gobiiformes were associated with their biological and ecological parameters, including their habitat types (benthic, pelagic or benthic-pelagic; freshwater, estuarine, or marine environments). For the chromosome arm number (Nombre fundamental, NF) determination, the m/sm chromosomes were considered bi-armed whereas the st/a chromosomes were considered to have a single arm. The karyotypes of the homogametic sex were considered as the standard for the species when sex chromosome systems were present.

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Author contributions SASS: conceptualization, methodology, writing- original draft preparation, data curation. WFM: conceptualization, methodology, writing- original draft preparation, funding acquisition, project administration, writing—reviewing and editing. GWWFC: investigation, validation. PAL-F, CCM-N: supervision, visualization. MBC, LACB: writing—reviewing and editing.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Animal and human rights statement The experimental work fulfilled all ethical guidelines regarding the handling of specimens. The experiments followed ethical and anesthesia conducts in accordance with the Ethics Committee on the Use of Animals (#044/2015) at the Federal University of Rio Grande do Norte (UFRN). The procedures used in this study adhere to the tenets of the Declaration of Helsinki.

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