American Genetic Association

Journal of Heredity, 2017, 239–253
doi:10.1093/jhered/esx003
Original Article
Advance Access publication February 9, 2017

OXFORD

Original Article

Chromosomal Evolution and Cytotaxonomy in Wrasses (Perciformes; Labridae)

Leandro A. H. Almeida, Lorena A. Nunes, Jamille A. Bitencourt, Wagner F. Molina, and Paulo R. A. M. Affonso

From the Department of Biological Sciences, State University of Southwestern Bahia, Jequié, BA, Brazil (Almeida, Nunes, Bitencourt, and Affonso); and Department of Cell Biology and Genetics, Universidade Federal do Rio Grande do Norte, Natal, RN, Brazil (Molina).

Address correspondence to Paulo R. A. M. Affonso at the address above, or e-mail: paulomelloaffonso@yahoo.com.br.

Received October 8, 2016; First decision November 7, 2016; Accepted January 21, 2017.

Corresponding Editor: Horacio Schneider

Abstract

The wrasses (family Labridae) represent a suitable model to understand chromosomal evolution and to test the efficacy of cytotaxonomy since they display a remarkable karyotypic variation, rarely reported in marine Perciformes, as well as a high number of species and complex systematics. Therefore, we provided new chromosomal data in 5 labrids from South Atlantic (*Doratonotus megalepis*, *Halichoeres dimidiatus*, *Halichoeres penrosei*, *Thalassoma noronhanum*, and *Xyrichtys novacula*) and carried out a detailed comparative analysis of karyotypic data in Labridae using multivariate approaches. Basal diploid values ($2n = 48$) were observed in most of species studied in the present work but *D. megalepis* ($2n = 46$), along with distinct karyotype formulae. Single 18S rDNA sites interspersed with GC-rich heterochromatin were also commonly reported except for both *Halichoeres* species (2 18S rDNA-bearing pairs), following a species-specific pattern. These data show the high rates of chromosomal evolution in wrasses, ranging from microstructural rearrangements to centric fusions. A revision of chromosomal data in Labridae based on multivariate analysis of 74 taxa allowed inferring karyoevolutionary trends within tribes and genera of wrasses. The dendrogram obtained was in agreement with recent systematic hypotheses. In spite of the independent occurrence of some chromosomal rearrangements, karyoevolutionary trends could be identified within tribes of Labridae. Moreover, the karyotypic features are also suitable as cytotaxonomic markers of wrasses.

Subject area: Genomics and gene mapping
Keywords: cytogenetic markers, reef fish, multivariate analysis

The wrasses (Labridae) represent the third largest family in the order Perciformes (Nelson 2006), encompassing 82 genera and more than 600 reef-associated species (Parenti and Randall 2011). The species richness in labrids was increased after the inclusion of 2 formerly distinct families (Scaridae and Odacidae) as tribes within Labridae based on molecular phylogenetic studies (Westneat and Alfaro 2005). This monophyletic group is characterized by a remarkable adaptive radiation in tropical and subtropical oceans (Parenti and Randall 2000) mostly driven by diversification and multiple origins of trophic novelties (Cowman et al. 2009).

The systematic uncertainties of Labridae misled the idea that the reef ichthyofauna along the Western South Atlantic was a depauperate extension of the Caribbean Province. However, over the last decades, detailed taxonomic studies often supported by genetic

evidence have identified a significant number of endemic species from South Atlantic. For instance, *Sparisoma frondosum*, *Sparisoma amplum*, *Sparisoma axillare*, *Scarus trispinosus* (Moura et al. 2001), *Halichoeres dimidiatus*, and *Halichoeres penrosei* (Rocha 2004) have been validated in the Brazilian Province, the largest biogeographic region in Western South Atlantic. Moreover, new species of Labridae were described along the coast and oceanic islands of Brazil, such as *Halichoeres rubrovirens* (Rocha et al. 2010), *Halichoeres sazimai* (Luiz et al. 2009), *Sparisoma tuiupiranga* (Gasparini et al. 2003), *Sparisoma rocha* (Pinheiro et al. 2010), *Halichoeres bivittatus*, *Lachnolaimus maximus*, and *Xyrichtys martinicensis* (Garcia et al. 2015). These reports reveal that the diversity of Labridae in South Atlantic has been overlooked as well as the importance of detailed integrative taxonomic studies to infer the richness of reef fishes in South Atlantic.

Under a cytogenetic viewpoint, the karyotypic reports in Labridae are limited to about 12% of valid species, being concentrated in the tribes Julidini and Labrini. Nonetheless, these studies have shown an unusual interspecific chromosomal variation and accentuated karyoevolutionary rates when compared to most marine Perciformes. Apparently, pericentric inversions have played a major role in the karyotype diversification of wrasses, leading to distinctive chromosome formulae while the basal chromosomal number ($2n$ = 48) remained invariable. This trend is particularly noticeable within Julidini and Hypsigenyni, a sister group of all other labrids (Westneat and Alfaro 2005; Cowman et al. 2009). On the other hand, the chromosomes of some tribes and genera seem to have undergone centric fusions as observed in *Sparisoma* (Sena and Molina 2007b; Paim et al. 2014), some representatives of Novaculini (Ueno and Takai 2000) and Labrini (López et al. 1989).

Unfortunately, refined chromosomal analyses are even scarcer than karyotype reports in Labridae since most studies are based only on chromosomal morphology and number, and location of nucleolar organizer regions (NORs) and/or heterochromatin (Table 1). Indeed, the mapping of genes by fluorescence in situ hybridization (FISH), considered useful markers to cytotaxonomy and to infer reliably karyoevolutionary pathways, is restricted to *Coris julis* (Mandrioli et al. 2000) and some species of Hypsigenyni and Scarini of South Atlantic (Molina et al. 2012; Paim et al. 2014).

Because of the scarcity of cytogenetic reports and the lack of consensus between morphological and molecular systematics of Labridae, we provided new chromosomal data in four genera of wrasses from Western South Atlantic and carried out a detailed revision of cytogenetic reports in this family. Using a multivariate approach, we categorized and evaluated the distribution of chromosomal rearrangements in Labridae to infer the karyotypic evolutionary trends among tribes and genera to test their reliability to phylogenetic reconstruction.

Materials and Methods

Sampling

Four genera and 5 species of Labridae were used in cytogenetic studies, as follows: *H. dimidiatus* (n = 2), *H. penrosei* (n = 15), *Thalassoma noronhanum* (n = 6) belonging to the tribe Julidini (Figure 2A a, b, and c), *Xyrichtys novacula* (n=8) and *Doratonotus megalepis* (n = 11), both from the tribe Novaculini (Figure 2A d and e). The samples were collected along Todos os Santos Bay in the coast of Bahia, northeastern Brazil (13°38′S, 38°31′W) (Figure 1) as licensed by ICMBio/SISBIO (#19135-1, 131360–1 and 27027-2). All specimens were identified by Dr. Flávia Borges Santos and stored in the fish collection at Universidade Estadual do Sudoeste da Bahia.

Cytogenetic Analysis

After mitotic stimulation for 24–48 h (Molina et al. 2010) and euthanasis in iced water (Blessing et al. 2010), the mitotic chromosomes were obtained from cells of the anterior kidney according to Netto et al. (2007). The slides with the chromosomal preparations were stained in 10% Giemsa solution and the best metaphases were photographed using an epifluorescence photomicroscope (Olympus BX-51) equipped with digital system of image capture (Image-Pro® Plus v. 6.2, Media Cybernetics).

The chromosomal pairs were arranged by decreasing size order in karyotypes and classified as metacentric (m), submetacentric (sm), subtelocentric (st), and acrocentric (a) as commonly used in fish cytogenetics (Molina et al. 2012, 2013 among others). The number of chromosomal arms (fundamental number; FN) was calculated taking into account that m/sm chromosomes were bi-armed while st/a chromosomes were one-armed.

The active NORs were identified by silver nitrate staining (Howell and Black 1980) and heterochromatic regions were visualized by C-banding (Sumner 1972). The GC- and AT-rich sites were detected by base-specific fluorochrome staining using chromomycin A_3 (CMA_3) and 4′-6-diamino- diamidino-2-phenylindole (DAPI), respectively (Schmid 1980).

The FISH experiments (Pinkel et al. 1986) were performed under 77% of stringency using probes of 18S rDNA obtained from the genomic DNA of *X. novacula* via polymerase chain reaction (PCR) with the primers NS1 (5′-GTAGTCATATGCTTGTCTC-3′) and NS8 (5′-TCCGCAGGTTCACCTACGGA-3′) (White et al. 1990). The probe was labeled via nick translation using biotin-16-UTP (BioNick Labeling System, Invitrogen®) and the signals were detected with fluorescein isothiocyanate-avidin conjugate (Sigma–Aldrich®). Chromosomes were counterstained using DAPI (0.2 mg/mL) in Vectashield Mounting Medium (Vector®).

Statistical Analysis

The descriptive statistics (frequencies) and the multivariate analysis were used to infer karyoevolutionary trends in Labridae based on previous reports and the present data, totaling 74 nominal species (Table 1). Using the software Past v. 2.17c (Hommer et al. 2001), we built a matrix to generate the clusters based on Jaccard's similarity index by scoring 1 or 0 to the presence or absence of rearrangements in chromosomal pairs of all studied Labridae species (Supplementary Table S1).

The rearrangements were inferred from the putative basal karyotype of marine Perciformes ($2n$ = 48; FN = 48) (Galetti et al. 2006). All species with 24 acrocentric pairs were regarded as free of macrostructural rearrangements. Bi-armed pairs were interpreted as derived from pericentric inversions in species with basal $2n$ or FN values; from centric or Robertsonian fusions when $2n$ <48; or else from in tandem fusions/deletions in species with $2n$ <48 but NF incompatible with centric fusions. The consensus grouping was evaluated by the coefficient of cophenetic correlation (Sokal and Rohlf 1962; Bertan et al. 2006).

Afterwards, the inferred macrostructural rearrangements related to the karyotypic changes in each species were organized in 8 categories to the multivariate analysis and construction of another dendrogram. The categorization was based on the diploid ($2n$) and fundamental (FN) numbers, the karyotype formulae and

Table 1. Cytogenetic data of different tribes in Labridae, modified from Arai (2011)

Species	2*n*	NF	Formula	Ag-NORs	C-bands	GC-rich sites	18S rDNA	5S rDNA	Locality	References
Cheilini										
Cheilinus abudjubbe	40	54	8m + 2sm + 4st + 26a						Red Sea	Abu-Almaaty et al. (2014)
Cheilinus fasciatus	48	60	12sm + 36st/a						—	Ojima (1983)
Cheilinus digrammus	40	52	6m + 4sm + 2st + 28a						Red Sea	Abu-Almaaty et al. (2014)
Cheilinus lunulatus	40	54	10m + 4sm + 26a						Red Sea	Abu-Almaaty et al. (2014)
Cheilinus mentalis	44	60	6m + 6sm + 4st + 28a						Red Sea	Abu-Almaaty et al. (2014)
Cheilinus trilobatus	38	54	10m + 6sm + 22a	pair 7 (sm-p)					Thailand	Kaewsri et al. (2014)
Epibulus insidiator	48	60	4m + 8sm + 36st/a						Japan	Ojima and Kashiwagi (1980)
Oxycheilinus bimaculatus[a]	32	38	4m + 2sm + 26st/a						—	Ojima and Kashiwagi (1980)
Hypsigenyni										
Bodianus axillaris	48	86	8m + 30sm + 10st/a						Japan	Ojima and Kashiwagi (1980)
Bodianus insularis	48	78	4m + 12sm + 14st + 18a	pair 9 (st-t-p)	c-pr	pair 9 (st-t-p)	pair 9 (st-t-p)	pairs 16 (a-t-q), 19 (a-t-p)	Brazil	Molina et al. (2012)
Bodianus loxozonus	48	82	8m + 26sm + 14st/a						Japan	Ojima and Kashiwagi (1980)
Bodianus mesothorax	48	74	8m + 18sm + 22st/a						Japan	Ojima and Kashiwagi, (1980)
Bodianus pulchellus	48	78	4m + 12sm + 14st + 18a	pair 9 (st-t-p)	c-pr	pair 9 (st-t-p)	pair 9 (st-t-p)	pair 16 (a-q-t)	Brazil	Molina et al. (2012)
Bodianus rufus	48	80	6m + 12sm + 14st + 16a	pair 10 (st-t-p)	c-pr	pair 10 (st-t-p)	pair 10 (st-t-p)	pair 17 (a-q-t)	Brazil	Molina et al. (2012)
Choerodon azurio	48	56	6m + 2sm + 40st/a						Japan	Arai and Koike (1980)
Julidini										
Coris aygula	48	60	6m + 6sm + 36st/a						Japan	Ojima (1983)
Coris dorsomacula	48	62	6m + 8sm + 34st/a						Japan	Ojima and Kashiwagi (1979)
Coris gaimardi	48	60	2m + 10sm + 36st/a						Japan	Ojima and Kashiwagi (1980)
Coris julis	48	58	10m/sm/st + 38a	3 a (p-t)+1 m (i) pairs	c		3 a (p-t)+1 m (i) pairs	1 a (q-t)+1 (p-t) sm pairs	Italy	Mandrioli et al (2000)
Gomphosus varius	48	48	48st/a						Japan	Ojima and Kashiwagi, (1980)
Halichoeres brasiliensis	48	48	48a	1 (a-pr) pair	c-pr				Brazil	Sena and Molina (2007a)
Halichoeres dimidiatus	48	48	48a	pairs 14, 24 (a-p)	c-pr	pairs 14, 24 (a-p)	pairs 14, 24 (a-p)		Brazil	Present study
Halichoeres melanochir	48	50	2m + 46st/a						Japan	Ojima and Kashiwagi (1980)
Halichoeres penrosei	48	48	48a	pairs 5, 15 (a-p)	c-pr	pairs 5, 15 (a-p)	pairs 5, 15 (a-p)		Brazil	Present study
Halichoeres poecilopterus	48	54	4m+2sm+42st/a						Japan	Ojima and Kashiwagi (1980)
Halichoeres poeyi	48	52	4m + 44st/a	1 (a-pr) pair	c-pr				Brazil (RJ)	Sena and Molina (2007a)
Halichoeres poeyi	48	52	4m+44st/a	2 (a-pr) pairs	c-pr				Brazil (RN)	Sena and Molina (2007a)
Halichoeres prosopeion	48	50	2m + 46st/a						Japan	Ojima and Kashiwagi (1980)
Halichoeres radiatus	48	48	48a	1 (a-pr) pair	c-pr				Brazil	Sena and Molina (2007a)
Halichoeres tenuispinnis	48	50	2sm + 46st/a						Japan	Ojima and Kashiwagi (1979)
Halichoeres tenuispinnis	48	50	2sm + 46st/a						Japan	Arai and Koike (1980)
Halichoeres trimaculatus	48	48	48st/a						Japan	Ojima and Kashiwagi, (1980)
Hemigymnus fasciatus	48	60	6m + 6sm + 36st/a						Japan	Ojima (1983)
Hologymnosus annulatus	48	52	2m + 2sm + 44st/a						Japan	Ojima and Kashiwagi (1980)
Stethojulis bandanensis	48	52	4m + 44st/a						Japan	Ojima and Kashiwagi (1980)

Table 1. *Continued*

Species	2*n*	NF	Formula	Ag-NORs	C-bands	GC-rich sites	18S rDNA	5S rDNA	Locality	References
Stethojulis interrupta	48	50	2sm + 46st/a						Japan	Ojima and Kashiwagi (1980)
Stethojulis strigiventer	48	50	2m + 46st/a						Japan	Ojima and Kashiwagi (1980)
Thalassoma amblycephalum	48	48	48st/a						Japan	Ojima and Kashiwagi (1980)
Thalassoma cupido	48	48	48st/a						Japan	Ojima and Kashiwagi (1980)
Thalassoma lunare	48	48	48a	pairs 4, 12, 18 (a-p-t)	c	pairs 4, 12, 18 (a-p-t)			India	Kushwaha et al. (2011)
Thalassoma lutescens	48	48	48st/a						Japan	Ojima and Kashiwagi (1980)
Thalassoma noronhanum	48	50	2sm + 46st/a	pair 1 (sm-p-s)	c-pr-s	pair 1 (sm-p-s)	pair 1 (sm-p-s)		Brazil	Present study
Thalassoma pavo	48	48	48a						Italy	Cano et al. (1982)
Thalassoma quinquevittatum	48	48	48st/a						Japan	Ojima and Kashiwagi (1980)
Labrichthyini										
Labroides dimidiatus	48	48	48st/a						Japan	Ojima and Kashiwagi (1979)
Labrini										
Ctenolabrus rupestres	48	74	4m + 22sm + 10st + 12a						Spain	Alvarez et al. (1986)
Labrus merula	48	48	48a						Italy	Vitturi et al. (1986)
Labrus mixtus[a]	48	48	48a						Italy	Vitturi et al. (1986)
Labrus viridis	48	48	48a						Italy	Vitturi et al. (1986)
Symphodus cinereus[a]	48	76	2m + 26sm + 20st/a							Klinkhardt et al. (1995)
Symphodus mediterraneus	46	52	6m/sm + 40st/a						Spain	Cano et al. (1982)
Symphodus mediterraneus	48	90	22m + 20sm + 6a						Italy	Vitturi et al. (1986)
Symphodus melops	46	92	2m + 42sm + 2a						Spain	López et al. (1989)
Symphodus melops	46	56	10m + 36st						Italy	Cataudella et al.(1973)
Symphodus melops[a]	46	56	10m + 36st						—	Cataudella et al. (1973)
Symphodus ocellatus[a]	48	84	36m/sm/st + 12a						—	Klinkhardt et al. (1995)
Symphodus roissali	38	76	10m + 28m/sm/st						—	López et al. (1989)
Symphodus roissali	38	74	32m + 4sm + 2a						Italy	Vitturi et al. (1986)
Symphodus roissali	38	74	36m/sm + 2a						Spain	López et al. (1989)
Symphodus roissali[a]	38	74	14m + 22sm + 2st						—	Klinkhardt et al. (1995)
Symphodus scina	48	86	2m + 36sm + 10st/a						—	Klinkhardt et al. (1995)
Symphodus tinca[a]	48	82	34m/sm/st + 14a						—	Klinkhardt et al. (1995)
Symphodus doderleini	48	78	24m + 6sm + 10st + 8a						Italy	Catalano et al. (1988)
Symphodus rostratus	48	88	40m/sm/st + 8a						Black Sea	Vasiliev and Polykarpova (1980)
Novaculini										
Cheilio inermis	48	72	12m + 12sm + 24st/a						Japan	Ojima and Kashiwagi (1979)
Cheilio inermis	48	66	8m + 2sm + 8st + 30a						Red Sea	Abu-Almaaty et al. (2014)
Doratonotus megalepis	46	56	8m + 2sm + 36st/a	pair 14 (a-q-i)	c-pr	pair 14 (a-q-p)	pair 14 (a-q-p)		Brazil	Present study
Iniistius dea	44	44	44a						Japan	Ueno and Takai (2000)
Iniistius dea[a]	44	44	44st/a						—	Ojima and Kashiwagi (1980)
Iniistius pavo[a]	44	44	44a						Japan	Ueno and Takai (2000)
Iniistius twistii	22	40	18m/sm + 4a						Japan	Ueno and Takai (2000)

Table 1. *Continued*

Species	2*n*	NF	Formula	Ag-NORs	C-bands	GC-rich sites	18S rDNA	5S rDNA	Locality	References
Novaculichthys taeniurus[a]	48	52	4sm + 44st/a						—	Ojima (1983)
Xyrichtys novacula	48	56	8sm + 40a	pair 23 (a-q-i)	c-pr				Italy	Vitturi et al. (1989)
Xyrichtys novacula	48	56	8sm + 40st/a	pair 21 (a-q-i)	c-pr	pair 21 (a-q-i)	pair 21 (a-q-i)		Brazil	Present study
Pseudocheilini										
Cirrhilabrus cyanopleura	34	46	10m + 2sm + 22st/a						Japan	Ojima and Kashiwagi (1979)
Cirrhilabrus temminckii	34	46	10m + 2sm + 22st/a						Japan	Ojima and Kashiwagi (1980)
Pteragogus aurigarius	44	56	2m + 10sm + 32st/a						Japan	Arai and Koike (1980)
Pseudolabrini										
Pseudolabrus eothinus	48	52	2m + 2sm + 44st/a						Japan	Ojima and Kashiwagi (1979)
Pseudolabrus sieboldi	42	70	20m + 8sm + 14st/a						Japan	Arai and Koike (1980)
Pseudolabrus sieboldi	42	70	4m + 24sm + 14st/a						Korea	Park et al. (1995)
Scarini										
Calotomus japonicus	48	66	8m + 10sm + 30st/a						Japan	Arai and Koike (1980)
Chlorurus sordidus	48	66	18m + 8sm + 30st/a						Japan	Arai and Koike (1980)
Scarus quoyi	48	56	4m + 4sm + 40st/a	pair 6 (a-p)					Thailand	Kaewsri et al. (2014)
Scarus trispinosus	48	88	6m + 10sm + 24st + 8a	pair 9 (st-p)	c-pr				Brazil	Sena and Molina (2007b)
Sparisoma axillare	46	70	6m + 14sm + 4st/22a	pair 11 (st-p)	c-pr				Brazil	Sena and Molina (2007b)
Sparisoma radians	46	84	24m/sm + 22st/a	pair 14 (st-p)	c-pr-t	pair 14 (st-p)	pair 14 (st-p)	pair 23 (a-i)	Brazil	Paim et al. (2014)

2*n*, diploid number; FN, fundamental number; m, metacentric; sm, submetacentric; st, subtelocentric; a, acrocentric; p, short arm; q, long arm; c, centromeric; pr, pericentromeric; i, interstitial; t, terminal; s, subterminal.
[a]Scientific names modified according to current classification (Russell and Choat 2010; Pollard 2014).

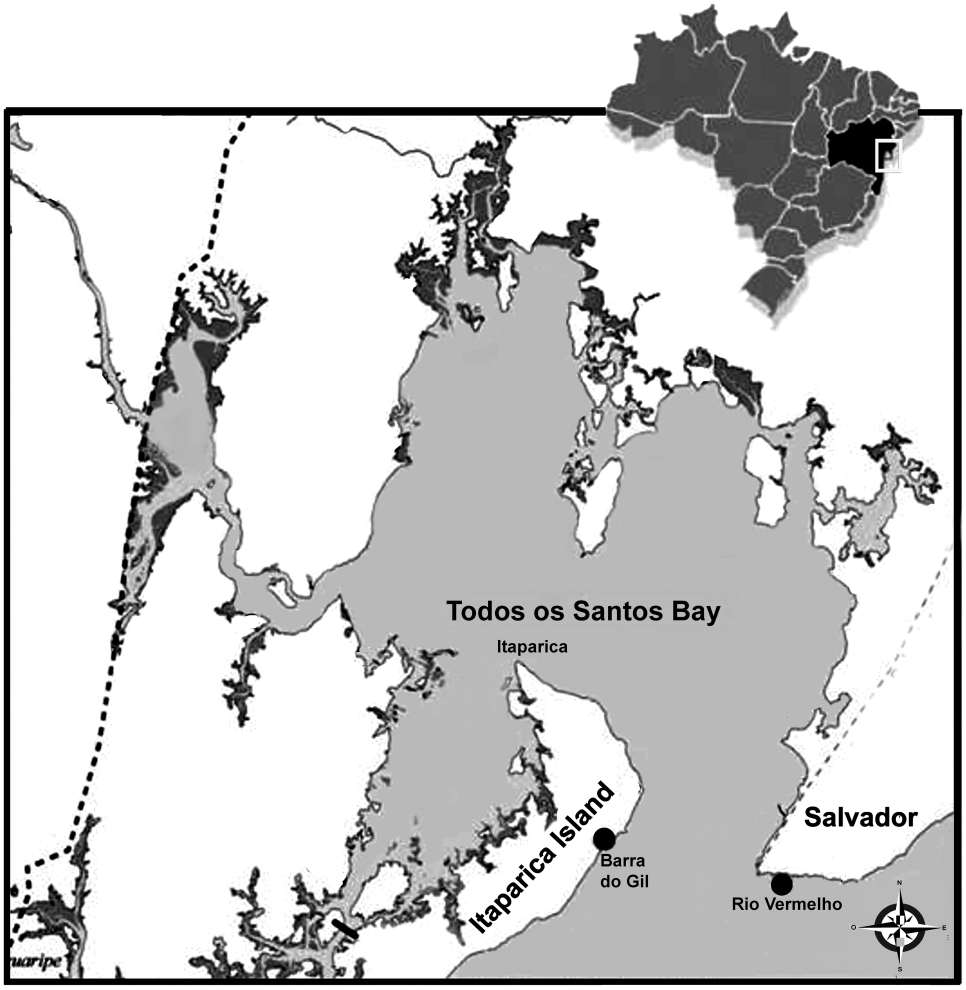

Figure 1. Map indicating the collection sites of Labridae in Todos os Santos Bay, coast of Bahia, northeastern Brazil.

the hypotheses of chromosomal evolution in marine Perciformes (Galetti et al. 2006; Molina 2007). Therefore, the Labridae species were divided into the following groups: 1) Lack of apparent macrostructural rearrangements: taxa with $2n = 48$ and FN = 48, following the plesiomorphic trait in Perciformes; 2) Pericentric inversions in less than 10 chromosomes: inversions are the main chromosomal rearrangements in Perciformes, including Labridae, inasmuch as species with relatively low number of biarmed chromosomes ($2n = 48$; $48 < NF < 58$) would bear slightly derived karyotypes; 3) Pericentric inversions in 10–20 chromosomes: taxa with increased number of pericentric inversions, leading to high FN values (60–68) in spite of the conservation of $2n = 48$; 4) Pericentric inversions in more than 20 chromosomes: taxa with remarkable high numbers of pericentric inversions ($2n = 48$, $NF > 68$), being quite distinctive from the pattern commonly found in other marine Perciformes; 5) Centric fusion in up to 8 chromosomal pairs. This group comprises taxa with $2n < 48$ and karyotypes bearing up to 16 large metacentric chromosomes; 6) Centric fusions in more than 8 chromosomal pairs: similarly to the category 5, this group includes species with $2n < 48$, but a high number of large bi-armed chromosomes (>16) derived from Robertsonian fusions; 7) In tandem fusions or deletions: considered a particularly unusual event in Perciformes, this group of species presents $2n < 48$, but lack large metacentric chromosomes, suggesting the occurrence of in tandem fusions or large deletions without diversification of chromosomal types (st-a); 8) Centric fusions and in tandem fusions/deletions: this category includes species with $2n < 48$, but the number of large metacentric chromosomes is not compatible with the reduction of diploid values, indicating the simultaneous occurrence of in tandem fusions or deletions.

It should be pointed out that the species in the categories 5–8 should also present pericentric inversions in some pairs, thus determining FN >48. However, because of the high frequency of pericentric inversions in wrasses, these categories were primarily discriminated by the occurrence of more peculiar rearrangements. Obviously, this categorization is somewhat arbitrary, but the goal here is to provide an overview of how the species of Labridae are related based on the main rearrangements observed in their karyotypes.

After categorizing each species according to the abovementioned pattern (Table 1), the grouping analysis was performed using Euclidean distance and neighbor-joining (NJ) as the hierarchical method in the software Past v. 2.17c (Hommer et al. 2001). The bootstrap values in the dendrogram were obtained after 10.000 replicates.

Results

The first chromosomal data were obtained for *H. dimidiatus*, *H. penrosei*, *T. noronhanum*, and *Doratonotus megalepis* while this is the first karyotypic reports for populations of *Xyrichtys novacula* from South Atlantic (Figure 2).

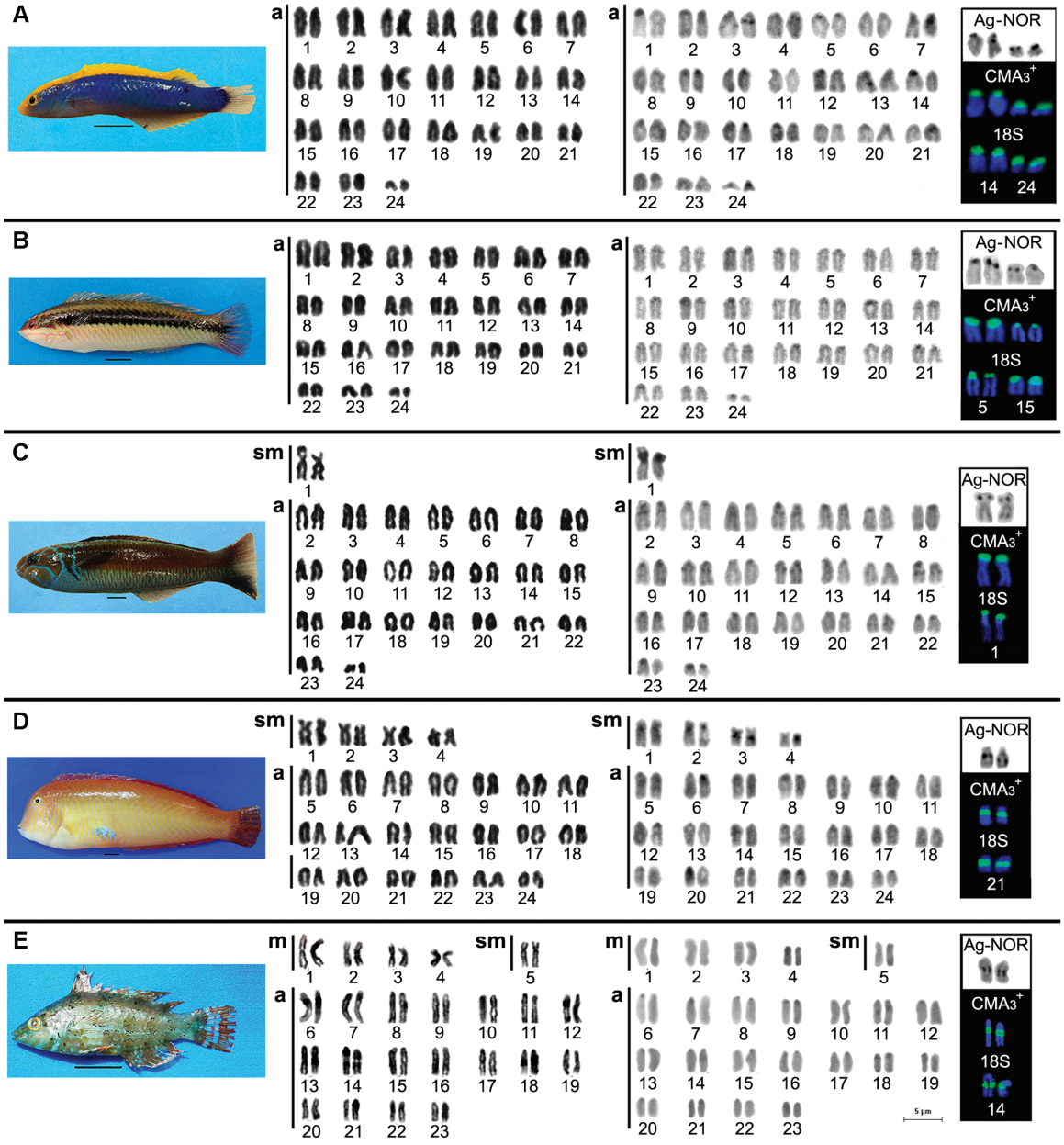

Figure 2. Specimens of *Halichoeres dimidiatus* (**A**), *Halichoeres penrosei* (**B**), *Thalassoma noronhanum* (**C**), *Xyrichtys novacula* (**D**), and *Doratonotus megalepis* (**E**) and their respective karyotypes after Giemsa staining (center) and C-banding (right). The NOR-bearing pairs after silver nitrate staining (Ag-NOR), CMA_3 staining (CMA_3^+), and FISH with 18S rDNA probe for each species are shown inbox (see online color version).

Both species of *Halichoeres* (*H. dimidiatus* and *H. penrosei*)—tribo Julidini—shared $2n = 48$, with apparent homogeneous karyotypes, entirely composed of acrocentric chromosomes (FN = 48) (Figure 2A, B). Similarly to the other Julidini analysed in the present study, *T. noronhanum* also presents $2n = 48$. However, the karyotype of this species includes both submetacentric and acrocentric chromosomes, with a karyotype formula of 2sm + 46a. In addition, a distinctive secondary constriction was observed on short arms of one chromosome in the sm pair, determining a size heteromorphism between homologous (Figure 2C).

Chromosomal variation was also identified among the 2 representatives of the tribe Novaculini. The karyotype of *X. novacula* from the Brazilian coast is composed of 48 chromosomes divided into 8sm + 40a (FN = 56) (Figure 2D). On the other hand, *D. megalepis* presented $2n = 46$ with a karyotype formed by 8m + 2sm + 36a (FN = 56). In this species, interstitial secondary constrictions were observed on the long arms of pair 14 (Figure 2E).

In general, heterochromatic blocks were restricted to pericentromeric and centromeric regions of all species (Figure 2). Nonetheless, the representatives of Julidini also presented interstitial C-bands in some chromosomal pairs (5 in *H. dimidiatus*; 1, 2, 7, 14, 15, and 17 in *H. penrosei*; 2, 5, 6, 10, 12, 15, and 21 in *T. noronhanum*) (Figure 2A–C, respectively). Moreover, a large heterochromatic block was observed close to secondary constriction in pair 1 of *T. noronhanum* (Figure 2C).

The active NORs were distributed onto a single or 2 chromosomal pairs (Figure 2, inbox). Besides the numerical variation, the location of NORs also varied among species. In *T. noronhanum*, single NORs

were visualized at subterminal region of pair 1 (sm) (Figure 2C, inbox). *Xyrichtys novacula* and *D. megalepis* also presented single NORs, but located at intersitital position in the acrocentric pairs 14 and 21, respectively (Figure 2D, E, inbox). In both *T. noronhanum* and *D. megalepis*, the NORs were coincident with the secondary constrictions observed in Giemsa staining (Figure 2C, E). On the other hand, multiple NORs close to centromeres were observed in 4 acrocentric chromosomes of *Halichoeres* (pairs 14 and 24 in *H. dimidiatus* and pairs 5 and 15 in *H. penrosei*) (Figure 2A, B, inbox). Invariably, the GC-rich sites (CMA_3^+/$DAPI^-$) and 18S rDNA as revealed by FISH were equivalent to the NORs observed by silver nitrate staining (Figure 2, inbox).

Another common feature observed in studied species, particularly in *Halichoeres*, was the non-random arrangement of chromosomes in metaphase plates. Therefore, acrocentric chromosomes were usually observed in association by the repetitive sequences and eventually NOR regions located at the satellites close to centromeres, assuming a radial configuration (Supplementary Figure S1).

To provide a comparative analysis, the chromosomal data in the five labrids from South Atlantic were organized in ideograms (Figure 3), revealing species-specific karyotypic patterns.

The consensus dendrogram based on Jaccard's similarity (Figure 4), resulted in clusters supported by a cophenetic correlation coefficient of 94%. These clusters are detailed as follows:

1. Several species and genera of Julidini (*Halichoeres, Thalassoma*, *Gomphosus*), *Labroides* (Labrichthyini), and 3 representatives of *Labrus* (Labrini) lacking macrostructure rearrangements;

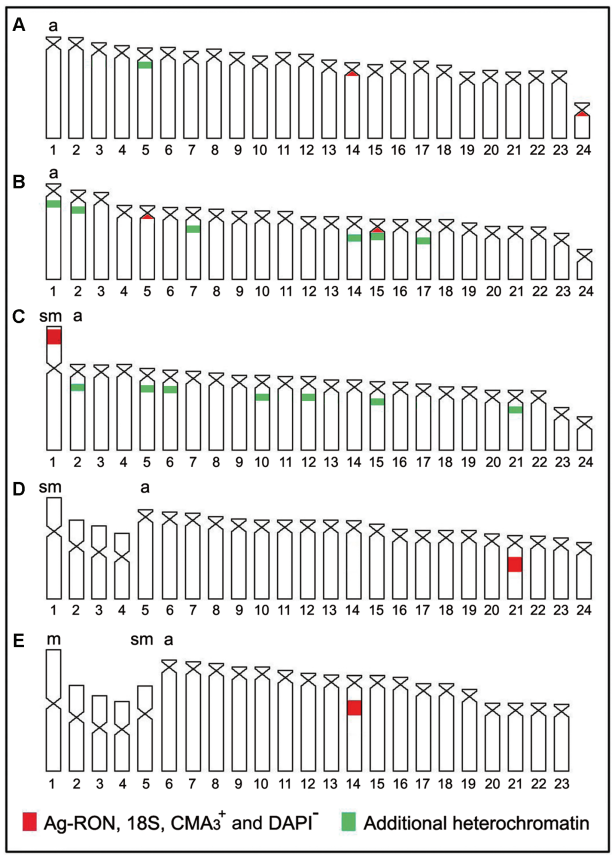

Figure 3. Ideogram representing the karyotypes and specific chromosomal regions of *Halichoeres dimidiatus* (**A**), *Halichoeres penrosei* (**B**), *Thalassoma noronhanum* (**C**), *Xyrichtys novacula* (**D**), and *Doratonotus megalepis* (**E**) (see online color version).

2. Species of the genus *Iniistius* (Novaculini), *Oxycheilinus bimaculatus* (Cheilini), 5 species of *Cheilinus* and 2 representatives of *Cirrhilabrus* characterized by derived karyotypes with a high number of centric fusions and/or in tandem fusions/deletions, being remarkably distinctive from the other labrids;
3. A cluster composed of *Symphodus melops*, *D. megalepis*, and *Pteragogus aurigarius* since they share a low number of centric fusions (≤8 chromosomes);
4. Several species sharing pericentric inversions, encompassing genera from distinct tribes, with particular emphasis for *Symphodus*, *Bodianus*, *Scarus*, and *Coris*; and
5. A group of some species with different diploid numbers, encompassing distinct mechanisms of karyotype changes (e.g., pericentric inversions or centric fusions with few inversions), such as *Calotomus japonicus* and *Sparisoma* species.

The categorization of macrostructure rearrangements based on the present data and previous reports (see Supplementary Table S1 and Figure 4) resulted in a consensus NJ tree with high bootstrap values (=100) for each cluster (Figure 5). Therefore, it was possible to define some groups based on karyotypic trends, as described below:

1. A basal and large cluster (n = 16), comprising several species and genera of Julidini (*Halichoeres, Thalassoma*, *Gomphosus*) as well as *Labroides* (Labrichthyini) and 3 species of *Labrus* (Labrini). These taxa share the putative basal karyotype of Labridae, with $2n$ and FN of 48;
2. A second group differentiated from the basal pattern by a few pericentric inversions in up to 10 chromosomes, which includes species of Julidini, Novaculini, one species of Pseudolabrini, and a karyomorph of *Cheilio inermis* (Novaculini), with a predominance in Julidini;
3. A cluster formed by taxa with intermediate number of pericentric inversions (10–20 chromosomes), represented by species of *Coris* and *Hemigymnus* (Julidini), and most of species of Cheilini and Scarini;
4. A large group (n = 17) formed by species with $2n$ = 48 and NF > 68, characterized by karyotypes with a high number of m and sm chromosomes derived from multiple pericentric inversions. This cluster includes all species of *Bodianus* karyotyped so far (Hypsigenyni), almost all species of *Symphodus* and 1 representative of *Ctenolabrus* (Labrini), 2 genera of Scarini (*Scarus* and *Chlorurus*) and another karyomorph of *Cheilio inermis* (Novaculini);
5. Characterized by the presence of centric fusions or Roberstonian rearrangements of some chromosomal pairs, this clusters encompasses species of *Cheilinus* (Cheilini) and *Sparisoma* (Scarini), 2 species of *Symphodus*, including a karyomorph of *Symphodus mediterraneus* (Labrini), one species of Novaculini (*D. megalepis*), Pseudocheilini (*Pteragogus aurigarius*) and Pseudolabrini (*Pseudolabrus sieboldi*). These species shared lowed diploid $2n$ values (40–46) along with the presence of large bi-armed chromosomes (probably derived from fusions) as well as other m/sm chromosomes related to pericentric inversions;
6. The sixth group shares a high number of chromosomes derived from centric fusions, resulting in particularly low diploid values ($2n$ = 34 or 38). The taxa in this group belong exclusively to *Symphodus roissali* (Labrini) and *Cirrhilabrus* (Pseudocheilini);
7. This cluster is composed of 2 *Iniistius* species (Novaculini). The presence of low diploid numbers ($2n$ = 44), but without the formation of m or sm pairs, indicates that the karyotypes of both species have evolved from in tandem fusions or deletions;

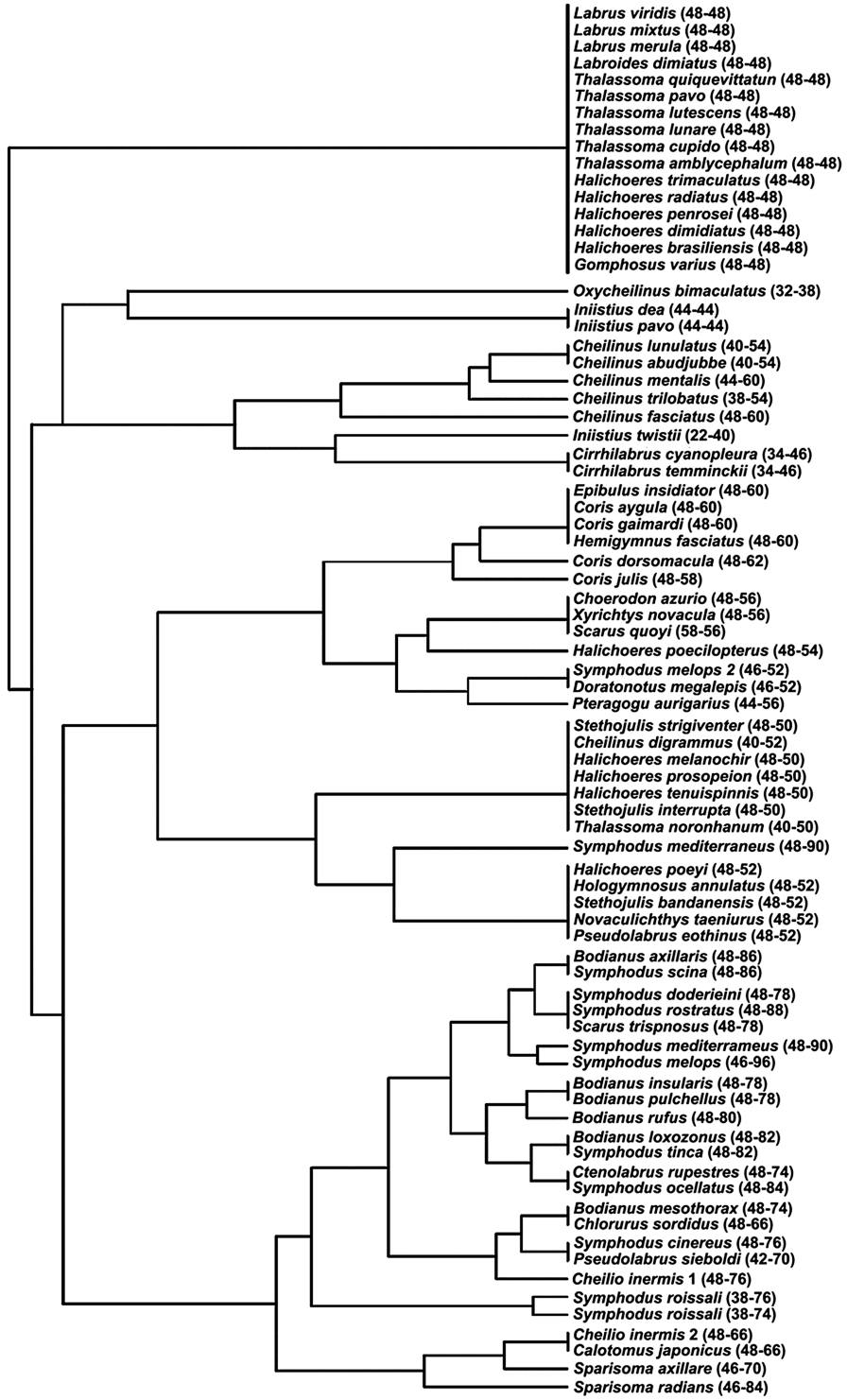

Figure 4. Dendrogram based on Jaccard's similarity of all chromosomal pairs and putative macrostructure rearrangements in 74 species of Labridae. The values between parentheses represent 2*n* and FN values, respectively.

8. The last groups includes only *Iniistius twistii* (Novaculini) and *Oxycheilinus bimaculatus* (Cheilini), since both present highly derived karyotypes (2*n* = 32 and 22, respectively). The rearrangements inferred to explain the origin of these karyotypes should involve centric fusions along with in tandem fusions/deletions.

Discussion

Macrostructure Chromosomal Changes in Labridae

The high dispersal potential, the formation of large aggregations and the weakness of biogeographical barriers have been inferred to explain chromosomal stasis in marine fish (Molina 2007;

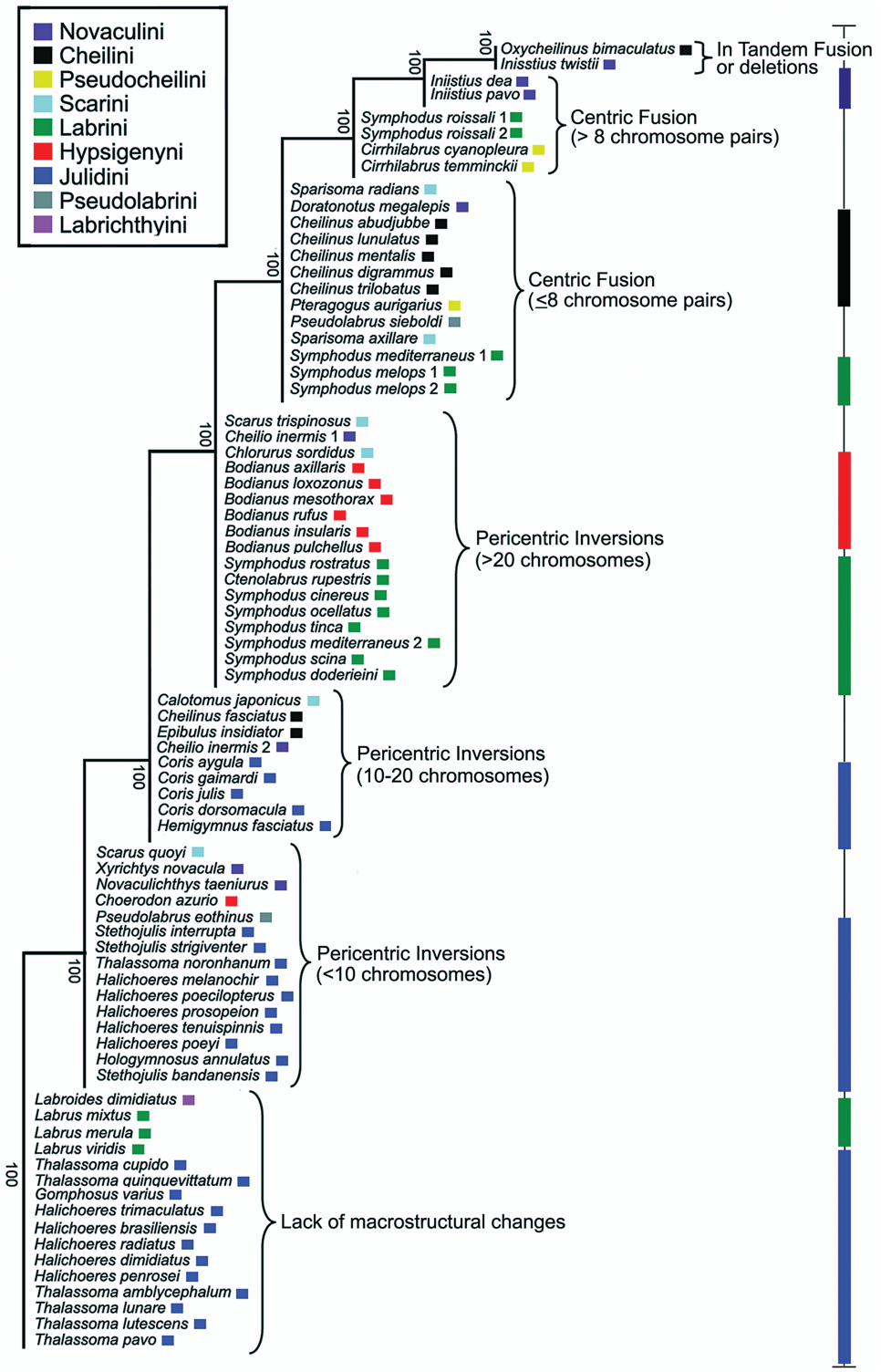

Figure 5. Neighbor-Joining (NJ) tree after categorization of chromosomal rearrangements in Labridae. The numbers indicate the bootstrap values and the circles represent the Labridae tribes proposed by Westneat and Alfaro (2005) and Cowman et al. (2009). The lateral bar indicates the more frequent rearrangements by tribe (see online color version).

Motta-Neto et al. 2011a, 2011b; Molina et al. 2013). Nonetheless, certain lineages have undergone more diversified karyoevolutionary pathways such as Acanthuridae (Affonso et al. 2014) and Pomacentridae (Molina and Galetti 2002, 2004). Moreover, some families of reef-associated fish, such as Pomacanthidae, might reveal intermediate rates of chromosome diversification, sharing conservative and highly derived karyotypes (Affonso et al. 2001; Affonso and Galetti 2005; Takai and Izutsu 2008).

In this perspective, the present data added new cytogenetic information in the large and diverse Labridae family, totaling 74 taxa (Table 1). The compilation of these data reinforces the remarkable chromosomal variation in wrasses. Even though the most labrids share a conserved diploid number of $2n = 48$, 52% of studied species present variation in chromosomal morphology, with FN > 48 (Supplementary Figure S2). This pattern indicates that pericentric inversions are the main mechanism of chromosomal evolution in Labridae, as also observed in other Labroidei (Alvarez et al. 1986; Molina et al. 2014a). Moreover, the karyotypes of some unrelated genera of wrasses, such as *Bodianus* (Molina et al. 2012) and *Coris* (Mandrioli et al. 2000) seem to have evolved essentially through inversions (Figures 4 and 5).

The present results in *T. noronhanum* ($2n = 48$, FN = 50) and *X. novacula* ($2n = 48$, FN = 56) are additional examples of karyotypic changes based on pericentric inversions. In the former, the pericentric inversion involved a single chromosomal pair, while 4 pairs have probably undergone inversions in *X. novacula*. Differently from Hypsigenyni (Molina et al. 2012), this rearrangement in Julidini is variable (present in ~50% of species), indicating independent events (Table 1, Figures 4 and 5). In fact, the presence of a sm pair in *T. noronhanum* (Figure 2C) represents the first report of asymmetric karyotype in *Thalassoma* (Table 1).

It should be pointed out that the chromosomal formula and NORs reported in *X. novacula* from the Brazilian coast (Figure 2D) is identical to that described by Vitturi et al. (1989) in populations from the Mediterranean. Such accentuated karyotypic homogeneity between geographically distant populations (about 8000 km) indicates the wide dispersal of this species as reinforced by their range and long duration of pelagic larvae (50–62 days) (Victor 1986; Lester and Ruttenberg 2005; Robertson et al. 2006). The only difference was related to the NOR-bearing pair, defined as pair 23 by Vitturi et al. (1989) and 21 in the present work. However, we believe that such divergence is rather related to the pair arrangement by distinct authors than to interpopulation chromosomal variation per se.

On the other hand, some cytogenetic reports in Labridae indicate intraspecific chromosomal polymorphism, particularly within *Symphodus* (Cano et al. 1982; Vitturi et al. 1986) and *Cheilio inermis* (Ojima and Kashiwagi 1980; Abu-Almaaty et al. 2014), as shown by the split of *C. inermis* and *S. mediterraneus* populations into distinct clusters (Figures 4 and 5). Even though limited to karyotype macrostructure and susceptible to taxonomic misidentifications, common in Labridae (Moura et al. 2001; Rocha 2004), these reports indicate the occurrence of cryptic species. Indeed, the taxonomic status of *Symphodus* species is highly controversial (Pollard 2014) while *C. inermis* has been regarded as *incertae sedis* (Westneat and Alfaro 2005). Thus, DNA barcoding is recommended to elucidate the taxonomic status of these wrasses since this approach has been successful to the molecular identification of several fish groups, including Labridae of South Atlantic (Paim et al. 2014; Brandão et al. 2016).

Numerical Chromosomal Changes in Labridae

Besides pericentric inversions, the wide variation of diploid numbers of wrasses, from $2n = 22$ in *Iniistius twistii* (Ueno and Takai 2000; Table 2) to $2n = 48$ in most species (Table 2, Supplementary Figure S2) reveals that numerical rearrangements have also played a significant role in the karyoevolution of Labridae.

Invariably, centric and in tandem fusions decrease the basal chromosomal number ($2n = 48$ in the case of Labridae). These rearrangements are particularly involved in the karyotype diversification of some tribes of Labridae, like Scarini (Sena and Molina 2007b; Paim et al. 2014), Cheilini (Ojima and Kashiwagi 1980), Labrini (López et al. 1989), and Novaculini (Ueno and Takai 2000) (Table 2, Figure 5).

Table 2. Distribution of diploid ($2n$) and fundamental (FN) numbers in Labridae

Tribes[a]	2n	FN
Cheilini	32–48	38–60
Hypsigenyni	48	74–86
Julidini	48	48–62
Labrichthyini	48	48
Labrini	38–48	52–92
Novaculini	22–48	40–72
Pseudocheilini	34	46
Pseudolabrini	42–48	52–70
Scarini	46–48	56–88

[a]According to Westneat and Alfaro (2005) and Cowman et al. (2009).

On the other hand, numerical rearrangements are less frequent phenomena in families of marine Perciformes (e.g., Affonso et al. 2014). Usually, cases of chromosomal fusions are identified in a polymorphic state, suggesting a relatively recent origin, as described in the genera *Chromis* (Pomacentridae) (Molina and Galetti 2002), *Uranoscoper* (Uranoscopidae) (Vitturi et al. 1991), and *Gobius* (Gobiidae) (Thode et al. 1985). Moreover, many marine species with derived karyotypes by numerical rearrangements present biological features that constrain dispersal and facilitate the fixation of chromosomal changes such as benthic eggs, parental care, reduced vagility, or short pelagic stages (Galetti et al. 2006).

However, similarly to the pattern observed in Acanthuridae (Affonso et al. 2014) and some Pomacentridae (Molina et al. 2014a), the labrids with reduced chromosomal numbers (Table 1) are not correlated to the abovementioned biological particularities. Alternatively, these groups seem to fit models of karyotype orthoselection and meiotic drive. According to this hypothesis, some phylogenetically related groups usually share peculiar chromosomal rearrangements (Molina 2007) because of preferential meiotic segregation of homologous in heterozygous pairs, causing fixation of specific rearrangements (Molina et al. 2014a, 2014b).

As observed in several tribes of Labridae, the present data include another case of karyotypes derived from Robertsonian translocations once *D. megalepis* presented $2n = 46$ with large metacentric chromosomes when compared to other pairs (Figure 2E). Such correspondence between wrasses with $2n < 48$ and the presence of large metacentric pairs support the hypothesis of centric fusions among formerly acrocentric chromosomes. In addition, the karyotype of *D. megalepis* reinforces the trend toward $2n$ reduction of Novaculini (Figures 4 and 5, Table 2).

Regardless of the decreased diploid number, the high FN value (56) in *D. megalepis* indicates that inversions have also taken place along with centric fusions, thus giving rise to additional metacentric and submetacentric pairs (Figure 2E). Indeed, as shown in cluster analyses (Figure 5), even species with karyotypes derived from Robertsonian rearrangements have also undergone pericentric inversions, reinforcing the importance of the latter to the chromosomal evolution in Labridae.

Surprisingly, some taxa in Labridae, like the species from the genus *Iniistius* and *Oxycheilinus bimaculatus* have followed a highly divergent karyoevolutionary pathway when compared to other marine Perciformes. The remarkable reduction of $2n$ values and FN < 48 in these representatives are not explained directly by centric

fusions, but indicates the occurrence of in tandem fusions and/or chromosomal deletions.

While in tandem fusions are well documented in mammals (Gibson 1984), these rearrangements have been rarely inferred in Perciformes and allies, being reported to some freshwater groups such as tilapias (Cichlidae) (Ferreira and Martins 2008). A few cases of in tandem fusions have been reported in marine species such as goby *Gobius paganellus* (Amores et al. 1990), surgeonfish *Acanthurus chirurgus* (Affonso et al. 2014), and damselfish *Dascyllus aruanus* (Getlekha et al. 2016).

It is also plausible that centric fusions followed by pericentric inversions have occurred in this particular species group, thus determining 1) the reduction of basal diploid values and 2) the modification of bi-armed into one-armed chromosomes. Even though this pathway is less parsimonious, this 2-step event has been reliably inferred to explain the origin of karyotypes in boxfishes of the family Ostraciidae (Tetraodontiformes) (Martinez et al. 2011). Anyways, the peculiar karyotype of *Iniistius* species represents additional evidence to discriminate these representatives from the genus *Xyrichtys* (Russell and Choat 2010) whose species usually present less modified karyotypes (Table 1).

Macrostructural Stasis Versus Microstructural Changes in Labridae

In spite of the unusual high rates of chromosomal changes in the family Labridae, some wrasses are characterized by symmetric karyotypes composed of 24 acrocentric pairs, suggesting a conservative evolution, as observed in *H. dimidiatus* and *H. penrosei* (Figure 2A, B). Phylogenetic molecular analyses revealed that both species are not closely related (Rocha et al. 2010) while *H. penrosei* is closer to *Thalassoma* than other *Halichoeres* species (Barber and Bellwood 2005). Accordingly, *H. dimidiatus* and *H. penrosei* presented distinct NOR-bearing pairs (Figure 2A, B, inbox) reinforcing their interspecific difference.

Moreover, multiple pairs bearing 18S rDNA sites are rarely reported in marine Perciformes (Gornung 2013), thus characterizing notorious karyoevolutionary novelties. This is considered a derived condition in teleosteans shared by other Julidini wrasses such as *H. poeyi* (Sena and Molina 2007a) and *C. julis*, which also presented multiple chromosomes bearing 5S rDNA (Mandrioli et al. 2000).

The distribution of NORs onto multiple pairs in *Halichoeres* might be related to the location of 18S rDNA sites close to centromeres of acrocentric chromosomes. In interphase, acrocentric chromosomes are usually arranged in chromosomal territories, being attached by their centromeres (Cremer and Cremer 2010), thus favoring the transposition of ribosomal sequences to other chromosomal pairs (Affonso and Galetti 2005; Gornung 2013). In fact, the centromeric association of acrocentric chromosomes has been reported in Perciformes (Molina and Galetti 2002), including *Halichoeres* species (Supplementary Figure S1).

The trend of microstructural diversification along with stable karyotype macrostructure in Julidini reinforces the idea that the apparent chromosomal stability of this group overlooks nondetectable rearrangements by conventional analyses (Accioly and Molina 2008). Unfortunately, most cytogenetic reports in labrids include only karyotyping and analyses of NORs by silver nitrate, thus failing in detecting inactive 18S rDNA sites and microrearrangements. Therefore, it is possible that a higher number of species in this family should bear multiple NORs after mapping ribosomal genes by FISH, indicating derived karyotypes. For instance, the variation in the number of Ag-NORs among populations of *H. poeyi* from northeastern and southeastern Brazil (Sena and Molina 2007a) could be rather related to differential expression of ribosomal cistrons than to structural differences.

On the other hand, *T. noronhanum* presented a single chromosomal pair bearing 18S rDNA located at subterminal region on short arms of sm chromosomes (Figure 3C, inbox), supporting the inference of a pericentric inversion on a former acrocentric pair with interstitial NORs on long arms. Therefore, the 18S rDNA would be relocated on short arms of a bi-armed pair, as also proposed for other marine groups with asymmetric karyotypes (Affonso et al. 2002, 2014). In addition, the NOR-bearing pairs seem to represent a potential cytotaxonomic marker for Julidini, revealing that mapping of rRNA genes is useful to differentiate groups with apparent stable karyotypes in Labridae (Molina et al. 2012).

Differently from Julidini, *X. novacula* and *D. megalepis* presented interstitial NORs on a single acrocentric pair (Figure 2D, E, inbox). This is regarded as a basal pattern for some reef fish like Pomacanthidae (Affonso and Galetti 2005) and Chaetodontidae (Molina et al. 2013) that has been maintained in Novaculini, suggesting homeologies in the NOR-bearing pairs within this tribe. Likewise, taxa of *Acanthurus* (Acanthuridae) from South Atlantic share the same NOR-bearing pairs in spite of their remarkable karyotype variation from $2n = 48$ to $2n = 34$ (Affonso et al. 2014; Fernandes et al. 2015).

On contrary, the analysis of heterochromatin distribution seems to be less informative in Labridae. C-bands located at pericentromeric and centromeric as well as GC-rich heterochromatic regions interspersed with NORs are widespread in most species of marine teleosteans (Accioly and Molina 2008; Molina et al. 2013), including wrasses (Table 1). Usually, the lack of conspicuous heterochromatic blocks is followed by reduced karyotypic diversification, thus contrasting with the remarkable karyotype variation observed in Labridae and other marine fish families like Acanthuridae (Fernandes et al. 2015). However, the lack of information about which repetitive DNA classes are present in these regions restrain further inferences about their role in chromosomal rearrangements of Labridae as reported in some fish groups (Molina and Galetti 2002; Getlekha et al. 2016).

Even though most labrids and other marine teleosteans share a low content of heterochromatin restricted to centromeres (basal pattern), the Julidini wrasses (*H. dimidiatus*, *H. penrosei*, and *T. noronhanum*) presented interstitial heterochromatic segments on long arms of some chromosomal pairs (Figure 2 a, b and c). Similarly to the multiples NORs in *Halichoeres*, these interstitial C-bands could have arisen from dispersal and transposition of repetitive DNA to equilocal position on acrocentric chromosomes during nonrandom arrangements in interphase (Affonso and Galetti 2005; Molina et al. 2012). Alternatively, these heterochromatic regions could result from heterochromatinization even though this phenomenon is usually associated with the evolution of sex chromosomes (Pokorná et al. 2011), what is unlikely to occur in Labridae.

Independently on their mechanism of origin, the presence of both interstitial heterochromatin and multiple NORs reinforce the microstructural variation in karyotypes of Julidini (Mandrioli et al. 2000; Sena and Molina 2007a; present study). Therefore, the application of banding methods is a *sine que non* for cytogenetic studies of Labridae to reveal derived and species-specific chromosomal features in apparent plesiomorphic karyotypes.

Trends of Chromosomal Evolution in Labridae

In spite of the scarcity of cytogenetic data, the unusual chromosomal variation of wrasses and the reliable reports about molecular phylogenies of Labridae (e.g., Westneat and Alfaro 2005; Cowman et al. 2009) allowed inferring general trends of chromosomal evolution in this family (Figures 4 and 5). According to phylogenetic studies, Hypsigenyni is a basal and monophyletic group in Labridae, while Labrichthyini and Julidini represent the most derived tribes. Moreover, Julidini comprises the highest number of species including several paraphyletic or polyphyletic genera, such as *Halichoeres*. Odacini are closely related to Hypsigenyni while Labrini is the sister group of both Scarini and Cheilinini, forming a clade. The tribes Pseudocheilini, Novaculini, and Pseudolabrini are also monophyletic (Westneat and Alfaro 2005; Cowman et al. 2009). When these data were compared to the chromosomal rearrangements detected in Labridae (Figures 4 and 5), we observed some trends that agree with the phylogenetic inferences along with incongruent results.

Taking into account that 48 acrocentric chromosomes is a basal karyotype for Perciformes (Brum and Galetti 1997), most taxa in Julidini (*Thalassoma*, *Halichoeres*, *Gomphosus*), *Labroides* (Labrichthyini) and some species of *Labrus* (Labrini) would be characterized by plesiomorphic karyotypes. While Labrini represents one of the most ancient groups of Labridae with early diversification about 52 million years ago (mya), Julidini and Labrichthyini are recent specialized groups (about 35 and 28 mya, respectively) (Cowman et al. 2009). Hence, the karyotypic data are not supported by phylogenetic inferences, indication apparent incongruence between the rates of molecular and chromosomal evolution.

However, as commented before, the cytogenetic data of these tribes are mainly focused on diploid number and karyotype formulae, representing the traits used in multivariate analyses. Most likely, variation in karyotype microstructure after refining cytogenetic studies in Labridae, as reported in the present study, might provide a new dataset of chromosomal traits to re-evaluate the karyoevolution of wrasses, particularly within Julidini.

In contrast, the species of Hypsigenyni, considered a basal tribe in Labridae presented highly derived karyotypes with a major role of pericentric inversions (Figures 4 and 5, Table 2). The predominance of inversions was particularly noticeable within *Bodianus* (Molina et al. 2012), but not restricted to them, since the same trend was reported in other taxa of Julidini, Novaculini, Pseudolabrini, Scarini, Cheilini, and Labrini (Figure 5). These results (Supplementary Figure S2) corroborate the pericentric inversions as the main evolutionary force in the karyotypic diversification of marine Perciformes (Molina, 2007).

Other rearrangements, such as centric or Robertsonian fusions, rarely reported in marine Perciformes (Affonso et al. 2014), seem to be frequent in Labridae, indicating putative apomorphies. For instance, even though certain species of *Cheilinus* (tribo Cheilini) might bear exclusively pericentric inversions (*Cheilinus fasciatus*), other congeneric species also have karyotypes derived from fusions involving up to 8 chromosomal pairs (*Cheilinus abudjubbe*, *Cheilinus lunulatus*, *Cheilinus mentalis*, and *Cheilinus digrammus*). The same trend was observed in *Symphodus* (Labrini) (Figure 5). Similarly, centric fusions seem to have taken place in other monophyletic groups such as Novaculi and Pseudocheilini.

Highly divergent karyotypic patterns were also identified within *Symphodus* (Labrini), *Iniistius* (Novaculini), and *Oxycheilinus bimaculatus* (Cheilini). Probably, these taxa have undergone both centric fusions and other rearrangements (in tandem fusions, deletions, or subsequent pericentric inversions), suggesting a remarkable dynamics of chromosomal evolution in certain lineages of Labridae (Figure 5). In these species, the analysis of other cytogenetic markers, such as mapping of transposable elements, is highly recommended to elucidate the genomic mechanisms underlying such high rate of chromosomal change.

In general, the NJ tree (Figure 5) was concordant with the clusters based on Jaccard's similarity (Figure 4), thus showing that most chromosomal rearrangements have taken place independently in Labridae, determining a scattered pattern of tribes through the trees based on karyotypic patterns. Therefore, the chromosomal macrostructure is not reliable to infer phylogenetic relationships in Labridae.

In fact, the independent evolution of chromosomal traits in fishes has been commonly reported, as verified for the origin of sex chromosome systems (Kitano and Peichel 2012; Almeida et al. 2015) or some chromosome rearrangements (Getlekha et al. 2016). Likewise, cytogenetic studies in some families of marine fish (e.g., Carangidae) have also shown that chromosomal variation might diverge from interspecific evolutionary relationships (Jacobina et al. 2013). However, it has been suggested that fish groups can preferentially accumulate particular chromosomal trends as a result of orthoselection process (Molina et al. 2014a). In fact, some general trends of chromosomal evolution are verified in Labridae (Table 2, Figures 4 and 5), as follows: 1) the apparent lack of macrostructure variation or the presence of a few pericentric inversions in Julidini; 2) the predominance of pericentric inversions in Hypsigenyni; and 3) the reduction of diploid number mainly in Cheilini, Labrini and Novaculini.

Conclusion

The present data reveal the high rate of chromosomal evolution in Labridae when compared to their families of marine Perciformes and related groups. It is noteworthy that 74.3% of studied species presented some rearrangement in karyotype macrostructure in spite of the high frequency of $2n = 48$ (Supplementary Figure S2). Moreover, a high variation of macro and microstructure rearrangements is reported in labrids, reinforcing their chromosomal plasticity. For instance, based only on five species of Labridae, we identified cases of pericentric inversions, Roberstonian fusions, and dispersal of ribosomal sites or heterochromatin.

Therefore, the compiled data provided potential cytogenetic markers for labrids (Paim et al. 2014). In fact, even though the intragroup karyotypic changes are not entirely suitable for extensive phylogenetic inferences, they are useful to identify preferential evolutionary trends among wrasses clades and, particularly, to cytotaxonomy purposes once the morphological identification in Labridae is often controversial and complex.

Supplementary Material

Supplementary data are found at *Journal of Heredity* online.

Funding

This work was supported by Conselho Nacional de Desenvolvimento Cientifico e Tecnológico, Ministério da Ciência e Tecnologia and Instituto Nacional de Ciência e Tecnologia em Ciências do Mar (SICONV 762228/2011), and Fundação de Amparo a Pesquisa do estado da Bahia (RED0009/2013).

Acknowledgments

The authors are grateful to Brazilian environmental agency ICMBio/SISBIO for the licenses in collecting samples. All experiments and euthanasia were approved by the Committee of Animal Ethics (CEUA/UESB) from Universidade Estadual do Sudoeste da Bahia (71/2014).

References

Abu-Almaaty AH, Regal MAA, Zeinab AM, Abdel-Basset ME. 2014. Cytogenetic characterization and molecular genetic variations of five marine species of Perciform fishes. *Ind J App Res*. 4:542–550.

Accioly IV, Molina WF. 2008. Cytogenetic studies in Brazilian marine Sciaenidae and Sparidae fishes (Perciformes). *Genet Mol Res*. 7:358–370.

Affonso PRAM, Guedes W, Pauls E, Galetti PM Jr. 2001. Cytogenetic analysis of coral reef fishes from Brazil (families Pomacanthidae and Chaetodontidae). *Cytologia*. 66:379–384.

Affonso PRAM, Guedes W, Pauls E, Galetti PM Jr. 2002. Close karyotypical relationship between two species of marine angelfishes from South Atlantic: *Pomacanthus arcuatus and P. paru* (Perciformes, Pomacanthidae). *Caryologia*. 55:323–329.

Affonso PR, Galetti PM Jr. 2005. Chromosomal diversification of reef fishes from genus *Centropyge* (Perciformes, Pomacanthidae). *Genetica*. 123:227–233.

Affonso PRAM, Fernandes MA, Almeida JS, Molina WF. 2014. Sequential steps of chromosomal differentiation in Atlantic surgeonfishes: evolutionary inferences. *Sci World J*. 2014:1–7.

Almeida JS, Migues VH, Diniz D, Affonso PR. 2015. A unique sex chromosome system in the knifefish *Gymnotus bahianus* with inferences about chromosomal evolution of Gymnotidae. *J Hered*. 106:177–183.

Alvarez MC, Garcia E, Thode G. 1986. Contribution to the karyoevolutive study of the Labridae (Perciformes). The karyotypes of *Ctenolabrus rupestris* and *Symphodus ocellatus*. *Caryologia*. 39:353–357.

Amores A, Giles V, Thode G, Alvarez MC. 1990. A tandem fusion in the fish *Gobius paganellus* (Gobiidae, Perciformes), a karyotypically polymorphic species. *Genome*. 33:57–59.

Arai R, Koike A. 1980. Chromosomes of labroid fishes from Japan. *Bull Natn Sci Mus Tokyo (A)*. 6:119–135.

Arai R. 2011. *Fish karyotypes. A check list*. Tokyo: Springer. p. 340.

Barber PH, Bellwood DR. 2005. Biodiversity hotspots: evolutionary origins of biodiversity in wrasses (*Halichoeres*: Labridae) in the Indo-Pacific and new world tropics. *Mol Phylogenet Evol*. 35:235–253.

Bertan I, Carvalho FIF, Oliveira AC, Vieira EA, Hartwig I, Silva JAG, Shimidt DAM, Valerio IP, Busato CC, Ribeiro G. 2006. Comparação de métodos de agrupamento na representação da distância morfológica entre genótipos de trigo. *R Bras Agrociência*. 12:279–286.

Blessing JJ, Marshall JC, Balcombe SR. 2010. Humane killing of fishes for scientific research: a comparison of two methods. *J Fish Biol*. 76:2571–2577.

Brandão JHSG, Bitencourt JA, Santos FB, Watanabe LA, Schneider H, Sampaio I, Affonso PRAM. 2016. DNA barcoding of coastal ichthyofauna from Bahia, northeastern Brazil, South Atlantic: high efficiency for systematics and identification of cryptic diversity. *Biochem Syst Ecol*. 65:214–224.

Brum MJI, Galetti PM Jr. 1997. Teleostei ground plan karyotype. *J Comp Biol*. 2:91–102.

Cano J, Thode G, Alvarez MC. 1982. Karyoevolutive considerations in 29 Mediterranean teleost fishes. *Vie Milieu*. 32:21–24.

Catalano E, Vitturi R, Valvo ML. 1988. Osservazioni morfologiche e cariologiche su *Symphodus doderleini* Jordan, 1891 del golfo di Palermo. *Atti Soc Ital Sci Nat Museo Civ Stor Nat Milano*. 129:261–271.

Cataudella S, Civitelli MV, Capana E. 1973. The chromosomes of some Mediterranean teleosts: Scorpaenidae, Serranidae, Labridae, Blenniidae, Gobiidae (Pisces - Scorpaeniformes, Perciformes). *Boll Zool*. 40:385–389.

Cowman PF, Bellwood DR, van Herwerden L. 2009. Dating the evolutionary origins of wrasse lineages (Labridae) and the rise of trophic novelty on coral reefs. *Mol Phylogenet Evol*. 52:621–631.

Cremer T, Cremer M. 2010. Chromosome territories. *Cold Spring Harb Perspect Biol*. 2:1–22.

Fernandes MA, Affonso PRAM, Cioffi MB, Bertollo LAC, Costa GWWF, Molina WF. 2015. Atlantic surgeonfishes bear only minor microstructural changes in highly derived karyotypes. *Zool Anz*. 254:62–66.

Ferreira IA, Martins C. 2008. Physical chromosome mapping of repetitive DNA sequences in Nile tilapia *Oreochromis niloticus*: evidences for a differential distribution of repetitive elements in the sex chromosomes. *Micron*. 39:411–418.

Galetti PM Jr, Molina WF, Affonso PR, Aguilar CT. 2006. Assessing genetic diversity of Brazilian reef fishes by chromosomal and DNA markers. *Genetica*. 126:161–177.

Garcia J Jr, Nóbrega MF, Oliveira JEL. 2015. Coastal fishes of Rio Grande do Norte, northeastern Brazil, with new records. *Check List*. 11:1659.

Gasparini JL, Joyeux JC, Floeter SR. 2003. *Sparisoma tuiupiranga*, a new species of parrotfish (Perciformes: Labroidei: Sciaenidae) from Brazil, with comments on the evolution of the genus. *Zootaxa*. 384:1–14.

Getlekha N, Molina WF, de Bello Cioffi M, Yano CF, Maneechot N, Bertollo LA, Supiwong W, Tanomtong A. 2016. Repetitive DNAs highlight the role of chromosomal fusions in the karyotype evolution of *Dascyllus* species (Pomacentridae, Perciformes). *Genetica*. 144:203–211.

Gornung E. 2013. Twenty years of physical mapping of major ribosomal RNA genes across the teleosts: a review of research. *Cytogenet Genome Res*. 141:90–102.

Howell WM, Black DA. 1980. Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method. *Experientia*. 36:1014–1015.

Hommer O, Harper DAT, Ryan PD. 2001. Past: paleontological statistics software package for education and data analysis. *Pal Ass*. 4:9.

Jacobina UP, Martinez PA, Cioffi MB, Garcia J, Bertollo LAC, Molina WF. 2013. Morphological and karyotypic differentiation in *Caranx lugubris* (Perciformes: Carangidae) in the St. Peter and St. Paul Archipelago, mid-Atlantic Ridge. *Helgoland Mar Res*. 68:17–25.

Kaewsri S, Tanomtong A, Getlekha N, Saenjundaeng P, Suksuwan R, Supiwong W. 2014. Standardized karyotype and idiogram of Quoy's parrotfish, *Scarus quoyi* (Perciformes: Scaridae) by conventional staining and Ag-NOR banding techniques. *Cytologia*. 79:429–435.

Kitano J, Peichel CL. 2012. Turnover of sex chromosomes and speciation in fishes. 94:549–558.

Klinkhardt M, Tesche M, Greven H. 1995. *Database of fish chromosomes*. Westarp Wissenschaften. Available from www.fishbase.org.

Kushwaha B, Kumar R, Nagpure NS, Srivastava SK, Basheer VS, Anil MK, Lakra WS. 2011. Chromosomal studies of three vulnerable marine fishes from west coast of India. *Indian J Geo Mar Sci*. 40:62–66.

Lester SE, Ruttenberg BI. 2005. The relationship between pelagic larval duration and range size in tropical reef fishes: a synthetic analysis. *Proc Biol Sci*. 272:585–591.

López JR, Alvarez MC, Thode G, Martinez G. 1989. Karyotype divergence in *Symphodus melops* and *Symphodus roissali* (Labridae, Perciformes): C-banded and Ag-NOR karyotypes. *Genome*. 32:35–39.

Luiz OJ Jr, Ferreira CEL, Rocha LA. 2009. *Halichoeres sazimai*, a new species of wrasse (Perciformes: Labridae) from the Western South Atlantic. *Zootaxa*. 2092:37–46.

Mandrioli M, Colomba MS, Vitturi R. 2000. Chromosomal analysis of repeated DNAs in the rainbow wrasse *Coris julis* (Pisces, Labridae). *Genetica*. 108:191–195.

Martinez PA, Jacobina UP, Molina WF. 2011. Comparative cytogenetics and heterochromatic patterns in two species of the genus *Acanthostracion* (Ostraciidae: Tetraodontiformes). *Mar Genomics*. 4:215–220.

Molina WF, Galetti PM Jr. 2002. Robertsonian rearrangements in the reef fish *Chromis* (Perciformes, Pomacentridae) involving chromosomes bearing 5S rRNA genes. *Genet Mol Biol*. 25:373–377.

Molina WF, Galetti PM Jr. 2004. Multiple pericentric inversions and chromosomal divergence in the reef fishes *Stegastes* (Perciformes, Pomacentridae). *Genet Mol Biol*. 27:543–548.

Molina WF. 2007. Chromosomal changes and stasis in marine fish groups. In: Pisano E, Ozouf-Costaz C, Foresti F, Kapoor BG, editors. *Fish cytogenetics*. Enfield: Science Publishers. p. 69–110.

Molina WF, Alves DE, Araújo WC, Martinez PA, Silva MF, Costa GW. 2010. Performance of human immunostimulating agents in the improvement of fish cytogenetic preparations. *Genet Mol Res*. 9:1807–1814.

Molina WF, Motta Neto CC, Sena DC, Cioffi MB, Bertollo LA. 2012. Karyoevolutionary aspects of Atlantic hogfishes (Labridae-Bodianinae), with evidence of an atypical decondensed argentophilic heterochromatin. *Mar Genomics*. 6:25–31.

Molina WF, Costa GWWF, Soares RX, Affonso PRAM, Cioffi MB, Araújo WC, Bertollo LAC. 2013. Extensive chromosome conservatism in Atlantic butterflyfishes, genus *Chaetodon* Linnaeus, 1758: implications for the high hybridization success. *Zool Anz*. 253:137–142.

Molina WF, Martinez PA, Bertollo LA, Bidau CJ. 2014a. Evidence for meiotic drive as an explanation for karyotype changes in fishes. *Mar Genomics*. 15:29–34.

Molina WF, Martinez PA, Bertollo LA, Bidau CJ. 2014b. Preferential accumulation of sex and Bs chromosomes in biarmed karyotypes by meiotic drive and rates of chromosomal changes in fishes. *An Acad Bras Cienc*. 86:1801–1812.

Moura RL, Figueiredo JL, Sazima I, 2001. A new parrotfish (Scaridae) from Brazil, and revalidation of *Sparisoma amplum* (Ranzani, 1842), *Sparisoma frondosum* (Agassiz, 1831), *Sparisoma axillare* (Steindachner, 1878) and *Scarus trispinosus* Valenciennes, 1840. *Bull Mar Sci*. 68:505–524.

Motta-Neto CC, Cioffi MB, Bertollo LAC, Molina WF. 2011a. Extensive chromosomal homologies and evidence of karyotypic stasis in Atlantic grunts of the genus *Haemulon* (Perciformes). *J Exp Mar Biol Ecol*. 401:75–79.

Motta-Neto CC, Cioffi MB, Bertollo LAC, Molina WF. 2011b. Molecular cytogenetic analysis of Haemulidae fish (Perciformes): evidence of evolutionary conservation. *J Exp Mar Biol Ecol*. 407:97–100.

Nelson JS. 2006. *Fishes of the World*. New York: John Wiley and Sons.

Netto MR, Pauls E, de Mello Affonso PR. 2007. A standard protocol for obtaining fish chromosomes under post-mortem conditions. *Micron*. 38:214–217.

Ojima Y, Kashiwagi E. 1979. A karyotype study of eleven species of labrid fishes from Japan. *Proc Jpn Acad Ser B*. 55:280–285.

Ojima Y, Kashiwagi E. 1980. Further studies of the chromosome of the Labridae (Pisces). A preliminary note. *Proc Jpn Acad Ser B*. 56:328–331.

Ojima Y. 1983. Fish Cytogenetics. In: Sharma AK, Sharma A, editors. *Chromosomes in evolution of eukaryotic groups*. Boca Raton: CRC Press. p. 254.

Paim FG, Brandão JH, Sampaio I, de Mello Affonso PR, Diniz D. 2014. Genetic identification of bucktooth parrotfish *Sparisoma radians* (Valenciennes, 1840) (Labridae, Scarinae) by chromosomal and molecular markers. *Genet Mol Biol*. 37:646–651.

Parenti P, Randall JE. 2000. An annotated checklist of the species of the Labroid fish families Labridae and Scaridae. *Ichthyol Bull*. 68:1–97.

Parenti P, Randall JE. 2011. Checklist of the species of the families Labridae. *Smith Bull*. 13:29–44.

Park IS, Kim HB, Lee YD. 1995. Karyotypic analysis of four labrid fishes from Korea. *Korean J Ichthyol*. 7:79–83.

Pinheiro HT, Gasparini JL, Sazima I. 2010. *Sparisoma rocha*, a new species of parrotfish (Actinopterygii: Labridae) from Trindade Island, South-western Atlantic. *Zootaxa*. 2493:59–65.

Pinkel D, Straume T, Gray JW. 1986. Cytogenetic analysis using quantitative, high-sensitivity, fluorescence hybridization. *Proc Natl Acad Sci U S A*. 83:2934–2938.

Pokorná M, Kratochvíl L, Kejnovský E. 2011. Microsatellite distribution on sex chromosomes at different stages of heteromorphism and heterochromatinization in two lizard species (Squamata: Eublepharidae: *Coleonyx elegans* and Lacertidae: *Eremias velox*). *BMC Genetics*. 12:90.

Pollard D. 2014. *Symphodus rostratus*. The IUCN Red List of Threatened Species. Available from http://10.2305/IUCN.UK.2014-3.RLTS.T187573A49025124.en.

Robertson DR, Karg F, Leao de Moura R, Victor BC, Bernardi G. 2006. Mechanisms of speciation and faunal enrichment in Atlantic parrotfishes. *Mol Phylogenet Evol*. 40:795–807.

Rocha LA. 2004. Mitochondrial DNA and color pattern variation in three Western Atlantic *Halichoeres* (Labridae), with the revalidation of two species. *Copeia*. 4:770–782.

Rocha LA, Pinheiro HT, Gasparini JL. 2010. Description of *Halichoeres rubrovirens*, a new species of wrasse (Labridae: Perciformes) from the Trindade and Martin Vaz Island group, southeastern Brazil, with a preliminary mtDNA molecular phylogeny of New World *Halichoeres*. *Zootaxa*. 2422:22–30.

Russell B, Choat H. 2010. *Iniistius twistii*. The IUCN Red List of Threatened Species 2010: e.T187539A8562216. Available from http://doi:10.2305/IUCN.

Schmid M. 1980. Chromosome banding in amphibia. IV. Differentiation of GC- and AT-rich chromosome regions in Anura. *Chromosoma*. 77:83–103.

Sena DCS, Molina WF. 2007a. Chromosomal rearrangements associated with pelagic larval duration in Labridae (Perciformes). *J Exp Mar Biol Ecol*. 353:203–210.

Sena DC, Molina WF. 2007b. Robertsonian rearrangements and pericentric inversions in Scaridae fish (Perciformes). *Genet Mol Res*. 6:575–580.

Sokal RA, Rohlf FJ. 1962. The comparison of dendograms by objective methods. *Taxonomy*. 11:33–40.

Sumner AT. 1972. A simple technique for demonstrating centromeric heterochromatin. *Exp Cell Res*. 75:304–306.

Takai A, Izutsu H. 2008. Diversified chromosomal characteristics in *Centropyge* fishes (Pomacanthidae, Perciformes). *Hydrobiologia*. 603:15–23.

Thode G, Giles V, Alvarez MC. 1985. Multiple chromosome polymorphism in *Gobius paganellus* (Teleostei, Perciformes). *Heredity*. 54:3–7.

Ueno K, Takai A. 2000. Chromosome evolution involving Robertsonian rearrangements in Xyrichthys fish (Labridae, Perciformes). *Cytobios*. 103:7–15.

Vasiliev VP, Polykarpova LK. 1980. The karyotypes of the Black Sea species of the genera *Crenilabrus* and *Symphodus* (Perciformes, Labridae) and the evidence of natural hybridization between *C. ocellatus* and *C. quinquemaculatus*. *Zool Zhurnal*. 59:1334–1342.

Victor BC. 1986. Duration of the planktonic larval stages of one hundred species of Pacific and Atlantic wrasses (family Labridae). *Mar Biol*. 90:317–326.

Vitturi R, Carbone P, Catalano-Macaluso M. 1986. Karyotypes of five species of Blennioidea (Pisces, Perciformes). *Caryologia*. 39:273–279.

Vitturi R, Catalano E, LoConte MR, Spampinato P. 1989. Ag-NORs and C-banding pattern of the labrid species *Xyrichtys novacula* L Pisces, Perciformes. *Biol Zent*. 1083:263–266.

Vitturi R, Catalano E, LoConte MR, Alessi AM, Amico FP, Colombera D. 1991. Intra-populational and intra-individual mosaicisms of *Uranoscoper scaber* L. (Perciformes, Uranoscopidae). *Heredity*. 67:325–330.

Westneat MW, Alfaro ME. 2005. Phylogenetic relationships and evolutionary history of the reef fish family Labridae. *Mol Phylogenet Evol*. 36:370–390.

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, editors. *PCR protocols: a guide to methods and applications*. New York: Academic Press. p. 315–322.