Molecular cytogenetic analyses of *Epinephelus bruneus* and *Epinephelus moara* (Perciformes, Epinephelidae)

Genus *Epinephelus* (Perciformes, Epinephelidae), commonly known as groupers, are usually difficult in species identification for the lack and/or change of morphological specialization. In this study, molecular cytogenetic analyses were firstly performed to identify the closely related species *Epinephelus bruneus* and *E. moara* in this genus. The species-specific differences of both fish species showed in karyotype, chromosomal distribution of nucleolar organizer regions (NORs) and localization of 18S rDNA. The heterochromatin (interstitial C-bands) and distribution pattern of telomere $(TTAGGG)_n$ in *E. bruneus* revealed the chromosomal rearrangements and different karyotypic evolutionary characteristics compared to those in *E. moara*. The cytogenetic data suggested that the lineages of *E. bruneus* and *E. moara* were recently derived within the genus *Epinephelus*, and *E. moara* exhibited more plesiomorphic features than *E. bruneus*. All results confirmed that *E. moara*, which has long been considered a synonym of *E. bruneus*, is a distinct species in the family Epinephelidae. In addition, molecular cytogenetic analyses are useful in species differentiation and phylogenetic reconstruction in groupers.

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19   **Abstract**

20   Genus *Epinephelus* (Perciformes, Epinephelidae), commonly known as groupers, are
21   usually difficult in species identification for the lack and/or change of morphological
22   specialization. In this study, molecular cytogenetic analyses were firstly performed to identify the
23   closely related species *Epinephelus bruneus* and *E. moara* in this genus. The species-specific
24   differences of both fish species showed in karyotype, chromosomal distribution of nucleolar
25   organizer regions (NORs) and localization of 18S rDNA. The heterochromatin (interstitial C-
26   bands) and distribution pattern of telomere (TTAGGG)$_n$ in *E. bruneus* revealed the chromosomal
27   rearrangements and different karyotypic evolutionary characteristics compared to those in *E.*
28   *moara*. The cytogenetic data suggested that the lineages of *E. bruneus* and *E. moara* were
29   recently derived within the genus *Epinephelus*, and *E. moara* exhibited more plesiomorphic
30   features than *E. bruneus*. All results confirmed that *E. moara*, which has long been considered a
31   synonym of *E. bruneus*, is a distinct species in the family Epinephelidae. In addition, molecular
32   cytogenetic analyses are useful in species differentiation and phylogenetic reconstruction in
33   groupers.

34

35   **Keywords:** cytogenetics, fish, chromosomes, taxonomy, species-specific, evolution

Introduction

The family Epinephelidae comprises approximately 163 grouper species in 16 genera (Craig, Sadovy de Mitcheson & Heemstra, 2011). These species are of considerable economic value, especially in the coastal fisheries of tropical and subtropical areas (Heemstra & Randall, 1993). Taxonomic confusion in the Epinephelidae often occurs due to similarities of color patterns and ontogenetic changes in color (Heemstra & Randall, 1993; Craig, Sadovy de Mitcheson & Heemstra, 2011). *Epinephelus bruneus* (Bloch 1793) and *E. moara* (Temminck & Schiegel 1842) are two important aquaculture and commercial fish species. However, *E. moara* has long been considered a synonym of *E. bruneus* due to their similarities in coloration and overlapping in geographical distributions (Heemstra & Randall, 1993; Craig, Sadovy de Mitcheson & Heemstra, 2011). Designation of correctly identified new species is important not only to the study of phylogenetic relationships, but also to the identification of fry and parent in grouper aquaculture. Based on morphological characteristics and molecular comparisons, *E. moara* has been suggested as a valid species (Guo et al., 2008, 2009; Liu et al., 2013). The interspecific differences between them were identified mainly based on the skeleton system as well as the meristic and morphometric characteristics (Guo et al., 2008). Gene differentiation (Guo et al., 2009) and mitogenome analyses (Liu et al., 2013) partially provided molecular information confirming their taxonomic status. Chromosomes are the carriers of genetic information, and chromosomal recombination plays a vital role in genetic diversity. Therefore, more other evidences are needed to support the hypothesis that *E. moara* is a valid species, such as molecular cytogenetic analyses.

Chromosomes are hereditary elements of the complete nuclear genome. Molecular cytogenetic studies on chromosomes constitute important approaches for characterizing species and reconstructing phylogenetic relationships (Galetti, Aguilar & Molina, 2000; Ocalewicz, Woznicki & Jankun, 2008; Coiffi, Martins & Bertollo, 2010; Ruiz-Herrera, Farre & Robinson, 2012). Karyological features indicate the evolutionary distance between species of different taxonomic categories (Dobigny et al., 2004). The Nucleolar organizer regions (NORs) were particularly significant in chromosomal evolutionary analyses (Miller et al., 1976; Fujiwara et

al., 1998). Heterochromatin corresponding to C-bandings is normally associated with rearrangements, quantitative variation, and formation of new karyotypes (Miklos & Gill, 1982; Rocco et al., 2002). Many taxonomic studies were based on the variations and polymorphism of the chromosomes containing major 18S rDNA (both active and non-active) (Cioffi, Martins & Bertollo, 2010; Britton-Davidian, Cazaux & Catalan, 2012), and minor 5S rDNA (Fujiwara et al., 1998; Mazzei et al., 2004) by fluorescent in situ hybridization (FISH). Location of telomeric sequence $(TTAGGG)_n$ provides direct evidence for cytotaxonomic studies and chromosomal evolution in fishes (Sola et al., 2003; Scacchetti et al., 2011). Therefore, molecular cytogenetic information has provided important contributions to the characterization of biodiversity and the evolution of ichthyofauna (Jesus et al., 2003; Vicari et al., 2008). The analyses of different methods above present a complete karyotypic picture for organisms.

The cytogenetic information providing by a variety of approaches will make us more fully to explain the taxonomic and evolutionary status, and reveal the inherent differences of *E. bruneus* and *E. moara*. In this study, the karyotypic techniques, including Giemsa-staining, Ag-staining, C-banding and localization of 18S rDNA and telomere $(TTAGGG)_n$ by FISH, were used to investigate the molecular cytogenetic characteristics of *E. bruneus* and *E. moara*. Cytogentic data provided a better definition of the specific epithet for these cryptic species. Molecular cytogenetic analyses were found to be applicable in differentiating between closely related species and reconstructing phylogenetic relationships in groupers.

Materials and methods

Fish collection and identification

Twenty-five individuals of *E. bruneus* (standard length, L_S, 140-550 mm) and 24 individuals of *E. moara* (L_S, 188-650 mm) were collected alive from the coastal waters of Fujian, China, and reared in laboratory for one week before analyses. Specimen identification was based on external coloration (Fig. 1), skeleton and morphological characteristics established in previous studies (Guo et al., 2008).

Chromosome preparation, karyptyping and staining analyses

91   Fishes were injected with colchicine (3 μg/g weight, Sigma) for 30min. Mitotic

92   chromosomes were obtained from cell suspensions of anterior kidney after the fishes were

93   anesthetized with tricaine methanesulfonate (MS222, 100 mg/L, Sigma), using the conventional

94   air-drying method (Ojima, Hitotsumachi & Makino, 1966). Chromosomes were stained using

95   Giemsa and classified as metacentric (m), submetacentric (sm), subtelocentric (st), or acrocentric

96   (a) based on the arm ratios (Levan, Fredga & Sandberg, 1964). The nucleolar organizer regions

97   (NORs) were visualized by Ag-staining (Howell & Black, 1980). Heterochromatin was identified

98   by C-banding using barium hydroxide method (Sumner, 1972). After the acquisition of anterior

99   kidney, tissue samples (mostly dorsal muscle) were collected and stored at -80℃. All

100   experiments were conducted in accordance with the guidelines approved by the Institutional

101   Animal Care and Use Committee at the Xiamen University.

Chromosomal probes preparation

103   Genomic DNA of all specimens was extracted from muscle tissue using the phenol-

104   chloroform method (Sambrook, Fritsch & Maniatis, 1989). 18S rDNA and 5S rDNA probes for

105   chromosome hybridization were prepared as follows: The partial coding region of 18S rDNA

106   were amplified using the primers 18S rDNA-F (5'-GTAGTCATATGCTTGTCTC-3') and 18S

107   rDNA-R (5'-TCCGCAGGTTCACCTACGGA-3') as described by White et al. (1990). The

108   coding region of 5S rDNA were obtained using the primers 5S rDNA-F (5'-

109   TACGCCCGATCTCGTCCGATC-3') and 5S rDNA-R (5'- CAGGCTGGTATGGCCGTAAGC

110   -3') indicated by Martins & Galetti (1999). PCR reactions were performed as following: 94 °C

111   for 4 min, followed by 35 cycles of 94 °C for 30 sec, 54 °C (for 18S rDNA) or 62 °C (for 5S

112   rDNA) for 1 min, and 72 °C for 1 min, and a final extension at 72 °C for 5 min. The nucleotide

113   sequences of 18S rDNA and 5S rDNA were obtained after cloning into the pMD-18T vector

114   (Takara, Japan), and subjected to Blastn in NCBI database (http://www.ncbi.nlm.nih.gov).

115   Telomere probes for chromosome hybridization were prepared as follows: telomeric repeat

116   sequences (TTAGGG)$_n$ were amplified by PCR using (5'-TTAGGG-3')$_5$ and (5'-CCCTAA-3')$_5$

117   as primers (Ijdo et al., 1991). All probes were labeled with biotin-16-dUTP (Roche, Germany) by

118   nick translation according to the manufacturer's instructions.

Fluorescence in situ hybridization (FISH)

120   FISH and probe detection were conducted using methods as described previously (Wang et

121   al., 2010). Briefly, avidin-fluorescein isothiocyanate (FITC) (Sigma, USA) was used for signal

122   detection of probes 18S rDNA, 5SrDNA and telomere (TTAGGG)$_n$ based on the manufacturer's

123   instruction. Chromosomes were counterstained with 1 μg/ml 4', 6'-diamidino-2-phenylin-dole

124   (DAPI) (Roche, USA) in anti-fade solution of 70% glycerol, 2.5% DABCO [1,4-Diazabicyclo

125   (2.2.2) octan], and $1 \times$ standard saline concentration (SSC) at pH 8.0. Hybridization signals were

126   observed and analyzed under a fluorescence microscope Leica DM-400CCD.

127   **Results**

128   *Karyotypes and banding patterns*

129   A total of 172 metaphases of *E. bruneus* and 156 metaphases of *E. moara* were analyzed to

130   determine the karyotype structure. All specimens of *E. bruneus* and *E. moara* invariably showed

131   the same diploid number of chromosomes, 2n=48. The karyotype formulas of *E. bruneus* and *E.*

132   *moara* were 2m+4sm+42a, giving a fundamental number (NF) equaled to 54 (Fig. 2A and B),

133   and 4sm+44a, NF=52 (Fig. 2C and D), respectively. Chromosomes pairs were numbered based

134   on the relative length. The smallest chromosomes pairs No.24 were submetacentric

135   chromosomes (sm-3 for *E. bruneus* and sm-2 for *E. moara*, Fig. 2B and D). Chromosome pairs

136   No.9 in length were sm-2 for *E. bruneus* and sm-1 for *E. moara*. Chromosome pairs No.2 were

137   metacentric chromosomes m-1 for *E. bruneus*. Other chromosomes were acrocentric (a)

138   chromosomes for both *E. bruneus* and *E. moara*.

139   Active NORs were identified on the terminal position of short arms or sub-centromere

140   regions of those biarmed chromosomes. In *E. bruneus*, five actively transcribed NORs were

141   located on the metacentric and submetacentric chromosomes (Fig. 3A). In *E. moara*, four Ag-

142   NORs were found on the submetacentric chromosomes (Fig. 3B).

143   The constitutive heterochromatin was observed in the centromeric and/or pericentromeric

144   region of most chromosomes for both *E. bruneus* and *E. moara*. And the biarmed chromosome

145   pairs with positive Ag-NORs were coinciding with the positive heterochromatin C-bandings.

146   While three pairs of acrocentric chromosomes were almost indiscernible in both fish species

147   (Fig. 4A and B). However, the significant differences of heterochromatin were the

148   heterochromatic blocks found in the interstitial region of the long arms of one pair of medium-

149   sized acrocentric chromosome in *E. bruneus* (Fig. 4A).

150   ***Sequences analyses***

151   Sequences of 18S rDNA (GenBank accession nos. **FJ176793** and **FJ176794**) and 5S rDNA

152   (GenBank accession nos. **FJ176796** and **FJ176795**) were amplified from genomic DNA of *E.*

153   *bruneus* and *E. moara*. *Sequence* of 18S rDNA contained partial DNA of gene 18S rRNA. Partial

154   DNA sequence of 5S rDNA included the encoding and non-transcribed spacer (NTS) region for

155   both fish species. The determined sequences were highly conserved. The nucleotide similarities

156   of partial 18S rDNA and 5S rDNA were 100% and 99.99%, respectively, for both fish species.

157   The phylogenic neighbor-joining (NJ) trees based on partial sequences of 18S rDNA and 5S

158   rDNA strongly support the closed relationship of *E. moara* and *E. bruneus* (high bootstrap values

159   of 92 and 100). And genera of the order percomorpha were mostly reconstructed the

160   phylogenetic relationship by partial sequences of 18S rDNA but not 5S rDNA (data not shown).

161   ***FISH analyses***

162   Multiple sites of 18S rDNA by FISH confirmed the data obtained by Ag-staining for NORs.

163   In *E. bruneus*, six positive signals (both active and non-active) were identified, corresponding to

164   metacentric (m) and submetacentric (sm) chromosomes (Fig. 5A). Four hybridization signals

165   were observed on the short arms of submetacentric chromosomes in *E. moara* (Fig. 5B). 5S

166   rDNA and 18S rDNA were found on different chromosomes. Two 5S rDNA sites were located on

167   the arms of a medium-sized acrocentric chromosome pair in both *E. bruneus* and *E. moara* (Fig.

168   6A and B).

169   Telomeric repeats of $(TTAGGG)_n$ showed the typically telomeric signals on both telomeres

170   and/or centromeric region of all chromosomes in *E. bruneus* and *E. moara*. No positive signal

171   was detected at interstitial sites (Fig. 7A and B). Ten chromosome pairs of *E. bruneus* were

172   significantly stronger than the signals of the others (Fig. 7A). However, *E. moara* were

173   characterized by uniform telomeric signals in strength and size (Fig. 7B).

Discussion

175   Our previous study has distinguished *E.bruneus* and *E. moara* to be two species based on

176   the morphometric and skeleton characteristics (Guo et al., 2008). The species-specific

177   differences showed obviously on the bars of the body and stable skeleton characteristics of adult.

178   And the pyloric caeca indicates their different feeding habits and digestive function, which

179   means they could have different ecological niches. Mitogenome and molecular comparisons

180   confirmed *E. moara* to be a valid species of the family Epinephelidae (Liu et al., 2013). Further,

181   we developed a molecular method to differentiate both fish species (Guo et al., 2009). However,

182   the cytogenetic backgrounds and evolutionary situation, which is very important to the

183   cultivation and protection of fish resources, remains unclear for *E. bruneus* and *E. mo*ara. We

184   here comprehensively analyzed the cytogenetic backgrounds, and reconstructed their

185   phylogenetic relationships using molecular cytogenetic analyses.

186   For *E. bruneus* and *E. moara*, species-specific characteristics presented in karyotype,

187   NORs, C-banding and telomere distribution patterns. Karyotype variation appears to parallel

188   speciation events in many groups of vertebrates (Morescalchi et al., 2007; Ruiz-Herrera, Farre &

189   Robinson, 2012). Variations of NOR constituted a strong cytotaxonomic character in fishes

190   (Fujiwara et al., 1998; Galetti, Aguilar & Molina, 2000). Many species in genus *Epinephelus*

191   showed the same karyotypic characteristics, such as karyotype formula and NORs (Wang et al.,

192   2012). However, karyotype formula and NORs were different between *E. bruneus* and *E. moara*

193   (Table 1). Further, interstitial C-bandings were observed in *E. bruneus,* but not in *E. moara*.

194   Similar interstitial heterochromatin was also found in *E. coioides* (Wang et al., 2010) and

195   *Diplectrum radiale* (de Aguilar, 1997). The distribution patterns of $(TTAGGG)_n$ were different

196   obviously between *E. bruneus* and *E. moara*. Cytogenetic differences were inter-specific,

197 because *E. bruneus* and *E. moara* showed a similar geographical distribution (Heemstra &
198 Randall, 1993; Guo et al., 2009), and coupled with heterogeneously morphological
199 characteristics (Guo et al., 2008) and chromosomal structure.

200   The cytogenetic analyses suggested that the lineages of *E. bruneus* and *E. moara* recently
201 derived within the genus *Epinephelus*. Both fish species share a uniform number of
202 chromosomes to other species in the genus *Epinephelus* (Wang et al., 2012). However, they
203 contained more biarmed chromosomes such as metacentric and/or submetacentric chromosomes
204 (Table 1). In fishes, 48 uni-armed chromosome types like acrocentric chromosomes represented
205 the ancestral complement of diploid origin (Ohno, 1974; Vitturi et al., 1991; Sola et al., 2000). In
206 addition, most species in genus *Epinephelus* showed a conserved, NOR-bearing chromosome
207 pair No. 24 (Table 1), while *E. bruneus* and *E. moara* showed additional NORs on chromosome
208 pairs. For most vertebrates, the presence of a single NOR pair seems to be an ancestral character
209 state (Hsu & Pardue, 1975; Schmid, 1978; Galetti, Molina & Affonso, 2006). Both *E. bruneus*
210 and *E. moara* show even more constitutive heterochromatin (related to chromosomal
211 rearrangements or variation) than other species in *Epinephelus* (Sola et al., 2000; Molina et al.,
212 2002; Phillips & Rab, 2001; Wang et al., 2012).

213   Moreover, *E. moara* exhibited more plesiomorphic features than *E. bruneus*. The
214 fundamental number (FN) of *E. bruneus* is larger than that of *E. moara*. Species with a larger FN
215 are more derived in evolutionary terms (Martinez et al., 1989; Ghigliotti et al., 2007).
216 Chromosomal rearrangements and genomic modifications were more obviously in *E. bruneus*
217 compared to those in *E. moara*. Interstitial C-bandings appeared in *E. bruneus* imply the
218 karyotypic rearrangement (Galetti, Aguilar & Molina, 2000), robertsonian rearrangements and/or
219 reciprocal translocations (Eler et al., 2007). In despite of the conservation of (TTAGGG)n
220 sequence and location, slight changes in the telomeric sequences have occurred during vertebrate
221 evolution (Meyne et al., 1990). Uniform telomeric distribution in *E. moara* is similar to other
222 species in *Epinephelus* (Table 1) (Sola et al., 2000; Wang et al., 2012). However, remarkable
223 high repetitions of telomere sequence seems to exist on ten chromosome pairs with stronger
224 signals in *E. bruneus*, which appears to involve in complex homologous or/and non-homologous

225   recombination.

226   18S rDNA could be simultaneously applicable in the taxonomic and evolutionary analyses
227   of grouper. The 5S rDNA seems to be not suitable in the phylogenetic resolution, because the
228   order percomorpha in the NJ trees were not recovered as monophylum. Distribution patterns of
229   5S rDNA of *E. bruneus* and *E. moara* are similar to other species, while that of 18S rDNA were
230   different among species in genus *Epinephelus* (Sola et al., 2000; Wang et al., 2010, 2012). In
231   addition, the different distributions of highly conserved18S rDNA and telomere suggest the
232   distinct genomes and evolutionary situation of the closely related species *E. bruneus* and *E.*
233   *moara*.

234   In summary, many useful cytogenetic charateristics are available to distinguish *E. bruneus*
235   from *E. moara*, such as karyptypes, NORs, C-banding, 18S rDNA and telomere $(TTAGGG)_n$
236   distribution patterns. Moreover, the lineage of *E. bruneus* and *E. moara* seems to be derived
237   recently, and *E. moara* exhibits more plesiomorphic features than *E. bruneus*. Molecular
238   cytogenetic analyses could be applicable in identification of closely related species and
239   reconstruct their phylogenetic relationships in groupers.

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387

Figure 1

Specimen of adult *E.bruneus* and *E.moara.*

Figure 1 Specimen of adult *E. bruneus* (Bloch, 1973) [standard length (LS), 550 mm]
and *E. moara* (Temminck & Schiegel, 1842) (LS, 650 mm) (Xiamen, Fujian, China.
M.L.Guo).

Figure 2

Chromosome metaphase and corresponding karyotype of *E. bruneus* and *E. moara.*

Figure 2 Chromosome metaphase (left, Giemsa staining) and corresponding karyotype (right) of *E. bruneus* (A and B) and *E. moara* (C and D). Scale bar=5 μm. a: acrocentric, m: metacentric, sm: submetacentric.
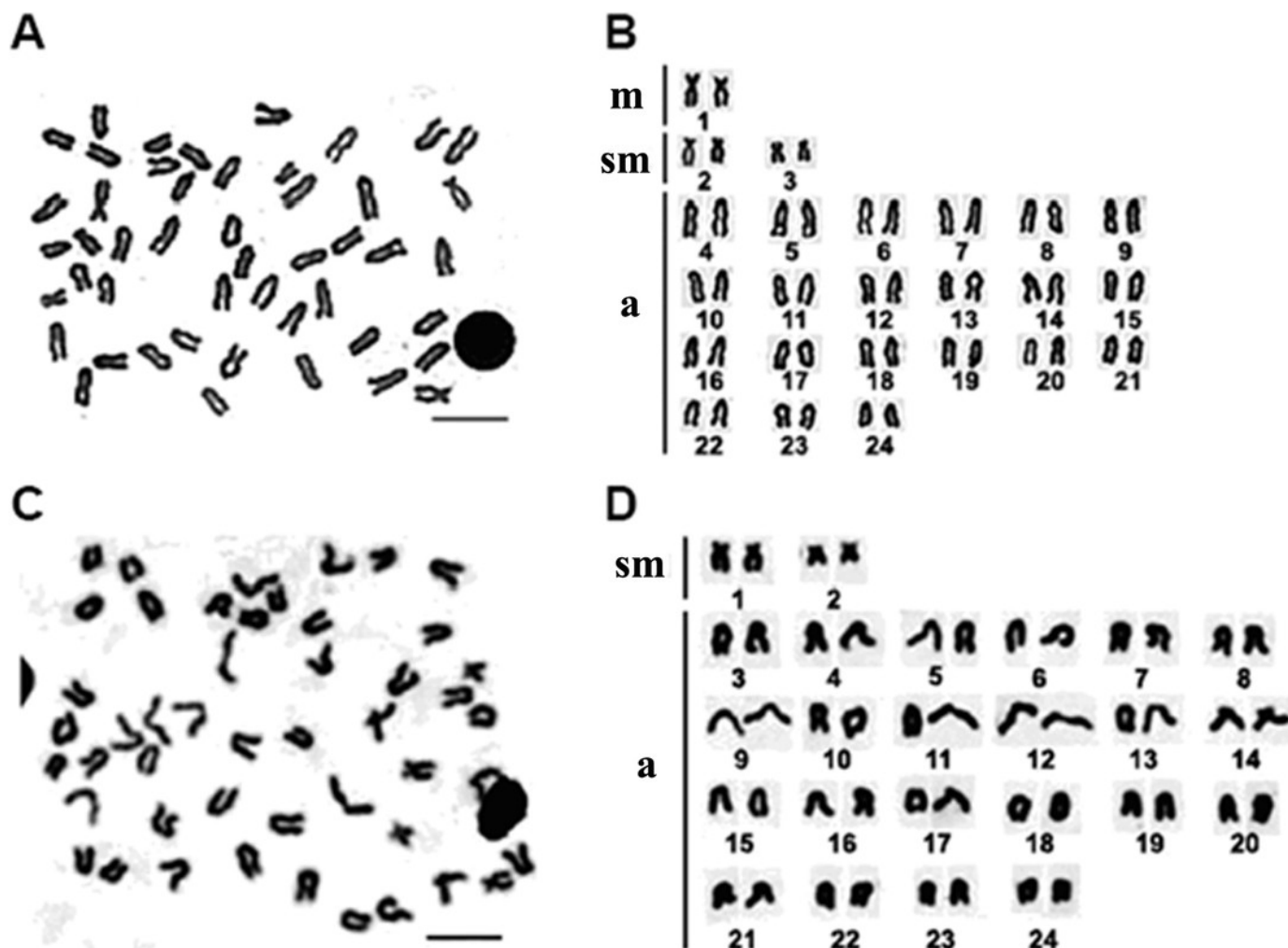
Figure 3

Ag-NORs characteristics of *E. bruneus* and *E. moara* with silver staining.

Figure 3 Ag-NORs characteristics of *E. bruneus* (A) and *E. moara* (B) with silver staining. Thick black arrows indicate the chromosomes No.9 in length, thin black arrows represent the chromosomes No.24 in length, and hollow arrows show the chromosomes No.1 in length. Scale bar=5 μm.
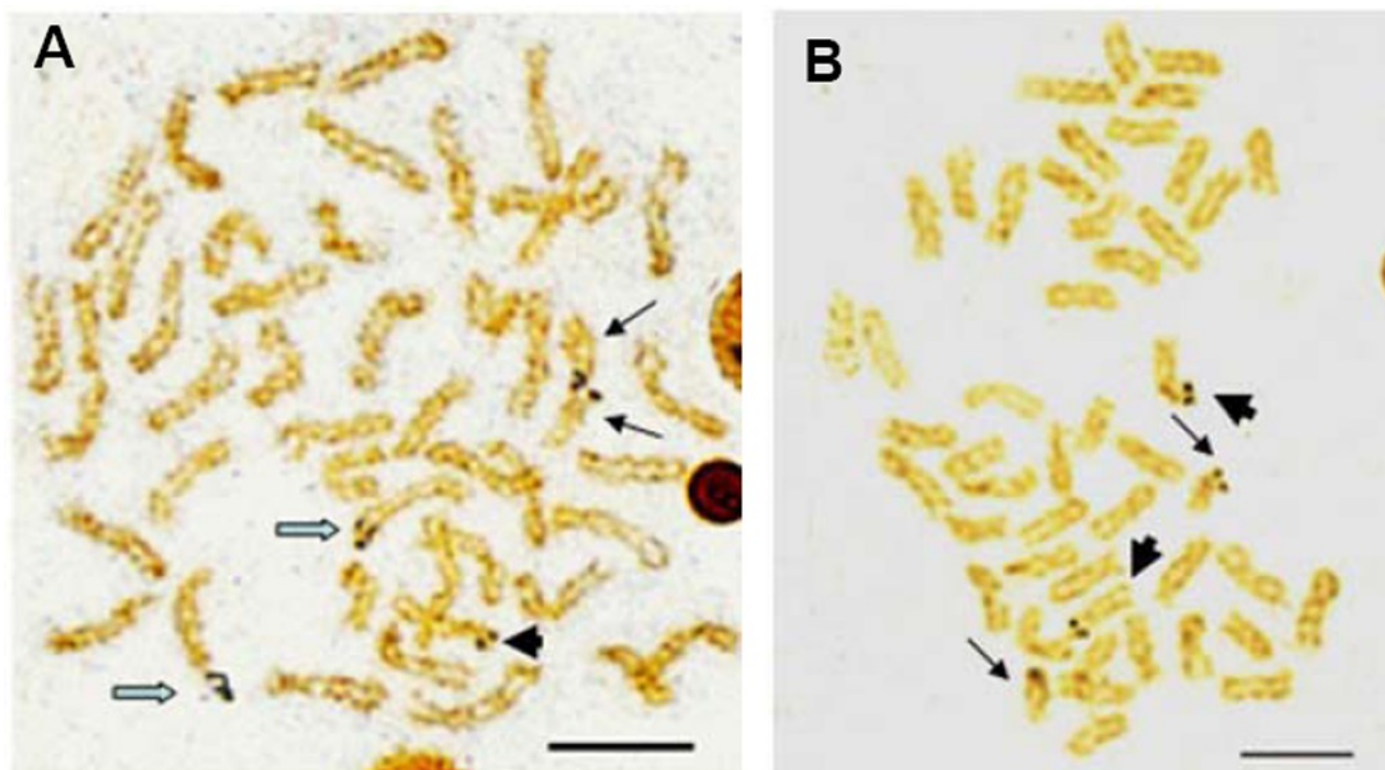
Figure 4

C-banding patterns of *E.bruneus* and *E.moara*.

Figure 4 C-banding patterns of *E.bruneus* (A) and *E.moara* (B). Heterochromatic blocks were observed in the interstitial region of the long arms of acrocentric chromosome pair No.12 in *E. bruneus* (white hollow arrows). Heterochromatin C-bands was consistent with the positive Ag-NORs sites on chromosome pair No.2 in both fish species (Thick black arrows). Heterochromatin C-bands were indiscernible for three pairs of chromosomes (Red arrows). Other arrow annotated with Fig.3. Scale bar=5μm.
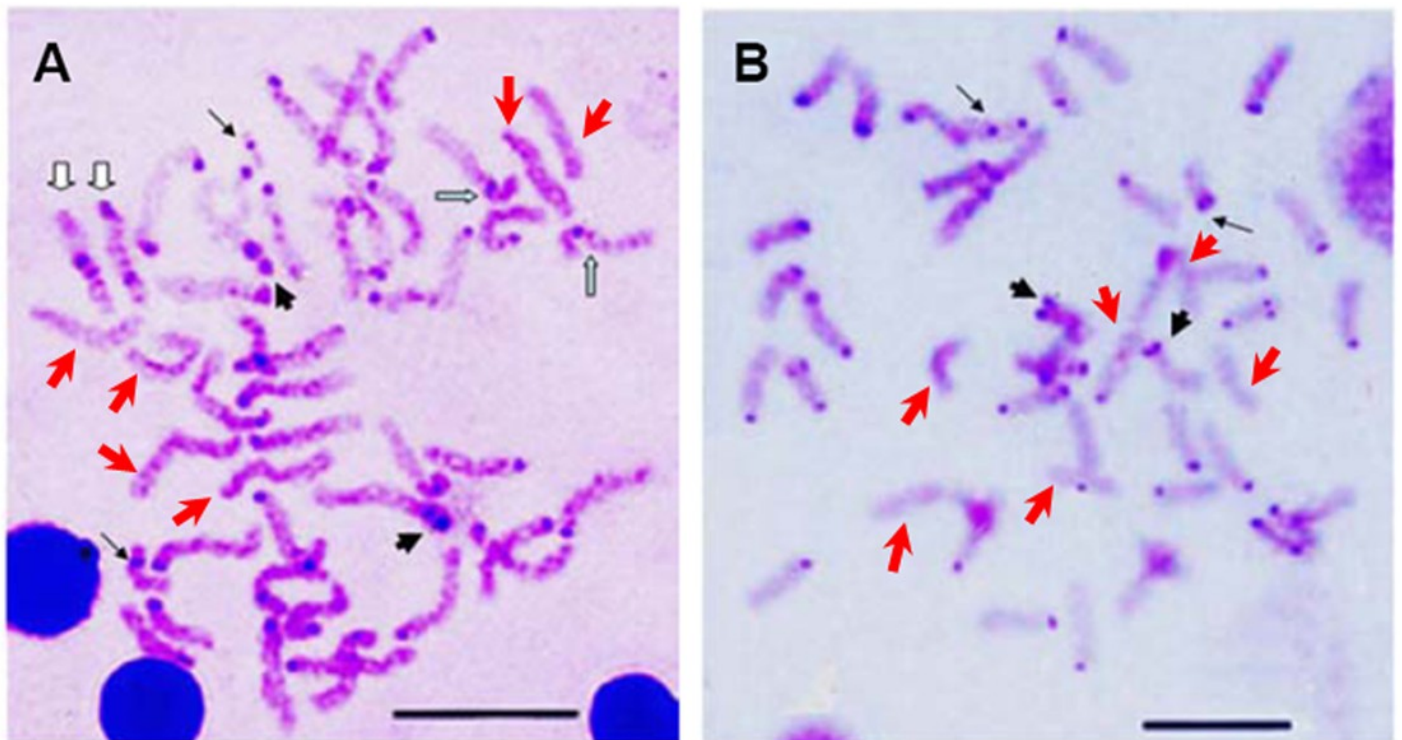
Figure 5

Distribution of 18S rNDA on chromosomes of *E. bruneus* and *E. moara* by FISH.

Figure 5 Distribution of 18S rNDA on chromosomes of *E. bruneus* (A) and *E. moara* (B) by FISH . White arrows indicate the biarmed chromosome, pairs No.2, No.9 and No.24 in length, in both fish species. Scale bar=5μm.
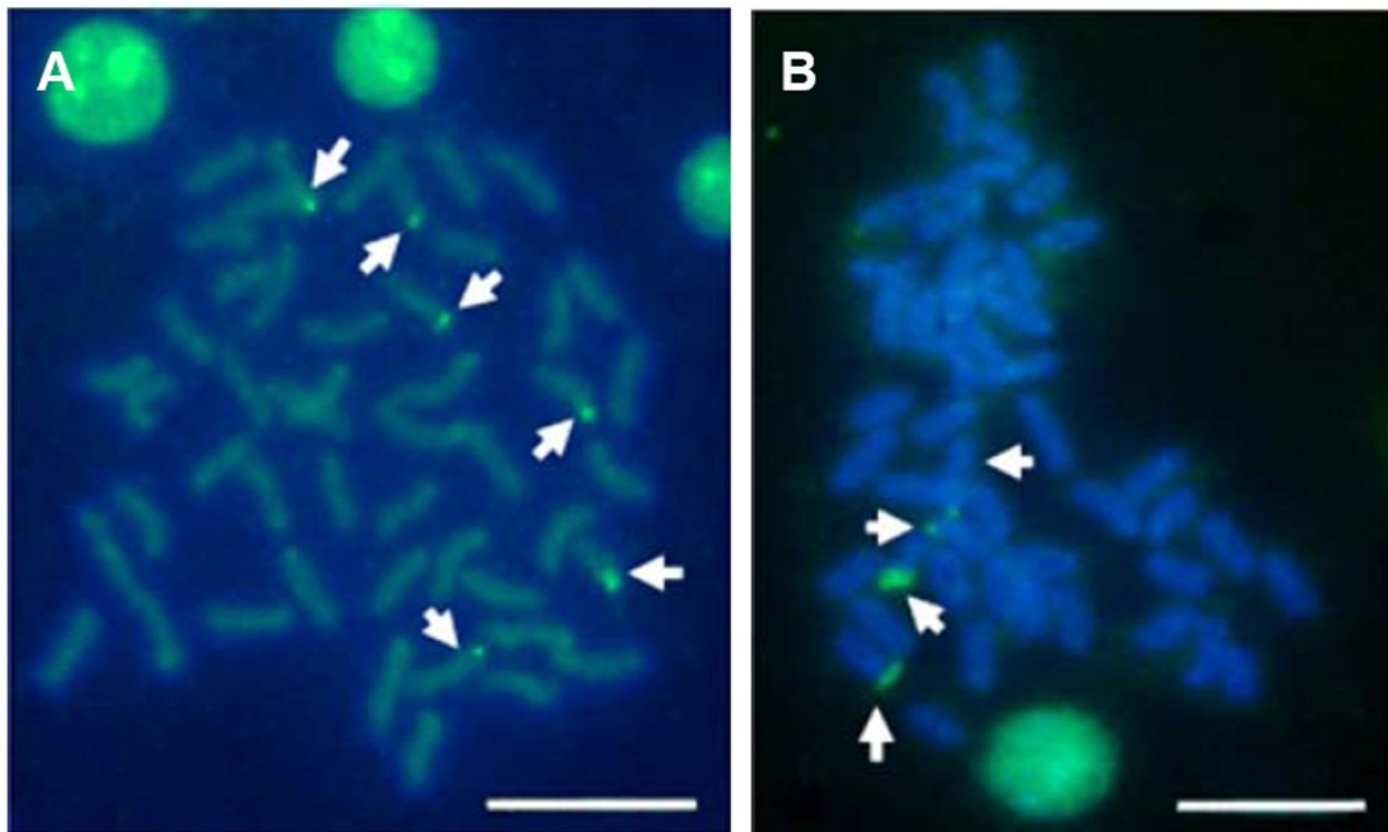
Figure 6

Localization of 5S rNDA on chromosomes of *E. bruneus* and *E. moara* by FISH.

Figure 6 Localization of 5S rNDA on chromosomes of *E. bruneus*(A) and *E. moara* (B) by FISH. White arrows indicate the two 5S rDNA clusters located on the arms of one of acrocentric chromosome pair. Scale bar=5μm.
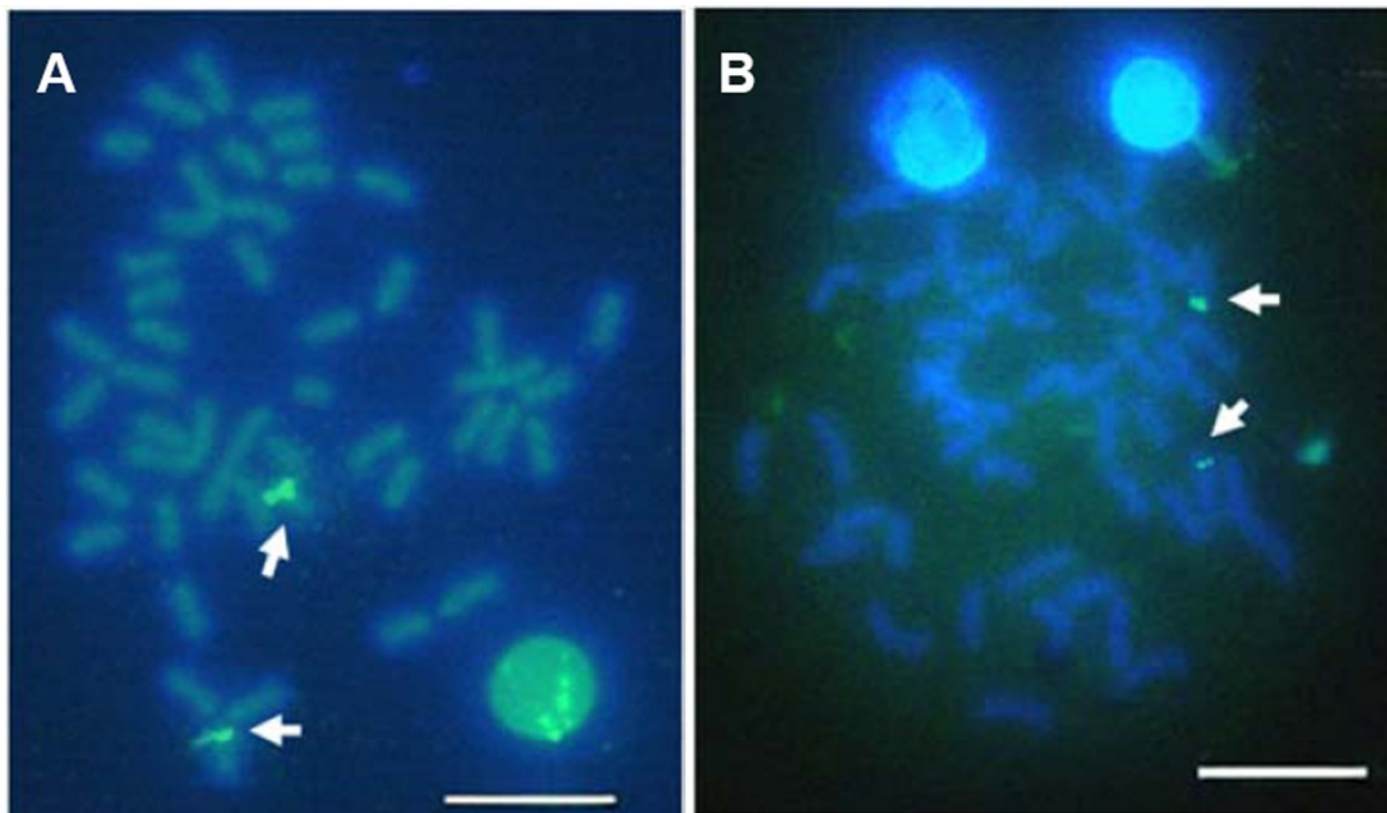
Figure 7

Distribution of telomeric (TTAGGG)n sequence on chromosomes of *E. bruneus* and *E. moara* by FISH

Figure 7 Distribution of telomeric (TTAGGG)n sequence on chromosomes of *E. bruneus* (A) and *E. moara* (B) with telomeric (TTAGGG)n sequence using FISH. Red arrows indicate chromosomes with significantly stronger and larger telomeric signals than others in *E. bruneus*. Scale bar=5μm.

Availble cytogenetic data of the genus *Epinephelus*.
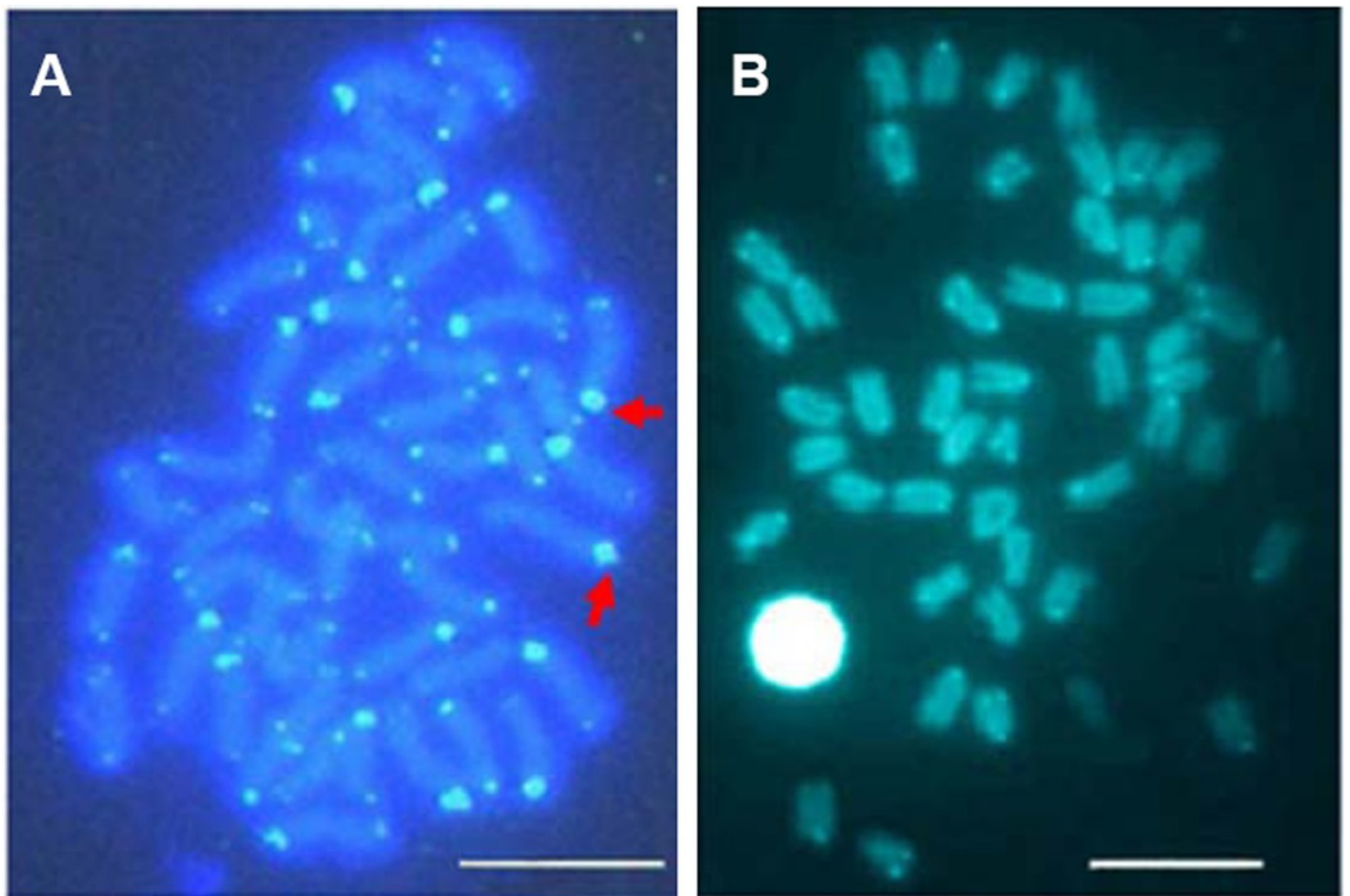
Table 1. Available cytogenetic data of the genus *Epinephelus*: diploid numbers (2 n), karyotype formula, fundamental number (FN), nucleolar organizer regions (NORs) and C-banding.

Species	2n	Karyotype formula	FN	NORs	C-banding	Reference
E. adscencionis	48	48a	48	SCR(24)* TR(2)	C(1-24)	Molina, Maia-Lima & Affonso, 2002
E. akaara	48	5st+43a	48	/	/	Wang et al., 2004
E. alexandrinus	48	48a	48	SCR(24) *	NC(1-23), SCR(24) *	Martinez et al., 1989
E. awoara	48	48a	48	SCR(24)	NC(1-23), SCR(24) *	Wang et al., 2012
E. bruneus	**48**	**2m+4sm+42a**	**54**	**SCR(24, 9, 2)**	**NC (?)** **C (?)** **SCR(24,9,2)** **SA(2)** **IR (?)**	**Present study**
E. caninus	48	48a	48	SCR(24)	/	Rodríguez-daga, Amores & Thode, 1993
E. coioides	48	2sm+46a	50	EA(24)	C(1-11, 13-24) SCR(5,12) EA(24)	Wang et al., 2010
E. diacanthus	48	2sm+46a	50	/	/	Natarajan & Subrahmanyan , 1974
E. fario	48	4m+6sm+4st+34a	62	/	/	Zheng et al., 2005
E. fasciatomaculosus	48	48a	48	SCR(24)	/	Li & Peng, 1994
E. fasciatus	48	48a	48	SCR(24)	/	Li & Peng, 1994
E. fuscoguttatus	48	2sm+46a	50	/	/	Liao et al., 2006
E. guaza	48	48a	48	SCR(24) *	NC(1-23) SCR(24) *	Martinez et al., 1989
E. guttatus	48	48a	48	/	/	Medrano et al., 1988
E. lanceolatus	48	4st+44a	48	/	/	Wang et al., 2003
E. malabaricus	48	48a	48	SCR(24) * ? (5)	C(1-24) EA(24)	Zou et al., 2005
E. marginatus	48	48a	48	SCR(24), TR(2)	C(1-24) SCR(24) * TR(2)	Sola et al., 2000
E. merra	48	4m+6sm+4st+34a	62	/	/	Zheng et al., 2005
E. moara	**48**	**4sm+44a**	**52**	**SCR(24, 9)**	**NC(?)** **C(?)** **TR(?)**	**Present study**
E. sexfasciatus	48	2sm+46a	50	/	/	Chen et al., 1990

E. tauvina	48	2sm+46a	50	/	/	Raghumath & Prasad , 1980

Notes.

2n, diploid number; a, acrocentrics; C, centromeric; EA, nearly the entire arm; FN, fundamental number; IR, interstitial region; m, metacentrics; NC, almost indiscemible; NORs, nucleolar organizer regions; SA, short arm; sm, submetacentrics; st, subtelocentrics; SCR, subcentromeric region; TR, telomeric region; /, not available; ?, not mentioned or measured; Numbers in parentheses, the number of chromosome pairs; * Data estimated from illustrations and text in the respective papers.